Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Histological and Histochemical Characters of the Pancreas in the Adult Indigenous Gazelle (*Gazella subgutturosa*).

Luay O. Hamza*

Department of Anatomy and Histology, College of Veterinary Medicine,University of Baghdad,Iraq

Received: 18 Mar 2018 Revised: 20 Apr 2018 Accepted: 02 May 2018

***Address for correspondence**
Luay O. Hamza
Department of Anatomy and Histology,
College of Veterinary Medicine, University of Baghdad,Iraq
E.mail: luay.hamza@hotmail.com / Luay.o@covm.uobaghdad.edu.iq

ABSTRACT

The investigation of histological and histochemical characters of the pancreas in indigenous Gazelle was carried out during April into December 2017.The tissue samples were processed by paraffin technique and stained with the hematoxilin and eosin,Masson's trichrom, combined Periodic Acid Schiff and Alcian blue stains and Gomori stain. The pancreas is covered by a thin connective tissue capsule mainly of blood vessels and few collagen fibers, as well as, rich in adipose tissue that very huge quantity to the septa. The secretory units of the exocrine portion are tubulo-acinar units with centeroacinar cells and separated by thin connective pad, it consist of a simple pyramidal cells converging toward a central narrow lumen, which resting upon a basal lamina supported by thin fibers. The duct system of pancreas is divided into intercalated duct, intralobular duct and interlobular duct.The islets of Langerhans appear as a pale area within the secretory units, oval or irregular, some islets were small others were large. The beta cells when stain with Gomori's are appear as faint blue which were medium size and dispersed mostly in the periphery of the Islet, while the alpha cells stain with red color by Gomori's stain which were smaller in size and distributed mostly in the interior of Islets.The present experimental results conclude that the histological observation had the same structure with other domestic ruminant except that the secretory units in the gazelle pancreas are tubulo-acinar with centeroacinar cells, the position of the beta cells in the islets of Langerhans is peripheral and that of alpha cells is mostly in the interior of Islets.

Keywords: Histology, Histochemical, gazelle, pancreas, islets of Langerhans.

INTRODUCTION

The pancreas in donkey was covered by connective tissue capsule which consisted of collagen fibers mainly and a few elastic fiber and many connective tissue septa extended from the capsule into the parenchyma of the pancreas dividing it into complete and incomplete lobules (Masaad, 2007). The pancreas is encapsulated and lobulated compound tubulo-acinar gland encompassing of exocrine and endocrine secretary portions in domestic animals

(Stinson and Calhoun, 1981). In camel, the pancreas was tubulo-acinar with the acinar portion more prominent which enclosed by a thick connective tissue capsule that rich in adipose tissue, reticular fibers, collagen fibers, elastic fibers, blood vessels and nerve fibers (Sultan, 1999; Mustafa, 2007). In domestic animals the pancreas identified by three types of endocrine cells in the pancreas, alpha cells (A cells), beta cells (B cells) and delta cells (D cells) (Bloom and Fawcett, 1994). In some species the granules of beta cell were only lightly different in size and electron density from those of alpha cell but in the dog and some other species they showed crystalloid structure of variable shapes embedded in a pale matrix (Dellmann and Brown, 1981; Bloom and Fawcett, 1994). The *Gazella subgutturosa* is widespread species; free living in northern Azerbaijan, eastern Georgia, some portion of every Iran and in the north to southern regions of Iraq (Murtskhvaladze et al., 2012). The *Gazella subgutturosa* has saved in several nature reserves located in many provinces including, the district of AL Madaen in the outskirts of the capital Baghdad, an area of (157) acres, where ksiab-reservoir to save the species and varieties from the risk of Extinction (Hamza and Al-Mansor, 2017). A present study was aimed to investigate the histology of the acinar and endocrine parts (islets of Langerhans) and special histochemical identification for staining reaction to the cells and gland in each part of the pancreas in the indigenous gazelle *Gazella subgutturosa*.

MATERIALS AND METHODS

Six male adult animals were obtained from (AL-Madaen Animal Reservoir) in Baghdad-Iraq. The study was conducted in department of anatomy, histology at College of Veterinary Medicine-University of Baghdad, during a period extended from April into December 2017. After animal slaughtering small pieces of tissue were taken from three regions of every pancreas; the body, the right lobe and the left lobe were taken and fixed in 10% buffered formalin saline (Carson and Hladik, 2009). The specimens were processed upgrading with ethanol alcohol for paraffin technique and sectioned serially at (5-7) µm. The prepared sections were stained with the Hematoxylin and Eosin, Masson's trichrom, combined PAS and Modified Alcian blue stains and Aldhyde fuchsine stain (Vacca, 1985; Bancroft et al., 2008). The histological observations were pictured by using the color digital camera (Kiernan, 2008).

RESULTS AND DISCUSSION

The present study showed that in gazelle the pancreas was composed of exocrine and endocrine parts enclosed by a thin connective tissue capsule which was rich in adipose tissue, blood vessels and few collagen fibers (Figure 1). Very thick connective tissue septa were extended from the capsule into the parenchyma of the pancreas making the gland as highly lobulated organ (Figure 1). The adipose tissue was very huge quantity to the septa (Figure 1). This observation is similar to that in other animals and man (Stinson and Calhoun, 1981; Bloom and Fawcett, 1994; Sultan, 1999; Dhoolappa et al., 2004). The exocrine portion of the pancreas was made up of secretory units and duct system. The secretary units were tubulo-acinar units with centeroacinar cells and separated by delicate connective pad (Figure 2 &3). The secretory units of the pancreas of the gazelle as seen in camel (Sultan, 1999) are tubuloacinar with the acinar portion more prominent. This is slightly different from the exocrine portion of the ruminant pancreas (Stinson and Calhoun, 1981) since the later showed dominance of the tubular portion. However, according to the present study the secretory units of the pancreas of the donkey and horse (Sisson and Grossman, 1964) were tubulo-alveolar types. The present result showed that the secretary units consisted of a simple pyramidal cells converging toward a central narrow lumen. These cells were resting upon a basal lamina supported by delicate fibers (Figure 2 &3). The nuclei of secretory cells were spherical in shape and located near the basal surface and the cytoplasm of the cell contained zymogen granules, the exocrine part of pancreas were positive for PAS stain and negative for Alcian blue (Figure 3). These results are similar to the observation of Stinson and Calhoun (1981) in goats, Mukherjee et al. (1986) in sheep, Sultan (1999) in the camel and Dhoolappa et al., (2004) in the Indian donkey. The present investigation agrees with the previous worker (Singh, 1980; Singh and Singh, 1980, and Mukherjee et al., 1986) that in pancreas there are three acinar cell types: active, exhausted and resting acinar cells. The cells located in the lumen of the acini were identified as centeroacinar cells (Figure 3) that beginning of the intercalated duct which constituted the

first part of the duct system. Within parenchyma of pancreas the duct system included intercalated duct, intralobular duct and interlobular duct (Figure 4, 5& 6), this result was similar to the findings of Bloom and Fawcett (1994) in humans, Lone et al., (1988)in sheep, Sultan (1999) in the camel and Dhoolappa et al., (2004) in the Indian donkey. Gemmel and Heath (1973); Sultan (1999); Dhoolappa et al., (2004) and the present study reported that the intercalated duct lined by low cuboidal cells. This disagrees with the findings of Lone et al., (1988) in sheep. Both the intra lobular and inter lobular ducts were lined by simple cuboidal cells supported by a basal lamina and collagen fibers, the interlobular duct was found between the lobules in the connective tissue septa (Figure 4, 5& 6).

Similar observation recoded by Stinson and Calhoun (1981); Lone et al., (1988) and Sultan (1999). Nevertheless, Gemmel and Heath (1973) have found that the intralobular duct is lined by columnar cells and not cuboidal cells. Lone et al. (1988) and Dhoolappa et al., (2004) stated that in the interlobular duct there are goblet cells interspersed amongst the columnar lining cells in sheep and Indian donkey respectively. On the other hand, Bloom and Fawcett (1994) have reported that in addition to the goblet cells, small mucous glands are present in the connective which supported the inter lobular duct. In the present investigation, neither the goblet cells nor the small mucous glands were observed in relation to the lining epithelium or the surrounding connective tissue of the interlobular duct of the pancreas of the gazelle, this is in accord with the previous work of Masaad (2007) in dog, cat, monkey and guinea pig, Sultan (1999) and Siddig (2002) in the camel and Dhoolappa et al., (2004) in the Indian donkey and Mustafa (2007); Gemmel and Heath (1973) observed the presence of goblet cells in the ventral pancreatic duct of the donkey and Mcminn and kugler (1961) in the monkey and guinea pig. Absence of goblet cells from the pancreas of the gazelles not a unique feature of this species alone but is also observed in cats and dogs (Mcminn and kugler, 1961).The islets of Langerhans which composed of the endocrine part of the gazelle pancreas appeared as many of pale stained areas among the exocrine part of pancreas which were round, oval or irregular, some islets were small others were large (Figure 1, 8, 9 & 10).

The islets had a rich vascular supply, some blood capillaries were enlarged forming a large sinus-like structure which with Gomori's stain, the cells of islets were stained faint blue and other red in color. The blue color cells representing beta cells which were medium size with prominent nucleus and were dispersed mostly in the periphery of the Islet. Those cells of red color representing the alpha cells which were smaller in size and distributed mostly in the interior of Islets (Figure 9 &10). This result confirms the findings of Alani (1987) and Sultan (1999) in the camel and Mukherjee et al., (1988) in sheep. Sultan (1999) claimed that there are interlobular islets of Langerhans in the camel pancreas. On the other hand the pancreas of the donkey showed no such interlobular islets of Langerhans. I believe that this unusual phenomenon does occur only occasionally and not on a regular basis. Another extraordinary feature of the pancreas of the camel was the presence of some ducts close to the islets of Langerhans (Al-Ani, 1987; Sultan, 1999).

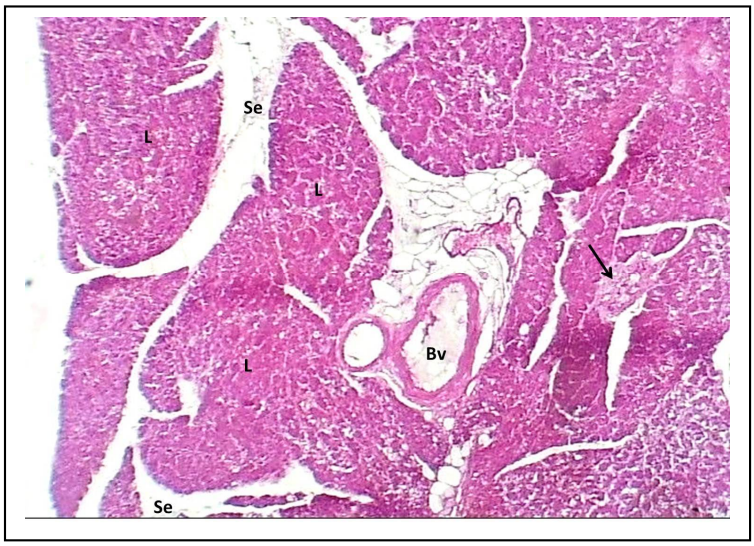
ACKNOWLEDGEMENTS

Author would like to acknowledge all technical staff your assistances in department of Anatomy, Histology &Embryology-College of veterinary Medicine at University of Baghdad.

REFERENCES

1. AL-Ani M I. (1987). Light microscopy of the camel (Camelus dromedaries) islets of langerhans. Journal of Sugar Beet Research. 18(1): 75-80.
2. Bancroft JD, Marilyn G. (2008). Theory and practice of histological technique 6th Ed., Elsevier Limited, London.168-173.
3. Bloom W. and Fawcett D W. (1994). A text book of histology. 10th. Edn., Philadelphia, W. B. Saunders Co., PP: 726-742.

4. Carson F L, and Hladik Ch. Histotechnology. (2009). A Self-Instructional Text. Third Ed., Chicago ASCP Press, P: 400.
5. Dellmann and Brown (Eds). (1981). Second Edition. Lea and Febiger: Philadelphia. Dellmann and Brown (Eds) Second Edition. Lea and Febiger: Philadelphia.
6. Dhoolappa M., Pawar Ashok Ramakarishna V and Gadre K M. (2004). Gross and histomorphology of pancreas in donkey. Indian Journal of Animal Science. 74(9): 926-929.
7. Gemmel R T. and Heath T. (1973). Structure and function of the biliary and pancreatic tracts of the sheep. Journal of Anatomy. 115(2): 221-236.
8. Hamza L O. and Al-Mansor N A. (2017). Histological and histochemical observation of the small intestine in the indigenous gazelle (Gazella subgutturosa). Journal of Entomology and Zoology Studies. 5(6): 948-956.
9. Kiernan J A. (2008). Histological and histochemical methods. 4th Ed., Scion Publishing Ltd. P: 606.
10. Lone T K., Prasad G. and Sinha R D. (1988). Histological studies on the duct system of chatangpuri sheep. Indian Journal of Animal Sciences. 58(10):1190-1193.
11. Masaad, G. A. M. (2007). A comparative morphological study on the pancreas of the Dromedary (Camelus Dromedarius) and the Donkey (Equus Asinus). Msc thesis, Faculty of Veterinary Medicine.University of Khartoum.
12. Mcminn R M H. and Kugler J H. (1961). The glands of the bile and pancreatic ducts. Auotoradiographic and histochemical studies. Journal of Anatomy. 95: 1-11.
13. Mukherjee G., Singh L P., Barnwal A K., Roy A K. and Sharan A. (1986). Acinar cell-types of sheep pancreas. Indian Journal of Animal Sciences. 56 (9): 930-934.
14. Mustafa G A. (2007). A comparative morphological study on the pancreas of the dromedary (Camelus dromedarius) and the donkey (Equus asinus). A thesis Submitted in partial fulfillment of the Requirement for the degree of master of Veterinary Science.
15. Murtskhvaladze M, Gurielidze Z, Kopaliani N, and Tarkhnishvili D. (2012). Gene introgression between Gazella subguturrosa and G. marica: limitations of maternal inheritance analysis for species identification with conservation purposes. Acta. Theriologica. DOI 10:1007.
16. Siddig, R. A. (2002). Morphology and morphometry of liver of the camel. (Camelus dromedarius). M. Sc. Thesis, University of Khartoum. 2002.
17. Singh L P. (1980). Note on acinar cell-type in the pancreas of domestic animals. Indian Journal of animal Science. 50 (9): 769-772.
18. Singh R. and Singh L P. (1980). Note on the exocrine parenchyma of the pig pancreas. Indian Journal of Animal Science. 50 (9): 790792.
19. Sisson S and Grossman J D. (1964). The Anatomy of the Domestic Animals. Fourth Edition. W. B. Sunders Company. Philadelphia and London.
20. Stinson Al W. and Calhoun M L. (1981). Digestive system. In: Textbook of Veterinary Histology. Dellmann, H. D. Endocrine system. In: Textbook of Veterinary Histology.
21. Sultan H S. (1999). Some morphological studies on the pancreas of the camel (Camelus dromedarius). M. Sc. Thesis, University of Khartoum.
22. Vacca L L. (1985). Laboratory Manual Histochemistry, raven press. Book, Ltd, New York.



Figuer1. Histological section of pancreas shows: Capsule (Ca), lobule (L), septum (Se), blood vessel (Bv) & island of Langerhans (Arrows). 40x. H&E stain

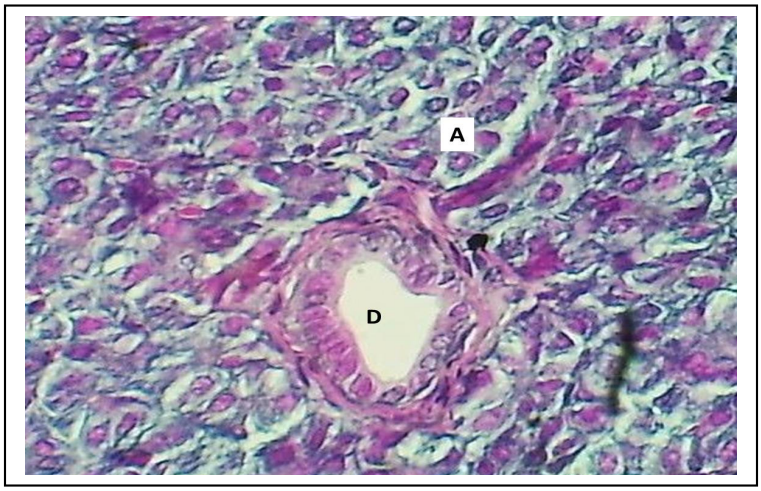


Figure 2. Magnified histological section of pancreas shows: Acini (A) & intra lobular duct (D). 400x. Gomori stain

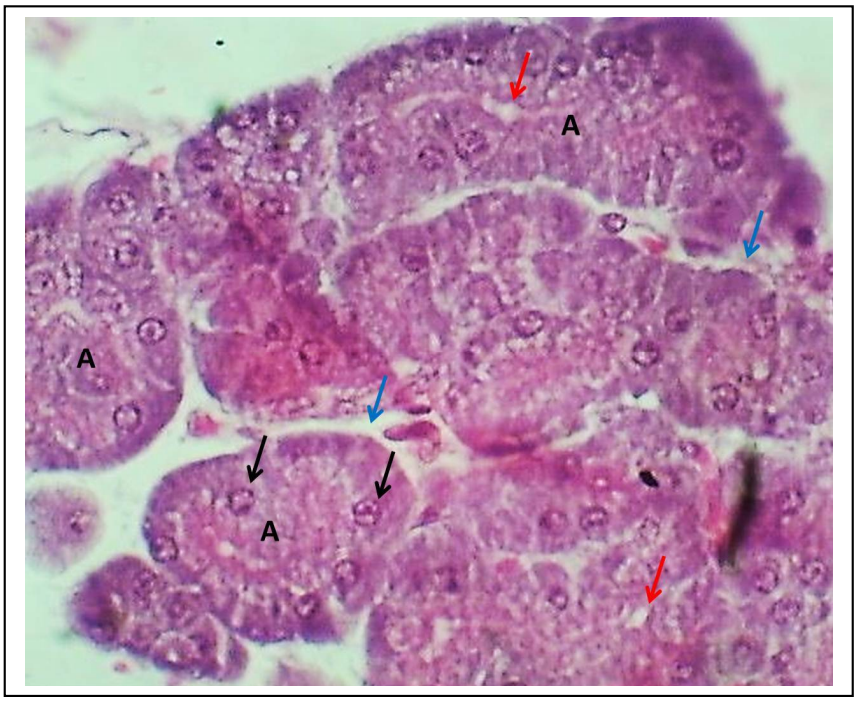


Figure 3. Magnified histological section of pancreas shows: Acini (A), nuclei (black arrow), delicate C.T. (blue arrow) & centeroacinar cells (Red Arrows). 400x. PAS stain

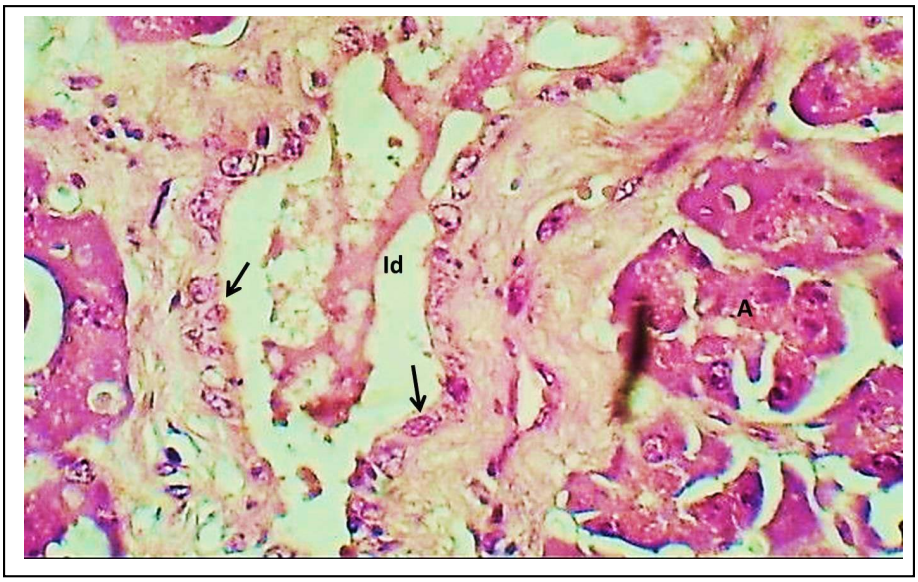


Figure 4: Magnified histological section of pancreas shows: Acini (A), simple cuboidal epithelium of intra lobular duct (black arrow), lumen of intra lobular duct (Id). 400x. H&E stain

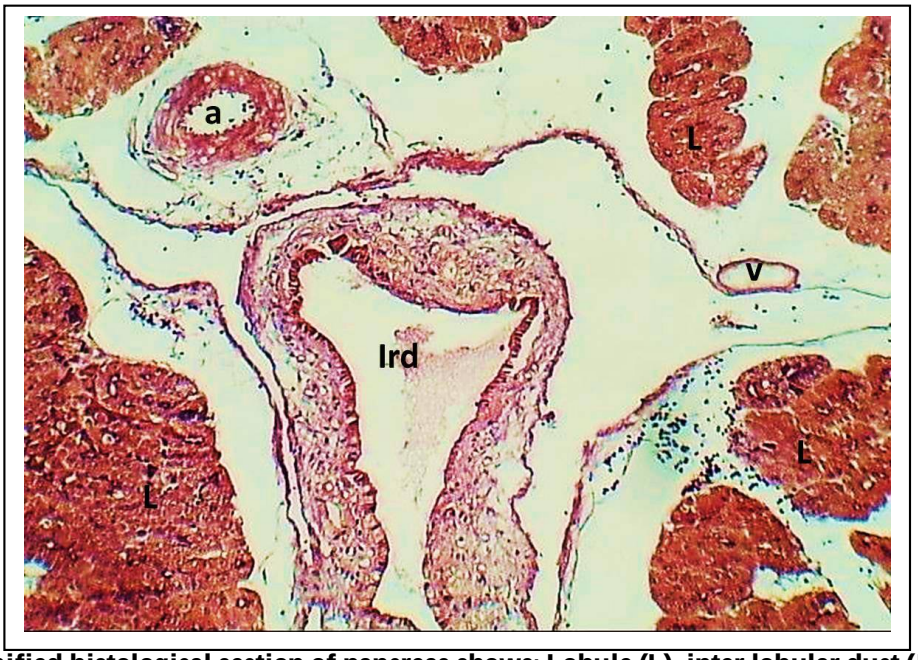


Figure 5. Magnified histological section of pancreas shows: Lobule (L), inter lobular duct (Ird), artery (a) & vein (v). 400x. Gomori stain

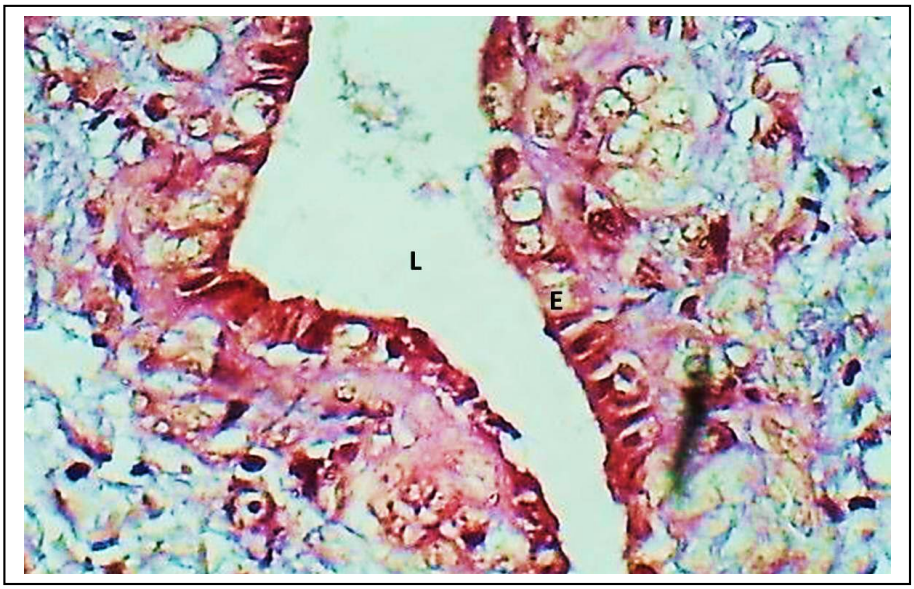


Figure 6. Magnified section of inter lobular duct shows: lumen of duct (L) and epithelium (E). 400x. Aldehyde fuchsine stain

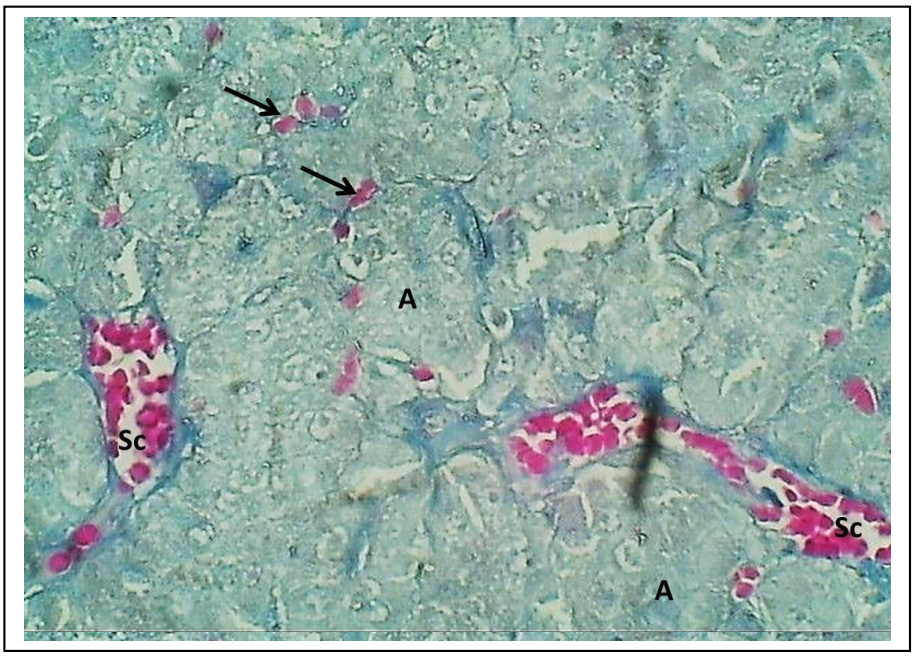


Figure 7. Magnified histological section of pancreas shows: Acini (A), , sinusoidal capillary (Sc) & capillaries (arrows) . 400x. Masson Trichrom stain

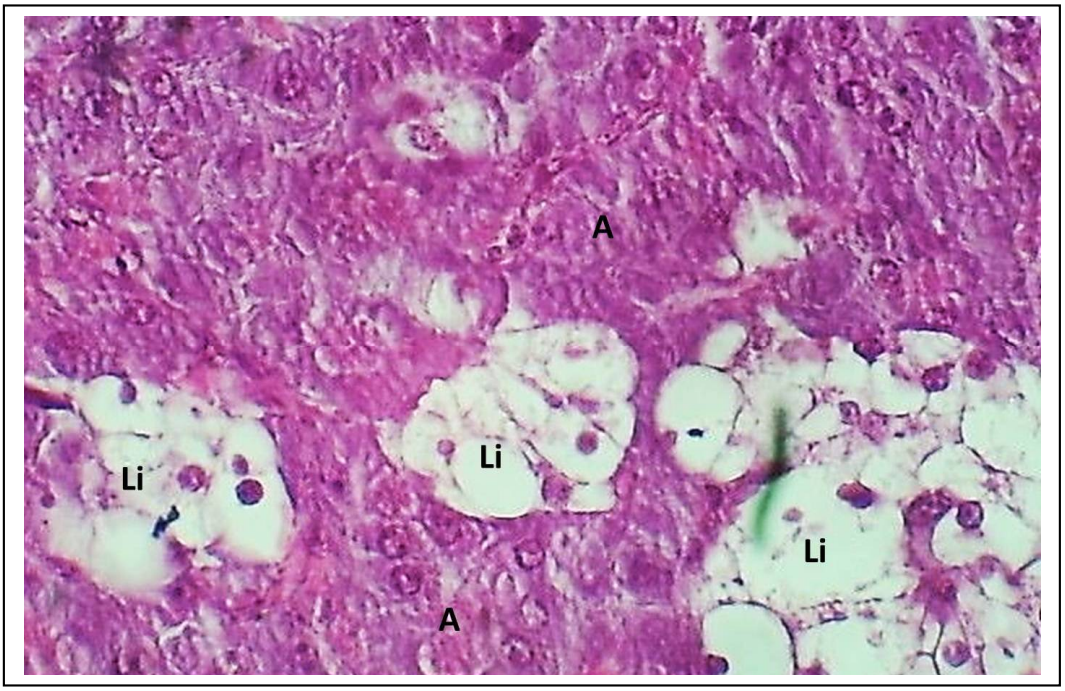


Figure 8. Magnified section of pancreas shows: Acini (A) & Langerhans islet (Li). 400x. H&E stain

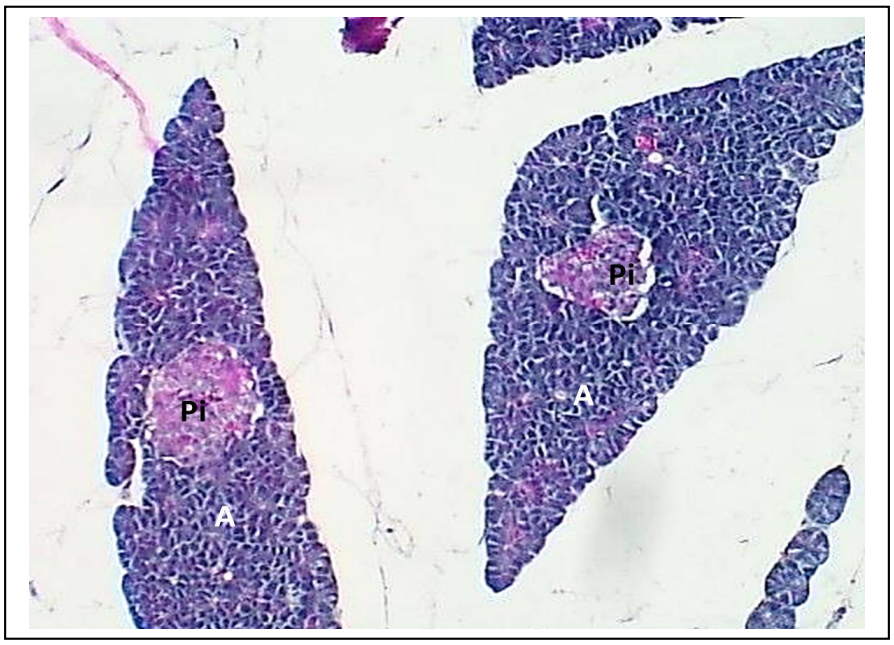


Figure 9. Transverse histological section of pancreas shows: Acini (A) & Langerhans islet (Pi). 100x. Gomori stain.

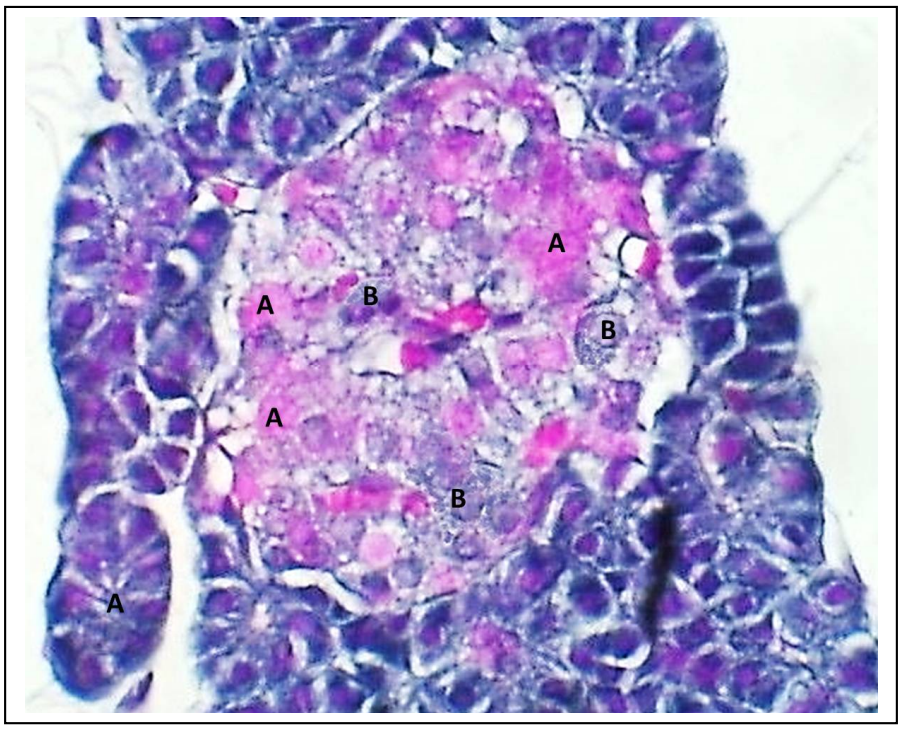


Figure 10. Magnified section of pancreas shows: Acini (A) & Alpha cells, Delta (A) & beta cells (B). 400x. Gomori stain

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Pharmacokinetic and Pharmacodynamics Interactions between Orally Glimepiride and Gemfibrozil in Healthy Dogs

Mays U.Hashim and Orooba M.S.Ibrahim*

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.

Received: 18 Mar 2018 Revised: 20 Apr 2018 Accepted: 02 May 2018

***Address for correspondence**
Orooba M.S. Ibrahim
Department of Physiology and Pharmacology,
College of Veterinary Medicine,
University of Baghdad,Iraq
E.mail: oroobam2000@gmail.com

ABSTRACT

This study is the first to examine the possibility of interaction in the blood circulation using glimepirid and gemfibrozil as representative in healthy dogs model, and identify changes. The current study design required the dogs(n=6) for Pharmacokinetics and pharmacodynamics.In group A, the Pharmacokinetics of glimepiride (0.05mg/kg/p.o.) was studied. Group B were administered with gemfibrozil (8.5 mg/kg/p.o.) and glimepiride (0.05mg/kg/p.o.) 30 minutes later. Group C, the animals were administered with gemfibrozil (8.5 mg/kg/p.o.) for 7 consecutive days. On the 8th day of 30 minutes after the gemfibrozil (8.5 mg/kg) administration, Glimepiride (0.05mg/kg/p.o.) was administered. In all groups the blood samples were collected at time intervals of 0, 0.15, 0.30,1, 2, 4, 6, 8, 12, 24 hours and the drug concentrations were estimated using HPLC assays and glucose levels estimated using GOD-POD method. Increase in AUC and C_{max} in group B and C (3.66 and 4.72 µg/ml) and (33.25 and 47.46 µg/ml*h) respectively, indicates the improved bioavailability of glimepiride in presence of gemfibrozil. Furthermore (CL) and (Vd) appeared reduce in the presence of gemfibrozil (149.4 and 104.07 ml/min) and (51.91 and 39.92 ml) in group B and C respectively. We studied the influence of gemfibrozil on the pharmacodynamics of glimepiride in healthy dogs. Interaction of glimepiride with single and multiple dose of gemfibrozil produced a high percentage of glucose reduction at 4 hr. In conclusion the combination of glimepiride with gemfibrozil led to the enhancement of bioavailability of glimepiride by inhibiting the CYP2C9 enzyme lead to produce hypoglycemic effect with peak plasma concentration effect at 4 hours in dogs.

Key words: gemfibrozil**,** glimepiride, interaction**,** glucose, reduction

INTRODUCTION

Drug-drug interaction (DDIs) is a pharmacological or clinical reaction to the administration of a drug regimen different from that predictable from the known effects of the two agents when given separately [1,2,3]. In

polypharmacy, it is an important to find out the prevalence and rate of occurrence of drug interactions furthermore serious effects in hospitalized patients. As well, it is extra significant to detect and observed agents that are the majority to give harmful interactions [4]. As per the survey, about 3 to 5% of the patients taking a few drugs, drug-drug interaction can occur whereas it is nearly 20% in patients receiving a lot of drugs [5,6]. About 8.3% of the adult population worldwide has diabetes, according to the International diabetes Federation, and it's been growing within the last decade [7]. The most important object for mortality in diabetes is cardiovascular disease, and adults with diabetes have a two- to four fold higher risk of disorder than that among non-diabetic adults [8]. Exclusively, diabetic hyperlipidemia attributes to about 80% of mortality due to cardiovascular problems[9]. Hyperlipidemia in patients with diabetes mellitus have necessary to treated [8]. Therefore, lipid-lowering drugs and anti-diabetic medication are require at the same time in these patients.

Thus, it's vital to study potential drug-drug interactions between dyslipidemia drugs and anti-diabetic medication. glimepiride is third-generation sulfonylurea that is widely used in diabetes mellitus type 2[9]. Absorption of glimepiride is totally done once oral administration, and its oral bioavailability is close to 100% it's metabolized completely occurs via the hepatic cytochrome P450 2C9 by oxidative biotransformation to a major metabolite, cyclo hexyl hydroxyl methyl derivative [10,11, 12,13, 14].

Fibric acid derivatives are first medication prescribed for the prevention and treatment of diabetic complications like cardiovascular disease and diabetic dyslipidemia [15]. Gemfibrozil may be a comparatively potent and tolerable fibrates and is often prescribed for hyperlipidemia in patients with both types of diabetes [16]. It's well absorbed after oral administration, and its oral bioavailability is almost to one hundred [17]. The medicine in clinically relevant concentrations may be a potent inhibitor of CYP2C9 [18, 13]. Healthcare providers ought to take responsibility for the harmless prescribing of medications bearing in mind both possible adverse reactions as well as drug interactions. It is acknowledged that having diabetes and hyperlipidemia at the same time can be challenging in patient management To assess the selection, combination and possible drug-drug interactions of pharmacological agents used in the management of both hyperlipidemia and diabetes. It's necessary to check the doable effects of gemfibrozil on some pharmacokinetics and pharmacodynamics parameters of glimepiride.

MATERIALS AND METHODS

Chemical Reagents and Apparatus

Glimepiride oral tablet 2mg was purchased from Sanofi-Aventis Company (Germany). Internal standard was employed for the glimepiride assay by HPLC was purchased from (Sanofi-Aventis company). Gemfibrozil oral tablet 600 mg was purchased from Pfizer company (Germany).Acetonitrile (HPLC grade), Diethyl ether and methanol and other reagents were obtained commercially. HPLC HPLC equipped with UV-visible detector series 200. The analyte were separated using prontosil C18 column (250 x 4.6 mm, 5 μm particle size).

Pharmacokinetic Studies

Eighteen dogs (2.5-3.5 Kg) were housed under controlled conditions. The animals were kept in individual cages, fed and water ad libitum. They were submitted to experimentation after a 14-day adaptation period. A cannula was surgically inserted into the right jugular vein of each study animal one day prior to blood sampling. These animals were fasted overnight before drug dosing on the next day. In the single dose interaction group (n=6), dogs were received an oral 8.5 mg/kg B.W. of gemfibrozil which was followed by an oral single dose of glimepiride at 0.05 mg/kg B.W. after 30 minute of administration. In multiple dose interaction group (n=6), dogs were received gemfibrozil (8.5 mg/kg B.W.) oral administration for 7 consecutive days, on the 8th day 30 minutes after the gemfibrozil, glimepiride (0.05mg/kg B.W.) was administered. Dogs receiving only the same oral dose (0.05 mg/kg B.W.) of glimepiride were used as controls (n=6). The aqueous drug solutions were freshly prepared prior to dosing.

Blood was withdrawn via the cannula just prior to glimepiride administration (t=0) and at 0.15, 0.30,1, 2, 3, 4, 6, 8, 12 and 24 hr. after dosing. From these samples, plasma was immediately separated by centrifugation at 10,000 g for 5 min and was divided into two 100 mL aliquots. Plasma sample were stored at -80oC until subsequent glimepiride analyses by HPLC assays and blood glucose levels estimated using GOD-POD method The hypoglycemic activity of glimepiride at any time 't' was calculated as the percentage reduction of blood glucose at that time with respect to initial blood glucose levels according to Percentage reduction in blood glucose formula**.**

Preparation of the Mobile Phase

Methanol: water (85:15 v/v) was used as mobile phase, and flow rate was maintained at 1ml.min. The volume of injection was 20µl. The mobile phase was filtered through 0.45µm membrane filter. The UV-visible detector adjusted at 230 nm [19].

Calibration Curve

Stander solution (100µg/ml) of glimepiride, used as internal standard, was prepared in mobile phase and the diluted accordingly with the same solvent system. Calibration curve was constructed in the range of 5ng to50 ng/ml in mobile phase and 10ng to70 ng/ml in dog plasma. Calibration curve was plotted by taking area of sample/area of internal standard on y axis and concentration of solution on x-axis. The method was applied for known sample solution and was found to be satisfactory for analysis of tablet dosage forms [19].

Linearity

Fresh aliquots were prepared from the stock solution (100µg/ml) in different concentrations. The samples were scanned in HPLC equipped with UV-visible detector adjusted at 230 nm against reagent blank. It was found that the selected drug shows linearity between the 5-50ng/ml.

Accuracy

Accuracy of the method was confirmed by studying recovery at 3 different concentrations 50,500, and 5000 ng/ml of these expected, by replicate analysis (n=6). Standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured.

Precision

Precision (intra-day) and (inter-day) precision studies were done by injecting 3 serial dilutions in developed chromatographic conditions, method was evaluated by carrying out the six independent test samples of the glimepiride. Peak areas were calculated for % RSD values.

Extraction Procedure

Plasma samples were stored at –20°C and allowed to thaw at room temperature before processing. 100 µL plasma, 100µL aliquot of working standard solution of glimepiride was added in a polypropylene centrifuge tubes and was added with 300 µL of acetonitrile and 5 mL of diethyl ether. Then tubes were centrifuged for 10 min at 3000 rpm. The clear supernatant layer was transferred into another conical glass tube and organic layer completely evaporated at room temperature. After evaporation the residue was dissolved in mobile phase. Resultant samples were injected in developed chromatographic conditions [19].

Estimation of Pharmacokinetic Parameters for Glimepiride

Maximum concentration in plasma and the time required to reach this concentration were obtained from the observed data for each dogs. The area under the plasma concentration- versus-time curve was calculated by using a linear trapezoidal estimation. The plasma concentration-time data of glimepiride were assessed by compartmental analysis. Least square regression analysis was employed directly on the data in both the distribution and terminal elimination phases for the estimation of apparent distribution (α) and elimination (β) rate constants, respectively.

Estimation of Pharmacodynamics Parameter

Glucose levels estimated using GOD-POD Method. The hypoglycemic activity of glimepiride at any time 't' was calculated as the percentage reduction of blood glucose at that time with respect to initial blood glucose levels according to Percentage reduction in blood glucose formula Percentage Reduction in Blood Glucose at Time (Chinnala *et al.,* 2015). T= a – b / a× 100 Where 'a' is initial blood glucose level and 'b' is blood glucose level at time't'

Quantitation of plasma glucose

Plasma glucose concentrations were measured by the glucose oxidase method using a Spinreact (Spain). Briefly, sample plasma (10 μL) was added to a clean glass tube, and then 1 mL of reacting solution for glucose was added to the tube. The glass was then incubated at 37°C for 5 min, and the absorbance was measured at 505 nm within 30 min.

RESULTS

Linearity and Accuracy

The linearity of the calibration curves was verified from 5ng to 50ng/ml for glimepiride in mobile phase and 10ng to 70ng/ml in dog plasma. The linear curve was observed in the concentration range of the respective samples. And the regression (r^2) value calibration curve in plasma samples and mobile phase was 0.9941, 0.9849 respectively.The regression equations show good intercept and slopes values Figures (1) and (2).

Accuracy

Accuracy of the method was confirmed by studying recovery at 3 different concentration 50, 500 and 5000 ng/ml of these expected, by replicate analysis (n=6). Stander drug solution was added to a pre analyzed sample solution and percentage drug content was measured. The percent recoveries are given in Table (1) the present method makes it easier for the determination of glimepiride in pharmaceutical dosage forms in a routine manner.

Limit of detection (LOD) and limit of quantification (LQD)

The limit of quantitation of the assay was evaluated as the concentration equal to ten times the value of the single –to –noise ratio. This was taken as the lowest concentration in the calibration range. The limit of detection (LOD) for plasma is 0.5 $ng.ml^{-1}$ while limit of quantitation (LOQ) for dog plasma was 10 ng/ml^{-1}.

Pharmacokinetic Data in Healthy Dogs

The plasma concentrations of glimepiride in normal dogs before and after treatment with single and multiple dose of gemfibrozil and plots of time course of Mean±SD Plasma concentrations of glimepiride following oral route of administration vs. time were shown in tables (2) and figures (3,4,5) respectively.

Pharmacodynamics Parameters in Healthy dogs

The percentage of glucose reduction by glimepiride in normal dogs before and after treatment with single and multiple dose of gemfibrozil were shown in tables (3) and figures (6) respectively. Hence the therapy with the combination was found not to be safe. The percentage of glucose reduction decrease gradually in proportion to the concentration of drug until the drug is completely eliminated from the body.

DISCUSSION

These experiments confirm that the present method for the determination of glimepiride in dogs plasma samples was simple, sensitive, accurate and requires relatively small volume of plasma (100µL). The calibration curve was linear in the concentration range 10 and 70 ng/ml and hence the method is suitable for conducting pharmacokinetic studies.

The absorption of drug was clearly evidence by observed absorption rate constant and absorption half-life. These finding indicated that glimepiride was completely absorbed from the gastrointestinal tract of fasting dogs. There was significant rise in pharmacokinetic parameters like AUC and Cmax of glimepiride with single and multiple dose treatment of gemfibrozil. These findings satisfy the result of [20,21,22] who stated that glimepiride is completely absorbed from the gastrointestinal tract after oral administration and time to reach Cmax at 2–3 hours. When administered with a meal, the mean (Tmax) was significantly increased (12%) and the mean peak plasma concentration (Cmax) and area under the concentration-time curve (AUC) were significantly decreased (8% and 9%, respectively). The increase in AUC and Cmax indicates improved availability of glimepiride in presence of gemfibrozil. The increased bioavailability cannot be due to improved absorption, since absorption rate and absorption half-life of glimepiride were not altered. There might not be interaction at absorption level because both the drugs are absorbed rapidly after oral administration. The absorption half-life (t1/2 Ka) and elimination half-life (t 1/2 Kel) were not altered in the presence of gemfibrozil indicating that the enhanced plasma glimepiride levels might be due to either inhibition of metabolism or alteration in the distribution of glimepiride in the presence of gemfibrozil. The study indicates that, the interaction observed was pharmacokinetic interaction. In the presence of gemfibrozil, the peak plasma glimepiride levels were observed at 3.45h and the peak activity was at the same interval [21.23]. The drug profile of Glimepiride shows that it is a highly protein bound drug i.e. about 99.5% was bound to plasma proteins. Further glimepiride was reported to interact with highly protein bound drugs. Gemfibrozil is 98.6% or greater bound to plasma proteins, mostly albumin.

Since glimepiride and gemfibrozil are highly protein bound drugs they may compete with each other for the same protein binding site and hence the free blood levels of glimepiride was more. So the increase in the free blood levels of glimepiride might be responsible for the increase in its activity**.** Gemfibrozil is metabolized in the liver by CYP 450 2C9 [18]. Glimepiride is mainly metabolized by CYP 450 2C9[22]. Hence the interaction may be occurring at the level of the metabolism also. Gemfibrozil acts as a substrate for the enzyme CYP 450 2C9 and delay the metabolism of glimepiride leading to its enhanced effect in addition to being displaced from protein bound sites [24]. The drug glimepiride is mainly eliminated through urine (60%) and 40% is eliminated through feces [25,26]. Approximately 70% of the administered dose of gemfibrozil is excreted in the urine, mostly as the glucuronide conjugate with < 2% excreted as unchanged gemfibrozil [27**]**. Such an increase in exposure mirrored the significant decrease ($P<0.05$) in clearance with no significant decrease ($P>0.05$) in elimination rate constant and half-life. This similar to that investigated by [22,23,28, 29]. Moreover in the combination treatment at the elimination phase secondary peak of glimepiride is not observed. Thereby the interaction between these two drugs at elimination level is negligible. Hence the enhancement of glimepiride response in the presence of gemfibrozil might be because of pharmacokinetic mechanisms namely inhibition of its metabolism coupled with displacement from protein binding sites. Furthermore, volume of distribution was significantly decrease ($p<0.05$) in animals receiving oral gemfibrozil. These occurred in the presence of gemfibrozil as the drug profile shows that it is a highly protein-bound drug, and about 98.6% or

greater was bound to plasma proteins. Further, glimepiride was reported to interact with highly protein bound drugs**.** Glimepiride in combination with gemfibrozil produced peak hypoglycemic activity at 4hr in healthy dogs indicating the presence of interaction in pharmacokinetic parameters, since there was change in absorption parameters of glimepiride like AUC, Cmax, Tmax and clearance and also elimination parameters of glimepiride likeT1/2, Vd, and MRT in the presence of gemfibrozil. The glimepiride is metabolized by CYP 2C9 and gemfibrozil being metabolized by the same isoenzymes CYP2C9 there was significant changes in the absorption and elimination of glimepiride by gemfibrozil was produced in the single and multiple dose treatment[23,30]. Hence the therapy with the combination was found not to be safe. The percentage of glucose reduction decrease gradually in proportion to the concentration of drug until the drug is completely eliminated from the body.

REFERENCES

1. Tornio, A.; Niemi, M.; Neuvonen, P.J. and Backman, J.T. (2012). Drug interactions with oral antidiabetic agents: pharmacokinetic mechanisms and clinical implications. Trends In Pharmacological Sciences, 33(6): 312-322.
2. Theodosios, D.F.; Evangelos, N.L. and Moses, S.E. (2015). Dapagliflozin in patients with type 2 diabetes mellitus. Ther Adv Endocrinol Metab., 6(1): 29-41.
3. May, M. and Schindler, C. (2016). Clinically and pharmacologically relevant interactions of antidiabetic drugs. Therapeutic advances in endocrinology and metabolism, 7(2): 69-8.
4. Matheny, C.J.; Lamb, M.W.; Brouwer, K.L. and Pollack, G.M. (2001). Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 21(7): 778-796.
5. Janadri, S.; Ramachandra, S.S. and Kharya, M.D. (2009). Influence of itraconazole on antidiabetic effect of thiazolidinedione in diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences, 1(1): 119-24.
6. Parameshappa, B.; Rao, N.V.; Gouda, T.S.; Sen, S.; Chakraborty, R.; Basha, M.A. and Kumar, S.S. (2010). A study on drug-drug interaction between anti-hypertensive drug (propranolol) and anti-diabetic drug (glipizide). Annals of Biological Research, 1(3): 35-40.
7. Guariguata, L.; Whiting, D.R.; Hambleton, I.; Beagley, J.; Linnenkamp, U. and Shaw, J.E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Research And Clinical Practice, 103(2): 137-149
8. Dake, A.W. and Sora, N.D. (2016). Diabetic dyslipidemia review: an update on current concepts and management guidelines of diabetic dyslipidemia. The American Journal Of The Medical Sciences, 351(4): 361-365.
9. Vyas, M. and Galani, V.J. (2010). In vivo and in vitro drug interactions study of glimepride with atorvastatin and rosuvastatin. Journal of Young Pharmacists, 2(2): 196-200.
10. Langtry, H.D. and Balfour, J.A. Glimepiride (1998). A review of its use in the management of type 2 diabetes mellitus. Drugs, 55: 563-84.
11. Renwick, A.B.; Watts, P.S.; Edwards, R.J.; Barton, P.T.; Guyonnet, I.; Price, R.J. and Lake, B.G. (2000). Differential maintenance of cytochrome P450 enzymes in cultured precision-cut human liver slices. Drug Metabolism and Disposition, 28(10): 1202-1209.
12. Kyrklund, C.; Backman, J.T.; Neuvonen, M. and Neuvonen, P.J. (2003). Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. Clinical Pharmacology & Therapeutics, 73(6): 538-544.
13. Hinton, L.K.; Galetin, A. and Houston, J.B. (2008). Multiple inhibition mechanisms and prediction of drug–drug interactions: status of metabolism and transporter models as exemplified by gemfibrozil–drug interactions. Pharmaceutical Research, 25(5): 1063-1074.
14. van de Steeg, E.; Greupink, R.; Schreurs, M.; Nooijen, I.H.; Verhoeckx, K.C.; Hanemaaijer, R. and Verwei, M. (2013). Drug-drug interactions between rosuvastatin and oral antidiabetic drugs occurring at the level of OATP1B1. Drug Metabolism and Disposition, 41(3): 592-601.

15. Snow, V.; Aronson, M.D.; Hornbake, E.R.; Mottur-Pilson, C. and Weiss, K.B. (2004). Lipid control in the management of type 2 diabetes mellitus: a clinical practice guideline from the American College of Physicians. Annals Of Internal Medicine, 140(8): 644-649.
16. Lai, S.W.; Lin, C.L. and Liao, K.F. (2015). Rosuvastatin and risk of acute pancreatitis in a population-based case–control study. International Journal Of Cardiology, 187: 417-420.
17. Miller, D.B. and Spence, J.D. (1998). Clinical pharmacokinetics of fibric acid derivatives (fibrates). Clinical pharmacokinetics, 34(2): 155-162.
18. Wen, X.; Wang, J.S.; Backman, J.T.; Kivistö, K.T. and Neuvonen, P.J. (2001). Gemfibrozil is a potent inhibitor of human cytochrome P450 2C9. Drug Metabolism and Disposition, 29(11): 1359-1361.
19. Tripathi, A.S.; Dewani, A.P.; Chandewar, A.V. and Mazumder, P.M. (2016). Approaches Development and validation of RP-HPLC method for estimation of glimepiride in rat plasma and application to Pharmacokinetic Studies. Eurasian J Anal Chem., 11(2): 55-61.
20. Helmy, S.A.; El Bedaiwy, H.M. and Mansour, N.O. (2013). Dose Linearity of Glimepiride in Healthy Human Egyptian Volunteers. Clinical Pharmacology In Drug Development, 2(3): 264-269.
21. Anitha, N.G.; Gurajapu, K.C. and Mamatha, T. (2014). study of pharmacokinetic and pharmacodynamic drug–drug interaction between rosuvastatin and glimepiride in normal rabbits. World Journal of Pharmacy and Pharmaceutical Sciences, 4(01): 1218-1230.
22. Zalat, Z.A. (2015).Study of Pharmacokinetic Drug-Drug Interaction between Glimepiride and Gemfibrozil in Healthy Subjects. Current Science International, 4(3):387-392.
23. Niemi, M.; Neuvonen, P. J. and Kivistö, K. T. (2001). Effect of gemfibrozil on the pharmacokinetics and pharmacodynamics of glimepiride. Clinical Pharmacology & Therapeutics, 70(5): 439-445.
24. Sultanpur, C.M.; Reddy, N.S.; Kumar, S.; Satyanarayana, S. and Kumar, K.E. (2010). Drug-drug interaction between pravastatin and gemfibrozil (antihyperlipidemic) with gliclazide (antidiabetic) in rats. Journal of Young Pharmacists, 2(2): 152-155.
25. Rosenkranz, B. (1996). Pharmacokinetic basis for the safety of glimepiride in risk groups of NIDDM patients. Hormone And Metabolic Research, 28(09): 434-439.
26. Rosenkranz, B.; Profozic, V.; Metelko, Z.; Mrzljak, V.; Lange, C. and Malerczyk, V. (1996). Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. Diabetologia, 39(12): 1617-1624.
27. Davidson, M. H. (2006). Statin/fibrate combination in patients with metabolic syndrome or diabetes: evaluating the risks of pharmacokinetic drug interactions. Expert Opinion On Drug Safety, 5(1): 145-156.
28. Appiah-Opong, R.; Commandeur, J.N.; Istyastono, E.; Bogaards, J.J. and Vermeulen, N.P.E. (2009). Inhibition of human glutathione S-transferases by curcumin and analogues. Xenobiotica, 39(4): 302-311.
29. Al-Jenoobi, F.I. (2010). Effects of some commonly used Saudi folk herbal medications on the metabolic activity of CYP2C9 in human liver microsomes. Saudi Pharmaceutical Journal, 18(3): 167-171.
30. Chinnala, K.M.; Elsani, M.M.; Santhosham, K. and Aukunuru, J. (2015). Influence of Lansoprozole on the pharmacokinetics and pharmacodynamics of Glimepiride in normal and diabetic rats. Der Pharmacia Lettre, 7 (4): 192-1

Table 1: Result of accuracy for glimepiride

Sample No.	Quality control samples glimepiride (ng/ml)	Recovered amount glimepiride (ng/ml)	Accuracy (%) Glimepiride
1	50	45	90%
2	500	490	98%
3	5000	4985	99.7%

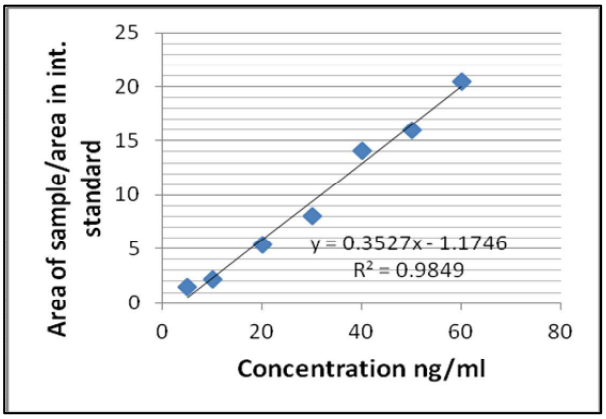


Figure 1: Calibration curve of glimepiride in mobile phase as standard

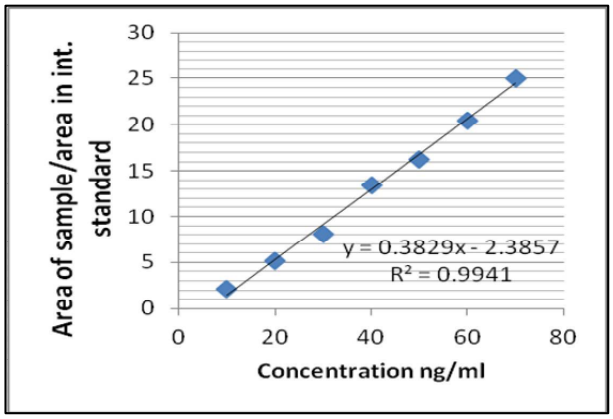


Figure 2: Calibration curve of glimepiride in plasma phase as standard

Table 2. Pharmacokinetic parameters of glimepiride after single oral administration of glimepiride (0.05mg/kg B.W.) only, with single and multiple doses of Gemfibrozil (8.5 mg/kg B.W.) in healthy dogs.

Pharmacokinetic parameters	Glimepiride	Glimepiride + Gemfibrozil (Acute)	Glimepiride + Gemfbrozil (Chronic)
$T_{1/2}$ ka (hr)	2.07 ± 0.58 B	2.25 ± 0.23 AB	2.52± 0.17 A
$T_{1/2}$ ke (hr)	2.17 ± 0.45 B	2.40 ± 0.20 AB	2.65 ± 0.27 A
C_{max} (µg/ml)	2.54 ± 0.58 C	3.66 ± 0.43 B	4.72± 0.34 A
T_{max} (hr)	3.11± 0.54 B	3.45 ± 0.28 AB	3.83± 0.15 A
Vd (ml)	73.97 ± 0.66 A	51.91 ± 0.65 B	39.92 ± 0.21 C
CL (ml/min)	235.87 ± 0.75 A	149.4 ±0.80 B	104.07± 0.73 C
AUC 0-t (µg/ml*hr)	21.12 ± 0.14 C	33.25 ± 0.04 B	47.46 ± 0.19 A
MRT (hr)	6.12 ± 0.64 B	6.72 ± 0.63 B	7.47± 0.35 A
Ka (1/hr)	0.33 ± 0.33 A	0.30 ± 0.29 A	0.27± 0.14 A
Ke (1/hr)	0.31± 0.61 A	0.28 ± 0.61 A	0.26 ± 0.33 A

All values are expressed as mean ± SE (N=6). Different capital letter due to significant differences p<0.05

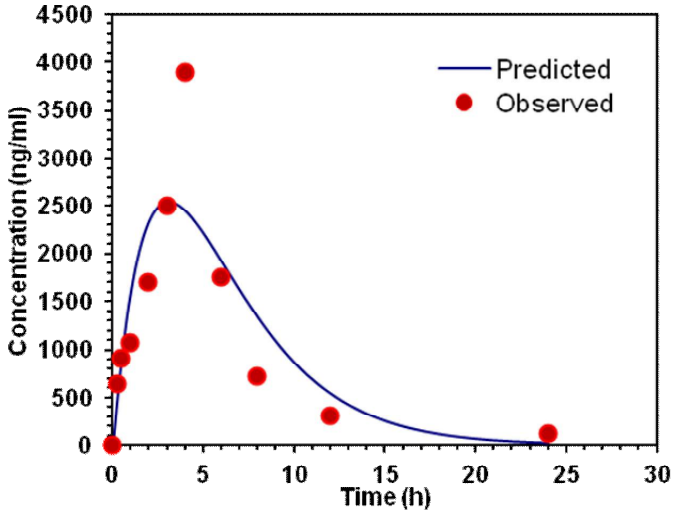


Figure 3: Plasma glimepiride concentration (mean ± SE) after single oral administration (0.05mg/kg B.W.). (n=6)

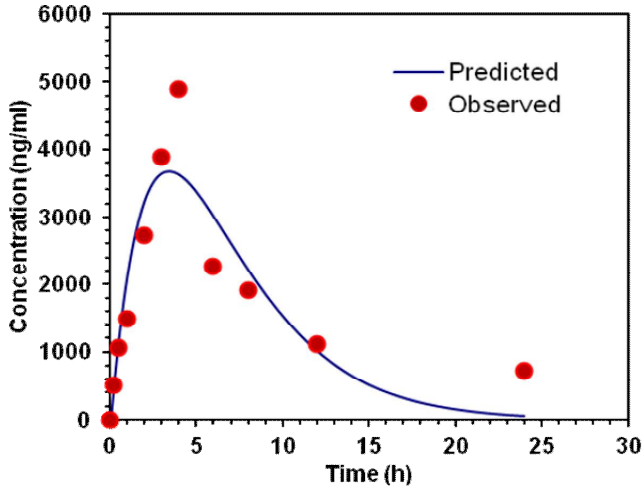


Figure 4: Plasma glimepiride concentration (mean ± SE) after oral administration of glimepiride (0.05 mg/kg B.W.) with single doses of gemfibrozil (8.5mg/kg B.W.) at the same day. (n=6)

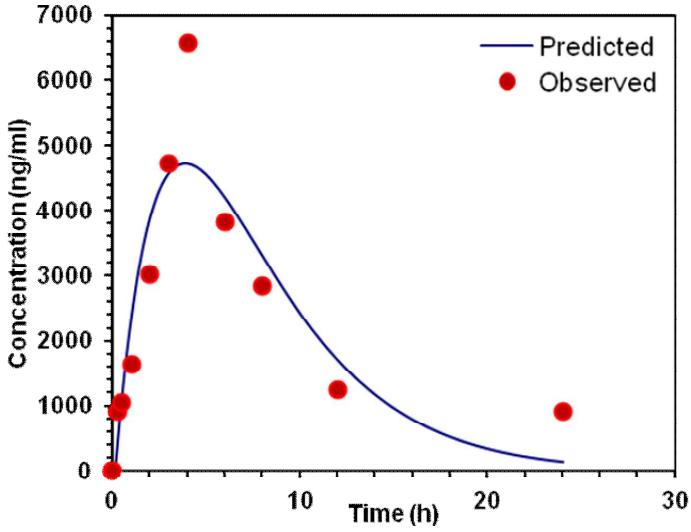


Figure 5: Plasma glimepiride concentration (mean ± SE) in after single oral administration of glimepiride (0.05mg/kg B.W.) with multiple doses of gemfibrozil (8.5mg/kg B.W) for 8 days. (n=6)

Table 3: Mean percentage blood glucose reduction in healthy dogs after oral administration of glimepiride,gemfibrozil, and their combinations (SDI and MDI)

Time (hr)	Glimepiride	Glimepiride + Gemfibrozil Acute	Glimepiride + Gemfibrozil Chronic
0	0 ± 0 A e	0 ± 0 A d	0 ± 0 A d
1	12.05 ± 0.17 B d	22.41 ± 0.32 A c	25.89 ± 0.17 A c
2	25.53 ± 0.28 B c	34.23 ± 0.21 A b	39.19 ± 0.28 A b
4	40.46 ± 0.24 B a	47.21 ± 0.27 A a	52.81 ± 0.24 A a
6	32.10 ± 0.54 C b	38.64 ± 0.08 B b	44.48 ± 0.54 A b
8	27.20 ± 0.23 C bc	34.06 ± 0.21 B b	40.15 ± 0.23 A b
12	16.86 ± 0.12 C d	21.40 ± 0.05 B c	31.76 ± 0.12 A c
24	9.85 ± 0.86 B d	19.26 ± 0.16 A c	23.21 ± 0.86 A c

All values are expressed as mean ± SE (N=6)

Means having with the different big letters in same rows and small letter in the same column differed significantly. ($P<0.05$).

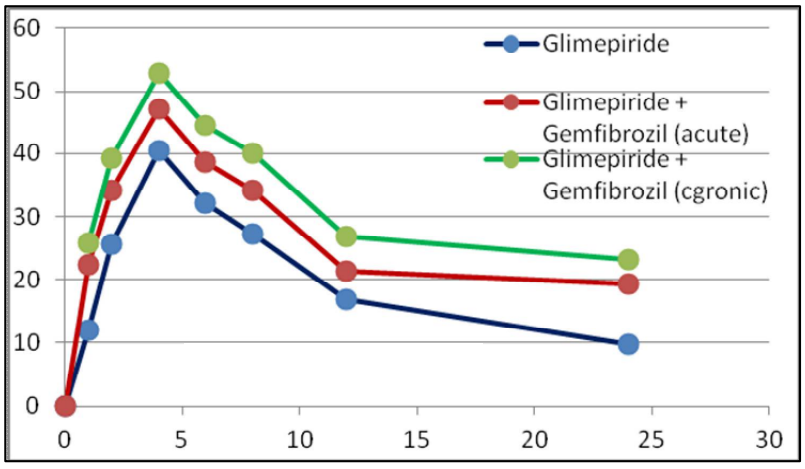


Figure 6: Comparative % glucose reduction in healthy dogs

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Vitamin D Supplementation Role in Pregnant Rats: Relation to Serum Prolactin Concentration

Shatha Q. Jaber* and Tahani S.S.Al-Azawi

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.

Received: 18 Mar 2018 Revised: 20 Apr 2018 Accepted: 03 May 2018

***Address for correspondence**
Shatha Q. Jaber
Department of Physiology and Pharmacology,
College of Veterinary Medicine,
University of Baghdad, Iraq
E.mail: tahanialazawi@yahoo.com / shathakasim2@gmail.com

ABSTRACT

This study aimed to investigate the effect of oral administration of Vitamin D3 on fertility indices in female rats. A total of 15 adults female rats 5-6 months old and weighting 200-250 g were divided into three equal groups : G1- control rats were received saline solution , G2- rats were received (5000 IU/ daily) of the vitamin orally and daily before pregnancy for 2 weeks . G3- rats were received the same dose for two weeks during gestation (8-21days). There was a significant increase in fertility, viability indices of rats received Vitamin D for two weeks prior to or during gestation (G2 &G3) as compared to non-supplemented rats (G1). Lactating index was increased in rats that supplemented with the vitamin D during pregnancy (G2) compared with G1 and G2. On conclusion, the administration of Vitamin D at dose (5000 IU/ daily) for two weeks prior to or during gestation seems to be safe with no abortion and the concentration of serum calcium and inorganic phosphorus remain within normal limit in all three groups. The most upper safety limit of serum 25(OH) D and Prolactin to maintain a normal healthy pregnancy was also determined in this experiment.

Key words: Vitamin D, Fertility, Lactation, Prolactin.

INTRODUCTION

Vitamin D refers to a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate. The most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol) as mentioned by [1].Vitamin D3 or cholecalciferol, after formation in the skin, and vitamin D2 from dietary sources, undergo hydroxylation in the liver, resulting in formation of 25-

hydroxyvitamin D or 25 (OH) D, the chief circulating form. 25(OH) D subsequently undergoes hydroxylation in the kidney under the influence of 1α- hydroxylase enzyme to yield the biologically active, dihydroxylated form of vitamin D, calcitriol or 1,25 (OH)2 D. This acts through specific vitamin D receptors (VDR_s) to regulate not only calcium metabolism, but also differentiation and division of various cell types. It has been suggested that in addition to its pivotal role in bone mineralization, calcium homeostasis, and its related disorders, vitamin D may play a role in muscle strength [2], pathogenesis of psoriasis [3], certain cancers [4], multiple sclerosis [5], diabetes [6] and blood pressure [7].

During the past decade, investigations have established that optimal vitamin D and its status are considered essential for overall health and well-being. However, vitamin D deficiency is emerging as a global problem and be a risk for wide range of diseases [8].This is not surprising, because VDR_s are expressed in different tissues such as skin, heart, bone, brain, breast, immune cells and gonads [9].Now, it is interested to find whether Vitamin D administration before or during gestation has any effect on fertility indices. Therefore, the aim of this experiment was to evaluate the effect of 5000 IU/ daily (human dose) of Vitamin D before or during pregnancy on fertility, viability and lactating indices. Serum calcium, phosphate, 25(OH) D and prolactin concentration were also determined in pregnant rats.

MATERIALS AND METHODS

This experiment was carried out at the animal house of the College of Veterinary Medicine / University of Baghdad from 15 November – 15 February. A total of 15 adult female Albino Wister rats weighing (200-250g).Their ages ranged between (5-6 months) were divided after 2 weeks acclimatization into three equal groups as follow: G1- control received saline solution , G2- received vitamin D (5000 IU) orally and daily for 2 weeks before pregnancy. G3- received the same dose for two weeks during gestation (8-21 days). Each rat was received 71.5 mg/ 0.5 ml/100 g B.Wt.by stomach tube. The females of these groups were subjected to Synchronization of estrus cycle by daily vaginal smears according to the procedure mentioned by [10].

At proestrus phase females were kept with healthy males for mating which was confirmed after 24 hours by the presence of seminal plug [11]. To confirm pregnancy, the females were subjected to daily vaginal smear test for five successive days where they stay in the diestrus. The following parameters were studied on pregnant female rats and newborns according to [12]: Fertility index, Gestation index, number of newborns and fetal absorbance, Viability index and Lactating index. Immediately after parturition, blood samples were collected from each rat by cardiac puncture for serum calcium, phosphate, 25(OH) D and Prolactin concentration determination according to [13] Data were subjected to One-way ANOVA and LSD was applied to assess the significant differences among means SAS (2010).

RESULTS

Effect of oral vitamin D_3 supplementation on fertility and gestation index of pregnant female rats

The total number of mated, pregnant and non-pregnant females for three groups are represented in Table (1).Vitamin D administration in rats for 15 days prior and during the gestation period showed positive result on fertility index compare with control. The table shows that the fertility index in control group is 60% whereas that rats received the 5000 IU dose of vitamin D for 15 days prior to or during gestation shows fertility index of 80% compared with control Moreover, the infertility is 20% in groups of rats that received the vitamin dose of vitamin D prior to or during gestation period for 15 days compared to control 40%.

The same table reveals that the gestation index for rats received distilled water prior to and during gestation time (G1) is 100% compared with 50% for these received vitamin D for 15 days prior to gestation only (G2) and 25% for

rats administered with the same dose during gestation. At the meantime, the number that gives alive newborn in G1 was three from total number of three pregnant females. The data represented in The data represented in table (1) shows that female give absorbance fetus, the number that give absorbance fetus I ,G3 was three from total number of four pregnant females, whereas the number that give absorbance fetus in G2 was two from total number of four pregnant compared with control was zero.

Effect of oral vitamin D_3 supplementation on total number of alive newborns, viability and lactating index of female rats

Table 2 represents the total alive newborns for three groups of rats in this experiment. There is a decrease in number of alive newborns in both vitamin D supplemented groups prior to or during gestation. The total number of alive newborn are (22) for control, (9) and (8) for G2 & G3 respectively. The table also shows the number of dead newborns for each group during the days after parturition and viability index.However, the viability index for rats that received 71.5 IU/0.5ml/100g B.Wt prior and during gestation (G2, G3) was 100% compared with control 95%.

Total number of alive newborns until the fifth and twenty one days of age for each group are represented in table 2. The number of alive newborns until five day is (21), (9) and 8 for control, G2 and G3 respectively. Table 2 shows a decrease in lactating index of group of rats that received vitamin D prior to gestation, which was (44.44%) as compared to control, and G3 was (100%).

Effect of oral vitamin D_3 supplementation on serum prolactin, Po_4, Ca^{++} and 25 OH D

A.Group of rats received D.W before and during pregnancy

It is illustrated in figure (1) that rats which have give birth to alive pups have a mean values of 0.39, 8.2, 10.5 and 16.7 for their serum prolactin, Po_4, Ca^{++} and 25 OH D_3 respectively. While the mean values for rats in same group which had reabsorbed fetuses are 0.97, 5.4, 9.2 and 8.8 for serum prolactin, Po_4, Ca^{++} and 25 OH D_3 respectively.

B.Group of rats received oral dose of 5000 IU/day vitamin D_3 for 15 days before pregnancy

Figure (2) represents that rats of this group which have resorbed fetuses show a mean value of o.64, 8.95, 10.45 and 14.85 for serum prolactin, Po_4, Ca^{++} and 25 OH D_3 respectively. On the other hand, rats in the same group which deliver a new pups have a mean value of 0.42, 11.15, 10.1 and 18.5 for serum prolactin, Po_4, Ca^{++} and 25 OH D_3 respectively.

C.Group of rats received oral dose of 5000 IU/day of vitamin D_3 for 15 days during pregnancy

Rats that have resorbed fetuses in their uterus have a mean value of 0.769, 7.46, 10.13 and 34.26 for serum prolactin, Po_4, Ca^{++} and 25 OH D_3 concentrations respectively. These values are compared with 0.42, 10.15, 9.7 and 16.0 for serum prolactin, Po4, Ca++ and 25 OH D3 concentrations respectively (figure 3).

Detection of vitamin D in ROC curve of control and groups of rats received oral dose of 5000 IU/day of vitamin D_3 for 15 days prior to or during pregnancy

ROC curve creating true positive rate (TPR) against false positive rate (FPR) of groups supplemented vitamin D prior to or during pregnancy that show the best marker detection and the best value (safety) of each parameters. However, sensitivity value was higher (100%) and in each prolactin and PO_4, while the specificity value was higher (100%) in

each prolactin, vit D and Ca. The cutoff point is different which was (> 0.45, > 27, ≤ 9.6 and ≤ 9.4) for prolactin, vit D, Ca and Po_4 respectively (figure 4).

DISCUSSION

The results of this experiment showed that supplementation of vitamin D at a dose of 5000 IU daily to rats for 15 days prior or during gestation time has the best effect on fertility index than the group of rats which received distilled water. However, there is some evidence indicate the role of vitamin D in modulating reproductive processes in female and male beside the role of sex steroid hormones. Vitamin D receptors (VDRs) have been shown to be expressed in the ovaries [**1**4]. Moreover, CYP27B1 (encoding 1α- hydroxylase) and VDR had been expressed in human placenta [15].The present experiment showed that administration of vitamin D prior or during pregnancy maintains a normal level of serum calcium and phosphate. Vitamin D has been well known for its function in calcium and phosphate homeostasis in the body.

The biologic action of vitamin D are mediated through VDRs which are distributed a cross various tissues including skeleton and parathyroid glands as well as reproductive tissues [16]. Moreover, previous studies have demonstrated that calcitriol promotes calcium transport in the placenta [17] and stimulate placenta Lactogen expression [17]. It also regulates HOXA10 expression in human endometrial cell [18]. This gene is important for the development of the uterus and essential for endometrial development, allowing uterine receptivity to implantation [19]. This is documented by increasing the fertility and decreasing the infertility index in the supplemented groups of the current experiment. From the other hand, the role of calcium in oocyte activation and maturation had been reported [20]. It has been shown that vitamin D deficiency reduces mating success and fertility in female rats [21].Because both 1,25 (OH)2 D3 and prolactin serum levels are increased during lactation [22] , prolactin may act together with 1,25 (OH)2 D3 for some of its effects related to calcium homeostasis. Animal studies indicate that prolactin and placental lactogen might stimulate intestinal calcium absorption. Rats and mice have a much shorter gestation period about 22 and 19 days respectively. They have to transfer large amounts of calcium during pregnancy and lactation to developing 10-12 fetuses and suckling neonates respectively [23]. In the present experiment, the viability and lactating index recorded 100% for both supplemented groups. Moerover, 25 (OH) D , the storsage form of vitamin D had been reported to be readily traverses the hemochorial placentas of rats [24].

Thus, the neonates to born with adults normal 25 (OH) D concentrations, their mothers must be vitamin D- sufficient. Passage of 25 (OH) D from mother to fetus could reduce maternal levels, especially if the mother is deficient in vitamin D. Therefore, to bring plasma vitamin D levels into the range considered to be sufficient, daily supplementation with vitamin D3 up to 5000 IU is safe and may be model in certain cases [25]. It was suggested that reduces reproductive capacity function in vitamin D deficiency are not because of hypocalcemia, but are due to a lack of vitamin D or 1,25 dihydrocholecalciferol. Whether the defect in the vitamin D-deficient male is the result of hypocalcemia or vitamin D per se is not known at the present time [26]. [27] demonstrated that vitamin D deficiency in male rats also reduces fertility and reproductive capacity in female supplemented vitamin D that cause complication during pregnancy and at parturition which resulted in diminished fertility included fetal resorption and pseudo pregnancy and mother giving birth to dead pus. However, until this moment, no clinical study has applied to adjust the safe serum 25 (OH) D and prolactin levels during pregnancy. The current experiment and for the first time reported that up to 27 ng/ml and 0.45ng/ml serum level of 25 (OH) D and prolactin respectively are safe during pregnancy. Above these levels, pregnancy facing some problems which attributed to absorbed fetuses. ROC curve revealed that the prolactin was the best marker for detection of vitamin D supplementation.

REFERENCES

1. Holick, M. F. (2006, March). High prevalence of vitamin D inadequacy and implications for health. In *Mayo Clinic Proceedings* (Vol. 81, No. 3, pp. 353-373). Elsevier.
2. Pludowski, P., Holick, M. F., Pilz, S., Wagner, C. L., Hollis, B. W., Grant, W. B., & Soni, M. (2013). Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—a review of recent evidence. Autoimmunity reviews, 12(10), 976-989.
3. Walsh, P., May, R & Otto, C. (2004). Unions and union membership in New Zealand: annual review for 2003. *New Zealand Journal of Employment Relations*, *29*(3), 83.
4. Garland, C. F., Garland, F. C., Gorham, E. D., Lipkin, M., Newmark, H., Mohr, S. B., & Holick, M. F. (2006). The role of vitamin D in cancer prevention. American journal of public health, 96(2), 252-261
5. Barkhof, F., Filippi, M., Miller, D. H., Scheltens, P., Campi, A., Polman, C. H., ... & Valk, J. (1997). Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain: a journal of neurology*, *120*(11), 2059-2069.
6. Pittas, A. G., Lau, J., Hu, F. B., & Dawson-Hughes, B. (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *The Journal of Clinical Endocrinology & Metabolism*, *92*(6), 2017-2029.
7. Qi, C. R., Su, H., Mo, K., & Guibas, L. J. (2017). Point net: Deep learning on point sets for 3d classification and segmentation. *Proc. Computer Vision and Pattern Recognition (CVPR), IEEE*, *1*(2), 4.
8. Lerner, A., Shapira, Y., Agmon-Levin, N., Pacht, A., Shor, D. B. A., López, H. M., & Shoenfeld, Y. (2012). The clinical significance of 25OH-vitamin D status in celiac disease. *Clinical reviews in allergy & immunology*, *42*(3), 322-330.
9. Insel, T. R., & Scolnick, E. M. (2006). Cure therapeutics and strategic prevention: raising the bar for mental health research. *Molecular psychiatry*, *11*(1), 11.
10. Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian journal of Biology*, *62*(4A), 609-614.
11. Gromko, M. H., Gilbert, D. G., & Richmond, R. C. (1984). Sperm transfer and use in the multiple mating system of Drosophila. *Sperm competition and the evolution of animal mating systems*, 371-426.
12. Klaassen, S., Probst, S., Oechslin, E., Gerull, B., Krings, G., Schuler, P., ... & Gramlich, M. (2008). Mutations in sarcomere protein genes in left ventricular noncompaction. *Circulation*, *117*(22), 2893-2901.
13. Fluttert, M., Dalm, S., & Oitzl, M. S. (2000). A refined method for sequential blood sampling by tail incision in rats. *Laboratory Animals*, *34*(4), 372-378.
14. Agic, A., Xu, H., Altgassen, C., Noack, F., Wolfler, M. M., Diedrich, K., & Hornung, D. (2007). Relative expression of 1, 25-dihydroxyvitamin D3 receptor, vitamin D 1α-hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. *Reproductive Sciences*, *14*(5), 486-497.
15. Barrera, D., Díaz, L., Noyola-Martínez, N., & Halhali, A. (2015). Vitamin D and inflammatory cytokines in healthy and preeclamptic pregnancies. *Nutrients*, *7*(8), 6465-6490.
16. Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, *357*(3), 266-281.
17. Tuan, Y. F. (1991). Language and the making of place: A narrative-descriptive approach. *Annals of the Association of American geographers*, *81*(4), 684-696.
18. Du, H., Daftary, G. S., Lalwani, S. I., & Taylor, H. S. (2005). Direct regulation of HOXA10 by 1, 25-(OH) 2D3 in human myelomonocytic cells and human endometrial stromal cells. *Molecular Endocrinology*, *19*(9), 2222-2233.
19. Bagot, C. N., Kliman, H. J., & Taylor, H. S. (2001). Maternal Hoxa10 is required for pinopod formation in the development of mouse uterine receptivity to embryo implantation. *Developmental Dynamics*, *222*(3), 538-544.
20. Homa, S. T. (1995). Calcium and meiotic maturation of the mammalian oocyte. *Molecular reproduction and development*, *40*(1), 122-134.
21. Kwiecinski, G. G., Petrie, G. I., & DeLuca, H. F. (1989). Vitamin D is necessary for reproductive functions of the male rat. *The Journal of nutrition*, *119*(5), 741-744.

22. Halloran, B. P., & Deluca, H. F. (1980). Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. *The Journal of nutrition*, *110*(8), 1573-1580.
23. German, J. B., & Dillard, C. J. (2006). Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. *Critical reviews in food science and nutrition*, *46*(1), 57-92.
24. Haddad, A., Smith, M. D., Herscovics, A., Nadler, N. J., & Leblond, C. P. (1971). Radioautographic study of in vivo and in vitro incorporation of fucose-3H into thyroglobulin by rat thyroid follicular cells. *The Journal of cell biology*, *49*(3), 856-882.
25. Wimalawansa, S. J. (2012). Vitamin D in the new millennium. *Current osteoporosis reports*, *10*(1), 4-15.
26. Kwiecinksi, G. G., Petrie, G. I., & DeLuca, H. F. (1989). 1, 25-Dihydroxyvitamin D3 restores fertility of vitamin D-deficient female rats. *American Journal of Physiology-Endocrinology And Metabolism*, *256*(4), E483-E487.
27. Walters, M. R., Hunziker, W., & Norman, A. W. (1980). Unoccupied 1, 25-dihydroxyvitamin D3 receptors. Nuclear/cytosol ratio depends on ionic strength. *Journal of Biological Chemistry*, *255*(14), 6799-6805.

Table1: Fertility, Infertility and Gestation index (%) of adult female rats received oral and daily dose of vitamin D (5000 IU/day) two weeks prior to or during gestation period.

Group/Parameter	G1	G2	G3
Total NO. of mated females (n)	5	5	5
No.of pregnant females (n)	3	4	4
Fertility index (%)	60	80	80
No. of non-pregnant female (n)	2	1	1
Infertility index (%)	40	20	20
No. of rats that give alive newborns (n)	3	2	1
Gestation index (%)	100	50	25
No. of rats that give absorbance (n)	0	2	3

G1: control group received orally distilled water daily before and during all pregnancy period.
G2: received oral dose of 5000 IU/day of vitamin D3 for 15 days before pregnancy. G3: received oral dose of 5000 IU/day Vitamin D3 for 15 days during pregnancy.

Table 2 : Total number of alive newborns, viability and Lactating index (%) of newborns from rats that received oral daily dose of vitamin D (5000**IU/day) two weeks prior to or during gestation period.**

Groups / parameter	G1	G2	G3
TotalNumber of newborns	22	9	8
No. of alive newborns until day five of age	21	9	8
No. of alive newborns until day twenty one of age	21	4	8
Viability index (%)	95	100	100
Lactating index (%)	100	44.44	100

G1: control group received orally distilled water daily before and during all pregnancy period.
G2: received oral dose of 5000 IU/day of vitamin D3 for 15 days before pregnancy. G3: received oral dose of 5000 IU/day Vitamin D for 15 days during pregnancy.

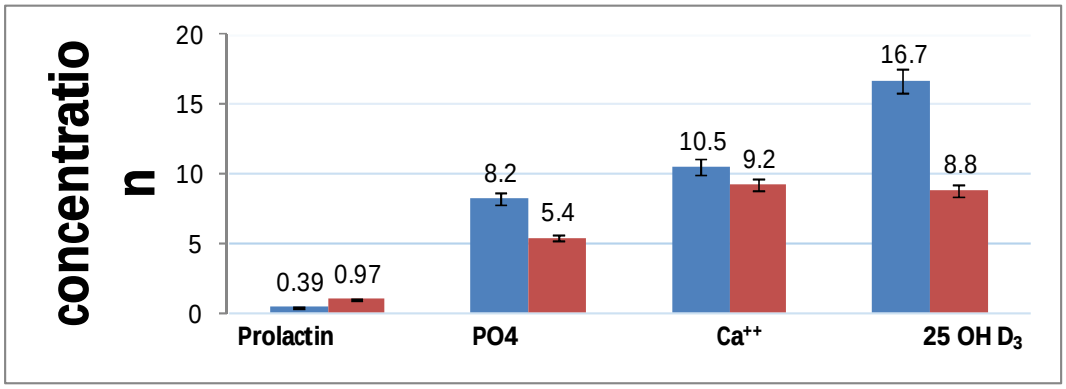


Figure 1: Mean serum prolactin (ng/ml), PO_4 (mg/dL), Ca^{++} (mg/dL) and 25 OH D_3 (ng/ml) concentration in-group control group received oral D.W daily before and during pregnancy.

Rats had normal delivery pups.

Rats had resorbed fetuses in their

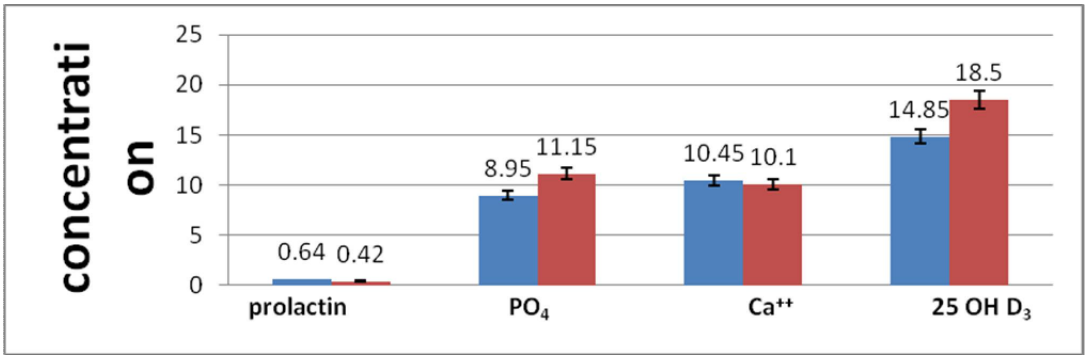


Figure2 : Mean serum prolactin (ng/ml) , PO_4 (mg/dL), Ca^{++} (mg/dL) and 25 OH D_3 (ng/ml) concentration in group of rats received 5000 IU/day before pregnancy for 15 days.

Rats had normal delivery pups.

Rats had resorbed fetuses in their uterus.

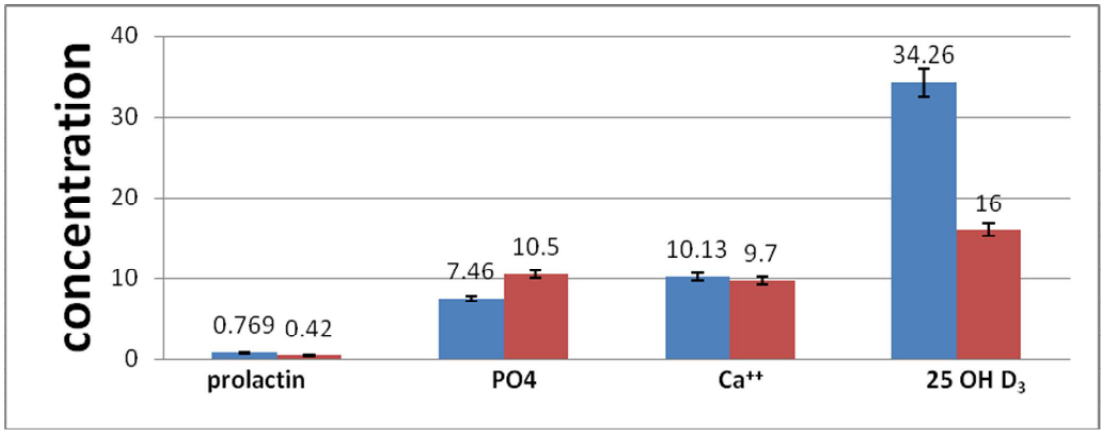


Figure 3 : Mean serum prolactin (ng/ml), PO_4 (mg/dL), Ca^{++} (mg/dL) and 25 OH D_3 (ng/ml) concentration in group of rats received 71.5 IU/0.5ml/100g B.Wt during pregnancy (8-21 days).

Rats had normal delivery

Rats had resorbed fetuses in their uterus.

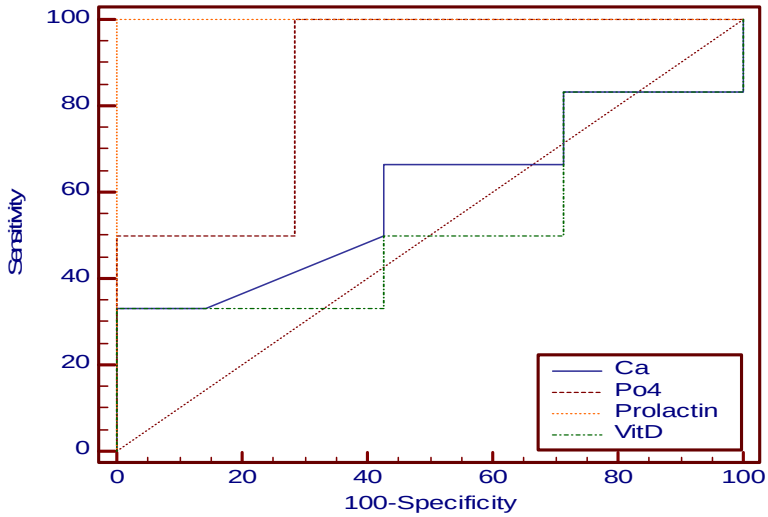


Figure 4: Roc curve showed the specificity and sensitivity for prolactin (ng/ml), PO_4 (mg/dL), Ca^{++} (mg/dL) and 25 OH D_3 (ng/ml) concentration in all group.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Dietary Incorporation of '*Dhanwantharam thailam*' Residue on Haemato Biochemical Parameters in Cross Bred Calves

C.R.Chithrima Seethal*, P.Gangadevi, K.Ally, K.Shyama and K.S.Anil.

Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy – 680 651, Thrissur, Kerala, India.

Received: 20 Mar 2018 Revised: 22 Apr 2018 Accepted: 05 May 2018

*Address for correspondence
Dr.C.R.Chithrima seethal
MVSc scholar,
Department of Animal Nutrition,
College of Veterinary and Animal Sciences,
Mannuthy – 680 651, Thrissur, Kerala, India.
E.mail: chithrimaseethal90@gmail.com

ABSTRACT

An experiment was conducted in crossbred calves for a period of three months to assess the effect of dietary incorporation of Dhanwantharam thailam residue on their haematological and biochemical parameters. Eighteen healthy crossbred calves of three to four months of age were selected and divided into three groups of six each, as uniformly as possible with regard to age, sex and body weight and were allotted randomly to experimental rations. All the experimental animals were fed as per ICAR, 2013 standards.Experimental rations were T1 (control), T2 (calf starter containing 20 per cent Dhanwantharam thailam residue) and T3 (calf starter containing 30 per cent Dhanwantharam thailam residue).Data on haematological and biochemical parameters such as haemoglobin, serum total protein, albumin, globulin, glucose, BUN, calcium, phosphorus, total cholesterol, HDL-cholesterol, ALT and AST were the criteria employed for evaluation and they did not show any significant difference among treatment groups ($P > 0.05$) except haemoglobin and serum phosphorus. Haemoglobin ($P<0.05$) and serum phosphorus ($P<0.01$) levels were significantly higher in T2 and T3 compared to T1, however values were within the normal range. The results of the present study indicated that Dhanwantharam thailam residue can be incorporated in the calf starter up to 30 per cent level without any adverse effects.

Keywords: *Dhanwantharam thailam* residue, calf starter, crossbred calves, blood.

INTRODUCTION

Good nutrition and scientific management plans are necessary for the success of a dairy industry. So provision of nutrients in optimal quantity and quality get prime importance. However in the present scenario, a major threat for animal production system is the scarcity of fodder and feed stuffs. This inconsistent year round supply of feed and fodder resources adversely affect growth and production performance of livestock population. Ayurvedic pharmaceutical industries produce a large quantity of residues after preprocessing and extracting or distillation of active ingredients from medicinal herbs. These solid residues are presently being discarded as waste or incinerated. Since these byproducts are rich source of energy, protein and fibre, they may be economically diverted as animal feed commodity. However systematic studies are required for the effective utilisation of these byproducts in animal feed. Dhanwantharam thailam residue (DTR) is one of such ayurvedic byproduct obtained after the preparation of Dhanwantharam thailam which is made from forty different medicinal herbs, cow milk and gingelly oil. The residue of this product has high energy content and is available in plenty locally. Hence, this research work is planned to determine the effect of feeding Dhanwantharam thailam residue on haemato biochemical parameters in crossbred calves.

MATERIALS AND METHODS

Eighteen healthy weaned crossbred female calves of three to four months of age, selected from University Livestock Farm and Fodder Research and Development Scheme (ULF&FRDS), Mannuthy formed the experimental subjects for the study. The calves were housed individually in well ventilated, clean and dry pen in the same shed with facilities for feeding and watering. The calves were divided into three groups of six each as uniformly as possible with regard to age, sex and body weight and each group was allotted randomly to one of the three dietary treatments,T1(calf starter without Dhanwantharam thailam residue),T2(calf starter with 20 per cent Dhanwantharam thailam residue) and T3 (calf starter with 30 per cent Dhanwantharam thailam residue). All the calves were dewormed before the commencement of the study. All the experimental calves were maintained under identical conditions of feeding and management throughout the experimental period of 90 days. All the calves were fed as per ICAR standards (ICAR, 2013).Weighed quantity of calf starter and good quality green grass were offered to all the experimental animals during the forenoon and afternoon periods and data on quantities of calf starter and green grass offered daily were recorded. The left over portion of the calf starter and green grass were weighed daily and their moisture content was analyzed to calculate the dry matter intake.

Data on daily dry matter intake from calf starter and green grass with respect to each calf were maintained throughout the experimental period. Body weights of all the calves were recorded at fortnightly intervals. Based on the body weight, feed and fodder allowances were revised fortnightly. Clean drinking water was made available to all calves throughout the experiment. Proximate analysis of Dhanwantharam thailam residue, calf starter and green grass were done as per the standard procedures (AOAC, 2012). Blood samples were collected from all animals at the end of the feeding experiment. Blood haemoglobin was estimated by cyanomethaemoglobin method. The serum was separated after centrifugation at 3000 rpm for 10 minutes. Serum samples were used to determine glucose (GOD-PAP method), total protein (Jong and Vegeter,1950),albumin(Bromocresol green method), globulin (difference between total protein and albumin), total cholesterol (Lie et al., 1976), calcium(Modified OCPC methodology), phosphorus (Bernhart and Wreath, 1955), alanine amino transferase (ALT) , aspartate amino transferase(AST) (IFCC methodology) and blood urea nitrogen (modified Berthlot method) using Semi Automated Biochemical Analyser (Master T).The standard biochemical kits used for these assays were purchased from M/s. Agappe Diagnostics Limited, Ernakulam, Kerala. Data obtained on different parameters during the course of the experiment were subjected to statistical analysis using Analysis of Variance (ANOVA) (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Proximate composition of Dhanwantharam thailam residue is presented in Table 1. The ingredient and chemical composition of experimental rations are presented in Table 2 and 3 respectively. The haematological and serum biochemical parameters of the experimental calves such as haemoglobin, total protein, albumin, globulin, glucose, total cholesterol, HDL-cholesterol, blood urea nitrogen, calcium, phosphorus, ALT and AST estimated at the end of the experiment are listed in Table 4. The average blood haemoglobin concentrations of experimental calves fed dietary treatments T1, T2 and T3 were 10.43, 11.42 and 11.52 g/dl, respectively. The average total protein concentrations of the calves maintained on three dietary treatments T1, T2 and T3 were 7.48, 7.65 and 7.69 g/dl respectively. The average albumin and globulin concentrations of calves maintained on three dietary treatments T1, T2 and T3 were 3.16 and 4.32, 3.26 and 4.39, 3.20 and 4.49 g/dl, respectively. The serum glucose level of calves estimated in the present study was 53.75, 54.23 and 53.57 g/dl for the three dietary treatments T1, T2 and T3, respectively. The serum total cholesterol concentration for the dietary treatments T1, T2 and T3 was 124,151.12 and 135.18 mg/dl, respectively. The serum HDL-cholesterol concentration for the dietary treatments T1, T2 and T3 was 50.82, 57.52 and 52.77 mg/dl, respectively. The blood urea nitrogen concentration for dietary treatments T1, T2 and T3 was 26.14, 25.77 and 25.36 mg/dl, respectively. The average serum calcium and phosphorus value of calves maintained on three dietary treatments T1, T2 and T3 at the end of the feeding trial were 10.54 and 6.56, 11.19 and 7.50, 7.18 and 11.06 mg/dl, respectively. The serum ALT and AST concentrations for the dietary treatments T1, T2 and T3 were 17.84 and 70.63; 17.60 and 71.79; 17.96 and 72.13 IU/L, respectively.

Statistical analysis of the data revealed that there was no significant difference (P>0.05) in all the haemato biochemical parameters except haemoglobin and serum phosphorus. But the calves fed T2 and T3 had significantly higher blood haemoglobin (P <0.05) and serum phosphorus concentration (P<0.01) compared to T1. However, the values recorded in the present study falls in the normal range reported for the species (Kaneko *et al*., 2008). Similar values for haematobiochemical parameters in calves fed with standard ration were also reported by various authors (Jith 2004, Asitha,2006, Vinu 2013, Jini 2014 and Rajkumar 2016). Present results are in agreement with Roshma *et al*. (2014) who reported that incorporation of Ksheerabala residue at 0, 10 and 20% level in the diet of Malabari kids showed no significant difference between treatments in haematological parameters such as total protein, serum calcium and total cholesterol. Also, Rani *et al*. (2016) found that incorporation of nutraceutical residue at 0 and 20 per cent levels in the diet of crossbred calves did not produce significant difference between treatments in haematological parameters such as total protein, albumin, globulin, glucose, cholesterol and blood urea nitrogen. The data on haemato biochemical parameters of the present study revealed that all the values were within the normal range which indicates all the experimental animals were in good nutritional status. Hence incorporation of Dhanwantharam thailam residue up to 30 per cent level in calf starter did not produce any deleterious effect on haemato biochemical parameters in crossbred calves.

REFERENCES

1. ICAR. Nutrient requirements of Animals - cattle and buffalo. (3rd Ed.).Indian Council of Agricultural Research, New Delhi. 2013. p. 24.
2. AOAC. Official Methods of Analysis, Association of official analytical chemists.19 th ed. Washington: D.C; 2012.
3. Jong, H.H. and Vegeter, J.J. Determination of serum proteins with the biuret test. Pharm. weekbl. 1950., 85: 755764.
4. Lie, R.F., Schmitz, J. M., Pierre, K. J. and Gochman, N.Cholesterol oxidase based determination, by continous-flow analysis, of total and free cholesterol in serum. Clin. Chem.1976.,22: 1627-1630.
5. Bernhart, D.N. and Wreath, A. Colorimetric determination of phosphorus by modified phospho molybdate method. Analyt.Chem. 1955. 27: 440-441.
6. Snedecor, G.W. and Cochran, W.G. Statistical Methods.8thEd.The Iowa State University press, Ames, IA. 1994., p.314.

7. Kaneko, J.J., Harvey, J.W. and Bruss, M.L. Clinical biochemistry of domestic animals. 6 th ed.USA: Elsevier academic press; 2008.p.916.
8. Jith, J.M. Effect of replacing dried fish with lysine and methionine in the calf starter on the growth performance of crossbred calves. M.V.Sc thesis, Kerala Agricultural University, Thrissur, 2004., 56p.
9. Asitha, T.V. Effect of supplementation of dried yeast (NEUTRAMIX-MDY) on growth performance in pre-ruminant calves. M.V.Sc thesis, Kerala Agricultural University, Thrissur, 2006.,64p.
10. Vinu, M.N. Dietary supplementation of organic selenium in crossbred calves.M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode, 2012.,p.97
11. Jini, K. S. Effect of phase feeding on growth in pre-ruminant cross bred calves. MVSc thesis, Kerala veterinary and animal sciences university, Pookode.. 2014.,p. 47.
12. Roshma, T. J., Effect of dietary incorporation of Ksheerabala residue on growth performance in Malabarikids. MVSc thesis, Kerala veterinary and animal sciences university, Pookode. 2014., p.48.
13. Rani,J.K., Seethal,C.C.R., Bunglavan,S.J., Ally, K. and Shyama.K. Effect of dietary incorporation of nutraceutical residue (Alfalfa Pellet) on growth performance and haematological parameters in cross bred calves. Indian J. Natu.Sci. 2016., 6(35):10747-10752..

Table I. Proximate composition of Dhanwantharam thailam residue (On dry matter basis)

Parameter	Nutritive value (%)
Dry matter	96.40
Crude protein	9.29
Ether extract	26.16
Crude fibre	18.37
Total ash	6.11
Nitrogen Free Extract	40.07
Acid insoluble ash	0.45
Neutral detergent fibre	59.01
Acid detergent fibre	41.06

Table II. Ingredient composition of calf starters offered to calves maintained on three experimental rations,%

Ingredient	Dietary treatments		
	T_1	T_2	T_3
Maize	40.00	20.00	10.00
Dhanwantharam thailam residue	00.00	20.00	30.00
Soya bean meal	40.00	40.00	40.00
Dried fish	05.00	05.00	05.00
Wheat bran	14.00	14.00	14.00
Salt	01.00	01.00	01.00
Total	100.00	100.00	100.00
Feed supplement(g/100 kg feed)			
Nicomix $AB_2D_3K^*$	20.00	20.00	20.00

Table III. Chemical composition of calf starter and green grass fed to calves maintained on three experimental rations, %

Nutrients , %	Dietary treatments			Fodder
	T1	T2	T3	
Dry matter	89.88	89.13	89.86	19.02
Crude protein	25.05	25.40	25.53	9.43

Ether extract	3.77	4.62	5.30	1.86
Crude fibre	4.96	5.98	6.86	35.57
Total ash	8.54	9.86	10.59	10.52
Nitrogen Free Extract	57.68	54.14	51.72	42.62
Acid insoluble ash	2.16	2.60	2.87	2.76
Neutral detergent fibre	21.83	22.91	26.54	65.03
Acid detergent fibre	6.03	7.63	8.86	38.19

Table IV. Haematological parameters of calves maintained on three experimental rations at the end of experiment

Parameters	T1	T2	T3
Blood haemoglobin*, g/dl	10.43 ± 0.19^{b}	11.42 ± 0.29^{a}	11.52 ± 0.33^{a}
Total protein, g/dl	7.48 ± 0.16	7.65 ± 0.22	7.69 ± 0.16
Albumin, g/dl	3.16 ± 0.17	3.26 ± 0.11	3.20 ± 0.10
Globulin, g/dl	4.32 ± 0.23	4.39 ± 0.26	4.49 ± 0.14
Glucose,mg/dl	53.75 ± 2.40	54.23 ± 1.36	53.57 ± 1.01
Total cholesterol, mg/dl	124 ± 7.08	151.12 ±6.94	135.18 ±8.50
HDL- Cholesterol,mg/dl	50.82 ± 1.87	57.52 ± 2.61	52.77 ± 2.36
BUN, mg/dl	26.14 ± 1.11	25.77 ± 1.29	25.36 ± 0.99
Serum Calcium,mg/dl	10.54 ± 0.22	11.19 ± 0.22	11.06 ± 0.29
SerumPhosphorus**,mg/dl	6.56 ± 0.15^{b}	7.50 ± 0.14^{a}	7.18 ± 0.15^{a}
ALT, IU/L	17.84 ± 0.55	17.60 ± 0.48	17.96 ± 0.41
AST , IU/L	70.63 ± 0.78	71.79 ± 1.68	72.13 ± 2.47

T1, T2, T3- Average of six values

a,b- Means with different superscripts within the same row differ significantly.

*significant at 5% level, **significant at 1% level.

RESEARCH ARTICLE

Study of the Effect of Aqueous Extraction for *Eryngium creticum* on the Histological Structure of Testes in the Albino Mouse (*Mus musculus*)

Thekra Atta Ibrahim[1*] , Nisreen Sabbar[2], Aiymen Ahmed A.K.AL-Abassi[2] and Mohammed Nsaif Abbas[3]

[1]Department of Biology, College of Education for Pure Science, Diyala University, Iraq.
[2]Education Directory of Diyala, Iraq.
[3]Environmental Engineering Department, Faculty of Engineering, Mustansiriyah University, Iraq.

Received: 15 Mar 2018 Revised: 18 Apr 2018 Accepted: 05 May 2018

[*]**Address for correspondence**
Thekra Atta Ibrahim,
Department of Biology,
College of Education for Pure Science
Diyala University, Iraq.
E.mail: thekra.atta@gmail.com

ABSTRACT

The results obtained from this study showed that all animals of two experiment groups that treated with extraction for Eryngium creticum indicated a change in the thickness of seminiferous tubule wall of the testes and contraction in them where its appearance became irregularly and crooked as well as observed atrophy in some seminiferous tubule and depletion of some germ cells and collected in the cavity of the seminiferous tubule. Also an increase in the area between Sertoli cells was observed.

Keywords: testis, Sertoli cells, Eryngium creticum, seminiferous tubules, Leydig cells, *Mus musculus*

INTRODUCTION

Now, medicinal plants occupy a distinct position in agricultural and industrial production as the main source of pharmaceuticals, which are the source of the active substances involved in the preparation of many medicines and treatments in the form of extracts used as raw material to produce some chemical compounds that represent the nucleus of chemical synthesis of some generic medicines Cortisone, sex hormones, alternative blood plasma and others (1). The World Health Organization has given great attention to the use of medicinal plants for the purpose of general human health care if great attention is paid to the development of scientific research in the world in general and in third world countries, especially as a treasure to be discovered in the light of modern science to be the first step of self-sufficiency in developing countries to raise and improve the level of public health Per capita and community (2). Therefore, the study of medicinal plants in terms of the nutritional and medicinal value of great

the vital organs of the living organism, through which the organism returns itself to the species that belong to the other important mechanism, so it is able to maintain the survival of the species and prevent it from extinction through the inheritance of life from generation to generation. Therefore, many medicinal plants have been used to improve fertility and reproduction in the animals (3). Eryngium creticum is a family of Apiaceae, a perennial plant that reaches between 30-50 cm or more. Its leaves are many and far apart.

The basal leaves fade rapidly and have a long, elongated trunk. They are divided in a medium binary, with sharp teeth, (3 - 8) Spatially divided into long, spindly parts with sharp teeth and pinned parts, cuneiform sheets coated 5 axial stripes with two flakes at the base and sometimes along the edge 3-4 times longer than 8 mm length of the main bulb (4) This plant is considered by the scientists of nutrition and medicine for the containment of chemical compounds effective in the treatment of many diseases, and this plant is anti-snake and anti-scorpion, anti-bacterial and fungal, and has anti-inflammatory effects is also a reduction in blood sugar, Blood sugar (5). The plant contains many chemical compounds that act as antioxidants, carcinogens and swelling,These substances include volatile and fixed oils, flavonoids, phenols, tannins, kumarins, terpenes, carotenoids, saturated fatty acids, fatty acids, vitamins dissolved in mucus, fats, mineral elements and plant estrogens that qualify for fertility(6). Due to the paucity of information about the importance of this plant in fertility, this experiment was designed to identify Study of the Effect of aqueous extraction for Eryngium creticum on the histological structure of Testes in the albino mouse (*Mus muscu.*

MATERIALS AND METHODS

Preparation of aqueous extract *Eryngium creticum*

Gradelet and others were used (7) for the preparation of the extract, where 50 g of Eryngium creticum plant powder was milled in 1000 ml of distilled water at 60 ° C and then left in the Vibrator for a whole day at 37 ° C Then the mixture was sprayed with medical gauze and placed in test tubes. The tubes were transported to the centrifuge at 3000 cycles per minute for 10 minutes. After that, filtrate again with filter paper. Then, take the starter and place in a glass petri dish inside the oven at 57 ° C. Scrap the extract where the weight of the extract obtained 2 g and save the extract in a container until the court use.

Laboratory Animals and Histological Study

Twenty-two male mice of Swiss mice (Mus musculus) were obtained from an animal house in Biology Department-College of Education for Pure Science/Diyala University. The average weights and ages were between 20-38 g and 8-10 weeks respectively. The experiments animals were divided randomly into three groups as follow: 1st group (is the control group and has 7 male mice and 2nd group is experimental group and has 20 male mice. The second group is subdivided into two groups of 7 male mice for each one. The animals in one of this sub-group were treated with 100 mg/kg of extraction for Eryngium creticum while the mice in the other sub-group (EG2) were treated with 140 mg/kg of extraction for Eryngium creticum. The animals were treated once daily for three weeks. At the end of the last day of dosage, all mice were anesthetized with chloroform, post-mortem to the eradication of **Testes**, fixed with formalin for 24 h, washed with tap water and transferred to 70% alcoholic solution for keeping. Histological sections were prepared depending on (8). The sections were (Dehydration) with an ascending chain of ethyl alcohol, then immersed in xylene for (clearing) and embedding in paraffin wax. The wax molds were cut using rotary microtome with 7µm thickness. The sections were stain used hematoxylin and eosin stain according to (9) and finally loaded with Canada balsam, tested and photographed using light microscope supplied by a digital camera.

RESULTS AND DISCUSSION

The testicle (testis) the most important in the reproductive system male organ Male reproductive system. It is characterized by two main functions is the production of steroid hormone Steroid Hormone and sperm composition Spermatozoa (10). There are many different factors that affect sperm formation, among which are chemical agents such as drugs, pesticides, and toxic chemical agents that are polluting the environment.[11).The results of the present study showed that mice that were injected with a concentration of 100 mg / kg of aqueous extraction for Eryngium creticum showed significant changes in the thickness of the walls of semeniferous tubules and their shrinking, where their general appearance was wavy and irregular As well as the occurrence of atrophy in some sperm tubes, as well as irregularity of the semeniferous epithelium as shown in Figure (1). This finding correlates with the findings of Richardson and his colleagues (1989) [12] where they stated that the affected basal plate plays an important role in maintaining the transfer of materials between the interstitial tissues and the germ epithelium Spermatogonic and maintaining the shape, structure and function of these tissues.

Zheng et al. [13] pointed out that increasing the thickness of the wall in the spermatozoa weakens the relationship between it and the interstitial tissue.As the thickness of the wall increases, many disorders of the testicle appear, especially in the function of Sertoli cells, which affect the differentiation of bacterial cells and inhibition of sperm formation While Winters [14]. Sertoli cells secrete Collagen Fibers IV collagen fibers, which cause thickening of the walls of the spermatozoa, thus leading to poor sperm formation. The results of the study also showed the absence of Spermatozoa sperm in some seminal vesicles, as well as the appearance of vacula in some areas of the testis. It clearly shows the wide distance between the germ cells, their dissociation of the epithelial tissue and their accumulation in the seminal cavity, the degeneration of Sertoli cells and increases the area between neighboring Sertoli cells as shown in Figures (2, 3). The results of this study also showed the emergence of large Phagocyte within the spermatozoa cavity as shown in Figure (3). Monsees et al. [15] has suggested that a disturbance in sertoli cells will inevitably affect The germ cells and eventually lead to dysfunction in testicular tissue Reis et al. [16] stated that the Sertoli cell plays a necessary role in the development of germ cells by forming the blood testicular barrier that protects germ cells, and the transfer of nutrients and hormones to germ cells. All of these pathological signs are believed to be due to a malfunction in the structure and function of sertoli cells.

The results of the current study revealed that the aqueous extraction for Eryngium creticum had an effect on the Leydig Cells and Interstitial tissue, which was evident through the emergence of decay and necrosis and also the emergence of vaculation in the interstitial tissue. As shown in Figure(2)The results of the present study are consistent with the findings of Kaur et al. [17] that Lidig cells are a center of fertility regulation through the production of Testosterone Hormone. While Papaioannou et al. [18] reported that Lidig cells are stimulated by the hormone LH Luteinizing Hormone, the arachidonic acid catalyst and the hormone testosterone. The results of the current study showed that the dosage of rats with a concentration of 140 mg / kg of aqueous extraction for Eryngium creticum has increased the shrink of seminiferous tubules and depletion of depletion in some layers of germs of seminiferous tubules_At the same time, it led to degeneration and vaculation, apoptosis in the spermatogonia, primary sperm cells, Spermatid, mature sperm, the return of Spermatidand mature sperm into the somniferous tubules On the other hand, the results showed that many somniferous tubules were empty of germ cells as shown in Figs. (4,5). This is thought to be due to a malfunction of the Sertoli cells, This imbalance will in turn affect the essential proteins required in the synthesis process that are needed to differentiate germ cells, These proteins are excreted at the highest level during the differentiation stage of Spermatid. This finding is consistent with Foley's [19] findings that the regressive movement of Spermatid and sperm in the seminiferous tubule wall may be due to the toxicity of the testis by aqueous extraction for Eryngium creticum.

REFERENCES

1. Benayad Z., Gomez- Cordoves C. and Es- Safi N. (2014). "Characterization of Flavonoid Glycosides from Fenugreek (*Trigonella foenum–graecum*) Crude Seeds by HPLC- DAD- ESI\MS Analysis". *International Journal of Molecular Sciences*. Vol. 15, No. 11, pp: 20668-20685.
2. Chen W. C., 1977. Proceeding of seminar on the use of medicinal plants in health care, World Health Organization (regional office, fro the western pacific), Japan, 13-17 sept.
3. Carreau S., Bourguiba S., Lambard S., Galeraud-Denis I., Genissel C., Levallet J., (2002), "Reproductive system: aromatase and estrogens" , *Molecular and Cellular Endocrinology*, Volume 193, Issues 1–2, 31 July, pp: 137–143
4. Kikowskaa, M , Dworacka, M.b Kedziora.I¸ a. Thiem, B.2016. Eryngium creticum – ethnopharmacology, phytochemistry and pharmacological activity. A review. Revista Brasileira de Farmacognosia (26) 392–399.
5. Erdem, S.A., Nabavi, S.F., Orhan, I.E., Daglia, M., Izadi, M., Nabavi, S.M., 2015. Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus Eryngium. DARU J. Pharm. Sci. 23 (53), 1–22.
6. Rammal, H., Fahran, H., Jamaleddine, N., El Mestrah, M., Nasser, M., Hijazi, A., 2015. Effects of altitude on the chemical composition and on some biological properties of Lebanese Eryngium creticum L. J. Chem. Pharm. Res. 7, 887–893.
7. Gradelet, S.;Bon, A.; Berges,R.;Suschetet, M. and Astorg . P.(1998). Dietary carotenoids inhibit Aflatoxin BL,African J. Biotech.(2):46-49.
8. Bancroft J. D., and Gamble M., (2008), "Theory and Practices of Histological Technique", 6th edition. Churchill Livingstone, Elsevier, Philadelphia
9. Suvarna S. K., Layton C. and Bancroft J. D., (2013), "Bancroft's Theory and Practice of Histological Techniques", Churchill Livingstone, Elsevier, e-Book ISBN: 9780702058172. Hardcover ISBN: 9780702042263.
10. Carreau S., Bourguiba S., Lambard S., Galeraud-Denis I., Genissel C., Levallet J., (2002), "Reproductive system: aromatase and estrogens", *Molecular and Cellular Endocrinology*, Volume 193, Issues 1–2, 31 July, pp: 137–143.
11. Yano C. L. and Dolder H., (2002), "Rat testicular structure and ultrastructure after paracetamol treatment", *Contraception*, Volume 66, Issue 6, December, Pages 463-467.
12. Richardson L. L., Kleinman H. K. and Dym M. (1998), "Altered basement membrane synthesis in the testis after tissue injury", Journal of Andrology, Volume 19, Issue 2, March-April, pp: 145-155.
13. Zheng Y., Zhang X., Zhon J., Cheng F, Rao T. and Yao Y., (2002), "Expression of extracellular matrix proteins and vimentin in testes of azoospermic man: an immunohistochemical and morphometric study", Asian Journal of Andrology, Volume 4, Issue 1, pp:55-60.
14. Winters S. J., (2004), "Male Hypogonadism: Basic, Clinical, and Therapeutic Principles", Humana Press Inc., Totowa, NJ. Chapter 2: Leydig Cell Function in Man by Dong Q. and Hardy M. P., pp:23-43.
15. Monsees T. K., Franz M., Gebhardt S., Winterstein U.,Schill W. B. and Hayatpour J., (2000), "Sertoli cells as a target for reproductive hazards", Androlgia, Volume 32, Issue 4-5, September, pp: 239–246.
16. Reis M. M., Moreira A. C., Sousa M., Mathur P. P., Oliveira P. F. and Alves M. G., (2015), "Sertoli cell as a model in male reproductive toxicology: Advantages and disadvantages", *Journal of Applied Toxicology*, Volume 35, Issue 8, August, pp: 870–883.
17. Kaur G., Thompson L. A. and Dufour J. M., (2014), "Sertoli cells--immunological sentinels of spermatogenesis", Seminars in Cell & *Developmental Biology*, Volume 30, June, pp: 36-44.
18. Papaioannou M. D., Pitetti J., Ro S., Park C., Aubry F., Schaad O., Vejnar C. E., Descombes P., Zdobnov E. M., McManus M. T., Guillou F., Harfe B. D., Yan W., Jégou B. and Nef S., (2009), "Sertoli cell Dicer is essential for spermatogenesis in mice", *Developmental Biology*, Volume 326, Issue 1, 1 February, pp: 250–259.
19. Foley G. L., (2001), "Mechanisms of Male Reproductive Organ Toxicity", *Toxicologic Pathology*, Volume 29, No 1, pp 49 –63.

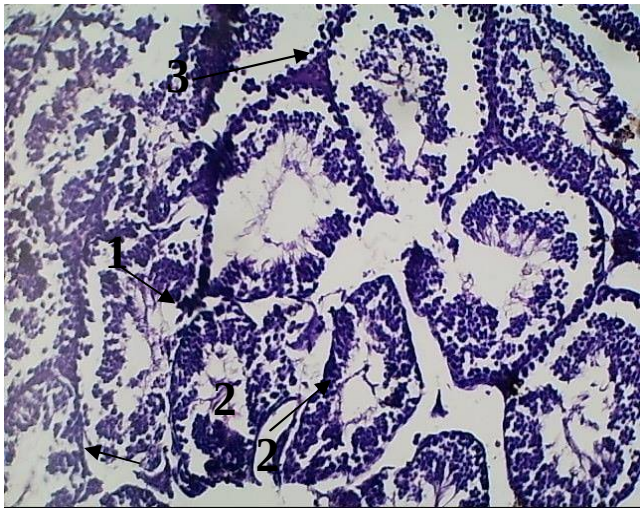


Figure 1.Parasagittal section of the Testis of mice receiving with 100 mg/kg extraction for Eryngium creticum for one month showing 1) irregularity of the semeniferous epithelium ,2 vaculation 3, necrosis of the semeniferous epithelium). H&E, 40X.

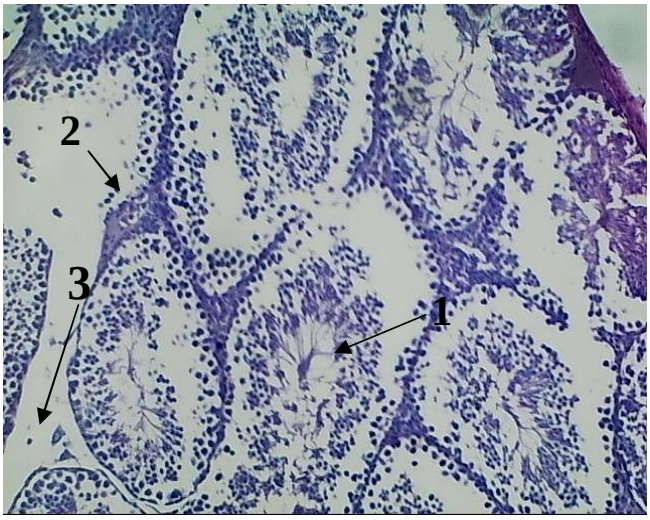


Figure 2.Parasagittal section of the Testis of mice receiving with 100 mg/kg extraction for Eryngium creticum for one month showing 1) aggregate the germinal cells in the lumen of Seminiferous tubules, 2, necrosis of the semeniferous epithelium) 3 degeneration in Leydig cells . H&E, 40X.

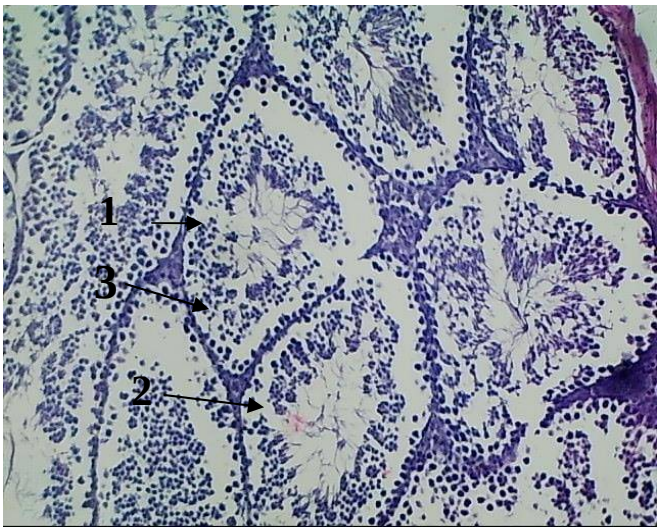


Figure 3.Parasagittal section of the Testis of mice receiving with 100 mg/kg extraction for Eryngium creticum for one month showing 1) degeneration in Sertoli cells, 2, necrosis of the semeniferous epithelium) 3, The emergence of large cells phagocyte within the cavity semeniferous. H&E, 40X.

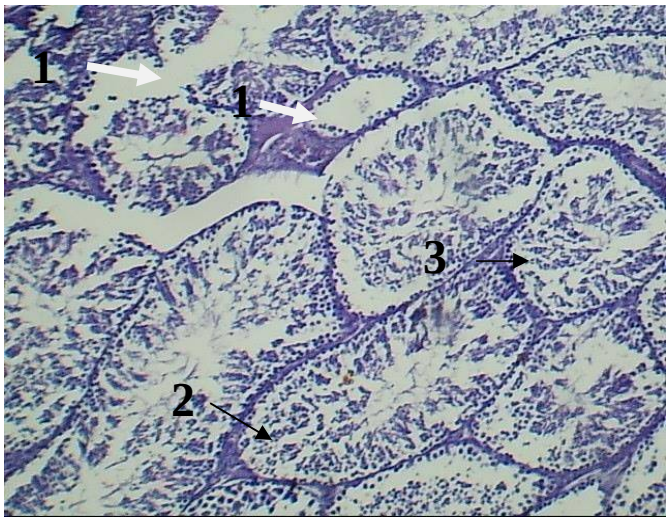


Figure 4.Parasagittal section of the Testis of mice receiving with 140 mg/kg extraction for Eryngium creticum for one month showing 1), Empty the Seminiferous tubules from germinal cells 2, necrosis of the semeniferous epithelium) 3, degeneration in Sertoli cells. H&E, 40X.

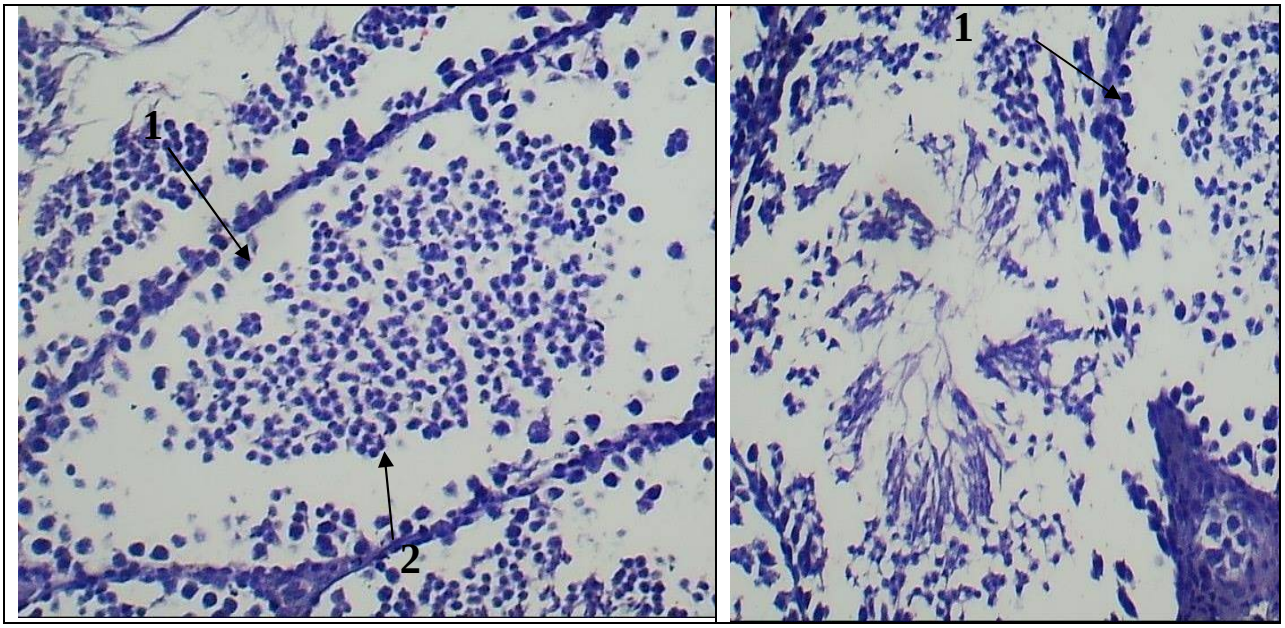


Figure 5.Parasagittal section of the Testis of mice receiving with 140 mg/kg extraction for Eryngium creticum for one month showing 1), Empty the Seminiferous tubules from germinal cells 2, degeneration in Sertoli cells. H&E, 40X.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Salty Feeding on ALT, AST Enzymes Activity and Cortisol Hormone in Blood Plasma of *Cyprinus carpio* L.

Dhamyaa Oleiwi Abdulhussein Al- Saadi * and Mohammed Shaker Al- Khshali

Department of Animal Production, College of Agriculture, University of Baghdad. Iraq.

Received: 17 Mar 2018 Revised: 22 Apr 2018 Accepted: 11 May 2018

*Address for correspondence
Dhamyaa Oleiwi Abdulhussein Al-Saadi
Department of Animal Production,
College of Agriculture,
University of Baghdad. Iraq.

ABSTRACT

This study was conducted to investigate the effect of salt feeding on the ALT and AST enzymes activity and cortisol hormone in the blood plasma of common carp (*Cyprinus carpio*), by using NaCl salt in formation of the diets as 1, 3 and 5 % , as well as the control diet (free of salt) , and each diet represented an independent treatment included three replicates. Fish were distributed on 12 glass tank as 6 fish / tank at average weight of 30 $\pm$ 5 g. Fish were fed on diet with 30.7 % protein content during the experiment. ALT enzyme activity was increased to 51.48, 54.19 and 57.35 IU / in diets with salt ratio of 1, 3 and 5 %, respectively in comparison with the control treatment (363.90 IU / L). The concentration of the cortisol hormone in the blood plasma was elevated to 0.98, 1.86 and 2.31 ug / 100 ml in diets with salt ratio of 1, 3 and 5%, respectively as compared with the control treatment (0.56 ug / 100 ml).

Keywords: Salt feeding, AST and ALT Enzymes, Cortisol, Common carp

INTRODUCTION

Due to raise the salt concentrations in most of fresh water bodies generally and especially in Iraq in which there are dangerous risk on fish life. Also the other water biology and biological diversity may face challenges, so there is extreme need to rice fresh water fish ability to tolerate the high salt concentrations because they tolerate narrow ranges of salinity. This can be done by using salt feeding mechanisms which includes a certain ratio of sodium chloride in fish feeding diets to get ionic balance case between the internal environment (fish body) and the external environment (the surrounded water) and this gives enough time to the salt stress on fish reaching homeostasis case and decreasing the consumed used energy amount in osmotic organization process and then directs this energy for fish growth (Lawson and Alake, 2000). The salt feeding technique was successfully used with fresh water fish such as Tilapia fishes (Oreochromis niloticus species) (Fontainhas et al, 2000) and herbal common carp (Tenopharyngodon idella) (Al-Kashale, 2013). Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes are put with the

transaminases enzymes which contribute in operation of transport amine group from the amino acids to the ketones acids and this operation is considered from the important biological operations in amino acids metabolism (Bahjet and Shaban, 1985). ALT enzyme which is also called glutamic pyruvate transaminase (GPT) concentrates in liver tissues in great quantities and in the skeletal muscles in less degree and in kidney and heart tissues, and it is also present mainly in cell cytoplasm and it may be connected with mitochondria, it was noticed that its level in rainbow trout fishes ranged between 17-13, IU L^{-1} (Das et al, 2004). AST enzyme which is also called glutamic oxaloacetate transaminase (GOT) is concentrated mainly in heart, bones, kidney, liver and red bloods cells tissues and its natural level in blood plasma of rainbow trout fishes ranges between 51-443 IuL^{-1} (Gaudet et al , 1975). Cortisol hormone is secreted from the adrenal cortex gland which is in the bone fish buried inside the kidney and it is sometimes called internal tissues, and cortisol is considered from the main hormones that are responsible of increase of fish saline tolerance ability when they transport from fresh water to saline water due to its role in reduce hypo-osmotic body liquid (Morgan and Iwama, 1991). The important role of cortisol hormone appears in body liquids electrolytes organization in the bone fish through its direct effect in cell membrane permeability, increase number and size of mitochondria and activation of Na^{+}/k^{+} ATPase which is the main enzyme that control of salts movement inside chloride cells in bone fish branchia (Uchida et al, 1997). Cortisol hormone which is considered as glucocorticoids work on increase carbohydrate metabolism in liver releasing glucose and proteins metabolism average, these effects may increase nitrogen in blood plasma (Vijayan et al, 1996a). The aim of this study is to investigate effect of saline feeding on AST and ALT activity and cortisol hormone activity in the common Carp fishes blood plasma after nutrition by diets having different sodium chloride ratios.

MATERIALS AND METHODS

Two hundred of the common Carp fish which have weights ranged between 20-40 gram were brought from fish culture. After transferring the fish to the laboratory, they were put in glass fibers tank containing free chloride water and air pump. In the next day, the fish were immersed in 3% salt solution concentration for sterilization and getting rid of the expected external parasites. The fish then were distributed into glass tanks having free chloride water and good aeration. The experiment fish were adapted for 15 days and they were fed during these 15 days on a control diet which was free of salt and having 30.7 protein content at 3% of body weight.

Fish feeding and salt diet preparation

Salt diets were prepared laboratory by grinding known quantities of the commercial diet per fish diet (Table 1) and the protein ratio in the commercial diet was adjusted from 23.4 to 30.7 by using known ratio of fish paste. The salt diets were prepared by adding sodium chloride, Al-Menaa mark, at 1%, 3% and 5% and they were mixed together, and water was added at 400ml kg-1 to make suitable paste and the samples were minced by hand mincer with suitable openings to get discs, then they were air dried and chopped into suitable sizes (15mm length and 3mm diameter). Every salt diet represented treatment by itself beside presence of the control treatment which was free of salt. The fish were fed by a diet having 30.7 protein content at 3% ratio of fresh body weight during experiment time and the diet was put in certain location in the tank twice a day , first at nine o'clock morning and the second at four afternoon and the basins were being cleaned daily before morning feeding offering.

ALT (GPT) enzyme activity measurement

ALT enzyme activity was measured following Reitman and Frankel (1975) method and using ALT kit provided by the French Randox company. Alfa- glutamate reacts with L- alanine in presence of ALT and releasing L-glutamate and pyruvate ALT enzyme is measured by measuring pyruvate hydrazine concentration (2,4-dinitrophepyl hydrazine) and the absorptivity was read by spectrophotometer device at 546 nanometer wave length. Sample

absorptivity is taken against absorptivity of blank sample and ALT enzyme activity is calculated by using prepared certain tables for this purpose in measurement unit (IU L^{-1}).

AST (GOT) enzyme activity measurement

Doumas and Briggs (1969) method is followed to measure AST activity in blood plasma. AST kit that supplied by the French Randox company. Alfa- oxoglutarate is reacted with L- aspartate with presence of AST and releasing L-glutamate and oxaloacetate. AST enzyme activity is measured by measuring oxaloacetate hydrazine concentration (2,4-dinitrophenyl hydrazine) and absorptivity was read by spectrophotometer device at 546 nanometer- wave length and AST enzyme activity is calculated by using prepared certain tables for this purpose in unit (IUL^{-1}).

Cortisol hormone activity determination

Cortisol level in blood plasma was measured by using Ria kit according to radio immune assay. Cortisol measurement depends on measuring the remaining range in the isotope standard cortisol I^{123} after putting it in an incubator. Plasma samples and the standard solutions were incubated in monoclonal bottles treated with antibiotics and the isotope binding ability was measured by drawing the standard curve of cortisol and then cortisol values are estimated.

Statistical analysis

The data were statistically analyzed by using CRD design and the significant differences between treatments means were obtained by using Duncan (1955) test at 0.05% probability.

RESULTS AND DISCUSSION

Water temperature ranged between 23-25 C^{o} during the measuring of AST and ALT enzymes activity, and averages of the dissolved oxygen ranged between 5.8 to 6.2 mgL^{-1} and PH between 7.3 to 7.6. All measurements are within the natural levels of the common Carp fishes living (Hattingh et al, 1975). Table -2- illustrated the presence of increase of ALT enzyme activity in the common Carp fishes blood plasma with increase of sodium chloride ratio in the diets and the statistical analysis results showed significant differences ($p<0.05$) between control treatment and third and fourth treatments and AST enzyme activity increased with the increasing of sodium chloride ratio in the diets and significant differences were recorded between control treatment and other treatments. It may be noticed a slight increasing in ALT and AST enzymes values in the common Carp fishes blood plasma in which the fishes were fed by using 1.3 and 5% NaCl saline diets and this rise may be attributed to the stress case in which fishes were faced due to the saline feeding to substitute the destroyed cells but when high destruction or damage were at high ratios in cells especially in liver cells which are the center of nutrients metabolism so, the ALT and AST concentrations were unnatural due to its role in the non-essential amino acids formation process needed by body to substitute the damaged cells by the stress or excessiveness in nutrients material consumption that may stressed liver work.

The non-essential amino acids formation processes from transamination processes of pyruvic acid to from alanine acid (Das et al, 2004), so any increase of these enzymes over the natural level in blood plasma is a result of these tissues destruction due to physiological, pathogenic and nutritional cases and it is believed that facing the fish to stress may lead to increases AST and ALT enzymes leak to blood (Bahjet and Shaban, 1985). These results was explained by similar studies results such as Salaei (2006) study which pointed that ALT and AST enzymes activity values in the common Carp fishes (*Cyprinus carpio*) blood plasma in which they were nursed in soils tanks were highly increased over their recorded values in the carps fishes that were nursed in glass tanks and it is believed that it does not only depend on treatment effect but it is related to hunting nature in the soils tanks that may stressed

largely the fish and increase these values. Soltan (2007) noticed that ALT and AST enzymes activity in blood plasma of golden alsham (*Acanthopagrus latus*) fishes with salinity degree rise and it was attributed to salt stress effect and kidney function turbulence. Vijayan et al (1996a) mentioned that transferring Bolte fishes from fresh water to sea water for two weeks caused significant rise in ALT and AST enzymes activity in liver, and increase of liver protein destruction processes was noticed in fish that presented in the salt water more than those fish which presented in fresh water and this explains the increasing need to energy. Destruction processes are associated with increase of ALT and AST enzymes activity in liver and it was noticed that their activity in Salmo gairdneri fishes kidney in saline water (20 gm L^{-1}) were higher than the fresh water (Jurss et al ,1983). Barman et al (2012) indicated also to ALT and AST enzymes activity rise in Milk fishes that were fed by saline diets. It is shown in table (3). The rising of cortisol hormone concentrations in the common Carp fish blood plasma in which they were fed by saline diets at 1,3 and 5% NaCl and the hormone concentrations reached 0.098, 1.86 and 2.31 microgram L^{-1} respectively. Statistical analysis results showed non-significant differences between control treatment and the second treatment (1% - NaCl), while there were significant differences ($p<0.05$) between the control treatment and the third and fourth treatments (3 and 5% - NaCl) and between the third and fourth treatments. Cortisol and prolactin hormones and growth hormone that are responsible of osmotic regulation processes in fish and the growth hormone stimulates fresh water fish adaption to tolerate the salt -water while, prolactin hormone stimulates salt water fish adaptation in the fresh water.

The cortisol hormone participates and interacts with both of hormones (growth and prolactin hormones) and it has paired function that can stimulate the fresh and salt water fish to adapt in salt water and fresh water respectively (McCormick, 2001). Rise of cortisol hormone concentration in this study indicates getting stress in the common Carp fish that are fed at different levels of NaCl concentrations. Feeding the fresh water fish by salt foods increases cortisol hormone secretion which is considered from the important saline sensitive hormone and it is also responsible of saline concentration adjusting between the internal ambient and the internal environment of body through its role in body liquids concentration decline and it represents the a physiological responses that are followed by fish as a result of osmotic regulation processes occurrence under harmonic control (McCormick, 1995). Many studies referred that cortisol hormone is associated to osmotic regulation processes in fresh water fish adaptation on the salt water and this may increase their ability to tolerate salts (Al-Gafar, 1999) and the reason of that may be to the cortisol hormone role in increasing number and size of chloride cells that are responsible in ionic exchange in branchia , and activation Na+/K+ ATPase enzyme which is responsible of ions movement and increase of mitochondria size and number of chloride cells (Uchida et al, 1997). Cortisol hormone combined in strong relation with the salt diet as an internal tool for adaptation in marine environments, and Fontainnas et al (2000) studied the relation between salt feeding and cortisol hormones levels in blood plasma in which Nile Tilapla fish (*Oreochromis niloticus*) were classified into two parts, the first fish group were fed on free salt die and then they were transferred to sea water (15g L^{-1}Concentration) and the second fish group on salt diet and then they were transferred to sea water (20 g L^{-1}) and levels of cortisol in blood plasma of these two groups were measured after 6,12 ,24 and 48 hours .

The results showed rise of cortisol level in fish blood plasma of the second group compared with the first group fish and the two groups of the transferred fish to sea water (15 and 20 g L^{-1}) compared with the fresh water control treatment.Utida et al (1972) referred that cortisol hormone had important role in significant increase occurrence in water flux and pumping averages through the intestines of adapted Eel fish in fresh water while prolactin hormone had adverse action to cortisol work in reduction of water flux in Eel fish in the sea water. In study of Chhorn et al (2006) about cortisol hormone level in Nile Tilapia fish that were fed on different NaCl levels nutrient diets observed cortisol level rise with increase of diet salt level and also with nutrition period on these diets. On other side, Shirashi et al (1997) indicated that the embryonic and larval growth stages of Tilapia Mozambican fish (Oreochromis mossambicus) were associated by chloride cells growth and rise of blood cortisol hormone levels when they were transferred to saline water. Madsen and Bern (1992) indicated that injection some of Salmonidae family species by cortisol hormone caused increase of numbers and size of chloride cells and increase Na+/ K+ ATPase enzyme activity . In study about immigration of young of some Salmon kind species to the sea, it was noticed increase cortisol level in blood plasma with increased activity of Na+/K+ ATPase enzyme in branchia and it was associated with decline in

thyroxine level. This result explained that the thyroxine may activate cortisol receptors in the branchia (Ayson et al, 1995). Hyde et al (2004) mentioned that cortisol hormone participates with growth hormone (GH) in saline tolerance rise during cortisol receptors regulation processes in branchia of the adopted fish on the saline water and the cortisol hormone has an important role in increase of ions uptake average besides its reaction with prolactin hormone during the adaptation in the fresh water (Pelis and McCormick, 2001).

REFERENCES

1. Al-Jafar, L.M.A. 1999 . Effect of cortisol and thyroxine on the osmotic regulation of the common Carp *(Cyprinus carpio L*) Agriculture, University of Basra, p;63
2. 2. Al- Khshali, M.S .2013. Effect of salt feeding on mechanism and efficiency of ions exchange of the *Grass Carp*. The Egyptian journal of applicable sciences. 28(5): 95-106.
3. Ayson , E G ; Kaneko , T ; Hasegawa , S . and Hirano ,T . (1995) . Cortisol stimulates the size and number of mitochondrian – rich cells in the yolk – sac membrane of embryos and larvae of Tilapia (*Oreochromis mossambicus*) in vitro and in vivo . J.EXP . Zool . , 272 : 419 – 425 .
4. Barman , U.K ; Garg , S. K . and Bhatnagar , (2012) . Effect of different salinity and ration levels on growth performance and nutritive physiology of milk fish , *Chanos chanos* (Forsskal) – field and laboratory studies . Fish . Aqua .J .Vol . FAJ-53 India
5. Behgat, A.M. and A.M. Shaban .1985. The clinical chemistry foundation of technical education .Foundation of technical education. Institute of medical technology. Baghdad. First ed. P: 256
6. Chhorn, L; Mediha Y.A. ; Thomas , W & Karen , V. (2006) .Effect of feeding suration of sodium chloride - containing diets on growth performance and some osmoregulatory parameters of Nile Tilapia , *Oreochromis niloticus* ,After transfer to water of different salinities .J. Applied Aquac .,18 (4) : 234 – 242 .
7. Das,P.C ; Ayappan S.J ; jena J.k & Das B .K. (2004) . Acute toxicite of ammonia and sub lethal effects on marigal (*Cirrhinus mrigala*) Hamilton ,Aquate .Res ., 35 : 134 – 143 .
8. Doumas , B .T . and Briggs ,H.G. (1969) .Clinical Chemistry .Acta , 25 : 57 – 87 .
9. Duncan ,D.B . (1955) . Multiple range and multiple F test *Biometerics* , 1:11-19 .
10. Fontainhas – Fernades , A . ; Russell – pinto , F.; Gomes , E .; Reis – Henriques M.A and combra , J. . (2001) , the effect of dietary sodium Chloride on some Osmoregulatory parameters of teleost , oreochromis niloticus , after transfer form fresh water to sea water . Fish physiol .Biochem . 23 : 307 : -316 .
11. Gaudet , M. ; Raciocot , J . G . and Leray , C. (1975) . Enzyme activities of Plasma and selected tissues and rainbow trout *Salmo gairdneri.* J.Fish Biol ., 7:505 – 512 .
12. Hattingh , J.; Le Roux Fourie , F and Van Vuren , J.H.S (1975) ,. The transport of freshwater fis .J.Fish Biol ., 7:447-449 .
13. Hyde , G.N . ; Seale ,A . p ; Grau , E.G . and Borski , R. J (2004) . Cortisol rapidly suppresses intracellular calcium and voltage – gated calcium channel activity in prolactin cells of the Tilapia (*Oreochromis mossambicus*). Ame . J.Physiol Endocrinol . Metab ., 286:626-633.
14. Jurss K .; Brttorf , T.h ; V okler , T.H and wacke , R . (1983) . influence of nutrition on biochemical seawater adaptation of the Rainbow trout salmogarideeri . comp . Biochem . physiol ., 75 B: 713 – 717 .
15. Lawson ,E. O .; A lake , S .A . (2011) . Salinity adaptation and tolerance of hatchery reared comet Goldfish *Carassus auratus* (Linnaeus 1758) . Int . J . Zool . Res ., 7: 68-76 .
16. Madesn ,S.S and Bern H.A . (1992) .Antagonism of prolactin and growth hormone : impact on seawater adaptation in two salmonids *Salmo trutta and Oncorhynchus mykiss* . Zool . Sc I ., 9:775 -784
17. McCormick , S.D. .(1995) .Hormonal control of gill Na + , K+ - ATPase and chloride cell function in : (Wood C.M. and Suttleworth , T.J.) (Eds) . Fish physiology I osmoregulation : cellular and molecular approaches to fish ionic regulation . San Diego : Academic press . (14) : 285 – 315 .
18. McCormick ,S.D (2001) . Endocrine control of osmoregulation in teleost fish .Am . Zool ., 41:781 – 794 .

19. Morgan, J.D. and Iwama, G. k. (1991). Effects of salinity in growth, metabolism, and ion regulation in juvenile rainbow trout (*Oncorhynchus mykiss*) and fall Chinook salmon (*Oncorhynchus tshawytscha*). Can .J. Fish Aqua. Sci., 48 (11) : 2083 – 2094 .
20. Pelis, R and McCormick , S.D . (2001). Effects of growth hormone and cortisol on NA+ , k+ contrasporter localization and abundance in the gills of atlantic salmon . Ame . Zool , 42:421 -436 .
21. Reitman , S. and Frankel , S. (1957) . Colorimetric method for the determination of serum glutamic oxalacetic method for the determination of serum glutamic oxalacetic and glutamic pyruvic .
22. Salaei , V.S.M (2006) . the effect of substitution of soybean meal by cotton seed cake in common carp *Cyprinus carpio* diet Degree of Master . University of Salahaddin – Arbil ., 63 pp .
23. Shirashi, K; kaneko , T . ; Hasegawa, S . and Hirano , T . (1997) . Development of multicellular complex of chloride cells in the yolk – sac membrance of tilapia *Oreochromis mossambicus* embyo and larvae in seawater Cell Tissu . Res ., 288:583-590 .
24. Sultan, F.A. 2007. Effect of saline adaptation on some physiological and nutrition aspects in young of golden houttyn, Acanthopagrus latus, 1982. Ph.D. Thesis. College of Agriculture. University of Basra. p: 162,
25. Uchida K. ; Kaneko , T.; Yamauchi , A.; Ogasawara , T. and Hirano , T . (1997) . Reduced hypo – osmoregulatory ability and alteration in gill chloride cell distribution a mature chloride cell distribution a mture chum salmon (Oncorhynchus Keta) migration up stream for spawning Mar Biol ., 129: 247 – 253 .
26. Utida , S.; Hirano , T ., Oide , H .; Ando , M .; Johnson , D . W . , and Bern , H. A (1972) . Hormonal control of the intestine and urinary bladder in teleost osmoregulation .Gen . Comp . Endocrinol . Suppl . 3:317 – 327.
27. Vijayan ,M.M.; Morgan , J.D .; Sakamoto , T .; Gran , E.G, and iwama , G.K. (1996 a) . Food deprivation effects seawater acclimation in tilapia: hormonal and metabolic changes. J.EXP.Biol. 199:2467-2475.

Table-1- The chemical analysis of the commercial diet

Protein	Fat	Moisture	Carbohydrate	Fibers	Ash
8.20	8.12	48.04	5.91	5.88	23.40

Table-2- Means of AST and ALT enzymes activity (IU L^{-1}) in blood plasma of the common Carp fishes which were fed on different saline diets.

Treatments		AST	ALT
T1	Without NaCl addition	363.90±5.77 c	50.26±0.57 b
T2	1% NaCl	384.80±2.30 a	51.48±0.27 b
T3	3% NaCl	385.10 ±4.61a	54.19± 1.15 a
T4	5% NaCl	375.30± 8.66 b	57.35±0.57 a

Table -3-Means of the Cortisol hormone concentration (microgram L-1) in blood plasma of the common carp fishes those were fed with different salt diets.

Treatments		Cortisol hormone concentration
T1	without -NaCl	0.56±0.42 c
T2	1%-NaCl	0.98±0.27 c
T3	3%-NaCl	1.86±0.18 b
T4	5%- NaCl	2.31±0.34 a

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Toxicopathological and Accumulative Titer of Endosulfan on the Testicular Tissue of Swiss Albino Mice after Ingested Fish Flesh in Iraq

Bushra I. AL-Kaisei*

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Iraq.

Received: 15 Mar 2018 Revised: 20 Apr 2018 Accepted: 12 May 2018

***Address for correspondence**
Dr. Bushra I. AL-Kaisei
Assitant Professor
Department of Pathology and Poultry Diseases,
Collages of Veterinary Medicine,
University of Baghdad. Iraq
E.mail: bassimpatho@yahoo.com

ABSTRACT

Despite the advancements in the field, there is a lack of data when it comes to determine the toxicopathological effects with accumulative titer of the tioxic substance that transported from water to the fish, which can cause tissue damage in the body of the organism after ingestion. Therefore, this study was designed to address this issue. Swiss albino mice experimentally were taken**,** then eatingfish flesh, that poising in the rivers via *Endosulfan*. The experimental groups were 30 mice, were randomly divided into three group, the 1st treated group [T1] contain 10 animals daily intake 250 gramfish flesh from *Common Carp* that contain about[0.00008ppm.]of *Endosulfan* in the muscle that sacrificed after 4 weeks, and the 2nd treated group [T2] contain 10 animal daily intake daily intake 250 gram fish fleshfrom *Common Carp* that contain about [0.00008 ppm.] of *Endosulfan* in the muscle that sacrificed after 8 weeks, while control group [C] contain 10 animals feed on normal fish flesh. All results showed that *Endosulfan* compounded has accumulative effect in the muscle of *Common Carp Cyprinus carpio*.There are severe histo &clinical pathological effect with infertility male reproductive system of swiss albino mice after ingested 250 gramfish flesh from *Cyprinus carpio* that contains accumulative level from *Endosulfan*.The present study revealed that the residual assay *Endosulfat* by [GCs] in muscle of *Common Carp* also the toxico &clinical pathological effect of *Endosulfan* on the testicular tissue of mice after ingested 250 gramfish flesh from *Common Carp Cyprinus carpio* that contain accumulative titer from *Endosulfan* compound in the muscle, and to recording its potential role in infertility male reproductive system.

Keywords: *Endosulfan, Gas Chromatographic, toxico & clinical pathological accumulative titer*.

INTRODUCTION

Pesticides have become a type of organochlorine chemicals compound that are used in wide range to kill not only insects but also fungi and weeds for better yields of our agriculture from the time 1960s , one of organochlorine insecticides is *Endosulfan* which is consider as one of environmental chemicals material, that increasing fear from both natural and man-made , having estrogenic possessions may lead the listed with high variety of male reproductive system disorders specially after intake directly or indirect intake (5,8). *Endosulfan* is very toxic to human and other lab. Animal besides fish, to be specific as important meal in many different countries when, equal importance of other organochlorine substances but hardly than other pesticides such as dichlorodiphenyltrichloroethane [DDT] (9, 10, 11). The poisoning severity of *Endosulfan* standing on the type of isoform for that two categories of *Endosulfan* toxicity which included [isomer-α and isomer-β], the isomer-β represented a widespread use in agriculture alongside forestry to surveillance a wide variety of insect pests and on non – food crops such as tobacco, coffee and cotton, while the type of isomer-α being the more toxic pattern of technical *Endosulfan* (16, 20). For that many confusion and disruption of endocrine strategy in the cortico steroidogenic cells of endocrinal function are thought to be effected on reproductive system specially on the spermatid, spermatogenesis, seminiferous tubule, level of testosterone hormones in human and animal which caused by ingestion of *Endosulfan*(16). Thus allowing study the toxicopathological effect of ingestion the toxic muscle of *Common CarpCyprinus carpio* experimentally via *Endosulfan* in Iraq after taken by male mice on male reproductive system as a models and how can statistical alteration in level of testosterone hormone, which we aim to it.

MATERIALS AND METHODS

Experimental animal and management

30 Swiss mice with 35±2 g. Bw. were used in this study, were represented the first generation of lab. Animal, [after inserted removal from any infection cases] are housed in the animal house of department of pathology/ Collage of Veterinary Medicine /Baghdad University in a room 6X4X3 m³ under 12 hours light / 12 hours dark at 20± 2Cº. For 60 day. And all experimental groups were provided identical management protocol.

Preparation of half lethal concentration (LC-50) of *Endosulfan in Cyprinus carpio*

The LC50 of *Endosulfan* in *Cyprinus carpio* that widely distributed in Iraqi water bodies and fish farming was determined according to (Al-Kuraizi. 2010) which measured about [0.0012µg/L] after 48 hour (9).Whereas the residual accumulated of *Endosulfan*toxicity in muscle of *Common Carp Cyprinus carpio*, was detected through used the Gas Chromatographic analysis [GC] method; in Ministry of science and Technology/Center of Technologic Environment and Water Research CTEWR., according to whom [17]

Toxication of lab. Animal

Tthe Swiss albino mice were taken, then animals were randomly divided into three group., Control group [C] contain 10 animals feed on healthyfish flesh, in the male of 1st treated group [T1] contain 10 animals daily intake 250 gram fish fleshfrom *Common CarpCyprinus carpio* that contain about [0.00008 ppm.] of *Endosulfan* in the flesh that sacrificed after 4 weeks , while 2nd treated group [T2] contain 10 animal daily intake daily intake 250 gram fish fleshfrom *Common Carp Cyprinus carpio* that contain about [0.00008 ppm.] of *Endosulfan* in the muscle that sacrificed after 8 weeks.All animal were sacrificed for post mortem examinationin addition, blood samples were collection Via cardiac puncture technique, collected in test tubes without anticoagulant which allowed to stand and coagulate for 15 minutes in refrigerator, The serum was separated from coagulated blood samples by centrifugation at 2000 rmp. For 15 minutes, a spirited and frozen at -20 ˚C until usingfor further determination the titer serum testosterone level in

mice by using special enzyme-linked immunosorbent assay *ELISA* method Testosterone kit of LILAC Medicare [P] Ltd., Mumbai was utilized for the experiment. On the other side, post mortem examination was done and any gross lesions were reported.

Pathology technique

The testes organ were taken for fixed in 10% formalin for 72 hour and processed according to whom (18), and the histopathological changes were observed under light microscope.

Sperm Analysis and Evaluation

The excised testis was taken to determined the sperm viability that included [dead, live ratio and sperm density] in right testis of male mice were calculated according to whom(12) and observed [$1x10^{10}$], through density and viability percentage respectively. From other hand the ratio of abnormal sperm was scored in 10 - 20 speared fields according to technique of whom (13), by use 1% trypan blue. Whereas the technique was made special ELISA kit occur by used the normal range was calibrated and then 25 µl serum samples were taken in the well plates. 100 µl of enzyme conjugate was added in each well. Then left for incubation at 37°C. in incubator for 60 minute. Afterward, washed by used 300 µl distilled water for at least 4 times and blotted. Next, 100 µl TMB solution was added as substrate in each well plate and was again put in the incubation for 15 minutes for the color. Atlest, 100 µl stop solution was added in each well to stop the reaction. Reading was taken at 630nm. Through Merck ELISA reader in ng/ml value.

Statistics analysis

Data were expressed as [Mean ± Stander Division]. The statistical analysis was carried out by one way analysis of variance (ANOVA) and made the comparison by Dunnett's T-test. ($P \leq 0.001$) was considered statistically significant.

RESULTS

There were no clinical signs, gross lesions, or mortality in the uninfected control mice.

Residual assay of endosulfan in fish flesh

The results of residual assay *Endosulfan* by gas chromatography [GCs] in fish fleshof*Common CarpCyprinus carpio*, appear in peak number nine(Figure: 1). While the (Figure:2) show the stander assay of *Endosulfan*, and from this figure the [Ret. Time] of stander was (3.831), while the [Ret. Time] and [area under peak] of *Endosulfan*that acumination in fish fleshof*Common CarpCyprinus carpio*Ret. Time was (3.821), while the area & area% under the peak was (633450; 2.6916%) respectively. Which it's important fordescribe the substantial the item, also important for concentration of these item and percentage in sample. Via used the equation

$$\text{Concentration sample} = \frac{[\text{Concentration stander}] \times [\text{Area of stander}]}{\text{Area of sample}}$$

$$\text{Concentration of Endosulfan In Muscle} = \frac{[\text{Concentration sample}] \times [\text{Volume of sample}]}{\text{Weight of organ}}$$

Which equal (0.0008 ppm) that conceder a highly concentration in muscle tissue.

Clinical Pathological Analysis

The results of titration serum testosterone level in mice by using special *ELISA* kit used are listed in (table:1) and (Figure:3)The normal level of testosterone hormone appear in control group of mice having (8.7707 ± 0.06081), while after group (T1), daily ingested 250 gram fish fleshfrom *Common Carp Cyprinus carpio*, that contain about (0.00008 ppm.) of *Endosulfan* for 4 weeks, was (5.9243 ± 0.03890) also the (T2) showed (5.2879 ± 0.03888) after daily ingested 250 gram fish fleshfrom *Common Carp* that contain about (0.00008 ppm) of *Endosulfan* for 8 weeks.

Sperm quality

The feature of sperm quality that appear in table of that included (%Sperm Abnormality, Sperm Density and % Sperm Viability) were significant decrease in (T1) and (T2) group respective when compared with control after ingestion 250 gram from fish muscle contain (0.0008 *Endosulfan* compound). From these result, *Endosulfan* causes pathophysiological effect in mice due to decrease in the level of testosterone hormones, with decline of sperm count and infertility disorderswhich characterized by seminal ejaculatory that causes azoospermic condition in Swiss albino male mice.

Histopathological examination

Before made the histopathological examination, mice were euthanized using an ether inhalation inside anesthesia room champers, and the necropsy was performed immediately after the mice were euthanized, and the pathological lesions were examined, with special attention paid to testes and epididymis in male reproductive system. The specific structural lesions observing in the parenchyma of the testes which The morphology of testes of the group T1 & T2 was characterized by the presence of interstitial exudates, degeneration, and necrosis of spermatogenic and interstitial [Leydig cells] with focal arcas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, and abnormal distribution of spermatozoa showed in Lumina of the seminiferous tubules, associated with increase of interstitial tissue with infiltration of MNCs with reduction in number of maturing spermatogenic cells occasionally with loss of architecture in some lesion or thinner wall of somniferous tubule. Epididymal epithelium of epidydymal tissue of T1 show enclosed a lumen containing spermatozoa. The interstitial space in between the epididymal tubules was filled with sparse stroma.

The pseudostratified epithelium was composed of principal cells with nuclei situated at the base. Epidydymal tissue of T2 showed a decrease in the number of sperm, the pseudostratified epithelium was composed of principal cells with nuclei situated at the base appear severe exaggerated features, increase in interstitial space, lumen of without sperm, and disappearance of the muscle layer. The (A) normal testes,figure (B,C,D and E) show the histopathological picture of testes mice in (T1) note the mild exudate formation into and between interstitial space companies with vacuole and degeneration of spermatogenic cells with sever necrotic tissue formation . The figure (F,G,H and I) described the histopathological picture of testes mice in (T2) show increase of interstitial tissue with infiltration of MNCs with reduction in number of maturing spermatogenic cells occasionally with loss of architecture in some lesion or thinner wall of somniferous tubule The figure (a,b.c and e) show the histopathological picture of epididymis mice in (T1) appear irregular of circular tubules caused a decrease in stereocilia, disorganized or confused pseudostratified columnar epithelial cell that separated from the connective tissue by an intact basemen with reduction in sperm density flowed with cellular debris in lumen of epididymis. The figure (e,f.g and h) show the histopathological picture of epididymis mice in (T1) appear severe distraction & desquamation of the pseudostratified columnar epithelial cell and disappear of the muscle layer with dilation of lumen space, without sperm containing.

DISCUSSION

There are view of literature suggests that some *Endosulfan*can affect the male reproductive functions including sperm counts motility and morphology spermatogenesis (5). Also, no any data reported that ingestion of the *Endosulfan*can causes defect in the male reproductive system. In the present study, our results clearly demonstrated at first the *Endosulfan*compounded that accumulative in muscle of the fish during exposure to these compounded these result agreement with (10, 17) that founded level of *Endosulfan*in the fish organs. Also *Endosulfan*compounded can seriously change in the testes and reproductive tract in male mice especially after ingestion muscle toxic by *Endosulfan*with alteration in the hormonal of testicular tissue. This reduction in sex function and hormone was accompanied by an alteration of the normal histological structure in the testicular organ of male mice. The decreasing level of the testosterone hormone in the blood of male mice after ingestion 250 gram fish fleshfrom *Common Carp Cyprinus carpio* that contain 0.0008ppm. Occur due to severe distraction and degeneration that occur in the testicular tissue especially in spermatide and somniferous tubule, This result agreed with (1,4 and 21) when reported the important role of *Endosulfan*on testosterone level and seminiferous tubule of testis of mice. The infertile of male mice caused by ingestion of 250 gram *Common Carp Cyprinus carpio*fish fleshfrom that contains 0.0008 ppm. Interfere with disorders seminal ejaculatory may occur because azoospermic condition in mice due to abnormal spermatids that present with changes in epididymal, including epithelial damage and degeneration in the interstitial tissue which indicated that the epididymal change may be an important contributory factor in infertility.

This idea is in consistence with idea mentioned by (22) who investigated that significant DNA damage because oxidative stress of *Endosulfan* that lead to fragmentation of DNA sperm, and with (23 and 21) which predicted that the mechanism toxicity of *Endosulfan* may stand on the antagonist effect of androgen receptor that stimulation the lagan binding site and reduces the positive of androgen receptor in the sertoli cells. The pathological alteration in testis histology that resulting structural disorder in spermatids and sperm male mice with insufficient or incomplete and imperfect in primary and secondary spermatocyte that see in these study which exhibited that *Endosulfan*compound may be causes disordered arrangement of germ cells with decrease spermatogenic cell layer in the seminiferous tubules particulary degenerating effect on latter phase of spermatogenesis. This evidence was in consistence with (19) who predicted that reported present atrophy of testicular tissue and with infertility and reduced in male sperm count after exposure to *Endosulfan*can result. In summary, we have verified that present study investigated that the endosulfant compounded that accumulative in *Common Carp Cyprinus carpio* muscle that exposure to an organochlorine insecticide that lead to reduction in sex function and hormone reduced was accompanied with alteration in the normal histological structure of testicular organ that causes toxicopathological effect associated with infertile of male mice after ingestion 250 gram fish fleshfrom *Common Carp Cyprinus carpio* muscle that contain 0.0008 ppm.

Conflict of Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement

This study was not funded by any funding agency and was conducted at the expense of the researcher to combat the rising problem resulting from exposure to toxic material which may infect *Common Carp Cyprinus carpio* that may be the main source of poisoning of the organismof the country.

Animal Rights Statement

The experiments and procedures involving Swiss albino mice were approved by scientific committee of department of pathology and poultry disease; in the College of Veterinary Collages of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, and were conducted according to the guidelines of the committee.

REFERENCES

1. Singh J.K., Nath A., Arun Kumar, Ali Md. and Ranjit Kumar.:Study on the effect of *Endosulfan* on testosterone level and seminiferous tubule of testis of mice. Mahavir Cancer Institute and Research Centre, Phulwarisharif, Patna (Bihar), India – World Journal of Environmental Pollution,2011, 1 (1): 01-04.
2. Ranjit Kumar, Ali Md, Singh J.K., Nath A., and Arun Kumar: Bio-remedial impact of withania somnifera on *Endosulfan* exposed spermatozoa of mice. Mahavir Cancer Institute and Research Centre, Phulwarisharif, Patna (Bihar), India – International journal of pharmaceutical science and research, 2012, Vol.3 (5): 1343-1347.
3. Nath, Priyanka, Aseem Kr. Anshu, Mona Hassan, Chandan Kumar Singh, Sachhidanand Behera and J.K. Singh: Abnormality in microtubules due to effect of *Endosulfan* toxicity leads to asthenozoospermia in swiss albino Mice. S.S. Hospital and Research Institute. Kankarbagh, Patna, India. Int. J. Pharm. Sci. Rev. Res., 41(1), November - December 2016, Article No. 21, Pages: 104-109.
4. Singh JK, Nath A, Kumar A, Md. Ali, Kumar R.: Study on the effect of *Endosulfan*on testosterone level and seminiferous tubule of testis of mice. World Journal of Environmental Pollution, 2011, 1(1), 01-04.
5. Khan S.: Study of histopathological changes induced by *Endosulfan*in kidney of albino rats, Journal of Drug Delivery & Therapeutics, 2014, 4(3), 125-128.Çok I,"Durmaz TC, Durmaz E, Satıroglu MH, Kabukcu C. Determination of organochlorine pesticide and polychlorinated biphenyl levels in adipose tissue of infertile men. Environ Monit Assess, 162, 2010, 301–309."
6. Casabar RC, Wallace AD, Hodgson E, Rose RL.: Metabolism of *Endosulfan*-alpha by human liver microsomes and its utility as a simultaneous in vitro probe for CYP2B6 and CYP3A4. Drug Metab Dispos, 2006, 3(4), 1779–1785.
7. Singh SK. and Pandey RS.: Effect of sub-chronic *Endosulfan* exposures on plasma gonadotrophins, testosterone,testicular testosterone and enzymes of androgen biosynthesis in rat. Indian Journal of Experimental Biology, 1990, 28(10), 953-956.
8. Risk Management Evaluation *Endosulfan*, Long version – UNECE Context, Prepared by BiPRO GmbH, Germany, Mai, 2010.
9. Al-Kuraizi, Mohammed Hilal Kadhim: Pathological and hematological effects of *Endosulfan* pesticide on common carp *Cyprinus carpio*.Thesis for requirements for the degree of Master of Science in veterinary medicine. Pathology& Poultry Diseases, veterinary medicine at the University of Baghdad. Baghdad, Iraq, 2010
10. Naqvi SM, Vaishnavi C.: Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals, Comp Biochem Physiol C, 1993, 105,347-361.
11. Sebastian R and Raghavan SC.:*Endosulfan* induces male infertility, News and Commentary. Cell Death and Disease,6; e2022; doi:10.1038/cddis.2015.368.
12. Prasad MR, Chinoy NJ. and Kadam KM.: Change in succinate dehydrogenase treated level in rat epididymis under normal and altered physiological conditions. Fertil Steril , 1972, 23 (3): 186- 90.
13. Talbot P and Chacon RS.: A triple stain technique for evaluating normal acrosome reaction of human sperm. J exp Zool, 1981, 21 (2): 201-8.
14. Leather IJ.: Endocrine and reproductive function in great lakes salmon in: chemically induced alterations in sexual and functional development "the wildlife/human connection [Colborn T, Clement C,eds]. Princeton,NJ PrincetonScientific Publishing, 1992, 129-145.
15. Reijnders PJH.: Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature, 1986, 324,456-457.

16. Goebel, H.; Gorbach, S.; Knauf, W.;Rimpau, R.H. and Huttenbach, H.: Properties, effects, residues and analytics of the insecticide endosulfan. Residue Rev, 1982, 83: 5–16.
17. Arunee Therdeteppitak and Kittima Yammeng.: Determination of organochlorine pesticides commercial fish by gas chromatography with electron captuer detector and confirmation by chromatography-mass spectrometry. Science Asia, research article, 2003, 29:127-134.
18. Luna, L.G.: "Manual of histologic staining methods of the armed force institute of pathology" 3rd Ed. McGraw-Hill, New York. USA. , 1968.
19. Sebastian R. and Raghavan SC.: Exposure of *Endosulfan* can result in male infertility due to testicular atrophy and reduced sperm count. Cell Death Discovery, 2015,1, 1-10.
20. Toxicological profile for *Endosulfan*, U.S. Department of mhealth and human services Public Health Service. Agency for Toxic Substances and Disease Registry, 2015.
21. Sebastian R. and Raghavan SC.: Exposure of *Endosulfan* can result in male infertility due to testicular atrophy and reduced sperm count. Cell Death Discovery, 2015, 1, 1-10.
22. Subramani E. and Chaudhary K.: Detrimental effects of oxidative stress on sperm membrane and DNS Integrity in normozoospermic infertile men. Proceeding of 2010 International Conference on Systems in Medicines and Biology, IIT Kharagpur, India,2010, 198-202.
23. Viswanath G., Chatterjee S., Dabral S., Nanguneri SR., Divya G. and Roy P. : Anti-androgenic endocrine disrupting activities of chloropyrofos and piperophos, J. Steroid Biochem. Mol. Biol, 2010, 122, 22.

Table 1: Titration serum testosterone level in mice

Mice Group	Mean ± SE.	P. value
Control	8.7707 ± 0.06081	< 0.001
T1	5.9243 ± 0.03890	< 0.001
T2	5.2879 ± 0.03888	< 0.001

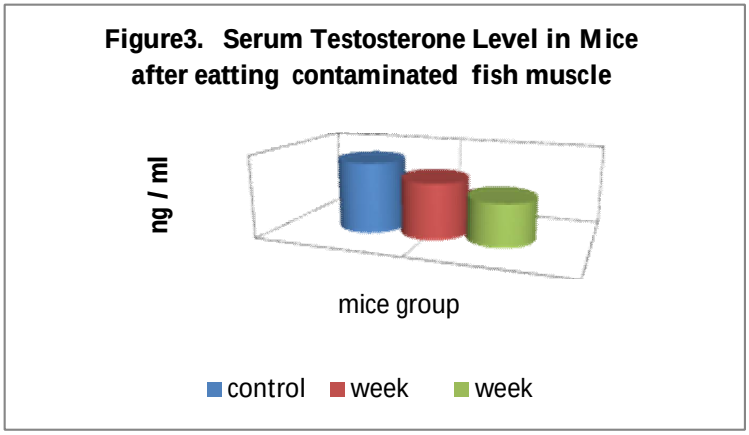


Table 2: Effects of toxic fish fleshon sperm quality in adult mice

Groups	Sperm Abnormality Ratio %	Sperm Density 106/l	Sperm Viability %
C	14.12 ± 1.01	15.73±0.29	80.11±2.77
T1	22.55 ± 2.19 •	9.97±0.35•	55.44±3.02•
T2	39.99 ± 2.97••	7.89±0.49••	42.97±6.63••

Levels of significance values are[mean ± SEM],[n=10],[• p<0,05, •• p<0,001] compared with control mice.

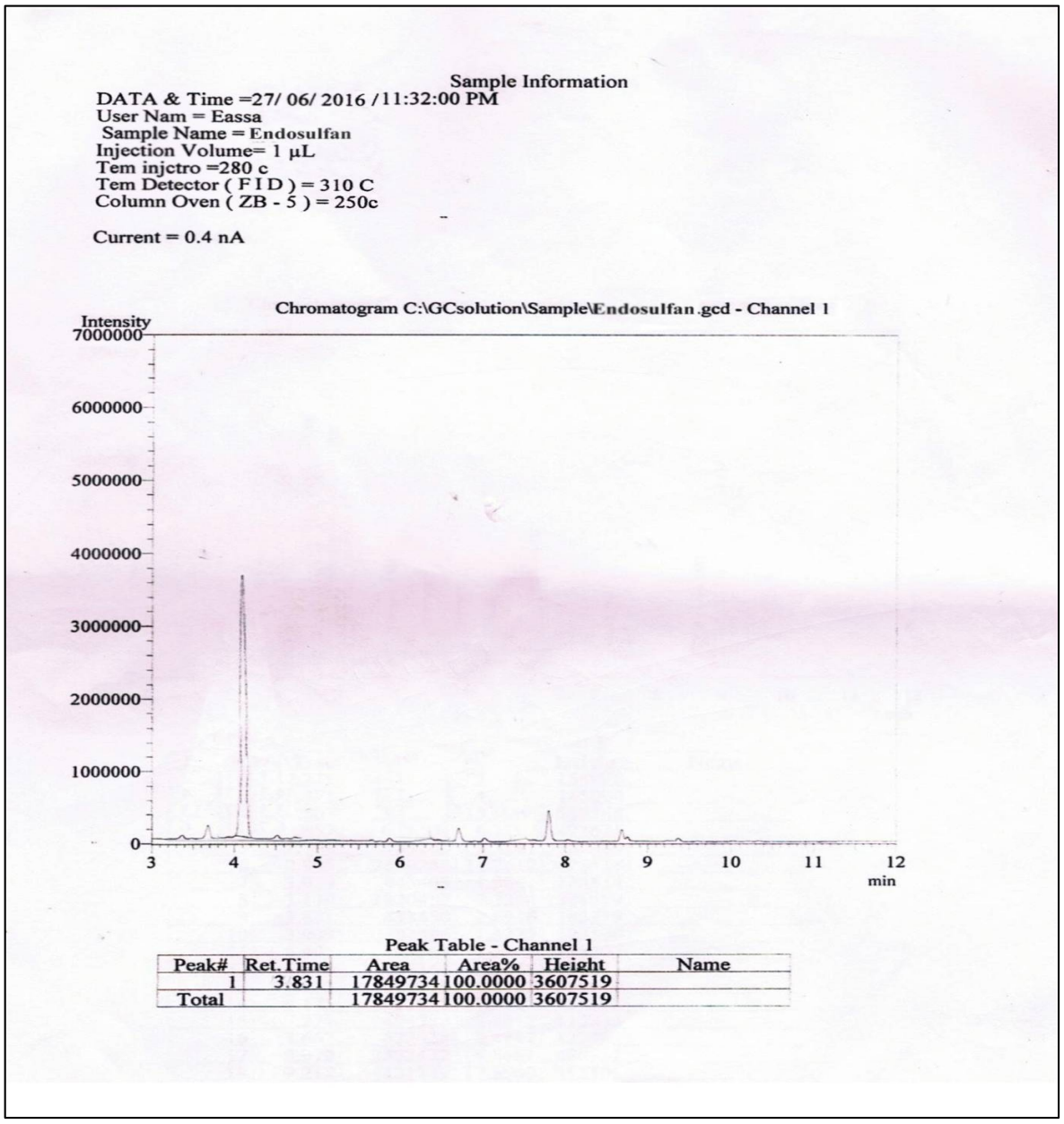
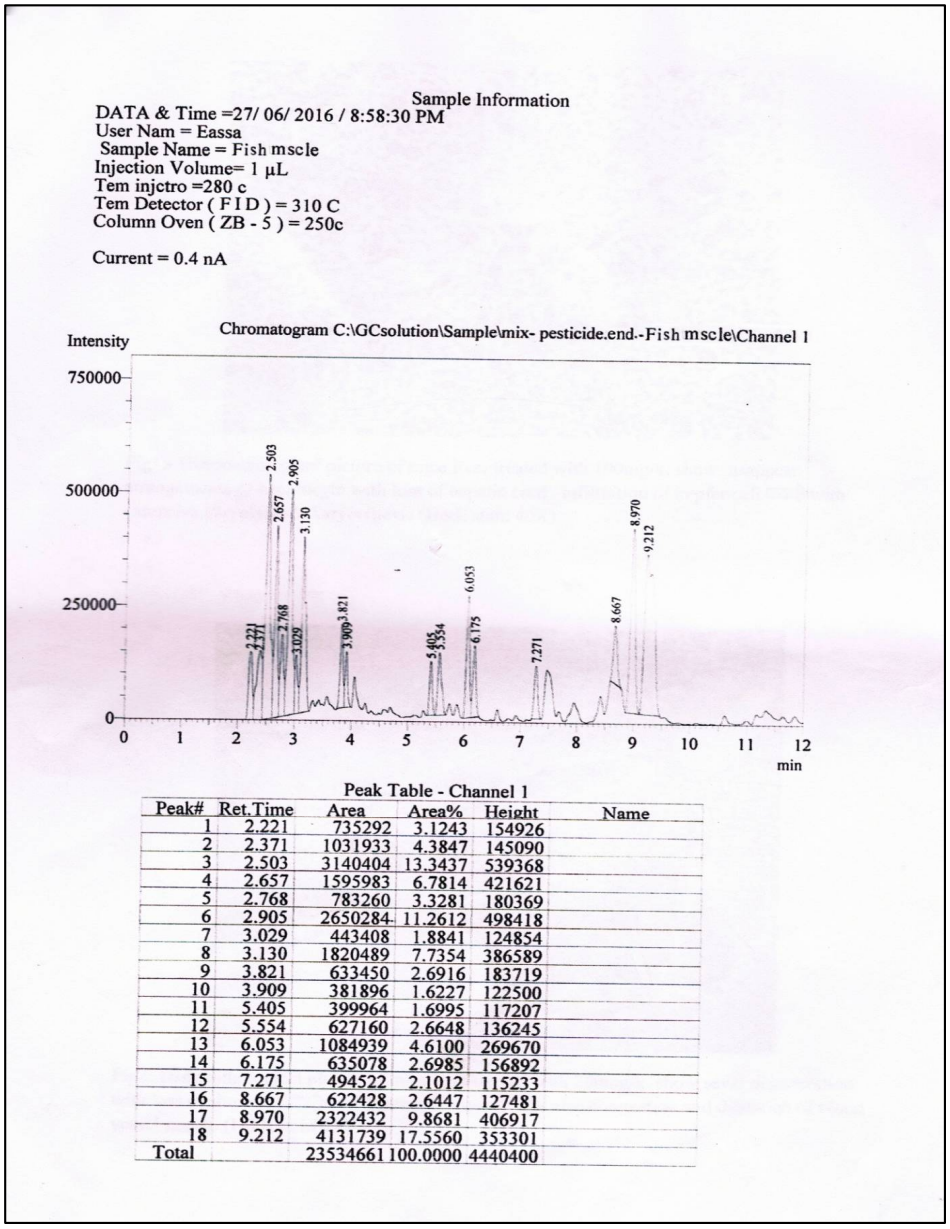


Figure: 1:- Normal carves of stander *Endosulfan* by use gas chromatographic analysis.



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	2.221	735292	3.1243	154926	
2	2.371	1031933	4.3847	145090	
3	2.503	3140404	13.3437	539368	
4	2.657	1595983	6.7814	421621	
5	2.768	783260	3.3281	180369	
6	2.905	2650284	11.2612	498418	
7	3.029	443408	1.8841	124854	
8	3.130	1820489	7.7354	386589	
9	3.821	633450	2.6916	183719	
10	3.909	381896	1.6227	122500	
11	5.405	399964	1.6995	117207	
12	5.554	627160	2.6648	136245	
13	6.053	1084939	4.6100	269670	
14	6.175	635078	2.6985	156892	
15	7.271	494522	2.1012	115233	
16	8.667	622428	2.6447	127481	
17	8.970	2322432	9.8681	406917	
18	9.212	4131739	17.5560	353301	
Total		23534661	100.0000	4440400	

Figure:2:- Residual carves of *Endosulfan* in fish fleshby using gas chromatographic analysis

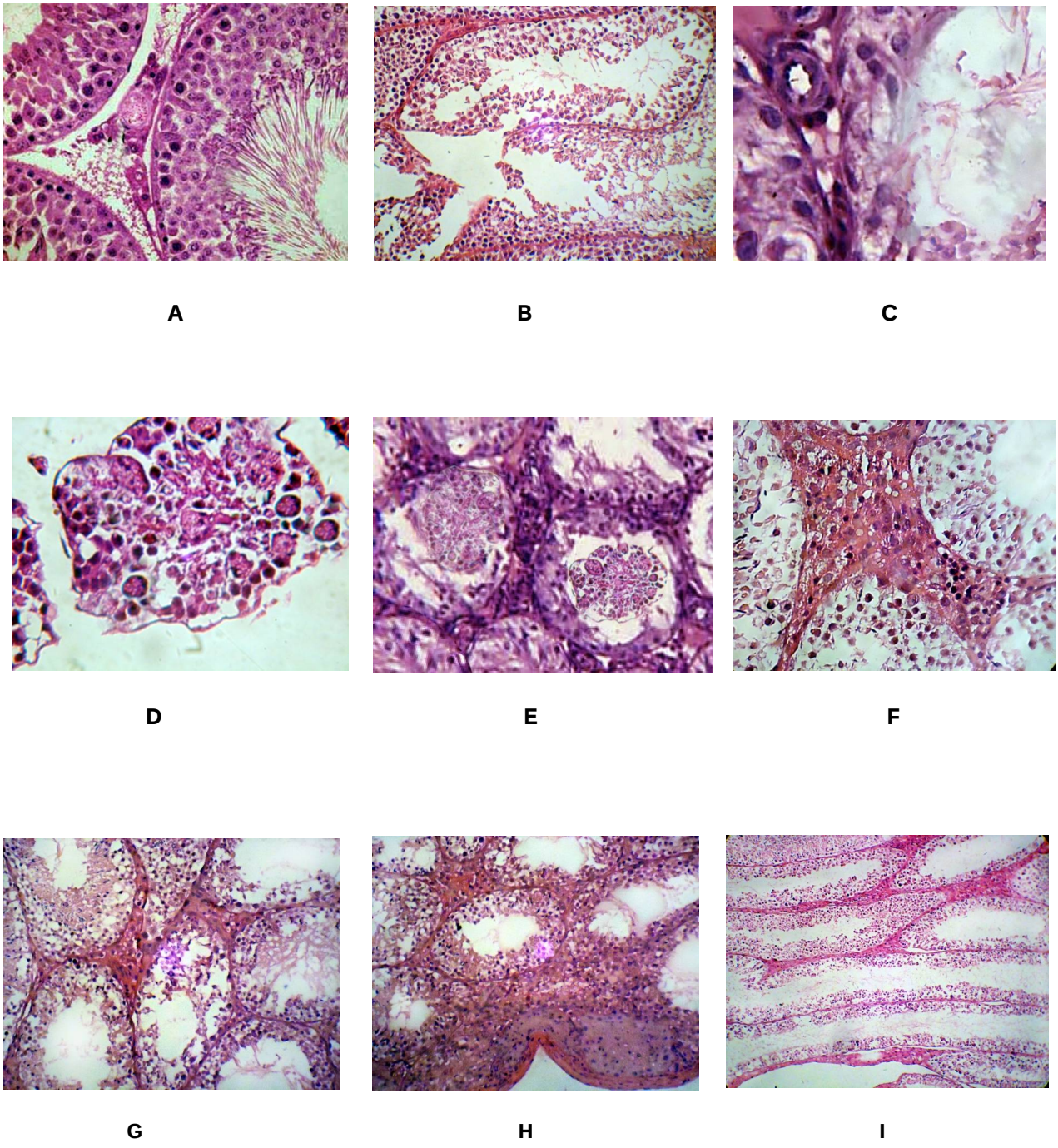


Figure 4. Show the histopathological picture of testes of mice

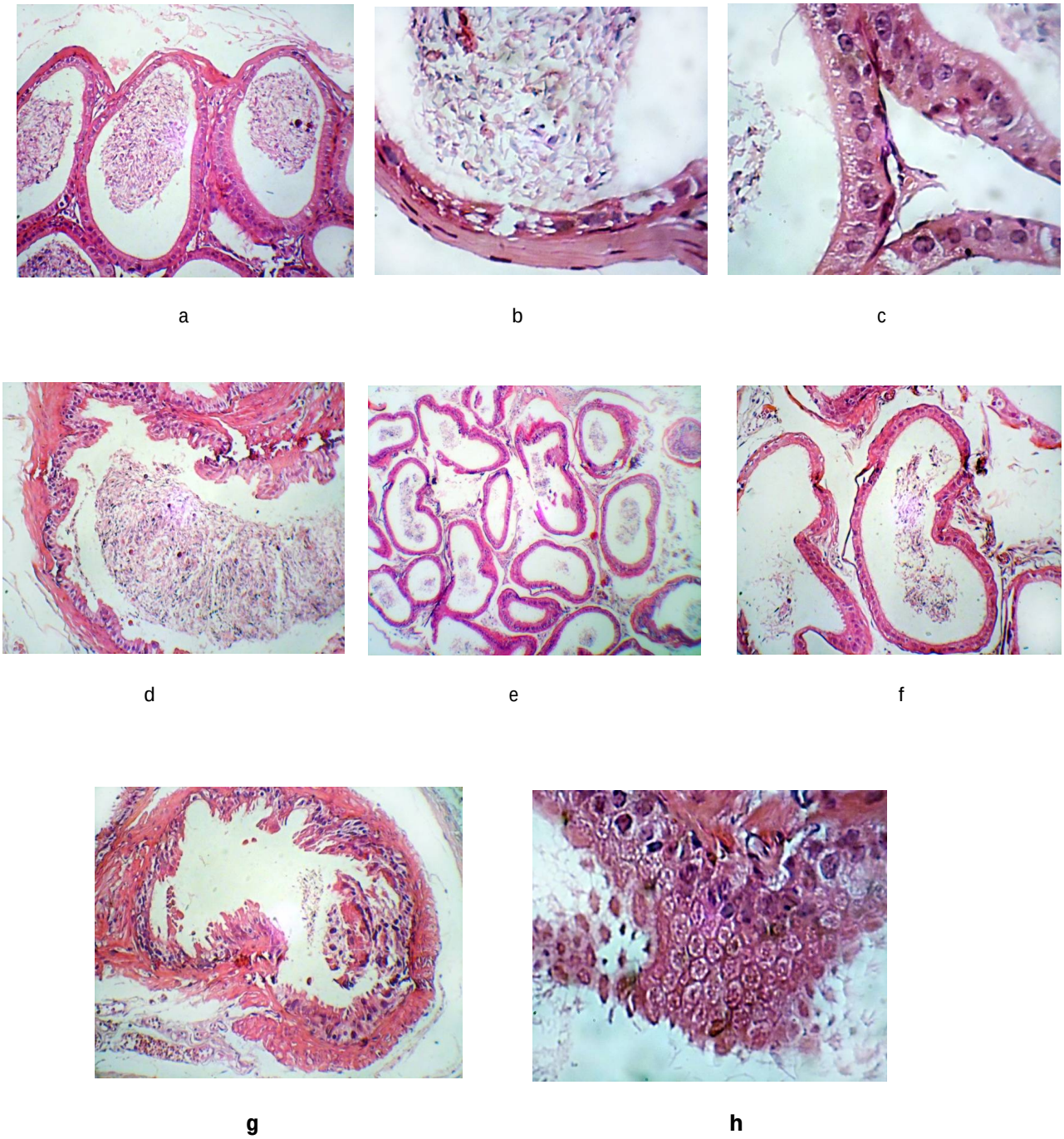


Figure 5. Show the histopathological picture of epididymis mice

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Allelopathic Potential of Two Sunflower Cultivars (*Helianthus annuus* L.) on Weed Companion

Ali Kareem Sarbout *and MajidHanoon Sharhan

Department of Biology, College of Science, Wasit University, Kut, Iraq.

Received: 17 Mar 2018 Revised: 18 Apr 2018 Accepted: 13 May 2018

***Address for correspondence**
Ali Kareem Sarbout
Department of Biology,
College of Science, Wasit University, Kut, Iraq.

ABSTRACT

Two experiments were conducted during 2015-2016 in Iraq Wasit province, to test the allelopathiceffect of sunflower (*Helianthus annuus*L) two cultivars (Asgrio and Flamme)to screened allelopathic of root exuded against weeds in sunflower field. Sunflower cultivars significantly reductionof total number and biomass of companion weed and this reduction is genotype dependent. Resuls show the asgarocultivars was the higher mostallelopathic cultivars with reduction on the total weed number by 68.6% compared with control and weed biomass by 61.0% compared with control respectively. Flamme was the slightest allelopathic genotypes. Asgaro Genotypes, which restraint of add up to weed number and weed biomass by 24% and 35.4% contrasted and control separately. A staircase explore demonstrated that root exudates of the Asgaro genotype was stifled weeds more than Flamme genotype this givin extra confirmation for the predominance of the Asgaro genotype in its allelopathic weed concealment. Substance examination demonstrated the organization of phenols compound in root exudates the two genotypes with a fundamentally higher sum in the Asgaro genotype (0.403 mg/g) contrasted and Flamme genotype (0.220 mg/g) and that aggregate phenolic began to increment at 28 day at that point declined at end season. Phenolics compound by Chromatographic investigations (HPLC) demonstrated the nearness of a few allelochemicals, viz, protocatechuic, vanillic, syringic, ferulic and p-coumaricacid.Chromatographic examinations showed the nearness of phytotoxins in the root radiates of the tried sunflower genotypes. All mixes appeared to have changed maintenance time and were distinguished as phenolic mixes.

Keywords: Allelopathic, Sunflower, *Helianthus annuus* **L.**

INTRODUCTION

Weeds cause a worldwide agronomic issue that lessens the profitability of yields. Weeds challenge with developed harvests for the available dampness, supplements and light. Thus, weeds essentially diminish either edit yield or quality. Control of weeds is imperative to keeping up the generation of monetary yields. Farming world is utilizing

around 3 million tons of herbicides for each year, and herbicide-safe weeds have turned out to be far prolific, which has more extended the utilization of herbicides (Shibayama, 2001). The overemployment of agrochemicals has created natural contamination, weed resistance and human wellbeing concerns. To take care of these issues, it is important to create workable weed administration frameworks that may lessen herbicide reliance, cheap, simple to utilize and supportive in keeping up the biological system security (Khanh*et al.,* 2013). Allelopathy assumes a most critical part in nature environments by deciding vegetation al designing, plant strength, plant progression and plant biodiversity, anticipating seed rot and influencing seed lethargy (Rice, 1984). Be that as it may, allelopathy has a noteworthy part in farming environments. It assumes a critical part in weed-trim, edit weed, edit harvest, ranger service and supplement cycling (Singh *et al.,* 2012). Several genotypes of sunflower have been introduced to Iraq for cultivation. Preparatory field notes uncovered that development and populace of friend weeds were adaptable among the stands of chosen genotypes. Likewise, fluctuation development and populace variety were experiential on weeds developed in the field after sunflower collect (Sarbout, 2010). This recommends allelopathy have system in charge of the hindrance of weeds development and populace and the contrasts between stands could be because of contrasts in the allelopathic capability of the examination cultivars (Tawifiq and Alsaadawi, 2014). In this way, it was mulled over in the investigations to Screen two sunflower genotypes for their allelopathic inclination to control development and populace of friend weeds keeping in mind the end goal to characterize the very allelopathic genotypes and assess the impact of allelopathic on partner weed in populace and development.

MATERIALS AND METHODS

Site experiment

The examination was coordinated at Research station of State Board of Agricultural Extension, Ministry of Agriculture, Wasit, Iraq. The dirt of field site was calcareous earth topsoil. Natural carbon, pH and EC were 0.8%, 7.7 and 7.4 dS m-1, individually. Normal yearly precipitation isn't as quite a bit of "50 mm and day/night temperatures amid the developing season were 30-40/15-30 ºC

Seeds procurements

Sunflower cvAsagri and Flammecv. Were got from Department of Crop Production, College of Agriculture, Baghdad University.

Weeds identification

Weeds found in companion with sunflower were surveyed.Their identification was performed by the help ofWasituniversity Herbarium and specialists of State Board of Agriculture Extension at Wasit Province.

Effects of sunflower genotypes on companion weed.

Field test was directed amid summer 2015 of every a field situated in Wasit territory 180 km south to Baghdad". Field plots (2.5m x 2.5m) were made haphazardly in field vigorously with weeds. The plots were furrowed by spade to the profundity of 30 cm and got Nitrogen as NPK (46%N) at 240 kgha– 1 (half before planting and half following two weeks of planting) and P as triple superphosphate(46% P2O5) at 240 kg ha– 1 at planting time (5). Seeds of two sunflower genotypes (Asagri and Flamme) were sown physically on their separate plots in lines with a separation of (25) cm amongst seeds and (50) cm between lines .The plots were chosen in plan (RCBD) with three replications. Toward the finish of yield development, the plants of every sunflower genotype were gathered. Number of each

weed species was computed, the over the ground add up to weed biomass was collected, air-dried for a few days under daylight amid July of 2015 and measured utilizing advanced adjust.

Bioassay of root exudates by stair case procedure

This examination was finished under field conditions to test the differential allelopathic capacity of the test sunflower cultivars against weeds between sunflower plants and weeds. The test was affected using a stair to case device delineated by (Alsaadawi and Rice, 1982). Seeds of sunflower cultivars and the weed of Echinochloacolonum, one of the accomplice weeds found in sunflower field was planted freely in plate stacked with destructive washed sand (4% HCl) to prepare seedlings for the look at device. Two "seedlings of Asagri and Flamme cultivars and Echinochloacolonum were transplanted in their different plastic pots each contained (0.5)kg destructive washed sand. Treatment game plan involved pots of sunflower cultivars substituted with pots of Echinochloacolonum the stair case structure while a control course of action included pots of Echinochloacolonum turned with pots containing destructive washed sand so to speak. Complete supplement game plan (HoaglandandArnon, 1950), was secured at provisions settled at the most noteworthy purpose of each course of action and allowed to pass the pots of each game plan by spilling in conclusion accumulated at the base stores of each game plan, where it was reused every day .The game plan was changed Echinochloacolonum plants were allowed to create for 30 days, by then procured, confined to roots and shoot and took a gander at in view of oven dried weight at 70 °C for 3 days. All prescriptions were imitated three times and were sorted out in a randomized arrangement. The data were accurately poor down using Fischer's examination of progress using least essential refinement (LSD) test at $P \leq 0.05$ (Steel*et al.,* 1997).

Determination of total phenolics in sunflower residues amended soil

FolinDenis was used for phenolics analysis (A.O.A.C. 1990) the process are

1. Ferulic acid phenol was used as standard since it is an allelopathic agent present in sunflower plant (Haslam, 1988)
2. Soil samples were taken from soil of plots amended with all treatments at a depth of 30 cm at 1, 4, and 6 weeks after sowing (WAS).
3. The soils were mixed thoroughly and allowed to dry at room temperature for 3 days. Samples of 250 g dry soil were extracted separately in 250 ml of distilled water by shaking for 24 h at 200 rpm (Ben-Hammouda*et al*., 1995).
4. Soil suspensions were filtered through Whatman No. 2 filter paper under vacuum. Folin-Denis (0.5 ml) and Na_2CO_3 (one ml) were added to one ml of soil water extract and left to stand for 30 minutes".
5. Absorbance was determined at 750 nm on a spectrophotometer (Blum *et al*., 1991). The total phenolic content was obtained by standard curve using different concentrations of ferulic acid.

Separation, identification and quantification of phytotoxins from sunflower

1 Water concentrate of the sunflower of high allelopathic cultivars (Asgaro and Flamme) were readied(Harborne, 1973) with some alteration.

2 One gram of soil of test genotypes were absorbed 100 ml hot refined water fermented with one milliliter of acidic corrosive. The blend was warmed delicately, blended altogether by ultrasonic mechanical assembly to bar air rises from the buildups and permitted to remain for 4 hrs.

3 The blend of each example was separated by channel paper under vacuum condition and kept in icebox until utilize.

4 For distinguishing proof, 50 µl of the concentrate of each example was infused in High Performance Liquid Chromatogram (HPLC Shimadzu-C-6A) utilizing methodology laid out by Hartley and Buchan.

5 The pinnacles were distinguished by UV indicator. Gauges of suspected phytotoxinswere run comparatively for distinguishing proof and measurement. The investigation was done in the labs of Ministry of Science and Technology. Centralization of each segregated compound was controlled by the accompanying condition:

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{Concentration of standard} \times \text{Dilution factor}$$

Statistical analysis

The Data collected was analyzed by Fishers analysis of variance technique. Least significant difference (LSD) test was applied at 0.05 Probability level to compare treatment means (Steel, 1984).

RESULTS

Allelopathic potential of sunflower genotypes against number of companion weed

Field observations observed 60% of weeds species grown in field were broad leaf *viz*. *Beta vulgaris* L,*Malvarotundifolia* L., *Ducuscarota,*Plantagoovata., and the remain was grass weeds, namely *Avenafatua* L.,*Loliumtemulentum*L. And *Phalaris minor* L. (Table 1). The effect of test sunflower genotypes appeared to be non-selective. genotypes such as Asagri showed higher inhibitory effect on narrow and broad leaves weeds while others such as Flamme does not have inhibitory effects on particular weed species. The results also showed that weed species responded differently to allelopathic potential of the sunflower genotypes. For example, Genotype Asagri appeared to be more inhibitory to all weeds except *Beta vulgaris.* In contrary, Flamme genotype exhibited higher ability to reduce the number of all test weeds except *Avenafatua*, L.*Plantagoovata* and *Beta vulgaris* which were slightly affected.

Test of allelopathic potential of sunflower genotypes against growth of companion weed

Outcome present in table 2 indicated the presence of weed species grown in the field of sunflower genotypes. However, the numbers of the recorded weed species are different between among the test sunflower genotypes Asgaro inhibition the total number of weeds grown in the field by 68.6% of control where ether Flamme genotype slightly reduced the number of weed compared to the other genotypes causing a reduction of 24.0 % of control (Table 2) Results demonstrated that the aggregate over the ground biomass of weeds were altogether decreased by all test genotypes of sunflowe (Table 2). The extent of the diminishment was genotype subordinate.Asagri proved to be the inhibitory genotypes to weed biomass with a reduction up to 61.0%. Other genotypes showed a reduction ranged from 35.4% in"Flamme compared with control.

Impact root exudates of sunflower cultivars on development of the test weed by stair case procedure

Root exudates of both uniforms fundamentally held root and shoot development of Echinochloacolonum weed contrasted with the control (Table 3), with the upside of Asagri cultivar over Flamme cultivar in the concealment of entire plant of the test weed by 68.9% in Asagri and 21.4 % in fire contrasted and control. The consequence of staircase explore bolsters the aftereffects of field test in that root exudates of the two cultivars. Essentially hindered root and shoot development of the test weed (Table 3) with the predominance of Asagri over Flamme cultivar in the concealment of root, shoots and entire plant of the test weed 75.8 % and 53.3 % individually. No endeavors were made to distinguish the allelochemicals in root exudates.

Determination of total phenolics compound in field of sunflower residues amended soil

All treatments, including control, showed no significant differences in total phenolics at the beginning of the experiment (Table 4). The release of total phenolics started after two weeks of agriculture, reached show that at 4-week and remained at higher concentration then decreased until reach the lowest concentration at 6-week of decomposition. Soil from plots amended with sunflower root showed higher amount of total phenolics and the concentration increased with at 4-week after agriculture".weedy check showed lower concentration of total phenolics at all decomposition periods

Aftereffects of HPLC investigations demonstrated the nearness of 5 phytotoxins in the dirt root radiates of the examination sunflower genotypes (table 5). All the separated mixes look as though have changed maintenance times and recognized as phenolic mixes. The profile of each compound gave off an impression of being diverse among the test genotype. The grouping of the separated mixes" was found in the accompanying request: ferulic, protocatecheic acid, syringic acid, vanillic acid, p-coumaric acid in Asagriprotocatecheic acid, syringic acid, p-coumaric acid, vanillic acid, ferulic in Flamme. The "aggregate convergence of phytotoxins appeared to be substantially higher in Asagri genotypes than in Flamme genotypes.

DISCUSSION

Allelopathy has been reported to offer a significant role in weed control (Sodaeizadeh and Hosseini, 2012). Several non herbicidal weed control strategies in which Allelopathy is involved has been explored such as rotational crop, cover crop, smother crop, intercropping, crop mixtures, water extract and use of allelopathic crop residues as mulch or incorporated in field soil (Mohammadi, 2013).The effect of test sunflower genotypes appeared to be non-selective. genotypes such as Asagri showed higher inhibitory effect on narrow and broad leaves weeds while others such as Flamme, this result can be show No attempts were made to find out the reason; however, it "could be attributed" to the root exudates contain several inhibitory compounds of different mechanisms of effect. Therefore, it would be fruitful to investigate the inhibitory effect of the root exudates compounds separately or in combination to know if the compounds have selective effects on weeds (Alsaadawi *et al.,* 2007). The inhibitory effect of the sunflower genotypes against the total number of weeds could be attributed to the allelopathy and competition exerted by sunflower genotypes (Alsaadawi*et al.,* 2013). Be that as it may, the change restraint in weedsnumber among the test cultivar of sunflower genotypes could be ascribed chiefly to the differences in the allelopathic capability of the test genotypes through root exudation since all plots of sunflower genotypes got equivalent agrarian administrations as far as water and manure rehearses.

The "distinctions in allelopathic capability of a few allelopathic trims other than sunflower have been accounted for and all around reported by a few laborers (Alsaadawi*et al.,* 2007) found significant differences in allelopathic potential against weeds among the test sorghum genotypes(Reberg-Horton,*et al.,* 2005) examined ten different cultivars of cerel rye and found significant differences among them in the amount of one common allelochemical (DIBOA). Dilday*et al.* (Dilday*et al*., 1998) revealed that out of 16000 accessions screened for their allelopathic ability, many were found to suppress ducksalad (*Heterantheralimosa*) and redstem (*Amemeniacoccinea* weeds drastically. Estimations of weed dry weight is additionally affirmed the allelopathic capability of the test genotypes. As a rule, the diminishment in complete weeds dry weight seemed, by all accounts, to be parallel with the lessening" in weeds number, and the sunflower genotype (Asagri) which indicated higher decrease in weeds number have additionally higher diminishment potential in weed biomass.

The proposes are that the root oozes contain phytotoxic mixes which could be discharged in to the dirt condition by the activity of microorganism and impact on root. Rice (Rice, 1984) demonstrated that few classes of soil microorganisms include in the disintegration of plant deposits and freedom of the allelopathic mixes. Chou and Lin (Chou*et al.,* 1976) found that few microorganisms contribute in deterioration of rice deposits in soil and unusual a

few allelopathic mixes including phenolics and short unsaturated fats. Blum (Blum *et al.,* 1991) demonstrated that few types of microorganisms (i.e., green growth, microbes, actinomycetes, and parasites) in soil have an expansive scope of metabolic exercises that can disintegrate natural issue and amalgamation/discharge/loss of potential allelopathic specialists.The consequence of staircase test bolsters the aftereffects of field analyze in that root exudates of the two cultivars altogether hindered root and shoot development of the test weed with the strength of Asagri over Flamme cultivar can be expected the nearness of a few allelochemicals of phenolics in nature .Tawifiq and Alsaadawi(Tawifiq and Alsaadawi, 2014) reported the presence of several allelochemicals of phenolics in nature such as neochlorogenic acid,5–*O*–P–Coumaroylquanic acid,chlorogenic acid,neochlorogenic acid, caffeoylquinic acidand neochlorogenic acidacids. These phenolic acids are reported to interfere with several physiological processes including respiration, photosynthesis, ions uptake, hormones biosynthesis and cell division and others (Einhellig*et al.,* 2004). The presence and release of phytotoxins from the root exudes of plant incorporated into soil is further confirmed by phenolics dynamic determination in soil. The release of phenolics started after two weeks of agriculture, reached its at 4-week and remained at higher concentration then declined until reach the lowest concentration at 6-week of decomposition. This suggests that phenolics released from sunflower root in soil are the main cause of suppressive activity of weeds. Meanwhile, these results give explanation for the poor growth of weeds observed during the first two months from cowpea sowing.

Similar results were also reported when the residues of sorghum were added in broad bean field (AL-Bedairy, 2011). No attempt was made to isolate and identify the phenolic acids in the decomposed sunflower residues in soil; however (Al-Temimi, 2010) and Sarbout.,(Sarbout, 2010) were able to isolate and identify phenolic acids, namely Chlorogenicacid, isolchlogenicacid, caffeicacid, gallicacid, syrinigicacid, hydroxybenzoicacid, p- coumaricacid, ferullicacid, vanillicacid and Catechol from the soil containing sunflower residues and they reached their maximum beak at 4th week and vanished at 2nd month from incorporation of residues in to the field soil. These phytotoxins are reported to have inhibitory effects on several metabolic processes such as inhibition of chlorophyll biosynthesis (Alsaadawi*et al*., 1986a.: Weir *et al*., 2004), ions uptake (Olmsted, and Rice, 1970,Alsaadawi, *et al.,* 1986a), photosynthesis (Hejl*et al.,* 1993), inhibition of activity of plasma H^+-ATPase which leads to decreased ions and water absorption by guard cells of leaves and causing close of stomata (Hejl and Koster, 2004), inhibition of photosystem II and thus decreases the production of ATP and $NADPH_2$ required for CO_2 fixation in dark reaction (Barkosky*et al.,*2000) , inhibition of oxidation phosphorylation (Koeppe and Miller 1974), inhibition of activity of several enzymes involved in essential metabolic processes (Politycka and Gmerek 2008), interfering with hormones metabolism in plant. Inhibition of stomata opening (Rai and Ayub, 2003). Also phenolic acids are reported to reduce the number of mitochondria and disrupt the membranes surrounding nuclei, mitochondria and dictyosomes(Lorber and Muller, 1976). Our results revealed that inhibition of ions uptake is one of the above mechanisms by which sunflower residues suppress the of test weeds. Results of HPLC analyses showed the presence of 5 phytotoxins in the soil root exudes of the examination sunflower genotypes.

CONCLUSION

Theallelopathic potential is changed among the test sunflower genotypes and Concentration of the aggregate phenolic phytotoxins confined "from the deposits seemed, by all accounts, to be in charge of the allelopathic capability of the test genotypes.

ACKNOWLEDGEMENTS

Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for supporting.

REFERENCES

1. A.O.A.C. (1990).Official methods of analysis of the association of official analytical chemists.*Tannin.15th ed. Washington*, D.C.p. 746.
2. Al-Bedairy, N,R., Al saadawi, I. S., and Shati, R.K. (2011).Effect of combination of Sorghum Bicolor L. (MOENCH) Residues and Trifluralin herbicide on broad bean and its weeds. Iraqi Journal of Agriculture 94-102
3. AL-Bedairy, N. R., (2011).Allelopathic effects of sorghum (*Sorghum bicolor* (L.)Moench) on companion weeds and next crop.*phD.Sc. thesis, Field Crop Sciences, College of Agriculture, University of Baghdad, Iraq.*
4. Alsaadawi, I. S. and Rice.E.L. (1982).Allelopathic effects of *polygonumaviculare* L. 1.Vegetataional Patterning. J.*Chem.Ecol*. 8:993 – 1009.
5. Alsaadawi, I. S., Al-Ekeelie, M. H. S. and Al-Hamzawi, M. K. (2007).Deferential allelopathic potential of grain sorghum genotypes to weed.*Allelopathy Journal* 19:153–160.
6. Alsaadawi, I. S., Al-Hadithy, S. M. and Arif, M. B. (1986a).Effect of three phenolic acids on chlorophyll content and ions uptake in cowpea seedlings.*Journal of ChemicalEcology*12: 221-227.
7. Alsaadawi, I. S., Al-Hadithy, S. M. and Arif, M. B. (1986a).Effect of three phenolic acids on chlorophyll content and ions uptake in cowpea seedlings.*Journal of ChemicalEcology*12: 221-227.
8. Alsaadawi, I. S., Al-Hadithy, S. M. and Arif, M. B. (1986a).Effect of three phenolic acids on chlorophyll content and ions uptake in cowpea seedlings.*Journal of ChemicalEcology*12: 221-227.
9. Barkosky, R.R., Einhellig, F.A., Butler, J.L. (2000).Caffeic acid-induced changes in plant-water relationships and photosynthesis in leafy spurge *Euphorbia esula*.*Journal of Chemical Ecology* 26: 2095-2109
10. Ben-Hammouda, M., Robert, J. K., Harry, C. M. and Sarwar, M. (1995).A Chemical basis for differential allelopathic potential of sorghum hybrids on wheat.*Journal of Chemical Ecology* 21:775-786.
11. Blum, U., Wentworth, T. R., Klein, A. D., King, L. D., Gerig, T. M.andLyu, S. W. (1991). Phenolic acid content of soils from wheat-no till, wheat-conventional till and fallow-conventional till soybean cropping systems.*Journal of Chemical Ecology* 17:1045-1067.
12. Blum, U., Wentworth, T. R., Klein, A. D., King, L. D., Gerig, T. M.andLyu, S. W. (1991). Phenolic acid content of soils from wheat-no till, wheat-conventional till and fallow-conventional till soybean cropping systems.*Journal of Chemical Ecology* 17:1045-1067.
13. Chou, C.H.andLin,H.J.(1976).Autointoxicationmechanism of*Oryzasativa*.1.Phytotoxiceffects of decomposing rice residues in soil. *Journalof ChemicalEcology* 2:353-367.
14. Dilday,R.H.;Yan,G.W.;Moldenhauer,K.A.K.andGravois,K.A.1998).Allelopathicactivityinriceforcontrollingmajora quatic weed.InOlofsdotter,M.Allelopathyinrice.Proc.Workshopon AllelopathyinRice;Manila,Philippines.pp. 25-27.November1998. IRRI, Manila, Philippines.
15. Einhellig, F. A. (2004).Mode of allelochemical action of phenolic compounds. In: MACIAS, F. A. et al. (Eds.). Allelopathy: chemistry and mode of action of allelochemicals. *London: CRC Press, LLC*, 2004. p. 217-238.
16. Harborne, J. B. (1973).Phytochemical Methods.*Chapman and Hall Press. London, UK.*
17. Haslam, E. (1988). Plant polyphenols (syn. Vegetable tannins) and chemical defense- are appraisal. *Journal of Chemical Ecological* 14:1789-1805
18. Hejl AM, Einhellig FA, Rasmussen JA (1993). Effects of juglone on growth, photosynthesis and respiration.*Journal of Chemical. Ecology* 19: 559-568.
19. Hejl, A. M. and Koster, K. L. (2004). The allelochemicalsorgoleone inhibits root H+-ATPase and water uptake. *Journal of Chemical Ecology* 30:2181- 2191.
20. Hoagland, D.R.andArnon,D.I. (1950).Thewater-culturemethodfor growing plantswithout soil. CaliforniaAgriculturalExperiment Station *CircularNo. 347. University of California,Berkeley*
21. Khanh ,T.D., Linh, L.H. Ham, Xuan,T.D(2013). Rapid and high-precision marker assisted backcrossing to introgress the *SUB1* QTL into the Vietnamese elite rice varietyJ Plant Bred Crop Sci, 5 (2) (2013), pp. 26-33
22. Koeppe, D. E. and Miller, R. J. (1974) Kaempferol inhibition of corn mitochondrial phosphorylation.*Plant Physiology* 54: 374-378.

23. Lorber, P. and Muller, W. H. (1976). Volatile growth inhibitors produced by *Salvia leucophylla*:*Allelopathy Journal*54: 374-378
24. Mohammadi, G. R. (2013). Alternative weed control methods: A Review.
25. Olmsted, C. E. and Rice, E. L. (1970). Relative effects of known plant Inhibitors on species from first two stages of old field succession.*Southwestern Naturalist* 15:165–173.
26. Politycka, B. and Gmerek, J. (2008).Effects of ferulic and *p*-coumaric acids on the activity of hydrolytic enzymes and the growth of radicles in germinating seeds of cucumber and pea.*Allelopathy Journal* 21: 227-238.
27. Rai, V.R., Lokesh, S.andAyub, K.(2003).Occurrence and management of some seed-borne fungal pathogens of maize and sorghum *in vitro.Seed Science Research* 30: 112-117.
28. Reberg-Horton,S.C; Burton,J. D; Danehower,D.A; Ma,G.;Monks, D.W,Murphy,J.P.; Ranells,N.N;WilliamsonJ. DandCreamer,N.G.(2005).Changesovertimeintheallelochemicalscontentoftencultivars of rye (*Secalecereale*). *Journalof ChemicalEcology*31:179-192.
29. Rice, E. L. (1984). Allelopathy. 2nd Ed. *Academic Press, Orlando, Florida*, USA.
30. Sarbout, A. K. (2010).Study the Allelopathic Potential of Sunflower Cultivars on Weeds and Wheat. *MSc* thesis, *Biology Department, College of Science, University of Baghdad, Iraq*.
31. Shibayama H. (2001).Weeds and weed management in rice production in Japan. *Weed Biology and Management 1 53–60.*
32. Singh A.K., Kumar, P., N. Rathore, R.M. (2012).GadeAllelopathy: A sustainable alternative and eco-friendly tool for plant disease management.J Plant Dis Sci, 7 (2), pp. 127-134
33. Sodaeizadeh1, H. and Hosseini, Z. (2012).Allelopathy an environmentally friendly method for weed control.*International Conference on Applied Life Sciences*. Turkey, September 10-12.
34. Steel, R. G. D.; Torrie, J. H. andDickey, D. (1997). Principlesand Procedures of Statistics: A Biometrical Approach(3rd.ed.).McGraw HillBookCo.Inc., *NewYork, USA*.pp. 172-177.
35. Tawifiq, A. A. and Alsaadawi, I. S. (2014).Allelopathic effect of root exudates of tow sunflower cultivars on companion weeds. Submitted for publication to *The Iraqi Journal of Science*
36. Weir, T. L., Sang-Wook, P., Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* 7:472–479
37. Yang, X., Scheffler, B. E. and Weston, L. A. (2004). SOR1, a gene associated with bioherbicide production in sorghum root hairs. *Journal of Experimental Botany* 55: 2251–2259.

Table 1.Weed species grown companion with Sunflower field.

Common name	Scientific name	Family
Broad leaf – weeds		
Common beet	*Beta vulgaris* L.	Chenopodiaceae
Mallow	*Malvarotundifolia* L.	Malvaceae
Wild carrot	*Ducuscarota* L.	Umberlliferae
blond plantain	*Plantagoovata*	Plantaginaceae
	Narrow- leaf weeds	
Wild oat	*Avenafatua* L.	Poaceae
Rye grass	*Loliumtemulentum*L.	Poaceae
Canary grass	*Phalaris minor* L.	Poaceae

Table 2.Allelopathic effect of sunflower genotypes on growth of companion weed under field Conditions.

Genotypes	Dry weight of total weeds/plot (g) *	Reduction % inhibition control	total number weed m^2	Reduction % inhibition
Asgaro	99.3	61.0	32.7	68.6
Flamme	164.6	35.4	79.3	24.0
Control	254.7	……	104.3	……
LSD=0.05	19.66		7.21	
Standard deviation	68.01		31.62	
Standard error of mean	22.67		10.54	

Table3. Effect o f root exudates of sunflowercultivars on growth of *Echinochloa colonum* weed.

Treatments	Dryweight (g)*		
	Shoots	Roots	Whole plant
Asgaro	0.07	0.017	0.087
Flamme	0.15	0.070	0.22
Control	0.19	0.090	0.28
LSD≤0.05	0.037	0.019	----------
Standard deviation	0.0337	0.0552	0.0876
Standard error of mean	0.0112	0.0184	0.0292

*Average of 4 replicates

Table 4.Concentration of total phenolics released from root exudes of sunflowerat different periods of decomposition.

Treatments*	Total Phenol concentration**	
	28 day	52day
Weedy check (Control)	0.103	0.227
Asgaro	0.403	0.107
Flamme	0.220	0.150
LSD ≤ 0.05	0.0869	0.0649
Standard deviation =	0.142	0.0702
Standard error of mean =	0.0474	0.0234

*Each number is an average of 4 replicates. **Total phenolic acid is expressed in ferulic acid equivalents per gram of soil.

Table 5.Concentration of phytotoxins in the root exudes of test sunflower genotypes

Phytotoxins		Concentration (ppm)			Total
	Sunflower genotypes				
	Protocatechuic	Vanillic	syringic acid	p-coumaric acid	ferulic
Asgaro	127.4	111.2	123.7	109.6	138	609.9
Flamme	147.9	60.0	83.7	77.6	55	424.3

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Haematological, Plasma Biochemical and Pathological Changes in Rabbits Experimentally Infected *Trypanosoma evansi*

AzharAli Faraj*

Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Received: 18 Mar 2018 Revised: 20 Apr 2018 Accepted: 15 May 2018

***Address for correspondence**
AzharAli Faraj,
Department of Parasitology,
College of Veterinary Medicine,
University of Baghdad, Iraq.

ABSTRACT

The aim of this experiment was to study the hemo-biochemical and histological changes in domestic rabbits infected with *Trypanosoma evansi* parasites. Eight male rabbits were purchased from the local markets in Baghdad city anddivided into two groups (5 infected and 3controls). The rabbits in the infected group were injected with 1 x 10^5 µl parasites by intraperitoneal route.Results showed that the parameters of Hb, PCV concentration, RBC counts, and MCV values were significantly (P<0.05) lower in the infected animals as compared with control group, while WBC counts and MCHC were significantly (P<0.05) higher in infected rabbits. Serum chemistry revealed that the parameters of ALT, AST and ALP were significantly (P<0.05) higher in infected animals than uninfected animals. Microscopically, the liver revealed lesions from congestion of central vein and enlargement of hepatocytes. Spleen showed depletion of lymphoid follicles and presence of megakaryocytes.The biochemical changes were correlated with hematological changes. In conclusion: the*Trypanosomaevansi* infection caused major alteration in the hematological, biochemical parameters and histopathology change in the rabbits. These changes were reason for the devastating effects of the disease on the animals.

Keywords: Rabbits, hematology, serum chemistry, histological change, *Trypanosomaevansi,* Iraq.

INTRODUCTION

Trypanosomosis caused by *Trypanosoma evansi*, resulting in a disease commonly known as “surra”. Surra is particularly serious diseases in equids and camels, infections and clinical cases have been reported in most domesticated mammals and some wild species (Mbaya et al., 2010).The impact of clinical and sub-clinical form of this mechanically transmitted disease by biting flies such as tabanids is difficult to assess(Claes et al., 2004; Desquesnes et al., 2013). Animals become infected with trypanosomosis lead to physiology alters (Biryommumaisho et al., 2003).

This is due to the blood biochemicalchangesand hematological aberrations that occur (Katunguka-Rwakishaya, 1996). The evaluation of blood parameters helps to know the health status of animals and also to determine of damage to hosts tissues (Otestile et al., 1991, Allam et al., 2011**)**. The present study was conducted to estimate the hemo-biochemical changes and microscopical pathology in rabbits infected with *Trypanosoma evansi* in for the purpose of knowing the pathological bases for trypanosome infection of the animal.

MATERIALS AND METHODS

Inoculation of animals

Eight maledomestic rabbits (*Oryctolaguscuniculus*), aged 6-9wks, were used for this experiment, which divided for two groups. Group oneincluded of 5 rabbits,the other three were in control group. Each rabbit in the group 1 was infected with *T. evansi*strain that infects Iraqi camels (*Camelusdromedaus)* by inoculation with 1 x 10^5µ lintraperitoneally (Sivajothiet al., 2015). After 28 days,all animals were sacrificed and blood and tissue samples were obtained for hematological and histopathological analysis.

Haemo- biochemical parameters

About three ml of blood was collected from each rabbit per group from heart using sterile needle and syringe.1 ml of blood sample was collected in EDTA tubes for hematological analysis. While the other 2ml of blood was transferred into gel tube (without anticoagulant) and allowed to stand in refrigerator for 15 min at speed of up to 3500/rpm for 5 minutes. Serum was aspirated by a pipette and transported into small clean tubes kept and frozen until be used for estimation of activity of the following enzymes.Various enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by UV enzymatic colorimetric test(Winn-Deen et al.,2005).

Histopathological Examination

specimensof tissue (liver and spleen) were collected in 10 % buffered neutral formalin.Paraffin sections offive µm thickness were prepared and stained with haematoxylin and eosin (H&E) for recording the histopathological change (Luna, 1968).

Statistical methods

The t-test statistical analysis was used to analyze all the results obtained ($P \leq 0.05$) (Steel andTorrie, 1980).

RESULTS

Table (1) showed a significant decreasing ($P<0.05$) in the means of some blood parameters (PCV, Hb, RBC, and MCV) in the infected animals as compared with control group, while WBC counts and MCHC were significantly ($P<0.05$) increasedin the infected rabbits compared with control group. Table (2) results revealed that the parameters of ALT, AST and ALP were significantly ($P<0.05$) increased in infected rabbits compared with the control group. The pathological lesions of liver characterized by congestion of central vein and enlargement of hepatocytes leading to absence of some sinusoids with vaculation and presence of mitotic figures in addition to infiltration of inflammatory cells in liver parenchyma including around central vein and portal area (Figure ,1). The spleen revealed lesions from depletion of lymphoid follicles and presence of megakaryocyte (figure 2).

DISCUSSION

The results revealed a significant (P<0.05) decreasing in the RBCs level, Hb concentration, total erythrocyte counts, and MCV in the infected animals whereas a significant increasing (P<0.0) was shown in the MACH and WBC counts in rabbits infected with *T. evansi* as compared with the uninfected rabbits.The increasing of these parameters could be considered as indicatior for anemia (Padmaja, 2012; Ekanem et al., 2008). Anemia and leukocytosis are common features of trypanosomosis (Gutierrez et al, 2006). Anemia appears to be predominantly caused by hemolytic associated with decrease life span of erythrocytes and extensive erythrophagocytosis (Habila et al., 2012). Kramer, (2000) reported that the severe anemia was due to erythrogenesis depression which might be a result of chronic liver inflammation. In addition, there was a strong correlation between the level of infestation and the values of RBCs numbers, PCV% and Hb concentration (Ekanem et al., 2008).

The lower counts of lymphocytes observed in the infected group may be attributed to the immunosuppressive actions of Trypanosome infection (Abubakar et al 2005, Ekanem et al., 2008). Sulaima andAdeyemi, (2010) reported that the lymphocytosis may be occur due to generalized lymphoid tissue hyperplasia, characteristic of the acute form of the disease, while in chronic infection, the immune system becomes depleted of lymphoid cells. Results obtained from the present stydy showed an increasing in the means of neutrophil count, monocyte count and eosinophils count of the infected animals. The reduction in leukocyte levels could be a result of immuno-suppression. In this regards Abd El-Baky and Shaymaa, (2011) observed asignificant leucocytosis, neutrophilia, monocytosis and eosinophilia in naturally infected camels with Trypanosomiosis. The eosinophilia could be also occurred as a result a feature of parasitic infections .Results showed that the increased of ALT, AST and ALP is agreed with the results obtained by Taiwo et al., (2003) in sheep infected with *T. brucei*. Also agreed with results of goats infected with *T. congolense* obtained by Adah et al., (1992) who reported that the tissue breakdown (necrosis) could lead to the elevated values of ALT, AST and ALP enzymes.

The histopathological lesion of liver may occur due to trypanosome infection. Adah et al., (1992) recorded anecrosis and hemorrhage within the sinusoids of the liver, with fatty degeneration in hepatic cells of the goats infected with *T. evansi*. The changes were destructive and irreversible. Hepatomegaly was seen by Biswas et al., (2001). Dargantes et al., (2005) reportedthat thecongestion in the liver following necropsy in goat infected with *T.evansi*. Necrotic foci in the liver and destruction of hepatocytes with infiltration by inflammatory cells in the liver of goats. Also it was observed that the spleen was haemorrhagicwith pronounced haemosiderosis and RE cell proliferation (Virmani et qal. 2004). In other study Sivajothi et al., (2015) found that the stimulation given by the presence of *T. evansi* or their toxic metabolites resulteddifferent degrees of anaemic anoxia, which may induce splenic damage. Similar results were found by Suryanarayana et al., (1986). In conclusion: theTrypanosomiosisinfection caused major alteration in the hematological and biochemical parameters in the rabbits. These changes were responsible for the devastating effects of the disease on the animals.

REFERENCES

1. Abd El-Baky, AA, Shaymaa I S: Clinico pathological and Cytological Studies on Naturally Infected Camels and Experimentally Infected Rats with *Trypanosomaevansi*. World Applied Sciences Journal. 2011; 14: 42-50.
2. Abubakar A, Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, Shamaki BA, Afolayan DO, Ogbadoyi EO:Antitrypano-somal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biokemistri. 2005; 17:95-9.
3. Adah MJ, Otesile EB, Joshua RA: Changes in level of transaminases in goats: experimentally infected with *T. congolense.* Rev d' Elev. Me'd Vet. Pays Trop. 1992; 45: 284-286.

4. Allam L, Ogwu D, Rowland IS , Anthony K B S: Hematological and serum biochemical changes in gilts experimentally infected with *Trypanosomabrucei*. Vet. Arhiv. 2011;81 : 597-609.
5. Biryomumaisho S, Katunguk-Rwakishaya E, Rubaire - Akiiki C M: Serum biochemical changes in experimental *Trypanosomacongolencse* and *Tryapanosomabrucei*infection in small east African goats. Vet. Arhiv.2003; 73: 167-180.
6. Biswas D, Choudhury A, Misra KK: Histopathology of *TrypanosomaTrypanozoonevansi* infection in bandicoot rat. I. Visceral organs. Exp Parasitol.2001; 99(3):148-59.
7. Claes F, Radwanska M, UrakawaT, Majiwa PA, Goddeeris B ,Buscher P: Variable Surface Glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosomaevansi* infections. Kinetoplastid.Biol. Dis. 2004; 3:1-6.
8. Dargantes AP, Campbell RS, Copeman DB, Reid SA: Experimental *Trypanosomaevansi* infection in the goat. II.pathology. J. Comp. Pathol. 2005;133:267-276.
9. Desquesnes M, Holzmuller P, Lai D, Dargantes A, Lun Z , JittaplapongS:*Trypanosomaevansi* and Surra: A review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. Bio.Med. Res. Intl. 2013; 33: 166-180.
10. Ekanem JT, Kolawole OM , Abbah OC:Trypanocidal potential of methanolic ex- tract of Brideliaferrugineabenth bark in Rattusnovergicus. Afr. J. Biochem. Res . . 2008; 2:45-50.
11. Gutierrez C, Corbera JA, JusteDoreste FMC,Morales I: Clinical, hematological, and biochemical findings in an outbreak of abortion and neonatal mortality associated with *Trypanosomaevansi* infection in dromedary camels. Ann. N. Y. AcadSci . 2006; 1081: 325-327.
12. Habila N, Inuwa MH, Aimola IA, Udeh MU , Haruna E: Pathogenic mechanisms of *Trypanosomaevansi* infections. Res Vet Sci . 2012; 93: 13-17.
13. Katunguk-Rwakishaya, E: The prevalence of trypanosomosis in small ruminants and pigs in a sleeping sickness endemic area of Buikwe country Mukono district, Uganda. Revd' Elev. Me'd Vet. Pays Trop. 1996; 49: 56-58
14. Kramer JW:Normal hematology of cattle, sheep and goats. In, Feldman BF, Zinkl JG and Jain NC (Eds) Schalm's Veterinary Haematology, 5th ed. Lippincott Williams and Wilkins, Philadelphia. 2000. p. 1075-1084.
15. Luna LG: Manual of Histologic Staining Methods of the armed force institute of Pathology 3rd Ed. McGraw - Hill, NewYork. 1968. p. 36.
16. Mbaya AW, Ibrahim UI, Apagu ST: Trypanosomosis of the dromedary camel of the dromedary camel (*Camelusdromedariusamelusdromedarius*) and its vectors in the tsetse-free arid zone of northeastern, N nd its vectors in the tsetse-free arid zone of northeastern, Nigeria. Nig. Vet. J. 2010;31:195-200.
17. Ngeranwa JJ, Gathumbi PK, Mutiga ER, Agumbah GJ: Pathogenesis of *Trypanosoma* (*bruci*) *evansi* in small east African goats. Res. Vet. Sci. 1993;54:283-9.
18. Otesile EB, Fagbemi BO, Adeyemo O: The effect of *Trypanosomabrucei*infection on serum biochemical parameters in boars on different planes of dietary energy. Vet. Parasitol. 1991;40: 207-216.
19. Sivajothi, S, Rayulu VC, Sudhakara RB:Haematological and biochemical changes in experimental *Trypanosomaevansi* infection in rabbits . J Parasit Dis. 2015; 39:216–220.
20. Steel RGD,Torrie J H: Principles and Procedures of Statistics. Abiometrical Approach. 2ndEdn. McGraw Hill, Book Co., Inc.1980; p. 481.
21. Sulaima FA, Adeyemi OS: Changes in haematological indices and protein concentrations in *Trypanosomabrucei* infected rats treated with homidium chloride and diminazeneaceturate. EXCLI.J. 2010; 9: 39-45
22. SuryanarayanaC,Gupta SL, Singh RP, Sadana JR : Pathological changes in donkeys (*Equusasinus*) experimentally infected with *T. evansi*. Indian Vet.Med. J. 1986; 6:57–59.
23. Taiwo VO, Olaniyi MO, Ogunsanmia AO: Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro*peroxidation during experimental *T. congolense*and *T. brucei i*nfections in sheep. Israel J. Vet.Med. 2003;58: 1-10.
24. Virmani N, Sengupta P P, Panisup A S:Pathomorphological changes in rats following infection with *Trypanosomaevansi* isolated from a heterologous host. Indian J. Anim. Res. 2004; 38 : 29 – 32.
25. Winn-Deen ES, David H, Siglerand G, Chavez R: Determination of total and pancreatic α-amylase in human serum with 2-chloro-4-nitrophenyl-α-d-maltotrioside as substrate. Clin.Chem. 2005; 34: 19-88.

Table 1: Hematologic changes of T. *evansi* in infected rabbit and control group.

Parameters	Control animals	Infected animals
RBC (x10^6/μL)	9.90 ± 0.18a	8.06 ± 0.14b
HB (g/dL)	8.29 ± 0.14a	7.44 ± 0.12b
PCV (%)	26.65 ± 0.44a	24.21 ± 2.61b
WBC (x10^3/μL)	6.73 ± 0.24b	8.37 ± 1.65a
MCV (fL)	26.19 ± 0.41a	22.7 ± 1.30b
MCH (pg)	8.35 ± 0.14a	7.84 ± 0.51a
MCHC (%)	31.93 ± 0.28b	34.64 ± 1.99a
Lymphocytes(10^3/μL)	42.65 ± 1.48a	35.4 ± 7.47b
Monocytes(10^3/μL)	4.66± 0.17a	5.25 ± 0.53a
Neutrophils(10^3/μL)	52.13± 1.43b	55.9 ± 6.67a
Eosinophils(10^3/μL)	3.49 ± 0.17b	5.70 ± 0.72a
Basophils(10^3/μL)	0	0

Means with the a different letter in the same row significantly different (P≤ 0.05)

Table 2: Means of ALT, AST and ALP in infected rabbit sand control group.

Parameters	Control	Infected
ALT (μl)	5.05± 0.14b	10.02± 0.29a
AST (μl)	9.44± 0.29b	18.60± 0.31a
ALP (μl)	21.42± 0.43b	35.00±0.52a

Means with the a different letter in the same row significantly different (P≤ 0.05)

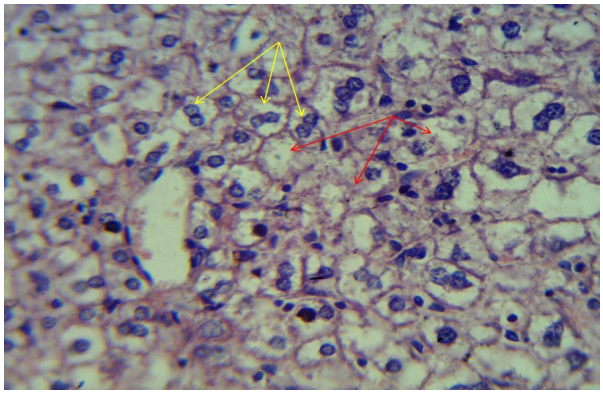


Figure1: liver showing enlargement of hepatocyte Leading to absence of sinusoids with vaculation (Red arrow) and presence of mitotic figures

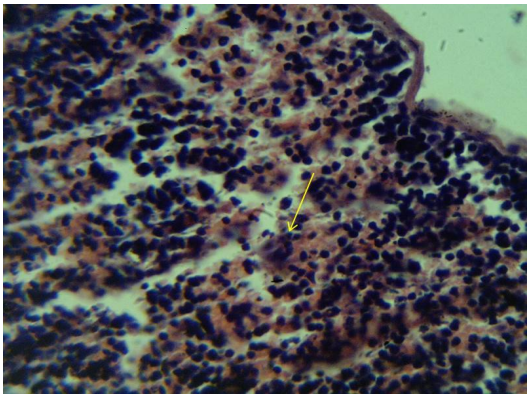


Figure2: The spleen showing depletion of lymphoid follicles and presence of megakaryocyte (yellow arrow)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Treatment of Recurrent Orogenital Herpes and Herpes Associated Erythema Multiforme with Topical Zinc Sulphate 2% and 5% Solution With Oral Acyclovir

Galawish Ahmed Abdullah*

Alkindy College of Medicine, University of Baghdad, Baghdad, Iraq.

Received: 17 Mar 2018 Revised: 21 Apr 2018 Accepted: 16 May 2018

*Address for correspondence
Galawish Ahmed Abdullah,
Assistant.Professor,
Alkindy College of Medicine,
University of Baghdad,
Baghdad, Iraq.

ABSTRACT

This study was carried out to evaluate the therapeutic efficacy of topical zinc sulphate 2% and 5% once weekly in recurrent orogenital herpes, and herpes associated erythema multiforme (EM) compared to control group. Ninety five patients with recurrent orogenital herpes divided randomly into 3 groups treated with 2% zinc sulphat (53 patients), 5% zinc sulphat (22 patients), and third group with distilled water as a control(20 patients). Patients were asked to mention number of attacks of herpes during 6 months before starting treatment, and record number of attacks during treatment (6 months), and 6 months post treatment. Also patients who had herpes associated EM(28 patients) were followed in the same manner. Results revealed that a statistically significant reduction ($P<0.05$) was detected in the numbers of attacks of herpes in both groups 2% and 5% during and post treatment compared to control group, also EM associated herpes also showed significantly reduction ($P<0.05$) between pre and during treatment and post treatment in both treated groups compared to control group. In conclusion: topical zinc sulphat 2% and 5% is an easy way in prevention of herpes reactivation and EM associated herpes. 5% causes slight irritation while 2% not.

Keywords: Herpes erythema multiforme Zinc sulphate.

INTRODUCTION

Herpes simplex virus (HSV) infections are common worldwide and are caused by closely related types of HSV. Their main clinical manifestations are mucocutaneous infections, with HSV type1(HSV-1) being mostly associated with

orofacial disease, whereas HSV type2(HSV-2) is usually associated with genital and perigenital infection.[1] Following primary infections, 30-50% of oral and 90% of genital herpes patients will experience recurrences. The actual frequency of recurrences varies widely between individuals. The mean number of episodes per year is about 1.6 [2,3]. Most cases of erythema multiforme (EM) are related to infections. Herpes virus is definitely the most common cause, principally in recurrent cases. Proof of causality of herpes is firmly established from clinical experience, epidemiology,[4] detection of HSV DNA in the lesions of EM,[5] and prevention of EM by suppression of HSV recurrences [6]. Clinically, a link with herpes can be established in about one-half of cases. In addition 10%-40% of cases without clinical suspicion of herpes have also been shown to be herpes related, because HSV DNA was detected in the EM lesions by polymerase chain reaction (PCR)[7]. Recurrences of herpes are often mild and infrequent, and most patients do not seek treatment. Drug therapy to prevent recurrences is available and effective, but because of cost and inconveniences issues, it is traditionally reserved for use in patients who have more than six out breaks per year [8]. Many drugs are in used for the treatment of primary as well as recurrent herpes, these include acyclovir, famciclovir, valacyclovir, ganciclovir, idoxuridine, trifluridine, foscarnet, cidofovir. But it should be noted that to date, there is no evidence that any antiviral drug can affect the natural history of herpes in human & curtail the recurrent nature of the disease [9]. In the past, topical zinc sulphate solution 0.5% to 20% was used for recurrent herpes simplex, especially for herpetic keratoconjunctivitis, but it was soon discontinued because of sever irritation and dryness of the skin & mucus membrane caused by the drug [10]. A number of studies have showed the efficacy of Zn^{2+} in inhibiting herpes viruses in vitro [11]. This study was undertaken to evaluate the therapeutic efficacy of topical zinc sulphate ($ZnSo_4$) in a 2% and 5% for recurrent oro genital herpes and EM associated herpes.

MATERIALS AND METHODS

Ninety five patients; 61 female(64.21%) & 34male(35.78%), their ages ranged between 3-50 year presented with recurrent herpes simplex labialis & genitalis to Private Clinic & Department of Dermatology and Venereology of Al-Kindy Teaching Hospital during the period from January2015 to August 2017.Twenty eight of them were associated with recurrent EM with each attack of herpes.

All patients full fill the following criteria

1- At least 2 attacks during the last 6 months before start study.
2- No history of drug hyper sensitivity, pregnancy, & breast feeding (at the time of study).
3- No medications other than the study drug.

Patients were randomly divided in to 3 groups. All patients during the attacks of herpes were treated with oral acyclovir 200mg 5 times daily for 5 days & topical antibacterial agents as fusiderm ointment and systemic steroid as prednisolone 20mg were added to those who had EM. Two percent zinc sulphate in DW was used for 53 patients, 5% zinc sulphate for 22 patients, and distilled water alone for 20 patients, applied once weekly/6 months at night time to areas were herpes recur. All patients were asked to mention number of attacks of herpes and EM during last 6 months before starting treatment and were seen every 3 months and asked to record at home the number of attacks of herpes during this period.

The data were collected the end of 6 months of treatment and 6 months post treatment. At each visit, the number of attacks, patient tolerance, and complications were recorded. Analysis of data was carried out using the available statistical package of SPSS-24 (Statistical Packages for Social Sciences- version 24). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference among means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or LSD test for difference among more than two independent means. The significant differences of different percentages

(qualitative data) were tested using Pearson Chi-square test (χ^2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

RESULTS

A total of 95 patients were enrolled in this study, divided in to 3 groups. First group (53 patients) were treated with 2% zinc sulphate, second group(22 patients) treated with 5% zinc sulphate, and third group (20 patients) as a control were treated with distilled water. Descriptive statistic for all patient , age, gender, and type of herpes for each group (table1). Table (2) showing 3 groups of patients, first group 53 patients were treated with 2%zinc sulphate, second group 22 patients were treated with 5% zinc sulphate, and third group 20 patients as a control. In this table the proportions of patients who had attacks of herpes for each group was statistically significant during and after 6 months from treatment between 3 groups while the three independent means was not significant between the 3 groups in the pre and during treatment and it was significant in the post treatment.

For each group the P value was significant between pre and during treatment, and between pre and post treatment. But it was not significant between during and post treatment (Table 3). Figure (1) showing total no. of patients who had herpes pre, during, and post treatment. Table (4) revealed no. of patients who had erythema multiforme associated with herpes as a causative agent in the 3 groups. Because of the small no. of patients who had erythema multiforme associated with herpes (17, 6,and 5) P value was not significant between the 3 groups, but it was significant in both groups treated with zinc sulphat 2% and 5% pre and during treatment, and pre and post treatment, while it was not significant in the control group (table 5). Although P value was not significant because of the small no. of patients, mean value was decreasing in both groups from 3.3 to 1.3 in both groups treated with zinc sulphat while in the control group it was decreased from 2.6 to 2. Figure-2 revealed the proportions of patients who had EM in the 3 groups. The no. of patients who had attacks of EM decrease dramatically compared to control group, even after 6 months of treatment.

DISCUSSION

Zinc ions inhibit the activity of HSV-specified DNA polymerase, like other well-known therapeutic agents (e.g. acyclovir), but the hypothesis that zinc might block HSV replication by selective intranuclear inhibition of viral DNA polymerase appears to have lost its validity. It is thought that zinc binds to sulfahydryl groups of viral glycoprotein B and that when zinc accumulates in the virion, glycoprotein functions are inhibited, leading to the dramatic antiviral effect.[11,12] Topical zinc sulfate solution 0.5% to 20% was used since 1948 for recurrent herpetic keratitis, labialis, and genitalis.[10,13,14] A study carried out by Brody showed that daily application of low concentration (0.025-0.05%)of zinc sulphate is effective in recurrent herpes at skin site and also prevent relapse of post-herpetic erythema multiforme.[15]In another study carried out by Wahba,4% zinc sulphate in water applied topically was found to be effective in recurrent herpes simplex. Eighteen patients were treated with 4% zinc sulphate in water.

In all patients, pain, tingling and burning stopped entirely within first 24hr of zinc sulphat application.[16] In this study single-blinded controlled clinical trial study was used for patients with recurrent herpes labialis or genitalis, and herpes associated EM with zinc sulfate solution 2% and 5% was applied once weekly to areas were herpes recurs in between attacks and avoided at time of attack of herpes to prevent irritation when applied to open wound. In spite of this maneuver still 5% zinc sulfate solution causing irritation (pain, tingling and burning) in most of patients but 2% was not. Whereas, therapeutic efficacy of 2% and 5% was equal in comparison to placebo.So topical zinc sulfate solution 2% once weekly to area where herpes recurs has been found to be an effective as 5% without causing irritation in the prevention of herpes labialis, genitalis and EM associated herpes.

CONCLUSION

Topical zinc sulphat 2% and 5% is an easy way in the prevention of herpes reactivation and herpes associated EM when applied on areas where herpes recur. To overcome irritation caused by zinc sulphat 2% and 5%, the solution was applied to area were herpes recur after healing of herpes lesion. The therapeutic efficacy of 2% and 5% was equal in comparison to placebo, 5% causes slight irritation while 2% not. So 2% solution is preferred than 5%.

*__Conflict of Interests:__ Author certifies that there is no actual or potential conflict of interest in relation to this article.

REFERENCES

1. Spruance SL, Overall JC Jr, Kern ER, Krueger GG, Pliam V, Miller W. The natural history of recurrent herpes simplex labialis: Implications of antiviral therapy. N Engl J Med. 1977; 297(2): 69-75.
2. Corey L. First-episode, recurrent, and asymptomatic herpes simplex infections. J Am Acad Dermatol. 1988;10:169-72.
3. Lafferty WE, CoombsRW, Benedetti J et al. Recurrence after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. N Engl J Med. 1987;1444-9
4. Auqier-Dunant A et al : Correlatons between clinical patterns and causes of erythema multiforme majus, Stevens-Johonson Syndrome & toxic epidermal necrolysis. Arch Dermatol.2002; 138:1019.
5. Weston WL : Herpes-associated erythema multiforme. J Invest Dermatol. 2005;124:xv.
6. Tatnall FM, Schofield JK, Leigh IM: A double-blind, placebo-controlled trial of continuous acyclovir therapy in recurrent erythema multiforme. Br J Dermatol.1995; 132:267.
7. Ng PP et al: Detection of herpes simplex virus genomic DNA in various subsets of Erythema multiforme by polymerase chain reaction. Dermatolgy.2003; 207:349,
8. Spruance SL. Prophylactic chemotherapy with acyclovir for recurrent herpes simplex labialis. J Med Virol. 1993;Suppl 1: 27-32.
9. Gupta S, Kumar B. Herpes Genitalis Simplex Infections. In: Sexually Transmitted Infections. 1st ed. New Delhi: Elsvier, 2005; p. 191-214
10. Graham RM, James MP, Bennett S. Low concentration zinc sulphate solution in the management of recurrent herpes simplex infection. Br J Dermatol. 1985; 112: 123-4.
11. Kumel G, Schrader S, Zentgraf H, et al. Therapy of banal HSV lesions: molecular mechanisms of the antiviral activity of zinc sulfate. Hautarzt. 1991;42:439-45.
12. Kumel G, Schrader S, Zentgraf H, et al. The mechanism of the antiherpetic activity of zinc sulfate. J Gen Virol. 1990; 71:2989-97.
13. Iraji F, Faghihi G. A randomized double blind placebo-controlled clinical trial of two strengths of topical zinc sulfate solution against recurrent herpes simplex. Arch. Iran Med. 2003; 6: 13-15.
14. Mahajan BB, Mohit D, Rajwinder S. Herpes genitalis- Topical zinc sulfate : an alternative therapeutic and modality. Ind. J sexually Trans. Disease & AIDS. 2013;34(1): 32-34.
15. Brodyl I. Topical treatment of recurrent herpes simplex and post-herpetic erythema multiforme with low concentration of zinc sulphate solution. Br J Dermatol.1981;104:191-194.
16. Wahba A. Topical application of zinc solutions: A new treatment for herpes simplex infections of the skin? Acta Derma Venereol. 1980; 60:175-77.

Table 1: Descriptive statistic of all patient (age, gender, and type of herpes)

		Treatment with 2% Zinc Sulphate lotion		Treatment with 5% Zinc Sulphate lotion		Control		P value
		No	%	No	%	No	%	
Age (years)	<10	12	22.6	2	9.1	3	15.0	0.945
	10---19	4	7.5	1	4.5	2	10.0	
	20---29	20	37.7	10	45.5	8	40.0	
	30---39	13	24.5	7	31.8	5	25.0	
	=>40	4	7.5	2	9.1	2	10.0	
	Mean±SDRange	**23.1±12.9 (3-50)**		**26.4±9.5 (6-42)**		**25.6±12.0 (5-45)**		**0.486**
Gender	Male	20	37.7	7	31.8	7	35.0	0.885
	Female	33	62.3	15	68.2	13	65.0	
Type	G	11	20.8	5	22.7	5	25.0	0.924
	OL	42	79.2	17	77.3	15	75.0	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.								
#Significant difference among three independent means using LSD test at 0.05 level.								

Table 2: Comparison among groups for the effect of Zinc Sulpate

		Treatment with 2% Zinc Sulphate lotion(G1)		Treatment with 5% Zinc Sulphate lotion(G2)		Control		P value
		No	%	No	%	No	%	
Have attack of Herpes previous 6 months	Yes	53	100	22	100	20	100	-
	No	-	-	-	-	-	-	
	Mean±SD (Range)	3.5±0.9 (2-5)		3.5±1.0 (2-5)		3.0±0.9 (2-5)		0.099
Have attacks of Herpes during treatment	Yes	11	20.8	5	22.7	16	80.0	0.0001*
	No	42	79.2	17	77.3	4	20.0	
	Mean±SD (Range)	1.4±0.5 (1-2)		1.8±1.3 (1-4)		2.0±0.9 (1-4)		0.183
Have attacks of Herpes after 6 months	Yes	27	50.9	13	59.1	18	90.0	0.009*
	No	26	49.1	9	40.9	2	10.0	
	Mean±SD (Range)	1.3±0.5 (1-2)		1.5±0.9 (1-4)		1.9±0.8 (1-4)		0.025#
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.								
#Significant difference among three independent means using LSD test at 0.05 level.								

Table 3: P values of the comparison between pre and during treatment, and between pre and post treatment

	G1	G2	Control
No of attacks of herpes previous 6 months – No of attacks during treatment	0.0001*	0.009*	0.005*
No of attacks of herpes previous 6 months – No of attacks after 6 months	0.0001*	0.0001*	0.0001*
No of attacks of herpes during treatment –No of attacks after 6 months from treatment	0.678	0.374	-

Table 4: No. of patients who had erythema multiforme associated with herpes as a causative agent in the 3 groups

		Treatment with 2% Zinc Sulphate lotion		Treatment with 5% Zinc Sulphate lotion		Control		P value
		No	%	No	%	No	%	
Have attacks of EM	Yes	17	100	6	100	5	100	-
	No	-	-	-	-	-	-	
	Mean±SD (Range)	3.3±0.8 (2-5)		3.3±0.8 (2-4)		2.6±0.5 (2-3)		
Have EM during treatment	Yes	7	41.2	3	50.0	5	100	0.067
	No	10	58.8	3	50.0	-	-	
	Mean±SD (Range)	1.4±0.5 (1-2)		1.3±0.6 (1-2)		2.2±0.4 (2-3)		
Have attacks of EM after 6 months of treatment	Yes	9	52.9	4	66.7	5	100	0.154
	No	8	47.1	2	33.3	-	-	
	Mean±SD (Range)	1.3±0.5 (1-2)		1.3±0.5 (1-2)		2.0±0.7 (1-3)		
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.								
#Significant difference among three independent means using LSD test at 0.05 level.								

Table 5: P values of the comparison between pre and during treatment, and between pre and post treatment

	G1	G2	Control
Number of attacks of EM – No of EM during treatment	0.004*	0.020*	0.178
Number of attacks of EM – No of attacks of EM after 6m of treatment	0.0001*	0.003*	0.208
No of EM during treatment – No of attacks of EM after 6m of treatment	-	-	0.374

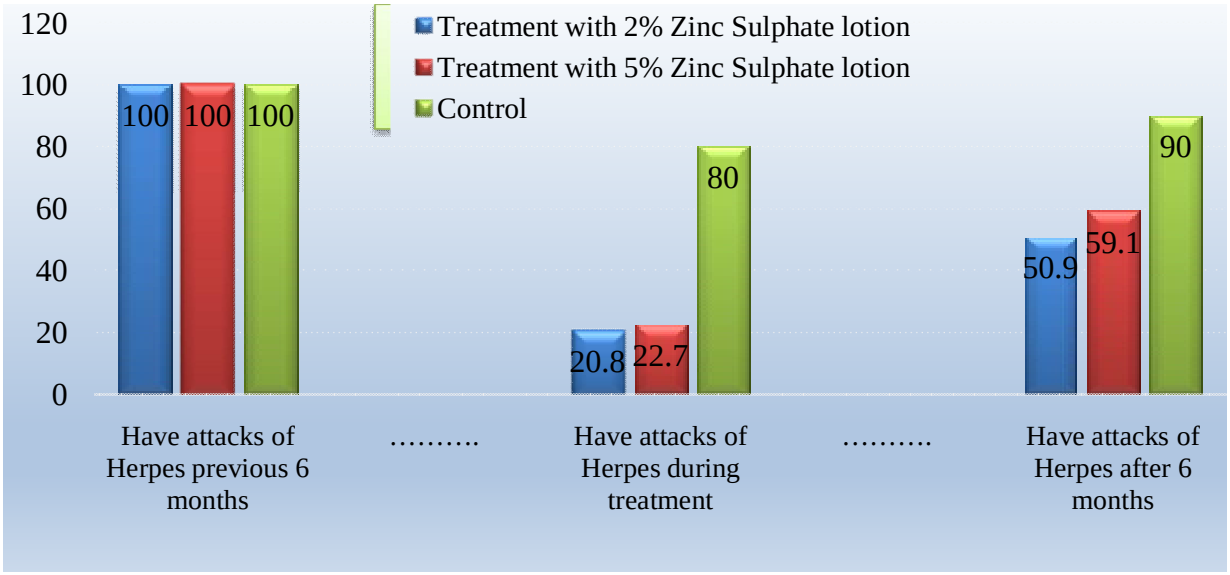


Fig. 1: Total no. of patients who had herpes pre, during, and post treatment.

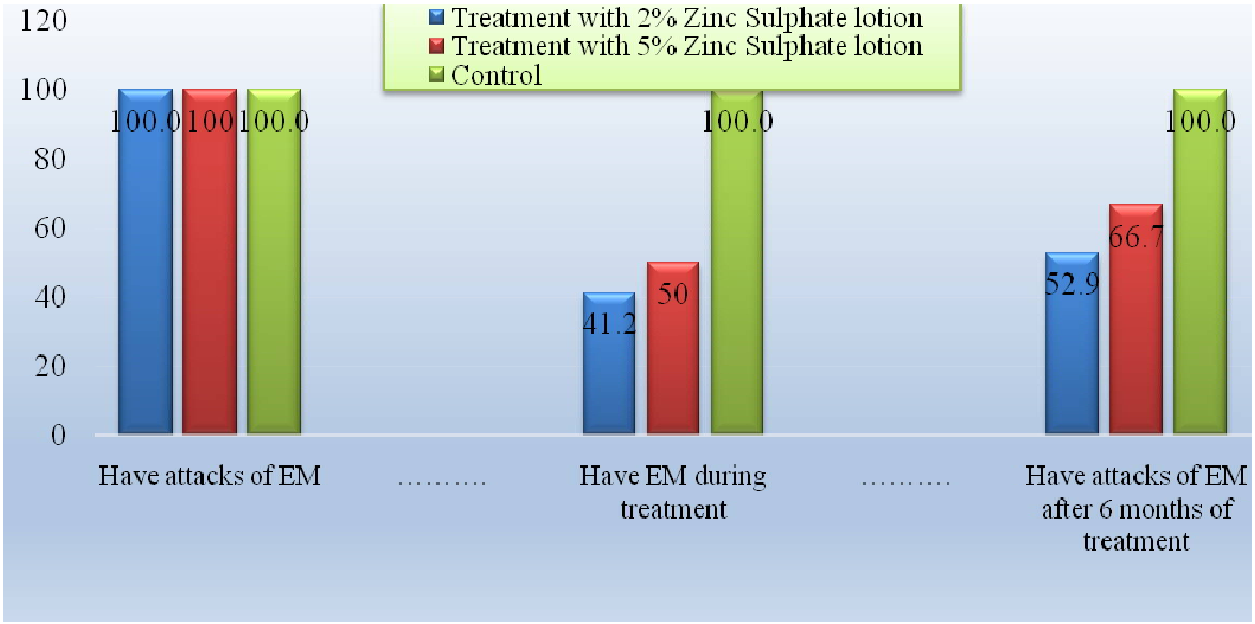


Fig. 2: The proportions of patients who had EM in the 3 groups

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of New Analgesic Combinations in Postoperative Patients

Zainab. G.Al-Jassim*

Department of Pharmacology, College of Dentistry, Baghdad University, Baghdad, Iraq.

Received: 16 Mar 2018 Revised: 21 Apr 2018 Accepted: 16 May 2018

*Address for correspondence
Zainab G. Al-Jassim,
Department of Pharmacology,
College of Dentistry,
Baghdad University, Baghdad, Iraq.
E-mail: ph.zainab86@yahoo.com

ABSTRACT

It is necessary to achieve postoperative pain management and patient comfort. Opioids and a few non-narcotic analgesics are available to control postoperative pain, but many of these medications can cause side-effects and have limited analgesic potency, it has been suggested that analgesic drug combinations may be useful to improve analgesia and limit side-effects. Nefopam is a centrally acting non-narcotic analgesic, which has been used in the surgical setting in many countries since 1976. It is chemically distinct and pharmacologically unrelated to any presently known analgesic agents. The present study was conducted to ascertain and evaluate in a large double-blind randomized study the analgesic effect, using the numeric rating scale (NRS-11), and tolerance of i.v.nefopam alone and when used in combination with morphine and diclofenac sodium after abdominal surgery. The results show that nefopam is able to control postoperative pain and when combined with other commonly used analgesic agents like morphine and diclofenac, the combination has the potential to control the postoperative pain sufficiently in most of the patients especially with morphine. Results revealed that there were significant ($P<0.05$) differences among NRS scores for all drug groups. The percentage of the highest pain relief (NRS score= 0) increased when using of nefopam in combination with morphine in about 33.3%, while using of nefopam alone led to 15.5% pain relief, and morphine alone 26.7%. Also, nefopam when used with diclofenac led to 27.8% pain relief compared with diclofenac alone, which resulted in 22.2%. Results also showed that the differences in the side effects (sweating, nausea, vomiting, sedation, tachycardia, and dyspnea) among groups were not significant except in the sedation as the differences were significant ($P=0.02$) in the morphine groups. In conclusion, due to its powerful analgesic action explained by its mechanism of action, nefopam is the appropriate analgesic in multimodal analgesia. It is powerful analgesic with antihyperalgesic properties. It is important to administer nefopam in IV slow infusion in order to decrease side effects. Nefopam is a useful non-opioid adjuvant to opioids and other analgesics like non-steroidal anti-inflammatory drugs.

Keywords: Postoperative; Pain; Opioids; Non-narcotic analgesics; Side-effects; Nefopam.

INTRODUCTION

Postoperative pain management is essential to achieve patient comfort and postoperative relief. Acute postoperative pain is seen in more than 80% of patients who undergo surgical procedures and approximately 75% of those with postoperative pain experience the severity of pain as moderate, severe, or extreme.[1-2]. Adequate or mild postoperative pain experienced in about less than half of patients who undergo surgery [1]. Uncontrolled postoperative pain can negatively affect patient quality of life, functional recovery, and the risk of post-surgical complications [3]. Opioids considered the most widely used analgesic group to control postoperative pain, but opioids alone can cause side-effects and have limited analgesic potency, it has been suggested that analgesic drug combinations may be useful to improve analgesia and limit side-effects [4-5]. Only a few non-narcotic analgesics are available (e.g. acetaminophen, non-selective non-steroidal anti-inflammatory drugs and selective inhibitors of cyclooxygenase [5-6]. Nefopam is chemically distinct and pharmacologically unrelated to any presently known analgesic [7]. It has been used in Europe for i.v. and oral administration since 1976, and has been available for i.v. administration in France since 1981 [8]. Nefopam is a racemic mixture of its two enantiomers [9] and is a centrally acting non-narcotic analgesic. Although its mechanism of action is not completely understood, it is a centrally acting, non-opioid analgesic that inhibits reuptake of serotonin, norepinephrine and dopamine, neurotransmitters involved in many central actions including pain signaling, and thus increases their level and activities [10].

Also, nefopam was found to modulate sodium and calcium channels, thereby inhibiting the release of glutamate, a key neurotransmitter involved in pain processing [10]. Nefopam has the advantage of not affecting platelet aggregation and having no central nervous system depressive effect [11]. There have been rare fatal overdoses with the oral form of the drug, characterized by convulsions and arrhythmia [12]. Its sympathomimetic action renders it contraindicated for patients with limited coronary reserve, prostatitis and/or glaucoma. Nefopam causes minor side effects (nausea, dizziness and sweating) in 15-30% of treated patients [13]. Despite its availability for many years, clinical data on the analgesic effect and tolerance of i.v. nefopam for postoperative pain relief is rare. Previous studies have evaluated the use of non-narcotic drugs and opioids in comparison and in combination postoperatively and analgesic efficacy was demonstrated in many clinical studies in different types of surgery, even the more painful surgeries such as orthopedic surgery [14]. Nefopam was found to provide a synergistic effect with many NSAIDs and with paracetamol [15-17]. Nefopam provides a powerful analgesic effect and due to its different and complementary mode of action comparing to morphine, the use of nefopam in the management of postoperative pain allows reduction in the consumption of morphine up to 50% in the first 24 hours and an improvement of pain (seen on Visual Analog Scale – VAS) more potent than the one obtained with morphine alone[18]. Therefore, Nefopam is the appropriate analgesic in the multimodal analgesia. The purpose of this study was to ascertain and evaluate in a large double-blind randomized study the analgesic effect and tolerance of i.v. nefopam alone, in comparison with morphine and diclofenac sodium and when used in combination with these pharmacologically different analgesic agents after abdominal surgery.

MATERIALS AND METHODS

Patient selection and randomization

After obtaining ethics committee approval and written informed patient consent, 150 patients scheduled to undergo or emergency performed appendectomy were included in this multicenter (n=12), double-blind, randomized study of nefopam vs morphine and diclofenac sodium. To be included, patients had to be between 18 and 60 yr old, have an ASA score of I±III and require performing appendectomy and the surgery performed under standard general anaesthesia. Contraindications for nefopam and morphine or diclofenac use, severe cardiac disease, renal or hepatic insufficiency, and preoperative use of analgesics (corticosteroids, other opioids, other NSAIDs). The Consolidated Standards of Reporting Trials (CONSORT) recommendations for reporting randomized, controlled clinical trials were followed (Figure 1.) The Numeric Rating Scale (NRS-11) is an 11-point scale for patient self-reporting of pain. It

is for adults and children 10 years old or older. This scale (NRS: 0= No pain, 1-3= mild pain, 4-6=moderate pain, 7-10=severe pain) was used in this study for all patients undergone the surgery [19].

Administration of the analgesic drug

According to the randomization assignment, patients received a dose of analgesic medication immediately after surgery (T0), 6 hours after surgery (T6), and 12 hours after surgery (T12). The medications used nefopam 20mg diluted in dextrose 5%, 100 ml i.v., morphine 50 mg diluted in dextrose 5%, 100 ml i.v., Diclofenac 75 mg i.m. or combination of either nefopam and morphine or nefopam and diclofenac. Upon arrival in the postanaesthesia care unit (PACU), pain was evaluated every 5 min using the Numeric Rating Scale (NRS). The scores: 0 = No Pain, 1-3 = Mild Pain (nagging, annoying, interfering little with activities of daily living (ADLs)), 4–6 = Moderate Pain (interferes significantly with ADLs), 7-10 = Severe Pain (disabling; unable to perform ADLs). Nefopam tolerance was comparable to that of the morphine and diclofenac, in addition to the drug combinations. The existence of pain in addition to the presence of side effects was evaluated every 5 minutes for the cumulative dose of medications given postoperatively by PCA during 12 h. The most common side effects that were observed and measured are sweating, nausea, vomiting, sedation, tachycardia, and dyspnea.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two ways ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. The proportions were compared using Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

During the period of the study, 150 patients were included for analysis. Characteristics of the patients included in the study are shown in table 1. Results revealed that there were significant ($P<0.05$) differences among NRS scores for all drug groups. In the nefopam group and diclofenac group, the highest number of postoperative patient lie in the moderate level of pain (NRS=4-6). In the morphine group, the highest level was in the mild level of pain (NRS=1-3). In the nefopam-morphine group, the highest level of patients was in the mild level of pain (NRS=1-3) and in the no pain level (NRS=0). In the nefopam-diclofenac group, number of patients was high in the no pain, mild pain, and moderate pain level. The results revealed that, the percentage of the highest pain relief (NRS score= 0) increased when we used nefopam in combination with morphine in about 33.3%, while using nefopam alone result in 15.5% pain relief, and morphine alone 26.7%. Also, nefopam when used with diclofenac result in 27.8% pain relief compared with diclofenac alone which resulted in 22.2%. Figure 2 shows the differences between the 5 groups of medications in NRS scores.

Figure 3 shows the differences between using nefopam and morphine in combination or either drug alone after giving the analgesic medicatios at time 0 (T0), after 6 hours (T6), and after 12 hours (T12). The results revealed that using nefopam with morphine reduce significantly more the pain than morphine alone or nefopam alone after 6h and 12h. On the other hand, Figure 4 shows the differences between using nefopam and diclofenac in combination or either drug alone after giving the analgesic medications at time 0 (T0), after 6 hours (T6), and after 12 hours (T12). The results revealed that using nefopam with diclofenac together is able to reduce significantly the pain than either drug alone after 6h and 12h. Table (3) shows the incidence of side effects between the different groups of analgesic medication assessed every 5 minutes for the cumulative effect of using the medication for 12 hours. The side effects assessed are sweating, nausea, vomiting, sedation, tachycardia, and dyspnea. Results show that nefopam group and nefopam-morphine group have the highest sweating, nausea and/or vomiting side effects. Sedation appears high in the morphine and nefopam-morphine group in a percent of (36.66), which agrees with the narcotic analgesic action of

morphine. In nefopam group it was (23.33%), which also agrees with that it's a centrally acting analgesic. Tachycardia and dyspnea side effects were relatively low among the five different groups, except that tachycardia occurrence was 20% for nefopam group. The results suggested that nefopam is able to control postoperative pain as demonstrated by this study and other previous studies and when combined with other commonly used analgesic agents like morphine and diclofenac, the combination has the potential to control the postoperative pain sufficiently in most of the patients especially with morphine, which agrees with many previous studies [7,18]. A previous study has found that cumulative 24 h morphine consumption was decreased by almost 30% when used in combination with nefopam. However, this outcome has to be interpreted with caution as it was reported in only three trials with data on 306 patients [7]. However, the degree of morphine sparing may be used to compare indirectly the efficacy of analgesic adjuvants. For instance, nefopam's morphine-sparing effect appeared to be more pronounced in comparison with acetaminophen [20-21] but similar to ketamine or non-steroidal anti-inflammatory drugs [20, 22]. Several combinations of opioids and anti-inflammatory drugs have been examined in various models of pain and inflammation. For example, in one study, the combination of morphine and diclofenac has been shown to be effective in a model of acute inflammatory pain [23].

A synergistic antiallodynic effect of spinal morphine administered with ketorolac in nerve injured rats has also been reported [24]. Recently, it has been shown that the antinociceptive activity of morphine combined with different NSAIDs (including nimesulide, meloxicam, diclofenac, naproxen, piroxicam, parecoxib, and ketoprofen) induced a synergistic interaction in the mice model of visceral acute pain [25]. In this study and other previous studies, the analgesic potency using the new drug nefopam have been examined in combination with morphine and diclofenac. Nefopam has been proved to be the appropriate analgesic in multimodal analgesia. It is a centrally acting non-opioid analgesic, which guarantees powerful analgesic effect with a significant reduction of consumption of morphine and diclofenac sodium. Nefopam's analgesic efficacy was demonstrated in many clinical studies in different types of surgery, even the more painful surgeries such as orthopedic surgery [14]. According to a different and complementary mode of action compared to morphine and diclofenac sodium, the use of nefopam in the management of postoperative pain allows the reduction in the NRS pain score more potent than the one obtained with morphine alone or diclofenac alone.Opioids are the drugs of choice for the treatment of moderate to severe pain, however, a large number of clinical studies have reported that opioids, particularly morphine, had weak post-operative analgesic efficacy and many side effects [26]. Nefopam, a nonopioid analgesic drug, can produce analgesia in animals and humans. It was reported that in CCI model of neuropathic pain, a single dose of nefopam, significantly reduced pain behavior. Moreover, it was shown that nefopam has preventive analgesic effects [27]. Acute administration of nefopam (intraperitoneal, subcutaneous, and oral) exhibited a dose-dependent attenuation of pain behavior in the hot plate and plantar tests [28]. In this study, the dose used of nefopam showed pain reducing effects, which is consistent with previous studies [7]. Diclofenac sodium is a nonsteroidal anti-inflammatory drug (NSAID) used for management of mild to moderate pain and management of moderate to severe pain alone or in combination with opioid analgesics [29].

When used with nefopam, there was a significant decrease in the NRS pain score compared to using diclofenac alone. Nefopam does not induce opioid nor NSAID side effects, as demonstrated by many previous studies, this is mainly due to its original mechanism of action [30-31]. Nefopam preserves respiratory function, gastrointestinal mucosa, and intestinal transit, without antipyretic or anti-inflammatory action [32]. It prevents and reduces the incidence and severity of postanesthetic shivering, which potentially prevents the cardiovascular side effects [11, 33, 34] Nefopam has an original mechanism of action. It is a nonmorphinic analgesic, which has a central analgesic action. It inhibits reuptake of monoamines and increases monoarninergic activity. Therefore, Nefopam potentiates the action of pain monoaminergic inhibitory pathways [8, 10, 35]. In conclusion, Due to its powerful analgesic action explained by its mechanism of action, Nefopam is the appropriate analgesic in multimodal analgesia. It is powerful analgesic with antihyperalgesic properties. It is important to administer nefopam in IV slow infusion (no direct IV bolus) in order to decrease side effects. Nefopam is a useful non-opioid adjuvant to opioids and other

analgesics like non-steroidal anti-inflammatory drugs. It has many advantages in comparison to other analgesic drugs explained by its mechanism of action and its adverse effect profile.

ACKNOWLEDGEMENTS

This article was financially supported by the author from Department of Pharmacology, College of Dentistry/ Baghdad University,

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

REFERENCES

1. Apfelbaum JL, Chen C, Mehta SS, Gan TJ. Postoperative pain experience: Results from a national survey suggest postoperative pain continues to be undermanaged. Anesth Analg. 2003; 97:534-540.
2. Gan TJ, Habib AS, Miller TE, White W, Apfelbaum JL. Incidence, patient satisfaction, and perceptions of postsurgical pain: Results from a US national survey. Curr Med Res Opin.2014; 30:149-160.
3. Kehlet H, Jensen T, Woolf C. Persistent postsurgical pain: Risk factors and prevention. Lancet. 2014; 367:1618-1625.
4. Pöpping DM, Zahn PK, Van Aken HK, Dasch B, Boche R, Pogatzki-Zahn EM. Effectiveness and safety of postoperative pain management: a survey of 18 925 consecutive patients between 1998 and 2006 (2nd revision): a database analysis of prospectively raised data. Br. J Anaesth. 2008; 832-840.
5. Buvanendran, Asokumar, and Jeffrey S. Kroin. Multimodal analgesia for controlling acute postoperative pain. Curr Opin Anesthesiol. 2009; 588-593.
6. Dilmen, Ozlem Korkmaz, et al. Efficacy of intravenous paracetamol, metamizol and lornoxicam on postoperative pain and morphine consumption after lumbar disc surgery. Eur J Anaesthesiol. 2010; 428-432.
7. Evans, MS, Lysakowski C, Tramer MR. Nefopam for the prevention of postoperative pain: quantitative systematic review. Br. J. Anaesth.2008; 101: 610–17.
8. Heel RC, Brogden RN, Pakes GE, Speight TM, Avery GS. Nefopam: a review of its pharmacological properties and therapeutic efficacy. Drugs. 1980; 19: 249-67
9. Mather GG, Labroo R, Le Guern ME, Lepage F, Gillardin JM, Levy RH. Nefopam enantiomers: preclinical pharmacology/toxicology and pharmacokinetic characteristics in healthy subjects after intravenous administration. Chirality. 2000; 12: 153-9
10. Girard P, Chauvin M, Verleye M. Nefopam analgesia and its role in multimodal analgesia: A review of preclinical and clinical studies. Clinical and Experimental Pharmacology & Physiology. 2016; **43**: 3–12.
11. Chanques G, Sebbane M, Constantin JM, Ramillon N, Jung B, Cissé M, Lefrant JY, Jaber S. Analgesic efficacy and haemodynamic effects of nefopam in critically ill patients. Br J Anaesth. 2011; 106:336–343.
12. Kerr, Deborah E., and Alan K. Fletcher. Fatal nefopam overdose. Emerg Med J. 2010; 27:407-408.
13. Aveline, Christophe, et al. Postoperative analgesia and early rehabilitation after total knee replacement: A comparison of continuous low-dose intravenous ketamine versus nefopam. Eur J Pain.2009; 613-619.
14. Du Manoir, B., et al. Randomized prospective study of the analgesic effect of nefopam after orthopaedic surgery. Bri. J anaesth. 2003; 836-841.
15. Mimoz O, Incagnoli P, Josse C, Gillon MC, Kuhlman L, Mirand A., Fletcher D. Analgesic efficacy and safety of nefopam vs. propacetamol following hepatic resection. Anaesth. 2001; 56: 520-525.
16. Girard P, Verniers D, Coppé MC, Pansart Y. Nefopam and ketoprofen synergy in rodent models of antinociception. Eur J Pharmacol. 2008; 263-271.

17. Hwang, BY, Kwon JY, Lee DW, Kim TK. A randomized clinical trial of Nefopam versus Ketorolac combined with oxycodone in patient-controlled analgesia after gynecologic surgery. Int J Med Sci. 2015; 12: 644–649.
18. Beloeil H, Delage N, Negre I, Mazoit JX, Benhamou D. The median effective dose of nefopam and morphine administered intravenously for postoperative pain after minor surgery: a prospective randomized double-blinded isobolographic study of their analgesic action. Anesth Analg 2004; 98: 395–400.
19. Salmore R. Development of a new pain scale: Colorado Behavioral Numerical Pain Scale for sedated adult patients undergoing gastrointestinal procedures. Gastroenterol Nurs.2008; **25** (6): 257–62.
20. Elia N, Lysakowski C, Trame`r MR. Does multimodal analgesia with acetaminophen, nonsteroidal antiinflammatory drugs, or selective cyclooxygenase-2 inhibitors and patient-controlled analgesia morphine offer advantages over morphine alone? Meta-analyses of randomized trials. Anesthesiol. 2005; 103: 1296–304.
21. Remy C, Marret E, Bonnet F. Effects of acetaminophen on morphine side-effects and consumption after major surgery: meta-analysis of randomized controlled trials. Br J Anaesth. 2005; 94: 505–13.
22. Elia N, Trame`r MR. Ketamine and postoperative pain—a quantitative systematic review of randomised trials. Pain. 2005; 113: 61–70.
23. Fletcher D, Benoist JM, Gautron M, Guilbaud G. Isobolographic analysis of interactions between intravenous morphine, propacetamol, and diclofenac in carrageenin-injected rats. Anesthesiol. 1997; 87: 317-26.
24. Lashbrook JM, Ossipov MH, Hunter JC, Raffa RB, Tallarida RJ, Porreca F. Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX1- and COX2- inhibitors in nerve-injured rats. Pain. 1999; 82: 65-72.
25. Miranda HF, Prieto JC, Puig MM, Pinardi G. Isobolographic analysis of multimodal analgesia in an animal model of visceral acute pain. Pharmacol Biochem Behav. 2008; 88: 481-6.
26. Maund E, McDaid C, Rice S, Wright K, Jenkins B, Woolacott N. Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: a systematic review. Br J Anaesth. 2011; 292-297.
27. Biella GE, Groppetti A, Novelli A, Fernández-Sánchez MT, Manfredi B, Sotgiu ML. Neuronal sensitization and its behavioral correlates in a rat model of neuropathy are prevented by a cyclic analog of orphenadrine. J Neurotrauma. 2003; 20: 593-601.
28. Girard P, Pansart Y, Coppe MC, Gillardin JM. Nefopam reduces thermal hypersensitivity in acute and postoperative pain models in the rat. Pharmacol Res. 2001; 44: 541-5
29. Mads K, Gögenur I, and Rosenberg J. Postoperative use of non-steroidal anti-inflammatory drugs in patients with anastomotic leakage requiring reoperation after colorectal resection: cohort study based on prospective data. Br. Med. J. 2012; 345: e6166.
30. Ju HL, Jae HK, Cheong YK. The analgesic effect of nefopam with fentanyl at the end of laparoscopic cholecystectomy. Korean J pain. 2013; 361-367.
31. Richebé P, Picard W, Rivat C, Jelacic S, Branchard O, Leprouts S, Cahana Alex, Janvier G. Effects of nefopam on early postoperative hyperalgesia after cardiac surgery. J Cardiothorac Vasc anesth. 2013; 427-435.
32. Delage N, Maaliki H, Beloeil H, Benhamou D, Mazoit JX. Median effective dose (ED50) of nefopam and ketoprofen in postoperative Patients: a study of interaction using sequential analysis and isobolographic analysis. Anesthesiol. 2005;102:1211-6.
33. Alfonsi, Pascal, et al. Nefopam and alfentanil additively reduce the shivering threshold in humans whereas nefopam and clonidine do not Anesthesiology: J Am. Soci. Anesthesiol .2009; 102-109.
34. Taniguchi, Yoshie, et al. The effects of nefopam on the gain and maximum intensity of shivering in healthy volunteers. Anesthesia & Analgesia. 2010; 409-414.
35. Gregori-Puigjané, Elisabet, et al. identifying mechanism-of-action targets for drugs and probes. Proceedings of the National Academy of Sciences. 2012; 11178-11183.

Table 1: Characteristics of the patients included in the study (n=150).

Age (yr)	28 (19–37)
Sex (F/M)	53/97
Body mass index (kg)	74.2 (57–91)
Height (cm)	167.1 (155-178)

Table 2: Numeric rating scale (NRS) pain scores during 12 h following abdominal surgery in patients receiving patient-controlled analgesia with nefopam, morphine, diclofenac, nefopam-morphine combination or nefopam-diclofenac combination.

NRS score	Nefopam (n=30)	Morphine group (n=30)	Diclofenc (n=30)	Nefopam + Morphine (n=30)	Nefopam + diclofenac (n=30)
No pain (0)	C 4.66±2.51 c	AB 8.00±1.00 b	BC 6.66±1.15 b	A 10.00±2.00 a	AB 8.33±2.08 a
Mild pain (1-3)	BC 8.33±1.15 b	A 11.00±1.00 a	C 6.00±1.00 b	A 11.66±1.52 a	AB 10.33±0.57 a
Moderate pain (4-6)	A 11.00±1.00 a	C 6.66±0.57 b	AB 9.66±1.52 a	BC 7.33±2.51 b	AB 9.00±1.00 a
Intense pain (7-10)	AB 6.00±2.00b c	BC 4.33±0.57 c	A 7.66±1.15 ab	CD 1.00±1.00 c	C 2.33±0.57 b
LSD	2.3627				

Mean±SD. Means with different small letters in the same column significantly different (P<0.05). Means with different capital letters in the same row significantly different (P<0.05).

Table 3: Incidence of Adverse Events among the 5 different groups of analgesic medication.

Medication/Side effects	Sweating	Nausea or vomiting	sedation	Tachycardia	Dyspnea
Nefopam group (n=30)	9(30%)	10(33.33%)	7(23.33%)	6(20%)	1(3.33%)
Morphine group (n=30)	6(20%)	8(26.66%)	11(36.66%)	1(3.33%)	2(6.66%)
Diclofenac group (n=30)	3(10%)	2(6.66%)	2(6.66%)	5(16.16%)	0(0%)
Nefopam + Morphine (n=30)	9(30%)	7(23.33%)	11(36.66%)	5(16.16%)	1(3.33%)
Nefopam + diclofenac (n=30)	5(16.16%)	4(13.33%)	6(20%)	4(13.33%)	0(0%)
P	0.24	0.07	0.02	0.36	0.80

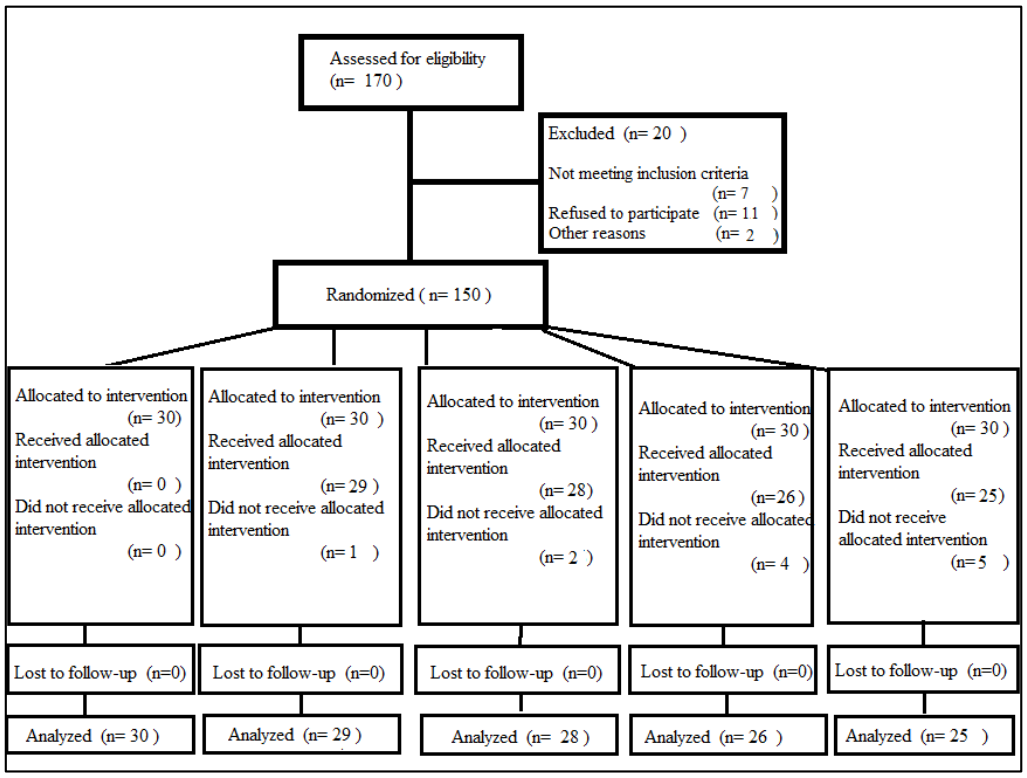


Fig 1. Consort flow diagram

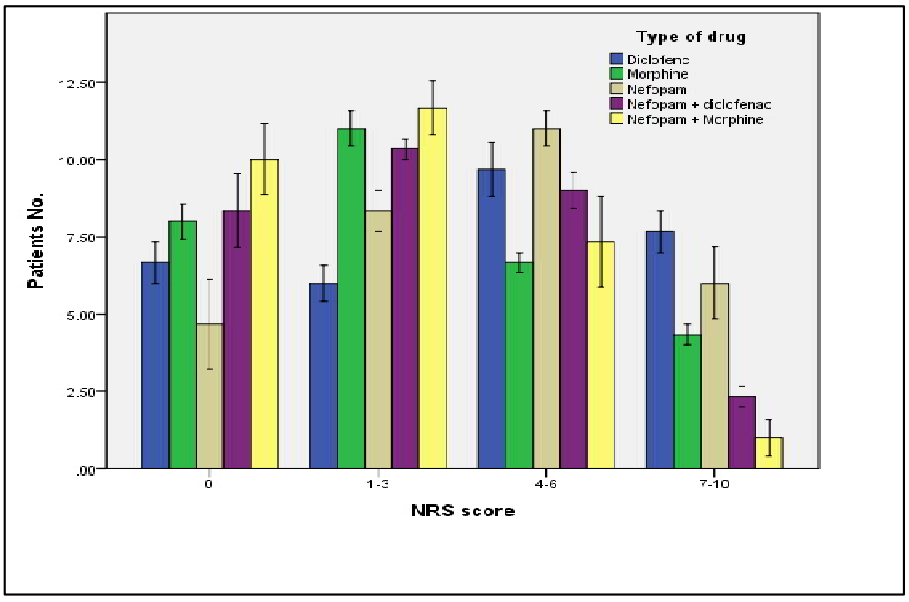


Fig. 2: NRS pain score assessed during the 12-h for the mean + SD of patient number for the five different drug groups. *P<0.05.

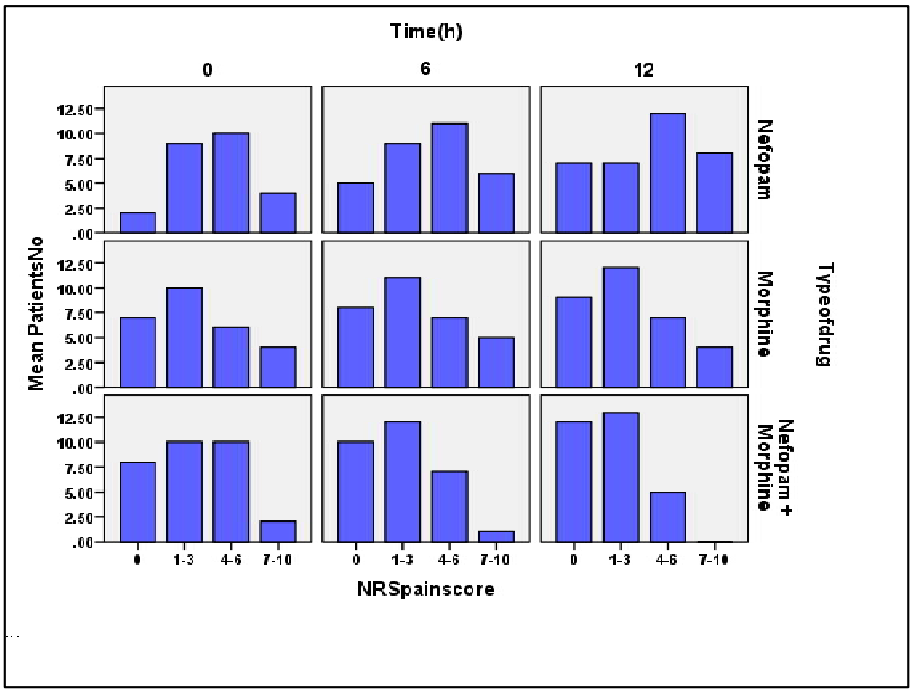


Fig 3: NRS score (mean and SD) assessed at different times during the 12-h. PACU (post-anaesthesia care unit) assessment: *P<0.05. 0, 6, 12 are time points (h) after the preoperative administration of the drug doses.

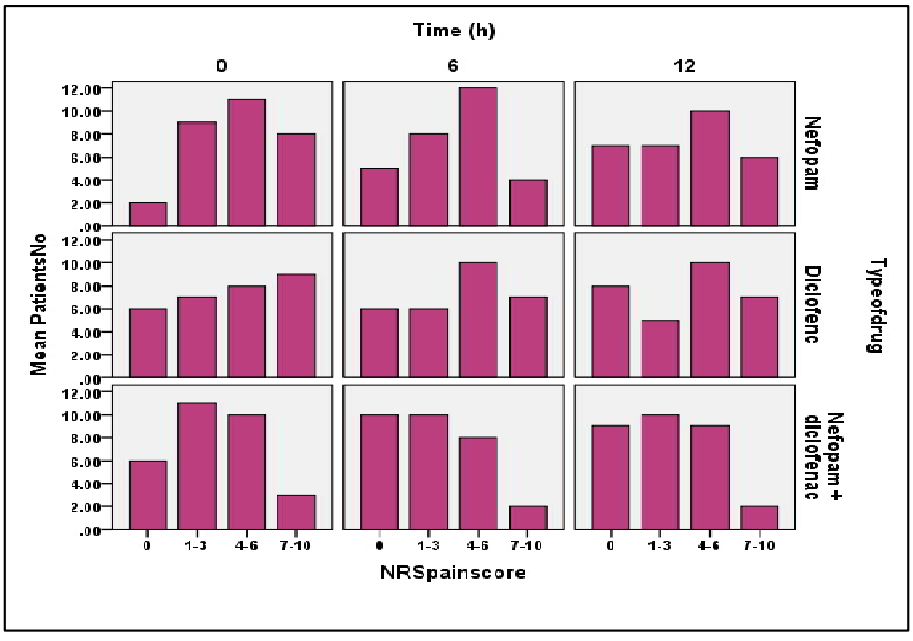


Fig 4: NRS score (mean and SD) assessed at different times during the 12-h. PACU (post-anaesthesia care unit) assessment: *P<0.05. 0, 6, 12 are time points (h) after the preoperative administration of the drug doses.

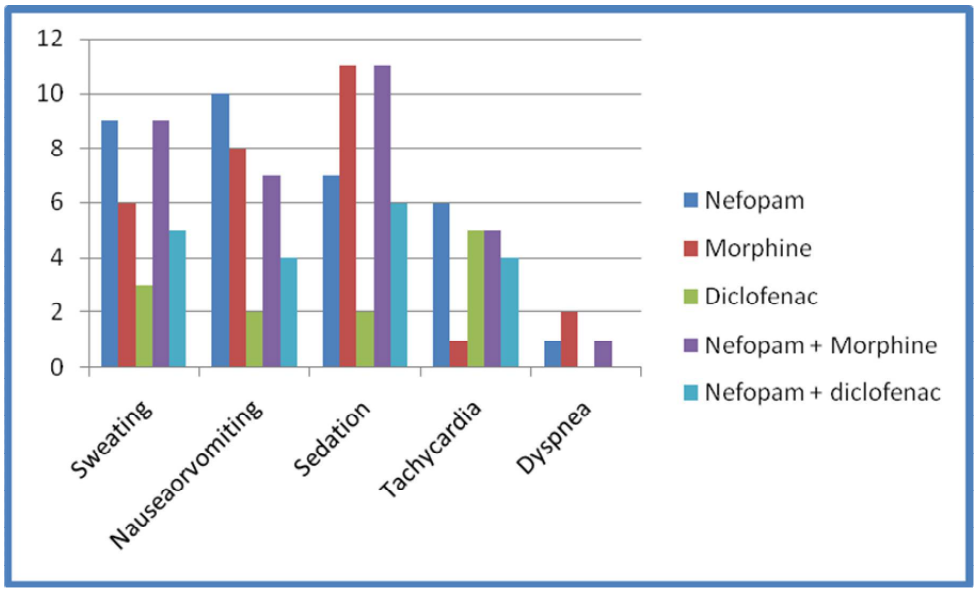


Fig 5: The differences in the incidence of side effects among the 5 different groups of analgesic medication.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Molecular Detection and Phylogenetic Tree Analysis of *Cryptosporidium parvum* From Stool Samples in Wasit Province, Iraq.

Zahraa Ali Faieq[1*] and Baraa Abdulsalam Hraiga[2]

Department of Biology, College of Science, Wasit University, Kut, Iraq.

Received: 15 Mar 2018 Revised: 22 Apr 2018 Accepted: 17 May 2018

[*]**Address for correspondence**
Zahraa Ali Faieq
Department of Biology,
College of Science,
Wasit University, Kut, Iraq..

ABSTRACT

The motivation behind the present examination was to assess PCR for molecular detection of *C. parvum* in feces tests, evaluation the genetic characterization relationship and DNA sequencing based on phylogenetic tree investigation of small-subunit rRNA gene in *C. parvum*. In this study, the PCR was done by designed specific PCR primers using NCBI-GenBank (AF159112.1) to amplified 573bp PCR product. The results showed 18 (36%) stool samples were positive, where 32 (64%) were negative for *C. parvum* by PCR. The various arrangement investigation and neighbor joining phylogenetic tree examination (MEGA 6.0 version) of small-subunit rRNA gene in two local *C. parvum* isolates were show closed related to NCBI-Blast Cryptosporidium parvum Iran isolate K2 small subunit ribosomal RNA gene (AF159112.1) at (99%) homology sequence identity by using NCBI-BLAST. This study represented the most sensitive and most specific molecular method to detect obtains *C. parvum*. Moreover, is considered the first on use the molecular phylogeny *C. parvum* in wasit province, Iraq.

Keywords: *Cryptosporidium parvum,* phylogenetic, Molecular.

INTRODUCTION

Cryptosporidium is associate degree living thing extra cytoplasmatic protozoa parasite with a monoxnous life cycle, wherever all nonsexual and sexual improvement happens within a number. The parasite contaminates the microvillus fringe of the channel and metabolism animal tissue of an in depth type of vertebrate hosts, together with individuals, inflicting regular ailments. It's been accounted for to cause waterborne and nourishment born flare-ups overall [1,2]. Animal disease and individual to-individual transmission, however, ar likewise acknowledged [3,4]. A comprehension of it's the study of unwellness transmission has be hampered by poor learning of the species structures and general eudaemonia significance of various Cryptosporidium species and genotypes. In cultivate

creatures, Cryptosporidium parasites cause issue of the stomach related and respiratory frameworks, that's prompt weakness of tainted creatures and critical financial misfortunes. Conversely, a current report proposed that all Cryptosporidium parasites, including these form bring down vertebrates, ought to be viewed as perilous too people [5]. There are eight validly named species of Cryptosporidium [6] of which *C. Parvum* is infectious for man and virtually all other mammals [7,8,9]. Other species of interest include *C.bailyi* [10]. *C. Muris* [11] and *C.Wrari*, which are infectious to chicken, the house mouse and guinea/pigs, respectively [12, 13] other species of cryptosporidium cause infection in the domestic cat, turkeys, fish and snakes [14, 15, 16, 17]. Which have been separated through succession polymorphisms the little subunit ribosomal RNA [18, 19, 20], the acetyl CoA synthetize [21], warm stun proten70, and also the Cryptosporidium-oocyst macromolecular protein (COWP) characteristics, and named once they were resolved [22]. The parasite is transmitted to people via polluted drinking water [23], attach tainted creatures, and attach contaminated people [24]. In the immunocompetent, cryptosporidiosis shows itself as self-restricted looseness of the bowels, here and there joined by queasiness, stomach spasms, fever, and heaving. In the immunodeficent, in any case, cryptosporidiosis might be serious, endless, and hazardous [25]. In this correspondence, we display the consequences of grouping portrayal and phylogenetic examination of different Cryptosporidium disconnects from human feces tests.

MATERIALS AND METHODS

Sample Collection

50 stool tests were gathered form patient with diarrhea in Wasit hospital, Iraq. The fecal sample was placed to a clean, dry plastic container and transported to the laboratory for analysis.

Stool DNA Extraction

DNA was removed form feces tests by utilizing (Stool DNA extraction Kit, Bioneer. Korea). The removing was finished by organization guidelines by utilizing stool lysis convention technique with Proteinase K [26]. From that point forward, the extricated gDNA was checked by Nano drop spectrophotometer, and after that put away at -20C at fridge until utilized as a part of PCR enhancement.

Polymerase-chain-reaction

PCR test was performed for identification of *C. parvum* from human feces tests in view of intensification of little subunit ribosomal RNA quality. The PCR test was completed by [26]. The groundworks were outlined in this investigation utilizing (GenBank: AF159112.1) and Primer3plus. The groundworks were given by (Bioneer organization. Korea) (Table-1). At that point PCR ace blend was set up by utilizing (AccuPower® PCR PreMix pack. Bioneer. Korea). The PCR premix tube compose of solidify dried pelt off (Taq "DNApolymerse1 U, dNTPs250µM, TrisHCl (pH 9.0) 10mM, KCl30mM, MgCl2 1.5mM, stabilizer, following color). the PCR pro mix reaction was" set up according to unit rules [26], in 20µl total by included 5µl of cleansed genomic DNA and 1.5µl of 10pmole of forward preparatory and 1.5µl of 10pmole of switch foundation, by then complete the PCR premix tubes by deionizer PCR water into 20µl and immediately mixed by XSpin vortex rotator (Bioner. Kora) (Table2). The response was performed in a thermos-cycler (T100 Thermal cycler, Bio Rad. USA) by set up the accompanying thermos/cycler conditions [26]; beginning denaturation-temperature of 95 °C for 5 minutes took after by 30 cycles at denaturation 95°C for 30. s, toughening 58/°C for 30.s, and augmentation 72°C for 1min. moment and afterward last augmentation at 72°C for 5.m. The PCR items (573bp) were inspected by electrophoreses in a 1% agarose gel, recolored with ethidium bromide, and envisioned under UV enlightenment (Table-3)

DNA Sequencing

DNA sequencing technique was act for evaluation the genetic characterization relationship between neighborhood *C. parvum* isolates and NCBI submitted C. parvum isolates in view on small subunit rRNA gene. Through using Phylogenetic tree analysis. The small-subunit rRNA gene. 573bp PCR object was refined from agarose gel through utilising (EZ-10 Spin Column DNA Gel Extraction Kit, Biobasc. Caneda). The purged little subunit rRNA pleasant PCR item tests have been despatched to Macrogen Company in Korea for played out the DNA sequencing making use of little subunit rRNA forward preliminary through (AB DNA sequencing framework). The phylogenetic investigation used to be carried out in view of NCBI-Blast Alignment distinguishing proof and Neighbor Distance Phylogenetic tree examination (Mege version 6) [27].

Statistical analysis

Statistical analysis: The statistical analysis was performed using SAS (Statistical Analysis System - version9.1) [28] .

RESULTS

Polymerase Chain Reaction

The PCR was performed in a total of 50stool samples, which the results were 18 (36%) stool samples were positive, where 32 (64%) were negative for *C. parvum*. The PCR created and assessed in the present examination which demonstrated the extent of indicative sections of PCR items that was (573 bp) (Fig-1).

DNA Sequencing

The partial small-subunit rRNA gene sequence in local *C. parvum* isolates were obtained No.1 and No.2 based Clustal alignment analysis (Fig-2). The Phylogenetic tree analysis used for evaluation of genetic relationship between local Cryptosporidium parvum and NCBI-BLAST isolates. The local *C. parvum* isolates No.1 and No.2 were show closed related to NCBI-Blast *C. parvum* Iran isolate K2 small subunit ribosomal RNA gene (AF159112.1). Whereas, the NCBI-Blast *C. parvum* isolates were show different and out of tree (Fig-3).

DISCUSSION

Phylogenetic examinations of the SSU rRNA quality progressions of Cryptosporidium parasites have been compelled, and the couple of examinations that have been done have added to the perplexity concerning the systematics of Cryptosporidium parasites. A fundamental SSU rRNA gathering examination revealed more than 99% character between C. parvum and C.muris [29]. Current phylogenetic examinations in like manner fail to separate C.parvum, C.muris, and C.baileyi [30]. Test misidentification and gathering blunder, in any case, may have affected the aftereffect of these examinations [31]. To limit the commitment of grouping mistakes, we utilized just *C. parvum* SSU rRNA groupings created in this examination in the phylogenetic investigation. DNA purification treatments, removed PCR inhibitors before PCR amplification. This isolation and purification of DNA was used for evaluation of sensitivity of PCR methods for *C. parvum* detection. In current study, a total of 50 stool samples, which the results were 18 (36%) stool samples were positive for PCR this result is agree with [32] which observed 21% (9/43) were *C. parvum* positive by PCR. Similar reports of prevalence of *C. parvum* have been reported in other developing countries including Brazil [33], Peru [34, 35], Kenya [36], Vietnam [37], Iran [38] and Haiti [39] and India [40, 42,43] which agree with our finding. In this study, using sequence examination the partial SSUrRNA gene, 18 *Cryptosporidium* isolatesfrom diarrheic children were identified as *C. parvum*, which reliable with considers from some creating and

created nations, for example, Malaysa, Kuwat, Yeman, Swedan, United Kingdam, Nethrland, Frence, Portugl, Nicargua [33, 36, 37, 38, 40, 41, 42, 43, 44]. The Neighbor-Joining phylogenetic tree constructed clearly discriminated the previously described lineages of Cryptosporidium spices and subtypes. The Neighbor-Joining phyletic tree designed clearly unintegrated the already delineate ancestries of Cryptosporidium flavors and subtypes.

CONCLUSION

PCR is considered as an alternative tool in epidemiological studies and the diagnosis of *C. parvum.* The results showed that The Local Cryptosporidium parvum isolates No.1 and No.2 were show closed related to NCBI-Blast Cryptosporidium parvum Iran isolate K2 small subunit ribosomal RNA gene (AF159112.1).

ACKNOWLEDGEMENTS

Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for supporting

REFERENCES

1. Millard, PS, Gensheime, KF, Addis, DG, Sosin, DM, Beckett, GA, Houck-Jankoski, A, and Hudson A. An outbreak of cryptosporidiosis from fresh-pressed apple cider. The Journal of the American Medical Association. 1994; 272:1592–1596.
2. Smith, HV, and Rose, J B. Water borne cryptosporidiosis: current status. Parasitology Today .1998; 14:14–22
3. Awad-El-Kariem, FA, Warhurst, DC, and McDonald, V. Detection of *Cryptosporidium* oocysts using a system based on PCR and endonuclease restriction. Parasitology. 1994; 109:19–22.
4. Cordell, RL, and Addiss, DG. Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. The Pediatric Infectious Disease Journal. 1994; 13:311–317.
5. Tzipori, S, and Griffiths, JK. Natural history and biology of *Cryptosporidium parvum*. Advance Parasitology. 1998; 40:5–36.
6. Fayer R. General Biology of Cryptosoridim. In: Dupey jp, Speer Ca, Fayer R, eds. Cryptosporidiosis of man and animals. Boca Raton, FL, CRC Press. 1990.
7. O· Donoghue, PJ. Cryptosporidium and cryptosporidiosis in man and animals. International Journal of parasitology 1995; 25: 139-195.
8. Nime, FA, Burek, JD, Page, DL, Holscher, MA, Yardley, JH. Acute enterocolitis in a human being infected with the protozoan Cryptosoridium. Gasroenterology. 1976; 70: 592-598.
9. Mesisel, JL, Perera, DR, Meligro, C, Rubin, CE, Overwhelming watery diarrhea associated with a cryptosporidium in an immunosuppressed patient. Gastroenterology 1976; 70: 1156-1160.
10. Current, WL, Upton SJ, Haynes TB. The life cycle of Cryptosporidium baileyi n. sp. (Apicomplexa, Cryptosporidiidae) infecting ckicken. The Journal of Protozoology. 1986; 33: 289-296.
11. Tyzzer, EE. Cryptosporidium parvum. Sp. nov., a coccidium found in the small intestine of the common mouse. Arch Protistenk. 1912; 26: 394-412
12. Tyzzer, EE. An extracellular coccidium, Cryptosporidium muris (gen. et. Sp. nov.) of the gastric glands of the common mouse. The Journal of Medical Research. 1910; 23: 487-509.
13. Vetterling, JM, Jervis, HR, Merrill, HG, Sprinz, H, Cryptosporidium Wrairi sp. N. From the guniea pig Cavia porcellus with an emendation of the genus. The Journal of Protozoology. 1971; 18: 243-247.
14. Iseki, M. Cryptosporidium felis sp. n. (protozoa, Eimeriorina) from the domestic cat. Japanese Journal of Parasitology. 1979; 28: 285.
15. Slavin, D. cryptosporidium meleagridis (sp. nov.). Journal of Comparative Pathology. 1955; 65: 262-266.

16. Hoover, DM, Hoerr, FJ, Caelton, WW, Hinsman, EL, Ferguson, HW. Enteric cryptosporidiosis in a Naso tang. Naso lituratus Bloch and Schneider. Journal of Fish Diseases. 1981; 4: 425-428.
17. Levine, ND. Some corrections of coccidian (Apicomplexan: protozoa) nomenclature. Journal of parasitology. 1980; 66: 830-834.
18. Xiao, L, Morgan, UM, Limor, J, Escalante, A, Arrowood, M, Shulaw, W. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Applied and Environmental Microbiology. 1999; 65:3386-91
19. Perz, JF, Le Blancq, SM. *Cryptosporidium parvum* infection involving novel genotypes in wildlife from Lower New York State. Applied and Environmental Microbiology. 2001; 67:1154-62.
20. Morgan, UM, Sargent, KD, Deplazes, P, Forbes, DA, Spano, F, Hertzberg, H. Molecular characterization of *Cryptosporidium* from various hosts. Parasitology 1998; 117 (Pt 1):31-7.
21. Sulaiman, IM, Morgan, UM, Thompson, RC, Lal, AA, Xiao, L. Phylogeneticrelationships of *Cryptosporidium* parasites based on the 70-kilodalton heat shock protein (HSP70) gene. Applied and Environmental Microbiology. 2000; 66:2385- 91.
22. Xiao, L, Limor, J, Morgan, UM, Sulaiman, IM, Thompson, RC, Lal, AA. Sequence differences in the diagnostic target region of the oocyst wall protein gene of *Cryptosporidium* parasites. Applied and Environmental Microbiology. 2000; 66:5499-502.
23. MacKenzie, WR, Schell, WL, Blair, KA, Addiss, DG, Peterson, DE, Hoxie, NJ. Massive outbreak of waterborne Cryptosporidium infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clinical Infectious Diseases. 1995; 21:57-62.
24. Navin, TR. Cryptosporidiosis in humans: review of recent epidemiologic studies. Europa Journal of Epidemiology. 1985; 1:77-83.
25. Arrowood, MJ. Diagnosis. In: Fayer R, editor. Cryptosporidium and cryptosporidiosis. Boca Raton (FL): CRC Press; 1997. p. 43-64.
26. Haque, R, IKM, A, Akther, S, Petri, WA. Comparison of PCR, isoenzyme analysis and antigen detection for diagnosis of Entamoeba histolytica infection. Journal of Clinical Microbiology. 1998; 36: 449-452.
27. Xiao, L, Escalante, L, Yang, C, Sulaiman, I, Anannias, AE, Richard, J M, Ronald, F and Altaf, A L. Phylogenetic Analysis of *Cryptosporidium* Parasites Based on the Small-Subunit rRNA Gene Locus. Applied and Environmental Microbiology. 1999; p. 1578–1583
28. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010
29. Cai, J, Collins, MD, McDonald, V, and Thompson, D E. PCRcloning and nucleotide sequence determination of the 18S rRNA genes andinternal transcribed spacer 1 of the protozoan parasites *Cryptosporidium parvum* and *Cryptosporidium muris*. Biochimica et Biophysica Acta. 1992; 1131:317–320.
30. Tzipori, S, and Griffiths, JK. Natural history and biology of *Cryptosporidium parvum*. Advance in Parasitology. 1998. 40:5–36.
31. Xiao, L, Sulaiman, I, Fayer, R, and Lal, AA. Species and strain specific typing of Cryptosporidium parasites in clinical and environmental samples. Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 1998: 93:687–691.
32. Shehla, K, Bijay, RM, Ashutosh, P, Yogita, S, Govind, M, Jaishree, P. Cryptosporidium species subtypes and associated clinical manifestations in Indian patients. Research Institute for Gastroenterology and Liver Diseases. Gastroenterology and Hepatology From Bed to Bench. 2017
33. Bushen OY, Kohli A, Pinkerton RC, Dupnik K, Newman RD, Sears CL. Heavy cryptosporidial infections in children in northeast Brazil: comparison of Cryptosporidium hominis and Cryptosporidium parvum. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007; 101:378-84.
34. Nahrevanian H, Azarinoosh SA, Esfandiari B, Ziapoor SP, Shadifar M, Amirbozorgy G, Hayati E, Davoodi J. Current situation of *Cryptosporidium* and other enteroparasites among patients with gastroenteritis from western cities of Mazandaran province, Iran, during 2007-2008. Gastroenterology and Hepatology from Bed to Bench. 2010; 3(3):120-25.
35. Tahvildar-Biderouni F, Salehi N. Detection of *Cryptosporidium* infection by modified ziehl-neelsen and PCR methods in children with diarrheal samples in pediatric hospitals in Tehran. Gastroenterology and Hepatology from Bed to Bench. 2014; 7(2):125-30.

36. Nyamwange CI, Mkoji G, Mpoke S, Nyandieka HS. Cryptosporidiosis and its genotypes among children attending Moi teaching and referral hospital in Eldoret, Kenya. East African Medical Journal. 2012; 89:11-9.
37. Gatei W, Greensill J, Ashford RW, Cuevas LE, Parry CM, Cunliffe NA. Molecular analysis of the 18S rRNA gene of Cryptosporidium parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. Journal of Clinical Microbiology. 2003; 41:1458-62
38. Zavvar M, Sadraei J, Emadi H, Pirestani M. The use of a nested PCR-RFLP technique, based on the parasite's 18S ribosomal RNA, to characterize Cryptosporidium isolates from HIV/AIDS patients. Annals of Tropical Medicine and Parasitology. 2008; 102:597-01.
39. Gatei, W, Das, P, Dutta, P, Sen, A, Cama, V, Lal, AA. Multilocus sequence typing and genetic structure of Cryptosporidium hominis from children in Kolkata, India. Infection, Genetics and Evolution. 2007; 7:197-05.
40. Raccurt, CP, Brasseur, P, Verdier, RI, Li, X, Eyma, E, Stockman, CP. Human cryptosporidiosis and Cryptosporidium spp. in Haiti. Trop Med Int Health. 2006; 11:929-34.
41. Muthusamy, D, Rao, SS, Ramani, S, Monica, B, Banerjee, I, Abraham, OC. Multilocus genotyping of Cryptosporidium sp. isolates from human immunodeficiency virus-infected individuals in South India. Journal of Clinical Microbiology. 2006; 44:632-34.
42. Rao Ajjampur, SS, Asirvatham, JR, Muthusamy, D, Gladstone, BP, Abraham, OC, Mathai, D. Clinical features & risk factors associated with cryptosporidiosis in HIV infected adults in India. Indian Journal of Medical Research. 2007; 126:553-57.
43. Sulaiman, IM, Hira, PR, Zhou, L, Al-Ali, FM, Al-Shelahi, FA, Shweiki, HM, Iqbal, J, Khalid, N, Xiao, L. Unique endemicity of cryptosporidiosis in children in Kuwait. Journal of Clinical Microbiology. 2005; 43(6):2805-09.
44. McLauchlin, J, Amar, C, Pedraza-Díaz, S, Nichols, GL. Molecular epidemiological analysis of Cryptosporidium spp. in the United Kingdom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. Journal of Clinical Microbiology. 2000; 38(11):3984-90.

Table 1: List of Primer that used in PCR amplification

Primer	Sequence		Amplicon
C. parvum	F	CGGGTAACGGGGAATTAGGG	573bp
	R	TCCTTGGCAAATGCTTTCGC	

Table 2: The compound of PCR Master mix in PCR

PCR master Mix	Volume
DNA template	5µl
Forword Primer (10 pmol)	1.5µl
Reverse Primer (10 pmol)	1.5µl
PCR water	12µl
Total	20µl

Table 3: The PCR cycles

PCR step	Temperature	Time	Repeat cycle
Initial Denaturtion	95.°C	5/min	1
Denaturtion	95.°C	30/sec	30
Annealing	58.°C	30/sec	
Extansion	72.°C	1/min	
Final Extansion	72.°C	5/min	

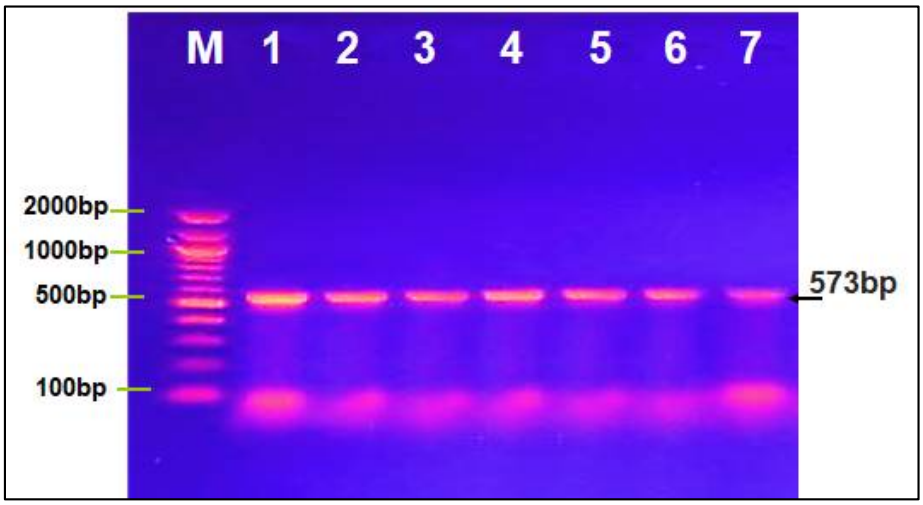


Figure 1: Agarose-gel-electrophoreses image which show the PCR item of small-subunit rRNA gene that detection Cryptosporidium parvum in Human stool samples. Where M: Marker (100-2000bp), lane (1-7) some positive samples at 573bp PCR product size.

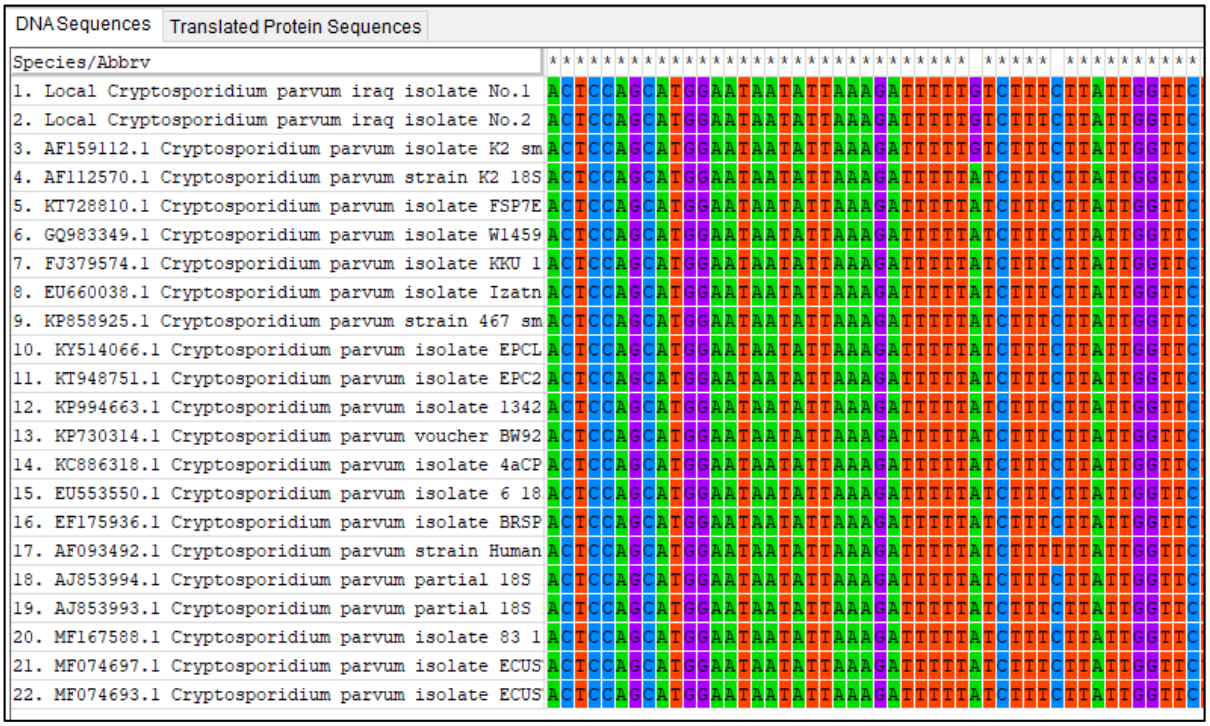


Figure 2: Multiple sequence alignment examination the partial small subunit rRNA gene sequence in local *C .parvum* isolates No.1 andNo.2 based Clustal W alignment analysis through (MEGA 6.0,

multiple alignment analysis tool). That demonstrate the numerous arrangement examination similitude (*) and differences in small-subunit rRNA gene nucleotide sequences.

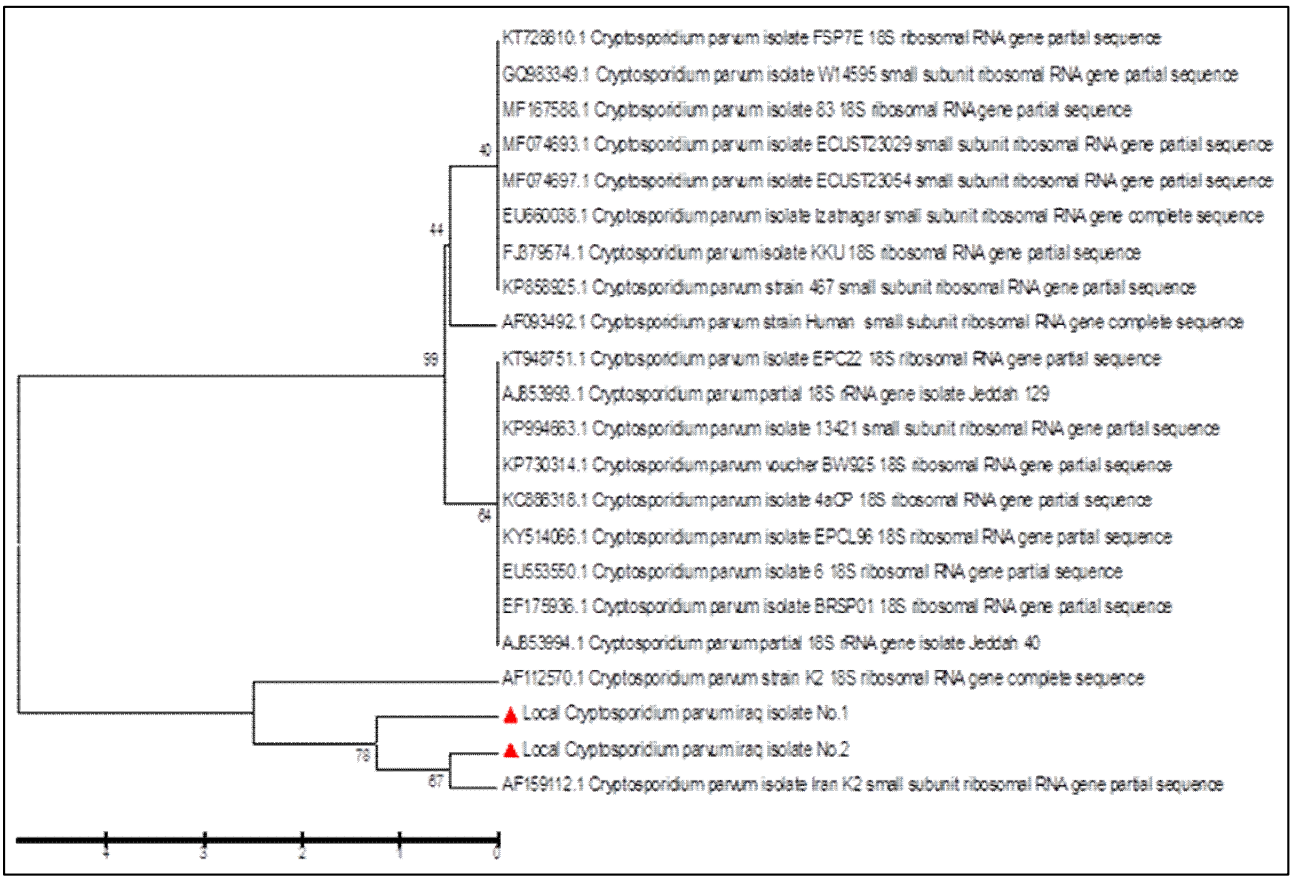


Figure3: Phylogenetic-tree-analysis in view on the small-subunit rRNA gene partial sequence that used for evaluation of genetic relationship between local *C. parvum* and NCBI-BLAST isolates. The Phylogenetic tree was constructed using Maximum Likelihood tree method in (Mega6.0 version).

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Inhibitory Effect of *Lactobacillus Spp*, on Pathogenic Bacteria in AL-Qadisiyah Province, Iraq.

Hind HamzahAbdulhussein*

Department of Biology, College of Science, University of AL- Qadisiyah, Iraq.

Received: 20 Mar 2018 Revised: 17 Apr 2018 Accepted: 22 May 2018

***Address for correspondence**
Hind HamzahAbdulhussein
Department of Biology ,
College of Science,
University of AL- Qadisiyah, Iraq.

ABSTRACT

Lactic acid bacteria (LAB) are noteworthy to human wellbeing because of the generation of some antimicrobial substances and capacity to restrain pathogenic microscopic organisms. Moreover, the microscopic organisms are likewise utilized as a part of the creation of different nourishment items. The point of this examination was disengagement and portrayal of Lactobacillus species from dairy items and concentrates the antimicrobial impact on numerous pathogenic microbes. Thirty (30) strains of lactic acid microscopic organisms (LAB) secluded from fifty (50) test of Milk, Yogurt, Cheese, Bovine drain and raw drain which arbitrarily gathered from business sectors in AL- Qadisiyah, Iraq between October 2017 to January 2018. The *Lactobacillus* spp disconnected from tests utilizing MRS soup and agar refined media. Strains disengaged were described by infinitesimally and biochemical properties. Other portrayal like deliver antimicrobial substances dynamic against chose pathogens disconnects (clinical separate). Discoveries from this investigation bolster the likelihood to investigate the tried lactobacilli and their CFSs as characteristic bio-additives, alone or in mix with affirmed bacteriocins in sustenance and pharma plans in the wake of approving their wellbeing.

Keywords: *Lactobacillus* spp, Milk, yoghurt, cheese, Pathogenic Bacteria.

INTRODUCTION

Notwithstanding the extensive innovative and business significance of their part in the assembling and safeguarding of numerous matured nourishment items, lactic corrosive microbes (LAB) including Lactobacillus spp likewise assume a vital part in the assurance of the intestinal and urogenital tracts (1,2). The sort Lactobacillus comprises of a hereditarily and physiologically differing gatherings of Gram-positive, bar molded, catalase negative and non-spore shaping microscopic organisms (3). A large number of Lactobacillus spp. are utilized as a part of starter societies for

nourishment and sustain maturations, and a few species are as often as possible experienced in the human gastrointestinal tract [4]. Lactobacilli are throughout the board in nature, severa kinds have discovered packages in the sustenance business. Lactobacilli are located wherein wealthy, carbohydrates containing substrate are available, and along those traces, in an collection of residing spaces, as an instance, mucosal movies of human beings and creatures, (mainly in oral cavity, digestive device, and vagina) and on plant cloth and maturing nourishment, for instance, cheddar (5,6) lactobacilli are entirely fermentative, air tolerant to anaerobic, aciduric or acidophilic and they have complicated nutritional requirements (7) lactobacilli can deliver diverse antimicrobial parts together with natural acids (lactic, acidic, propionic acids), hydrogen peroxide, carbon dioxide, low-atomic weight antimicrobial materials, bacteriocins and grip inhibitors and consequently have picked up important satisfactory as probiotics (eight) and (nine). One approach that brought on the decrease and, in diverse condition, quit of gut pathogenic microorganisms in humans, creatures contains the ingestion of probiotics inside the journal abstain from food (10, eleven). Probiotics are stay microorganisms that, when administered in first-class sums, provide useful influences on the host through modifying indigenous microbiota and stopping illnesses (12). Lactic corrosive microbes (lab) with probiotic homes, for example, bifid bacterium spp. What is greater, lactobacillus spp. Have been utilized to preserve a few intestinal pathogenic contaminations and to invigorate the host's invulnerable framework in the two people and creatures (13, 14, 15). It's miles all round archived that lactobacillus spp. With probiotic properties hold the development and poison generation of microorganisms, for instance, campylobacter jejuni, listeria monocytogenes, helicobacter pylori, salmonella, shigella and escherichia coli (sixteen, 17, 18, 19).

The foe movement of probiotics on pathogenic micro organism might be associated with the opposition for dietary supplements and locales of grip within the mucosa of the small digestive system and the generation of carbon dioxide, hydrogen peroxide and di-acetyl (11). Except, the inhibitory impact at the improvement of a few enter pathogenic microorganisms is possibly connected with the antimicrobial mixes produced with the aid of lactic corrosive microbes, for instance, bacteriocin and lactic, acetic and other short-chain natural acids, which can be chargeable for a decrease within the intestinal ph (18, 20). Lactic corrosive speaks to the essential antimicrobial compound present in cultures of lactic corrosive microscopic organisms (21, 22, 23, 24, 25). Feeble acids have better antimicrobial pastime than solid acids, which ionize definitely in a watery arrangement (26).The nonseparated types of natural acids can work as protonophores, inducing the the cell's inward pH influences the deluge of protons through the cell film, which dissipates the proton-thought process constrain, decreasing cell vitality (ATP) and influencing substrate take-up in the cell (26, 27). A few in vitro and in vivo thinks about exhibited the antagonism of various strains of Lactobacillus, including L. delbrueckiivardelbrueckii, L. plantarum, L. acidophilus, L. reuteri and L. casei, against an assortment of pathogens (16, 17, 18,19). Disregarding numerous point by point examines concerning the hostile impacts of these microscopic organisms on pathogens, there is as yet a requirement for new bacterial strains with antimicrobial properties for clinical and business benefits (28).The main objective of this work was to evaluate theperformance of *Lactobacillus* isolated Milk, yoghurt, cheese, bovine milk and Raw milk with respect to their inhibitory effect on the growth of *Escherichia coli*, *Salmonella sppShigellaspp, Proteus spp, Pseudomonas auerogenosa*, *Enterobacterspp, Staphylococcus aures.*

MATERIALS AND METHODS

Sample Collection

An aggregate of thirty (50) distinctive secluded (Milk, Yogurt, Cheese, Bovine drain and Raw drain) tests were gathered from nearby markets in the AL-Qadisiyah territory, Iraq between October 2017 to January 2018. The examples were put away aseptically at 4°C to anticipate pollution instantly after gathering.

Samples preparation and bacterial cultivation

Ten grams (10 g) of each example were independently weakened in 90 ml of clean ordinary saline. Tests were enhanced in MRS juices (PH6.2) for 24 h at 37°C under anaerobic conditions. Tests were then taken and streaked on to the MRS agar (PH6.2) plates and were hatched in an anaerobic jug at 37°C for 72 h. Suspected states were decontaminated and streaked on MRS agar for encourage distinguishing proof (29).

Preliminary identification of the isolates

Recognizable proof of the *Lactobacillus* spp. was performed by their morphological, social, physiological and biochemical attributes. Perceptible appearance of the considerable number of states was analyzed for social and morphological qualities. Estimate, shape, shading, and surface of the provinces were recorded. Disengages were portrayed in light of Gram's stain response, cell morphology, the nearness of case or endospore, motility, catalase response, oxidase response and by development at 15°Cand 45°C as depicted by Benson (30). Trial of, nitrate diminishment, sulfide, and indole creation, and CO2 from glucose and H2S generation were performed by (31).

Antimicrobial activity assay

Antimicrobial action of the chose probiotic separates was checked by utilizing the agar-spot test. *Escherichia coli*, *Salmonella* spp, *Shigella*spp, *Proteus* spp, *Pseudomonas auerogenosa*, *Enterobacter* spp, *Staphylococcus aureus* (clinical seclude) as the marker microorganisms. Quickly, the segregates were refined in MRS soup overnight at 37 °C and cell free supernatants (CFS) were set up by centrifuging the way of life juices at 8000×g for 15 min. The supernatants were changed in accordance with pH 6.5 and separated through 0.22 μm film filtration, and after that 50 μl of each filtrate was added to 7 mm distance across wells, made in the Mueller-Hinton agar plates (Sigma-Aldrich, USA), which before were brooded overnight by pointer pathogens at 37 °C (14). After overnight brooding, the antimicrobial movement was tested in view of the measurement of the unmistakable zones around of the each well (hindrance zone) (32).

Statistical analysis

The statistical analysis was performed using SAS (Statistical Analysis System - version 9.1) (33).

RESULTS

Identificationof *Lactobacillus* strains

The confines were contemplated for their morphological portrayal. The settlements seemed little and extensive in their shape. The shade of settlements ran from grayish, sparkling white to smooth white. In view of Gram recoloring and different biochemical tests, 30 confines had been chosen (Table-1) and (Figures-1 and 2).

Testing for antibacterial activity

The opposing impact of the secluded LAB species on some normal pathogenic microorganisms was assessed utilizing the agar-spot strategy (34). Results in the table (1) demonstrated that all LAB species display the opposing impact on both Gram-positive and Gram-p) negative microscopic organisms.

DISCUSSION

Probiotic microorganisms are as of late utilized as a part of various wellbeing related regions, for example, the control of aggravation and contaminations, administration of unfavorably susceptible sicknesses, anti-microbial related looseness of the bowels gastroenteritis, clogging, lactose narrow mindedness a preventive part in the beginning of tumors (35). Lactobacilli are broadly spread in nature and the most generally utilized as probiotics in the nourishment business (36, 37). Customary matured dairy nourishments, for example, different cheeses or yogurts are great stores for finding new probiotics specifically the variety of Probiotic microorganisms are as of late utilized as a part of Lactobacilli (36).Lactic corrosive microbes are notable maker of antimicrobial mixes particularly bacteriocins which have high antimicrobial movement (38). *Lactobacillus* spp. is known for their creation of different antimicrobial mixes. The creation of these mixes by intestinal microflora is a standout amongst the most critical systems in charge of the adversarial wonder and in this way it is fundamental to look at this property in confines that possibility for probiotic (39). The great probiotics should introduce their antimicrobial activities especially to the pathogens in the GI framework (40). In this examination, Escherichia coli, Salmonella sppShigellaspp, Proteus spp, Pseudomonas auerogenosa, Enterobacterspp, Staphylococcus aures. were utilized as the test microbes since they are incidentally found as sustenance borne microorganisms that may cause gastroenteritis and another ailment. The possibly probiotic Lactobacillus segregates were subjected to antibacterial action examine. The outcomes are appeared in table that the most antibacterial intensity to *Enterobacter* spp and *E. coli* while, the antibacterial intensity against *Salmonella* spp, *Shigella* spp, *Proteus* spp*, Pseudomonas auerogenosa* were weaker.

The creation of natural corrosive and hydrogen peroxide by Lactobacilli was accounted for to hinder both gram positive and gram negative microscopic organisms, though bacteriocin influences just the development of gram positive microbes (40). Our outcome concurs with (41) which demonstrated the most antibacterial strength to S. aureus and E. coli. The (42) demonstrated that all LAB species display the hostile impact on both Gram-positive and Gram-negative microorganisms which are like our finding. Our examination is likewise concurrence with (21) which found that most Lactobacillus strains create substances that repress pathogenic, non-pathogenic and decay creatures in maturing nourishments and drinks. As a rule, the antimicrobial movement of lactobacilli might be because of lactic corrosive, acidic corrosive, formic corrosive, phenyllactic corrosive, caproic corrosive, natural acids, ethanol natural acids, hydrogen peroxide, bacteriocins or other inhibitory metabolites. Lactic corrosive and acidic corrosive are especially vital mixes, repressing a wide scope of microorganisms (43). Our discoveries are as per (44), who likewise evaluated the inhibitory capability of human drain lactobacilli against various pathogens. Dairy items alongside meat and eggs are the most well-known reasons for Salmonella spp. intervened nourishment borne diseases (45). Curiously, CFSs of a large portion of our detaches showed opposing action against Salmonella Typhi (46) and (47) additionally announced solid to-frail inhibitory action of Lactobacillus supernatants against Salmonella spp. Furthermore, we have additionally watched the adversarial action of Lactobacillus CFSs against P. aeruginosa, one of the disturbing crafty pathogen in hospitalized, immuno-bargained, and cystic fibrosis patients. P. aeruginosa-intervened contaminations are regularly hazardous and muddled to treat, because of constrained powerlessness to usually honed antimicrobial medications (48). Our outcomes are as per the discoveries of (49), who additionally showed opposing action of lactobacilli CFS against P. aeruginosa.

CONCLUSION

Our discoveries bolster the theory that these LAB disconnects may have application as common antimicrobial operators' sustenance framework, and the metabolites delivered by these strains could be investigated as elective pharmaceutical mixes with promising restorative records, after recognizable proof of their dynamic part, testing their cytotoxic impacts and approving security under in vitro and in vivo models.

ACKNOWLEDGEMENTS

Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, University of AL-Qadisiyahfor supporting.

REFERENCES

1. Wood BJB. The lactic acid bacteria in health and disease. The lactic acid bacteria. Appl Sci. 1992; 1.
2. Gharaei-Fathabad E, Eslamifar M. Isolation, and applications of one strain of lactobacillus paraplantarum from tea leave (*Camellia sinensis*). Am J Food Technol. 2011; 6: 429-434.
3. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd edn. Lippincott Williams and Wilkins: USA. 2000.
4. Vaughan EE, de Vries MC, Zoetendal EG, Ben-Amor K, Akkermans AD, de Vos WM. The intestinal LABs. Antonie Van Leeuwenhoek. 2002; 82: 341-352.
5. Hammes WP, Weiss N, and Holzapfel WH. The genera Lactobacillus and CarnobacteriumIn the prokaryotes. In: Handbook on the Biology of Bacteria: Ecophysio-logy, Isolation, Identification, Applications. Balows A, Truper HG, Dworkin M, et al, eds. 2nd ed. New York, Springer 1991; 1535-1594.
6. Pot B, Ludwig W, Kersters K, et al. Taxonomy of lactic acid bacteria: Bacteriocins of lactic acid bacteria: Genetics and Applications. De Vuyst L. and Vandamme EJ. Eds. Chapman and Hall, Glassgow, UK: 1994; 13-89.
7. Desai A. Strain identification, viability and probiotics properties of Lactobacillus casei. Thesis, school of Biomedical and health sciences. Victoria University, Werribee campus Victoria, Australia 2008: 48-53.
8. Stiles, M.E. and W.H. Holzapfel. Lactic acid bacteria of foods and their current taxonomy. International Journal of Food Microbiology, 1997. 36: 1-29.
9. Quwehand, A.C. and S. Vesterlund (2004): Antimicrobial components from lactic acid bacteria. Lactic Acid Bacteria Microbiological and Functional Aspects, Marcel Dekker Inc., New York, USA.
10. Gopal P.K., Prasad J., Smart J. & Gill H.S. 2001. In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacteriumlactis* DR10 strains and their antagonistic activity against enterotoxigenic*Escherichia coli*. Int. J. Food Microbiol. 67:207-216.
11. Guarner F. & Schaafsma G.J. Probiotics. Int. J. Food Microbiol. 1998. 39: 237-238.
12. FAO/WHO. 2001. Joint FAO/WHO Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.
13. Perdigón G., Vintiñi E., Alvarez S., Medina M. & Medici M.Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. J. Dairy Sci. 1999. 82:1108-1114.
14. Fang H., Elina T., Heikki A. & Seppo S. Modulation of humoral immune response through probiotic intake. FEMS Immunol. Med. Microbiol.2000. 29:47-52.
15. NakazatoG., Paganelli F.L., Lago J.C., Aoki F.H., Mobilon C., Brocchi M., Stehling E.G. &Silveira W.D. *Lactobacillus acidophilus* decreases *Salmonella* Typhimurium invasion *in* vivo. J. Food Saf. 2011. 31:284-289.
16. Jin L.Z., Ho Y.W., Ali M.A., Abdullah N. &Jalaludin S. Effect of adherent Lactobacillus spp. on in vitro adherence of salmonellae to the intestinal epithelial cells of chicken. J. Appl. Bacteriol. 1996. 81:201-206.
17. Kalantzopoulos G. Fermented products with probiotic qualities. Anaerobe. 1997. 3:15-19.
18. Servin A.L. &Coconier M.H. Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Pract. Res. 2003. 17:741-754.
19. Poppi L.B., Mancilha I.M., Ferreira A.J.P. & Leal D.D.M. Evaluation of the antagonistic effect of Lactobacillus on *Listeria monocytogenes* in vitro. Braz. J. Food Technol. 2008. 11:113-119.
20. Cheng H.Y., Yu R.C. & Chou C.C. Increased acid tolerance of *Escherichia coli* O157:H7 as affected by acid adaptation time and conditions of acid challenge. Food Res. Int. 2003. 36:49-56.

21. Earnshaw R.G. 1992. The antimicrobial action of lactic acid bacteria: natural food preservation systems, p.211-232. In: Wood B.J.B. (Ed.), The Lactic Acid Bacteria: the lactic acid bacteria in health and disease. Chapman and Hall, London, UK.
22. Navarro L., Zarazaga M., Sáenz J., Ruiz-Larrea F. & Torres C. Bacteriocin production of by lactic acid bacteria isolated from Rioja red wines. J. Appl. Microbiol. 2000 88:44-51.
23. Todorov S.D. & Dicks L.M.T. 2005. *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme Microb. Technol. 36:318-326.
24. Rossland E., Langsrud T., Granum P.E. &Sorhaug T. Production of antimicrobial metabolites by strains of *Lactobacillus* or *Lactococcus*co-cultured with *Bacillus cereus* in milk. Int. J. Food Microbiol. 2005. 98:193-200.
25. Moraes P.M., Perin L.M., Silva Jr A. & Nero L.A.. Comparison of phenotypic and molecular tests to identify lactic acid bacteria. Braz. J. Microbiol. 2013. 44:109-112.
26. Axe D.D. & Bailey J.E. Transport of lactate and acetate through the cytoplasmic membrane of *Escherichia coli*. Biotechnol. Bioeng. 1995. 47:8-19.
27. Diez-Gonzalez F. & Russell J.B. The ability of *Escherichia coli* O157:H7 to decrease its intracellular pH and resist the toxicity of acetic acid. Microbiol. 1997. 143:1175-1180
28. Chaucheyras-Durand F. & Durand H. Probiotics in animal nutrition and health. Beneficial Microbes.2010. 1:3-9.
29. Hadeel H. Alrubaye*, Saad S. Fakhry, Zahraa A. Jebur, and Farqed Farhan. Biochemical and Molecular Characterization of Lactobacillus spp. Isolated from Dairy Products. JSM Microbiology .2018. 6(1): 1048.
30. Benson HJ. Microbiological Application, Laboratory Manual in General Microbiology. WMC Brown Publishers: Dobuque, USA. 1994.
31. Merck KA. Microbiology Manual Darmstadt. Germany. Microbiology. WMC. Brown Publishers: Dobuque, USA. 1997.
32. Zeinab F, Bahram PG, Amir S, Maryam S and Ahmad Y.The Investigation of the Diversity of *Lactobacilli* Spp. and Assessment Their Some Probiotic Properties in Traditional Dairy Products in East Azerbaijan Province in Iran.Iranian Journal of Pharmaceutical Research 2017, 16 (4): 1538-1545.
33. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010.
34. MarianelliC, Cifani N, Pasquali P. Evaluation of antimicrobial activity of probiotic bacteria against Salmonella enterica subsp. Enteric serovar typhimurium 1344 in a common medium under different environmental conditions. Res Microbiol. 2010; 161: 673-680.
35. Uccello M, Malaguarnera G, Basile F, D□Agata V, Malaguarnera M, Bertino G, Vacante M, Drago F and Biondi A. Potential role of probiotics on colorectal cancer prevention. *BMC Surg*. 2012. 12: S35.
36. Hassanzadazar H, Ehsani A, Mardani K and Hesari J. Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. *Vet. Res. Forum* 2012. 3: 181-5.
37. Coeuret V, Gueguen M and Vernoux JP. *In-vitro* screening of potential probiotic activities of selected lactobacilli isolated from unpasteurized milk products for incorporation into soft cheese. *J. Dairy Res.* 2004. 71: 451-60.
38. Aween MM, Hassan Z, Muhialdin BJ, Noor H. and El-jamel YA. Evaluation on Antibacterial Activity of Lactobacillus acidophilus strains isolated from Honey. American Journal of Applied Sciences .2012. 9(6): 807-817.
39. Bilkova A, Sepova, HK, Bukovsky M and Bezakova L. Antibacterial potential of Lactobacilli isolated from a lamb. VeterinarniMedicina. 2011 7(56): 319–324
40. Klayraung S, Viernstein H, Sirithunyalug, J and Okonogi S. Probiotic properties of Lactobacilli isolated from Thai traditional food. Scientia Pharmaceutica 2008. 76(3): 485-503
41. VasieeA R., Tabatabaei Y F, Mortazavi A and Edalatian MR. Isolation, identification and characterization of probiotic Lactobacilli spp. from Tarkhineh. International Food Research Journal 2016: 2487-2492 .
42. Hadeel H. Alrubaye, Saad S. Fakhry, Zahraa A. Jebur, and Farqed Farhan. Biochemical and Molecular Characterization of Lactobacillus spp. Isolated from Dairy ProductsFood Contamination Research Center, Ministry of Science and Technology, Iraq18 April 2018
43. Helander IM, Von Wright A, Mattila-Sandholm MT. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends in Food Sci. Technol. 1997; 8: 146-150

44. Diba FS, Hossain KM, Azim MA, Hoque M (2013) Isolation characterization and determination of antimicrobial properties oflactic acid bacteria from human milk. Jordan J BiolSci 116-6:111
45. Arques JL, Rodriguez E,Langa S, Landete JM, Medina M. Antimicrobial activity of lactic acid bacteria in dairy productsand gut: effect on pathogens. BioMed Res Int. 2015
46. Abdel-Daim A, Hassouna N, Hafez M, Ashor MS, Aboulwafa MM.Antagonistic activity of *Lactobacillus* isolates against Salmonella typhi in vitro. BioMed Res Int. 2013.
47. Bahri F, Lejeune A, Dubois-Dauphin R, Elmejdoub T, BoulahroufAThonart P (2014) Characterization of Lactobacillus strainsisolated from Algerian children faeces for their probiotic.
48. JamalifarH, Rahimi HR, Samadi N, Shaverdi AR, Sharifian Z. Hosseini F, Eslahi H, Fazeli MR. Antimicrobial activity ofdifferent *Lactobacillus* species against multi-drug resist antclinical isolates of *Pseudomonas aeruginosa*. Iran J Microbiol–properties. Afr J Microbiol Res. 2011. 3:21 8:297–30325.
49. Aminnezhad S, Kermanshahi RK, Ranjbar R. Evaluation ofsynergistic interactions between cell-free supernatant of Lactobacillus strains and amikacin and genetamicin against Pseudomonas aeruginosa. Jundishapur J Microbiol. 20158:e16592.

Table 1: The inhibition effect of the LAB on the pathogenic isolated bacteria

No	Pathogenic Bacteria Isolate	Diameter of Inhibition Zone
1	*Enterobacterspp*	22 mm
2	*E. coli*	20 mm
3	*Shigellaspp*	18 mm
4	*Pseudomonas auerogenosa*	15 mm
5	*Salmonella spp*	15 mm
6	*Staphylococcus aureus*	11 mm
7	*Proteus spp*	10 mm
8	*Control 1	--
9	**Control 2	--

*Control 1 (MRS broth)
**Control 2 (Pathogenic Bacteria in MRS broth without Lactobacillus)

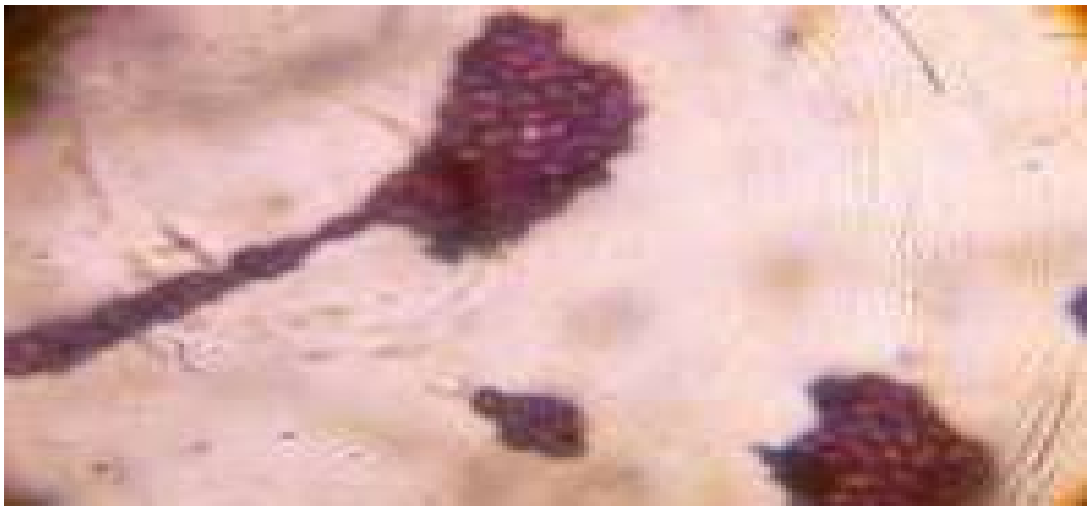


Figure 1: - Gram-positive *Lactobacillus* spp

Figure 2: - Rod shape *Lactobusllius* spp

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Detection of *Toxoplasma gondii* in Goats and Women in Al-Suwirah District, Wasit Province, Iraq.

Huda Atia Ali[1]*, Haider Mohammed Ali Al-Rubaie[2], RebahNajahJabbar[3] and FareedJasim Mohammed[4]

[1]Zoonosis Unite ,College of Veterinary Medicine , University of Baghdad, Iraq. .
[2]Parasitology Department , College of Veterinary Medicine , University of Baghdad, Iraq.
[3]Biotechnology Research Center , AL-Nahrain University , Iraq. .
[4] Gynaecology Department , Collage of Veterinary Medicine , University of Baghdad,Iraq.

Received: 20 Mar 2018 Revised: 19 Apr 2018 Accepted: 25 May 2018

***Address for correspondence**
Huda Atia Ali
Zoonosis Unite,
College of Veterinary Medicine,
University of Baghdad. Iraq.

ABSTRACT

Ninety six goats aged between one and > 4 years and ninety two adult women aged between 15-to 45 years old were used to detect the prevalence of toxoplasmosis by using Latex Agglutination Test-LAT, Enzyme Linked Immunosorbent Assay - ELISA and Polymerase Chain Reaction-PCR analysis (60 goats and 60 women) in Al- Suwirah District, Wasit Province during the period from 1/11/ 2016 – 1/8/2017. The results were showed that the total infection rates of infection in goats by LAT, ELISA and PCR 18.75% , 4.16% , and 00.00% respectively. A high infection rates (38.46%,7.69%) were recorded in the age group >4 years by LAT and ELISA respectively ,followed by 16.98% in the age group between 2-4 years ,and finally the age group between 1-2 years (13.33%) by LAT ,while by ELISA the age group between 1-2 years was a high infection rate (6.66%) than the age group between 2-4 years (1.88%).In women ,the infection rates were recorded 30.43% , 13.04 % , and 10% by using LAT , ELISA , and PCRrespectively .A high infection rate(40.47%) in the age group between 25-35 years old ,followed by the age group 15-25 years old (23.25%) ,and finally the age group >35 years old (14.28%) by LAT , while in ELISA a high same infection rate (14.28%) was found in the age groups 25-35 and >35 years old and the low infection rate (11.62%) in the age group between 15-25 years old. In the aborted women , A high infection rate was recorded in aborted women (50.00%) by using LAT ,followed by ELISA (36.66%) , and finally PCR (13.33%), and a high infection rate was found in women had one abortion (50.00%) ,followed by two (30.00%) ,third (13.33%) and>4(6.66%) abortions.

Keywords: *Toxoplasma*, Toxoplasmosis, Goats, Latex, ELISA, Women, PCR, Abortion.

INTRODUCTION

Felines are the definitive host for *Toxoplasma gondii* (both domestic cats and most other species of felids) and any other warm-blooded animals (other mammals including humans) and birds, get the infection and acts (only) as intermediate hosts (Dubey, 20091).The infection is common in many animals that used for food consumption, such as sheep, pigs and rabbits, but the infection in cattle, water buffaloes and horses is less and the tissue cysts of this parasite may survive in food animals for years and virtually, all edible portions of an animal can harbor the viable parasite(Dubey *et al* ., 19952). Parasite can be transmitted in several ways, it has adapted to be transmitted most efficiently by carnivorism in the cat and by the fecal–oral (oocysts) route in other hosts (Dubey *et al*., 19963).The main way of horizontal infection is either throughingestion of undercooked meat with tissue cysts and via cat oocysts contaminated soil, water or food and vertical infection from the primary infection (tachyzoites) to the fetus, that potentially causing abortion or serious fetal infection (Tenter *et al*., 20004). It has been identified that water was a potential source of the infection in both humans and animals (Bowie *et al*., 19975 and Lin *et al*., 20086).

In dairy goats toxoplasmosis caused abortion and neonatal mortality (Dubey, 19817 and Dubey *et al*., 19818, 19869). In human, the infection is widespread, and the prevalence varies widely from place to place (Dubey and Beattie, 198810). In the adult population 35-40% have been infected by *Toxoplasma* in the world (Smith, 1991) ,andthe infection has a wide variety of manifestations in the fetus and infant such as spontaneous abortion, still-birth, a live infant with a classic signs of congenital toxoplasmosis (hydrocephalus or microcephalus , chorioretinitis , cerebral calcifications) and fails to thrive or has central nerves system involvement or normal infant apparently , but develops chorioretinitis or symptoms of central nerves system later in life (McAuley *et al*., 199411 and Remington *et al*., 200612).The diagnosis of toxoplasmosis in the intermediate hosts can be done by using different common serological tests, such as Sabin Feldman Dye Test, Indirect Fluorescent Antibody Test (IFAT), Complement Fixation Test (CFT), Indirect Heamagglutination Test (IHT), Modified Agglutination Test (MAT), Latex Agglutination Test (LA) and Enzyme linked Immunoabsorbent Assay (ELISA) and other tests such as Intradermal Test (IDT) Histology,andImmunohistochemical staining (Lunde, and Jacobs,196713;Frenkel*et al* ., 197014; Camargo and Leser, 197615 ; Voller*et al.* ,1976 16; Holliman *et al* .,198917; Remington *et al*.,199518and Dubey, 2008 19,20091).Due to the importance of this disease in women and goats ,this study was conducted to detect the prevalence of this parasite in these host .

MATERIALS AND METHODS

Animals and humans

Ninety six goats aged between one year to > 4 years and ninety two adult women aged between 15-to 45 years old were used for detect the prevalence of toxoplasmosis Al-Suwirah District, Wasit Province during the period from 1/11/ 2016 – 1/8/2017.

Blood Samples

About ten ml of blood were collected from the jugular vein from each goats, and from radial vein of each woman, which divided into 8 ml (96 goats and 92 women) for sera collection (Coles ,198620)for Latex Agglutination Test-LAT *and Enzyme Linked Immunosorbent Assay-ELISA **(LAT and ELISA were done according to theinstructions of manufacturer's) and 2 ml (60 goats and 60 women) was added to the anti-coagulant tube-EDTA for molecular analysis by using conventional polymerase chain reaction-PCR ***and the primers were designed and used to detect *Toxoplasma gondii*as *SPINREACT,S.A.U. -ctra .santacoloma ,7 E - 17176 SANT ESTEVE DE BAS - (Girona) SPAIN . **Human Gesellschaft fur Biochemica und Diagnostic ambH .Max- planck-Ring 21.65205 Wiesbaden. Germany.

I D vet, 310, rue Louis Pasteur - Garbels - FRANCE. ***FAVORGEN, BIOTECH CORP. KOREA. The kit was used for DNA extraction from blood samples is FATGK 001-2(300 preps) from Favorgen Biotech corporation Taiwanand DNA was specifically amplified by PCR reaction using ready to use lypholized master mix from Bioneer Corp., Korea. Reactionmixture was added to final volume of 20 µl. **3-8 Statistical Analysis the Statistical Analysis System-SAS (201221) program was used to effect of difference factors of study parameters .Chi-square test was used to significant compare between percentages in this study.

RESULTS

The infection rate of toxoplasmosis in goats

The total infection rates of toxoplasmosis in goats by using LAT, ELISA and PCR were 18.75 %(18/96), 4.16% (4/96) and 0.0%(0/60) respectivelywith a significant (P< 0.01) difference. (Table, 1).

The infection rate of toxoplasmosis by latex agglutination test in goats according to age

The total infection rates in goats sera by LatexAgglutination Test according to the age were 13.33% (4/30),16% (9/53) and 38.48% (5/13) in the age groups 1-2 ,2-4 and >4 years respectively with a significant (P< 0.01) difference .(Table,2)

The infection rate of toxoplasmosis by ELISA in goats according to age

The total infection rates in goats by ELISA were 6.66% (2/30), 1.88% (1/53) and 7.69% (1/13) in the age groups 1-2 ,2-4 and >4 years respectively with a significant (P< 0.05) difference .(Table, 3)

Infection rate of toxoplasmosis in women

The total infection rates of toxoplasmosis in women by LAT, ELISA and PCR were 30.43% (28/92) ,13.04% (12/92) and 10.0%(6/60) respectively with a significant (P< 0.01) difference. (Table, 4, Figure,1, 2).

The infection rate of toxoplasmosis in women according to age by LatexAgglutination Test

The total infection rates of toxoplasmosis in women according to age by Latex Agglutination Test were 23.25% (10/43) , 40.47%(17/42) and 14.28% (1/7) in the age groups 15-25,25-35 and >35 respectively with a significant (P< 0.01) difference. (Table, 5).

The infection rate of toxoplasmosis in women according to age by ELISA

The total infection rates of toxoplasmosis in women according to age by ELISA were 11.62% (5/43) , 14.28 %(6/42) and 14.28% (1/7) in the age groups 15-25,25-35 and >35 respectively without significant (P>0.05) difference. (Table, 6).

The infection rate of toxoplasmosisby PCR in women according to age

The total infection rates of toxoplasmosis in women blood according to age by PCR were 3.84% (1/26) , 17.85 %(5/28) and 00.00% (0/7) in the age groups 15-25, 25-35 and >35 respectively with a significant (P< 0.01) difference. (Table,7)

The relationship between toxoplasmosis and abortion in women

Table (8) was show a high infection rate (50%) in the women that had one abortion previously followed by two (30%) ,three (13.33%) and finally Four <(6.66%)with significant (P<0.01) difference .These cases were distributed according to diagnostic methods in the table (9) that showed a high infection rate (50.00%) was recorded in the latex agglutination test ,while the lower infection rate(13.33%) was recorded in the PCR technique with a significant (P< 0.01) difference.

DISCUSSION

The diagnosis of toxoplasmosis is made by biological, serological, histological, or molecular methods, or by some combination of them, because the clinical signs of disease are non-specific and are not sufficiently characteristic for a definite diagnosis.The evaluation of serological tests becomes important in order to use sensitive and specific tests in serological surveys (Ugglа*et al.,* 198322and Moreno *et al.,* 199123). In goats, a wide variations were recorded in the present study by using different diagnostic methods (LAT, ELISA, and PCR). A high infection rate was recorded by LAT ,followed by ELISA and finally PCR,these results explain that the LAT is less sensitive for detection toxoplasmosis than ELISA and PCR, which are more sensitive and accuracy .Previously reported thatthe direct agglutination test -DAT has development as simple test aided tremendously in the serological diagnosis of disease in humans and other animals, because in this test no special equipment or conjugates are needed that may give a false positive results compare with the recent developed assays (ELISA and PCR),also,the modified agglutination test - MAT has been used extensively for the diagnosis of toxoplasmosis in animals andthe sensitivity and specificity of MAT has been validated by comparing serologic data and isolation of the parasite from naturally and experimentally infected animals (Dubey *et al*., 199524 and Dubey, 199725). LAT is a rapid screening test for determining early antibody response (Khalaf, 2012 26and AbouZeid *et al .,* 2010 27). On one hand, *T. gondii* in the goats induced a mild clinical signs in infected animals and detection of anti- *Toxoplasma* antibodies play an important role in the diagnosis of this disease (Malik *et al*., 199028).

The results was showed a high infection rate by using ELISA than PCR that may be due to the circulating antibodies specially the IgG , that represent chronic or dormant infections by tissue cysts (bradyzoites) rather than tachyzoites , also, there is no non-pathogenic strain of parasite and mayhave no clinical relevance of disease in humans and livestock , due to the different behavior of wild felids compared to that of domestic cats and the number of possible intermediate hosts, that may be suggest a complex ecology of this parasite in environment and leading to a high genetic diversity (Boothroyd,200929).The different distribution of antibody titer may be ascribed to variance in periodic exposure of different animals to the source of infection (Plant *et al* ., 1982 30and Abd-Al- Hameed , 200731). There is likely to be a wide variation in *T. gondii* prevalence within regions .The region is generally drier and oocysts may not survive as well in the soil of this climate. However, due to variations in weather, cat populations and human behavior. A high prevalence of toxoplasmosis was recorded in the south provinces (Basrah 51.11% , Mesan 25.00% and Al-Muthanna 16.06%) of Iraq that might be attributed to the high temperature and elevation of relative humidity (Khalaf , 201226), andthe infection rate of sheep infection by LAT with 2-mercaptoethanol amounted to 33.33%, 31.33% with chronic infection and 2% with acute infection (AL-Sray , 201432). Khalaf (201226) who found in experimentally infected goats developing an antibody titer of 1/32 or more after 14 days of infection, and Innes *et al*. (200933)was referred that develop an effective immune response in infected animals, which will protect against disease in subsequent pregnancies.

Also, higher percentages were recorded in small ruminants (sheep) in different regions of the world by ELISA , it is recorded in Thyqar 12.71% (Khadi *et al* .,200934) ; In Canada 57.64% in Ontario (Waltner-Toews *et al*.,199135); 27.6 % in Morocco (Sawadogo*et al* ., 200536); 29.4% in Brazil (Clementino *et al* ., 200737) ; 72.6% in Iran (Hamidinejat*et al* .,200838). The variation in the infection rates of the previous reports compared with our results that may be attributed

to host parasite relationship, which depends on the virulence of parasite in the area and the presence of cats, which is considered as the main source of infection for ruminants and human (Gamarra *et al* ., 200839).On the same hands, Abouzeid *et al* . (201027) noticed that the prevalence of infection varied from region to region. This variation may be attributed to the difference of the environmental and ecological conditions, which affect the biology of the parasite or the system of breeding and hygienic measures inside farms, also prevalence of toxoplasmosis varies among countries, depending on traditions customs and life styles of the inhabitants (Smith ,199140).Moreover, stray cats may easily enter to the environment of goats (Malik *et al* ., 1990 28and Lunden *et al* ., 199341). Most *T.gondii* infections occur through the ingestion of sporulated oocysts, a stage of the parasite, which is very stable and can survive in favorable conditions in the environment for over 12 months, which contaminating pasturefoods and drinking water (Innes *et al*., 200933). The infection rate of *T. gondii* increased with the age of the animal ,that was agreement with Khalaf (201226) who found a higher infection rate among adult goats compared with the young one . Also AL-Sray (201432)found in sheep most positive cases were among the ages equal to or more than 3 years (≥3 years) with significant (P<0.05) difference ; that infection may be attributed to the feeding habit of goats, which usually graze short grasses and lick soil around them thus are liable to contract the infection with oocysts (Khalaf , 201226) . Many serological and PCR methods are used in an attempt to diagnose the toxoplasmosis in pregnant women (Remington *et al.,* 200612).It was found a high percentage of human and goats infection by *T. gondii*, that may be indicated the goat were considered an important source of infection to humans (Ragozo *et al* ., 2010 42and Khalaf , 201226). Also, AL-Sray (201432) showed that 17.8% of women were positive by ELISA (17 % with chronic infection and 0.8% with acute infection), that results were differ from our results of LAT , ELISA and PCR in women 30.43% , 13.04% ,and 10% respectively, that may be commonly acquired the infection in human by the oral ingestion of tissue cysts containing bradyzoites or ingestion of sporulated oocysts containing sporozoites . Classically, consumption of undercooked meat has been ascribed to be the major risk factor for acquisition of toxoplasmosis and improved animal husbandry practices as well as increased awareness of the risks of consuming undercooked meat have resulted in decreased prevalence of toxoplasmosis world-wide (Tenter *et al* ., 20004).

On the same hand ,ingested of sporulated oocysts are thought to become infected for life, with detectable and persistent IgG antibody levels specific for parasiteand this has aided the assessment of infection levels in many host species (Dubey and Jones, 200843), and among pregnant women in Europe identified ingestion of raw or undercooked meat (meat-products), containing tissue cysts, as the major source of infection, while infection through oocysts, from environmental contamination, plays only a minor part (Cook *et al*., 200044), that may the effects in the prevalence of infection in the present study andthe highest infection rate was recorded in women in the age group ranged between 20-29 years old (19.9%)without significant(P>0.05)difference from other age groups(AL-Sray ,201432), was agreed with the results of present study , the infection rate in women was increase in age group between 25-35. First reported detection of *T. gondii* DNA from a single tachyzoite using the B1 gene byPCR(Burg *et al* .,198945). Several subsequent PCR tests have been developed using different gene targets. Overall, this technique has proven very useful in the diagnosis of clinical toxoplasmosis.A highly sensitive method using a real-time PCR and fluorogenic probe was found to detect DNA from as few as four bradyzoites(Jauregue*et al* ., 200146), that disagree with the results of the present study that recorded a low infection rate by conventional PCR 10% compared with other diagnostic methods (LAT and ELISA),also, were disagree with Powell *et al* .(200147), Costa and Langoni(201048)andDudey*et al* .(201449) ,who diagnosed the parasite by using PCR techniques,that may be due to the size of samples that were collected from the dormant parasite, which found in the tissues as tissue cysts (bradyzoite). Also ,the sensitivity of the testis effect by the gestation periods , that an overall sensitivity 64%, a negative predictive value of 87.8%, a specificity of 100%, and a positive predictive value of 100% and the sensitivity of test is significantly higher, when maternal infection occurs between 17 and 22 weeks gestation (Romand *et al* ., 200150).

REFERENCES

1. Dubey, J.P. (2009). Toxoplasmosis in animals and humans. 2nd ed. Florida; CRC Press.

2. Dubey, J.P.; Thulliez, P. and Powell, E.C.(1995). *Toxoplasma gondii* in Iowa sows: comparison of antibody titers to isolation of *T. gondii* by bioassays in mice and cats. J. Parasitol; 81: 48–53.
3. Dubey, J.P.; Mattix, M.E. and Lipscomb, T.P. (1996). Lesions of neonatally induced toxoplasmosis in cat. Vet. Pathol., 33:290-295.
4. Tenter, A.M.; Heckeroth, A.R. and Weiss, L.M. (2000). *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol., 30: 1217-1258
5. Bowie, W.R.; King, A.S.; Werker, D.H.; Isaac-Renton, J.L.; Bell, A.; Eng, S.B. and Marion, S.A. (1997) .Outbreak of toxoplasmosis associated with municipal drinking water. Lancet, 350: 173-177.
6. Lin, Y. L., Liao, Y. S., Liao, L. R., Chen, F. N. and Kuo, H. M. He. S. (2008). Seroprevalence and sources of *Toxoplasma* infection among indigenous and immigrant pregnant women in Taiwan. Parasitol. Res.; published online.
7. Dubey, J.P.(1981). Epizootic toxoplasmosis associated with abortion in dairy goats in Montana. J. Am. Vet. Med. Assoc., 178: 661–670.
8. Dubey, J.P.; Sundberg, J.P.; Matiuck, S.W.(1981). Toxoplasmosis associated with abortion in goats and sheep in Connecticut. Am. J. Vet. Res., 42: 1624–1626.
9. Dubey, J.P.; Miller, S.; Desmonts, G.; Thulliez, P. and Anderson,W.R.. (1986). *Toxoplasma gondii*-induced abortion in dairy goats.J. Am.Vet. Med. Assoc.,188: 159–162.
10. Dubey, J.P. and Beattie, C.P.(1988). Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, FL : 1–220.
11. McAuley, J.; Boyer, K.M.; Patel, D.; Mets, M.; Swisher, C.; Roizen, N.; Wolters, C.; Stein, L.; Stein, M.; Schey, W.; Remington, J.; Meier, P.; Johnson, D.; Heydeman, P.; Holfels, E.;Withers, S.; Mack, D.; Brown, C.; Patton, D. and McLeod, R.(1994). Early and longitudinal evaluations of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago Collaborative Treatment Trial. Clin. Infect. Dis., 18: 38–72.
12. Remington, J.S.; McLeod, R.; Thulliez, P. and Desmonts, G. (2006). Toxoplasmosis. In: Infectious Diseases of the Fetus and Newborn Infant. Remington, J.S., Klein, J.O., Wilson, C.B. and Baker, C.J. Eds. Elsevier Saunders, Philadelphia : 947–1091.
13. Lunde, M.N. and Jacobs, L. (1967). Evaluation of Latex Agglutination Test for Toxoplasmosis. J. Parasitol. 53: 933-936.
14. Frenkel ,J.K.; Dubey, J.P. and Miller, N.L. (1970). *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. Sci., 167: 893–896.
15. Camargo, M.E. and leser, P.G. (1976).Diagnostic information from serological tests in human toxoplasmosis, II: Evaluated study of antibodies and serological pattern on acquired toxoplasmosis as detected by haemagglutination and compliment fixation. Rev. Inst. Med. Trop. Saopaulo, Brazil, 18:277.
16. Voller, A.; Bid well, D.E.; Bartlett, A.; Fleck, D.G.; Perkins, M. and Oladehin, B. (1976): A micro plate enzyme-immunoassay for *Toxoplasma* antibody. J. Clin. Path., 29: 150-153.
17. Holliman, R.E.; Johnson, J.; Duffy, K. and New, L. (1989):Discrepant *Toxoplasma* latex agglutination test results. J. Clin. Pathol., 42: 200-203.
18. Remington, J.S.; McLeod ,R. and Desmonts, G. (1995). Toxoplasmosis. In: Infectious Disease of the Fetus and Newborn Infant .Remington, J.S. and Klein, J.O. Eds.. Philadelphia: W.B. Saunders Company:140–267 Dubey, J. P. (2008).The history of *Toxoplasma gondii*—The first 100years.J. Eukaryote. Microbiol., 55(6): 467–475.
19. Dubey, J. P. (2008).The history of *Toxoplasma gondii*—The first 100years.J. Eukaryote. Microbiol., 55(6): 467–475.
20. Coles ,E.H. (1986).Veterinary clinical pathology.4th ed. W. B. Saunders Company: 46-47.
21. SAS(2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
22. Uggla,A. ;Beskew ,P.; Shwan ,O.; Bergguist, N.R. and Waller ,T. (1983).Ovine toxoplasmosisin Sweeden .Acta Vet. Scand. ,24 (1) :113-119.
23. Moreno, T.; Gomz, F.M.; Rodriguez, S.H.; Cruz, M.D.M. and Moreno, A.M. (1991).The seroprev -alence of ovine toxoplasmosis in Cordoba, Spina. Ann. Trop. Med. Parasitol., 85: 287–288.
24. Dubey, J. P., Thulliez, P., Weigel, R. M., Andrews, C. D., Lind, P. and Powell, E. C. (1995). Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am. J. Vet. Res., 56:1030–1036.

25. Dubey, J. P. (1997). Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Vet. Parasitol., 71:307–310.
26. Khalaf, A.H.(2012). Evaluation of Three Serological Tests for Diagnosis of Toxoplasmosis in Goats in Diyala Province .MScthesisCollege of Veterinary Medicine , University of Baghdad .
27. Abouzeid, N.Z.; Amer,H.A.; Barakat ,T. M.;Selim, A.M. and El-Balkemey, F.A. (2010).Toxoplasmosis in naturally and experimentally infected goats .J. Am. Sci;6(11):122-129.
28. Malik, M. A.; Deesen ,D. W. and Cruz, A. (1990). Toxoplasmosis in sheep in Northeastern United States. J. Am. Vet. Med. Assoc.,196:263-265.
29. Boothroyd, J.C. (2009) .Expansion of host range as a driving force in the evolution of *Toxoplasma*. Mem. Inst. Oswaldo. Cruz., 104: 179-184.
30. Plant, J.W.; Freeman, P. and Sounders, E.(1982). Serological survey of the prevalence of *Toxoplasma gondii* antibodies in rams in sheep flocks in New South Wales. Aust .Vet.J.,59 :87-89.
31. Abd-Al Hameed, A.S.(2007). Seroepidemiological study on ovine toxoplasmosis in Baghdad and Diyala provinces .MSc.thesis ,College of Veterinary Medicine, University of Baghdad ,Iraq.
32. Al-Sary, H. K. (2014). Molecular and Serological detection of *Toxoplasma gondii*in Human and Sheep in Wasit Province. MSc. thesis College of Veterinary Medicine, University of AL-Qadisiyah, Iraq.
33. -Innes, E. A.; Bartley, P. M.; Buxton, D. and Katzer, F. (2009). Ovine Toxoplasmosis. Parasitol; 136: 1887-1894.
34. Khadi,J.A.; Thamer, M. K.and Al-Amin,A.T.(2009).Prevalence of antibodies to *Toxoplasmagondii* in aborted ewes in south of Iraq .Iraqi J.Vet.Sci.,23(Suppl.1P):199-201.
35. Waltner-Toews,D.; Mondesire,R. and Menzies, P.(1991). The seroprevalence of *Toxoplasma gondii* in Ontario sheep flocks .Can. Vet. J., 32:734-737.
36. Sawadogo, P.; Hafid ,J.; Bellete ,B.; Sung , R. T. ; Chakdi , M.; Flori, P.; Raberin , H.; Amouni, I. B.; Chait , A . and Dalal, A .(2005). Seroprevalence of T. *gondii* in sheep from Marrakech, Morocco .Vet. Parasit. 130:89-92.
37. Clemention, M.; Souza, M.F. and Andrade -Neto, V. F. (2007). Seroprevalence and *Toxoplasma gondii* –IgG avidity in sheep from Lajes, Brazil.Vet. Parasitol; 146: 199-203.
38. Hamidinejat, H.; Goraninejad, S.; Ghorbanpoor, M.; Nabavi, L. and Akbarnejad, F. (2008) .Role of *Toxoplasma gondii* in abortion of ewes in Ahvaz, Southern-West Iran. Bull.Vet. Instit. Pulawy, 52:369-371.
39. Gamarra, J.A.; Cabezon, O.; Pabon, M.; Arnal, M. C.; *et al .* (2008). Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet. Parasitol, 153:152-156.
40. Smith, J.L. (1991). Food borne toxoplasmosis. J .Food. Safety; 12 :17-57.
41. Lunden, A.; Nasholm, A. and Uggla, A. (1993).Long-term study of *Toxoplasma gondii* infection in a Swedish sheep flocks . Acta Vet. Scandinavica, 35:273-281.
42. Ragozo, A. M. A.;Pena, H.F.J.; Yai ,L.E.O.; Su, C. andGennar ,S.M. (2010). Genetic diversity among *Toxoplasma gondii* isolates of small ruminants from Brazil: novel genotypes revealed .Vet. Parasitol.,170 : 307-312
43. Dubey, J. P. and Jones, J. L. (2008). *Toxoplasma gondii* infection in humans and animals in the United States. Int. J. Parasitol., 38 : 38: 1257–1278.
44. Cook, A.J.C.; Gilbert, R.E.; Buffolano, W.; Zufferey, J.; Petersen, E.; Jenum, P. A.; Foulon, W.; Semprini, A. E. and Dunn, D. T. (2000). Sources of *Toxoplasma* infection in pregnant women: European multicentre case control study. Br. Med. J ; 321: 142–147.
45. Burg, J. L., Grover, C. M., Pouletty, P. and Boothroyd, J. C. (1989). Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain-reaction. J. Clin. Microbiol. 27:1787–1792.
46. Jauregue, L.H.; Higgins, J.A.; Zarlenga, D.S. *et al.* (2001).Development of a real-time PCR assay for the detection of *Toxoplasma gondii* in pig and mouse tissues. J. Clini. Microbiol; 39: 2065–2071.
47. Powell, C.C., Brewer, M. and Lappin, M.R. (2001). Detection of *Toxoplasma gondii* in the milk of experimentally infected lactating cats. Vet. Parasitol. 102:29-33.
48. Costa, V.M. and Langoni,H. (2010). Detection of *Toxoplasma gondii* in the milk of experimentally infected Wistar female rats. J.Venom Anim. Toxins Incl.Trop. Dis;16 (2):1678.

49. Dubey,J.P.; Verma,S.K.; Ferreira,L.R.; Oliveira,S.; Cassinelli,A.B.;Ying,Y.;Kwok,O.C.; Tuo,W.;Chiesa,O.A.andJones,J.L. (2014). Detection and survival of *Toxoplasma gondii* in milk and cheese from experimentally infected goats . J. Food Prot;77(10):1747-1753.
50. Romand, S.; Wallon ,M.; Franck, J.; Thulliez, P.; Peyron, F. and Dumon, H. (2001). Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. Obstet. Gynecol; 97 (2):296-300.

***Toxoplasma gondii*as follows**

No.	Primer name	Primer sequence 5' to 3'		Amplicon size
1	T1 – F	Forward primer	TGTTGGATATCCTGCGCTGC	250
	T1 - R	Reverse primer	AGTTTAGGAAGCAATCTGAAAGCAC	250
2	T2 - F	Forward primer	ACATGAGTTTGCATCTCTCTCCA	323
	T2 - R	Reverse primer	AGCAATCTGAAAGCACATCGAG	323
3	T3 – F	Forward primer	AGAGATTTGCATTCAAGAAGCGT	286
	T3 – R	Reverse primer	AAGCACATCGAGAGAGATGC	286
4	T4 – F	Forward primer	TGGATATCCTGCGCTGCTTC	329
	T4 – R	Reverse primer	ACATTGCGTTTCGCAGTTCG	329
5	T5 – F	Forward primer	GAAGCCAGTGCAGGTATCCG	262
	T5 - R	Reverse primer	GCGAGCCAAGACATCCATTG	262
6	T6 – F	Forward primer	CAGCGAAGGGGCTCAATTTC	274
	T6 - R	Reverse primer	CACATTGCGTTTCGCAGTTC	274

The following program was used to amplify 18sRNA of *T. gondii* in DNA samples extracted

Step	Temperature ^{0}C	Time	No. of cycles
Initial denaturation	94	5 min	1
Denaturation	95	45 sec	35
Annealing			
Extension	72	45 sec	
Final extension	72	10 min	1
Hold	4		

Table 1.The total infection rate of *Toxoplasma gondii* in goats according to different diagnostic methods .

Tests	No. of samples examined	Positive	Percentage(%)
Latex Agglutination Test	96	18	18.75
ELISA	96	4	4.16
PCR	60	0	00.00
P-value	---	---	0.0037 **

**** (P< 0.01)**

Table2.The total infection rate of Toxoplasmosisin goats by LatexAgglutination Test according to age of the animal

Years	No. of samples examined	Positive	Percentage (%)
1-2	30	4	13.33
2-4	53	9	16.98
> 4	13	5	38.46
Total	96	18	18.75
P-value	---	---	0.0002 **

** (P < 0.01)

Table 3. The total infection rate of *Toxoplasma gondii* in goats seraby ELISA according tothe age

Years	No. of samples examined	Positive	Percentage (%)
1-2	30	2	6.66
2-4	53	1	1.88
> 4	13	1	7.69
Total	96	4	4.16
P-value	---	---	0.0493 *

* (P< 0. 05)

Table 4.The total infection rate of Toxoplasmosisin women according to different diagnostic tests.

Tests	No. of samples examined	Positive	Percentage (%)
Latex Agglutination test	92	28	30.43
ELISA	92	12	13.04
PCR	60	6	10
P-value	---	---	0.0094 **

**** (P < 0.01)**

Table 5.The infection rate of Toxoplasmosisin women sera by LatexAgglutination Test according to age.

Years	No. of samples examined	Positive	Percentage (%)
15-25	43	10	23.25
25-35	42	17	40.47
> 35	7	1	14.28
Total	92	28	30.43
P-value	---	---	0.013 **

** (P< 0.01)

Table6.The total infection rate of *Toxoplasma gondii* in women sera byELISA accordingto age.

Years	No. of samples examined	Positive	Percentage (%)
15-25	43	5	11.62
25-35	42	6	14.28
> 35	7	1	14.28
Total	92	12	13.04
P-value	---	---	0.483 NS

NS: Non-Significant

Table 7.The infection rate of Toxoplasmosisin women blood by PCRaccording to age

Years	No. of samples examined	Positive	Percentage (%)
15-25	26	1	3.84
25-35	28	5	17.85
> 35	6	0	00.00
Total	60	6	10
P-value	---	---	0.0087 **

** (P < 0.01)

Table 8 .The relationship between toxoplasmosisand the number ofwomen abortions

No. of aborted samples	Positive	Abortions			
		One	Two	Three	>Four
43	30	15 (50%)	9 (30%)	4 (13.33%)	2 (6.66%)
P-value	---	0.0001 **			

**** (P <0 .01)**

Table 9.The infection rate of toxoplasmosis in aborted women according to different diagnostic methods.

Diagnostic methods	Number of aborted women	Positive (%)
Latex Agglutination Test	30	15 (50.00)
ELISA		11 (36.66)
PCR		4 (13.33)
P-value	---	0.0001 **

** (P <0 .01)

Figure 1.The positive result of women Toxoplasmosis in sera by ELISA.

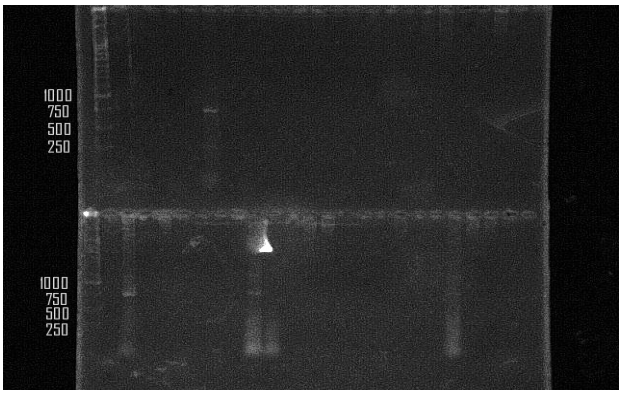


Figure 2.The positive result of human Toxoplasmosis in blood by PCR.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Therapeutic Effects of Silver Nanoparticles on Infected Fractured Bone in Rabbits Comparing to Cefotaxime

Ali H. Saliem[1]*, Orooba M.S. Ibrahim[1] and Sarwa Ibrahim Salih[2]

[1]Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Baghdad, Iraq.

[1]Departement of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad,, Iraq

Received: 20 Mar 2018 Revised: 19 Apr 2018 Accepted: 28 May 2018

***Address for correspondence**
Ali H. Saliem,
Department of Physiology,
Pharmacology and Biochemistry,
College of Veterinary Medicine,
University of Baghdad,, Iraq.
E-mail ; alisaliem977@gmail.com

ABSTRACT

The present study was carried out to evaluatethe healing activity of silver nanoparticles and cefotaximeon induced infected fracture by *staphylococcus aureus*in rabbit model after inducing infected transvers diaphyseal ulnar fracture using pathogenic *Staphylococcus aureus*(0.025ml from 2.7 $\times 10^6$ cfu/ml) directly and healing activity after inducing non infected fracture of silver nanoparticles and cefotaxime in the rabbit ulnadirectly.This experiment was carried out on 40 rabbits and included four groups (ten rabbits for each) as following: - Group (A) was the positive control group (infected untreated), group (B) was the negative control group (not infected untreated), group (C) infected group was treated with cefotaxime (0.5gm locally) and group (D) infected group was treated with silver nanoparticles (0.5gm locally). The results showed that the administration of 0.5gm silver nanoparticles locally at the site of fracture was succeeded in prevent severity of the symptoms of infection within the first day. The same dose and rout of cefotaxime showed reducing the symptoms of infection. While treatment with 0.5gm of silver nanoparticles and 0.5gm of cefotaxime succeeded in treating osteomyelitis or reducing *Staphylococcus aureus* in the infected rabbits, preventedinfection and promote fracture healing. Radiographic results reflected both of rate and extent of bone reconstruction and remodeling, after radiographic examination of ulna. In (-ve) control (group B) the results showed normal bone anatomy in all period of experiment, while in (+ve) control (group A) showed radiographic changes after 3 weeks post infection. Furthermore after 6 weeks post infection there was clear signs of osteomyelitis in all infected animals. After 6 weeks of treatment using silver nanoparticles (group D) and cefotaxime (group C) most signs of osteomyelitis disappeared, whereas the radiographic pictures showed normal bone

appearance and all signs of osteomyelitis disappeared.The powerful bioactivity demonstrated by the silver nanoparticles leads towards the clinical use as antimicrobial and acceleration of bone healing.

Keywords: Silver nanoparticles, infected, bone.

INTRODUCTION

Nanoparticles define as a group of molecules extending between 1 and 100 nm in size which shows the best properties such size, distribution and morphology than the bigger particles of the mass materials from which the nanoparticles are made[1]. SNPsare used in wide applications like catalysis, optoelectronics, recognition, analytic, and therapeutics[2]. Silver is perceived as a compelling antimicrobial agent that displays less dangerous effect on people and has variable applications in both *in vivo* and *in vitro[*3]. Silver-based topical dressings are broadly used for treating contaminated open injuries and chronic ulcers [4]. One of the most importantapplication of silver and SNPs is in medicinal industry, as in topical ointment to prevent infection[5]. Biomaterials are usually applied in regenerative treatment and tissue building of bone. Local application of nanoparticles in bone injury may be suitable for various potential uses as for the change of tissue recovery, the upgraded osseous integration of implants and the prevention of infections [6]. This present study aimed to studying the SNPs activity in bone healing processof infectedfracture bone thatis caused by *staphylococcus aureus* in rabbits comparing to cefotaxime.

MATERIALS AND METHODS

Activation and Maintenance of Bacterial Isolates

Bacterial cultures were activated in screw capped tubes containing 10 ml of brain heart infusion agar slants and incubated for 24 hours at 37oC. For maintenance of isolates, nutrient and brain heart infusion agar were stored at 4oC, and were sub -cultured once every two-weeks [7].

Experimental Animals

Forty healthy male local rabbits aged between (8-12) weeks, their weights between (1.5-2) Kg were used in the present study. Rabbits were housed in metallic cages 90**×**50 **×**70 cm dimensions (2 rabbits /cage) , placed in a special housing room belongs toCollege of Veterinary Medicine/University of Baghdad for acclimatization before experiment. Standard rodent diet (Commercial feed pellets) and water from the tap were freely accessible. The conditions of housing were (20-25 ^{o}C)in conditioned room, and the room quality was changed constantly by ventilation vacuum utilizing, while the cycle of light and dark was (14**/**10) in the housing place.

Inducing Ulnar Fracture

After adaptation period (2 weeks), the anesthetization of rabbits was by injection a mixture of Ketamine hydrochloride (35 mg/kg) intramuscularly, 5 mg/kg of Xylazine and 0.75 mg/kg of Acepromazine[8].The right ulna was prepared for aseptic surgery. Two centimeter of was made inthe lateral aspect of the right ulnadiaphysis was made, then subcutaneous tissues was reflected bluntly to expose ulnar bone. A transverse diaphyseal ulnar fracture was made by using surgical saw.

Inducing Bone Infectionand Application of SNPs andCefotaxime

He challenge dose which induced infection was 2.7 x 10^6cfu/ml of *Staphylococcus aureus* suspension[7]. The inoculum preparation standardized according to the viable counting method-pour plate technique by using serial ten-fold dilutions. 0.025 ml of bacterial suspension was injected locally at the fracture site by micropipette, simultaneously addition of the respective silver nanoparticles, and cefotaxime powder locally in the bone fracture of rabbits according to the each group. After that thesubcutaneous tissue closed by simple interrupted suturesusing 3/0catgut, the skin was closed by simple interrupted sutures using3/0 silk.

Experimental Design

Forty rabbits were divided equally into four groups (ten rabbits in each group), treatment begin directly post inducing of fractures and infection, as following tables

Group (A) Positive control

Induced fracture of right ulna and infected locally with *Staphylococcus aureus* of dosing 2.7 x 10^6cfu/ml(0.025ml) and not treated group.

Group (B) Negative control

Induced fracture of right ulna not infected and not treated.

Group (C)

Induced fracture of right ulna, infected locally with *Staphylococcus aureus* of dosing 2.7 x 10^6cfu/ml (0.025ml) and treated with (0.5gmcefotaxime) locally at the site of fracture.

Group (D)

Induced fracture of right ulna infected locally with *Staphylococcus aureus* of dosing 2.7 x 10^6cfu/ml (0.025ml) and treated with SNPs (0.5gm) locally at the site of fracture.

Assessment of bone Fracture

Animals were continuously observed for clinical signs including, fever, bone pain, swelling, and redness over the fracture site, unusual movement, pus formation and mortality rate, radiographic examination was done for all animals of study (3weeks and 6 weeks) post infection and treatment.

RESULTS

A-Clinical Signs

All animals were healthy before induction of fracture and infection, presented with normal activity, normal food and water intake.Two days after induce of fractures and infection, the animals of group (A) were suffering from fever (40 ^oC), swelling at the site of fracture. All animals in group (A) exhibited clinical signs of illness characterized byredness over the site of infection and recurring infections in the soft tissue above the bone, and an open, draining wound with pus and laceration of tissue and underlying tissues 10 days post infection (Figure: 1). All animals in this group A

presented with low activity, crowding and limited movement in the fractured bone. The animals of group (C) which received cefotaxime showed some clinical signs that mentioned previously, while animals of group (D) which treated with SNPs exhibited complete recovery without signs of illness, inflammation and abscess formation post treatment (Figure: 2), while the animals of the group B characterized by normal signs.The clinical signs that appeared in animals of groups are abbreviated in table (1).

B-Bone Radiography

Theradiographic image of fractured ulna of group (A) at 3 weeks post fracture shows severe bone destruction. The infection had even extended to the cortex (signs of osteomyelitis). While 6 weeks post fracture shows the extension of bone destruction with presence of caseated pus that referred to osteomyelitis. (Figure: 3) and (Figure: 4). the radiographic examination of ulna in group (B) showed normal bone anatomy in all periods of experiment. Radiographic image of fractured ulna of group (B) at 3 weeks post fracture shows complete disappearance of fracture line (boney union) ,but the periosteal reaction still exist. While at 6 weeks post fracture shows complete union of the fractured bone and the ulna returned to its normal anatomical shape (Figures:5 and 6). The radiographic image of fractured ulna of group (C) at 3 weeks post fracture showedthe existence of fracture line and the boney callus is filling the site of fracture but not completely mineralized. While at 6 weeks post fracture showed complete disappearance of the fracture line and the boney union is clear (Figures: 7 and 8) and in the treatment with cefotaxime most features of osteomyelitis disappeared. In group (D), results with SNPs were better than cefotaxime. There were no changes in ulna in the 3 weeks and 6 weeks post infection with*staphylococcus aureus*, radiographic pictures showed normal bone anatomy and all features of osteomyelitis disappeared.Radiographic image of fractured ulna in group (D) at 3 weeks post fracture showed line of fracture is crossed by incomplete boney bridge connecting the fractured segments.While at 6 weeks post fracture there is complete boney union, medullary canal is almost clear as signs of early remodeling (Figures:9 and 10).

DISCUSSION

The clinical signs that appeared in group (A) are generally in line with the findings observed by Baltenspereg*et al*.,[8,9] which he referred to the infection of acute osteomyelitis may develop within two weeks of an injury, initial infection, or the start of an underlying disease. These finding evidences of the virulent *Staphylococcus aureus*are of the most significant bacterial pathogens and can cause diseases ranging from minor and surgical site infections[10].*Staphylococcus aureus* carries a formidable array of virulence factors allowing it to cause a wide variety of infectious syndromes and intoxications [11]. Panton Valaentine Leukocidin (PVL) toxin usually targets human and rabbit mononuclear and polymorphonuclear cells (PMNs). The toxic effect results from synergistic action of two separate exoproteins, namely, LukS-PV and LukF-PV, which are encoded by two genes (lukS-PV and lukF-PV[12].They are separated by a single thymine nucleotide and transcribed as a single mRNA molecules and they are carried on temperate bacteriophages.The two components of PVL, LukS-PV and LukF-PV are secreted from *S. aureus* before they assemble into a pore-forming heptamer on PMN membranes. High PVL concentrations cause PMN lysis and tissue necrosis which could result from release of reactive oxygen species (ROS) from lysed PMNs. Alternately, release of granule contents from lysed PMNs could eventually result in tissue necrosis[13].

The animals of group D which received single dose of SNPs showed complete recovery of clinical sign. These results confirm the effectiveness of SNPs on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, SNPs can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag+) released from SNPs can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur-containing proteins, leading to the inhibition of enzyme functions as confirmed by Matsumura *et al*., [14].The

treatment with the silver ions results in similar morphological changes in the Gram positive bacteria. The cytoplasmic membrane detaches from cell walls and an electron-light region containing condensed deoxyribonucleic acid molecules appears in the center of the cell. The inhibitory activity of silver ions is higher in case of Gram negative bacteria.

This might be due to the thickness of the peptidoglycan layer which may prevent the action of the silver ions[15]. While animals of group C which treated with Cefotaxime exhibited faster recovery (most signs of inflammation, abscess and radiologic signs disappeared post treatment, due to Staphylococci (*S. aureus* strain) reduced susceptibility to glycopeptide antibiotics and *in vitro* potency, pharmacologic data are important in assessments of the clinical usefulness of Cefotaxime. The results are in agreement with Hecker*et al*.,[16] who referred that the spectrum of activity includes Methicillin-resistant Staphylococcusaureus(MRSA) and staphylococci with reduced susceptibility to glycopeptide antibiotics; penicillin- resistant. Bone radiography showed that the bone returned to the normal state after treatment with SNPs. Andrea *et al*., [6]reported that biomaterials are commonly applied in regenerative therapy and tissue engineering in bone, Thereby, research approaches focus more and more on nanoparticles, which have great potential for a variety of applications in bone, locally applied nanoparticles may be suitable for numerous potential uses with respect to the improvement of tissue regeneration, the enhanced osseointegration of implants, and the prevention of infections. In osteomyelitis, the inflammatory changes may involve soft tissues and periosteum. Some or all of these tissues, as well as marrow, are affected in osteomyelitis, and their involvement (marrow) may cause bony changes that are more obvious than the initial soft-tissue inflammation [17].The radiologic signs in the group that infected and not treated was in agreement withkealy*et al.,[*18] who mentioned that osteomyelitis are seen by loss of trabecular pattern, which may be the first sign , lysis or destruction of bone appeared as an area of radiolucency within the bone ,there was periosteal reaction , the periosteum often becomes elevated and there was subperiosteal new bone formation and a sequestrum may be seen as fragment of cortical bone of normal opacity, the signs also appeared as more opaqueappearance than normal because it contrasted with the surrounding lucent zone; there was cortical destruction may be seen too.

Zhang *et al.,[*19] exhibited that the SNPs improve bone fracture healing and induced early closure of the fracture gap by promoting the formation of fracture callus. SNPs may promote the formation of the callus and the subsequent end joining of the fracture bone via either chemo-attraction of mesenchymal stem cells. Fibroblasts are migrate to the fracture site; induction of the proliferation and osteogenesis of mesenchymal stem cells or induction of osteogenic differentiation of mesenchymal stem cells via induction/activation of transforming growth factor-beta (TGF-β)/bone morphogenetic protein (BMP) signaling in mesenchymal stem cells. In addition, Shin-Woo *et al.,[*20]reported that the nanoparticles stimulate and promote the differentiation, mineralization of osteoblast and increase bone mineral density by stimulation of the autophagy. On other hand, Andreaet *al*.,6 showed that the nanoparticles support the osteo regeneration by direct influence of osteoblast differentiation and osteoclast behavior.Stylios*et al*., [21]and Nair and Laurencin,[22] reported that the SNPs induce bone ingrowth, cell migration, tissue ingrowth, and vascularization.

Mammalian macrophages are able to sense peptidoglycan [23]. Fracture healing requires the induction of mesenchymal stromal cells to differentiate along the osteoblastic lineage for new bone formation. Mesenchymal stem cell proliferation and differentiation are regulated by growth factors. The macrophages have a significant role in expression of inflammatory cytokines and growth factors [24]. Therefore, it has been hypothesized that the macrophage cell has a central role in fracture healing [25]. However, the induction of inflammatory cytokines and fibrosis promoting growth factors by stimulated macrophages suggests a prominent osteolytic effect as the macrophages loses its ability to synthesize BMP-2 under proinflammatory conditions[26]. On this basis, it has been suggested that osseous healing is inhibited by conditions that promote proinflammatory activity of the macrophage[25]. *Staphylococcus aureus* peptidoglycan has been shown to be a major stimulus to the inflammatory processes in tissue repair, and *staphylococcusaureus* peptidoglycans induced a striking increase in the local number

ofactivated macrophages in the tissue [27]. The number of neutrophils, mesenchymal cells, and new blood vessels also increase by local application of *Staphylococcus aureus* peptidoglycans.

The net effect was an increased accumulation of reparative collagen. These effects have been attributed to the fact that macrophages, when activated, release a number of growth factors, including platelet derived growth factor(s), basic fibroblast growth factor, transforming growth factor beta, and angiogeneisis factor released from activated macrophages [28]. On the other hand, healing is delayed when wound macrophages are depleted. Peptidoglycans are chemotactic for neutrophils and macrophages [29]. It has been shown that reparative tissue collagens were significantly higher with *Staphylococcus aureus* peptidoglycans, and that *Staphylococcus aureus* peptidoglycans induce greater inflammatory exudates [30]. In fracture, the initial inflammation is equivalent to other tissue responses to injury. However, during the first days there develops what has been termed the primary callus response, which appears to be a fundamental reaction to bone injury. This first callus response is poorly organized and composed of calcified cartilage and woven bone, ultimately remodeled into a mechanically competent bone structure. The biological events in fracture healing are finite, and an overshoot in fibroblast tissue regeneration may induce a shift from competent calcified tissue to incompetent fibrous tissue. *Staphylococcus aureus* peptidoglycans induce an alteration in the normal bone healing response towards a less calcified callus production. Mineral density and mineral content were significantly reduced in the bone fractures that infected with *Staphylococcus aureus* peptidoglycans locally[31].

CONCLUSION

Silver nanoparticles are highly effected for treating infected bone fractures with *Staphylococcus aureus* with good properties of bone fractures healing when compared with cefotaxime.

REFERENCES

1. Hassan K. and Siavash I. (2012). Silver Nanoparticles. The Delivery of Nanoparticles. Dr. Abbass A. Hashim (1st Ed.), Ch. One. Pp.1-35.
2. Sivakumar, P.; NethraDevi, C. and Renganathan, S. (2012). Synthesis of silver nanoparticles using *Lantana camara*fruit extract and its effect on pathogens. *Asian J. Pharm. Clin. Res*., 5 (3): 97-101.
3. Farooqui, M.D.A.; Chauhan, P.S.; Krishnamoorthy, P. and Shaik, J. (2010). Extraction of silver Nanoparticle from the leaf extracts of *Clerodendruminerme*. *Dig. J. Nanomat. Biostruct*., 5(1): 43-49.
4. Dipankar, C. and Murugan, S. (2012).The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresineherbstii*leaf aqueous extracts. *Colloid. Surfac. B*: *Biointerfaces*, 2 (98): 112- 119.
5. Ravikumar, S.; Gokulakrishnan, R.; Selvanathan, K. and Selvam, S. (2011). Antibacterial activity of metal oxide nanoparticles against ophthalmic pathogens. *Int. J. Phar. Res. Dev*., 3 (5): 122-127.
6. Andrea, T.; Anna, K. and Anita, I. (2012). Nanoparticles and their potential for application in bone. *Int. J.ofNanomed.*, 17(7):4545–4557.
7. Quinn, P.J.; Carter, M.E.; Markey, B. and.Carter, G.R. (2004). Clinical Veterinary Microbiology. Mosby. Edinburgh, London, New York, Oxford and Philadelphia (2nd Ed.),USA.Pp. 21-63.
8. Lipman, N.S.; Marini, R.P. and Erdman, S.E. (1990). A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. *Lab. Anim. Sci*., 40 (4):395-8.
9. Baltensperger, M.; Gratz, K.; Bruder, E.; Lebeda, R.; Makek, M. and Eyrich, G. (2004). Is primary chronic osteomyelitis a uniform disease? Proposal of a classification based on a retrospective analysis of patients treated in the past 30 years. *J. Cranio-Maxillofac. Surg.,* 32(1): 43-50.
10. Rennie, R. P.; Jones, R. N. and Mutnick, A. H. (2003). Occurrence and antimicrobial susceptibility patterns of pathogens isolated from skin and soft tissue infections. *Diag. Micro. and Inf. Dis.,* 45 (4):287–293.

11. Gordon, R.J. and Lowy, F.D. (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus*infection. *Clin. Infect. Dis.,* 46 (5): 350-409.
12. Denis, O.; Deplano, A.; De Beenhouwer, H.; Hallin, M.; Huysmans, G.; Garrino, M.G.; Glupczynski, Y.; Malaviolle, X.;Vergison, A. and Struelens, M.J. (2005). Polyclonal emergence and importation of community acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-ValantineLeukocidin genes in Belgium. *J. of Antimicrob. Chemoth.*, 56 (6): 1103-1106.
13. Holmes, A.; Ganner, M.; McGuane, S.; Pitt, T. L.; Cookson, B. D. and Kearns, A. M. (2005). *Staphylococcus aureus*isolates carrying Panton-ValantineLeucocidin genes in England.J. Clin. Microbiol.,43 (5):2384-90.
14. Matsumura, Y.; Yoshikata, K.; Kunisaki, S; and Tsuchido, T. (2003). Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl. Environ. Microbiol*., 69(7): 4278-4281.
15. Jung, W.K.; Koo, H.C.; Kim, K.W.; Shin, S.; Kim, S.H. and Park, Y.H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus*and *Escherichia coli*. *Appl. Environ. Microbiol.* 74(7):2171-2178.
16. Hecker, S. J.; Glinka, T. W. ;Cho, A.; Zhang, Z. J.; Price, M. E.; Chamberland, S.; Griffith, D. and Lee, V. J. (2000). Discovery of RWJ-54428 (MC-02,479), a new cephalosporin active against resistant gram-positive bacteria. *J. Antibiot.,*53(11):1272–1281.
17. Catcott, E. J. (1974). Canine surgery (2nd Ed.), American Vet. Publication, Pp. 917-920.
18. Kealy, J.K.; Mcallister, H. and John, P.G. (2011). Diagnostic Radiography and Ultrasonography of small animals (5th Ed.), Pp. 433-436.
19. Zhang, R.; Lee, P.; Lui, V.C.; Chen, Y.; Liu, X.; Lok, C.N.; To, M.; Yeung, K.W. and Wong, K.K. (2015).Silver nanoparticles promote osteogenesis of mesenchymal stem cells and improve bone fracture healing in osteogenesis mechanism mouse model.*Nanomedicine*, 11 (8): 1949-1959.
20. 20. Shin-Woo, H.; Neale, W.M. and George, R.B. (2014). Bioactive Silica Nanoparticles Promote Osteoblast Differentiation through Stimulation of Autophagy and Direct Association with LC3 and p62. *ACS Nano*., 8 (6): 5898–5910.
21. 21. Stylios, G.; Wan, T. and Giannoudis, P. (2007). Present status and future potential of enhancing bone healing using nanotechnology. *Injury*, 38 (1): 63–74.
22. 22. Nair, L.S. and Laurencin, C.T. (2008). Nanofibers and nanoparticles for orthopaedic surgery applications. *J. Bone Joint Surg. Am*., 90 (1):128–131.
23. 23. Giardin, S.E.; Travassos, L.H. and Herve, M. (2003). Peptidoglycan molecular requirements allowing detection by NOD1 and NOD 2. *J. Biol. Chem.*, 278 (43): 41702–41708.
24. 24. Danon, D.; Kowatch, M.A. and Roth, G.S. (1998). Promotion of wound repair in old mice by local injection of macrophages. *Proc. Natl. Acad. Sci*., 86(6): 2018–2020.
25. 25. Champagne, C.M.; Takebe, J. and Offenbacher, S. (2002). Macrophage cell lines produce osteoinductive signals that include bone morphogenic protein-2. *Bone,* 30(1): 26–31.
26. 26. Kovacs, E.J. and DiPietro L.A. (1994).Fibrinogenic cytokines and connective tissue production. *FASEJ.,* 8(11): 854–861.
27. 27. Kilcullen, J.K.; Ly, Q.P. and Chang, T.H. (1998). Nonviable Staphylococcus aureus and its peptidoglycan stimulate macrophage recruitment, angiogenesis, fibroplasias and collagen accumulation in wounded rats. *Wound Rep. Reg.*, 6(2):149–156.
28. 28. Riches, D.W.H. (1996). Macrophage involvement in wound repair, remodeling and fibrosis. In: Clark RAF, editor. The molecular and cellular biology of wound repair (2nd Ed), New York: Pleneum Press. Pp.95–141.
29. 29. Dziarski, R. (1986). Effects of peptidoglycan on the cellular components of the immune system. In: Seidl PH, Schleifer KH, editors. Biological properties of peptidoglycan. Berlin: De Gruytra, Pp. 229–247.
30. 30. Liu, X.; Chang, T.H and Levenson, S.M. (1997). Wound fluids from saline and S aureus peptidoglycan inoculated sponges induce expression of metalloproteinase-13 mRNA by cultured rat fibroblasts. *Wound Rep. Reg*.,5 (4):348–354.
31. 31. Olav, R.; Jacob, E.; Wang, S.; Foster, J. and Stein, E.U. (2006).*Staphylococcus aureus* Peptidoglycan Impairs Fracture Healing: An Experimental Study in Rats. *J. of orth. Res.,* 25(2): 262-266.

Table (1): Clinical signs that appeared in animals of the groups post three and six weeks of fractures induction.

group	Clinical signs (three weeks)	Clinical signs to (six weeks)
A	Fever, swelling and redness at the site of fracture and soft tissue above the bone and pus formation.	Necrosis at the site of fractureoperation,sinus formation, low activity, crowding and limitedmovement in the fractured bone.
B	Normal signs of activity and movement.	Normal signs of activity and movement.
C	Some animalsappeared some clinical signs like redness and pus formation.	Some animalsappeared some clinical signs like limited movement.
D	Complete recovery without signs of illness, inflammation and abscess formation.	Complete recovery without signs of illness, inflammation.

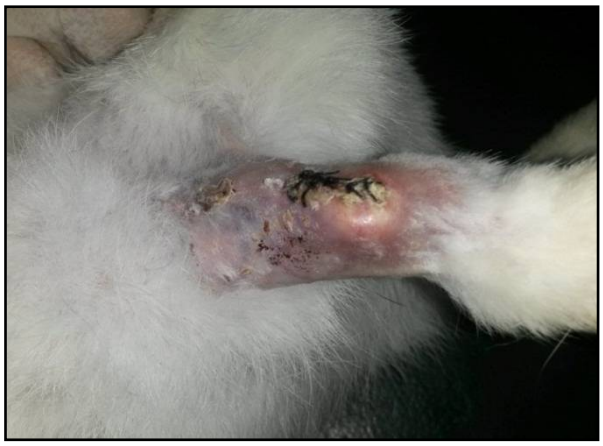


Figure (1):Shows the site of operation 10 days post infection with swelling and pus formation (group A)

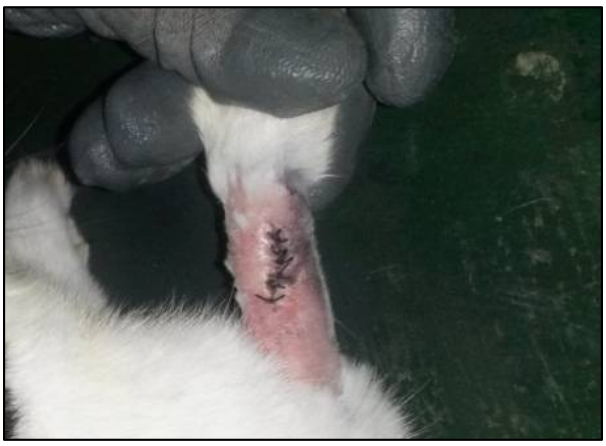


Figure (2): Site of operation ten days post infection with normal healing of skin and Subcutaneous tissue (group D).

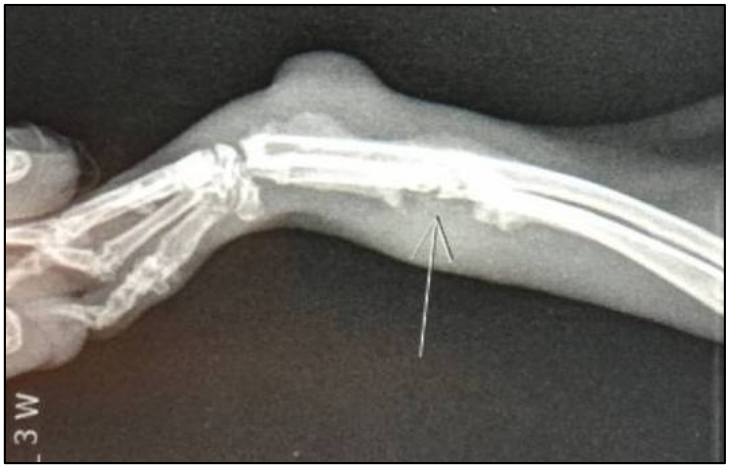


Figure (3): Radiographic image of fractured ulna, group (A) at 3 weeks post fracture shows severe bone destruction and the infection had even extended to the cortex (signs of osteomyelitis).

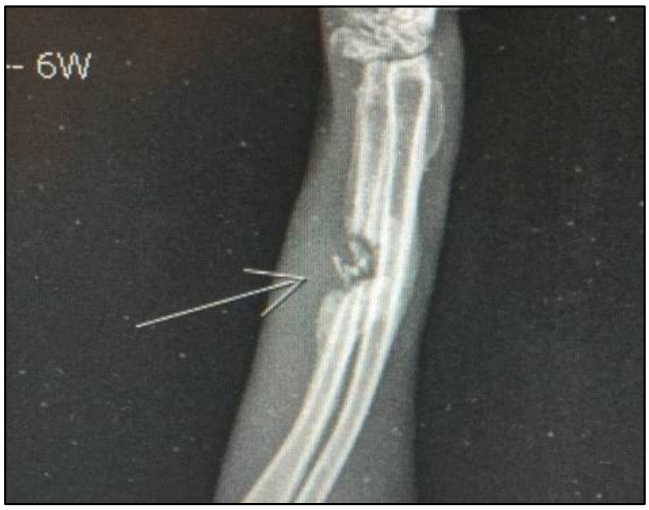


Figure (4): Radiographic image of fractured ulna, group (A) at 6 weeks post fracture shows the extension of bone destruction with presence of caseated pus (osteomyelitis).

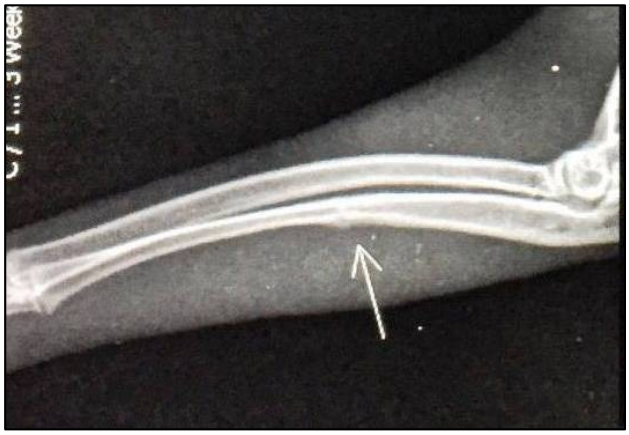


Figure (5): Radiographic image of fractured ulna, group (B) at 3 weeks post fracture shows complete disappearance of fracture line (boney union), but the periosteal reaction still exist (mediolateral view).

Figure (6): Radiographic image of fractured ulna, group (B) at 6 weeks post fracture shows complete union of the fractured bone, ulna returned to its normal anatomical shape (remodeled).

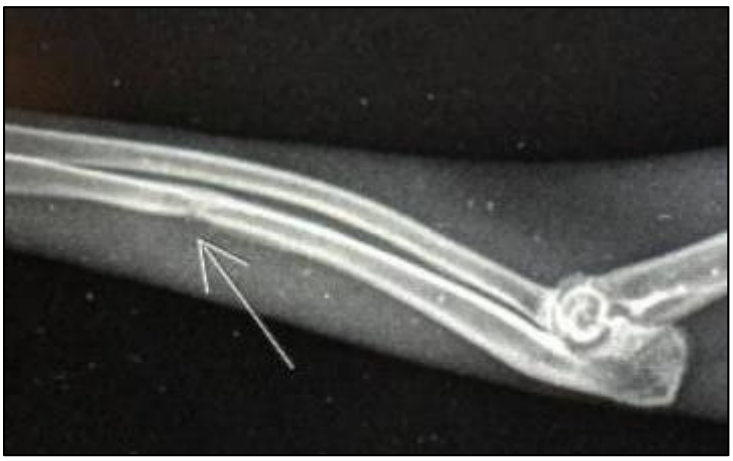


Figure (7): A mediolateral radiographic view of fractured ulna, group (C) at 3 weeks post fracture shows starting of callus formation. Line of the fracture can be identified, boney callus is filling the site of fracture but not completely mineralized.

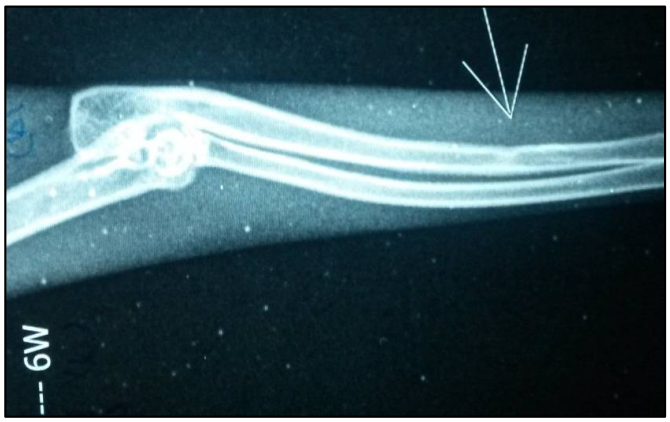


Figure (8): A mediolateral radiographic view of fractured ulna, group (C) at 6 weeks post fracture shows complete disappearance of the fracture line, boney union is complete.

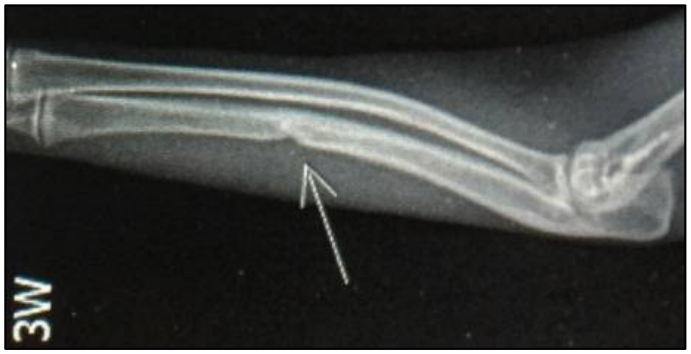


Figure (9): A mediolateral radiographic view of fractured ulna, group (D) at 3 weeks post fracture shows incomplete boney bridge connecting the fractured segments and the medullary canal still not clear at the site of fracture

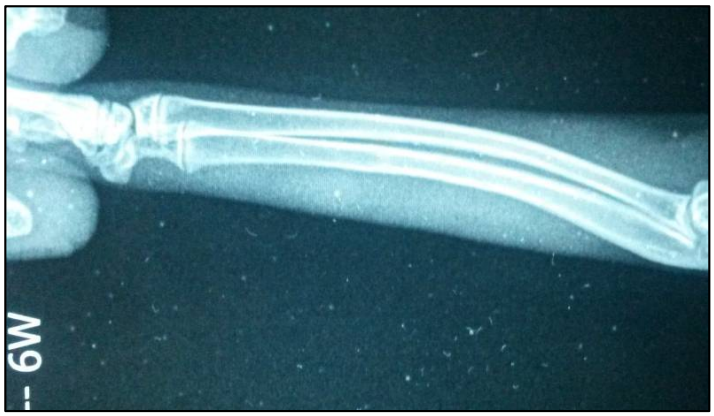


Figure (10): Radiographic image of fractured ulna, group (D) at 6 weeks post fracture shows complete boney union, medullary canal is almost clear (signs of early remodeling).

Connecting People with Nature

INDIAN JOURNAL OF NATURAL SCIENCES

ISSN: 0976-0997

MJL& Cited by Thomson Reuters for Zoological survey in 2012

ISI Impact Factor :1.233 , NAAS Rating :3.56,IC Value: 47.65

Tamil Nadu Scientific Research Organization is working for the promotion of society by transferring science & technology since 1997. We are very keen in appreciating and recognizing the contribution of every one of you. We are publishing **Indian Journal of Natural Sciences** - **IJONS** from **August 2010**. The Journal is peer reviewed International Journal for publication of Original Research papers /Reviews/ Short communications/Book reviews/Reports on conferences/Seminar, Important events, News of interest, Special articles, General Articles etc.

Call for Papers: Investigations related to science in all branches

Highlights

- International Quality
- Published Bi-Monthly
- Fast acceptance and Quick Publication
- Low price
- High Rank Editorial Board
- Online manuscript submission
- Publish original Research Works and Reviews

Subscription Fee (Annual 6 Issues)

- For Individuals Rs.1000/-
- For students Rs.750/-
- For Institutions Rs.1200/-

Publication fee for Authors

- **Rs.3000/- (**Processing fee)
- **USD100/-** for Foreign author

Send all correspondence to

----------Chief Editor ----------

Indian Journal of Natural Sciences (IJONS)

C/o TNSRO, 39 Mura Bhavan,Koodal Nagar,Rajagopalapuram Post

Pudukkottai-622003,TamilNadu,India.

Phone:04322-261088 Mobile: 99528 86637

E-mail: ijonstnsro@gmail.com.,**www.tnsroindia.org.in**

Plant More Trees ***Love Animals*** ***Conserve our Nature***

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Elastic Electron Scattering From ^{12}Be and ^{14}Be Halo Nuclei in the Framework of the Three Body Model

Ahmed N. Abdullah*

Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq.

Received: 20 Mar 2018 Revised: 18 Apr 2018 Accepted: 30 May 2018

*Address for correspondence
Ahmed N. Abdullah,
Department of Physics,
College of Science,University of Baghdad, Iraq.
E-mail:ahmednajim@scbaghdad.edu.iq

ABSTRACT

The ground state properties of two neutrons halo nuclei ^{12}Be and ^{14}Be are investigated by the three-body model of (Core+2n) with the single particle Gaussian and harmonic oscillator wave functions. The calculations are based on using different model spaces for the core and valence (halo) neutrons. The single particle Gaussian and harmonic oscillator wave functions are used with two different size parameters. The halo structure of above nuclei is emphasized through exhibiting the long tail performance in their calculated neutron and matter density distributions, where this performance is considered as a distinctive feature of halo nuclei. The elastic electron scattering form factors for these nuclei are studied via the plane wave Born approximation (PWBA).

Keywords: Three body model, unstable neutron-rich halo nuclei.. PACS number(s): 21.10.Gv, 25.30. Bf, 21.10.ft

INTRODUCTION

In the low-mass region of the nuclear chart, with the decrease of binding energy, some nuclei are characterized by the halo state, which exhibits an extended density distribution and a narrow fragment momentum distribution and so on. The investigation of the halo structure is a hot topic in the field of radioactive ion beam physics. Both on theories and experiments, studying the halo structure is very useful to well understand the structure of nucleus. There are two types of halo state: the two-body halos where one nucleon is surrounding the core nucleus, such as the one-neutron halos ^{11}Be and ^{19}C and the one-proton halo ^{8}B; and the three-body halos where two valence nucleons are around the core nucleus, such as ^{6}He, ^{11}Li and ^{14}Be. Up to now, all of the established three-body halo nuclei are Borromean nuclei where the three-body system is bound while its pairwise subsystems are unbound [1]. The electron scattering from nuclei is a powerful to investigate the electromagnetic structure in stable nuclei. This is because of the relatively weak interaction of electron with nucleus which is done through the well-known

electromagnetic force. Electron scattering from exotic nuclei is not presently available; the technical proposal for the construction of electron-ion collider at GSI/Germany [2] and RIKEN/Japan facility [3] will be a great opportunity to study the electromagnetic structure of these exotic nuclei in the near future. Ahmed [4] have been calculated the ground-state properties such as the neutron, proton and matter densities and the associated rms radii of proton-rich ^{8}B and ^{17}Ne halo nuclei using single-particle radial wave functions of Woods-Saxon (WS) potential. He found that the structure of the halo proton in ^{8}B is a pure $(1p_{1/2})$ and the structure of the two halo protons in ^{17}Ne is mixed configurations with dominant $(2s_{1/2})^2$. Elastic electron scattering form factors of these halo nuclei were also studied by the plane wave born approximation. Effects of the long tail behavior of the charge density distribution on the charge form factors of ^{8}B and ^{17}Ne were analyzed. He found that the difference between the charge form factors of ^{8}B and that of stable isotope ^{10}B (or of ^{17}Ne and that of stable isotope ^{20}Ne) is attributed to the influence of the charge density distributions of the last proton in ^{8}B (or of the last two protons in ^{17}Ne). Ahmed [5] have been used the single-particle radial wave functions of Woods-Saxon (WS) potential within the two-body model of (Core+n) [within the three-body model of (Core+2n)] to study the ground state densities and the associated root mean square (rms) radii of one neutron halo nuclei (such as ^{11}Be and ^{15}C) [of two neutrons halo nuclei (such as ^{16}C and ^{17}B)]. The halo structure of one neutron (^{11}Be and ^{15}C) and two neutrons (^{16}C and ^{17}B) halo nuclei was emphasized through exhibiting the long tail performance in their calculated neutron and matter density distributions, where this performance is considered as a distinctive feature of halo nuclei. The structure of the valence (halo) neutron in ^{11}Be and ^{15}C was found to be in a pure $2s_{1/2}$ also the structure of two valence (halo) neutrons in ^{16}C and ^{17}B were in a pure $(2s_{1/2})^2$.

THEROY

As the halo nuclei are oversized and easily broken systems consisting of a compact core plus a number of outer nucleons loosely bound and spatially extended far from the core, it is suitable to separate the ground state matter density distribution $\rho_m(r)$ into two parts. The first part is connected to the core nucleons $\rho_c(r)$ while the second is connected to the valence (halo) nucleons $\rho_v(r)$ [6], *i.e.*

$$\rho_m(r) = \rho_c(r) + \rho_v(r). \qquad (1)$$

Two density distributions are utilized for calculating the ground state properties of halo nuclei, these are G+G (Gaussian–Gaussian) and HO+HO (Harmonic Oscillator- Harmonic Oscillator).

In the G+G density distribution, the core and valence density distributions are described by the Gaussian distribution expressed as follows [7]

$$\rho_g(r) = \beta_g\, e^{-r^2/a_g^2}, \qquad g = c, v \qquad (2)$$

Where a_g and β_g are the size parameter and amplitude of the Gaussian shape, respectively. β_g in Eq. (2) is normalized by the mass number A_g.

Whereas in the HO+HO density distribution, the core and valence density distributions are described by the harmonic oscillator distribution expressed as follows [5].

$$\rho_c(r) = \frac{1}{4\pi}\sum_{n\ell} X_c^{n\ell}\left|R_{n\ell}(r, b_c)\right|^2 \qquad (3)$$

$$\rho_v(r) = \frac{1}{4\pi} X_v^{n\ell}\left|R_{n\ell}(r, b_v)\right|^2 \qquad (4)$$

Where $X_{c(v)}^{n\ell}$ represents the protons or neutrons occupation number in the shell $n\ell$ and b_c and b_v are the HO size parameters of core and valence nucleons.

The matter density of Eq. (1) may also be expressed as [6]

$$\rho_m(r) = \rho^p(r) + \rho^n(r), \quad (5)$$

where $\rho^p(r)$ and $\rho^n(r)$ are the ground state proton and neutron densities of halo nuclei expressed as

$$\rho^p(r) = \rho_c^p(r) + \rho_v^p(r) \quad (6)$$

and

$$\rho^n(r) = \rho_c^n(r) + \rho_v^n(r). \quad (7)$$

The normalization conditions for the ground state densities given in Eqs. (1-7) are

$$g = 4\pi \int_0^\infty \rho_g(r) r^2 dr, \quad (8)$$

and the corresponding rms radii are

$$< r^2 >_g^{1/2} = \frac{4\pi}{g} \int_0^\infty \rho_g(r) r^4 dr, \quad (9)$$

Where $\rho_g(r)$ corresponds to the one of the densities [$\rho_m(r)$, $\rho_c(r)$, $\rho_v(r)$, $\rho^p(r)$, $\rho^n(r)$] and g corresponds to the number of nucleon in each case. The elastic electron scattering form factors from considered nuclei are studied by the plane wave Born approximation (PWBA). In the PWBA, the incident and scattered electron waves are represented by plane waves. The elastic electron scattering form factor is simply given by the Fourier-Bessel transform of the ground state charge density distribution (CDD) obtained by HO+HO density [8], *i.e.*

$$F(q) = \frac{4\pi}{Z} \int_0^\infty \rho_{ch}(r)\, j_0(qr)\, r^2 dr, \quad (10)$$

Where $j_0(qr)=sin(qr)/qr$ is the zeroth order spherical Bessel function, q is the momentum transfer from the incident electron to the target nucleus and the ρ_{ch} (r) is the CDD of the ground state. Inclusion the corrections of the finite nucleon size $F_{fs}(q)=exp(-0.43q^2/4)$ and the center of mass $F_{cm}(q)=exp(b^2q^2/4A)$ in the calculations needs multiplying the form factor of Eq. (10) by these corrections.

RESULTS AND DISCUSSION

The ground state properties of two neutrons halo nuclei ^{12}Be and ^{14}Be are investigated by the three-body model of (Core+2n) with the single particle Gaussian and harmonic oscillator wave functions. The calculations are based on using different model spaces for the core and valence (halo) neutrons. The single particle Gaussian and harmonic oscillator wave functions are used with two different size parameters. The elastic electron scattering form factors for these nuclei are studied via the plane wave born approximation (PWBA). The nucleus ^{12}Be is formed by coupling the core ^{10}Be with the valence two neutrons. The nucleus ^{14}Be is formed by coupling the core ^{12}Be with the valence two neutrons. Tables (1) and (2) display, respectively, the values of the Gaussian and harmonic oscillator size parameters utilized in the present calculations for ^{12}Be and ^{14}Be halo nuclei. It is clear from these tables that the calculated rms matter radii for core and halo nuclei using these values are in very good agreement with those of experimental results [9, 10]. Table (3) shows the calculated proton and neutron rms radii for selected nuclei along with the experimental results [10, 11]. One can see from this table that the calculated results agree reasonably with the experimental ones within quoted error.

Figure 1 shows the calculated matter density distributions (solid curves) obtained by G+G density distributions [Figs. 1(a) and 1(c)] and HO+HO density distributions [Figs. 1(b) and 1(d)]. The top and bottom panels correspond to halo nuclei ^{12}Be and ^{14}Be, respectively. The contributions of the core (dashed curves) and the halo neutrons (dash-dotted curves) to the matter densities are also shown in these figures. The experimental matter densities of ^{12}Be and ^{14}Be [13] are displayed by shaded areas, for comparison. The solid curves in Fig. 1 agree well with the experimental. The long tail behavior (which is a distinctive feature of halo nuclei) is revealed in all solid curves of Figs. 1(a), 1(b), 1(c) and 1(d). Figure 2 demonstrates the results as in Fig. 1 but for the calculated proton and neutron density distributions displayed as dashed and dash-dotted curves, respectively. The long tail performance is clearly noticed in the dash-dotted curves. This performance is associated to the existence of the outer neutron in the halo orbits. The steep slope performance is obviously observed in the dashed curves due to the absence of protons in the halo orbit, where all protons of these nuclei are found in its core only. Figure 3 exhibits the calculated matter density distributions of the halo nuclei ^{12}Be (dash-dotted curves) and ^{14}Be (solid curves) compared with that of a stable nucleus ^{9}Be (dashed curves). The calculated densities in Fig. 3(a) [obtained by G+G density distributions] are compared with corresponding densities in Fig. 3(b) [obtained by HO+HO density distributions]. It is clear from these figures that the density distributions of the halo nuclei and stable nucleus are diverse. As the valence two neutrons in ^{12}Be and ^{14}Be are weakly bound, the density distributions of these nuclei have a longer tail than that of the stable nucleus. Figures 2 and 3 provide the conclusion that the halo phenomenon in ^{12}Be and ^{14}Be connected to the valence neutrons but not to the core nucleons.

The elastic charge form factors for ^{12}Be and ^{14}Be halo nuclei are studied through combining the charge density distribution obtained by HO+HO density distributions with the plane wave Born approximation (PWBA). Figure 4 illustrates the comparison between the calculated elastic charge form factors of ^{12}Be and ^{14}Be halo nuclei (solid curves) and that of stable nucleus ^{9}Be (dashed curves). For comparison the experimental data (denoted by the filled circle symbols) of stable nucleus ^{9}Be [14] is also shown in this figure. The calculated charge form factors in Figs. 4(a) and 4(b) correspond to (^{12}Be, ^{9}Be) and (^{14}Be, ^{9}Be) nuclei, respectively. The charge form factor is independent on detailed properties of the neutron halo. The major difference between the calculated form factor of the halo nuclei ^{12}Be and ^{14}Be and that of a stable nucleus ^{9}Be is the difference in the center of mass correction which depends on the mass number and the size parameter. In Fig. 4 each of the solid curves and the dashed curves have one diffraction minimum and one diffraction maximum. The location of the minimum of the halo nuclei has forward shift as compared with the minimum of a stable nucleus.

CONCLUSION

The ground state properties of two neutrons halo nuclei ^{12}Be and ^{14}Be are investigated by the three-body model of (Core+2n) within the single particle Gaussian and harmonic oscillator wave functions. The calculations are based on using different model spaces for the core and valence (halo) neutrons. The single particle Gaussian and harmonic oscillator wave functions are used with two different size parameters. The halo structure of above nuclei is emphasized through exhibiting the long tail performance in their calculated neutron and matter density distributions, where this performance is considered as a distinctive feature of halo nuclei. The elastic charge form factors for these halo nuclei are studied through combining the charge density distribution obtained by HO+HO density distributions with the plane wave Born approximation (PWBA). It found that the major difference between the calculated form factors of the halo nuclei ^{12}Be and ^{14}Be and that of a stable nucleus ^{9}Be is the difference in the center of mass correction which depends on the mass number and the size parameter.

REFERENCES

1. H. Z. Guo *et al*., *Science in China Series G: Physics*, *Mechanics & Astronomy* 51 (2008) 781.

2. Technical Proposal for the Design, Construction, Commissioning, and Operation of the ELISe setup, Haik Simon, GSI Internal Report, Dec. 2005.

3. T. Suda, K. Maruyama, Proposal for the RIKEN beam factory, RIKEN, 2001; M. Wakasugi, T. Suda, Y. Yano, Nucl.Inst. Meth. Phys., A 532 (2004) 216.
4. Ahmed N. Abdullah, *International Journal of Science and Research* (*IJSR*) 6 (3) (2017) 2083.
5. Ahmed N. Abdullah, *Australian Journal of Basic and Applied Sciences* 11(9) (2017), 136.
6. A. K. Hamoudi and A. N. Abdullah, *Iraqi Journal of Science* 57 4B (2016) 2664.
7. Tetsuaki Moriguchi, Ph.D.Thesis, University of Tsukuba (2011).
8. T. Stovall, J. Goldemberg and D. B. Isabelle, *Nucl.Phys*. 86 (1966) 225.
9. S. Ilieva *et al.*, *Nucl. Phys.* A 875 (2012) 8.
10. Z. Z. Ren and G. O. Xu, *Physics Letters* B 252 (1990) 311.
11. I.Tanihata *et al.*, *Phys. Lett. B* 206 (1988) 592.
12. Y. S. Shen and Z. Z. Ren, *Phys. Rev. C* 54 (1996) 1158.
13. S. Ilieve, *Investigation of the Nuclear Matter Density Distributions of the Exotic ^{12}Be,^{14}Be and ^{8}B Nuclei by Elastic Proton Scattering in Inverse Kinematics* (Johannes Gutenberg-University, 2008).
14. J. A. Jansen, R.T. Peerdeman and C.D. Vries, *Nuclear Physics* A **188** (1972) 337.

Table (1): The calculated and experimental rms radii of ^{12}Be and ^{14}Be halo nuclei together with parameters for a_c and a_v utilized in the G+G density distribution.

Halo nuclei	Core nuclei	a_c (fm)	a_v (fm)	$< r^2_{core} >^{1/2}_{cal}$ (fm)	$< r^2_{core} >^{1/2}_{\exp}$ [9]	$< r^2_{m} >^{1/2}_{cal}$ (fm)	$< r^2_{m} >^{1/2}_{\exp}$ [9,10]
^{12}Be	^{10}Be	1.91	3.96	2.33	2.33±0.02	2.91	2.91±0.09
^{14}Be	^{12}Be	2.03	4.16	2.48	2.48±0.03	3.0	3.1±0.3

Table (2): The calculated and experimental rms radii of ^{12}Be and ^{14}Be halo nuclei together with parameters for b_c and b_v utilized in the HO+HO density distribution.

Halo nuclei	Core nuclei	b_c (fm)	b_v (fm)	$< r^2_{core} >^{1/2}_{cal}$ (fm)	$< r^2_{core} >^{1/2}_{\exp}$ [9]	$< r^2_{m} >^{1/2}_{cal}$ (fm)	$< r^2_{m} >^{1/2}_{\exp}$ [9,10]
^{12}Be	^{10}Be	1.61	3.09	2.33	2.33±0.02	2.91	2.91±0.09
^{14}Be	^{12}Be	1.69	2.96	2.48	2.48±0.03	3.11	3.1±0.3

Table (3): The calculated proton and neutron rms radii along with the experimental results.

Halo nuclei	$< r^2_{p} >^{1/2}_{cal}$		$< r^2_{p} >^{1/2}_{\exp}$ [11]	$< r^2_{n} >^{1/2}_{cal}$		$< r^2_{n} >^{1/2}_{\exp}$ [11,12]
	G+G	HO+HO		G+G	HO+HO	
^{12}Be	3.34	2.28	2.49±0.06	3.15	3.19	2.75±0.11
^{14}Be	2.48	2.39	3.0±0.36	3.18	3.35	3.22±0.39

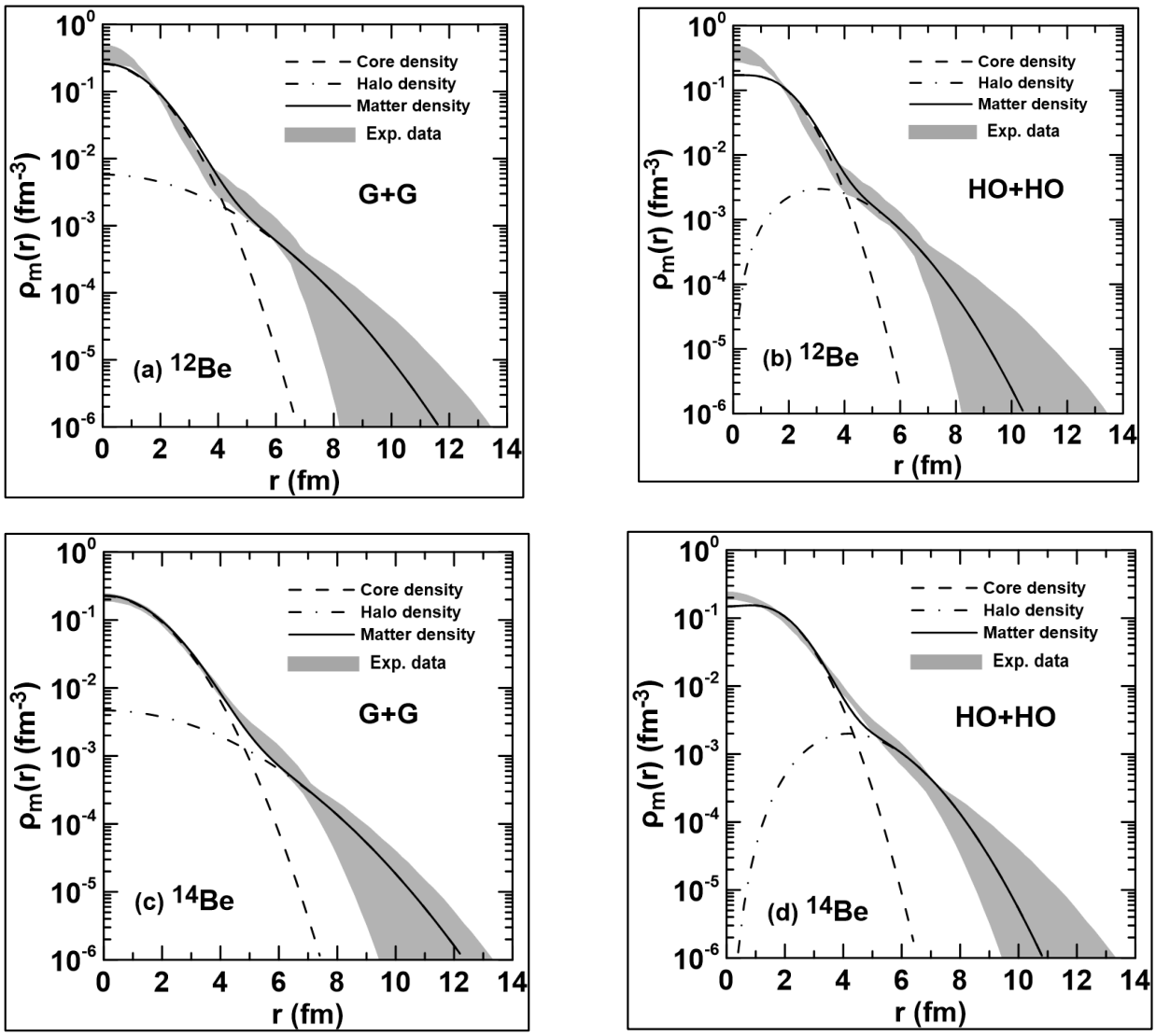


Figure 1: The calculated matter density distributions for halo nuclei ^{12}Be and ^{14}Be obtained via G+G and HO+HO density distributions.

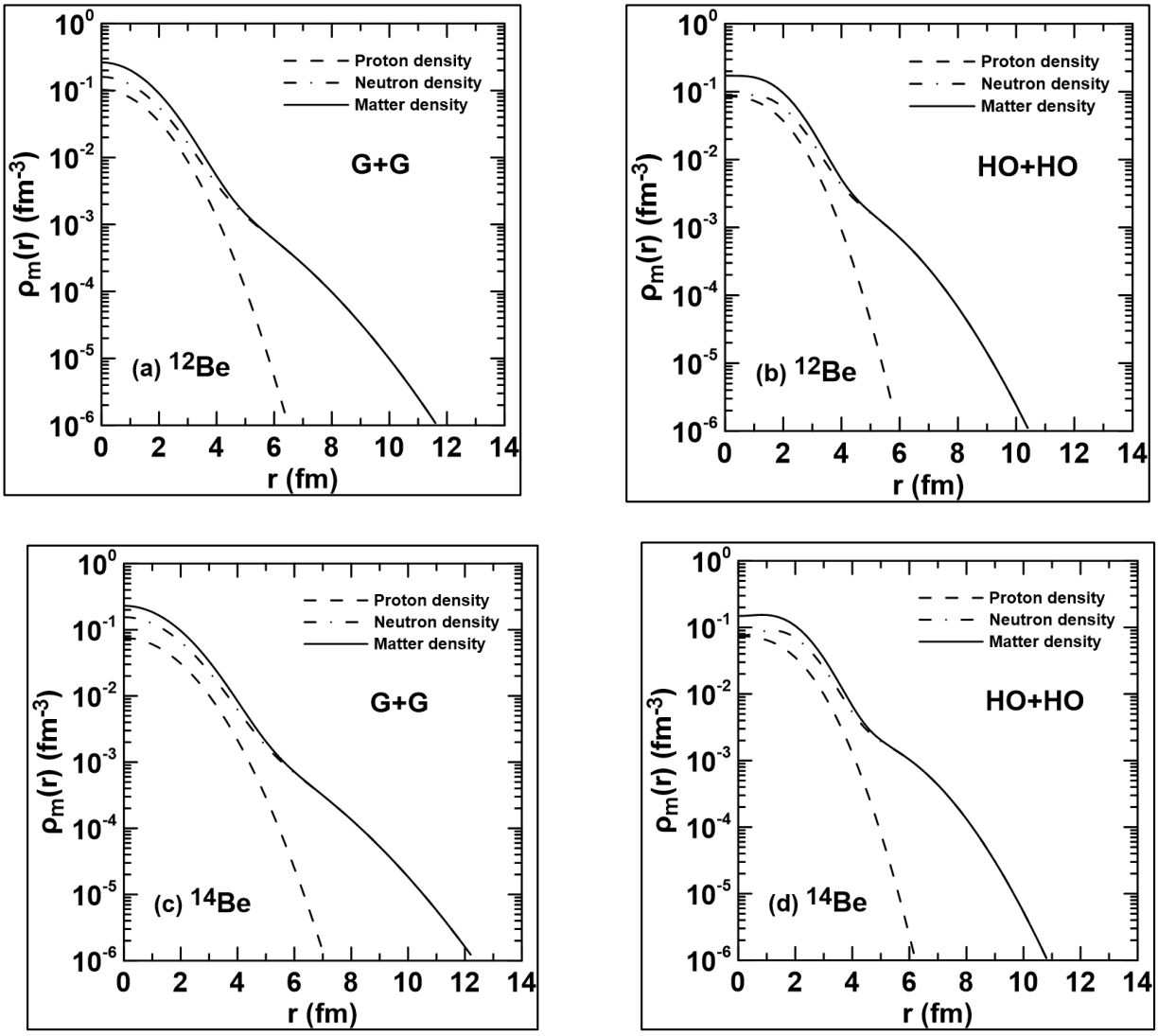


Figure 2: Neutron, proton and matter density distributions for halo nuclei ^{12}Be and ^{14}Be obtained via G+G and HO+HO density distributions.

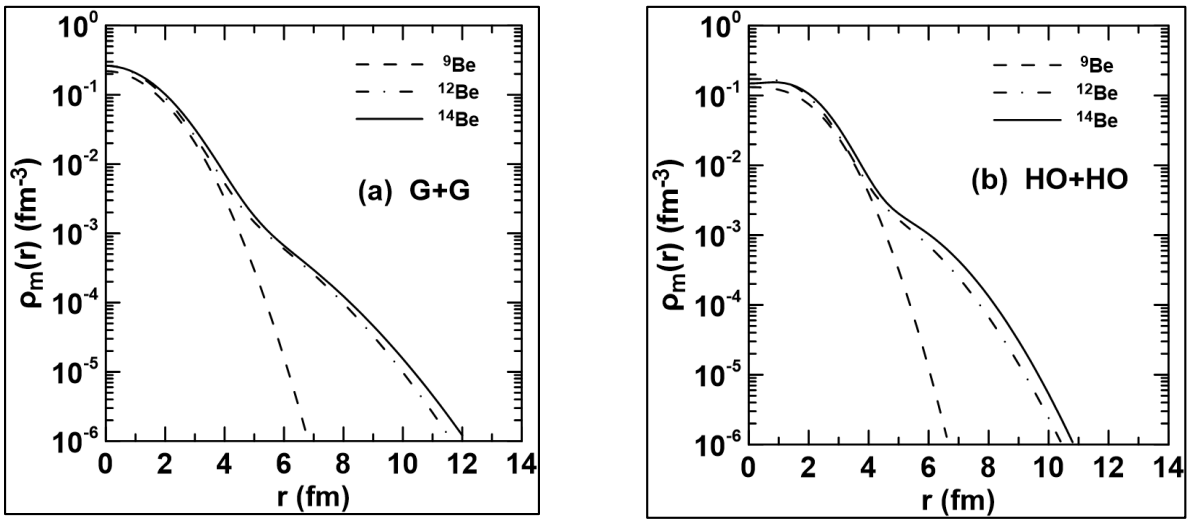


Figure 3: The calculated matter density of unstable nuclei (^{12}Be, ^{14}Be) compared with those of their stable isotope ^{9}Be obtained by G+G and HO+HO density distributions.

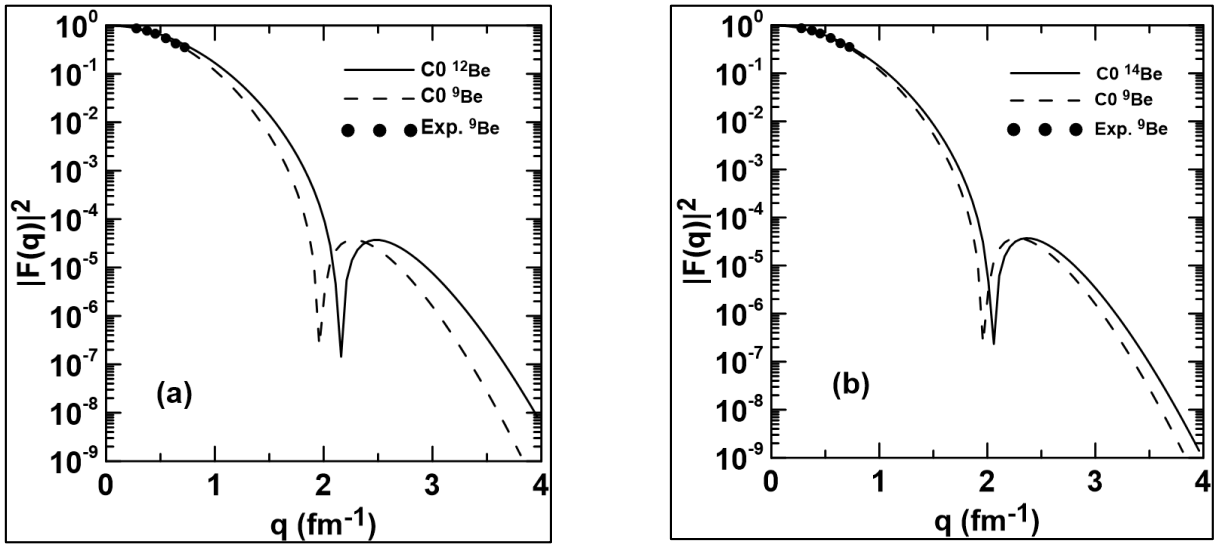


Figure 4: The charge form factors of unstable (halo) nuclei (^{12}Be, ^{14}Be) compared with those of their stable isotope ^{9}Be obtained by HO+HO density distributions.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Electrocardiographic Study Induced By Pericarditis in Goats in Iraq

Alaa Kamil Mahmood[1]* and Ziyad Mohammed Hassan [2]

[1]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad. Iraq.
[2] Minstry of Agriculture, Iraq.

Received: 18 Mar 2018 Revised: 19 Apr 2018 Accepted: 30 May 2018

***Address for correspondence**
Alaa Kamil Mahmood
Department of Internal and Preventive Veterinary Medicine,
College of Veterinary Medicine,
University of Baghdad. Iraq.

ABSTRACT

The aim of the present study was to determine the effects of injected of *Staphylococcus aureus* bacteria at a single dose in pericardium on some cardiac parameters in goats.In Wasit province .Ten local goats were used and a located randomly and equally into two groups. *Staph aureus* bacteria was injected into pericardial sac in five goats at dose 1ml ($97\times10^{*6}$CFU). Blood samples were taken from the jugular vein prior to administrations of bacteria, then at, 24, 48, 96, 144,192 and 240 hours after the injection. Clinical follow-up of the goats, In contrast, tachycardia, muffled heart sounds, distention of jugular vein ,increase of body temperature ,respiratory rate and heart rate. The ECG examination reflected that arrhythmias, tachycardia and duration and amplitude showed significant differences ($P< 0.05$) between the two groups concerning the increase of P amplitude, S-T segment elevations, P-R segment elevation, T wave inversions, and QRS wave is low compared with a control group.

Keywords: *Staph aureus,* Pericarditis, ECG, Iraqi goats

INTRODUCTION

Pericarditis, defined as inflammation of the pericardium with an accumulation of serous or fibrinous exudates,Pericarditis of haematogenous origin occurs less frequently ,it may be a concomitant finding in cattle with colibacillosis, pasteurellosis, salmonellosis or anaerobic infections, but the signs are usually masked by signs of septicaemia [1] Pericarditis is generally cause by foreign materials, fine, rough long (wire, nails, prick) that go into the reticulum, diaphragm and pericardial sac follow-on in traumatic pericarditis [2] Pericarditis attributable to hematogenous spread of infectious diseases (such as colibacillosis, pasteurellosis, salmonellosis and anaerobic infections) is much less common and is usually masked by signs of septicaemia[3] . unknown causes of pericarditis, which is observed in humans, dogs and horses, is un common in cattle[4] .Although reticulopericarditis isknown tooccur in cattle, it is rarely observed in small ruminants. [5] . The major clinical symptoms are tachycardia, without

heart sounds, a synchronous heart sounds such as friction or splashing sounds, jugular vein enlargement; the existence of all these symbols is path gnomonic for traumatic pericarditis. However, many cases do not show all of these signs and diagnosis may therefore not be straightforward. For example, the degree of difference diagnoses in acow with jugular vein enlargement and raise heart rate include right-sidedheart insufficiency related to valvular endocarditis, cardiomyopathy, cardiac leukosis and other diseases [1]. Electrocardiography is a non- persistent, inexpensive procedure that yields helpful information in categorized of arrhythmias, diagnosing transmission abnormality and it furthermore is an important aid in prognostic and therapeutic regard [6][7] .The electrocardiogram (ECG) provides a record and measure of the varying potential difference that occurs over the surface of the body as the result of electrical activity within the heart. This is associated with depolarization and repolarization of the myocardium. In the normal heart, depolarization and repolarization of the myocardium occur in a definite pattern and sequence and then the ECG can be used to measure and time these events. Thus discharge of the sinoatrial node results in a wave of depolarization over the atria to produce a P wave in the ECG. The delay in conduction at the AV node is registered by no electrical activity at the body surface and an isoeletric P-R interval on the ECG. Depolarization of the ventricles occurs with several sequential fronts to produce the QRS complex which is followed by another isoelectric period before repolarization represented by the T wave [6]

MATERIALS AND METHODS

Ten male goats were divided into two equal groups each group consisted of five goats.

Group 1

Consist of five goats were injected with normal saline in to pericardial sac 1ml/kg b.W

.

Group 2

Consist of five goats were injected with staph .aureus bacteria1 ml (97×10*6 CFU)

Experimental animals

Ten clinically healthy male goats, their ages ranged between (1-2 years).Ageing was determined by teeth eruption. The live weight was(10-25)Kg at the starting of an experiment were used in this investigation which was conducted for the period from mid-October 2017- November 2017. Goats were purchased from local livestock market and were housed within the farms in the Wasit governorate during all study period. The animals were fed three times a day. The meal was consisted of ordinary alfalfa, hay and concentrates supplements. They were given prophylactic doses of oxytetracycline 5% (Bremerpharma, Germany) in a dose of 10 mg/kg against bacterial infections. In addition, lambs have injected 3ml Co-Baghdad vaccine subcutaneously in a hairless area to avoid enterotoxaemia. The animals were ear tagged (numbered 1-10) and allowed two weeks preliminary period for observation and adaptation prior to the commencement of the experiment.

Preparations and administration of *Staphylococcus aureus* bacteria

Isolation of bacteria from any animal wound and grow on nutrient agar in incubator 37c for 24hr, after that take the sample from bacteria by the loop at planting these bacteria on blood agar to activation in incubator 37c for 24hr. Then need PBS solution to extenuation the bacteria, harvesting bacteria from blood agar after incubation period by the loop and input in tube contain 5ml buffer solution (7.2 – 7.4 PH) after that lay down in centrifuge at 3000 rpm for 15-20 minute, this protocol repeat three time until cleaning that suspension subsequently place in refrigerator in 4c.

After that taken10 tube without anticoagulant, each tube contains 9.5ml from buffer solution and add 0.5ml from bacteria stock add to each tube sequentially.For bacterial count needed three plates from blood agar for each dilution series, the surface of each plate allow 0.2 ml from bacterial stock and weigh ing 15-20 minute to absorb then put in incubation at 37c for 18 – 24 hr. Then calculate the number of colony forming unite (CFU) per ml from each plate and plus them after that divide on three plates, this method repeat on each tube.

CFU per ml = average number of colony for dilution ×50×diluent factor

Electrocardiography (ECG)

All the animals including healthy and induced pericarditis taken ECG. After induced pericarditis by injection of staphylococcus aureus bacteria in pericardium sac, taken ECG secondly and comparative between before and after induced. Recording the changes in electrodes and leads. The ECG that recorder on the bipolar limbs leads system in all animals using six leads in recumbence position .before using ECG that will be clipping and shaving hair of the area limbs after that using the ECG gel to applying the area to a good contact .Red electrode (RA) pointed on right forelimb. Yellow electrode (LA) pointed on the left forelimb, hind limb green color and black devoted to the left hind limb. The ECG is found with a single channel electrocardiographic tool. With a paper speed of 25mm/s and calibration of 10mm equal to 1 mv. The duration of all waves from were intended physically was each small square characterize 0.04s on the horizontally plan and 0.1v on the ventral plan.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). TwowayAnalysis of Variance (ANOVA) and Least significant differences (LSD) post hoc test were performedto assess significant differences among means. The $P< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

After Staphylococcus aureus bacteria injection into pericardial sac in five goats at infected doses 1ml (97×10*6CFU) the animals reflected firstly after 24he from injection mild signs including a decline of appetite, pyrexia, depression, these sign prolonged to48hr. after these time the clinical finding showed anorexia, congestion of mucous membrane watery nasal discharge, dyspnea, remittent diarrhea, abdominal respiration, lameness, in auscultation irregular heart rate ,irregularrespiration, absence of lung sound in ventral thoracic, heart rat sound hearing by auscultation in lung region,destention of jugular vein, increased of heart rate about 156/minute, increased of body temperature 40.6C^0, increased of respiratory rate38/minute. Moreover, all goats didn'tappearance any mortality ratio.

Electrocardiographic finding

The results mentioned to duration and amplitude in lead I which showed differences significant ($P< 0.05$) between the animals about the P and QRS as they significantly increased ($P<0.05$) at 24, 48, 96,144,192 and 240 hoursafter injection compared with zero time in amplitude wave. The leads of electrocardiography were see in (figure -1). the normal conditions that were recording on studied goatsat the electrocardiographic examination, decline in QRS wave seen in (figure 2), the left bindle branch block (figure 3), decrease in T wave in (figure 4), increase of ST wave (figure 5),the increase of T wave (figure 6),irregular rhythm (figure 7) and was sinus tachycardia (figure 8) in which the heart rate reached 160 beat/minute. Elevation of body temperature (pyrexia) was noticed in current study, which may be stimulation of thermal centre in the hypothalamus,by pyrogens released **in** addition to ncreasing basal metabolic rate. [8] . An elevation of heart rate is a significant factor in increasing cardiac output and maintaining circulatory equilibrium. It has been documented that cattle can increase their heart rate to four times their resting values [2]

(Radostits *et al*., 2007). Muffled heart sounds usually indicate pericardial effusion [9] .The heart sounds are muffled because of pericardial effusion and fibrinous changes in the pericardial sac and asynchronous abnormal heart sounds are characteristically present. These abnormal sounds depend on the type of lesions [1] .The electrocardiographic changes of acute pericarditis are same in some ways that seen with acute myocardial infarction [10] [11] .

ST Segment Elevation

Acute pericarditis is usually characterized by ST segment elevations due to alterations in ventricular repolarization in the early phase of pericarditis. This electrocardiographic pattern is due to the current of injury resulting from concomitant inflammation of the epicardium. ST elevation also occurs in acute myocardial infarction (MI) and early repolarization.

PR Segment Elevation

The repolarization of the atria is also affected by acute pericarditis, which occurs during the PR segment that is at the end of the P wave to the beginning of the QRS complex. Acute inflammation pericardium causes an atrial current of injury, which is reflected ECG by PR segment elevation in lead aVR and PR segment depression in other limb leads. Thus, PR segment and ST segment changes are typically in opposite directions in acute pericarditis. For example, in aVR, the PR segment is elevated (often by only 1 mm or so) while the ST segment is usually slightly depressed and other leads may show PR depression and ST elevation.

T Wave Inversion

The ST segment elevations, which are seen with acute pericarditis after a variable time is followed by T wave inversions, which may resolve completely with time with normalization of the ECG. But however, in some patients, the T wave inversions may persist for a prolonged period as in chronic pericarditis. This sequence of ST elevation followed by T wave elevation is the same as that described with myocardial infarction [12] .

QRS wave

Pericarditis usually the results to occurrence pericardial effusion. The major hemodynamic complication of pericardial effusion is cardiac tamponade. The most common ECG finding of pericardial effusion with or without cardiac tamponade is low voltage QRS complexes, probably due to short-circuiting of cardiac potentials by the fluid surrounding the heart. The presence of low voltage and sinus tachycardia should always raise concern about pericardial effusion with tamponade. Another ECG change that can occur with pericardial effusion and tamponade is electrical alternant. Electrical alternant with sinus tachycardia is a highly specific ECG finding of cardiac tamponade, but its absence does not exclude pericardial tamponade.

Low Voltage

This has always been considered to be a valuable sign and is thought to depend on the pressure of a lake of fluid about the heart causing a short circuiting effect. Low voltage is said to be present when the QRS amplitude is 5 mm or less in each lead.If the voltage remains low after the removal of fluid the decreased amplitude of theelectrocardiographic deflections is probably due to the insulating effect of fibrin [13].The decrease in cardiac chamber size and volume and changes in the generation and propagation of electrical currents in the myocardium, [14] . The several mechanisms have been proposed to explain the association between pericardial effusion and low QRS voltage. The mechanisms of low QRS voltage with pericardial effusion in an experimental approach have tried to clarify only by few investigators like [14].

T waves

The T-wave in pericarditis is usually is inverted.In typical cases of pericarditis, the T-wave becomes inverted in all standard leads with the exception of aVR and An incompletely inverted T-wave such as a diphasic wave or a notched T-wave is a characteristic feature of the electrocardiographic pattern in pericarditis [15] .T-wave abnormalities of pericarditis can be differentiated from T-wave abnormalities of myocardial infarction because of following factors: 1) The myocardial surface responsible for the abnormal T-wave vector is greater in pericarditis than in myocardial infarction.2). The muscle mass responsible for the T- wave vector is smaller in pericarditis than in myocardial infarction.3).The inflammatory changes associated with pericarditis appear to produce myocardial damage more slowly and insidiously than the ischemic changes associated with myocardial infarction (Figure 2).

However, in pericarditis, the T-waves are usually less deeply or less completely inverted than in myocardial infarction. An incompletely inverted T-wave such as a diphasic wave or a notched T-wave is a characteristic feature of the electrocardiographic pattern in pericarditis [15] .T-wave abnormalities of pericarditis can be differentiated from T-wave abnormalities of myocardial infarction because of following factors: 1) The myocardial surface responsible for the abnormal T-wave vector is greater in pericarditis than in myocardial infarction. 2) The muscle mass responsible for the T- wave vector is smaller in pericarditis than in myocardial infarction. 3) The inflammatory changes associated with pericarditis appear to produce myocardial damage more slowly and insidiously than the ischemic changes associated with myocardial infarction.

CONCLUSION

In conclusion *Staphylococcus aureus* bacteria injection into pericardium in dose 1ml (97×10*6CFU). Caused changes in clinical, and ECG changes.

REFERENCES

1. Grunder H D Krankheiten des Herzens und des Herzbeutels. In nere medizin und Chirurgie des Rindes. 4th edn. Eds G. Dirksen, H. D. Grün der, M. Stö ber. Berlin, Parey Buchverlag. (2002) pp 159-181.
2. Radostits O M , Gay C C , Blood, D C , Hinchcliff K W (2007).Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. W.B. Saunders, London, pp. 443–45.
3. Buczinski S Cardiovascular ultrasonography in cattle. Vet. Clin. North Am. FoodAnim. Pract. (2009) 25, 611–632.
4. Jesty S A, Sweeney, R W , Dolente B A , Reef V B Idiopathic pericarditis and cardiac tamponade in two cows. J. Am. Vet. Med. Assoc. (2005) 226, 1555–1558.
5. Akkoç A (2007).Traumatic Reticulopericarditis in a Saanen Goat. Turk J Vet Anim Sci; 31(4): 283-285.
6. Fregin, F.G. (1985). Electrocardiography. Vet. Clin. North Am. - Equine Pract., 1:419-432
7. Mendes, L.C.N. ; Camacho, A. A. ; Alves, A.L.G. ; Borges, A.S. ; Souza, R.C.A.and Ferreira, W.L.(2001). Standard electrocardiographic values in Holstein calves. Arq. Bras. Med. Vet. Zootec., 53,(6): 641-644.
8. Gupta S, Gupta S Fever . Indian J. Dermatol. Venereol. Leprosy, (2003). 69: 237-238
9. Roth L , King J M Traumatic reticulitis in cattle: a review of 60 fatal cases. *Journal of Veterinary Diagnostic Investigation*, (1991). *3*(1), 52-54.
10. Surawicz B , and Lasseter K C Electrocardiogram in pericarditis. *The American journal of cardiology*, (1970). *26*(5), 471-474.
11. Goldberger A L, and Goldberger E Clinical Electrocardiography, 5th ed. C V Mosby St Louis, Year Book,
12. Badiger S , Akkasaligar P T , & Biradar M S Electrocardiography–pericarditis, pericardial effusion and cardiac tamponade. *International Journal of Internal Medicine*, (2012) *1*(4), 37-41
13. Lepeschkin ,E.(1951). Modern Echocardiography. Baltimore, Williams and Wilkins; (1994). 1, pp. 453-458,.

14. Karatay C M , Fruehan C T , Lighty Jr G W , Spear R M & Smulyan, H. Acute pericardial distension in pigs: effect of fluid conductance on body surface electrocardiogram size QRS. *Cardiovascular research*, (1993). *27*(6), 1033-1038.
15. NOTH, P H , BARNES, A R Electrocardiographic changes associated with pericarditis. *Archives of Internal Medicine*, (1940). *65*(2), 291-320.

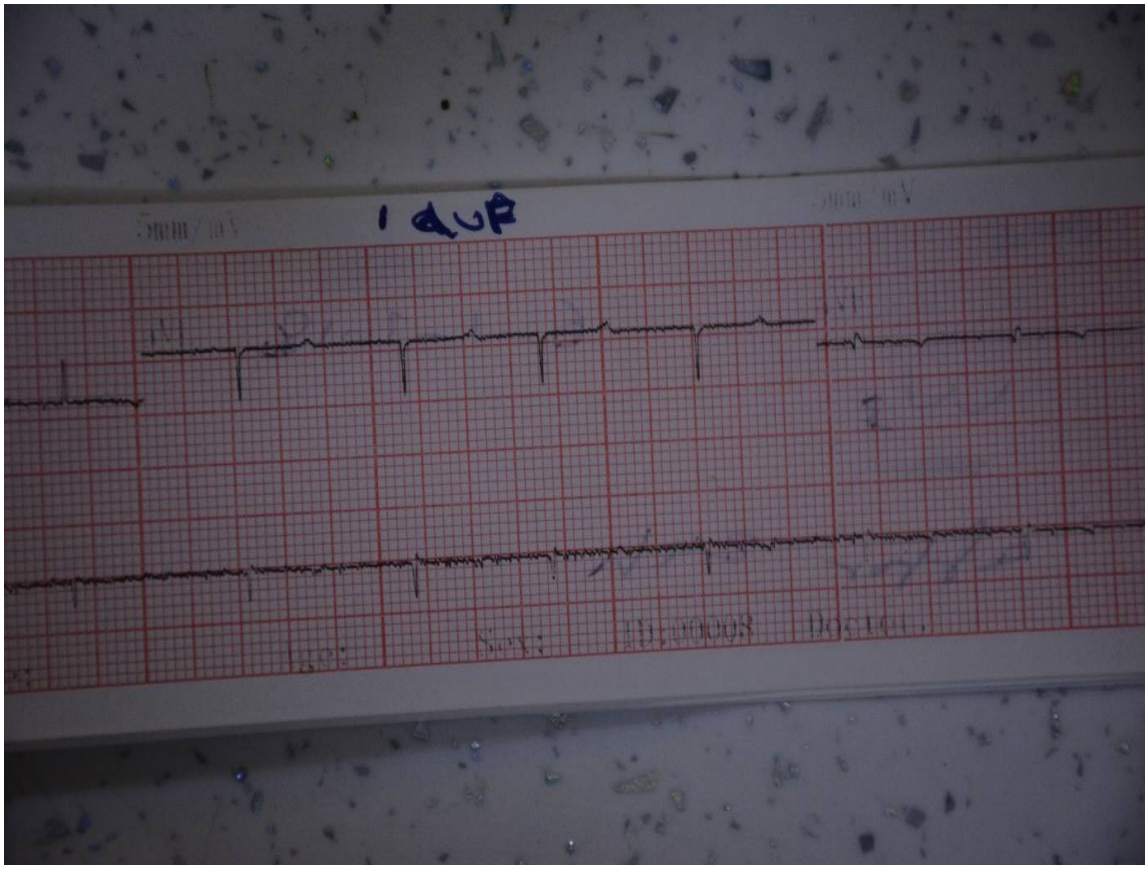


Fig 1.Shows normal electrocardiography in lead aVR (control)

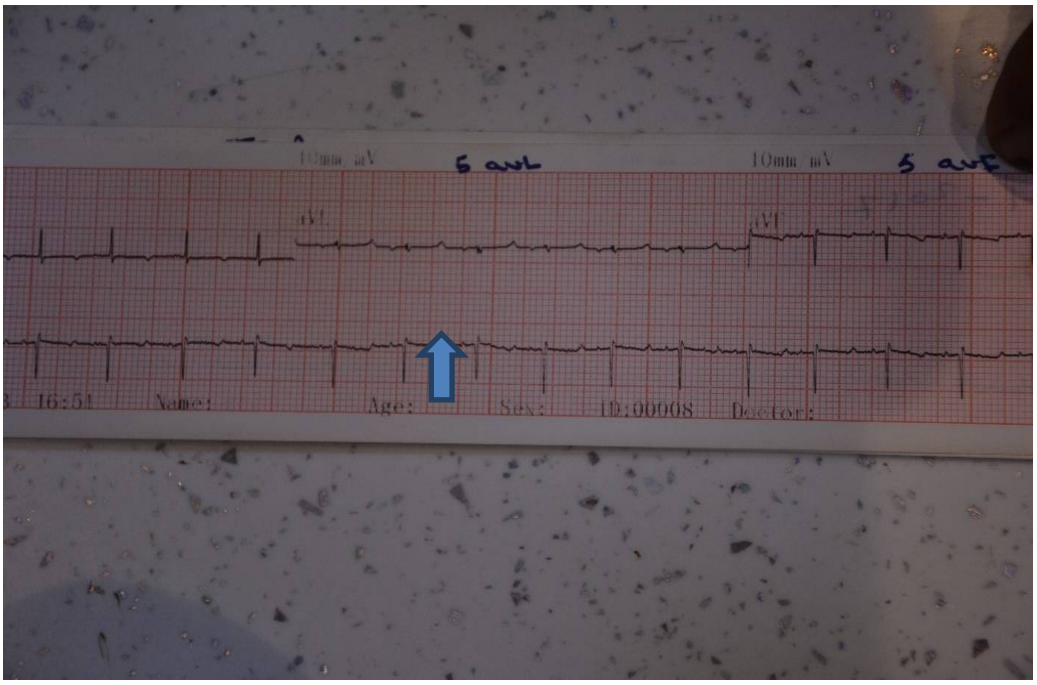


Fig 2. Shows electrocardiography (decrease QRS wave)

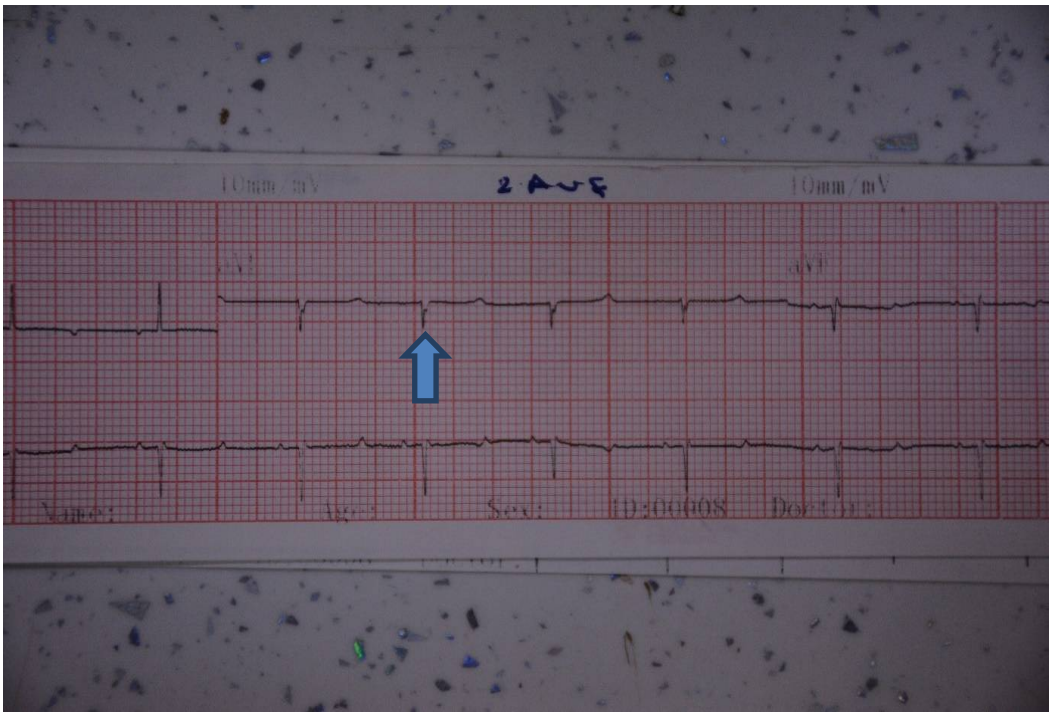


Fig 3.Shows electrocardiography (decrease R wave) (left bindle branch block)

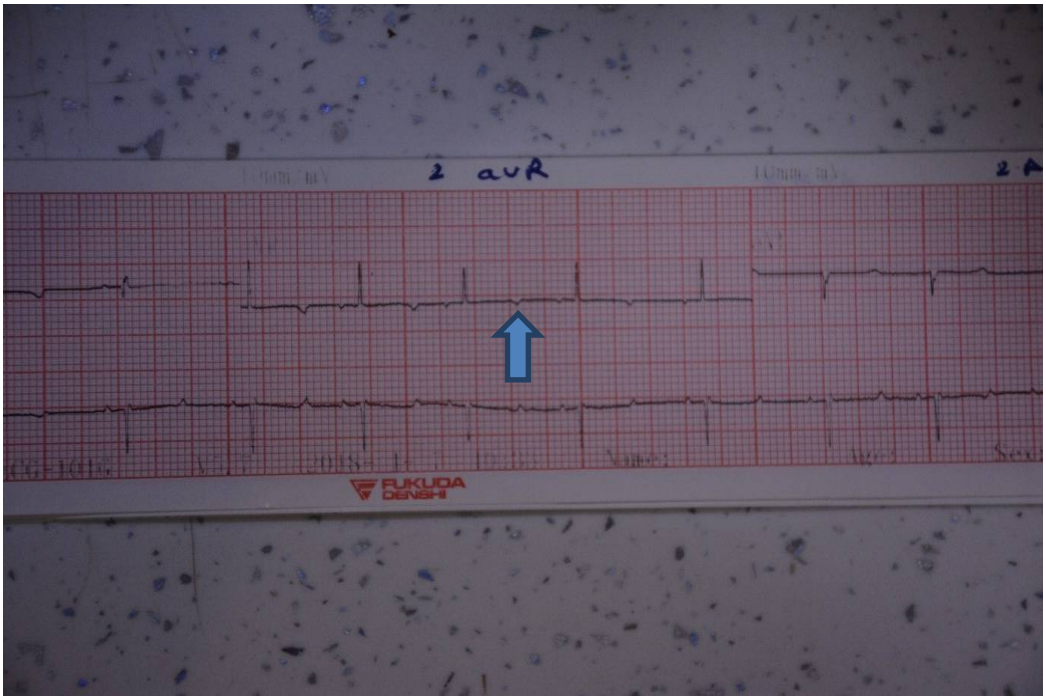


Fig 4.Shows electrocardiography (decrease or inversion T wave)

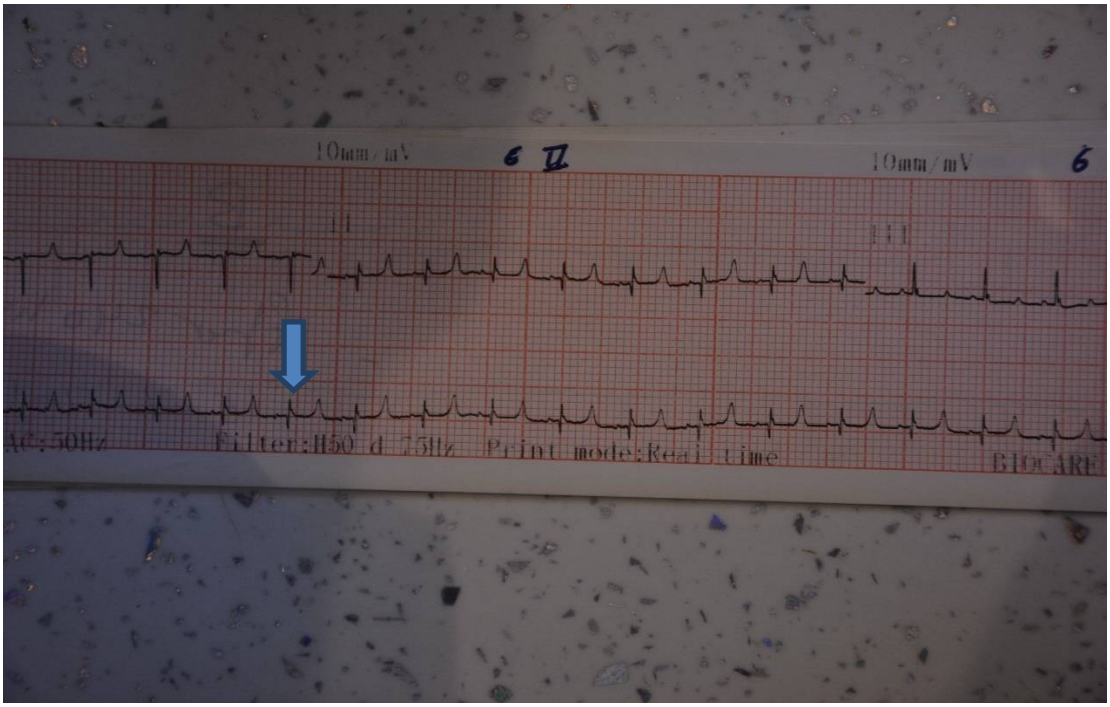


Fig 5.Shows electrocardiography (increase ST wave)

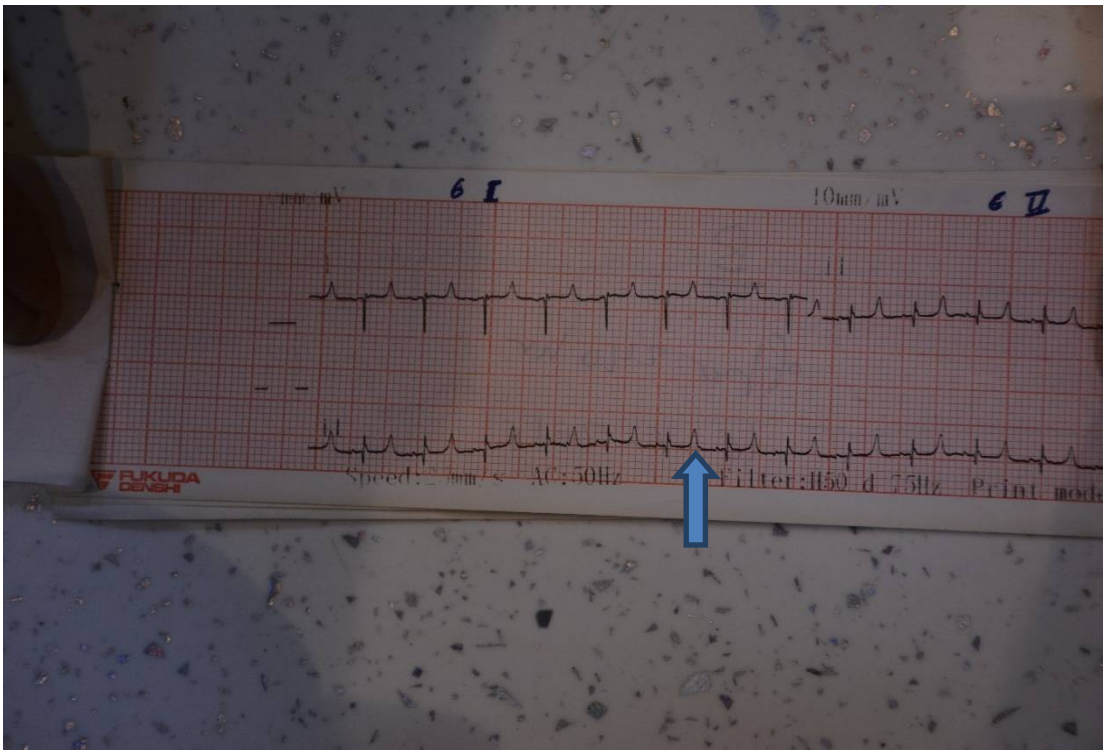


Fig 6.Shows electrocardiography (increase Twave)

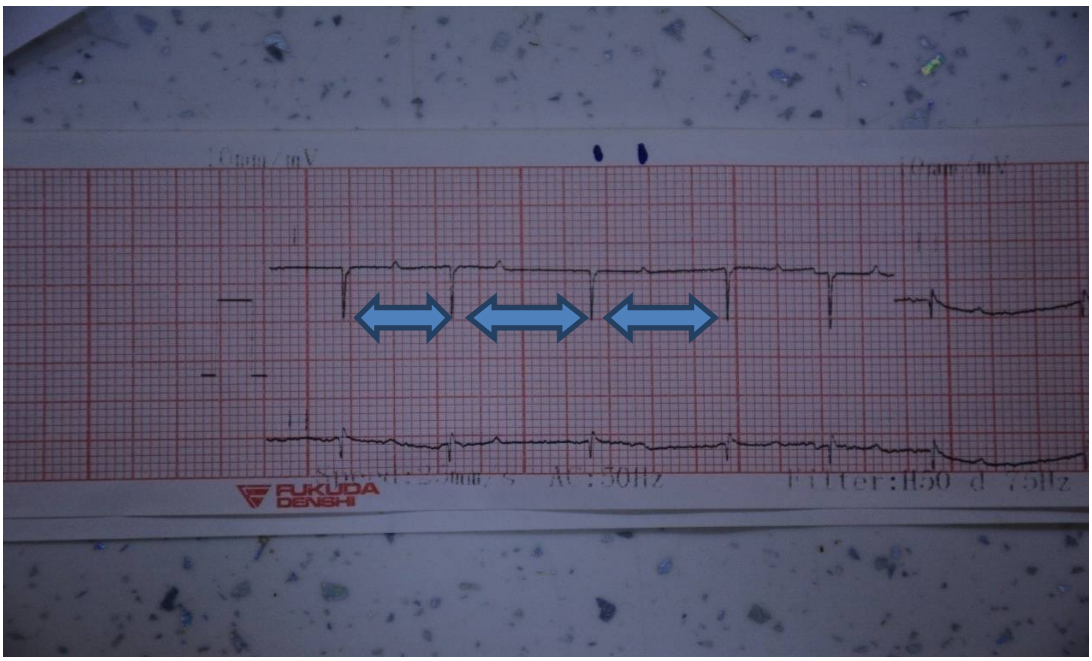
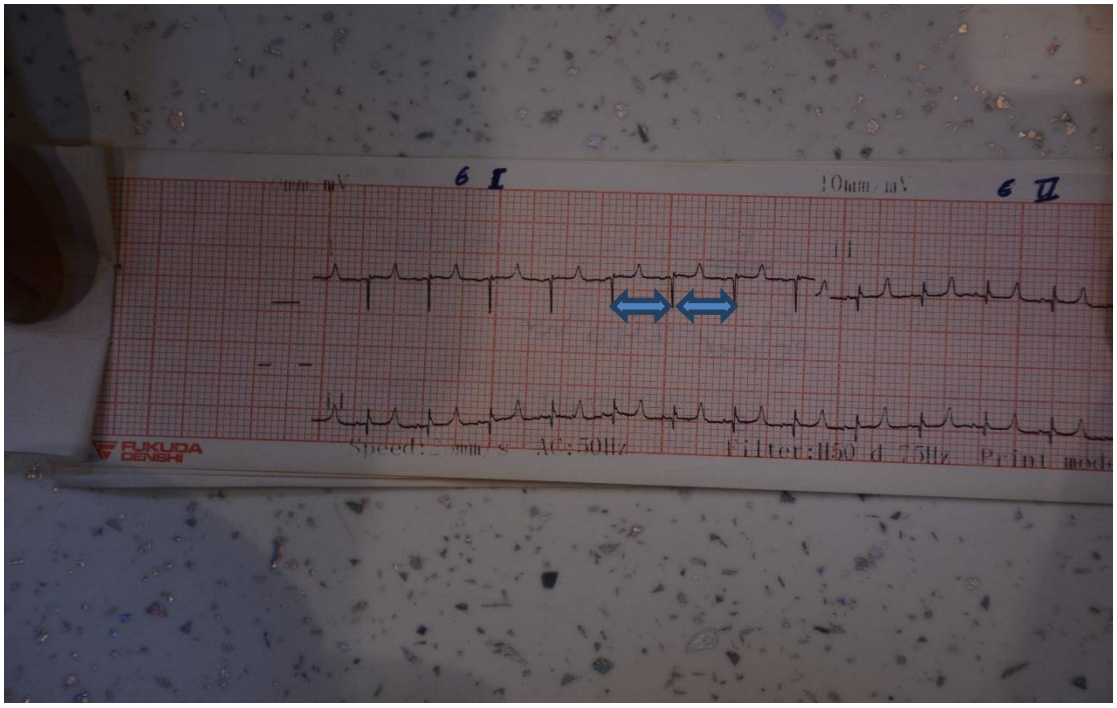


Fig 7.Shows electrocardiography irregular rhythm pulse (arrhythmia)



k

Fig 8.Shows electrocardiography (sinus tachy cardia)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Design and Development of Spray Dried Intranasal Mucoadhesive Microspheres of Risperidone by using Pectin as a Natural Polymer

P.S.Gangane * and P.S.Kawtikwar

Janta Shikshan Prasarak Mandal's, Sudhakarrao Naik Institute of Pharmacy, Pusad-445402, Dist. Yavatmal. (M.S.), India.

Received: 21 Mar 2018 Revised: 18 Apr 2018 Accepted: 30 May 2018

*Address for correspondence

P.S.Gangane

Janta Shikshan Prasarak Mandal's,
Sudhakarrao Naik Institute of Pharmacy,
Pusad-445402,
Dist.Yavatmal. (M.S.), India.
E-mail: p.gangane@gmail.com

ABSTRACT

The aim of this study was to design, develop and evaluate Nasal Mucoadhesive Microspheres of CNS acting drug like Risperidone. Risperidone is an atypical antipsychotic medication. It is most often used to treat delusional psychosis (including schizophrenia), but risperidone is also used to treat some forms of bipolar disorder and psychotic depression. It also has shown some success in treating symptoms of Asperger's Syndrome and autism. Risperidone is now the most commonly prescribed antipsychotic medication in the United States. The major objectives of designing Mucoadhesive Drug Delivery System were, To reduce nasal mucociliary clearance of the drug, To improve contact time of drug molecule with nasal mucosa, To increase systemic absorption of a drug candidate, To optimize the formulation based on the various evaluation tests, The dose of the drug is reduced and become a better option to the parenteral dosage form. Drug-Polymer interaction study such as FTIR, DSC and XRD revealed that both the drug and polymer were compatible with each other and no interaction was found in between them. Based on other parameters such as Particle Size 11.51 ± 0.12, Production yield 21.33± 0.43, Entrapment Efficiency 60.12± 1.96, Drug loading 16.22± 0.93, Mucoadhesion Study 79.10± 2.50 and Swelling index 0.612 ± 0.05 R1 batch was found as optimized batch which also showed good results for Drug release 96.07 at 240 min, Ex vivo permeation 81.15 at 240 min and microscopic observations for histological study indicated that the formulation has no significant effect on the microscopic structure of sheep nasal mucosa

Keywords: Mucosa, Nasal Microspheres, Mucoadhesive, Ex vivo, Permeability, histological, antipsychotic.

INTRODUCTION

Nasal drug delivery can be a better option as an alternative route for administration of drugs or biomolecules that are having susceptibility towards acidic or enzymatic degradation and showed gastrointestinal and hepatic presystemic metabolism[1]. Conventionally, the nasal route has been used for delivery of many drugs for the treatment of local diseases such as nasal allergy, nasal congestion, and nasal infection. In recent years the nasal route can be exploited for the systemic delivery of small molecular weight polar drugs, long chains of peptides and proteins, for rapid onset of action, is required and for therapeutic and recreational purposes. Intra-nasal administration of Active Pharmaceutical Ingredients (API) offers an interesting alternative for achieving systemic therapeutic effects that are equivalent to the parenteral route, which can be inconvenient during oral administration, which can result in low drug bioavailability.

Advantages of Nasal Drug Delivery System

1. It is the non-invasive, rapid and comfortable mode of drug administration.
2. It also bypasses the BBB and targets the CNS and thus reduces systemic side effects.
3. It avoids drug loss due to hepatic “first pass” metabolism and gut wall metabolism, allowing increased bioavailability.
4. Rapid absorption, higher bioavailability, therefore lowers doses.
5. The rich vasculature and highly permeable structure of the nasal mucosa greatly enhance drug absorption.
6. Good penetration of, especially lipophilic, low molecular weight (up to 1000 Da) drugs through the nasal mucosa [2, 3].

Limitations of Nasal Drug Delivery

1. Delivery is expected to decrease with increasing molecular weight of the drug.
2. Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.
3. Nasal congestion due to cold or allergies may interfere with this method of delivery[4, 5].

Brain and the central nervous system have the ability to exchange molecules with the disorders like schizophrenia, meningitis, migraine, interstitial fluid of the brain that contains parenchymatous cell for the passage of Parkinson’s disease and Alzheimer’s disease. The major problem in drug delivery to molecules and cells into the CSF is non-availability of the drug in the brain. The nasal route has a brain with the presence of the BBB that makes a promising approach for effective delivery of drugs to the target[6]. Macromolecular drugs like peptides and barrier that a systemically administered drug-protein, termed as “biologics” are too large and molecule encounters before entering the CNS is hydrophilic to penetrate the BBB from the systemic circulation. The major disadvantage with them is that they would be rapidly degraded by gastrointestinal enzymes or by the liver cytochromes if taken orally. It has been proved theoretically in the animal and human investigations that transport of exogenous materials directly from nose-to-brain is a potential route for by-passing the BBB. The drug transport from nose to CNS may occur via olfactory neuroepithelium and may involve paracellular or transcellular and/or neuronal transport. Olfactory pathway has potential to bypass BBB. Transport may occur via trigeminal nerve system also[7, 8, 9].

Drug absorption through the nasal mucosa

The first step in the absorption of the drug from the nasal cavity is a passage through the mucus. Small, unchanged particles easily pass through this layer. However, large or charged particles may find it more difficult to cross. Mucin, the principal protein in the mucus, has the potential to bind to solutes, hindering diffusion. Additionally, structural

changes in the mucus layer are possible as a result of environmental changes (i.e. pH, temperature, etc.). Subsequent to a drug's passage through the mucus, there are several mechanisms for absorption through the mucosa. These include transcellular or simple diffusion across the membrane, paracellular transport via movement between cell and transcytosis by vesicle carriers[10, 11].

Mechanism of the Olfactory nerve pathway

The olfactory epithelium is the entry gate for substance passing in the CNS and peripheral circulation. The neural connection between the nasal mucosa and the brain provide a unique pathway for providing direct access to the drugs to the CNS.

Possible transport pathway

Two mechanisms are involved in the nasal delivery of drugs to the CNS. First, a fast acting that depends on lipophilicity. Second, long-acting that depends on molecular weight [12].

Neuronal pathways

The neuronal connections between the nasal mucosa and brain provide a unique pathway for the non-invasive drug delivery of the drugs to the CNS, even to those drugs that do not cross BBB. The olfactory neuronal pathway provides an intraneuronal and extraneuronal pathway into the brain[43]. Besides, the trigeminal neural pathway may also be involved in the rapid delivery of protein therapeutic agents, such as insulin-like growth factor-1 to the brain and spinal cord following intranasal administration[13].

MATERIALS AND METHODS

Risperidone was obtained as a gift sample from Cipla, Mumbai, GENU Pectin (LM) was obtained from CP Kelco, and all other chemicals and Excipients were used of analytical grade.

IR spectral analysis

A weighed amount of the drugs Risperidone i.e. 1 mg were mixed thoroughly with potassium bromide (dried at 40°-50°C) which was then compressed under 10-ton pressure under the hydraulic press to form a pellet which was then scanned from 4000–400 cm-1 using FTIR spectrophotometer[14, 15 & 16].

Differential Scanning Colorimetry (DSC)

DSC determines the thermal change events of a given material by measuring enthalpy and onset of the thermal event, or by determining the melting point of the crystalline material and glass transition temperature of the amorphous material using Mettler Toledo. A weight between 3.5 to 4 mg was placed into an aluminum pan (40 µl) and the pan was sealed using the sealing press apparatus. The samples were heated at a rate of 10°C/min, and the scanning was performed between 35 and 300°C [17, 18].

X-Ray Diffraction Study

Physical states of Risperidone microspheres were determined by X-ray diffractometry (Brucker AXS D8 Advance). The powder samples were spread on metal sample holders, and glass slide was used to press and smooth the powder

surfaces. The diffraction intensity was recorded at 2-theta and a run time was 20 min. The current and voltage generator was set at 35 mA and 40 kV, respectively [19, 20]

Preparation of microspheres by spray drying

Formulations of Risperidone along with Pectin were formulated in three different ratios as shown in Table No. 1 respectively. The feed solutions in definite proportion were prepared by dissolving the polymer in distilled water with gentle heating using a magnetic stirrer to enhance the dissolution and the drugs were dissolved/dispersed in this polymeric solution. The microspheres were obtained by spraying the feed with a spray dryer (LU222, Labultima, India). The feed solution was atomized under 0.7 mm nozzle size, and inlet air temperature was set at 120oC. The outlet temperature was varying between 80 to 90oC. The feed rate was 3-7 ml/min, atomizing air flow rate and aspiration rate was kept at 357 L/h and 45% respectively. After evaporation of the solvent from atomized droplets, dried microparticles passed through the cyclone and were collected in the collecting vessel by the effect of gravity. The dried microspheres were collected from the apparatus collector [21, 22]. Similarly blank microspheres were prepared by dissolving the polymer seperately in distilled water with gentle heating using a magnetic stirrer and the microspheres were obtained by spraying the feed with a spray dryer by keeping processing parameters same.

Response Surface Methodology (RSM) is also widely employed to optimize formulations with the suitable experimental design because it permits a deeper understanding of a process or product and has important applications in establishing the robustness of that product. Full factorial designs, which have been widely used in response surface modeling and optimization. (Box, Wilson, 1951) [23]. RSM was used to establish the relative importance of two or more factors and also to indicate whether or not interaction occurs between the factors and thereby affects the magnitude of the response. The data were interpreted using response surface methodology (Design Expert Software Version 11.0.1.0, Stat-Ease, Inc.).

Validation of Results Based On Desirability

Validation of Results Based on Desirability was performed on the basis of results obtained for Drug loading, Entrapment efficiency and Particle Size for microspheres of Risperidone using Design Expert Software Version 11.0.1.0, Stat-Ease, Inc., and prediction of results was found. Overall desirability value is an indicator of the optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desired target response of independent variables[24].

Characterizations of Risperidone Mucoadhesive microspheres

Production Yield

The production yields of microspheres of various formulation batches were calculated using the weight of the final product after drying (practical mass) with respect to the initial total weight of the drug and polymer used for the preparation of microspheres (theoretical mass) and percent production yields according to the formula cited below [25],

$$\% \text{ Production Yield} = \frac{\text{Practical Mass (Microspheres)}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100$$

Drug Loading

The total amount of the drug within the microspheres was estimated by adding the weighed amount of microspheres in distilled water and kept overnight. The solution was then filtered through microfilter paper and quantified using

U.V Spectrophotometer (UV, Shimadzu,1800) to determine the drug loading (DL) present within the microspheres using equation[26].

$$\text{Drug Loading \%} = \frac{\text{wt of drug loaded in Microspheres}}{\text{Total weight of Microspheres}} \times 100$$

Particle size Analysis

The morphology and particle sizes were determined in a Motic, B1 Series, Systemic Microscope and computer controlled image analysis software (Motic images plus, 2.0 version). The microspheres were dispersed on a microscope slide. A microscopical field was scanned by a video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined m). The average particle diameter (particle size) was determined by using the Edmondson's equation[27].

$$\text{Average diameter} - \frac{\Sigma nd}{\Sigma n}$$

Where n = number of microspheres observed, d =Mean size range.

Entrapment Efficiency

A weighed amount of microspheres of Risperidone were separately dissolved in distilled water and kept overnight, then vortexed for 1 minute to extract the entrapped drug. The solution was then filtered through microfilter paper and quantified using U.V Spectrophotometer at 231 nm for 280 nm for Risperidone respectively [28].

$$\text{Percentage drug incorporation efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Swelling Property

The swelling ability of mucoadhesive microparticles formulations intended mucoadhesion in intranasal delivery. The swelling capacity of the microspheres was calculated by measuring the extent of swelling of microspheres to their equilibrium in phosphate buffer (pH 6.6). The experiment was performed by dispersing microsphere formulations (10 mg) on Millipore filter (0.22 µm) placed on a Franz diffusion cell (16 ml capacity) filled with buffer solution and kept for 3.5 min to ensure complete equilibrium of swelling. The degree of swelling was calculated as per following equation[29].

$$\alpha = \frac{Ws - Wo}{Wo} \times 100$$

Where α is a degree of swelling, Wo is the weight of microspheres before swelling and Ws is the weight of microspheres after swelling.

Scanning Electron Microscopy (SEM)

The surface morphology of microspheres was studied using a scanning electron microscopy (SEM, JSM 6390, JEOL DATUM Ltd., Tokyo, Japan). The powder of microspheres was dusted onto double-sided tape on an aluminum stub and coated with gold using a cold sputter coater in SEM chamber to a thickness of 400A°, and then photomicrographs were captured by operating at an accelerating voltage of 15 kV electron beam[30].

In-vitro mucoadhesion study

A freshly cut piece of sheep nasal mucosa (2cm^2) was obtained and then cleaned by washing with an isotonic saline solution. Accurately weighed amount of microspheres were placed on a mucosal surface which was fixed over polyethylene support at an angle of 45^0 relative to the horizontal plane. The mucosa was washed with pre-warmed (37^{0}C) phosphate buffer pH 6.6 at the rate of 5 ml/min. After 1h on the administration of microspheres, the concentration of drug in collected perfusate was determined by spectrophotometrically. The amount of microsphere corresponding to the drug amount in perfusate was determined. The amount of adhered microspheres was estimated as the difference between the applied microspheres amount and flowed microspheres amount [31].

$$\text{In vitro mucoadhesion}(\%) = \frac{\text{Amount of drug in washout liquid}}{\text{Actual amount of drug in applied microspheres}} \times 100$$

In vitro drug diffusion studies

An in vitro drug release test of the microspheres was performed using Franz diffusion cells with a dialysis membrane (cut-off Mol. Weight.12000). In which the donor compartment contained the microspheres while the receptor compartment was filled with phosphate buffer solution of pH 6.6 that was within the pH range in the nasal cavity. The donor chamber was placed in such a way that it just touched the diffusion medium in the receptor chamber. The temperature was maintained constant at 37 ± 0.5^0 C with the help of a circulating water bath. The microspheres equivalent to 15 mg of drug was dispersed on donor compartment and 3 ml of simulated nasal fluid was placed on it. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh pre-warmed buffer solution, and assayed using a UV spectrophotometer (UV 1800, Shimadzu, Japan)[32].

Ex-vivo permeation study

Drug permeation through nasal mucosa was studied for optimized formulation using Franz diffusion cell with sheep nasal mucosa. In this, the nasal mucosa was fixed between the donor and receptor compartment of the Franz diffusion cell. The weighed quantities of microspheres were placed in donor compartment and 3 ml SNF was added to it. The receptor compartment was filled with phosphate buffer solution (pH 6.6) at 37±0.5^{0}C. Samples were periodically withdrawn for 4 hours from the receptor compartment, replaced with the same amount of fresh pre-warmed buffer solution, and assayed using a UV spectrophotometer (UV 1800, Shimadzu, Japan)[33, 34]

Drug release kinetics

In order to investigate the drug release mechanism from microspheres, the drug release data were analyzed with following mathematical models and interaction of diffusion release mechanism.

Zero order kinetics $Q = Q_o - K_o t$ ……Eq .No 1

First order kinetics $Q = Q_o (1 - e^{-K_1 t})$ ……...Eq .No 2

Higuchi square root model $Qt = K_H t^{½}$ ……Eq .No 3

Hixson-Crowell cube root model $\sqrt[3]{Qo} - \sqrt[3]{Qt} = K_{HC} t$ ……Eq .No 4

Korsmeyer- peppas model $Qt/ Q_\infty = K_k t^n$……Eq .No 5

Where, Qt =amount of drug release d at time t.
Qo = initial amount of drug.

And Ko, K_1, KH, $K_{HC,}$ and K_K are the coefficients of equations. The most appropriate model was selected on the basis of regression values (r^2) and diffusion release exponent (n). Where value n is the release exponent characterizes the release mechanism of the drug. The release exponent 0.45 = n corresponds to a Fickian diffusion mechanism, 0.45 < n < 0.89 to non- Fickian transport, n = 0.89 to Case II (relaxational) transport and n > 0.89 to super case II transport. Drug release kinetics and best fit model for all the selected batches was found out with the help of Microsoft Excel. (Costa, Lobo, 2001; Samaha, Shehayeb, 2009; Yuksel, kanik, Baykaran, 2000, Costa FO 2003 et al) [35,36,37,38].

Stability Studies

The objective of stability study is to check how the quality of the drug product changes with time under the influence of elevated conditions of temperature, humidity, and light. The optimized batch was also subjected to long-term stability studies of 6 months. The vials filled with microspheres were sealed with rubber caps and kept under ambient temperature and moisture conditions (40ºC and 75% RH) for a period of 6 months in a stability chamber (CHM-10S, Remi Instruments, Mumbai, India). The samples were evaluated for Particle Size Size (µm), Swelling % and Mucoadhesion Potential % at the 1-month interval, using the same methods as mentioned earlier[39].

Histological study

The freshly isolated nasal mucosa was taken immediately after sacrificing a healthy sheep from the local slaughterhouse. The nasal mucosa was sectioned into pieces and cleaned by washing with a normal saline solution and treated for 8 hours with drug loaded-microspheres.Untreated piece of sheep nasal mucosa was used as a control. Samples were taken out and washed with NaCl (0.9%) and the tissue was fixed in 10% buffered formalin and embedded in paraffin wax for 4 h. Paraffin sections 7-5 mm were cut onto glass slides and stained with hematoxylin and eosin. Sections were examined by light microscopy to detect any damage during incubation. Examination of the tissue included the observation of all essential components of the respiratory epithelial cells such as goblet cells, ciliated cells, mucosal and submucosal layers, sero-mucinous glands. The possible epithelial necrosis was examined and sloughing of the epithelial cells and inflammatory cells were also studied [40].

RESULTS AND DISCUSSION

Drug-Polymer interaction study

From the FTIR spectra of Risperidone, Pectin and their formulation, in Figure 1, 2 and 3 it was found that there is no functional group change when Risperidone reacts with other Excipients. So they are found to be compatible with each other. The following characteristic peaks were observed for pure Risperidone: 2757.90 and 2360.04 cm^{-1} (aliphatic C-H stretching); C=O stretching (1641 cm^{-1}), 1534 cm^{-1} (C=N stretching, 1448 cm^{-1} (aromatic C=C stretching); CF stretching (1351 cm^{-1}), 817 cm^{-1} (C-Cl stretching). The presence of prominent peaks of pure Risperidone in thermograms as well as in selected Risperidone loaded microspheres reveals the compatibility between drug and carrier.

Differential Scanning Calorimetry

The DSC analysis was carried out to identify the compatibility between the drug and excipients. The DSC analysis of pure drug, a physical mixture of drug excipient was carried out using mettler Toledo. Samples (2-8 mg) were

accurately weighed and heated in sealed aluminum pans at a rate of 10º/ min between 0-300 ºC temp ranges under a nitrogen atmosphere. The thermogram of the pure drug figure 4 showed an endothermic peak at 172.71 º C. The physical mixture of drug and excipient shows a peak at 171.67 ºC. Results are shown in figure 5 and 6.

XRD Study Risperidone Microspheres

In order to characterize the physical state of the drug whether amorphous or crystalline before and after formulating it in floating microparticles, x-ray diffraction study was performed. The diffractograms of the pure drug, pure polymers and formulated microparticles Figure 7, 8 and 9 were performed. The diffraction spectrum of pure Risperidone showed that the drug is crystalline in nature as demonstrated by numerous distinctive peaks. The polymorphic structure of a drug is an important parameter that influences the dissolution rate and bioavailability of the drug. The prominent drug peak of high intensity at a 2θ value of 21.024 and peaks of lower intensities at 2 θ values of 14.032, 18.648, 19.532, 22.999 and 28.58 is in agreement with x-ray data found for Risperidone. Pectin diffraction patterns show no characteristic peaks indicating that they are amorphous in nature. The XRD profile of the physical mixtures Figure 9 is simply the superimposition of those of the pure components, demonstrated the crystalline peaks for Risperidone corresponding to 2 θ value of 21.024 and peaks of lower intensities 2 θ values at 14.032, 18.648, 19.532, and 22. This data leads to the fact that the drug maintained its crystalline form in the physical mixture which means that the presence of Risperidone in the drug-loaded microspheres has no influence on its physical state.

Characterization of mucoadhesive microspheres

Particle Size Analysis

The mean particle size of microspheres ranged from 11 to 22 μm (Table 2), indicating a narrow size distribution. Such particle size and narrow size distribution were considered favorable for intranasal administration. It was noted that increasing the concentration of polymer slightly increased the particle size of microspheres. Increase feed-flow rate also contributed to the increased particle size but not much significantly and this may be due to the narrower range being selected to focus this parameter. .

Production Yield

The yield of production was found in the range between 13–24 % (Table 2). These relatively low values may be due to the low quantity of feed used for the preparation of each batch and by the structure of the spray-dryer apparatus that lacked a trap to capture the smallest and lightest particles.

Entrapment Efficiency

Entrapment efficiency was found to be very high for spray drying technique. All microspheres had good entrapment efficiency of 50–68 % (Table 2). With increasing drug-polymer ratio, the entrapment efficiency was also found to be increased and this could be attributed to the higher availability of the drug to encapsulate the drug (Jain et al., 2009; Mahajan and Gattani, 2009) as the feed rate increased the entrapment efficiency decreased.

Drug Loading

The % drug loading found was in between 10 to 16.22 %. It was found that the drug loading goes on decreasing as the concentration of the polymer increases from R1 batch to R9 batch. As the feed rate was increased from 3 ml/min to 7 ml/min the drug loading may be decreased. The results are shown in table 2.

In-vitro mucoadhesive study

The mucoadhesive potential of Risperidone intra nasal microspheres was evaluated by in vitro method. The retention times showed by Risperidone intra nasal microspheres were 78.55 ± 1.10 to 87.43 ± 1.01, respectively. It was found that with increasing amount of pectin from batch R1 to R9 the mucoadhesive strength of microspheres was increased. From the results, it is clear that the microparticles remain adhered for a prolonged period. Thus it was hypothesized that the developed mucoadhesive preparation is able to increase the contact time between the dosage form and mucosal layers of nasal cavities. The results are shown in table 2.

Swelling Property

The swelling of the polymers is studied by their ability to imbibe water and swell enormously. In the present study polymer used in the formulation, Pectin has been reported to show good swelling properties. These polymers showed good swelling properties ranging from 0.597±0.08 to 0.744±0.16. This increase in swelling was possible only due to imbibitions and mucilage formation of polymers when it comes in contact with biological and or aqueous medium and due to which swelling took place. The results are shown in Table 2.

ANOVA Analysis of the Drug loading, Particle Size, and Entrapment efficiency

Evaluation and interpretation of research findings are important and the p-value serves a valuable purpose in these findings. ANOVA for the dependent variables Polymer Concentration (X_1) and Feed rate (X_2) was done. The coefficients of X1 and X2 were found to be significant at $p<0.05$, hence confirmed that both the variables have a significant effect on the selected responses. Overall both the variables caused a significant change in the responses. ANOVA and response surface analysis were done using Design Expert Software Version 11.0.1.0, Stat-Ease, Inc.

Response Surface Analysis

The quadratic model obtained from the regression analysis was used to build a 3-D graph in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots presented in Figures 10, 11, 12, 13, 14, 15 and 16. Contour plots (Figure 10, 12 and 14) are two-dimensional representations of the responses for the selected factors and shows that as the concentration changes of both the variables the Drug loading, Particle Size and Entrapment efficiency also varies. i:e, as the concentration goes on increasing the particle size, is increased. Three dimensional (3-D) surface plots (Figure 11, 13, and 15) for the obtained responses were drawn based on the model polynomial functions to assess the change of the response surface.

These plots explain the relationship between the dependent and independent variables i:e the effects of two factors on the response at one time. The response surface analysis for Polymer Concentration (X_1) and Feed rate (X_2) was studied which showed significant results. The Model F-value of Drug loading (371.42), Entrapment efficiency (242.64) and Particle Size (1210.07) for Polymer Concentration (X_1) and Feed rate (X_2) implies the model is significant. Values of "P" less than 0.0500 indicate model terms are significant. The R^2 of 0.998 for Drug loading, 0.0.997Enrapment efficiency for and 0.999 for Particle Size is in reasonable agreement with the "Adj R-Squared" of 0.995 for Drug loading, 0.993 Entrapment efficiencies and 0.998 for Particle Size. The probability value i:e P-value found was also less than 0.0500. This model can be used to develop the design. The values are shown in Table 3,4,5,6,7 and 8.

Final Equation in Terms of Coded Factors for % Drug Loading, % Entrapment Efficiency and Particle Size the information conveyed was

1) R^2 was high indicating the adequate fitting of the Quadratic Model.
2) As all the three factors showed (+ ve coefficient) showed +ve sign it also indicated that the prepared

microspheres gained optimum % Drug Loading, % Entrapment Efficiency, and Particle Size.

Scanning Electron Microscopy (SEM) of Risperidone loaded Microspheres

Regarding the morphology of the prepared microspheres, SEM photomicrographs of Risperidone-loaded microspheres are displayed in Figure 17 A, B, C. The drug-loaded microspheres based on spray-drying method showed the regular shape and smooth surface with no free drug.

In vitro drug diffusion studies

The in vitro release study of Risperidone loaded microspheres was carried out using Franz diffusion cells with a dialysis membrane (cut-off Mol. Weight.12000). On the basis of results of other parameters only R1 batch was taken for in vitro drug diffusion study. R1 batch showed Zero order release of drug and thus considered it as an optimized formulation. The release profile of Risperidone loaded from R1 batch of microsphere at pH 6.6 phosphate buffer is shown in table 9. The release pattern of the optimized formulation (R1) revealed that drug was released by zero order from microspheres showing consistent release.

Drug release kinetics

Kinetic study of drug release is often useful in obtaining one or two physically meaningful Parameters which are employed for comparative purposes and relating the release parameter. Moreover, a kinetic parameter can be used to study the influence of formulation factors on the drug release for statistical optimization. The drug release kinetics was studied by plotting the data obtained from the *in-vitro* drug release in various kinetic models. To establish the mechanism involved in drug release from the tablets, data of percentage drug release versus log time were plotted according to Korsmeyer–Peppas equation as drug release exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight line was found to be n= 0.674. If the exponent n= 0.45, then the drug release follows the Fickian diffusion and if 0.45< n <0.85 then it is said to be non-Fickian or anomalous release. The mechanism of release for the above formulations was determined by finding the R^2 value for each kinetic model viz. zero-order, first-order, Higuchi, and Korsmeyer–Peppas corresponding to the release data of each formulation. From most of the formulations, the R^2 value of Zero order plot is very near to one than the R^2 values of other kinetic models. Thus, it can be said that the drug release follows Zero order plot mechanism and n value was found **n** = 0.674 hence it can be postulated that formulation R-1 followed non-Fickian or anomalous release. The results are shown in Table 10 and Figure 19, 20, 21, and 22.

Ex-vivo permeation study

Permeation study was performed in Franz diffusion cells, using the sheep nasal mucosa. The percentages of cumulative permeation of the Risperidone incorporated into formulation were plotted against time in table 11. The results obtained revealed more tissue permeation of microsphere. More permeation may be because of the small size of the microsphere. At 240 min the permeation was found 81.15%.

Histological study

The morphologic changes in the nasal mucosa caused by drugs, enhancers, or other formulation additives, may result in damage to the ability of the nasal mucosa to carry out its normal defense functions. In addition, chronic infection may occur when recovery or regeneration of the normal epithelium cannot be achieved. Thus, it is important to study the histology of the nasal mucosa with the formulation. The histology of control and treated nasal mucosa is shown in Figure 24. The microscopic observations indicated that the formulation has no significant effect on the microscopic structure of sheep nasal mucosa. The surface epithelium lining and the granular cellular structure of the nasal

mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged. Neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after diffusion study as compared with control mucosa treated with phosphate buffer pH 6.6. Thus, the microsphere formulation seems to be safe with respect to nasal administration.

Accelerated stability studies

Formulation sample (R 1) showing optimum particle size, Swelling and mucoadhesion were subjected further to accelerated stability studies as per ICH [Q1A (R2)] guidelines at 40°C ± 2°C/75% RH ± 5% RH for a period of 6 months. The changes were negligible enough to conclude that the drug was retained within the microspheres and formulation was found to be stable throughout the stability period. The results are shown in table 12.

Validation of Results of Drug Loading, Entrapment Efficiency and Particle Size Based On Desirability

Overall desirability value is an indicator of the optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desired target response. From figure 16 for % Drug Loading, % Entrapment Efficiency and Particle Size it is clear that the results of % Drug Loading, % Entrapment Efficiency and Particle Size which were obtained from formulation has optimum concentration of polymers. (Design expert software version 11.0.1.0, Stat-Ease, Inc). The desirability found was 1 and hence it can also be concluded that results actually obtained matches with the software prediction and hence the formulation is also validated.

CONCLUSION

By considering the result of all the above mentioned factors and results it was concluded that out of all the nine batches, batch R1 was found to be the optimized batch as it has following optimum parameters which are required for intranasal microspheres, Particle Size 11.51 µm, Production Yield 21.33%, Entrapment Efficiency 60.12 %, Drug Loading 16.55 %, Mucoadhesive Strength 79.10, Swelling Index 0.612 and Response surface analysis was also found significant for all the three factors. It was also concluded that all the objectives o the formulation were meet.

REFERENCES

1. Bijal Prajapati, Rakesh Patel and Dr. Abhay Dharamsi, 2017 Intranasal Drug Delivery System “An Overview”, ejpmr , 4 (07), 277-283.
2. Pagar Swati Appasaheb, Shinkar Dattatraya Manohar, Saudagar Ravindra Bhanudas, 2013 A Review on Intranasal Drug Delivery System, JAPER , Vol 3 Issue 4, 333-346.
3. Yıldız Ozsoy, Sevgi Gungor and Erdal Cevher, 2009 Nasal Delivery of High Molecular Weight Drugs, Molecules, 14, 3754-3779.
4. Rahisuddin, Pramod K Sharma, Garima Garg, and Mohd Salim, 2011. Review on Nasal Drug Delivery System with Recent Advancemnt, Int J Pharm Pharm Sci, Vol 3, 2, 611.
5. M. Parvathi, 2012 Intranasal Drug Delivery To Brain: An Overview, IJRPC, 2(3), 889-895.
6. P. Shabana, Brahmaiah Bonthagarala, Abbaraju Lakshmi Harini and Varun Dasari, 2015 Nasal Drug Delivery: A Potential Route for Brain Targeting, IJASR; 1(02): 65-70.
7. Siva Reddy P, Sreekanth Nama, P. Srihari, K. Sasikanth, Prasanna Kumar Desu, 2013 Nasal Drug Delivery: A Potential Route For Brain Targetting, IJPSR, Vol 3, Issue 2, 50-55.
8. Mitchel J. R. Ruigrok and Elizabeth C. M. de Lange, 2015 Emerging Insights for Translational Pharmacokinetic and Pharmacokinetic-Pharmacodynamic Studies: Towards Prediction of Nose-to-Brain Transport in Humans, The AAPS Journal,Vol. 17, No. 3, 493-505.

9. https://health.howstuffworks.com/medicine/medication/understanding-central-nervous-system-medications-ga.htm.
10. Ram Chand Dhakar, Sheo Datta Maurya, Vijay K Tilak, Anish K Gupta, 2011 A review on factors affecting the design of nasal drug delivery system, IJDD, 3 194-208.
11. Ram C Dhakar, 2011 Non-invasive Systemic Drug Delivery via Nasal Route: A Review, The Afr. J Pharmaceutical Sciences and Pharm; 2:1, 114-144.
12. S. Talegaonkar, P. R. Mishra, June 2004 Intranasal delivery: An approach to bypass the blood brain Barrier, Indian J Pharmacol, Vol 36, Issue 3, 140-147.
13. J. Born, 2002. Demonstrating Intranasal Delivery Of Neuropeptides to the CSF of Humans in Nature Neuroscience.:5(6):514-516.
14. Tae-Joon Park, Dong-Hyun Ko, Young Ju Kim and Yongae Kim, 2009. Polymorphic Characterization of Pharmaceutical Solids, Donepezil Hydrochloride, by 13 C CP/MAS Solid-State Nuclear Magnetic Resonance Spectroscopy Bull. Korean Chem. Soc., Vol. 30, No. 9.
15. Amani M. El Sisi, Rasha M. Kharshoum, Adel A. Ali, Khaled M. Hosny, Ahmed Abd-Elbary, 2015 Preparation and Evaluation of Risperidone Oro-dispersible Tablets, Int. J. Pharm. Sci. Rev. Res., 30 (2); Article No. 01, 1-6.
16. Shukla D, Chakraborty S, Singh S,Mishra B, 2009. Preparation and in-vitro characterization of Risperidone-cyclodextrin inclusion complexes as a potential injectable product, DARU, Vol. 17, No. 4, 226-235.
17. Bharat V. Jain and Dr. Neelesh Choubey, 2018. Effect Of Process Variables On Complexation Of Bitter Drug Using Ion Exchange Resins, IAJPS, 05 (02), 1219-1225.
18. Shukla D, Chakraborty S, Singh S, Mishra B, 2009 Preparation and in-vitro characterization of Risperidone-cyclodextrin inclusion complexes as a potential injectable product DARU Vol. 17, No. 4, 226-235.
19. Sanjay Patil, Anil Babbar, Rashi Mathur, Anil Mishra, and Krutika Sawant, 2010 Mucoadhesive chitosan microspheres of carvedilol for nasal administration, Journal of Drug Targeting, 18(4): 321–331.
20. Poonam Mogal, Deeliprao Derle, 2017. Use of Solid Dispersions and Inclusion Complexation for Enhancing Oral Bioavailability of Ziprasidone in Treating Schizophrenia, JDDMC; 3(3): 37-48.
21. S. S. Shah, D. Y. Gohil, D. N. Pandya, D. B. Meshram, 2015. Preparation and evaluation of spray-dried mucoadhesive microspheres for intranasal delivery of prochlorperazine using factorial design, AJP, 178-189.
22. R. P. Patel, M. P. Patel and A. M. Suthar, 2009 Spray drying technology: an overview, INDJST, Vol.2 No.10.
23. Box GPE, Wilson KB, 1951. On the experimental attainment of optimum conditions. J Royal Stat Soe Ser B. 13: 45.
24. Pawar H, Dhavale R, 2014 Development and evaluation of gastroretentive floating tablets of an antidepressant drug by thermoplastic granulation technique. BJBAS, 3: 122- 32.
25. Chandrakant V. Pardeshi, Pravin V. Rajput, Veena S. Belgamwar and Avinash R. Tekade, 2012 Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres as intranasal carriers for Valsartan, Journal of Microencapsulation,; 29 (2): 103–114.
26. Pankaj Nerkar & Surendra Gattani, 2012. Spray-Dried Buccal Mucoadhesive Microparticles of Venlafaxine Based on Cress Seed Mucilage: In Vitro, in Vivo Evaluation in Rabbits, Drying Technology: An International Journal, 30:9, 968-978, DOI: 10.1080/07373937.2011.607931.
27. Abhijeet D. Kulkarni, Deepak B. Bari, Sanjay J. Surana, Chandrakantsing V. Pardeshi, 2016 In vitro, ex vivo and in vivo performance of chitosan-based spray-dried nasal mucoadhesive microspheres of diltiazem hydrochloride, JDDST, 31, 108-117.
28. Mamdouh. Abdel Mouez, Noha M. Zaki, Samar Mansour, Ahmed S. Geneidi, 2014. Bioavailability enhancement of verapamil HCl via intranasal chitosan Microspheres, EJPS , 51, 59–66.
29. Desai Sapna, G. Vidyasagar, Desai Dhruv, 2010. Brain targeted nasal midazolam microspheres, Int J Pharm Biomed Sci, 1(2), 27-30.
30. Chandrakantsing V. Pardeshi, Veena S. Belgamwar, Avinash R. Tekade & Sanjay J. Surana, 2013. Novel surface modified polymer–lipid hybrid nanoparticles as intranasal carriers for ropinirole hydrochloride: in vitro, ex vivo and in vivo pharmacodynamic evaluation, J Mater Sci: Mater Med, 24:2101-2115.

31. Abbas Z, Marihal S, 2014. Gellan gum-based mucoadhesive microspheres of almotriptan for nasal administration: Formulation optimization using factorial design, characterization, and in vitro evaluation. J Pharm Bioall Sci, 6:267-77.
32. Baby H. Dandge and M H G Dehgha, 2009 Formulation And Evaluation Of Nasal Mucoadhesive Microparticles Of Diltiazem Hydrochloride, Int. Jornal Chem Tech Research, Vol.1, No.4, 1036-1042.
33. Flavia Chiva Carvalho, Michel Leandro Campos, Rosângela Gonçalves Peccinini, Maria Palmira Daflon Gremiao, 2013 Nasal administration of liquid crystal precursor mucoadhesive vehicle as an alternative antiretroviral therapy, EJPB, 84,219–227.
34. Vrushali Kashikar, Shashikant Dhole, Ujawala Kandekar, Prachi Khose, 2014. Study of mucoadhesive microsphere of pirfenidone for nasal drug delivery, AJP, 46-53.
35. Costa P, Lobo JMS, 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci., 13:123–133.
36. Samaha D, Shehayeb R, Kyriacos S, 2009. Modeling and Comparison of Dissolution Profiles of Diltiazem Modified-Release Formulations. Dissolut. Technol. 41- 46.
37. Yuksel N, Kanık AE, Baykara T, 2000. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and independent methods. Int. J. Pharm., 209: 57–67.
38. Costa FO, Sousa JJS, Pais AACC, 2003 Comparison of dissolution profiles of Ibuprofen pellets. J. Control. Release. 89:199-212.
39. Abhijeet D. Kulkarni, Deepak B. Bari, Sanjay J. Surana, Chandrakantsing V. Pardeshi, 2016 In vitro, ex vivo and in vivo performance of chitosan-based spray-dried nasal mucoadhesive microspheres of diltiazem hydrochloride, Journal of Drug Delivery Science and Technology, 31, 108-117.
40. Patil SB, Kaul A, Babbar A, Mathur R, Mishra A, Sawant KK, 2012. In vivo evaluation of alginate microspheres of carvedilol for nasal delivery Conflict of Interest: None. J Biomed Mater Res Part B: 100 B: 249–255.

Table 1: Formulation batches (RISPERIDONE: PECTIN)

Batch code	Feed rate (ml/min)	Risperidone (mg)	Pectin (mg)
R1 (1:2)	3	200	400
R2 (1:2)	5	200	400
R3 (1:2)	7	200	400
R4 (1:3)	3	200	600
R5 (1:3)	5	200	600
R6 (1:3)	7	200	600
R7 (1:4)	3	200	800
R8 (1:4)	5	200	800
R9 (1:4)	7	200	800

Table 2: Table showing various parameters like Average Particle Size, % Production Yield, EE (%), % Drug Loading, Mucoadhesion, Swelling index of Risperidone loaded microspheres batches

Sr. No.	Formulation Code	Average Particle Size* (µm±SD)	% Production Yield * (±SD)	EE (%)* (±SD)	% Drug Loading * (±SD)	Mucoadhesion* (%±SD)	Swelling Index* (% ± SD)
1	R1	11.51 ± 0.12	21.33 ± 0.43	60.12 ± 1.96	16.22± 0.93	79.10 ± 2.50	0.612±0.05
2	R2	15.00 ± 0.24	17.66 ± .028	48.78 ± 1.32	13.43 ± 0.64	78.55 ± 1.10	0.60±0.03
3	R3	18.60 ± 0.67	13.66 ± 0.63	44.42 ± 1.45	9.87 ± 0.37	80.11 ± 1.50	0.597±0.08
4	R4	15.93 ± 0.53	24.6 ± 0.53	65.23 ± 1.63	14.28 ± 1.37	80.74 ± 1.20	0.642±1.13
5	R5	18.60 ± 0.21	20.75 ± 0.31	53.60 ± 1.83	12.79 ± 0.73	84.56 ± 1.27	0.628±0.15
6	R6	22.01 ± 0.33	18.07 ± 0.18	50.20 ± 1.12	10.00 ± 1.52	84.21 ± 1.33	0.71±0.19
7	R7	16.73 ± 0.38	24.32 ± 0.15	68.22 ± 1.38	10.80 ± 1.76	86.88 ± 0.25	0.722±0.27
8	R8	19.20 ± 0.64	21.54 ± 0.53	55.24 ± 1.50	9.80 ± 1.23	85.34 ± 0.77	0.734±0.05
9	R9	21.79 ± 0.16	20.4 ± 0.56	50.23 ± 1.7	8.50 ± 0.53	87.43 ± 1.01	0.744±0.16

*Values expressed as Mean ± SD, n=3

Table 3: Response 1: (R1) Drug Loading

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	52.07	5	10.41	371.42	0.0002	significant
A-POLYMER CON	18.10	1	18.10	645.46	0.0001	
B-FEED RATE	27.86	1	27.86	993.87	0.0001	
AB	4.10	1	4.10	146.26	0.0012	
A^2	1.69	1	1.69	60.38	0.0044	
B^2	0.3120	1	0.3120	11.13	0.0445	
Residual	0.0841	3	0.0280			
Cor Total	52.15	8				

Table 4: Showing Standard deviation, mean and R- Squared for Drug loading

Std. Dev.	0.1674	**R^2**	0.9984
Mean	11.74	**Adjusted R^2**	0.9957
C.V. %	1.43	**Predicted R^2**	0.9863
		Adeq Precision	56.9315

Final Equation in Terms of Coded Factors

Table 5: Response 2: (R2) Entrapment Efficiency %

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	502.71	5	100.54	242.64	0.0004	significant
A-POLYMER CON	69.16	1	69.16	166.90	0.0010	
B-FEED RATE	395.61	1	395.61	954.74	< 0.0001	
AB	1.31	1	1.31	3.16	0.1733	
A^2	6.78	1	6.78	16.37	0.0272	
B^2	29.85	1	29.85	72.04	0.0034	
Residual	1.24	3	0.4144			
Cor Total	503.95	8				

Table 6: Showing Standard deviation, mean and R- Squared for Entrapment Efficiency %

Std. Dev.	0.6437	**R^2**	0.9975
Mean	55.12	**Adjusted R^2**	0.9934
C.V. %	1.17	**Predicted R^2**	0.9705
		Adeq Precision	43.8177

Final Equation in Terms of Coded Factors

ENTRAPMENT EFFICIENCE = +45.84+5.76 A-3.01B+3.05 AB+3.41 A^2+0.5350 B^2

Table 7: Response 3: (R3) Particle Size µm

Source	Sum of Squares	Df	Mean square	F-Value	p-value Prob > F	Observation
Model	88.80	5	17.76	1210.07	< 0.0001	significant
A-POLYMER CON	26.52	1	26.52	1807.09	< 0.0001	
B-FEED RATE	55.35	1	55.35	3771.15	< 0.0001	
AB	1.04	1	1.04	70.98	0.0035	
A^2	5.83	1	5.83	397.46	0.0003	
B^2	0.0539	1	0.0539	3.68	0.1510	
Residual	0.0440	3	0.0147			
Cor Total	88.84	8				

Table 8: Showing Standard deviation, mean and R- Squared for Particle Size

Std. Dev.	0.1211	**R^2**	0.9995
Mean	17.71	**Adjusted R^2**	0.9987
C.V. %	0.6840	**Predicted R^2**	0.9963
		Adeq Precision	105.0894

Final Equation in Terms of Coded Factors

PARTICLE SIZE=+18.74+2.10A+3.04B-0.5103AB-1.71 A^2+0.1642 B^2

Table 9: In-vitro Drug Release study of Risperidone loaded microspheres (Optimized Batch R1)

Time (minutes)	%Cumulative drug release (CDR) (%±SD)
0	0
15	12.88± 0.80
30	28.69± 1.34
60	36.38± 1.11
90	43.76± 1.82
120	52.26± 1.00
150	65.92± 1.03
180	79.38± 1.47
210	89.61± 1.86
240	96.07± 1.50

*Values expressed as Mean ± SD, n=3

Table 10: Drug release Kinetics for Best Fit Model for R-1 Batch

Formulation	Kinetic Models (R^2)			
R-1	Zero order	First order	Higuchi	Best fit model
	0.978	0.877	0.968	Zero order
	Korsmeyer Peppas equation			
R-1	R^2	K value	n value	Mechanism
	0.973	1.619	0.674	Case II diffusion

Table 11: Ex-vivo Drug Release study of Risperidone loaded microspheres (Batch R1)

Time (minutes)	%CDR (%±SD)
0	0 ± 00
15	11.19 ±0.18
30	24.84 ± 1.83
60	29.46 ± 1.56
90	37.57 ± 1.10
120	46.03 ± 1.75
150	57.69 ± 1.23
180	66.11 ± 1.63
210	73.61 ± 1.39
240	81.15 ± 1.80

Table 122: Stability parameters for Risperidone loaded optimized formulation sample (R-1)

Sr.No.	Time (Days)	Particle Size Size(µm)	Swelling %	Mucoadhesion Potential %
1	0	11.511	0.612±0.05	79.10 ± 2.50
2	30	11.511	0.612±0.05	78.69 ± 2.50
3	60	11.511	0.606±0.05	78.69 ± 2.50
4	90	11. 486	0.606±0.05	78.69 ± 2.50
5	180	11.470	0.600±0.05	80.69± 2.50

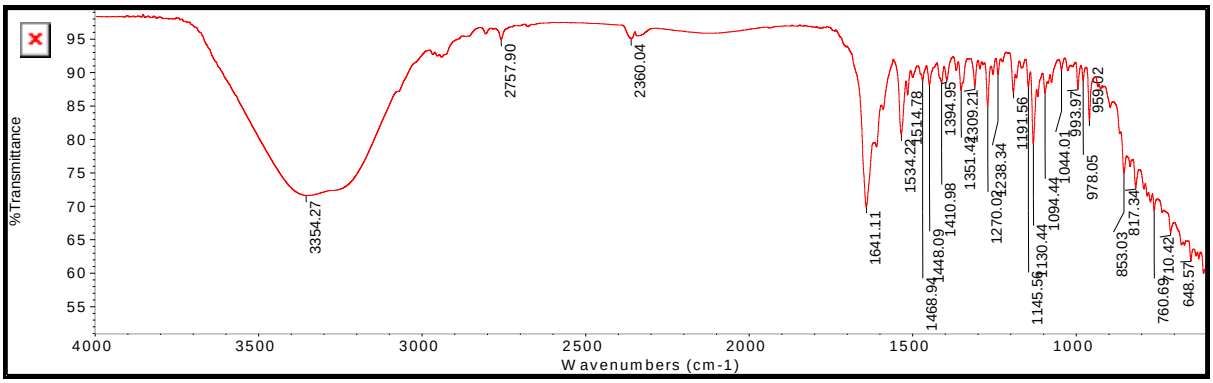


Figure 1: Infrared Spectroscopy of Risperidone

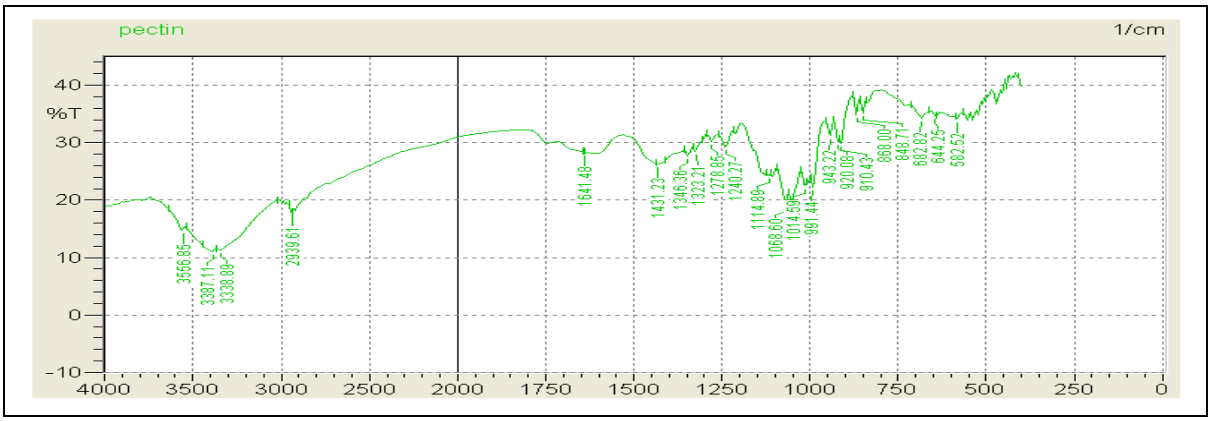


Figure 2: Infrared Spectroscopy of Pectin

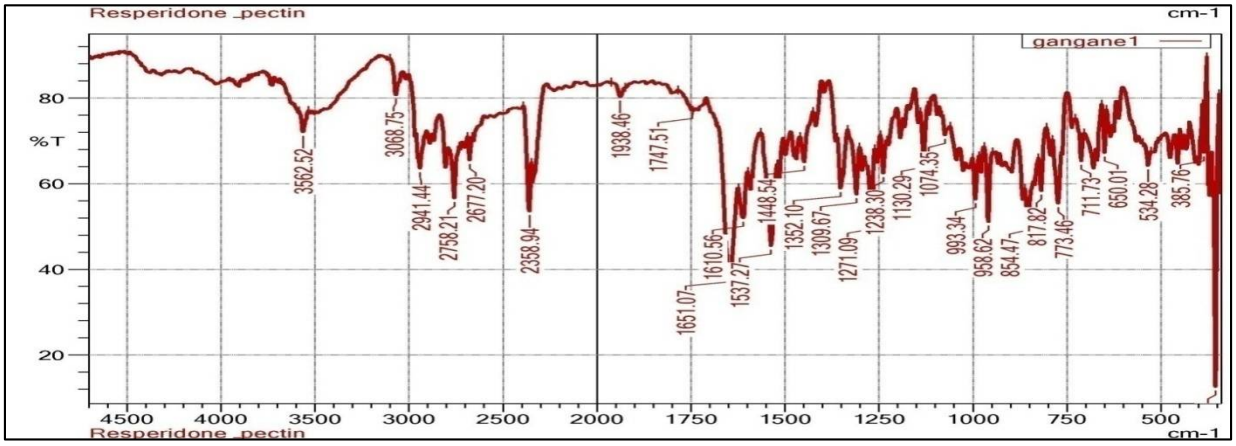


Figure 3: Infrared Spectroscopy of Risperidone and Pectin physical mixture

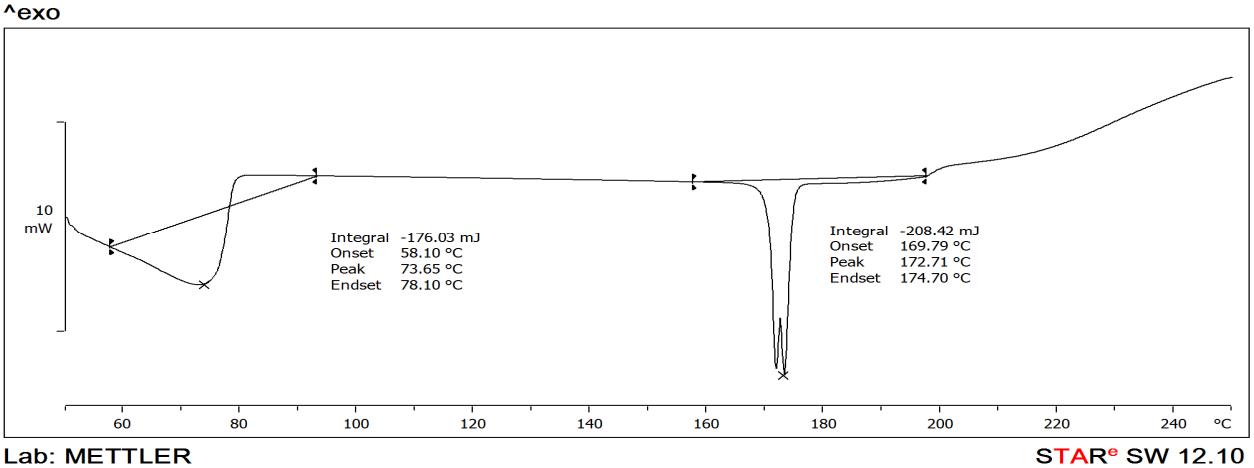


Figure 4: DSC of Risperidone

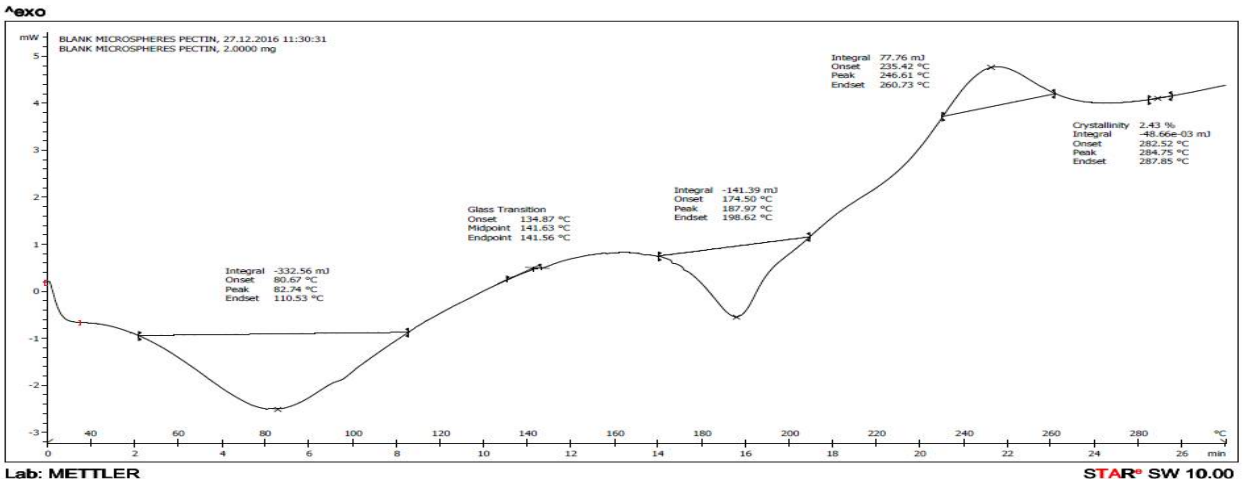


Figure 5: DSC of Blank Pectin Microspheres

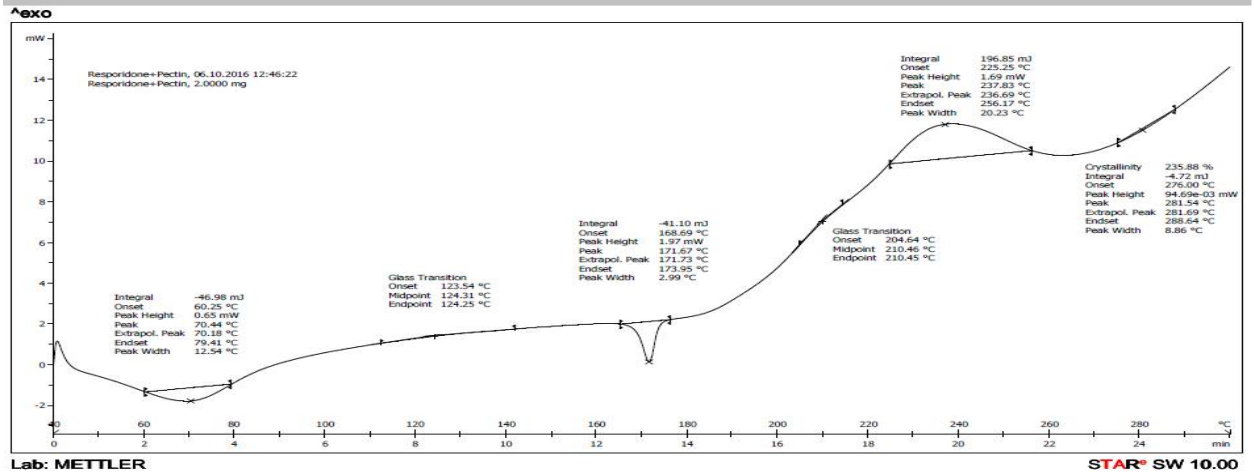


Figure 6: DSC of Risperidone loaded microspheres batches

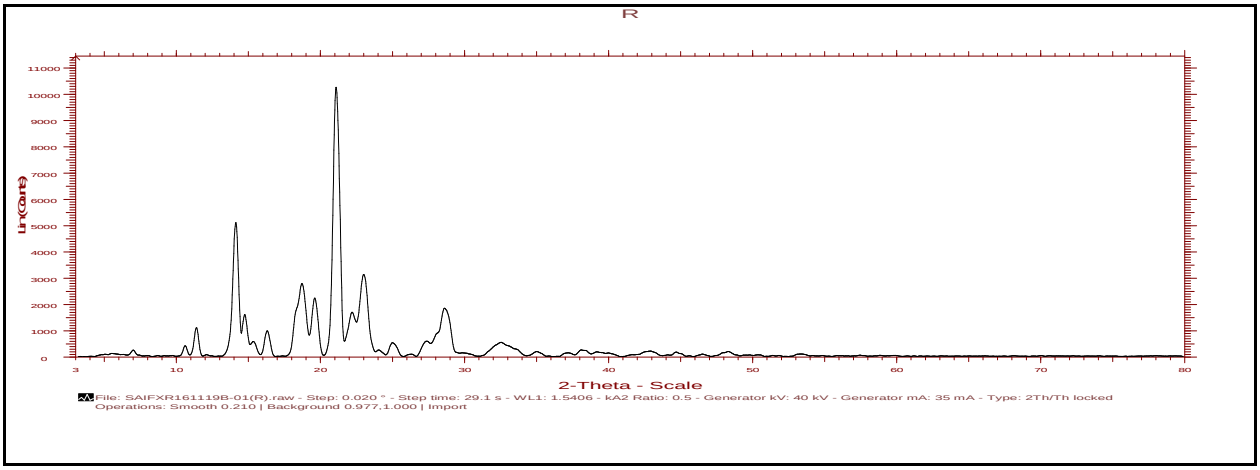


Figure 7: X-Ray Diffractograms of Risperidone

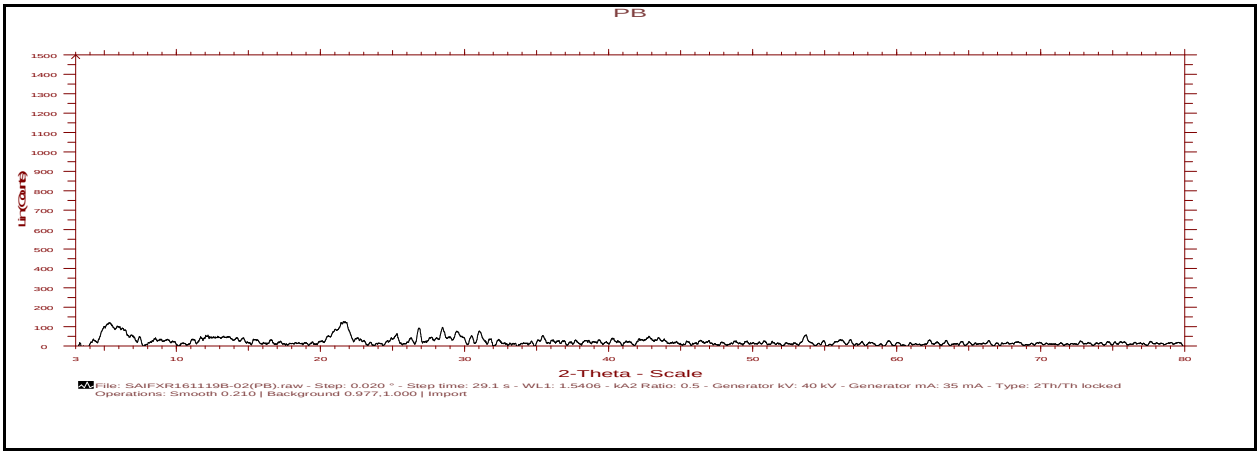


Figure 8: X-Ray Diffractograms of Pectin (Blank) Microspheres

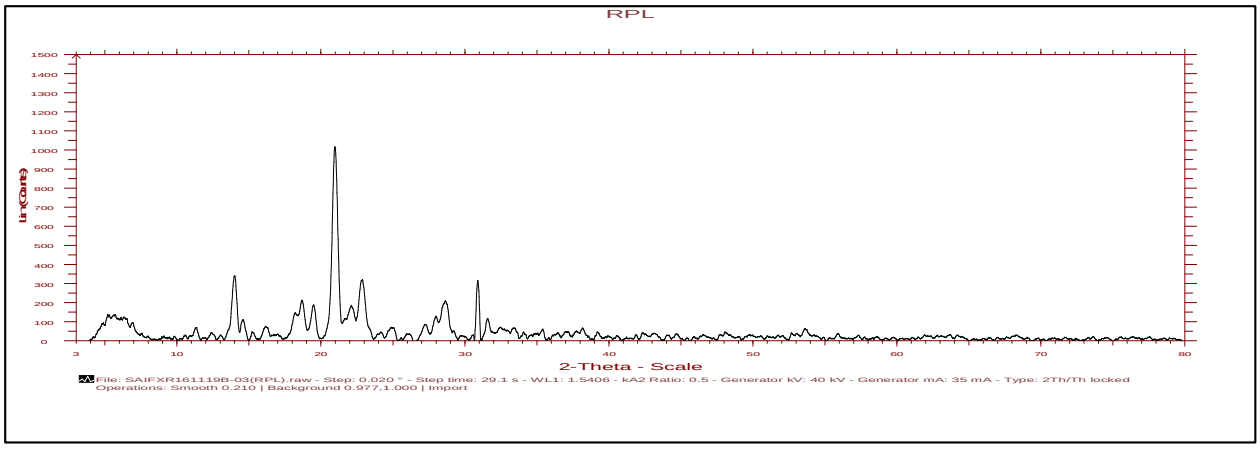


Figure 9: X-Ray Diffractograms of Risperidone loaded microspheres batche

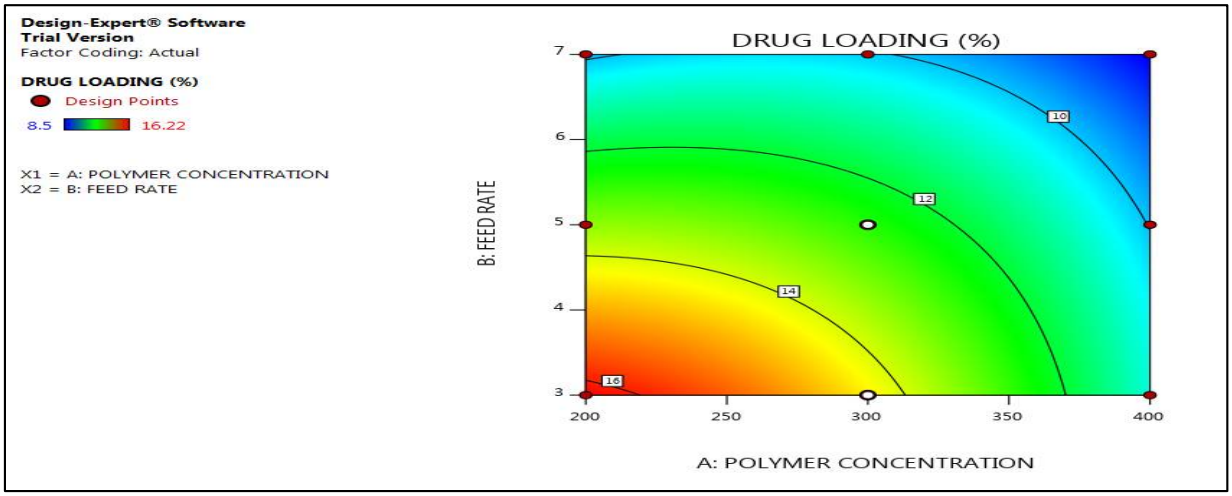


Figure 10: Response 1: (R1) Drug Loading % Counter Plot

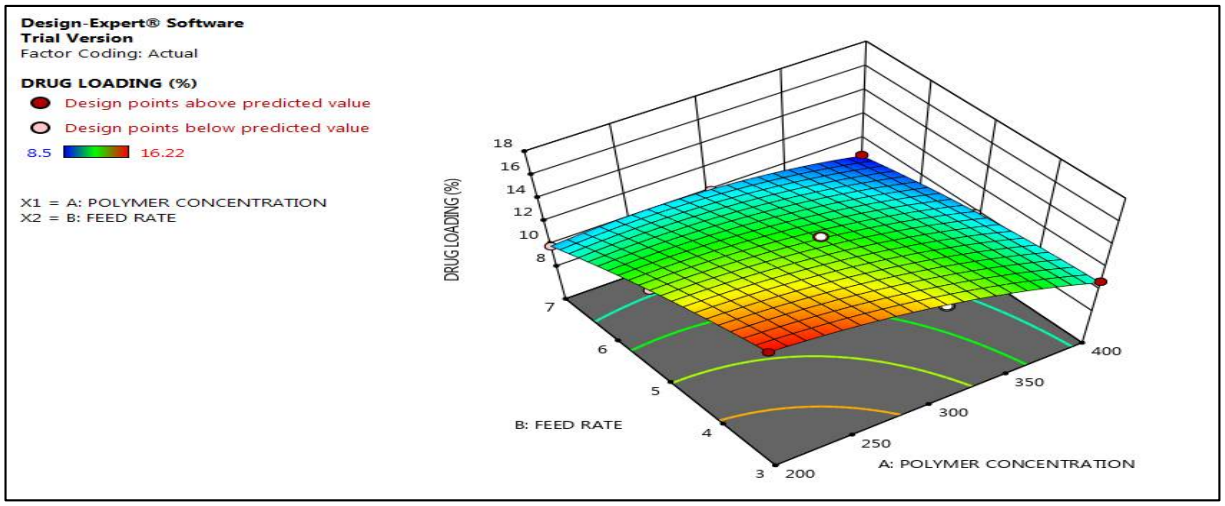


Figure 11: Response 1: (R1) Drug Loading % 3D Graph

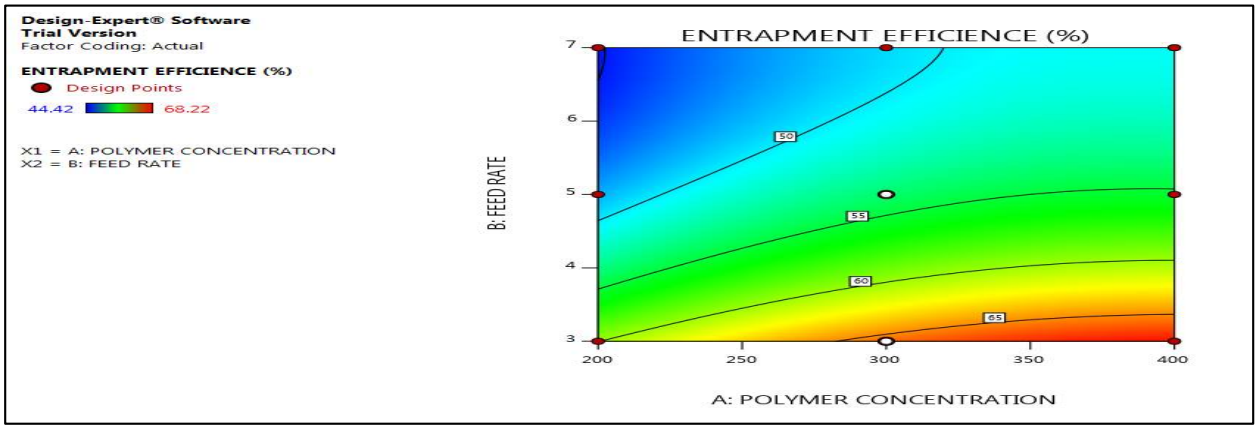


Figure 12: Response 2: (R2) Entrapment Efficiency % Counter Plot

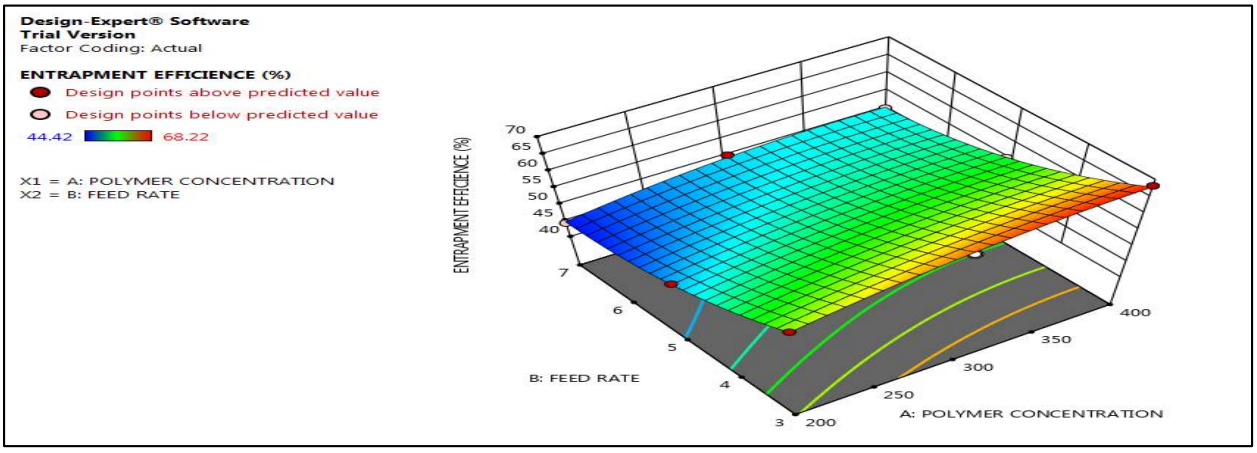


Figure 13: Response 2: (R2) Entrapment Efficiency % 3D Graph

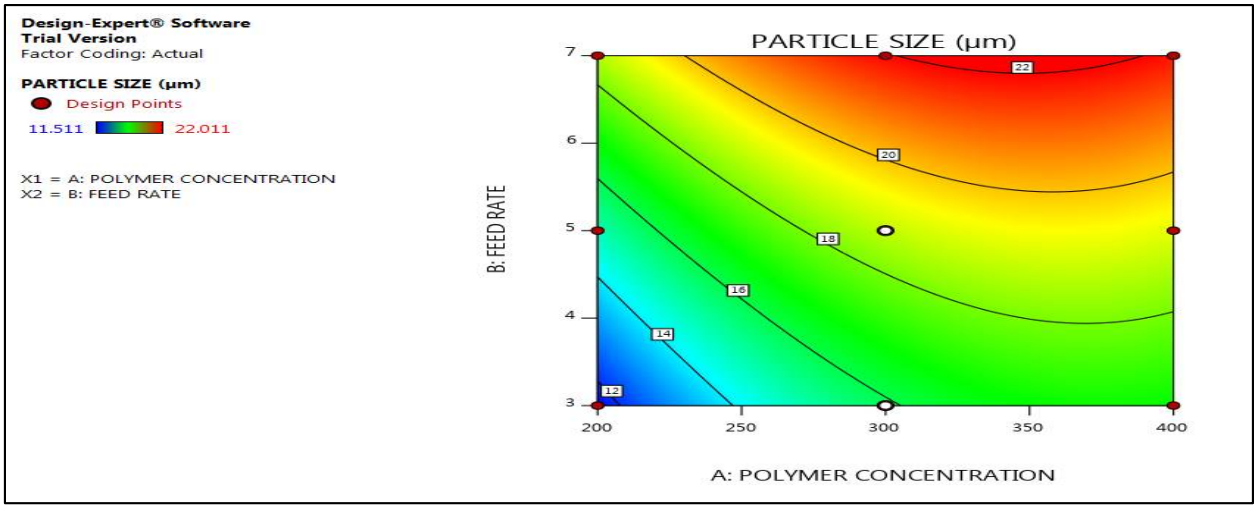


Figure 14: Response 3: (R3) Particle Size Counter Plot

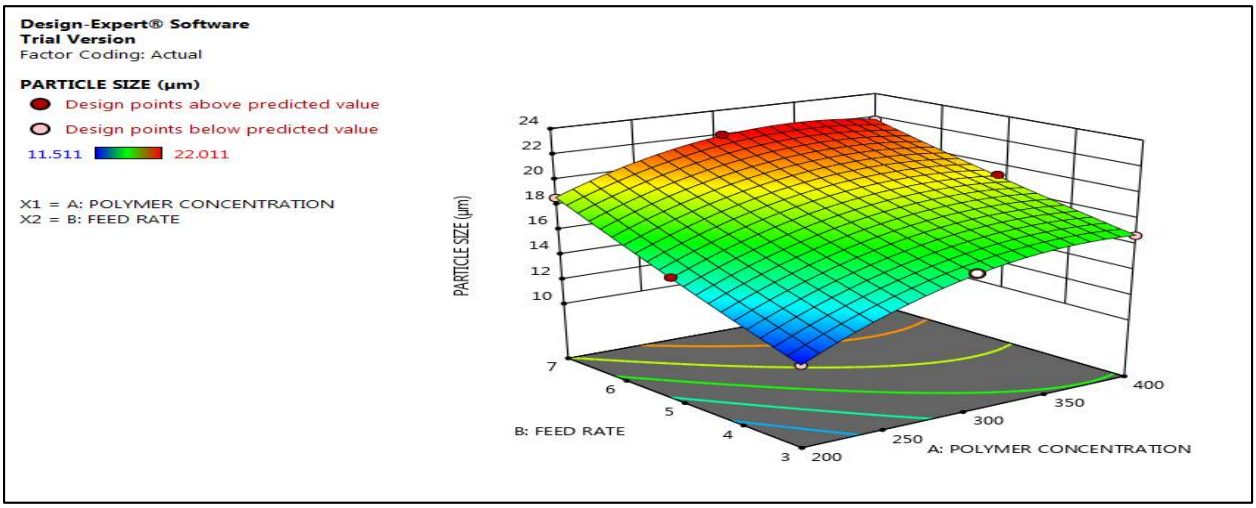


Figure 15: Response 3: (R3) Particle Size 3D Graph

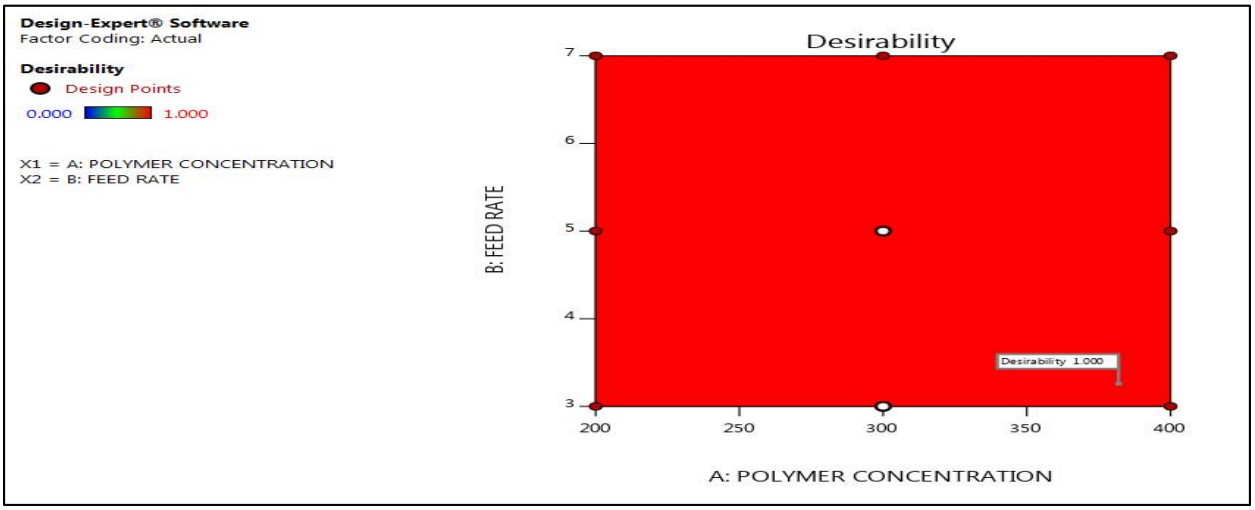
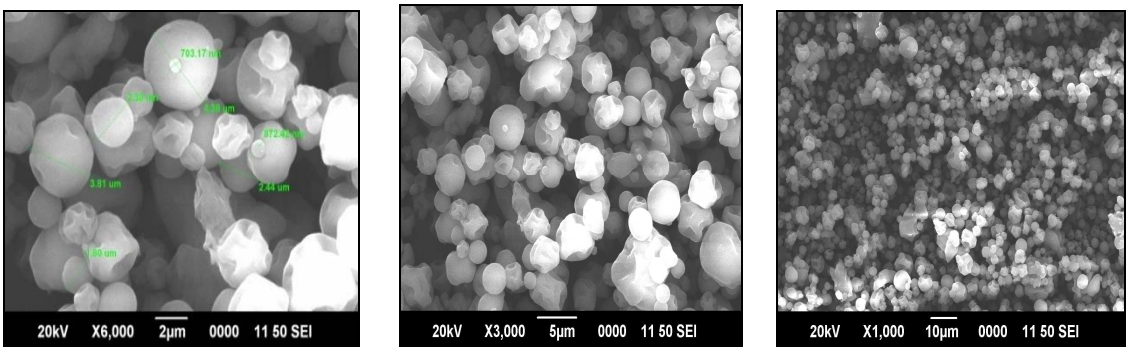


Figure 16: Desirability for all the three responses.



(A) (B) (C)

Figure 17: Scanning Electron Microscopy (SEM) of Risperidone Microspheres

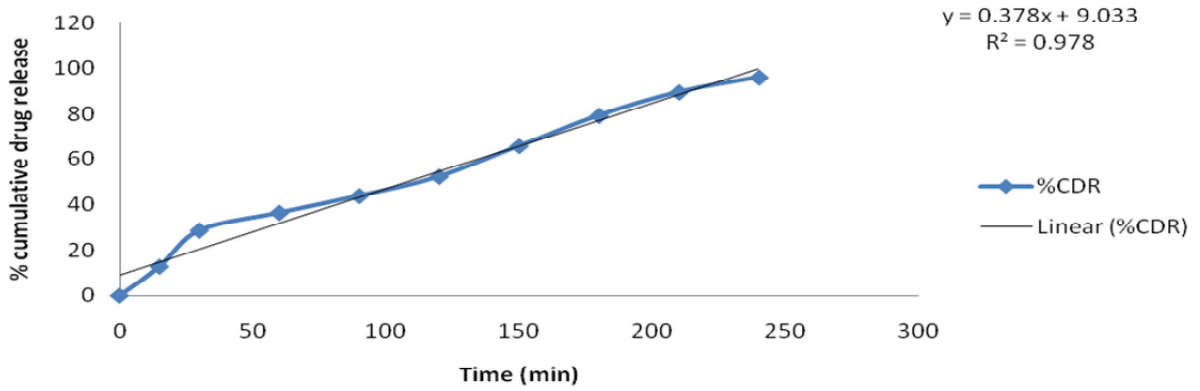


Figure 18: Zero order Plot of Risperidone loaded microspheres (Batch R-1)

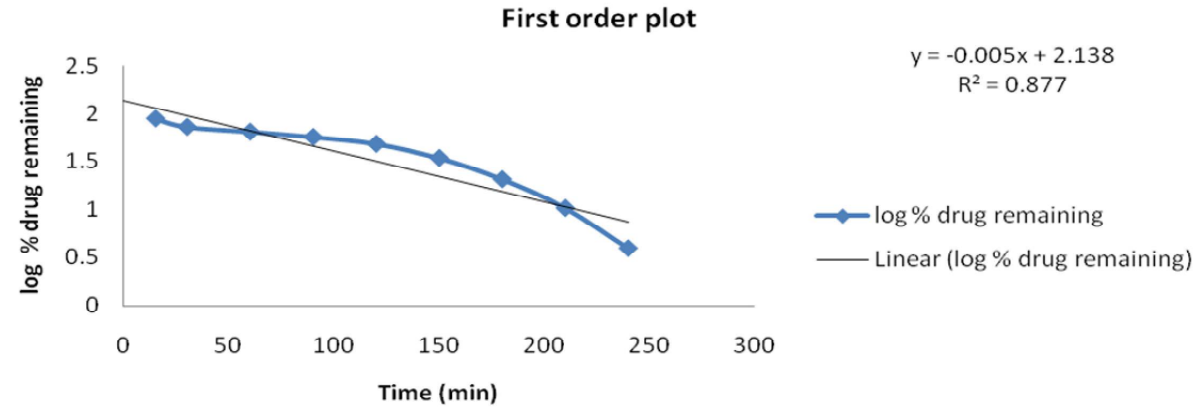


Figure 19: First order Plot of Risperidone R-1 Btach

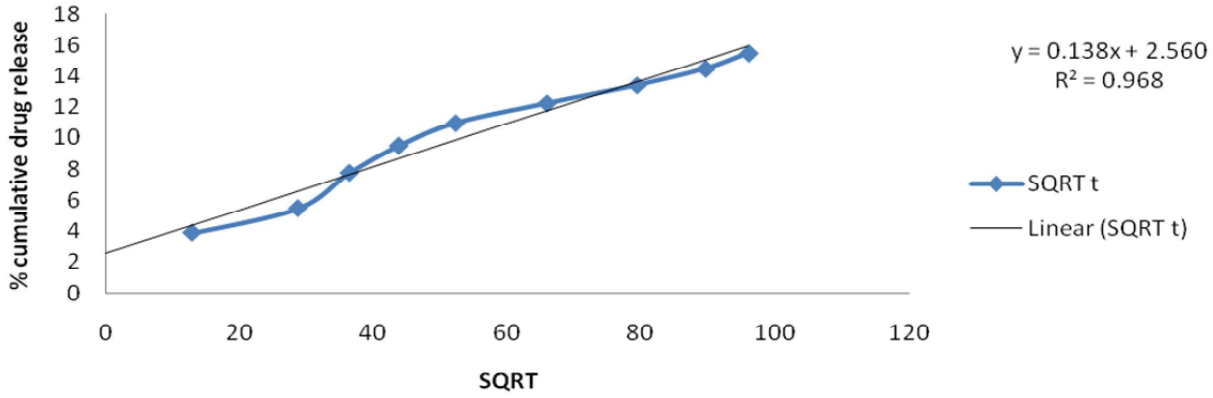


Figure 20: Higuchi Plot of Risperidone R-1 Btach

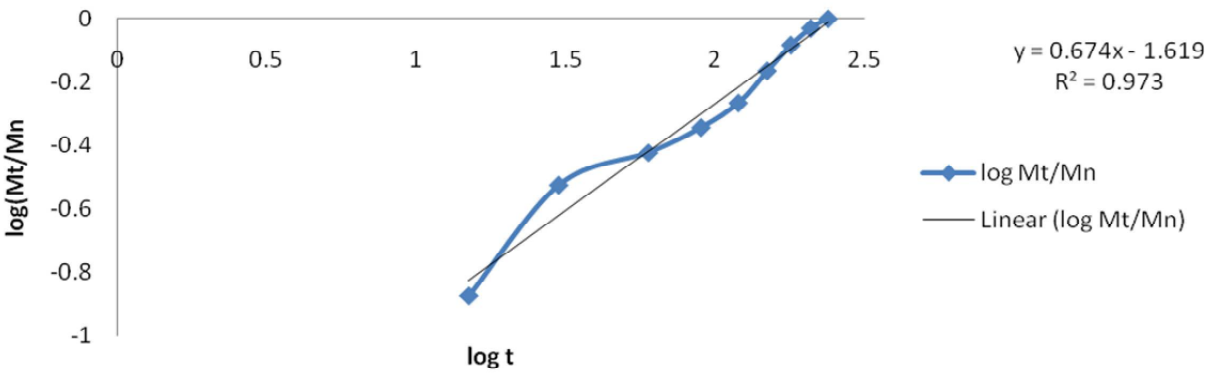
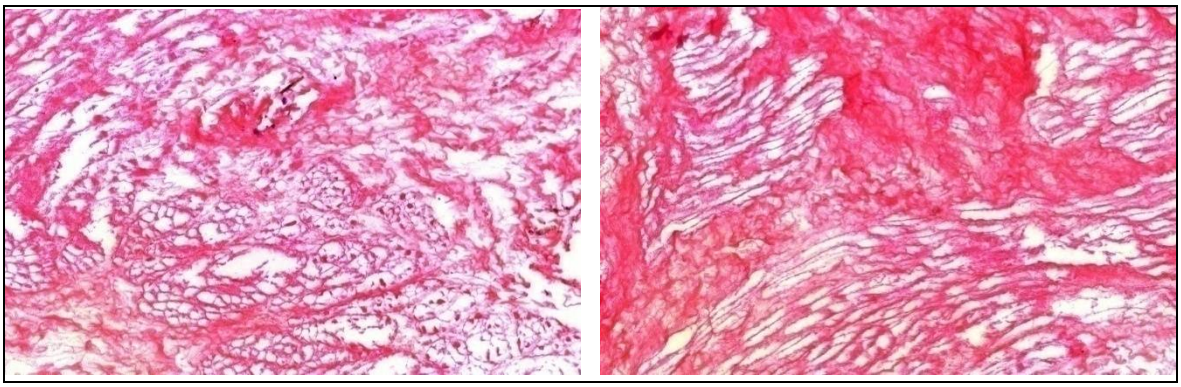


Figure 21: Korsmeyer-Peppas Plot of Risperidone R-1 Btach



a **b**

Figure 22: a) Histology evaluations of sections of untreated sheep nasal mucosa and b) sheep nasal mucosa after treatment with Risperidone loaded microspheres

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Detection of *COI* Gene in *Lucilia sericata* by PCR in Wasit Province, Iraq.

Algorany and Asmaa Fadhil Abdul Ridha*

Department of Biology, College of Science, Wasit University, Kut, Iraq.

Received: 17 Mar 2018 Revised: 20 Apr 2018 Accepted: 30 May 2018

***Address for correspondence**
Asmaa Fadhil Abdul Ridha
Department of Biology,
College of Science,
Wasit University, Kut, Iraq.

ABSTRACT

The mitochondrial cytochrome oxidase I (COI) quality has been proposed as standard DNA barcoding marker for the exposure of life forms. COI gives a perfect species recognizable proof marker in creepy crawlies, because of absence of introns, basic arrangement, restricted presentation to recombination and the accessibility of vigorous preliminary locales. Arrangement variety in this area for the most part demonstrates extensive interspecific, however little intraspecific difference. Lucilia sericata (Diptera: Calliphoridae) is the normal green jug fly found in many territories of the world, and the most understood of the various green jug fly species. L. sericata has much significance in the field of scientific entomology. We have intensified 600 bp part of cytochrome oxidase subunit I (COI) quality of L. sericata. Insect material or very young larvae—we propose to use it only in addition to the conventional methods.

Keywords: Detection, *COI* gene, *Lucilia sericata*, Wasit Province.

INTRODUCTION

Insect information can be connected in criminal examinations (1,2). Specifically, the estimation of the age of the hatchlings takes into consideration the assurance of the base posthumous period (PMImin), 3,4 which is frequently viewed as vital subtle elements of police examinations. The larval advancement relies upon the temperature (5,6), and every specie has a somewhat unique development rate (7,8). Hence, it is important to decide the sorts of hatchlings nourished by the remains appropriately to legitimately compute PMImin. To guarantee the distinguishing proof of the right species, the sub-atomic techniques built up were exchanged to the criminological field (9,10). Calliphoridae is one of the principal guests to contaminate a cadaver with their hatchlings (11). Mitochondrial DNA investigation (mtDNA) and unique, cytochrome oxidase (COI) has all the earmarks of being a helpful instrument in distinguishing species between subtypes of Calliphoridae (12, 13, 14, 15). MtDNA offers numerous focal points over DNA: the last experiences moderately ease back change rates contrasted with mtDNA, so it requires a more drawn out nucleotide arrangement assurance than is important with mtDNA. This improves mtDNA an instrument for recognizing contrasts in the succession of firmly related species and along these lines, valuable for atomic

distinguishing proof (16). The creepy crawlies associated with this approach are generally Dipterans, particularly those having a place with the Calliphoridae and Sarcophagidae families. L. sericata has a more noteworthy significance in criminological science. Like most Calliphorids, the creepy crawly has considered profoundly and has an all-around archived life cycle and propensities (17). As a result, the evolution stage of the insect is used to calculate a minimum period of colonization, so that it can be used as an aid in determining the time of death. Forensic science is an emerging field in forensic science and an important tool in criminal investigations. To determine the time it takes to die easily in an early post-mortem, but in later stages becomes the main problem. But by studying insect evidence, the post-mortem period can be determined in decomposed bodies. Flies were found at different stages of their development on fresh and decomposed bodies as evidence of insects. Therefore, the objective of our study was to study the applicability of the elementary and molecular methodology for the detection of L. sericata in Wasit Governorate, Iraq.

MATERIALS AND METHODS

The samples collection

60 larvae were collected during May, 2018 to June, 2018 in different area of Wasit, Iraq. The larvae were collected by hanging beef outside, and the larvae were placed in a clean, dry plastic container and transported to the laboratory for analysis.

Genomic DNA Extraction

The extraction of genomic DNA from hatchling tests was finished by organization guidelines by utilizing DNA Extraction convention with Proteinase K and chaotropic salt (gSYNCTM DNA extraction Kit, Geneald Biotech Ltd). From that point forward, the separated DNA was checked by spectrophotometer (A260/A280), and after that put away at - 20C at cooler until utilized as a part of PCR intensification.

PCR amplification

PCR assay was performed for detection of *L. sericata* based on one amplification PCR run by using primers for detect a region of approximately 600bp of the *COI* gene was amplified using the primers C1-J170 (5'-ATTGGGGGGTTTGGAAATTG-3`) and C1-N2353 (5'-GCTCGTGTATCAACGTCTATTCC-3`) (19). Primers were provided by (Bioneer Company. Korea) (Table-1). The PCR was set up by utilizing (AccuPower PCR PreMix unit. Bioneer. Korea). The PCR premix tube contains solidify dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and following color) and the PCR ace blend response was set up as per pack guidelines. Polymerase chain reaction (PCR) amplification was performed using 1µL of DNA in a 25µL reaction. Amplification times were 94 °C for 5 min denaturation, followed by 36 cycles of 94 °C for 30 seconds, 55 °C for 1 min, 72 °C for 30 seconds and a final extension period at 72 °C for 7 min (Table-2). PCR products were confirmed by gel electrophoresis stained in ethidium bromide (19).

Statistical analysis

The statistical analysis done SAS (Statistical Analysis System - version 9.1) (20).

RESULTS

analyzing DNA quality have been distinguished relying upon gel electrophoresis which demonstrated the presence of DNA in all examples (Fig-1), the PCR was performed in a total of 60 examples, the results were 49 (82%) examples were Positive, where 11 (18%) were negative. PCR made, evaluated in existent examination which exhibited degree off characteristic segments of PCR things (600bp) (Table-3), (Fig-2) (Fig-3).

DISCUSSION

The study used one gene of mitochondria, where most previous studies used either a combination of mitochondrial genes and one nuclear gene. COI in the mitochondrial genome has been shown to be an excellent source of information for a group of closely related families belonging to Diptera. The method successfully demonstrated was performed on 49 out of 60 positive samples, which showed PCR amplification of the COI gene molecule of L. *sericata* resulted in a single product of 600 years ago. Larvae were detected using published primates and DNA extraction from muscle tissue (22, 23). Although the selected *COI* gene was rather short, it was sufficiently specific to distinguish between the bloated specimens collected, and this result was consistent with 13, which similarly showed that short COI analysis was a suitable tool for distinguishing between important fly species from the genetic area in Western Australia. Our observations were also consistent with (24, 25, 26, 27) that raise the issue of the use of *COI* as a universal “barcoding” and is appropriate. Our findings were also consistent with 28 which established all three strains that were positively identified as L. sericata. Using the *COI* gene. The results showed that 11 samples out of 60 samples were negative. Our results agree with 29 and 30 that observed that the *COI* gene is present on mt-DNA, and because the mt-DNA of the sperm is decomposed into a fertilized egg, the mother's DNA does not transfer to the offspring, causing this gene to show additional analysis, using other genes, such as *L. sericata* and that *L. cuprina* had offspring that the genome of the genome (*COI*) could not detect. The results show that in some cases both nuclear and mitochondrial genes are needed for reliable species identification, this observe was agree with (12, 31,32,33). However, we suggest using only molecular methods in addition to conventional selection methods. The latter is faster and cheaper; moreover, it represents the basis for determining the correct molecular species.

CONCLUSION

DNA encoding is an excellent source of phylogeographic genetic variants. Change in nucleotides is a fundamental characteristic of all organisms that can be used to determine their identity and population status.

ACKNOWLEDGEMENTS

Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for supporting.

REFERENCES

1. Catts EP, Goff ML .1992. Forensic entomology in criminal investigations. Annu Rev Entomol 37:253–272
2. Benecke M.2008. A brief survey of the history of forensic entomology. Acta biol Benrodis 14:15–38
3. Leclercq M. 1983. Entomology and forensic medicine: dating of a death. Rev Med Liege 38:735–738
4. Goff ML, Odom CB, Early M .1986. Estimation of Post-Mortem interval by entomological techniques: a case study from Oahu, Hawaii. Bull Soc Vector Ecol, pp 242–246

5. Bowler K, Terblanche JS .2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol Rev Camb Philos Soc 83:339–355
6. Erzinclioglu YZ .1990. On the interpretation of maggot evidence in forensic cases. Med Sci Law 30(1):65–66
7. Davies L, Ratcliffe GG .1994. Development rates of some pre-adult stages in blowflies with erence to low temperatures. Med Vet Entomol 8(3):245–254
8. Richards CS, Crous KL, Villet MH .2009. Models of development for the blow fly sister species Chrysomya chloropyga and C. putoria. Med Vet Entomol 23:56–61
9. Wallman JF, Adams M .1997. Molecular systematics of Australian carrion-breeding blowflies of the genus Calliphora (Diptera: Calliphoridae). Austr J Zool 45:337–356
10. Benecke M .1998. Random amplified polymorphic DNA (RAPD) typing of necrophageous insects (diptera, coleoptera) in criminal forensic studies: validation and use in practice. Forensic Sci Int 98(3):157–168
11. Benecke M .2005. Arthropods and Corpses. In Tsokos M. (ed:(Forensic Pathology Reviews, vol. 2., Humana, pp. 207–240
12. Harvey ML, Gaudieri S, Villet MH, Dadour IR .2008. A global study of forensically significant calliphorids: implications for identification. Forensic Sci Int 177(1):66–76
13. Harvey ML, Mansell MW, Villet MH, Dadour IR .2003. Molecular identification of some forensically important blowflies of southern Africa and Australia. Med Vet Entomol 17(4):363–369
14. Wells JD, Williams D .2005. Validation of a DNA-based method for identifying Chrysomyinae (Diptera: Calliphoridae) used in a eath investigation. Int J Legal Med 121:1–8
15. Wells JD, Wall R, Stevens JR .2007. Phylogenetic analysis of forensically important Lucilia flies based on cytochrome oxidase I sequence: a cautionary tale for forensic species determination. Int J Legal Med Sci Law 121:229–233
16. Waugh J .2007. DNA barcoding in animal species: progress, potential and pitfalls. Bioessays 9(2):188–197
17. Joseph I, Mathew DG, Sathyan P, Varghese G et al. 2011. The use of insects in forensic investigations: An overview on the scope of forensic entomology. Journal of Forensic Dental Sciences, 3(2):89-91.
18. Goyal PK. 2012. An entomological study to determine the time since death in cases of decomposed bodies. Journal of Indian Academy of Forensic Medicine, 34(1):10- 12.
19. Kirstin A, Williams1,2, Jennifer L, Martin H. Villet2. 2017. Phylogenetic radiation of the green bottle flies Diptera, Calliphoridae, Luciliina. ZooKeys 568: 59-86.
20. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010
21. Priya Bhaskaran K P and Sebastian C D. 2015. Molecular barcoding of green bottle fly, Lucilia sericata (Diptera: Calliphoridae) using COI gene sequences. Journal of Entomology and Zoology Studies 3 (1): 10-12
22. Sperling FA, Anderson GS, Hickey DA .1994. A DNA-based approach to the identification of insect species used for postmortem interval estimation. J Forensic Sci 39(2):418–427
23. Wells JD, Sperling FA .2001. DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). Forensic Sci Int 120(1–2):110–115
24. Rubinoff D, Cameron S and Will K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. Journal of Heredity 97: 581-594.
25. Whitworth TL, Dawson RD, Magalon H and Baudry E. 2007. DNA barcoding cannot reliably identify species of the blowfly genus Protocalliphora (Diptera: Calliphoridae). Proceedings of the Royal Society B 274: 1731-1739.
26. Jordaens K, Sonet G, Richet R, Dupont E, Braet Y and Desmyter S .2012. Identification of forensically important Sarcophaga species (Diptera: Sarcophagidae) using the mitochondrial COI gene. International Journal of Legal Medicine 127: 491-504.
27. Sonet G, Jordaens K, Braet Y, Desmyter S .2012. Why is the molecular identification of the forensically important blowfly species Lucilia caesar and L. illustris (family Calliphoridae) so problematic? Forensic Science International 223: 153-159.
28. Tarone AM, Picard CJ, Spiegelman C, And Foran D R. 2011. Population and Temperature Effects on Lucilia sericata (Diptera: Calliphoridae) Body Size and Minimum Development Time. J. Med. Entomol. 48(5): 1062-1068

29. Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C and Schatten G.1999. Development: Ubiquitin tag for sperm mitochondria. *Nature*, 402, 371-372.
30. Katie SG, Edward BM, Scott HJ and Steven E. 2014. A Morphological and Genetic Analysis of Forensically Important Blow Flies, from Georgia: The Genus *Lucilia*. Department of Biology, University Honors Program Georgia Southern University.
31. DeBry R, Timm AE, Dahlem GA Stamper T. 2010. mtDNA-based identification of Lucilia cuprina (Wiedemann) and Lucilia sericata (Meigen) (Diptera: Calliphoridae) in the continental United States. Forensic Science International 202: 102 -109.
32. Wallman JF, Leys R and Hogendoorn K. 2005. Molecular systematics of Australian carrion-breeding blowflies (Diptera: Calliphoridae) based on mitochondrial DNA. Invertebrate Systematics 19: 1-15.
33. Tourle R, Downie DA and Villet MH. 2009. A morphological and molecular comparison of Lucilia cuprina and L. sericata (Diptera: Calliphoridae) in South Africa. Medical and Veterinary Entomology 23: 6-14.

Table 1: List of primers that used in PCR amplification

PCR	Primer	Sequence		Amplicon
Cycle	*COI* gene	C1-J1709	ATTGGGGGGTTTGGAAATTG	600bp
		C1-N2353	GCTCGTGTATCAACGTCTATTCC	

Table 2: - The Cycles of PCR.

PCR step	Temperature	Time	Repeat cycle
Initial Denaturation	94 °C	5 Min	1
Denaturation	94 °C	30 Sec	36
Annealing	55 °C	1 min	
Extenation	72 °C	30 Sec	
Finial Extension	72 °C	7 min	

Table 3: Results of Polymerase chain reaction PCR.

Result	Samples	Percentage %
Positive	49	82 %
Negative	11	18 %
Total	50	100 %

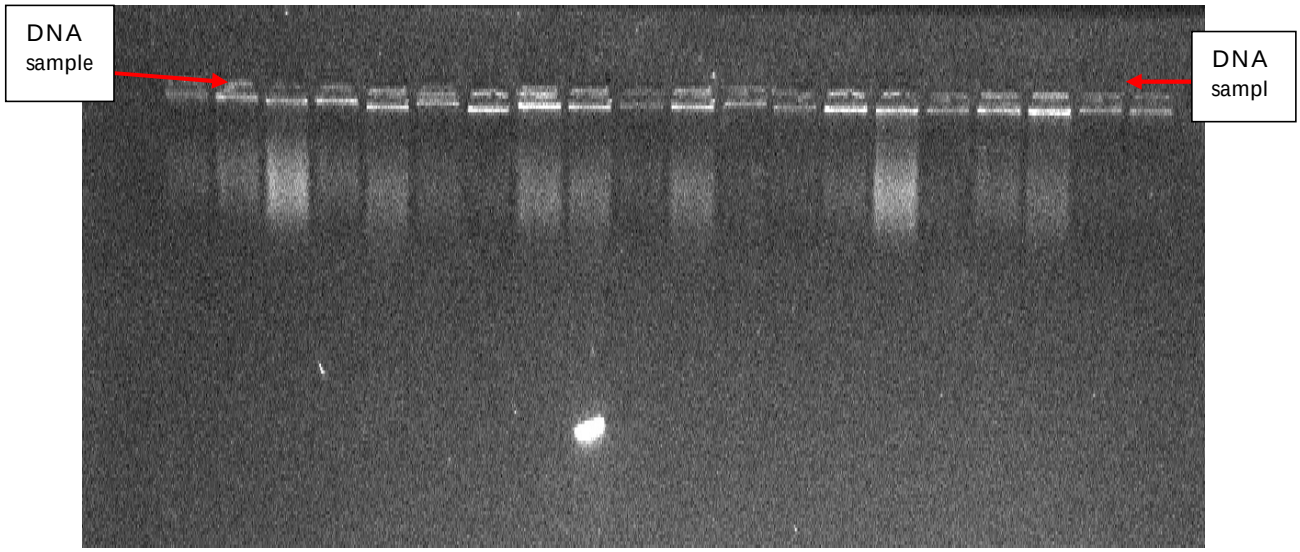


Figure 1: - Electrophoresis of genomic DNA. Up in the gel (Large in size), down in the gel (Small in size). Intense in color (High quality), down in the gel (Low quality).

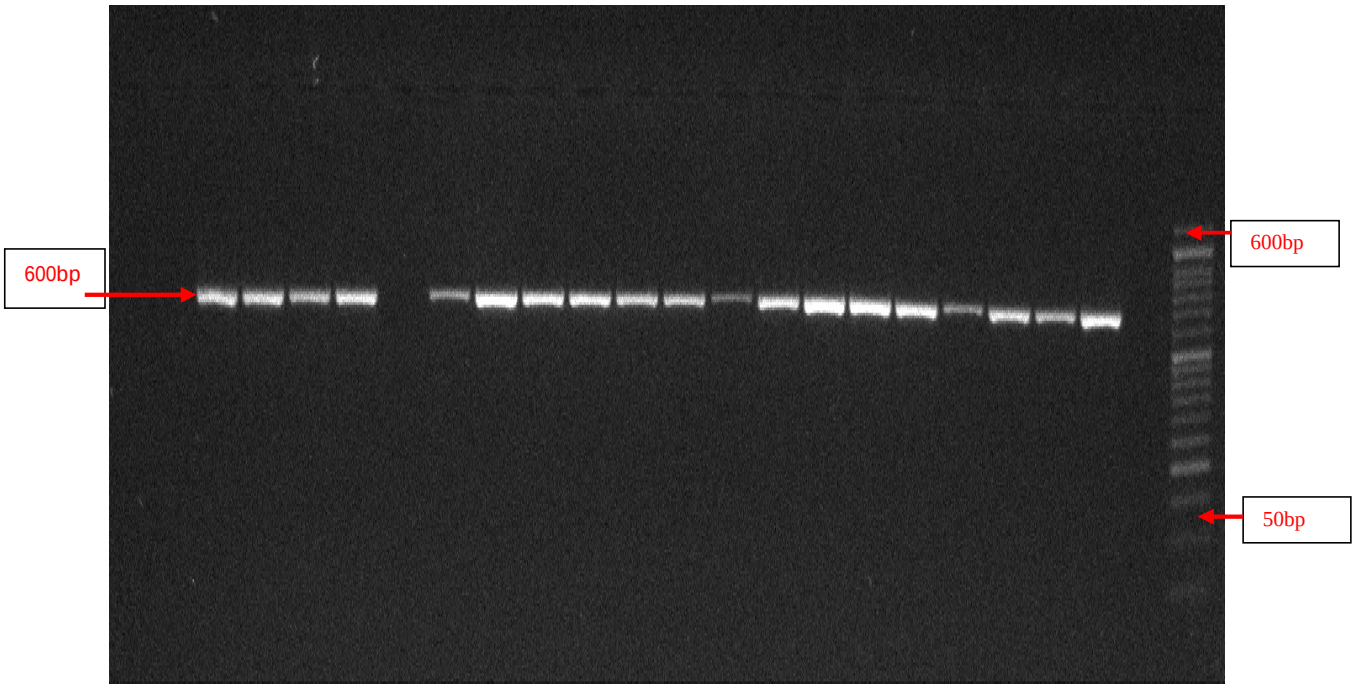


Figure 2: - Gel electrophoresis image that shown the PCR product of *COI* gene that using in detection L. *sericata* larvae. Where M: Marker (50-1000bp), Samples some positive at 600bp PCR product size.

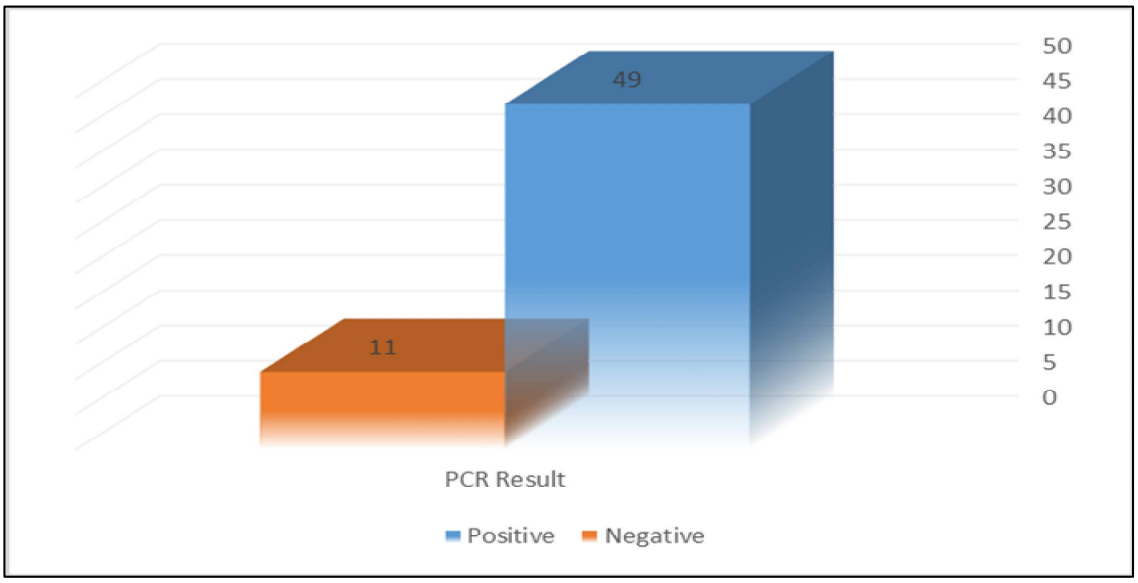


Figure 3: - PCR Result Showed that the Positive result 49 out of 60 while the negative result was 11 Out of 60 specimens.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study of Some Physiological and Immunological Variables in Patients with Celiac Disease in Wasit Province, Iraq

Maha Radhi Ahmed[1*] and Mohammad R.S.AL-Attabi[2]

[1]Directorate of Education Wasit, Iraq.
[2]Department of Biology,College of Science, Wasit University, Kut, Iraq.

Received: 17 Mar 2018 Revised: 20 Apr 2018 Accepted: 30 May 2018

[*]**Address for correspondence**
Maha Radhi Ahmed,
Directorate of Education Wasit,
Wasit University, Kut, Iraq.

ABSTRACT

The current study included the effect of Celiac disease on the function of thyroid gland and it's role in the occurrence of thyroid autoimmune disorders by measuring the level of thyroid hormone T4 , thyroid stimulating hormone TSH and measure the level of some indicators of thyroid immune disorders by determining the level of Anti-Thyroglobiolin antibodies(anti-Tg-Ab). The study was conducted at Al-Zahraa teaching hospital in Wasit Governorate, 46 celiac patients were diagnosed by using anti-gliadin-Ab (IgA, IgG) and anti-tTg-Ab (IgG, IgA) antibodies compared to 40 non-celiac patients were treated as a control group. The two groups were divided into three age groups (1-10 years), the second category (11-20 years) and the third (21 years). The results were as follows: anti-tTg-Ab was significantly increase (P ≤0.05) in the second and third groups of patients at 17.77 ± 6.84 ± 32.31 ± 18.21 compared to control, T4 was significantly decrease (P ≤0.05) in the second group of patients at 7.35 ± 1.45 compared to Control while TSH rises in second and third groups without statistically significant difference(P ≤0.05) in patients when compared with controls. Cileac disease is associated with autoimmune thyroid diseases and the risk of thyroid disease increases with the development of celiac disease, age progression and delayed diagnosis of celiac disease.

Keywords: Cileac disease, thyroid disorders, thyroglobin.

INTRODUCTION

Celiac disease is an immune disease of the small intestine affecting people who have genetic predisposition to be infected as a response to the digestion of gluten protein found in wheat, barley and oats and its prevalence about 0.5-1% around the world. Typical symptoms of celiac disease are diarrhea, weight loss, poor growth, and anemia caused by several reasons such as iron failure to regularly absorb, low blood volume, increased blood loss, lack of absorption of vitamins such as B12 and folic acid (24,9). As well as lack of absorption of other important nutrients in the

construction of the body and bone, also important in the functions of some body organs such as endocrine glands, the symptoms of non-typical of Celiac disease is fragile fragility and delayed puberty (27) celiac disease is diagnosed by serological tests or tissue biopsy of small intestine. The previous studies indicate that the serological tests are the first and most important step to the diagnosis. The presence of anti-gliandin antibodies, anti-tTG enzymes antibodies, and Endomysial antibodies (EMA) (35,33). The tTG or EMA antibody test also helps us to determine if a diagnose is needed by biopsy. If IgG antibodies appear to be very low or negative, with typical symptoms continuing, then the biobsy diagnosis is used (26). Treatment of Celiac disease is a long diet of gluten (31). The risk of the disease rise in untreated people. The development of celiac disease leads to lymphoma of the small intestine (10,12) and thyroid immune disorders (25,34). previous studies indicated high indices for thyroglobulin and thyroperoxidase antibodies, which are indicators that used for diagnose of thyroid disease in people with Celiac disease(40) .The first diagnosis of celiac disease in Iraq was in1975 by Al-Hassany (3) There have been studies in Iraq on the relationship of Celiac disease to some diseases such as diarrhea, type 1 diabetes, the association of celiac disease with anemia and the validity of serological examinations in the diagnosis of Celiac disease (2,23,38). Because of the lack of studies in Iraq about the relationship between celiac disease and thyroid autoimmune diseases the current study aimed to determining the effect of Celiac disease on the function of thyroid gland and its role in the occurrence of thyroid immune disorders.

MATERIALS AND METHODS

Design of the study

46 patients with Celiac disease were comparison to 40 people who were not infected and considered as a healthy control group, In Wasit Governorate. the patients and control groups were divided into three equal age groups, the first category (0-10) years, the second (11-20 years) The third category (21 years) after the information was collected about the condition of the patientsin terms of age, sex, family history of the disease and diet, we chose the peoples who did not diagnosed celiac disease and not diagnosed with pre-existing thyroid disorders and did not follow a gluten free diet. Intravenous blood was withdrawn from the two groups (patients and controls). Blood samples were kept in a deep freeze until the immunological tests were completed after the serum was separated using a device Centrifuge at 4000 cycles / min for 5 minutes and divide them into three tubes.

Diagnosis

Diagnosis of Celiac disease

The ELISA method was used for the diagnosis of celiac disease. The ratio of anti-gliadin antibodies and anti-tTg-antibodies was determined using immunoglobulin (IgA, IgG) for both diagnoses. This method was used Depending on the manufacturer's information (Aeskuliza, German company).

Diagnosis of thyroid immune diseases

(ELIZA) method, manufactured by the German company (Human), was used for diagnosis of thyroid diseases by determining thyroid hormone T4 and thyroid stimulating hormone (TSH) to detect thyroid function (19). The ELIZA method used for detecting the immune disorders of thyroid gland by determining the (anti-Tg-Ab) this method was used depending on the manufacturer's information, German company (De Medi Tec) (8).

Statistical analysis

The SPSS version 19 was used to perform a T.test to compare patients with controls with a probability level ($P \leq 0.05$) and LSD test used for compare age groups of patients with each other.

RESULTS

The results show that the first age group (0-10) is the most common type of Celiac disease. The rate of antibodies to each of the gliadins is increased in Table (1) and (2). Anti-tgT-Ab (IgA and IgG) are increased in Table (3) and (4) in all age groups of patients when compared to control groups. The results in Table (5) indicate that the concentration of T4 hormone significantly decrease ($P \leq 0.05$) in the second age group of patients when compared to control the rate was (7.35) for patients and (9.71) for control. While no significant difference ($P \leq 0.05$) was observed in T4 in the first and third groups when compared to control groups, TSH rises in the second and third age groups, but without significant difference ($P \leq 0.05$) when compared to control subjects table (6), and in Table (8) decrease the concentration of TSH with the increase of age groups with no significant statistical difference ($P \leq 0.05$) between the age groups of patients. Table (7) shows the percentage of anti-Tg-Ab, significant increase ($P \leq 0.05$) was observed in the concentration of anti-Tg- Ab in second and third groups of patients when compared to control and their rates ranged (17.77, 32.31) to patients and (8.62, 11.86) to controls respectively. There was no statistically significant difference ($P \leq 0.05$) in the first age group of patients when compared to control, when comparing the concentration of anti- Tg -Ab in Table (8), we note that the concentration of anti- Tg -Ab rises in the third age group ($P \leq 0.05$) compared to the first and second categories and the rate is (32.31) for the third category and (14.94,17.77) for the first and second categories respectively.

DISCUSSION

The results of age group (0-10) year (table 1) agree with (6,1). The reason is due to the change in the internal environment of the intestines and the result of the type of feeding and the nature of food, which explains the emergence of the disease in the early stages of life. The environmental change of the intestine is the presence of two types of intestinal bacteria, beneficial bacteria, Bifidobacterium and lactobacillus and other types of bacteria are not useful, represented by bacteroides, Gluten-free gisin reduces the number of these harmful bacteria. While the presence of bacteroides positively with the gluten in food,' where "the food is very rich in gluten causes an increase in the number of these bacteria, and thus the number is dominated by the number of beneficial bacteria Bifidobacterium and lactobacillus This case occurs in children, especially in the stage of breastfeeding or cow milk , and thus will be released zonulin (17), Which causes a change the structure of the strong protein ligaments of the small intestine tissue, leading to increase the gliadin infiltration from the small intestinal cavity to the endomesium where the lymphocyte receptors of (APC) are present and stimulates the release of antibodies and the results of Table (1) and (2) agree with AL-Mosawi (2012)(5). The diagnosis is used by measuring the level of anti-gliadin-Ab in the early stages of the disease, Neonatal and infants because the diagnosis by the (anti –tTg-Ab) is unclear and inaccurate at this age because of the absence of this antibodies in the early age of patients (11).

The results in (Table 3 and 4) agree with (5,26), because of the gluten in the grains containing it cause the release of zonulin, which in turn leads to the relaxation of the protein ligaments of the small intestinal tissue and thus causes an increase in intestinal infiltration of the gliadin. Hence the role of the enzyme tTg in immune activation by altering the chemical structure of the gluten by removing the amino group from it to be associated with the lymphocytic receptor (APC), and activated in the formation of cytokines and stimulate B cells to produce antibodies to gliadin and the enzyme tTg that show up high in the serum of people infected with celiac disease, though few were high (29, 26). The results in table (5) agree with (28,16) decrease the concentration of T4 hormone due to the inability of thyroid gland to produce normal amounts of T4 hormone. Previous studies have indicated the role of celiac disease in the emergence of symptoms of thyroid disorders, especially hypothyroidism, because, celiac disease causes a lack of absorption of important nutrients of the function of thyroid gland such as calcium and iodine (13,36). The results in table (6) and table (8) agree with (40,22). The reason is that the hormone TSH, which is produced from the pituitary gland is under the influence of the concentration of thyroid hormone secreted from the thyroid gland and secretion or inhibit the secretion of TSH hormone by negative feedback of the hormone thyroxine, where the pituitary respond to the

increase or decrease the proportion of thyroxine in blood, which leads to the suppression or secretion of hormone TSH (21). This study is expected to increase the height of thyroxine with the development of celiac disease because the increase of anti- tTg-Ab increases the possibility of development of thyroid autoimmune disorders, the more delayed diagnosis, especially in the second and third years due to the emergence of positive diagnosis of anti-Tg-Ab depending on the classification of Surks et al. (1990) (37) in determining the type of thyroid autoimmune disorders where he noted that the emergence of anti-thyroglobulin antibodies with the rise of TSH hormone and moral decline of the hormone T4 classified under hypothyroidism. The results in table (7) and (8) agree with agrees with (14), which indicated an increase in thyroid antibody obtained two to three years after the diagnosis of Celiac disease and a gastrointestinal difference. According to Previous study (39) the people who have a link between Celiac disease and autoimmune thyroid disease are older than people who suffer from Celiac disease alone. He also pointed out that people with untreated Celiac disease they have increased signs of autoimmune thyroid diseases and cause the development of these diseases, the study agrees with (18, 7). This is due to the fact that the broken mucous layer of the lower part of the small intestine leads to the leakage of the anti-tTg-Ab molecules of the broken layer and its circulation with the circulatory system and thus interact with the thyroid antigens and affect the tissue of the thyroid gland and stimulate it to generate Antibodies therefore the emergence of positive antibody diagnosis, which represent such as (Anti-TG) (15,32).

CONCLUSION

The current study concludes that celiac disease has effects on the function of thyroid gland this appear through decrease serum T4 and increase serum anti-thyroglobulin and normal TSH.

ACKNOWLEDGEMENTS

We would like to thank everyone who contributed and participated in the completion of this research.

REFERENCES

1. Abadie, V. ;Sollid, LM.; Barreiro, LB.; Jabri B ,2011, Integration of genetic and immunological insights into a model of celiac disease pathogenesis. Annu Rev Immunol 29: 493-525.
2. Abdulrazak, SH. ;Hasan Nadhim, Gh. ;Noaman, Mehdi, SH. Al-Zuheiry; Shahad, KH. Al- Qaisi .(2014), Serological detection of Celiac disease among children in Diyala province - Iraq , The Swedish Journal of Scientific Research (sjsr) ISSN: 2001-9211. Volume 1. Issue 6.
3. Al-Hassany, M. . Coeliac disease in Iraqi children. J Trop Pediatr 1975; 21: 178-9.
4. Aline Ventura; Marcelo Fernando; Ronsoni;Maria Beatriz; Cacese Shiozawa; Esther Buzaglo; Dantas-Corrêa; Maria Heloisa; Busi da Silva ; Canalli; Leonardo de Lucca ; Schiavon Janaína; Luz Narciso-Schiavon . (2014) .
5. revalence and clinical features of celiac disease in patients with autoimmune thyroiditis: cross-sectional study, Sao Paulo Med J, DOI: 10.1590/1516-3180. 1326725.
6. 5AL-Mosawi, A. .(2012) . Study the Correlation of Anti-gliadin IgA & IgG with Iron&Total Iron Binding Capacity & Albumin in sera of Patients with Celiac Disease . Journal of Kerbala University; 10(2):76-81.
7. Al-Saadi, H A.; Abid, A H. (2009). Celiac disease in karbala .J Karb Unv;7(2):51-61.
8. Ansaldi, N.; Palmas, T.; Corrias, A.; et al.,(2003). Autoimmune thyroid disease andceliac disease in children, Journal of Pediatric Gastroenterology and Nutrition, vol. 37, no. 1, pp. 63–66,.
9. Becker,W.; Reiners,C.; Börner,W.(1985). Immunologische Kriterien zur Differentialdiagnose vonSchilddrüsenerkrankungen. Therapiewoche ;35:1167 – 1176

10. Bergamaschi, G.; Markopoulos, K.; Albertini, R.; Di Sabatino, A.; Biagi, F.; Ciccocioppo, R.; et al.(2008) . Anemia of chronic disease and defective erythropoietin production Differentialdiagnose Differentialdiagnose Differentialdiagnose Differentialdiagnose patients with celiac disease. Haematologica ,93:1785-91
11. Chand, N.; Mihas, AA. (2006) .Celiac disease: current concepts in diagnosis and treatment. Clin Gastroenterol. ,40(1):3-14.
12. 11-Ciccocioppo, R.; Di Sabatino, A.; Ara, C.;Biagi, F. ; Perilli, M .; Amicosante, G .; Cifone ,MG. (2003) . Gliadin and tissue transglutaminase complexes in normal and coeliac duodenal mucosa. Clin Exp Immunol. 134: 516-24.
13. Collin, P.; Huhtala, H.; Virta, L.; Kekkonen, L.; Reunala, T.(2007) . Diagnosis of celiac disease in clinical practice: physician's alertness to the condition essential. J Clin Gastroenterol. ,41(2):152-6.
14. Dennis, M.; Doherty, C. (2010) .Supplements 101. In: Dennis M, Leffler D. Real Life With Celiac Disease: Troubleshooting and Thriving Gluten Free. Bethesda, MD: AGA Press : 98-109.
15. Derya Kalyoncu and Nafiye Urganci . (2015) . Antithyroid Antibodies and Thyroid Function in Pediatric Patients with Celiac Disease, Hindawi Publishing Corporation International Journal of Endocrinology Volume 2015, Article ID 276575, 4 pages
16. Duntas, LH.(2009) . Does celiac disease trigger autoimmune thyroiditis? Nat Rev Endocrinol.;5(4):190-191.
17. Erdogan Soyucen; Sema Yilmaz; Cokun Celtk;Ulfet Vatansever; Naci Oner;Serap KarasalThoglu.(2010) . Seroprevalence of autoimmune thyroiditis and celiac disease in children with insulin-dependent diabetes mellitus in the Thrace region of Turkey, Turk J Gastroenterol 2010; 21 (3): 231-235
18. Fasano, A.; Berti, I. Gerarduzzi, T.; Not, T.; Colletti, RB.; Drago, S. et al. (2004). Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med;163:286-92.
19. Forchielli, M.; Collina, A.; Zannarini, L. and Capelli, M.(2000). Celiac disease and thyroid abnormalities: another possible association. Journal of Pediatric Gastroenterology and Nutrition. vol31. supplement 2, p. S63,.
20. Gonzalez-Sagrado, M.; Martin-Gil, FJ. (2004) . Population-specific reference values for thyroid hormones on the Abbott architecti,2000, analyzer. ClinChem Lab Med;42:540.
21. Gujral, N.F.; Hugh, J.; Thomson, A.B.R.(2012) . Celiac disease: Prevalence, diagnosism pathogenesis and tratment World J. Gastroenterol., 18, 6036–6059.
22. Guyton and Hall . (2005) . Textbook of Medical Physiology, 11 eddition , saunderrs, eisevier, p.1152.
23. Hadithi, M.; de Boer, H.; Meijer, JW.; Willekens, F.; Kerckhaert, JA.; Heijmans, R. et al.(2007) . Coeliac disease in Dutch patients with Hashimoto's thyroiditis and vice versa. World J Gastroenterol. ,13(11):1715-22.
24. Hanaa Naji Abdullah ; Hanaa Jabbar Saber ;Median Ali Hussein.(2013) . Screening of celiac disease in Iraqi pataints with type 1diabetes mellitus, AL- Taqani ,Vol . 26 , No 3 ,2013.
25. Harper, JW.; Holleran, SF.; Ramakrishnan, R.; Bhagat, G.; Green PH.(2007) . Anemia in celiac disease is multifactorial in etiology. Am J Hematol,82:996-1000
26. Hollowell, JG.; Staehling, NW.; Flanders, WD.; Hannon, WH.; Gunter, EW.; Spencer, CA. et al.(2002) . Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab.,87(2):489-99.
27. Husby, S.; Koletzko, S.; Korponay-Szábó, IR.; Mearin, ML.; Phillips, A.; et al. (2012) . ESPGHAN guidelines for the diagnosis of coeliac disease in children and adolescents. An evidencebased approach 54: 136–160.
28. Ivanovski, P.; Nikolić, D.; Dimitrijević, N.; Ivanovski, I.; Perišić ,V.(2010) . Erythrocytic transglutaminase inhibition hemolysis at presentation of celiac disease. World J Gastroenterol, Nov 28;16(44):5647-50.
29. Laila, M. ;Teixeira; Renato Nisihara ; Shirley Ramos; da Rosa Utiyama; Ricardo, S. de Bem; Cristina Marcatto1; Michelli Bertolazo; Gisah, A.; de Carvalho1 .(2014). Screening of celiac disease in patients with autoimmune thyroid disease from Southern Brazil, Arq Bras Endocrinol Metab.;58/6.
30. Lammers, KM.; Lu, R.; Brownley, J.; Lu, B.; Gerard, C.; Thomas, K.; Rallabhandi ,P.; Shea- Donohue, T.; Tamiz, A.; Alkan, S.; Netzel-Arnett ,S.; Antalis ,T.; Vogel, SN.; Fasano, A. (2008) . Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology;135(1):194-204.e3
31. Logan, RFA. (1992) . problems and pitfalls in epidemiological studies of coeliac disease, Dyn Nutr Res 2:14-24.

32. Mac Culloch, K.; Rashid, M.(2014) . Factors affecting adherence to a gluten-free diet in children with celiac disease. Paediatr Child Health;19(6):305-309.
33. Naiyer, AJ.; Shah, J.; Hernandez, L.; et al.(2008) . Tissue transglutaminase antibodies in individuals with celiac disease bind to thyroid follicles and extracellular matrix and may contribute to thyroid dysfunction. Thyroid.;18(11):1171-1178.
34. Nakazawa, H.; Makishima, H.; Ito, T. et al. (2014) . Screening tests using serum tissue transglutaminase IgA may facilitate the identifica-tion of undiagnosed celiac disease among Japanese population Int J Med Sci 11(8):819-823.
35. Nisihara, RM. et al.(2005). Celiac disease in children and adolescents with Down syndrome. (Brazilian study) Jornal de Pediatria, 81:373-378.
36. Saneian, H.; Gorgani, AM. (2012) . Diagnostic value of serologic tests in celiac screening Int J Prev Med, 3(Suppl 1):58-63
37. Saturni, L.; Ferretti, G.; Bacchetti, T.(2010) . The gluten-free diet: safety and nutritional quality. Nutrients.;2(1):16-34.
38. Surks, MI.;Chopra, U.; Mariash, CN. et al. (1990) . American Thyroid Association guideline for use of laboratory tests in thyroid disorders. JAMA;263:1529–32
39. Tawfeeq, F.; AL-Auqbi, M.B.; Salah Aldeen Abdulnabi; Subhi Farhan, M.B.(2005). Prevalence of Asymptomatic Celiac Disease in low body weight Iraqis, Prevalence of Asymptomatic Celiac Disease, IJGE Issue 5 Vol 1 2005.
40. Tunbridge, WMG.; Evered ,DC.; Hall, R.; Appleton, D.; Brewis, M. ; Clark, F. et al. (1977) . The spectrum of thyroid disease in a community: The Whickham survey. Clin Endocrinol; 7: 481-93.
41. Ventura, A.; Neri, E.; Ughi, C.; Leopaldi, A.; Citta ,A.; Not, T.(2000) . Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease. J Pediatr,137:263-265.

Table (1): Concentration of gliadin IgA antibodies for cileac patiants compared to control

Groups / Gliadin - Ab(IgA)	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	30.94±16.91 a	0.000	≤ 0.05
Control		19	2.78±2.53 b		
Patients	(11-20 years)	9	23.01±11.87 a	0.001	≤ 0.05
Control		9	4.21±2.36 b		
Patients	(□20 years)	12	34.58±19.15 a	0.000	≤ 0.05
Control		12	3.31±2.04 b		

* The different letters indicate a significant difference at level ≤ 0.05
* Represents adjusted numbers ± standard deviation.

Table (2): Concentration of gliadin IgG antibodies for cileac patiants compared to control

Groups / Gliadin -Ab(IgG)	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	25.22± 21.37 a	0.000	≤ 0.05
Control		19	4.36±2.84 b		
Patients	(11-20 years)	9	14.83±9.26 a	0.006	≤ 0.05
Control		9	3.36±1.99 b		
Patients	(□20 years)	12	37.54±30.77 a	0.000	≤ 0.05
Control		12	4.79±3.69 b		

* The different letters indicate a significant difference at level ≤ 0.05
* Represents adjusted numbers ± standard deviation.

Table (3): Concentration of tTg antibodies (IgA)for cileac patiants compared to control

Groups / tTg- -Ab(IgA)	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	42.43±17.64 a	0.000	≤ 0.05
Control		19	1.75±1.43 b		
Patients	(11-20 years)	9	54.07±17.78 a	0.000	≤ 0.05
Control		9	2.59±1.76 b		
Patients	(□20 years)	12	34.84±9.17 a	0.000	≤ 0.05
Control		12	2.27±1.59 b		

* The different letters indicate a significant difference at level ≤ 0.05
* Represents adjusted numbers ± standard deviation.

Table (4): tTg antibody concentration (IgG) for cileac patiants compared to control

Groups / tTg-Ab (IgG)	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	41.69±28.27 a	0.000	≤ 0.05
Control		19	4.09±2.49 b		
Patients	(11-20 years)	9	28.22±15.49 a	0.001	≤ 0.05
Control		9	3.98±2.56 b		
Patients	(□20 years)	12	28.04±14.47 a	0.000	≤ 0.05
Control		12	2.34±0.44 b		

* The different letters indicate a significant difference at level ≤ 0.05
* Represents adjusted numbers ± standard deviation.

Table (5): concentration of thiroxine (T4) (µg / dl) for cileac patiants compared to control

Groups / T4 (µg/dl)	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	8.37±1.42 a	0.1	≤ 0.05
Control		19	9.66±3.07 a		
Patients	(11-20 years)	9	7.35±1.45 b	0.05	≤ 0.05
Control		9	9.71±3.10 a		
Patients	(□20 years)	12	7.77±2.48 a	0.50	≤ 0.05
Control		12	7.01±1.50 a		

* The different letters indicate a significant difference at level ≤ 0.05

* Represents adjusted numbers ± standard deviation.

Table (6): concentration of (TSH) for cileac patiants compared to control

Groups / TSH mlu/l	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	2.62± 1.16 a	0.71	≤ 0.05
Control		19	2.79 ± 1.18 a		
Patients	(11-20 years)	9	2.60 ± 1 a	0.51	≤ 0.05
Control		9	2.29 ± 0.94 a		
Patients	(□20 years)	12	2.36 ± 1.33 a	0.13	≤ 0.05
Control		12	1.39 ± 0.97 a		

* The different letters indicate a significant difference at level ≤ 0.05

* Represents adjusted numbers ± standard deviation.

Table (7): concentration of anti-thyroglobulin antibodies for cileac patiants compared to control

Groups / Thyroglobulin Ab ml/ul	Age	№	Mean ± SD	T-value	P-value
Patients	(0- 10years)	25	14.94 ± 7.79 a	0.60	≤ 0.05
Control		19	16.43 ± 10.50 a		
Patients	(11-20 years)	9	17.77 ± 6.84 a	0.008	≤ 0.05
Control		9	8.62 ± 5.96 b		
Patients	(□20 years)	12	32.31 ± 18.21 a	0.02	≤ 0.05
Control		12	11.86 ± 8.21 b		

* The different letters indicate a significant difference at level ≤ 0.05

* Represents adjusted numbers ± standard deviation.

Table (8): concentration of thyroid hormones T4, TSH and anti-thyroglobulin antibodies for cileac patiants

Groups / Parameters	Age (0-10) year	Age (11-20) year	Age (□ 21) year	LSD
	N= 25 Mean ± SD	N= 9 Mean ± SD	N= 12 Mean ± SD	
T4	8.37±1.42a	7.35±1.45a	7.77±2.48a	No sig.
TSH	2.66±1.16a	2.60±1 a	2.36±1.33a	No sig.
Anti thyroglobulin	14.94±7.79 b	17.77±6.84b	32.31±18.21a	14.54

* The different letters indicate a significant difference at level ≤ 0.05
* Represents adjusted numbers ± standard deviation.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Study for Serum Amyloid A (SAA) and Some Biochemical Markers in Iraqi Patients with Breast Cancer in Relation to Disease Progression.

Huda Jaber Waheed [1*] and Amal Rashid [2]

[1]College of Pharmacy, Mustansiriyah University, Baghdad.Iraq.
[2] Institute of Medical Technology,Al-Mansor, Baghdad, Iraq.

Received: 18 Mar 2018 Revised: 21 Apr 2018 Accepted: 30 May 2018

[*]**Address for correspondence**
Dr.Huda Jaber Waheed
College of Pharmacy,
University of Mustansiriyah,
Baghdad, Iraq.
Email: hudaiq84@yahoo.com.

ABSTRACT

The present study was design to evaluate some biomarkers (ALP, ferritin, TGF1β and SAA) and to assess the alteration of studied parameters in various stages. A total of (60) woman with breast cancer was enrolled in the study, for comparison (44) healthy women participated in the study. Patients group was divided to four sub-groups according to the Serum ferritin, SAA and TGF1β were measured by using ELISA technique. Alkaline phosphatase measured by reflatron.Results revealed that the serum SAA significantly elevated in patients when compared to control (p□0.05) and its increment association with the stage of disease. Serum ferritin and ALP elevated in all patients' stages when compared to control (p□0.05).In conclusion,all serum ferritin, ALP, SAA and TGF1β were significantly increased in patients. The level of SA showed a marked increase in the progression of the disease.

Keywords: Breast cancer, Serum amyloid A , Ferritin , ALP and TGF1β

INTRODUCTION

Breast cancer is one of the most common types of tumors after skin cancer, which has increased rapidly around the world, especially in Iraq (Al-Hashimi MM *et al.* 2014). Last years the survival rate has improved to 98% if the disease is diagnosed early and 39% if the person is diagnosed late (Redondo M *et al.* 2008). Early diagnosis is therefore an important way to survive from the disease. At present, the mammography examination depends in the diagnosis of disease, but this examination requires that the mass reach few millimeters in tumor size to be detected. This does not provide an early diagnosis, so there is a need to find a sensitive and specialized marker to diagnose the disease early (Tabar L et *al.* 2003 and Cocco E *et al.* 2010). High proteomic analysis predicts that new markers can be found to diagnose tumors through the imbalance in the concentrations of many proteins during tumor formation. Using this

technique a number of proteins have been identified that are associated with breast cancer (Guojun Zhang *et al*. 2012). Serum amyloid A (SAA) is proteins from apolipoprotein family that related to high-density lipoprotein (HDL) in plasma (Uhlar CM *et al.* 1999). The SAA expression is association with inflammatory stimuli (acute phase SAAs) (Manley PN *et al.* 2006). It also regulates by malignancies, as colon, hepatic, lung and pancreatic cancer. SAA also consider as inflammatory modulator which involved in the metabolism of cholesterol and has a role in tumors (Li Y, *et al.* 2011). Recent study has shown that the highest concentrations of ASS outside the liver are found in cancer tissues. Some studies have shown changes in SAA levels related to tumor found and the levels sensitive to the tumor developments (Mu Yang *et al.* 2016). ALP is an enzyme that responsible for elimination phosphate groups. ALP has several isoenzymes. Hyperalkalinephosphatasemia is present in several pathogenic conditions, such as malignant biliary obstruction, primary biliary cirrhosis, breast cancer, primary sclerosing cholangitis, hepatic lymphoma, and sarcoidosis (Singh AK *et al.* 2013).Recent study reported that ALP elevated in sepsis and malignant includes liver metastases but benign conditions are relatively less causes of hyperalkalinephosphatasemia (Bo Chen *et al.* 2016). Ferritin is protein act as iron storage protein. In breast cancer patients, ferritin levels in serum and tissue are rise (Alkhateeb AA *et al.* 2013). Transforming Growth Factor-β (TGF-β) is a multifunction protein which responsible for enhanced the epithelial mesenchymal transition (EMT),to effect on the immune system response in many disorders such as in malignant conditions (Joanna 2014). The objective of the current study to follow which of the studied parameters can be used to diagnose and monitor the development the disease.

MATERIALS AND METHODS

This study was carried out at the Consultant clinic at Baghdad Teaching Hospital. The study involved 60 breast cancer patients at different stages of the disease, aged (42-59 years). Forty four healthy individual also incorporate in the study for compression. About (5ml) of blood was taken from the patients in a tube and left for 30 minutes, then centrifuged to separate serum which store in eppendorf tube at -20 C until analysis day. Both serum amyloid A and ferritin were measured by ELISA kits supplied from (Biocompare co., USA). Serum TGF1β was measured by ELISA kit (Ray Biotech, USA), while ALP measured by using reflatron (Roch, Germany).

Statistical analysis

Statistical analysis of data was performed by SPSS (version 20). p□0.05 was considered statistically significant.

RESULTS

The characteristics of tumor and patients division according to cancer stage were described in table 1, where patient divided into four groups depend on the disease stage(1, 2, 3 and 4). Tumor size also recorded and divided toT1 (Tumor size ≥ 2 cm or smaller), T2 (tumor size ranged from 2-5 cm), T3 (Tumor size □ 5 cm) and T4 (Tumor at any size but it spread near the breast tissue to the chest surface). Current study showed that there was a significant difference in serum ferritin between patients and control as well as there was a significant difference among patients staging groups, but there was no significant difference between stage 2 and stage 3 of patients group, table (2). Table (2) showed the results of alkaline phosphatase where there was a significant difference between patients' sub-groups and control also there were significant differences among patients staging groups but there was no difference between stage 2 & stage 3 groups. Serum amyloid A level appear that there was a significant increase in all patients graduated with stage of disease as well as there was a significant increase when compare all patients stages with healthy individuals (Table 2). Serum TGFβ1 also increased in patients' stage group as compared with the control but there was no significant difference between stage 1 and stage 2 patients group. Also there was no significant difference between patients stage 3 and patients stage 4 (Table 2)

DISCUSSION

This study was designed to measure some parameters that have been shown to be affected by the present of the tumors such as ferritin, TGF1β, ALP and SAA to determine which is more sensitive to the development and deterioration of the disease. The present study showed a significant difference in the levels of alkaline phosphatase between the healthy group and the patients. Singh et a., [10] recorded that there were no significant difference in alkaline phosphatase levels in metastatic and non-metastatic breast cancer. Some studies have proved the importance of the examination of alkaline phosphatase in many cases of tumors in the event of spread of the tumor to the liver and to the bone, the levels rise significantly (Singh *et al*. 2013 and Mu Yang *et al.* 2016). Serum ferritin significantly elevated in patients groups when compare to healthy individuals and there was a significant difference between patients in stage 1 and other stages but there were no significant differences among other stages. Levels of the ferritin can increase in several conditions such as severe inflammation and tumors, the reason is not clear so far and it may be due to the increase in iron storage or imbalance in the protein syntheses and expression which usually association with tumors (Alkhateeb AA *et al*. 2013).

There is a significant difference in TGF1β levels between patients and healthy people, but there is no difference among different stages of the disease. TGF-β1 expression, enhanced of canonical and non-canonical signal pathways, in TGF-β1 gene mutations and its receptors are related to the oncogenic role of TGF-β1 itself. In the early stages breast cancer, TGF-β1 depressed the normal pathway for epithelial cells development and stimulates apoptosis, and appeared repressive actions in the tumor (Joanna 2014). There was a significant difference between all groups of patients and control group; the study also showed a significant increase in SAA levels among the stages of disease. The researchers were interested in SAA, where they received great interest in considering its relation with the tumor activity, where it was found that the concentration in the blood was significantly affected in patients with tumors, especially malignant and it was found to have an inverse relationship with the survival rate of the patient. Recent study suggested that an overall increment in SAA levels association with metastatic when compared to early stages tumor (Feng *et al*. 2016). In conclusion, current stud suggest that increase SAA concentration in breast cancer patients and correlated with the stage of tumor. Other studied parameters (ALP, ferritin and TGF1β) are suitable for diagnosis but not suitable in assessment of disease progression.

REFERENCES

1. Al-Hashimi MM, Wang XJ. Breast cancer in Iraq, incidence trends from 2000-2009. Asian Pac J Cancer Prev. 2014;15(1):281-6.
2. Redondo M, Rivas-Ruiz F, Guzman-Soler MC, Labajos C. Monitoring indicators of health care quality by means of a hospital register of tumours. J Eval Clin Pract. 2008;14:1026–1030.
3. Tabar L, Yen MF, Vitak B, Chen HH, Smith RA, Duffy SW. Mammography service screening and mortality in breast cancer patients: 20-year follow-up before and after introduction of screening. Lancet. 2003;361:1405–1410.
4. Cocco E, Bellone S, El-Sahwi K. et al. Serum amyloid A: a novel biomarker for endometrial cancer. Cancer. 2010;116:843–851.
5. Zhang G, Sun X, Lv H, Yang X, Kang X. Serum amyloid A: A new potential serum marker correlated with the stage of breast cancer. Oncol Lett. 2012;1; 3(4): 940–944.
6. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant". European Journal of Biochemistry. 1999; 265 (2): 501–23.
7. Manley PN, Ancsin JB, Kisilevsky R. Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A". Medical Hypotheses.2006; 66 (4): 784–92.
8. Li Y, Cai L, Wang H, *et al*. Pleiotropic regulation of macrophage polarization and tumorigenesis by formyl peptide receptor-2. Oncogene, 2011; 36: 3887-3899.

9. Yang M, Liu F, Higuchi K, Sawashita J, Fu X, Zhang L, Zhang L, Fu L, Tong Z, Higuchi K. Serum amyloid A expression in the breast cancer tissue is associated with poor prognosis. Oncotarget. 2016; 14; 7(24): 35843–35852.
10. Singh AK, Pandey A, Tewari M, Kumar R, Sharma A, Singh KA, Pandey HP, and Shukla HS. Advanced stage of breast cancer hoist alkaline phosphatase activity: risk factor for females in India. Biotech. 2013; 3(6): 517–520.
11. Chen B, Dai D, Tang H, Chen X, Ai X, Huang X, Wei W, Xie X. Pre-treatment serum alkaline phosphatase and lactate dehydrogenase as prognostic factors in triple negative breast cancer. J Cancer 2016; 7(15):2309-2316.
12. Alkhateeb AA, Han B, Connor JR. Ferritin stimulates breast cancer cells through an iron-independent mechanism and is localized within tumor-associated macrophages. Breast Cancer Res Treat. 2013 Feb;137(3):733-44.
13. Zarzynska JM. Two Faces of TGF-Beta1 in Breast Cancer. Mediators of Inflammation. 2014, Article ID 141747, 16 pages
14. Zang L, Ma M, Hu J, Qiu H, Huang B, Tongwei Chu T. The effects of lung and prostate cancer bone metastasis on serum osteoprotegerin levels: a meta-analysis. Sci Rep. 2015; 5: 18324.
15. Wang F, Ye H, Q Zhu Q, Qu G, Wang L, Zhao M, Wang P. Serum amyloid A expression is associated with breast cancer survival. Int J Clin Exp Pathol 2016;9(10):9853-9866.

Table 1: Clinical futures of breast tumor

	Classification	Patients (n=60)
Disease stage	1	19
	2	17
	3	15
	4	9
Tumor size	T1	21
	T2	15
	T3	14
	T4	10
Lymph node metastasis	N0	32
	N1	18
	N2	10
	N3	0

Table 2: The clinical parameters for patients and control groups

Parameters Mean±SD	control	Patients			
		Stage 1	Stage 2	Stage 3	Stage 4
Age	48.0±8.6a	51.2±10.2a	50.2±9.5a	52.7±10.7a	49.4±8.8a
Weight Kg	75.6±10.4a	78.3 ± 9.7a	77.5 ±10.6a	82.4 ± 8.4a	79.4 ±9.5a
Height cm	163.1 ± 13a	164.4±12 a	162.6 ± 14a	163.2 ± 16a	166.5 ± 12a
Serum ferritin (ng/ml)	12.4 ± 4.6 a	44.2 ±12.3b	52.8 ± 10.5c	55.6 ± 7.8 c	56.4 ± 5.7 c
ALP (IU/L)	90.1 ± 12 a	152.4 ± 92 b	243.0± 115c	267.3 ±140 c	416.5 ± 233 d
SAA (μg/ml)	2.43 ± 0.8 a	5.14 ± 1.4 b	11.48 ± 3.3c	17.8 ± 8.7 d	29.3 ± 10.9 e
TGFβ1 (ng/ml)	123.0 ± 22 a	214± 56 b	228.6 ± 49 b	323.7±64 c	309.7 ± 52 c

Means with a different letter in the same row significantly different (P<0.05)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Clinical Adverse Effect of Chemotherapy Protocol using 6-Mercaptopurine in Iraqi Patients with Acute Lymphocytic Leukemia during Maintenance Phase.

Mohammed Khudair Hasan[1*], Mowafaq Mohammed Ghareeb[2], Bassam Francis Matti[3], Qutaiba Ahmed Ibrahim Al Khames Aga[4]

[1]Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Iraq.
[2]Professor Assistant,Head of Pharmaceutics Department, College of Pharmacy, University of Baghdad, Iraq.
[3]Consultant Haematologist, Department of Hematology, Baghdad Teaching Hospital, Medical City Complex, Iraq.
[4]Professor Assistant, Department of Clinical Pharmacy, College of Pharmacy, University of Mosul, Iraq.

Received: 18 Mar 2018 Revised: 20 Apr 2018 Accepted: 30 May 2018

[*]**Address for correspondence**
Mohammed Khudair Hasan,
Department of Clinical Pharmacy,
College of Pharmacy
University of Baghdad, Iraq.
Email: msc.phclinc@yahoo.com

ABSTRACT

The study was aimed to find out the main clinical adverse effect of acute lymphocytic leukemia chemotherapy protocol. Acute lymphoblastic leukemia patients require long time continuation therapy to reduce the duration of moderately intensive chemotherapy to 12 to 18 months or less have given poor results in both children and adults. Thus, patients treated for two years or more. The most frequent adverse reaction to chemotherapy protocol is myelosuppression, hepatotoxicity, Hyperuricemia and hyperuricosuria, and immunosuppressive effect. Sixty ALL patients and sixty healthy subjects selected to participate in this study. The subjects involved two groups; group A Includes 60 patients with acute lymphoblastic leukemia on maintenance (continuation) phase receiving 6-mercaptopurine at least for a two-month duration on daily doses based on body surface area. The total number of patients was 60 (40 male and 20 Female); among them, 44 were less than 18 years old (mean ± SD was 9.348 ± 5.131), and 16 were more than 18 years old (mean ± SD was 29.058 ± 7.9173); and, group B Includes 60 healthy subjects' adults and children without any medical illnesses. Of this group there were (40 male and 20 Female), among them, 42 were less than 18 years old (mean ± SD was 8.926 ± 5.326), and 18 were more than 18 years old (mean ± SD was 28.645 ± 7.482). The mean ± SD of months using 6-MP was 13.94 ± 9.49 months, the mean ± SD of 6-MP dose for patients less than 18 years old was 43.77 ± 16.01 and for patients more

than 18 years old was 88.23 ± 30.76. The mean ± SD of weight of patients less than 18 years old was 35.45 ± 9.66 and for patients more than 18 years old was 65.423 ± 13.654. Patients characteristics (such as age, weight, gender, B.S.A, date of the beginning maintenance phase, date of blood sampling, adverse effects of 6-mp and presence of other diseases obtained through patient interview. Complete blood count (Hb, W.B.C count, R.B.C count, platelets count, absolute neutrophils count), liver function tests (AST, ALT, ALP and total serum bilirubin T.S.B), renal function tests (serum creatinine and blood urea) and blood sugar concentration obtained at the same day of blood sampling for patient and control subjects were done. There was no significant change between the male and female in patient and control subjects (p= 0.8675) in regard to age and gender. The main adverse effect of the protocol was seen with higher incidence of jaundice, fever, decreased appetite, unusual tiredness or weakness, headache, stomach pain, dark urine and pale skin. Whereas, the fewer incidences among the common adverse effect are hemoptysis, painful or difficult urination, diarrhea, nausea and vomiting, chest pain and sore throat. The mean serum creatinine and BUN increased significantly in patients ≤ 18 year (mean ± SD 0.97 ± 0.13) (mean ± SD 24.44 ± 6.92) and > 18 year (mean ± SD 0.98 ± 0.12) compared to the control subjects (0.64 ± 0.19 and 0.64 ± 0.22) (14.87 ± 3.64 and 15.21 ± 0.22) consequently. The mean serum ALT, AST, ALP and T.S. bilirubin increased significantly in the patients compared to the control subjects (60.66 ± 14.26 vs. 31.52 ± 5.51), (45.71 ± 23.04 vs. 11.48 ± 7.05), ALP (99.63 ± 38.21 vs. 60.61 ± 19.52) and (1.61 ± 0.58 vs. 0.40 ± 0.14 mg/dl) consequently. The mean RBS increased significantly in the patients compared to the control subjects (166.93 ± 87.56 vs.104.45 ± 16.74). The mean RBC and WBC count decreased significantly in the patients compared to the control subjects (3.68 ± 0.66 vs. 4.37 ± 0.94) and (3.48 ± 1.62 vs. 7.26 ± 1.70). The absolute neutrophil and absolute Lymphocyte count/μm3 were significantly less for patients than for control (1.36 ± 1.12 vs. 4.43 ± 1.27) and (0.81 ± 0.37 vs. 2.18 ± 0.64). The platelets counts *103 were significantly decreased in patients comparing to the control subjects (213.81 ± 83.78 vs. 344.07 ± 46.34). Hemoglobin mean were significantly less in patients comparing to the control subjects (11.43 ± 1.51 vs. 14.55 ± 1.17). In this study, our patients were higher in children and young study population with a male: female 2:1. In addition to the leukopenia, the adverse effect was recorded from the patients was jaundice, fever, decreased appetite, and weakness. The less commonly documented adverse effects of 6-MP have reported from our study was joint pain, heartburn, sores, and ulcer. There is an importance of long-term follow-up, especially for those diagnosed at a young age. Renal involvement is not uncommon in ALL. Renal involvement can present as an elevation in renal function test (creatinine and BUN). Although hematological malignancies commonly involve the liver, they rarely cause clinically significant hepatic disease. Metabolic disorders are common in ALL. Insulin resistance is emerging as a concern for long-term ALL survivors, as is diabetes. Diabetes, hypertension and cardiovascular events must evaluate.

Keywords: Acute lymphocytic leukemia ALL, 6- Mercaptopurine, Renal toxicity, Hepatic toxicity, Hematological malignancies, Metabolic disorders, Insulin resistance.

INTRODUCTION

Cancer subdivided into more than 100 different diseases that characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases (Clayton et al., 2016). Leukemia is a malignant hematopoietic disease characterized by an uncontrolled proliferation and block in differentiation of hematopoietic cells (Lee et al. 2007). These cells can spread to the lymph nodes, spleen, liver and other tissues. Leukemia broadly classified as acute or chronic referring to the cell affected and by the rate of cell growth and of myeloid or lymphoid according to the cell that is multiplying abnormally (Alitheen et al., 2011). Acute lymphoblastic leukemia (ALL) is a heterogeneous group

of lymphoid disorders that result from a monoclonal proliferation and expansion of immature B or T lymphocyte progenitor in the bone marrow, blood, and other organs (Jabbour EJ et al. 2005, Pui and Jeha 2007). Proliferation and accumulation of leukemic cells result in the suppression of normal hematopoiesis and involves various extramedullary sites especially liver, spleen, lymph nodes, thymus, central nervous system, and gonads. Acute lymphoblastic leukemia common in children but can occur in any age group. The healing rate of childhood acute lymphoblastic leukemia of more than 80% and the development of the treatment for this disease is one of the successes in the history of modern medicine (Onciu, 2009). Acute lymphoblastic leukemia mostly occurred in children, with an incidence of 3 to 4/100,000 persons 0 to 14 years of age and 1/100,000 in patients older than 15 years in the United States. In children, Acute lymphoblastic leukemia represents 75% of all acute leukemia cases (which in turn represents 34% of all cancers in this age group), with an incidence peak at two to five years of age. [(6)] The male slightly high affected in all age groups and a significant incidence among white children (Brisson et al. 2015). Varieties of genetic and environmental factors related to ALL, it occurs mostly in patients with Down syndrome, Bloom syndrome, neurofibromatosis type I and ataxia-telangiectasia. Exposure in utero to ionizing radiation, pesticides, and solvents has also related to an increased risk for childhood leukemia (Brisson et al. 2015). The signs and symptoms of ALL are sudden. Patients are suffering from a short history of fatigue or bleeding, malaise, lethargy, weight loss, fevers, and night sweats, patients with ALL suffering from one or more bone and joint pain, also present with asymmetric arthritis, low back pain, diffuse osteopenia, or lytic bone lesions (Faderl et al. 2007).

Anemia, abnormal leukocyte and differential counts, and thrombocytopenia are usually present at diagnosis, reflecting the degree to which bone marrow replaced with leukemic lymphoblast (Conter et al. 2004). The selection of treatment of ALL based on the clinical risk of relapse in the patient differs among subtypes of the disease. Three expected factors are essential in chemotherapy treatment which are; the age of the patient at a time of diagnosis, leukocyte count and the speed of response to treatment (Tubergen, 2010). Acute lymphoblastic leukemia patients require long time continuation therapy to reduce the duration of moderately intensive chemotherapy to 12 to 18 months or less have given poor results in both children and adults. Thus, patients treated for two years or more. A combination of methotrexate administered weekly and 6-mercaptopurine given daily constitutes the basis of most continuation regimens (Pui et al. 2006). Using intermittent pulses of vincristine and a glucocorticoid (prednisone) or dexamethasone to the methotrexate / 6-mercaptopurine combination is beneficial (Campana et al. 2011).

Once a complete hematologic remission obtained, maintenance therapy started, maintenance doses will vary from patient to patient. The usual daily maintenance dose of 6-mercaptopurine is 1.5 to 2.5 mg/kg/day as a single dose or ($75mg/m^2$) (Grossman et al. 2008). The most frequent adverse reaction to 6-mercaptopurine is myelosuppression. The induction of complete remission of acute lymphatic leukemia frequently is associated with marrow hypoplasia (Trissel, 2006). Six-mercaptopurine-induced hepatotoxicity is most common when doses exceed 2.5 mg/kg/day (Anonymous, 2006). Rapid onset of jaundice, cholestasis, ascites, hepatic encephalopathy and elevated liver enzymes often associated with hepatic necrosis and severe fibrosis, can occur (DeVita et al. 2001). Hyperuricemia and hyperuricosuria may occur in patients receiving 6-mercaptopurine because of rapid cell lysis accompanying the antineoplastic effect. Renal adverse effects minimized by increased hydration, urine alkalization, and the prophylactic administration of a xanthine oxidase inhibitor such as allopurinol. The dosage of 6-mercaptopurine should be reduced to one-third to one-quarter of the usual dose if treated with allopurinol concurrently (Anonymous, 2006).

Nausea, vomiting, stomatitis, and anorexia are uncommon during initial administration but may increase with continued administration. Mild diarrhea and sprue-like symptoms noted occasionally, but it is difficult at present to attribute these to the medication (Trissel, 2006). Six-mercaptopurine suppress cellular hypersensitivities and lower allograft repudiation. Inducement of immune reaction to infections and vaccines become subnormal in these patients; the antigen dose and a transitory relation to 6-MP will determine the grade of immunosuppression. This immunosuppressive effect carefully considered about infections and risk of subsequent neoplasia (Grossman et al. 2008). Other adverse effects of 6-MP include hypersensitivity (2-3%), fever (1-10%), alopecia (<1%),

Hyperpigmentation (1-10%), rash (1-10%), secondary malignancy leukemia and myelodysplasia), cysts and polyps (2-6%), increased risk of abortion if taken in first trimester of pregnancy (category D) and oligospermia (Trissel, 2005).

MATERIALS AND METHODS

Study design

A quasi-experimental study carried out at Medical City in Baghdad Teaching Hospital and a central teaching hospital of pediatrics under the supervision of consultant hematologist from December 2017 until June 2018. Sixty ALL patients and sixty healthy subjects selected to participate in this study. All patients complete the courses of the study successfully.

Patients Group

Includes 60 patients with acute lymphoblastic leukemia on maintenance (continuation) phase receiving 6-mercaptopurine at least for two-month duration on daily doses based on body surface area. The total number of patients was 60 (40 male and 20 Female), among them, 44 were less than 18 years old (mean ± SD was 9.348 ± 5.131), and 16 were more than 18 years old (mean ± SD was 29.058 ± 7.9173).

Control Group

Includes 60 healthy subjects' adults and children without any medical illnesses. PCR analysis made to thirty healthy subjects of them to compare thiopurine methyltransferase (TPMT) enzyme genes with patients, liver function, renal function, random blood sugar and complete blood count made to all sixty healthy persons to compare it with patients. Of this group there were (40 male and 20 Female), among them, 42 were less than 18 years old (mean ± SD was 8.926 ± 5.326), and 18 were more than 18 years old (mean ± SD was 28.645 ± 7.482).

Data Collection

Patients characteristics (such as age, weight, gender, B.S.A, date of the beginning maintenance phase, date of blood sampling, adverse effects of 6-mp and presence of other diseases obtained through patient interview. Plasma concentration of 6-mercaptopurine and 6-thioguanine in RBC measured by HPLC-UV. Genetic polymorphism of TPMT detected by polymerase chain reaction conventional (allele-specific method).Complete blood count (Hb, W.B.C count, R.B.C count, platelets count, absolute neutrophils count), liver function tests (AST, ALT, ALP and total serum bilirubin T.S.B), renal function tests (serum creatinine and blood urea) and blood sugar concentration obtained at the same day of blood sampling. Patient information sheet, including all the details that recorded for each patient who was acute ALL on maintenance phase.

Sample Collection and Preparation

Ten milliliters of a venous blood samples were drawn from each patient in the morning at 8:30 AM – 11:30 AM. These samples drawn from patients taking 6-MP at night on continues daily doses at least two consecutive months from the beginning of the maintenance phase. Five milliliters of blood sample divided into two EDTA containing a tube, in first tube 2ml put and stored in deep freeze at (–40 C^{o}) until time for genomic DNA analysis. In the second tube 3ml used to measure complete blood count (CBC) and the residue kept in 4-8 C^{o} until total sample collection in the same day, then centrifuged for 5–10 minutes at 4000 rpm to obtain and collect plasma in another tube for measuring 6-MP plasma concentration. The remaining RBC in the EDTA containing tube washed with sodium chloride 0.9% (normal saline) three times for each sample. Then a suspension of RBC was prepared and stored in deep freeze at (–40 C^{o})

until time for 6-thioguanine-concentration measurement. The other 5ml used to measure AST, ALT, ALP, B. urea; S. creatinine; T.S.B and R.B.S. CBC calculated in hematology department, the others were done in educational laboratories (Baghdad Medical City). In addition to the above, 10ml of blood collected from 30 healthy control subjects to study genetic polymorphism of TPMT and to measure AST, ALT, ALP, B. urea, S. creatinine, T.S.B, R.B.S, and CBC compare it with ALL patients.

Statistical analysis of data

Statistical analysis of the results obtained in this study included; mean ± standard deviation (SD), analysis of variance (ANOVA) single factor, and correlation coefficient (r). The results of the analysis with P value < 0.05 were considered significant. Statistical analysis and graphs were carried out by Microsoft Office Excel 2007 software.

RESULTS

Table 1 shows the distribution of the age within study group and Fig.1 shows the distribution of the study population by gender.

Adverse effects reported in the patients using ALL protocol

Fig 2 shows the main adverse effect of the protocol was seen with higher incidence of jaundice, fever, decreased appetite, unusual tiredness or weakness, headache, stomach pain, dark urine and pale skin. Whereas, the fewer incidences among the common adverse effect are hemoptysis, painful or difficult urination, diarrhea, nausea and vomiting, chest pain and sore throat.

Renal function test (serum creatinine and BUN)

Table 2 points out that the mean serum creatinine increased significantly in patients ≤ 18 year (mean ± SD 0.97 ± 0.13) and > 18 year (mean ± SD 0.98 ± 0.12) compared to the control subjects (0.64 ± 0.19 and 0.64 ± 0.22) consequently. Similar trends were found for BUN in patient's ≤ 18 year (mean ± SD 24.44 ± 6.92) and > 18 year (mean ± SD 27.33 ± 9.53) compared to the control subjects (14.87 ± 3.64 and 15.21 ± 0.22) consequently. Although these result were within the normal range value.

Liver function tests for the patients using 6-MP versus control subjects

Table 3 denotes that the mean serum ALT increased significantly in the patients compared to the control subjects (60.66 ± 14.26 vs. 31.52 ± 5.51). Similar trend were found for AST (45.71 ± 23.04 vs. 11.48 ± 7.05), ALP (99.63 ± 38.21 vs. 60.61 ± 19.52) and T.S. bilirubin (1.61 ± 0.58 vs. 0.40 ± 0.14 mg/dl).

Random blood sugar (RBS) for the control subjects versus the patients using 6- MP

Table 4 show that the mean RBS increased significantly in the patients compared to the control subjects (166.93 ± 87.56 vs.104.45 ± 16.74).

Hematological feature of control versus patients using 6-MP

Table 5 denotes that the mean RBC count decreased significantly in the patients compared to the control subjects (3.68 ± 0.66 vs. 4.37 ± 0.94). Similar trend were found for WBC count (3.48 ± 1.62 vs. 7.26 ± 1.70).

DISCUSSION

Background

Cancer incidence and mortality, in general, increase with age through much of adulthood, but earlier work has found that these rates decline among the elderly and adults. Leukemia is widespread cancer among children around the word. Acute lymphoblastic leukemia (ALL) participates to 76% of all leukemia cases, and 43% of all dooms of pediatric leukemia patients in the United States (Larson, 1995).

General characteristic of the study population

In this study, our patients were higher in children and young study population less than 18 years old (73.33%). The mean of the ALL patients less than 18 years old was 9.348 ± 5.131 years, and for those, more than 18 years old was 29.058 ± 7.9173 tables (3-1). With a male: female 2:1. The control subjects were selected randomly to reflect a reliable comparison with the patients. Sixty random volunteers were involved in this study. Sixty volunteers with gender-related to that of patients (40 male and 20 female). No significant difference in age of patients in comparison to the control subject, which excludes any effect of age on our study.

Genders

Of greater significance, boys were noted to present with high-risk B-precursor ALL four times more than girls (Snodgrass *et al.* 2018). In this study, we record that the higher proportion of ALL found in male (66%), a significantly higher percentage of patients was male for both more and less than 18 years old.

Characteristics of the patients

In this study, the mean period of using 6- MP was 13.94 months. All of the patients were in the maintenance stage of treatment to exclude the high dose used in induction stage. The dose of 6-MP for the patients in our study was within the precise dose/ m^2 of body surface area.

Adverse effects reported in the patients using ALL protocol

In this study, in addition to the leukopenia, the adverse effect was recorded from the patients was jaundice, fever, decreased appetite, and weakness. The less commonly documented adverse effects of 6-MP have reported from our study was joint pain, heartburn, sores, and ulcer. Next, to leukopenia, allergic reactions to 6-MP are the next prevalent, adverse effects. Whether nausea and malaise are also on an allergic basis has not been clear. Allergic reactions, fever, skin rash, joint pains, and back pain were the most common adverse effects (Rowe et al. 2005). Leukopenia was the most common adverse effect of AZA/6-MP treatment. Leukopenia and nausea/vomiting progressed in the early period of treatment of AZA/6-MP in patients with IBD (LI et al. 2015).

Clinical laboratory tests for the patients versus the control subjects using 6-MP

In this study, creatinine in serum and blood urea nitrogen was significantly elevated in patients compared to the control; in spite of it still at normal range, in patients ≤ 18 years (mean ± SD 0.97 ± 0.13) and > 18 years (mean ± SD 0.98 ± 0.12) when compared to the control subjects (0.64 ± 0.19 and 0.64 ± 0.22). Similar trends found for the blood urea nitrogen in patients ≤ 18 years (mean ± SD 24.44 ± 6.92), and > 18 years (mean ± SD 27.33 ± 9.53) compared to the control subjects (14.87 ± 3.64 and 15.21 ± 0.22) consequently. Which could a result of the kidney toxicity of 6-MP. In

acute leukemia, renal complications occur due to several factors including leukemic infiltration of the kidneys, therapy-related side effects such as tumor lysis syndrome, nephrotoxic drugs, and septicemias. Renal involvement can present as renal enlargement due to leukemic infiltrates or as a renal failure due to uric acid nephropathy. However, other causes such as nephrotoxic drugs, infections, and obstructive uropathy due to Para-aortic lymph nodes, retroperitoneal mass, urolithiasis, or ureteral clots can also occur (Dervieux et al. 1998). No significant differences in serum urea and creatinine levels in patients treated with the chemotherapeutic agents. There is mild increase after chemotherapy but not significant; this indicates that chemotherapy is not directly toxic to renal tubules (Onciu, 2015). In this study, a significant increase in serum creatinine and serum BUN were seen in both child and adult when compared with relative age with control subject, but they are still within normal range. In spite of weakly glomerular filtration rate (GFR) causing a condition of decreased urinary elimination, leads to retention and congregation of several nitrogenous waste products in the plasma and the body fluids, a variety in mean values of normal S.Cr level when evaluated in another age groups separately and also between males and females of various age groups (Wong et al. 2017).

Despite the common problem of hepatotoxicity with thiopurine drugs, there is a lack of studies with a significant number of well-characterized patients with this type of liver injury. Most studies have been limited to the irritable bowel syndrome patient population and focused on asymptomatic elevations of serum aminotransferase levels as the only evidence of liver injury (Sak, 2012). In another study, liver toxicity is caused by 6-MP uncommon in the adult patients mentioned in another study. Although hepatic lesion linked with the increased mean of 6-MMP concentration, the sensitivity, and specificity of 6-MMP for drug-induced liver toxicity was with little researches (Sambrook and Russel 2011). In our study, highly significant elevations of liver enzymes (AST, ALT, and ALP) in the patients compared with a control indicate the hepatotoxicity of 6- MP.

It is interesting that 6-MP, when used to treat leukemia in children, has resulted in unexplained symptomatic hypoglycemia. This low glucose level occurred by the action of a small molecule known as NR4A3 (nuclear orphan receptors) agonist, can elevate glucose in insulin target cells, although this occurs via both NR4A3-dependent and -independent actions; the last one is linked to an increment in phospho-AS160. Development of modern treatments strategies for insulin resistance established depending on these results (Zgheib *et al*. 2014). These findings were not matching with our result, in which, a significant elevation of RBS seen in the patients compared to the control subject, although, many research recognized pancreatitis effect caused by 6-MP. Hence this subject needs for more investigations to conclude the possible etiology of hyperglycemia whether it occurres as a result of the disease or drugs used like steroids.

CONCLUSIONS

In this study childhood, survivors of leukemia face many chronic side effects that can greatly impact their disease prognosis. It's currently an inevitable consequence of curative treatment received. There is an importance of long-term follow-up, especially for those diagnosed at a young age. Of considerable importance male was noted to exist with high-risk B precursor of acute lymphoblastic leukemia four times greater than female, proposing the requirements for more research into possible reasons behind this phenomenon. Renal involvement is not uncommon in ALL. Renal involvement can present as an elevation in renal function test (creatinine and BUN)

Leukemic blast cell infiltration may cause significant deterioration of kidney function and renal toxicity. However, other causes such as nephrotoxic drugs, infections, and obstructive uropathy due to Para-aortic lymph nodes, retroperitoneal mass, urolithiasis, or ureteral clots can also occur. Although hematological malignancies commonly involve the liver, they rarely cause clinically significant hepatic disease. In acute leukemia involvement of the liver is generally detectable clinically by the presence of hepatomegaly and high liver function test.

Hepatic infiltrations as the prodromal manifestation of acute leukemia are exceptional and carry a poor prognosis with a great danger of rapidly fatal progression in spite of relevant chemotherapy. Metabolic disorders are common in ALL. Insulin resistance is emerging as a concern for long-term ALL survivors, as is diabetes. Diabetes, hypertension and cardiovascular events must evaluate.

REFERENCES

1. Alitheen NB, Yeap SK, Faujan NH, Ho WY, B.K.Behand BK, Mashitoh AR, Leukemia and Therapy. Am J Immunol, 2011; 7 (4): 54-61.
2. Anonymous. Mercaptopurine: Drug Information. In: Rose BD. Up To Date Wellesley, Massachusetts, 2006; 14.2.
3. Clayton BD, Willihnganz M. Basic Pharmacology for Nurses- E-Book. Elsevier Health Sciences, 2016; 17th ed.
4. Campana D, Pui H, 2011; 6th ed. Childhood acute lymphoblastic leukaemia. In: Hoffbrand AV, Catovsky D , Edward GD , Tuddenham EG, Green AR, Postgraduate Haematology, U.K: Wiley –Blackwell. 25, p. 448 - 458.
5. Wong C. Natural Approaches to Chemotherapy Side Effects, 2017. https://www.verywell.com/chemo-side-effects-natural-remedies-88901.
6. Conter V, Rizzari C, Sala A, Chiesa R, Citterio M , Biondi A , 2004. Acute Lymphoblastic Leukemia . Orphanet Encyclopedia. pp 6.
7. Dervieux T, Boulieu R, 1998. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. Clinical chemistry 44: 551-555.
8. DeVita VT, Hellman S, Rosenberg SA., 2001. Cancer Principles & Practice of Oncology. 6th ed. Philadelphia:
9. Lippincott Williams & Wilkins. p. 404-11.
10. Brisson GD, Alves LR, Pombo-de-Oliveira MS, Genetic susceptibility in childhood acute leukaemias: a systematic review. E Cancer Medical Science.2015; 9: 539.
11. Grossman AB1, Noble AJ, Mamula P, Baldassano RN, 2008. Increased dosing requirements for 6-mercaptopurine and azathioprine in inflammatory bowel disease patients six years and younger. Inflamm Bowel Dis. 14 (6):750-5.
12. Pui H, Evans WE, Treatment of Acute Lymphoblastic Leukemia N. Engl J Med 2006; 354:166-178.
13. Jabbour EJ, Faderl S, Kantarjian HM, Adult acute lymphoblastic leukemia. Mayo Clin Proc. 2005;80: 1517-1527.
14. Sak K. Chemotherapy and Dietary Phytochemical Agents. Chemother Res Pract.2012; 282570.
15. Larson RA, Dodge RK, Burns CP, A five-drug remission induction regimen with intensive consolidation for children with acute lymphoblastic leukemia: cancer and leukemia group B study 8811. Blood.1995; 85 (8): 2025-37.
16. Lee SJ, Kim KH, Park JS, Kim DW, Kim KP, Comparative analysis of cell surface proteins in chronic and acute leukemia cell lines. Biochem. Biophys. Res. Comm.2007; 357: 620-626.
17. Onciu M, Acute Lymphoblastic Leukemia. Hematol Oncol Clin North Am. 2009;23(4):655-74.
18. Pui CH, Jeha S, New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. Nat Rev Drug Discov. 2007;6: 149-165.
19. Snodgrass R, Nguyen LT, Guo M, Vaska M, Naugler C, Kolvear FR, Incidence of acute lymphocytic leukemia in Calgary, Alberta, Canada: a retrospective cohort study. BMC Res Notes. 2018;11:104.
20. Rowe JM, Buck G, Burnett AK, Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. Blood.2005; 106(12): 3760-7.
21. Faderl SH, Kantarjian HM, Hematologic Malignancies: Acute Leukemias. Springer Science & Business Media. 2007; 21:110.
22. Sambrook J, Russel DW, 2001; 3rd edition. Molecular cloning: A laboratory manuals. Cold Spring Harbor laboratory Press, New York.
23. Li SY, Ye JU, Meng, FCF, and Mo Yang M, Clinical characteristics of acute lymphoblastic leukemia in male and female patients: A retrospective analysis of 705 patients. Oncol Lett 2015; 10 (1): 453–458.
24. Trissel LA, 3rd ed. Stability of Compounded Formulations.. Washington, District of Columbia: American Pharmacists Association. 2005; 268-9.

25. Tubergen DG. The Leukemias. In: Kliegman RM, Behrman RE Jenson HB, Stanton BF, 19th ed. Nelson Textbook of Pediatrics,., Philadelphia: Elsevier publishing. 2010;487, p.1694 – 1696.
26. Zgheib NK, Akra-Ismail M, Aridi C, Genetic polymorphisms in can-didate genes predict increased toxicity with methotrexate therapy in Lebanese children with acute lymphoblastic leukemia. Pharmacogenet Genomic. 2014; 24:387–396.

Table 1: The distribution of the age within study group.

Age range (years)	Number		% Total study population	Mean ± SD	P- value
≤ 18 (2-18)	Patient	44	73.33	9.348 ± 5.131	0.243
	Control	42	70.00	8.926 ± 5.326	
> 18 (18.5-42)	Patient	16	20.66	29.058 ± 7.917	0.385
	Control	18	30	28.645 ± 7.482	

Table 2: Serum creatinine and BUN for the patients versus the control subjects using 6-MP.

Serum parameter (mg/dl)	Group	Mean	± SD	P-value
Creatinine for patients ≤ 18 years	Patients	0.97	0.13	0.0007*
	Control	0.64	0.19	
BUN for Patients ≤ 18 years	Patients	24.44	6.92	0.00086*
	Control	14.87	3.64	
Creatinine for > 18 years	Patients	0.98	0.12	0.00065*
	Control	0.64	0.22	
BUN for > 18 years	Patients	27.33	9.53	0.00023*
	Control	15.21	3.61	

Table 3: Serum ALT, AST and ALP in the control subjects versus the patients using 6- MP.

Serum parameter (Normal value)	Group	Mean	± SD	P-value
ALT (U/L) (<55 U/L)	Patients	60.66	31.52	0.00006*
	Control	14.26	5.51	
AST (U/L) (<40U/L)	Patients	45.71	23.04	0.00019*
	Control	11.48	7.05	
ALP(U/L)(30-85 U/L)	Patients	99.63	38.21	0.00046*
	Control	60.61	19.52	
T.S.Bilirubin (0.3-1.2 mg/dl)	Patients	1.61	0.58	0.00724*
	Control	0.40	0.14	

*****Significant difference

Table 4: Random blood sugar (RBS) for the control subjects versus the patients using 6-MP.

Parameter (mg/dl)	Group	Mean	± SD	P-value
RBS >140	Patient	166.93	87.56	0.000108*
	Control	104.45	16.74	

*Significant difference

Table 5: Hematological feature of control versus patients using 6-MP

Hematological parameters (Normal range)	Group	Mean ± SD	P-value
RBC count (4.32-5.72)	Patients	3.68 ± 0.66	0.0004*
	Control	4.37 ± 0.94	
WBC count (3.5-10.5)	Patient	3.48 ± 1.62	0.00008*
	Control	7.26 ± 1.70	
Absolute neutrophil count//μm^3 (2.5-7)	Patient	1.36 ± 1.12	0.0004*
	Control	4.43 ± 1.27	
Absolute lymphocyte count//μm^3 (0.9-2.9)	Patient	0.8100 ± 0.37	0.00752*
	Control	2.1800 ± 0.64	
Platelets counts (150-400)	Patient	213.81 ± 83.78	0.00005*
	Control Patient	344.07 ± 46.34	
Hb mg/dl (12-17)	Patient	11.430 ± 1.51	0.00001*
	Control	14.550 ± 1.17	

*Significant difference

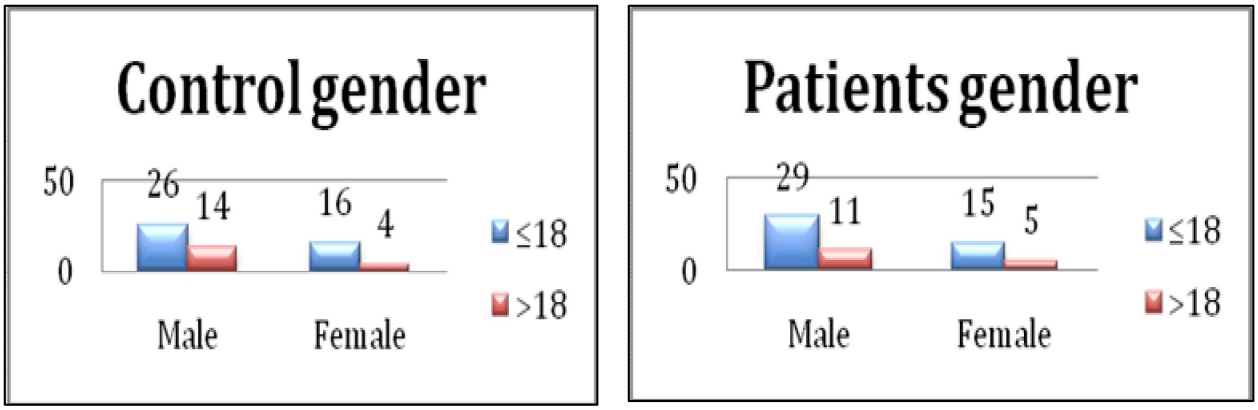


Fig. 1: The distribution of the study population by gender

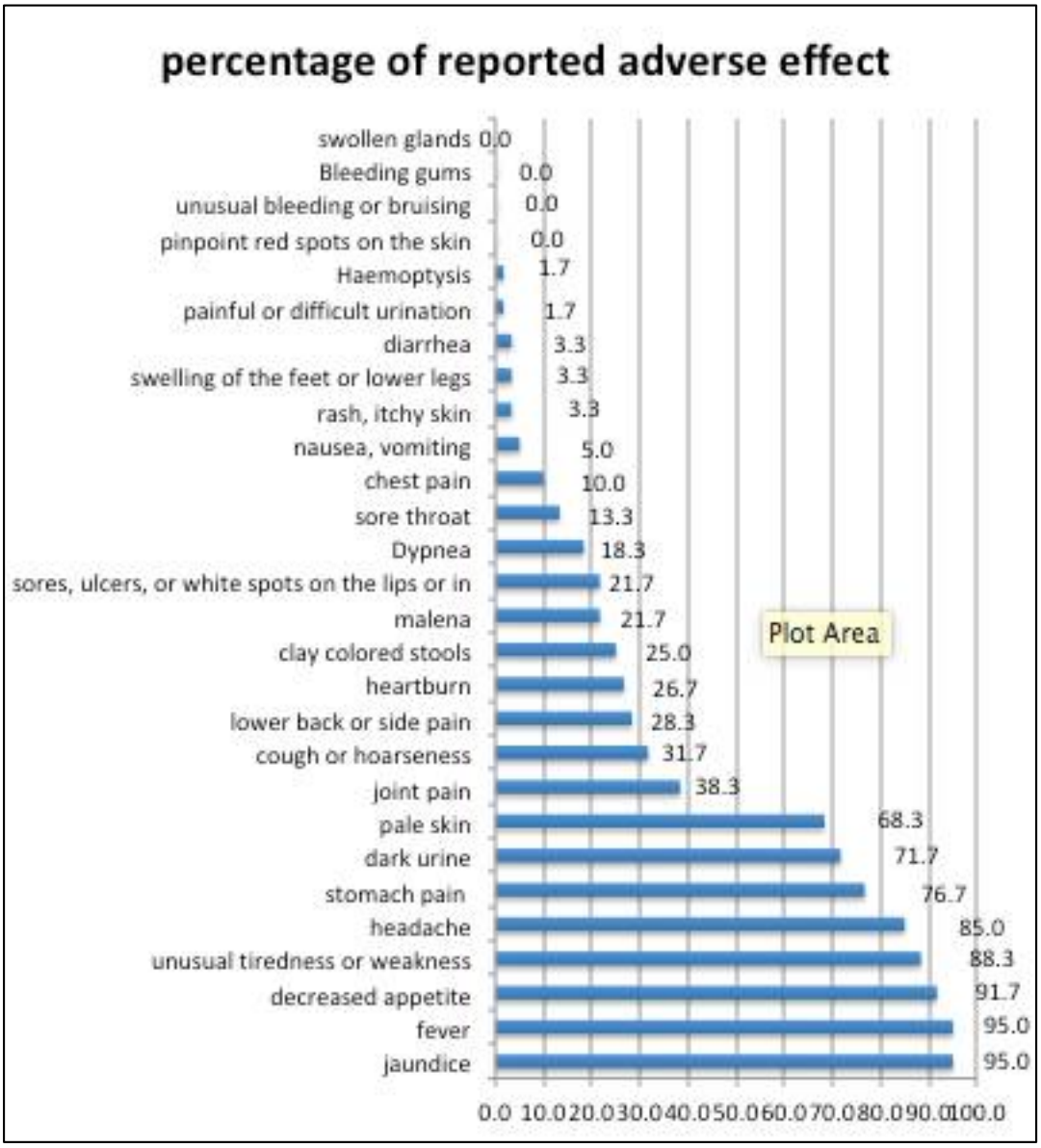


Fig. 2: Percentage of reported adverse effects in the patients using ALL protocol

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Antioxidant Role of Vitamin C and Zinc in Ovariectomized Rabbits With Metabolic Disorder

Sabreen M.Momammed Ali*, Muhammed R. Al-Kenany and Tahani S. Al-Azawi

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine , University of Baghdad,Iraq.

Received: 19 Mar 2018 Revised: 20 Apr 2018 Accepted: 30 May 2018

***Address for correspondence**
Sabreen M.Momammed Ali,
Department of Physiology, Biochemistry and Pharmacology,
College of Veterinary Medicine,
University of Baghdad, Iraq.

ABSTRACT

This experiment was aimed to investigate the antioxidant effect of vit C and zinc on some metabolic disorders after ovariectomy. Twenty female rabbits 7-8 weeks of age were divided into four groups.G1andG2 were left with the intact ovaries while, G3 and G4 were subjected to ovariectomy of both ovaries. VitaminC and zinc at dose of 10.166mg/kg B.wt was supplemented to G2 and G3 daily by stomach tube for 30 days. The results reveal a significant elevation in serum cholesterol in the ovariectomized groups (67.0 and 64.6) in comparison with the intact group(50.33 and 39.34) respectively. Vitamin C and zinc supplementation lowering serum cholesterol, triglyceride,LDL-C and glucose level accompaniend with elevating the HDL-C concentration. Moreover, the supplementation induces a significant decrease in serum level of cortisol, T3 and T4. The antioxidant effect of vitamin C and zinc and their vital role in metabolic had been discussed. In conclusion, this complementary antioxidant has an ameliorative effect against the metabolic disorder after ovariectomy.

Keywords: metabolic disorder, ovariectomy, Vitamin C, Zinc.

INTRODUCTION

The ovaries are vital organs. They produce hormones of female need, throughout her entire life in the quality are needed, at the time they are needed , in response to and as a part of the complex endocrine system(Challberg et al., 2011). There are many types of estrogen ,however ,all of them synthesized from androgens, specially testosterone and androstenedione, by the enzyme aromatase (Rebecca et al., 2013). Scientists are still learning about the actions of estrogen in the body. Studies have shown that estrogen affects almost every tissue or organ system (Bhupathy et al., 2010). There is growing concern regarding fat accumulation in tissues and organs in chronic diseases such as obesity, CVD, insulin resistance (Un and Myung, 2014). These metabolic syndroms are commonly increased at menopause and in ovariectomized humans and animals which could be reduced by hormonal replacement (Shinoda et al., 2002). However, recently the effect of some anti- oxidants on ameliorating reactive oxygen species in

ovariectomized rabbits have been studied. Supplementation of alpha- lipoic acid(10mg/Kg B.wt) to intact and ovariectomized rabbits had been associated with a protective action on some parameters related to metabolic disorders such as thrombosis, lipid profile and insulin resistance, with a significant decrease in some biological cardiac markers associated with cardiovascular disease (Al-Azawi and Rwayyih, 2017). Moreover, the role of complementary vitamin C and Zinc in ovariectomized rabbits induces a significant improvement of some cells and proteins related to immune system and decreasing the oxidative stress(Al-Azawi and Alkenany, 2017). Therefore, the aim of this experiment is to find out the effect of the complementary vitamin C and Zinc on some parameters associated with metabolism in intact and ovariectomized rabbits.

MATERIALS AND METHODS

Experimental animals and management

This experiment was carried out in the animal house at the college of veterinary medicine during the end of 2017 (lasted for 4 months).Twenty female Rabbits were used in this study, their ages were ranged between 7-8 weeks, and their weight was around 820-1050/gram. These animals were kept under suitable environmental conditions 20-25 0C in an air conditioned room and photoperiod of 12 hours daily. Fresh water and pellet diet were offered all over the period.

Ovariectomy surgical Operation technique

Ovariectomy for both ovaries was performed to ten rabbits according to the procedure by (Meredith et al., 1999).The other ten rabbits were left with intact ovaries. The animals were anesthetized with IM injection of ketamine (35mg/kg BWt) and xylazine (15mg/kg BWt). The operation was performed under sterile conditions. After the operation the animals were kept individually with post-operative care for at least 5 days.

Preparation of the complementary vitamin C and Zinc dose

The dose of this experiment was calculated according to the recommended dose for human 1-3 tablet/day that provided by MEDCELLPHARMA®CO(Netherlands). Each one contains three hundred and five mg (vitamin C 300mg + Zinc 5mg). Then each rabbit was received 1ml/1kgB.W (10.166 mg/kg B.W).The dose was administered daily to each female of two groups for thirty days using gastric intubation.

Experimental design for the experiment

After acclimatization for two weeks, the rabbits were divided equally into four groups, two groups was kept intact (G1, G2) and two groups were ovariectomized (G3, G4). Group two and four were administered daily with the complementary vitamin C and Zinc (10.166 mg/kgB.W). The other two groups (G1, G3) were received distilled water daily.

Blood samples collection

At the end of the period (30 day) of the experiment, fasting blood was obtained via jugular vein from each rabbit. Serum was isolated after centrifugation at a speed of 3000 revolution/minute (rpm) for 20 minutes. Serum samples were stored in freezer at -18 °C until use (Colak et al., 2013).

Parameters determination

Serum cholesterol, Triglyceride and HDL-C ,LDL-C were analyzed by enzymatic assay kits (Dimension®clinical chemistry) according to Burtis et al., (1999). Serum glucose concentration was enzymatically measured (enzymatic oxidation method) as described by Barnett and Owyang, (1988). The assay of serum insulin concentration was according to the competitive inhibition enzyme immunoassay technique (Alberti et al., 2006). Serum cortisol concentration was assayed according to Tietz, (2005). TheT4&T3 level in serum were evaluated by a method described by Tietz, (2005).

Statistical analysis

All data were subjected to one -way ANOVA and least significant differences (LSD) to assess the significant differences among means by using SAS, (2010).

RESULTS

There is a significant ($p \leq 0.05$) increase in cholesterol level in both ovariectomized groups as compared with intact animals. At the same time there is a significant elevation in each of (triglyceride and ,LDL-C, in (DW)intact group as compared with ovariectomized and supplemented groups. On the other hand, table (1) shows that, the oral supplementation of the complementary vitamin C and zinc induces a great significant decrease of serum cholesterol, triglyceride and LDL-C in the intact animals accompanied with increasing HDL-C compared with ovariectomized rabbits. Table (2) reveals a significant decrease ($p \leq 0.05$) in the level of glucose,cortisol,T3 and T4 in serum of ovariectomized groups as compared with intact animals. At the same time, there is a significant decrease in the serum cortisol, T3 and T4levels in both supplemented (ovariectomized and intact groups) in comparison with non-supplemented groups.

DISCUSSION

The studies were suggested that vitamin C may prevent the accumulation of sorbitol intracellular by inhibiting the reductase enzyme. Beveridge et al., (2008) illustrated that Vitamin C an aqueous phase antioxidant has been reported to improve whole body glucose disposal in healthy subjects and in diabetic patients and animals. Vitamin C plays important role in reducing the blood glucose through decreasing the oxidative stress in different ways including their protective effect on the blood vessels and preventing the lipid peroxidation. Also by their ability to regulate nitric oxide synthase that generates the nitric oxide a potent vasodilator which play a key role in controlling the cardiovascular system. Moreover, vitamin C has been reported to regulate the antioxidant enzymes including superoxide dismutase and glutathione and increased the tetrehydrobiopterin an important cofactor of nitric suggested that vitamin C supplementation in humans and animals regulates blood glucose due to decreasing its resistance to insulin and reducing the oxidative damage in the tissue by reducing free radicals and the decreasing of glycosylation to protein. The results revealed significant decrease ($p \leq 0.05$) in blood glucose concentration in ovariectomized group treated with vitamin C& zinc. This result is in agreement with Valko et al., (2006) who showed that the zinc is capable of modulating insulin action and improving hepatic binding to it. The ovariectomized treated group with vitamin C and zinc revealed a significant decrease in lipid profile (total cholesterol, triglyceride) compared with intact group. These findings are similar to results obtained by some researchers (Jariwalla et al., 1997; Beveridge et al.,2008). This could be attributed to the hypo cholesterolemia effect of vitamin c that protects LDL-cholesterol from oxidative damage and aids in degradation of cholesterol (Coles, 1986). Oxidative damage caused by toxic free radicals at early stage. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may partially prevent certain types of hepatic cellular damage (Walmsley

and White, 1988; Bruno et al., 2006). Moreover, vitamin C is required for regeneration of α-tocopherol, which may prevent LDL-C oxidation (Mawatari and Murakami, 2001). The results of the current study are documented by a previous findings (Frassinetti et al., 2006) who found that patients when treated with Zinc resulted in reduction of TC and TG and increase of HDL-C concentrations but did not decrease LDL-C concentration significantly which indicate that its treatment improved the individuals metabolic condition. Zinc plays an important role in the structure and function of biological membranes (Ortona et al., 2015). Zinc has also been shown to have an antioxidant potential through the non-enzymatic stabilization of biomembrane and biostructures. The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver as well as it exerts critical physiological role in regulating the structure and function of cells (Wintergerst et al., 2006). While Anderson et al., (1980) concluded that Zinc is required for the adequate formation and function of the antioxidant enzyme superoxide dismutase and various metallothionins. Also some authors concluded that ascorbic acid has both an adipogenic effect as a cofactor of an enzymatic process and a lipolytic effect as an antioxidant (Baltgalvis et al., 2010). Supplementation with ascorbic acid, a major source of nicotinamide adenine dinucleotide phosphate, inhibits adrenal suppression by promoting the turnover rate of 11- β -hydroxylase, thereby increasing the cortisol formation (Baltgalvis et al,m 2010). Vitamin C and zinc have a vital role in protection females body against the deleterious effect of estrogen deficiency either by ovariectomy or by normal physiological aged depression (6). It was concluded that vitamin C and zinc supplementation provide a complementary antioxidant effect to ameliorate the metabolic disorders associated with ovariectomy.

REFERENCES

1. Challberg J, Ashcroft LF, Lalloo B, Eckersley R, Clayton P, Hopwood P, Selby A, .Howell G, Evans DG. Menopausal symptoms and bone health in women undertaking risk reducing bilateral salpingo-oophorectomy: significant bone health issues in those not taking HRT. Br J Cancer.2011; 105: 22 – 27.
2. Rebecca VR, O'Donnell CI, Barón AE, Grunwald GK, Maddox TM, Bradley SM, Woning G, Wierman ME, Plomondon ME, Rumsfeld JS, Ho M. Testosterone Therapy and Mortality, MI, and Stroke.J A M A. 2013; 310(17):1829-1836.
3. Bhupathy P, Christopher CH, Leinwand LA. Influence ofsex hormones and phytoestrogens on heart disease in male and women.Womens Health. 2010;6(8):321-343.
4. Un JJ, Myung SC. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. Int J Mol Sci.2014; 15: 6184-6223.
5. Shinoda M, Latour MG, Lavoie JM. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. International Journal Obesity Related. 2002;26(3):335-343.
6. Al-Azawi TSS, Rwayyih S. Protective role of alpha Lipoic acid on someparameters related to metabolic disorders in intact and ovariectomizedrabbits. J Entomol Zool Stud..2017; 5(4): 1281-1285.
7. Al-Azawi TSS, Alkenany MA. Complementary effect of vitamin C and Zinc on some blood cells and serum proteins related to immunity in intact and ovariectomized rabbits. J Entomol Zool Stud. 2017;5(5): 1296-1303.
8. Meredith A, Redrobe S, Keeble E. Neutering pet rabbits.J Vet Rec. 1999;20:328.
9. Colak A, Toprak B, Dogan N, Ustuner F. Effect of sample type, centrifugation and storage conditions on vitamin D concentration. BiochemiaMedica. 2013; 23(3):321-5.
10. Burtis C, Ashwood E.Textbook of Clinical Chemistry.3rd ed. London. ; 1999;1145-1150.
11. Barnett JL, Owyang C. Serum glucose concentration as modulater integrity for digestive gastric motility. Gastroenterology. 1988; 94(3):739-744.
12. Alberti MK, Zimmet P, Shaw J. Metabolic syndrome – a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
13. Tietz NW. Textbook of Clinical Chemistry and Molecular Diagnostic, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Sounders CO.2005.

14. SAS SAS\STAT user Guide for Personal computer.Release 9.1…SAS institute Inc., cary, N.C, USA, 2010.
15. Beveridge S, Wintergerst ES, Maggini S. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Proc Nut Soc. 2008;67:E83.
16. Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M. (Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Bio Interact.2006; 160:1-40.
17. Jariwalla RJ, Harakeh S. Mechanisms underlying the action of vitamin C in viral and immunodeficiency disease. In: Packer L, Fuchs J, eds. Vitamin C in Health and Disease. New York: Marcel Dekker, Inc. 309-322.1997.
18. Coles EH. Veterinary Clinical Pathology. 4th ed. W.B. Saunders Co. Philadelphia, London. 1986:15-20, 53-56, 68,139.
19. Walmsley RN, White GH. A guide to Diagnostic Clinical Chemistry, 2th ed. Blackwell. Sci. pub 1, oxford,1988; 513.
20. Bruno RS, Leonard SW, Atkinson J. Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. Free Rad Bio Med.2006; 40(4):689-697.
21. Mawatari S, Murakami K. Effect of ascorbate on membrane phospholipids sand tocopherols of intact erythrocytes during peroxidation by t-butyl hydroperoxide. Lipids.AOCS Press.2001; 1(36):57-65.
22. Frassinetti S, Bronzetti G, Caltavuturo L, Cini M, Croce C. (2006).The role of zinc in life: a review. J Environ Pathol, Toxicol Oncol. 2006;25(3):597-610.
23. Ortona E, Delunardo F, Maselli A, Pierdominici M, Malorni W. Sex hormones and gender disparity in immunity and autoimmunity. Ital J Gend Specif Med. 2015;1(2):45-50.
24. Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions.Ann of Nutr Metab.2006; 50:85-94.
25. Anderson R, Oosthuizen R, Maritz R, Theron A, Van RensburgAJ. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. Am J Clin Nutr. 1980;33(1):71
26. Baltgalvis K, Greising S, Warren G, Lowe D. Estrogen Regulates Estrogen Receptors and Antioxidant Gene Expression in Mouse Skeletal Muscle. PLoS ONE. 2010;5(4):10164.

Table 1: The antioxidant role of vitamin C and zinc on serum cholesterol, triglyceride, LDL-C and HDL-C concentration.

	Intact		Ovariectomized		
parameter/Group	**G1**	**G2**	**G3**	**G4**	**LSD**
Cholesterol mg/dl	50.33±1.45b	39.34±1.45c	67.00±2.64a	64.66±2.66a	6.9818
Triglyceride mg/dl	114.33±0.98a	68.32±1.44b	80.00±1.73b	72.33±1.20b	12.129
LDL-C mg/dl	42.16±0.66a	17.0±0.08c	28.70±3.37b	32.63±3.48b	7.9782
HDL-C mg/dl	21.06±1.81b	25.30±0.80a	22.30±1.15ab	19.23±1.08b	4.1419

Values represent mean±SE, n=5

Different small letters denote significant difference between groups ($p \leq 0.05$).

Table 2: The antioxidant role of vitamin C and zinc on serum glucose, insulin, cortisol, T3 and T4 concentration.

	Intact		Ovariectomized		
parameter/Group	**G1**	**G2**	**G3**	**G4**	**LSD**
Glucose mg/dl	163.33±3.23a	124.33±2.40b	149.00±2.30b	121.33±2.33b	4.314
Insulin ulv/ml	1.73±0.03a	1.66±0.03a	1.70±0.05a	1.73±0.03a	0.1331
cortisol mg/dl	5.11±0.19a	0.63±0.03c	1.83±0.28b	0.73±0.3c	0.5684
T3nmol/L	1.60±0.27a	0.81±0.006c	1.29±0.01ab	0.97b±0.03bc	0.4567
T4nmol/L	66.96±1.03a	15.43±0.33c	40.86±1.62b	33.16±1.89b	9.1828

Values represent mean±SE, n=5

Different small letters denote significant difference between groups($p \leq 0.05$).

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Identification and Predictive Analysis of Water Quality using Evaluation of Rotifers and Zooplanktons

Rasha. M.Salman*

Department of Biology, College of Science, University of Waist, Kut, Iraq.

Received: 20 Mar 2018 Revised: 18 Apr 2018 Accepted: 30 May 2018

***Address for correspondence**
Rasha. M.Salman,
Department of Biology,
College of Science,
University of Waist, Kut, Iraq.

ABSTRACT

The evaluation of water quality and analysis of internal factors are quite important because it is the key element in nature. The quality of water includes physical, radiological, biological and chemical characteristics of the water which is always to be measured. In this research manuscript, the evaluation perspectives of water quality user rotifiers and zooplanktons are integrated with the measures for achieving the higher quality in the water.Rotifiers and Zookplanktons are widely used elements for the evaluation of water quality with assorted perspectives that is the key aspect in this manuscript.

Keywords: Evaluation of Water Quality, Water Quality Parameters, Rotifiers, Zooplanktons.

INTRODUCTION

Water Quality refers to the measurement and evaluation of the water condition with respect to the specific human need or biotic species (1, 2, 3). The prominent and widely used standards for assessing the water quality are associated with ecosystem, health and the drinking water.

Categories and the Taxonomy of water usage in different domains

- Human Consumption
- Industrial Usage
- Domestic Use
- Environmental Use

Following are the factors integrated with the quality of water for different domains and applications

- pH Value

- Color
- Alkanity
- Radon
- Pharmaceuticals
- Heavy Metals
- Taste
- Odor
- Dissolved Metals
- Metalliods
- Hormone Analogs
- Microorganisms

Chemical Indicators of Water Quality

- pH
- Chemical oxygen demand (COD)
- Heavy metals
- Dissolved oxygen (DO)
- Total hardness (TH)
- Pesticides
- Biochemical oxygen demand (BOD)
- Orthophosphates
- Nitrate
- Surfactants

Biological Indicators of Water Quality

- Mollusca
- Plecoptera
- Escherichia coli (E. coli)
- Ephemeroptera
- Trichoptera
- Coliform bacteria

Rotifiers

Rotifera is a sensibly assessed phylum of moment, relatively symmetrical, unsegmented creatures that live fabulously in freshwaters (4,5). The phylum name (Latin, rota, wheel; ferre, to hold up under), first utilized by Cuvier in 1798 (6, 7), recommends the chief end that in different species takes after a turning wheel in light of the dynamic beat of its cilia. Rotifers have been found on each landmass, including a differing gathering of basic condition, including marine, repulsive and freshwaters, and besides the film of water that coats normal greeneries and particles in moist soils. In lakes rotifers every now and again accomplish high masses densities (>1000 people per liter); similarly, as customers of modest living creatures, green advancement and protists they are normally fundamental in exchanging vitality to higher trophic levels. As rotifers are an OK sustenance for vigorous fishes, they are made in mass wholes in business aquaculture. They in like way fill in as models for investigate on creating, and as bioindicators for ecotoxicology (8, 9). Rotifers have been found in the inland waters (both crisp and saline) of each landmass, in the stickiness covering a few plants, for example, greeneries, and in wet soil. However everything considered not as

boundless as in region waters, rotifers a great part of the time flourish in nearshore marine waters and estuaries. Different rotifers are set up for experiencing gradualness, either as grown-ups or making lives, engaging them to scatter in space or time and routinely keep up a key detachment from severe conditions. Supporting by and large on other moment living animals, including microscopic living creatures, green improvement and protists, rotifers are fundamental basal customers in oceanic frameworks. Specifically lakes, rotifers acknowledge an essential part accomplice the microbial drift to the impressive sustenance web, whereby the split up ordinary carbon is taken up by the microscopic animals that are eaten up by rotifers, which thus are eaten up by higher trophic levels. In this manner, a segment of the essentialness in isolated consistent issue is come back to the sustenance web. As they are the fundamental sustenance for larval living animals, rotifers have been manhandled by aquaculturists to keep up monetarily essential fishes, shrimps and crabs.

As they are nearly unassuming and simple to culture and have all things considered short prospects, rotifers are utilized as a bit of creating contemplates and to evaluate the lethality of mixes, for example, significant metals, pesticides and pharmaceuticals.Several sorts of rotifers have particular natural prerequisites making them imperative as markers of water quality. The phylogenetic position of rotifers has not yet been settled, but rather they have each one of the stores of being identified with a party of pretty much nothing, jawed metazoans (Gnathifera). Acanthocephala, an absolutely parasitic phylum, are starting at now thought to be especially worsen rotifers. Amid the time rotifers have entered the aggregate culture ethos as subjects of verse, sci-fi and teenagers' organization, gifted specialists' workings in glass and blended media and surely understood music.

Zooplanktons

Zooplankton are unobtrusive animals that live in both fresh and marine waters. They skim in the water, skimming vivaciously or remain idly suspended in the water. The zooplankton eats the planktonic green progression and there is a dynamic connection between the zooplankton and the phytoplankton masses in a given conductor. The zooplankton store in new water is essentially managed of free-living protozoans, rotifers, cladoceraus and copepods (10, 11,12). The species coordinated accumulation and numbers help in finding water quality. ALS gives NATA guarantee examination of Zooplankton in waters (ground, surface and consumable), wastewaters, sewage and some bleeding edge effluents. By prudence of the short life cycle, zooplanktons respond quickly to environmental changes and could along these lines be an additional contraption to screen and track the alterations in the likelihood of the water condition. Unequivocally when zooplankton numbers climb to sporadic states, there is the likelihood to impact and effect water quality through taste and smell. The climb and fall of zooplankton masses prompts decay of their dead bodies which is appeared in scraping scents and conglomeration of trademark refuse cleaving down water quality, creating turbidity and shading, crippling disengaged oxygen and raising customary carbon levels. Further, extended zooplankton numbers are known to address a bugging by blocking channels and intruding with water treatment and purging procedures, accordingly diminishing the adequacy of plants.

Zooplanktons are considered as a champion among the most key sustenance source to the ocean living things particularly to planktivorous edge. Zooplankton social demand is the ensured course for criticalness change in the little fish based sustenance web impacts them to change into an essential bit in working of ocean regular frameworks (Santos-Wisniewski et al. 2006). Right when all is said in done, the properties of zooplankton add up to structure are portrayed by the trademark segments including surface zone, significance, trophic level, shade of water, and the run of the mill social event of the lake. Thus, these creatures can be an important gadget for the confirmation of customary status of a lake. Industrialisation and human activities are the fundamental driver of water pollution. Dirtied water contained compound substances affect algal create which roundaboutly makes issue ocean common structure, for instance, eutrophication. Zooplankton have cozy association with the disguising condition for the term they can envision cycles and they show brilliant changes in their masses while inciting influence happens, for instance, eutrophication. In this way, they are potential pointer species for water contamination. In this emerge condition, the present examination was embarked to see the three most standard sorts of freshwater zooplankton;

cladocerans, copepods, and rotifers in the eutrophic man-made lakes. We in like route hope to develop the level of relationship between physical normal elements and the species wealth in the zooplankton social affairs. Zooplankton are sans small skimming zone and water skilled microorganisms including shellfish, rotifers, untamed water dreadful minimal creature hatchlings and maritime bugs. The zooplankton group is made out of both basic customers, which eat free-floating green progression, and discretionary buyers, which eat up upon other zooplankton. The zooplanktons add up to is a basic piece of the maritime general lifestyle. These animals fill in as a judge animal groupings in the made lifestyle, trading centrality from planktonic green progression (essential creators) to the more noticeable invertebrate predators and fish who as needs be eat up upon them. Zooplanktons are exceedingly sensitive to changes in ocean trademark frameworks. The effects of consistent aggravations can be seen through changes in species creation, wealth and body measure scrambling. Information on the sorts of zooplankton that are found in the water, and the abundance of particular species as for each other, fills in as a measure of ordinary condition. Zooplankton are outstanding pointers of advance in supplement tainting after some time since they respond quickly to changes in supplement feeling of obligation with respect to the waterbody.

CONCLUSION

Evaluation of water quality is one of the utmost important tasks for industrial as well as domestic purposes. There are enormous approaches for evaluation of water quality including rotifiers and zooplanktons. The rotifers (Rotifera, dependably called wheel animals) make up a phylum of little and close minute pseudocoelomate animals. They were first portrayed by Rev. John Harris in 1696, and diverse structures were portrayed by Antonie van Leeuwenhoek in 1703. Most rotifers are around 0.1– 0.5 mm long (paying little personality to the way that their size can go from 50 μm to in excess of 2 mm), and are general in freshwater conditions all through the world with a couple of saltwater creature shapes; for example, those of class Synchaeta. A few rotifers are free swimming and to a great degree planktonic, others move by inchworming along a substrate, and some are sessile, living inside tubes or thick holdfasts that are associated with a substrate. Around 25 species are normal (e.g., Sinantherinasemibullata), either sessile or planktonic. Rotifers are a basic bit of the freshwater zooplankton, being an important foodsource and with various species moreover adding to the ruin of soil trademark issue. Most sorts of the rotifers are cosmopolitan, regardless there are moreover some endemic species, as Cephalodellavittata to Lake Baikal. Late barcoding certification, in any case, suggests that some 'cosmopolitan' species, for instance, Brachionusplicatilis, B. calyciflorus, Lecane bulla, among others, are truly species structures.

Zooplankton are heterotrophic (now and again detritivorous) little fish. Little fish are living things gliding in oceans, seas, and collections of new water. Particular zooplanktons are near nothing, yet a couple, (for instance, jellyfish) are more important and recognizable with the stripped eye. Zooplankton is a get-together intersection a level of living thing sizes including little protozoans and creature metazoans. It consolidates holoplanktonic life shapes whose aggregate life cycle exists in the tiny fish, and moreover meroplanktonicorganisms that spend some bit of their lives in the unpretentious fish before graduating to either the nekton or a sessile, benthicexistence. Notwithstanding how zooplankton are on a to a great degree principal level transported by merging water streams, diverse have speed, used to keep up a key separation from predators (as in diel vertical improvement) or to extend prey encounter rate. Ordinarily central protozoan zooplankton bundles join the foraminiferans, radiolarians and dinoflagellates (whatever is left of these are as much of the time as possible mixotrophic). Basic metazoan zooplankton join cnidarians, for instance, jellyfish and the Portuguese Man o' War; shellfish, for instance, copepods, ostracods, isopods, amphipods, mysids and krill; chaetognaths (jostle worms); molluscs, for instance, pteropods; and chordates, for instance, salps and juvenile fish. This wide phylogenetic range joins an in like way wide range in maintaining conduct: channel drawing in, predation and steady investment with autotrophic phytoplanktonas found in corals. Zooplankton eat bacterioplankton, phytoplankton, other zooplankton (from time to time brutally), waste (or marine snow) and even nektonic living things.

Thusly, zooplanktons are generally found in surface waters where sustenance resources (phytoplankton or other zooplankton) are flooding. In like way as any species can be compelled inside a land region, so is zooplankton. In any case, sorts of zooplankton are not scattered reliably or discretionarily inside an area of the ocean. In like path with phytoplankton, 'patches' of zooplankton species exist all through the ocean. Overlooking the way that couple of physical squares exist over the mesopelagic, specific sorts of zooplankton are all around bound by saltiness and temperature slants; while different species can withstand wide temperature and saltiness centers. Zooplankton unpleasantness can in like way be influenced by customary bits, and also other physical parts. Ordinary areas join raising, predation, party of phytoplankton, and vertical change. The physical factor that effects zooplankton task the most is mixing of the water segment (upwelling and downwelling along the float and in the untamed ocean) that impacts supplement availability and, hence, phytoplankton creation.

REFERENCES

1. Novotny, V., 1994. Water quality: prevention, identification and management of diffuse pollution. Van Nostrand-Reinhold Publishers.
2. Lenat, D.R. and Crawford, J.K., 1994. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. Hydrobiologia, 294(3), pp.185-199.
3. Lenat, D.R., 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society, 12(3), pp.279-290.
4. Sládeček, V., 1983. Rotifers as indicators of water quality. Hydrobiologia, 100(1), pp.169-201.
5. Begum, A., 2008. Study on the quality of water in some streams of Cauvery River. Journal of chemistry, 5(2), pp.377-384.
6. Segers, H., 1997. Contribution to a revision of Floscularia Cuvier, 1798 (Rotifera: Monogononta): notes on some Neotropical taxa. Hydrobiologia, 354(1-3), pp.165-175.
7. Rougier, C. and Pourriot, R., 2006. On some rare and new species of rotifers (Digononta, Bdelloida; Monogononta, Ploima and Flosculariaceae) in the Kaw River estuary (French Guiana). ZOOSYSTEMA-PARIS-, 28(1), p.5.
8. Marneffe, Y., Comblin, S. and Thomé, J.P., 1998. Ecological water quality assessment of the Bütgenbach lake (Belgium) and its impact on the River Warche using rotifers as bioindicators. In Rotifera VIII: A Comparative Approach (pp. 459-467). Springer, Dordrecht.
9. Duggan, I.C., Green, J.D. and Thomasson, K., 2001. Do rotifers have potential as bioindicators of lake trophic state?.InternationaleVereinigungfürtheoretische und angewandteLimnologie: Verhandlungen, 27(6), pp.3497-3502.
10. Lougheed, V.L., Crosbie, B. and Chow-Fraser, P., 1998. Predictions on the effect of common carp (Cyprinuscarpio) exclusion on water quality, zooplankton, and submergentmacrophytes in a Great Lakes wetland. Canadian Journal of Fisheries and Aquatic Sciences, 55(5), pp.1189-1197.
11. Sousa, W., Attayde, J.L., Rocha, E.D.S. and Eskinazi-Sant'Anna, E.M., 2008. The response of zooplankton assemblages to variations in the water quality of four man-made lakes in semi-arid northeastern Brazil. Journal of Plankton Research, 30(6), pp.699-708.
12. Chapra, S.C., 2008. Surface water-quality modeling. Waveland press.
13. Santos-Wisniewski M, Rocha O, Guntzel A, Matsumura-Tundisi T. 2006. Aspects of the life cycle of ChydoruspubescensSars, 1901 (Cladocera, Chydoridae) ActaLimnologica Brasiliensia.18(3):305–310.

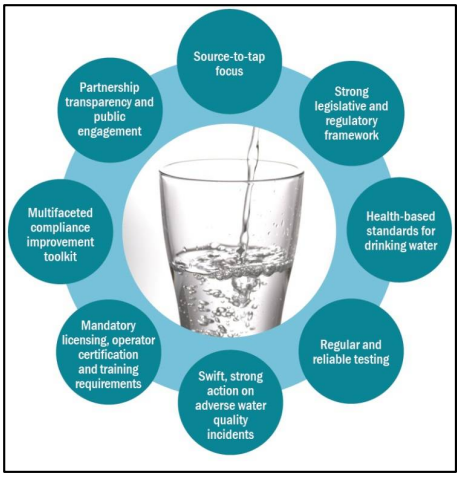
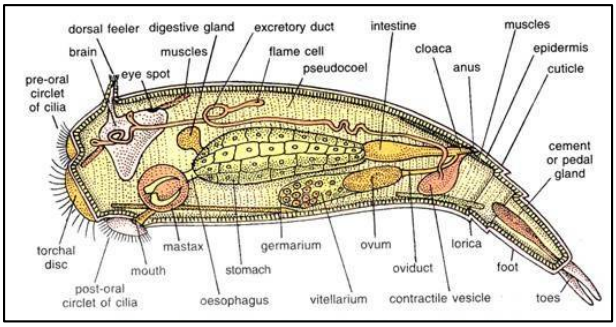


Figure 1. Assorted Perspectives of Water Quality



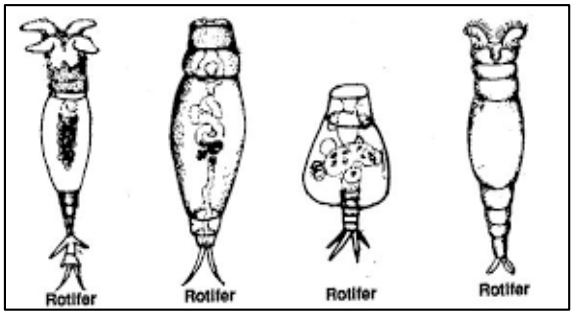


Figure 2. Rotifiers and Variants

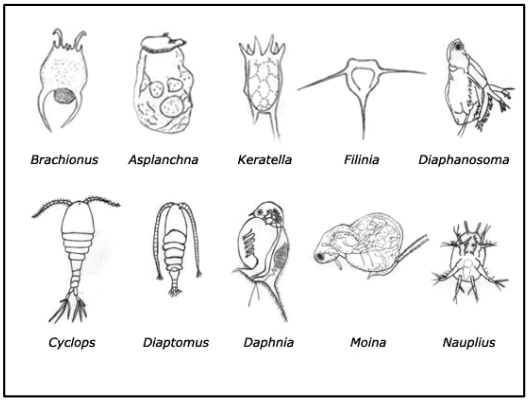


Figure 3. Zooplanktons and Variants

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Measuring the Wastewater of Three Sewage Treatment Plants in Wasit Province and their Effect on the Iraqi Waterway

Rasha. M.Salman*

Department of Biology, College of Science, University of Waist, Kut, Iraq.

Received: 19 Mar 2018 Revised: 20 Apr 2018 Accepted: 30 May 2018

***Address for correspondence**
Rasha. M.Salman
Department of Biology,
College of Science,
University of Waist, Kut, Iraq.

ABSTRACT

The objective of the present investigation the private water that was released from the wastewater treatment plants of the Wasit province in the waterway. Three sewage treatment Centers were selected in the Wasit province; the study was conducted between January 2017 and December 2017; two examples were given each month. Center one was AL-Kajia plants. The second Center was AL-Salam plants; The third Center is from AL-Anwar plants. Ten physical and compound parameters were chosen regarding the importance of these parameters. These ten parameters disappeared after: water temperature 13 to 33 ° C, pH 7 to 8, EC 1200 at 4800 µS / cm, BOD5 of 65 to 225 mg / l, TDS of 1000 to 2650 g / l, sulfate of 207 at 1020 mg / L, Cl-125 at 390 mg / L, NH314 at 67 mg / L, H2S5 at 69 mg / L, COD 102 at 615 mg / L. After examining the pulse, all wastewater treatment affects directly the qualities of the water of the waterway.

Keywords: Wastewater, Measuring, sewage treatment plants.

INTRODUCTION

Water is the most important asset on the planet, it is a key component in supporting all kinds of existence where there is no life and most works do not work (1). Approximately 20% of the total population needs potable water and a large part of the population needs adequate sanitation. This problem is intense in many producing countries, which release 95% of untreated wastewater into urban wastewater in Iraq, which is one of the nine Middle Eastern countries that do not have clean water. Water pollution is a global problem that is worth noting. It has been suggested that it is the main cause of exposure to diseases and illnesses. Surface water is more susceptible to pollution due to its simple availability in waste water transport. The term "waste water" refers to the irrigated water produced by the many exercises during the time devoted to the collection of its various life needs, which can be described as waste water from a group, which waste water refers to the expense or absorption of the waste water problem. water that has been disassembled or suspended. Domestic wastewater is known as domestic wastewater (5,6). The sewage of different houses and institutions (private and public) of the group constitute waste water of the city (7). The objective of the

current test is to know the limitations of the effect of the wastewater discharge in the Wasit Governorate on the complex physical properties of the effluents

MATERIALS AND METHODS

The examinArea

Wasit separates to right and left sectors through a water channel from north to south. Wasit extends to the internal borders of the provinces of Diyala, Baghdad, Babylon, Qadissiyah, DhiQar and Maysan.

Samples collections

Water tests were done to evaluate the physical examination using a 5 liter polyethylene bottle, which was washed with water twice before filling. The sample collected in surface water is approximately 20-40 cm, at this point it is held at 15 ° C on ice to the point where Example is analyzed(8).

Measure the water properties

The parameters include water temperature (using a precise mercury thermometer), hydrogen ion concentration (per PH meter), electrical conductivityand total dissolved solids (using the EC meter), the biological demand for oxygen and chemical oxygen (Winkler method), Ammonium, Sulphat (using titrimetric method), measured according to (9,10). In this study water properties were applied and tested at all sites in the waterway using Standard Iraqi Guidelines for wastewater discharged into the watercourse.

Statistical analysis

Statistical analysis: The statisticalanalysis done SAS (Statistical Analysis System version 9.1)(12).

RESULTS

Temperature

Indicates month to monthchangesinwatertemperature for the three chose Centers. Qualities went from 13 ºC in Center-2 to 33º C in Center-2 (Table-1).

Biological Oxygen Demands (BOD_5)

The adjustments in estimations of natural oxygen requests (BOD5) demonstrates the least esteem 65 mg/L was recorded in Center 2 and the most elevated 225 mg/L was in Center 2 (Table-1).

Chemical Oxygen Demand

The most minimal changes in estimations of the chemical oxygen request 102 mg/L was recorded in Center-3 and the most astounding 615 mg/L was in Center-3

Variation in PH Values

The variety in pH esteems fluctuated from the least esteem 7 was recorded in Center 2 and 3 though the most astounding worth 8 was in Center 3 (Table-1).

Values of Chloride

The adjustment in estimations of Chloride were recorded the most reduced 125 mg/L was estimated. from Center-2 and the most elevated 390 mg/L was seen in Center-3 (Table-1).

Values of Electrical Conductivity

The distinctive in estimations of electrical conductivity was recorded the most minimal 1200 μs/cm was estimated from Center 1 and the most noteworthy was 4800μs/cm that saw in Center 3 (Table-1).

Total Dissolved Solid

The adjustments altogether disintegrated strong were recorded the most minimal 1000 mg/L experienced in Center1, 2, 3. Though the most astounding quality 2650 mg/L was recorded in Center 3 (Table-1).

Values of Sulfate

The estimations of sulfate shifted from the low 207 mg/L were seen in Center 1, While as the higher 1020 mg/L was seen in Center 3 (Table-1).

Values of Ammonia

The variety in estimations of Ammonia changed from the most reduced 14mg/L was seen in Center 1, Whereas the most noteworthy esteem 67 mg/L was seen in Center3 (Table-1).

Values of Hydrogen

The variety in estimations of Hydrogen sulfide differed from the most reduced 3mg/L was seen in Center 3, Whereas the higher 69 mg/L was seen in Center 2 (Table-1).

DISUSSION

Water temperature is an imperative factor in any ocean situation influencing natural processes, this result was as investigations done in the past by (13), the application of natural oxygen is characterized as the amount of G that can oxidize the natural parts to water with the help of microorganisms - in a characterized test situation (14). For the most part, it has been shown that the expansion of BOD5, particularly at Center 3, may be due to the decomposition of natural, just-current problems for domestic wastewater. These results were slightly higher than those detailed up to (15, 16). The results indicated that the half-centralities-BOD5-exceeded levels, as admissible suggesting the standard rule of Iraq is an incentive for the western water released in the waterway in all destinations .

The application of the oxygen compound is characterized as a predefined oxidant which responds to the examples under controlled conditions (17) an estimate of poisons in waste water and regular water is additionally used as. The

COD-extended was exceeding the standard incentive for wastewater released into the waterway at all sites. The pH of the water is directly identified with the carbonate, and there are bicarbonate particles that are almost connected with the weight of CO2 and the ionic quality in the marine dispositions.It is noteworthy that pH is a critical parameter in the evaluation of the adjustment of the corrosive water base. The pH estimate of the water at the wastewater treatment plant that obtained a release from the paper zone was typically higher than that of the water in the stream. Water that has a pH more prominent than 8 contains carbonates and pH 4.5-8 contains bicarbonates and carbonic acids. Waters having a pH of less than 4.6 contain corrosive carbon dioxide. Chloride is a characteristic substance sample in all convenient waters and furthermore in waste water effluents such as metal salt. For the most part, the high centralization of the chloride shows a natural contamination in the water (18). Our result was as previous investigations carried out by (19,20) Electric conductivity used as a marker of water quality in view of disintegrated aggregate salts (21). The expansion the EC estimates in season 2 reflects the strong impact of domestic sewage flowing there. Likewise, the EC estimates recorded in the present investigation are in agreement with the findings of (19, 20). Total disintegrated solids (TDS) is the term used to describe inorganic salts and small measures of visualization of the natural problem in the disposal of water. These results were marginally lower than those reported by (22). The results indicated that the average convergences of electrical conductivity and disintegrated with added force within the permitted levels prescribed by the Iraq standard, which provides an incentive for wastewater discharged inland at all destinations.

Sulphate is widely distributed in nature and may be available in characteristic waters. The fundamental spring of Sulphat is the display of stones near bodies of water and the biochemical activity of anaerobic microorganisms (8). The results indicated that the average Sulphatcentering's were exceeding the permitted levels suggested by the standard Iraqi standard as an incentive for wastewater discharged into the conduit at all sites. The term aromatic salts incorporate non-ionized NH 3 and ionized NH 4 species. Larger alkaline sources offer overwhelming pollution control; odoriferous salt sources in wastewater reflect the impact of fluid leaks, high use of cleaners, and expansion of natural substances. Than early ammonification (23). An alkaline wastewater expansion is reused for water systems on agricultural land and can cause damage to plants and trees (24). The results mean that the average concentrations of ammonia within the acceptable levels prescribed by the Iraqi standard are an incentive for wastewater released into the canal at all locations. Hydrogen sulfide is a gas with a hostile odor of "spoiled eggs" that is noticeable with a low fixation when the sulfides hydrolyze in water in the most delicious way.Likewise, the edge of the sulfide odor throughout the air circulates through water or chlorinated water, and the levels of hydrogen sulfide in the hydrogen peroxide supplies are generally low, the ordinary waters are subject to a strange state They maintain a high concentration of hydrogen sulfide (25), hydrogen sulfide structures in wastewater without decomposed oxygen can arise due to the activity of microscopic organisms that is regularly present in wastewater and tracks natural problems that contain sulfur, indicative residual water test of sewage (H2S) fixation-serbeten from cleaners, sulfates and bacterial activities (26).

CONCLUSION

The summary to the current study;that wastewater be handling plants directly affect the nature of the waterway due to surpass admissible levels prescribed by Iraqi standard rule an incentive for wastewater released into the conduit in all locales.

ACKNOLEDGMENTS

Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for supporting.

REFERENCES

1. Cheepi, P. 2012. Musi River Pollution Its Impact on Health and Economic Conditions of Down Stream Villages-A Study. J. of Environmental Science, Toxicology and Food Technology,1 (4): 40-51, ISSN: 2319-2402.
2. Pimental D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E. and Nondagopal S. 2004. Water resources: Agricultural and environmental issues. Bioscience J., 54(10):909-918.
3. Yehia HM. and Sabae SZ. 2011. Microbial Pollution of Water in El-Salam Canal, Egypt. American-Eurasian J. Agric. Environ. Sci., 11: 305-309.
4. 4.Samarghandi M, Nouri J, Mesdaghinia AR, Mahvi.A, Nasseri S and Vaezi F.2007. Efficiency removal of phenol, lead and cadmium by means of UV/TiO2/H2O2 processes. Int. J. Environ. Sci. Tech., 4(1):19-25.
5. 5. Porteous A. 2000. Dictionary of Environmental Science and Technology; 3rd Edition, John Wiley and Sons Ltd., England.
6. EPA (Environmental Protection Agency).2012. Attains parent cause category summaries, adapted from doc. No.EPA-841-R-12-104.
7. Uwidia I.E and Ademoroti CM.2011. Characterisation of Domestic Sewage from an Estate in Warri, Nigeri a .International J. of Chemis.Vol. 3, No.3.
8. WHO (World Health Organization). 1996. Guideline for Drinking Water Quality Health Criteria and Other Supporting Information 2nd. Ed. Vol. 20.Geneva.
9. APHA. 1998. Standard methods for the examination of water and waste water, 20th ed.
10. APHA,AWWA and WFF. 2005. Standard Methods for the Examination of Water and wastewater, 21th ed. American Water Work Association and Water Environment Federation,USA.
11. Standard Specification of Drinking Water, Iraq. (2001). IQS (no.417), First modernization, the Council of Ministers, Central Organization for Standardization and Quality Control, 5pp.
12. SAS. SAS/STAT. Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010
13. Abumoghli I.A and Ghuneium NA. 1991. Manual of water and soil analysis. Jordan Univ. Jordan.
14. Fahad KK. 2005. Ecological survey for southern sector of Al-Garaf River, southern Iraq. Ph. D. Thesis. College of Agriculture, University of Basrah.:201p.
15. Sadek and Kamel. 2007.Seasonal variations in abiotic ecological conditions in Al-Garaf canal one of the main Tigris branches at ThiQarprovince.J. Coll. Sci. Univ., Basrah :3-6
16. Lind OT. 1979. Hand book of common methods in limnology. 2nd.Ed. London (109).
17. Abed Al-Razzaq HT. 2011. Effect of Domestic Wastewater from Pumping Center of Al-Kadimiya on Ecological Properties of Tigris River.MSc. Thesis. College of Science, Baghdad University.
18. Al-Kuraishi RA. 2011. A study of the effects of some ecological factors of Kut Dam on the Benthic Invertebrates of River Tigris. MSc. Thesis. College of Science, Baghdad university.
19. Rasheed RO. 1994. A limnological study on some water systems in Erbil province. Iraq. M.Sc. Thesis. Univ. of Salahaddin-Erbil, Iraq. 121pp.
20. Al-Saadi HA, Hadi RA and Al-Lami AA. 1989. Limnogical studies on some marsh areas in southern Iraq. Limnogica, 20 (2).
21. Joseph NC, Eddy AO, Elijah JP and Ikechukwu ON. (2011). Physicochemical evaluation of the effects of total suspended solids, total dissolved solids and total hardness concentrations on the water samples in Nsukka Town, Enugu State of Nigeria. Amr.J. Sci., 7 (5):827-836.
22. AL-Zamili HF. (2007). Monthly variations of some environmental factors of AL-Gharraf River, J. of DhiQar, 3 (3) :17-2.
23. Yuce G, Pinarbasi A, Ozce like S and Ugurlnoglu D. 2006. Soil and water pollution Derived from Anthropogenic Activities in the porsukRivre Basin,Turkey. Environ Geol.,49:359-375.
24. Priestly A.J.2002. Report on sewage sludge Treatment and Disposal. Environmental Problems and Research Needs from An Australian prospective CSIRO.Division of chemical and polymers,48pp.

25. Hem JD.1985. Study and Interpretation of the chemical characteristics of Natural water. USGS water supply paper 2254, 263pp.
26. Canada .2006. Guidelines for Canadian water quality Federal–Provincial, Territorial Committee on Drinking water.

Table 1:- Show the minimum and maximum, mean and standard deviation for the samples at every study stations

CentersParameters	Center-1	Center-2	Center-3
COD mg/L	204.8 ±108.8 (115 –399)	219.5±64 (130-315)	399.6 ± 213.6 (102-615)
Water Temp. °C	23.26 ±7.18 (14 – 31.5)	20.6 ± 6.77 (13 – 33)	22 ± 6. 22 (15 – 32)
E .C µS/cm	1951.8 ± 663 (1200 –3100)	2234 ± 864 (1552 –3650)	2860± 1207.64 (1860 – 4800)
H_2S mg/L	23.83 ± 8.23 (14 – 35)	38 ± 21.6 (13 – 69)	12 ± 4.4 (5 – 18)
TDS mg/L	1333± 280.47 (1000 –1700)	1466 ± 436.6 (1000 – 2100)	1808 ± 620 (1000 – 2650)
PH	7.316 ± 0.213 (7.1– 7.6)	7.5 ± 0.328 (7 – 7.9)	7.53 ± 0.417 (7 – 8)
NH_3 mg/L	27.16 ± 14 (14 – 53)	31.6 ± 12.81 (15 – 50)	39.6 ± 19.54 (18 – 67)
BOD_5 mg/L	125 ± 52 (75 – 188)	146.8 ± 53.8 (65 – 225)	139 ± 48 (80 – 220)
Cl^- mg/L	241 ± 80.5 (137 – 359)	223.6 ± 106.4 (125 –375)	305.8 ± 69 (205 – 390)
SO_4mg/L	440.6 ± 214.4 (207 – 695)	572.8 ± 204.6 (300 – 793)	673.6 ± 268.5 (390 – 1020)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antibacterial Activity of Magnesium Oxide Nanoparticle against *Staphylococcus aureus* in Raw Milk

Walaa Mahmood Mohamed* and Khulood K.Nazal

Department of Public Health, College of Veterinary Medecine, University of Baghdad, Iraq.

Received: 21 Mar 2018 Revised: 19 Apr 2018 Accepted: 12 May 2018

***Address for correspondence**
Walaa Mahmood Mohamed
Department of Public Health,
College of Veterinary Medecine,
University of Baghdad, Iraq.

ABSTRACT

In order to study the beneficial antimicrobial effect of Magnesium Oxide Nanoparticles (MgO NP) against *Staphylococcus aureus* isolated from locally produced cow's and ewe's raw milk samples.A total of (50) locally produced cow's and ewe's raw milk samples (25 for each) were collectedfrom the street hawkers in different streets of Diyala province.Samples were collected during the period fromOctober 2017 to the February 2018.The identification of *Staphylococcus aureus* was confirmed according to their cultural morphology and both the serological and biochemical properties.The laboratory studies of the cultural isolation during the period of the study revealed that there was no significance difference in the percentage of *Staphylococcus aureus* between the cow's and ewe's raw milk samples (52% and 48%, respectively).By using the Baird-Parker agar with both the biochemical and serological latex agglutination test kit in the current study, results showed that twenty five out of fifty (50%) milk samples were positive for the presence of *Staphylococcus aureus.* The mean values of *Staphyloccocus aureus* count (log cfu/ml)revealed that there were significance ($P<0.05$) differences in the average viable *Staphylococcus aureus* count after adding the 2nd and 3rd concentrations (250 and 500 µg MgO NP)30-40 nm in comparison to the control, while there was no significance differences between the 1st concentration (100 µg MgO NP) added raw milk sample incomparison to the control.The minimum inhibitory concentration for MgO NP against *Staphylococcus aureus* was verified by dilution method according to the national committee for clinical laboratory standards institute, The results revealed that the minimum inhibitory concentration of the MgO NP against *Staphylococcus aureus* was 1000 µg MgO NP/ ml.

Keywords: Magnesium Oxide Nanoparticles, *Staphylococcus aureus,* Cow, Ewe, raw milk**.**

INTRODUCTION

Milk and its derivate are recognized vehicles for *Staphylococcuaureus* infection in humans (Zecconi and Hahn., 2000).Staph.aureus causes a extensive variation of diseases in animals,and humans extending in severity from a mild skin infection to other severe diseases, for example pneumonia and septicemia (Fagundes*et al*., 2010). Antimicrobials can be synthetic, can be animal or plant origin, or can be chemically modified natural compounds (Von Nussbaum., *etal.,*2006).Lately, nanotechnology andnanosciences has been leading to a technological revolution in the world, which is concerned with materials with considerably improved and novel physical, chemical and biological properties Sundrarajan*et al*., 2012). In this respect, nanoparticles are accepted as antibacterial agents due to their surface properties and their size structure(Raghupathi*et al*., 2011). Therefor,nanotechnology proposals a way to advance the activity of inorganic antibacterial agent,Inorganic antibacterial agents have involved great attention for microbiological control (Jung *et al*., 2008).

The chief benefits of inorganic antibacterial agents, in comparison to organic antibacterial agents, are the enhanced stability under strict processing conditions(Makhluf*et al.,* 2005). Nowadays certain of the inorganic antibacterial materials, in specific inorganic metal oxides such as, Zinc Oxide, Magnesium Oxide and Calcium Oxide, have been studied (Huang *et al*., 2000; Sawai., 2003).Between the studied inorganic metal oxides, MgO is of specific attention since they are not only stable under severe process environments,but also commonly considered as nontoxic materials tohuman beings (Sundrarajan*et al.,* 2012). Continuously,there is need to progress new device and procedures that will assistance make these devices and processes more cost efficiently than their traditional counterparts (Colvin, 2003). Magnesium (Mg) nanoparticles andMgO nanoparticles consider very effective biocides against Gram-negative bacteriaand Gram-positive bacteriaand bacterial spores(Stoimenov*et al*,2002). Many researches about antibacterial activities of MgO NPalone or combination with other antimicrobial against food borne pathogen (*E-coli* O157-H7 and *Staphylococcus aureus*)were investigated, the result show that MgO NPhave strong bactericidal activities against the pathogens,achieving more than 7 Log reduction in bacterial count.The antibacterial activity of MgO NP increased as the concentrations of MgO NPincreased. Studies done in other countries with the antibacterial activities of MgO NPagainst food borne pathogens have been impressive,we need to repeat those studies in our country.

MATERIALS AND METHODS

This study carried out during the period began from end of October 2017 to the end of February 2018. A total of 50 raw milk samples were collected at weekly intervals (2 samples/week) in a sterile glass bottles from street hawker of Diyala province).The selective media used in this study for isolation and enumeration S. aureus is Baird Parker–egg youlkTellurite.Each milk sample diluted by tenfold serial dilution after that 1ml of each dilution transferred to plate of BairdParker by spreader to incubate for 24 hour at 37°C, (Baird Parker, 1962),in addition,biochemical(gram stain,catalase test,Dnase test) and Rapid method include Electronic RapIDTM staph. pluscode system. ERIC Rapid Method was used for Rapid biochemical Identification of Staphylococcus species in only 4 hours. This new system was based upon Microbial degradation of special substrate (Oxoid –Remel, 2013).

MgONP Stock preperation: MgONP 30-40 nm purchase from (naning technology) used in this study.To prepare different concentration,0.5 gram of MgOnanopowder (30-40nm) was dissolved in 100ml deionized distal water and placed on magnetic stirrer for homogenizationto get(100, 250 and 500 µg/ml) concentration of MgO NP.Raw milk sample inoculated with different concentration of MgONP wereleft at room temperature for 6 and 24hour to enumerate bacterial population by count of total Bacteria,coliform and Staph.aureus and compared with control(without addition).To determine Minimum inhibitory concentration (MIC)of Mgo 30-40 nm against *Staph.aureus* by macro dilution broth,0.1ml staph suspension which compared with (0.5 mc) Farland tube was inoculated in each10 tube of broth which contain different concentration of (MgO NP) then incubation aerobically for

24hr 37 °C. When comparative with control tube the tube has lowest concentration of MgONP showed diminution in turbidity was taken as MIC.

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way, Two ANOVA, unpaired t test and Least Significant Differences (LSD) post hoc test were performed to assess significant differences among means. Also Chi square was used to assess the differences among proportions P<0.05 was considered statistically.

RESULTS

Pure colonies of Staphylococcus aureus were identified from the locally produced raw milk samples that were collected from the street hawkers in different streets of Diyala province. The identification of *Staph.aureus*was confirmed according to their cultural morphology and both the serological and biochemical properties.The percentages of positive raw milk samples for *Staph.aureus* that were collected from both Cow's and Ewe's raw milk samples are shown in (Table, 1). The laboratory studies of the cultural isolation during the period of the study revealed that there was no significance (P<0.05) difference in the percentage of *Staph.aureus* between the Cow's and Ewe's raw milk samples (52% and 48%, respectively). By using the Baird-Parker agar with both the biochemical and serological latex agglutination test kit in the current study showed that twenty five out of fifty (50%) milk samples were positive for the presence of Staph.aureus (Table, 1).

The mean values of Staph.aureus count (log cfu/ml) in the both locally produced Cow's and Ewe's raw milk samples before and after adding of different concentrations of MgO NP are shown in (Table, 2 and 3). Figure, 1 showed the effect of different concentration of MgO NP inhibition on Staph.aureus bacteria after 24 hr. incubation of raw milk by using Baird-Parker Agar. The Statistical analysis revealed that there were significance (P<0.05) differences in the average viable *Staph. aureus* count after adding the 2nd and 3rd concentrations (250 and 500 µg/ml) of MgO NP in comparison to the control, while there was no significance difference between the averages after adding the 1st concentration (100 µg/ml) and control. The results in (Table, 2) showed that the Cow's raw milk samples after adding the 3rd concentration of MgO NP(500 µg/ml) recorded significantly (P<0.05) the lower *Staph. aureus* counts after 6 and 24 hours in room temperature (0.93±0.14 and 1.62±0.42 log cfu/ml, respectively) in comparison to the control that had significantly (P<0.05) the higher S.aureus counts (4.31±0.21 and 5.47±0.23 log cfu/ml, respectively).

The Ewe's raw milk samples recorded significantly (P<0.05) the lower *Staph. aureus* counts after adding the 3rd concentration of MgO NP after 6 and 24 hours in room temperature (0.77±0.11 and 1.36±0.20 log cfu/ml, respectively) in comparison to the control that had significantly (P<0.05) the higher *Staph. aureus* counts (3.24±0.19 and 4.42±0.31 log cfu/ml, respectively), (Table, 3). The minimum inhibitory concentration (MIC) for Magnesium Oxide Nanoparticles against *Staphylococcus aureus* was verified by dilution method according to the clinical laboratory standards institute (NCCLS), The isolates obtained from the locally produced Cow's and Ewe's raw milk samples were standardized to 0.5 McFarland turbidity. The results revealed that the minimum inhibitory concentration of the Magnesium Oxide Nanoparticles against *Staph.aureus* was 1000 µg MgO NP/ ml.

DISCUSSION

Staphylococcus aureus is an important food born pathogen that causes a massive losses in both dairy herds and the dairy industry (Artursson., 2010). It is the important cause of food borne intoxication in different food products that used for human consumption (Pelisser et al., 2009). Staphylococcus aureus is a pathogen that is often transmitted by improperly handling, storage and distribution of traditional dairy products, in the numerous countries, it was the most common pathogen responsible for outbreaks of food poisoning by consumption of the raw dairy products

(Verase*t al*., 2008).The results in (Table, 1) indicated that the raw milk were commonly contaminated with Staph. aureus and were in agreement with similar studies that indicated that enterotoxigenic Staph. aureus almost was found in the raw milk and dairy products (Bianchi et al., 2014).

Akineden et al., (2011) recorded that the higher prevalence rate of Staph. aureus occurred in the traditionally dairy products which could be associated with the milking cows because Staph. aureus was the major causative agent of sub-clinical mastitis in the dairy cows. Genetic difference of animal species may be a further reason for the difference in the occurrence of Staph. aureus in the farms dairying systems (Katsande*et al*., 2013).The current results were in disagreement with those studies by other researchers who indicated that lower prevalence rates of S. aureus was found in the bovine or caprine milk samples or raw dairy products (pelese*t al*., 2007).The prevalence of Staph.aureus was the highest in the cow's samples and followed by ewe's which could be attributed to the initial starting number of Staph. aureus in the raw milk and their multiplication during the transportation with improper cooling, also more Staph. aureus contamination occurs from human sources due to the poor personal and/or utensils hygiene during the production chain Cretenet*et al*.,2011) Our results were in agreement with (Kammil, 2017) in Diyala City who reveled that 25 out of 40(62.5%) raw milk sample were positive for the presence Staph.aureus. Another study in Baghdad City revealed that from 30 raw milk sample only18(60%) sample were contaminated with Staph.aureus(Mahmood,2017).

Investigation on other countries (Palestine) revealed that from 130 cow's raw milk samples only 48 (36.9%) samples were containing Staph. aureus(Farhan and Salk., 2007). Ekici*et al*., (2004) found that 18.18% were positive for Staph. aureus when studying 66 raw milk samples in Turkey. In Nowary and Italy 55%,43% sample of cow milk contaminated with staph aureus (Zelalem andBernara,2006). Another study in Malayer City in Iran found that 52% sample of raw milk contaminated with Staph. aureus (Pourhassan and Taravat,2011).The un hygienic practice and poor sanitation techniques in the milking process with improper handling,storage and distribution may introduced such organism in the milk and reflected on the high prevalence level of contamination with such organism which was responsible for many outbreaks of food poisoning by the consumption of the raw dairy products(Verase*t al*;2008).Higher Staph.aureus count (Table 2 and 3) indicates the poor hygienic quality under which such milk was produced and also may indicate udderinflammation as Staphylococcus spp. are the main etiological agents of small ruminant's intramammaryinfections, and the more frequent isolates being Staph. aureus (Bergoniere*t al*., 2003). Nearly similar findings were obtained byMuehlherr*et al*. (2003), relatively higher incidence was reported by Jakobsen*et al*. (2011).The high figures of contamination with the Staphaureus may be due to the rearing system of small ruminants, which still primitive in many countries and make it difficult both to minimize environmental bacterial contamination at the milking stage, and to carry out effective milk quality improvement programs.

Raw milk can becontaminated by Staph.aureus due to mastitic udder or poor hygienic condition such coughing,sneezing and dirty hand through handling the milking equipment and also farmers activities due to the colonization of Staph.aureus in nasal cavities of human being(fagundes and olivera 2004). The mean values of Staph.aureus count (log cfu/ml) in the both locally produced Cow's and Ewe's raw milk samples before and after adding different concentrations of MgO NP 30-40 nm are shown in (Table, 2 and 3).The Statistical analysis revealed that there were significance ($P<0.05$) differences in theaverage viable Staph. aureus count in MgO NP added raw milk sample in the 2nd and3rdconcentrations (250 and 500 µg/ml) in comparison to the control, while there was no significance difference between the averages after adding the 1st concentration(100µg/ml) and control.

Bacterial contamination continues to draw public attention. It is estimated that approximately 48 million cases of pathogenic diseases occur in the United States (Morris 2011; Jin and He, 2011). Therefore, in order to solve this problem, it is highly necessary to develop effective antimicrobial agents to control the bacterial population (Kumar et al., 2008; Li *et al*., 2006). As a result, inorganic antibacterial agents have attracted much interest for bacterial control (Jung *et al*., 2008). Among the studied inorganic metal oxides, MgO is of particular interest because it is not only stable under harsh process conditions, but also generally regarded as safe materials tohuman beings (Sundrarajan*et al*., 2012).Mgo NP have considerable potential as an antibacterial agent,Many reports have shown that the

antibacterial activity of MgONP is size-dependent. Huang et al. (2005) reported that antibacterial activity was increased with the decrease of the particle size of MgO. A relationship between the bactericidal efficacy against some pathogens and the particle size of nano-MgO was demonstrated.For particles in the size range ~ 45-70 nm, the bactericidal efficacy of nano-MgO increased slowly with decreasing particle size. Below ~ 45 nm however, the bactericidal efficacy showed a much stronger dependence on particle size.

Our result in agreement with (Zhang *et al*, 2011) whomfoundthat high MgONP concentrations resultedin greater bacterial inactivation.Generally, the specific surface area of MgONPincreases as the size of the nanoparticles decreases. The increase in surface area determines the potential number of reactive groups on the particle surface, which are expected to show high antibacterial activity (Pal et al., 2007). Many studies have indicated that the antibacterial mechanism of MgONPis due to the formation of ROS such as superoxide anion (O2-)(Yamamoto *et al*.,2010). It has been reported that the increase of the surface area of MgO particles leads to an increase of the O2– concentration in solution and thus results in a more effective destruction of the cell wall of the bacteria. The interaction of MgONPwith bacteria, subsequently damaging the bacterial surface, has been proposed to explain the antibacterial activity of MgONP. Stoimenov et al. (2002) suggested that the cell death was caused by the electro-static interaction between the bacteria surface and MgONP. Makhluf et al. (2005) demonstrated that nano-MgO exhibited high activity against bacteria due to the interaction of particles and bacteria. It was found that nano-MgO particles could take up halogen gases due to the defect nature of their surface and its positive charge,which resulted in a strong interaction with bacteria,which are negatively charged (Stoimenov*et al*,. 2002).

The alkaline effect has been considered as another primary factor in the antibacterial action of MgONP.The possible antibacterial mechanism was the adsorption of water moisture on the MgONPsurfaces, which could form a thin water layer around the particles(Sawai*et al*., 2001). The local pH of this thin water layer formed around the nanoparticles might be much higher than its equilibrium value in solution. When the nanoparticles are in contact with the bacteria, the high pH in this thin surface water layer could damage the membrane, resulting in cells death.(Pal *et al*., 2007). Depending on these factors, nanoparticles can have highly variable antibacterial properties (Merisko-Liversidge*et al*., 2003) which result in controversial results.

CONCLUSION

The results showed that MgO NPs have strong bactericidal activities against the *S.aureus* and its antibacterial activity was increased as the concentrations of MgO NP increased.

REFERENCES

1. Zecconi A, Hahn G. Staphylococcus aureus in raw milk and human health risk. Bull. IntDairy Fed. 2000 ;345:15-18
2. Fagundes H, Barchesi L, Nader Filho A, Ferreira L. M. , Oliveira C. A. F. Occurrence of S.aureus in raw milk produced in dairy farms in São Paulo state, Brazil. Brazilian J. Microbiol.2010; 41(2): 376-380.
3. Von Nussbaum F, Brands M, Hinzen B, WeigandS , Habich D. Antibacterial natural products in medicinal chemistry—Exodus or revival? Angew. Chem. Int. Ed.,2006;45:5072–5129.
4. Sundrarajan M, Suresh J, Gandhi R. R. Acomparative study on antibacterial properties of MgO nanoparticles prepared under different calcination temperature. Digest Journal of Nanomaterials and Biostructures,2012; 7(3): 983-989
5. Raghupathi R. K, Koodali R. T., Manna A. C. .Size-dependent bacterial growth inhibition and mechanism ofantibacterial activity of zinc oxidemnanoparticles. Langmuir,2011; 27: 4020-4028 .

6. Jung W. K., Koo H. C.,Kim K. W, Shin S, Kim S. H., Park Y. H. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Applied and Environmental Microbiology.,2008; 74(7):2171-2178.
7. Makhluf S, Dror R.,Nitzan Y.,Abramovich Y.,Jelinek R., Gedanken A. Microwave-assisted synthesis of nanocrystallineMgO and its use as a bacteriocide. Advanced Functional Materials.2005; 15(10):1708-1715
8. Huang Z., Maness P. C., Blake D. M,Wolfrum E. J., Smolinski S. L., Jacoby W. A . Bactericidal mode of titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology A: Chemistry,2000; 130(2-3): 163-170.
9. Sawai J., Kojima H.,Igarashi H., Hasimoto A., Shoji S., Sawaki T., Hakoda A., KawadaE,Kokugan T, Shimizu M. Antibacterial characteristics of magnesium oxide powder. World Journal of Microbiology and Biotechnology,2000; 16(2):187-194 .
10. Stoimenov P. K, Klinger R. L, Marchin G. L, Klabunde K. J. Metal oxide nanoparticles as bactericidal agents. Langmuir,2002; 18(17):6679-6686.
11. Colvin V. L.The potential environmental impact of engineered nanomaterials. Nature Biotechnology,2003; 21(10):1166
12. Baird Parker A. C. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. Journal of Applied Microbiology,1962; 25(1): 12-19.
13. Oxoid – Remel, (2013). Laboratory Manual for Media and Diagnostic Kits
14. National Committee for Clinical Laboratory Standards (United States).. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards.2003
15. Artursson K, Nilsson-Öst M, Persson Waller K.An improved method to culture Staphylococcus aureus from bovine milk.Journal of Dairy Science,2010. 93(4): 1534-1538
16. PelisserM.R,KleinC.S,AscolK.R,Zotti T.R.,Arisil A.C.M .Occurrence of Staphylococcus aureus and multiplex PCR detection of classic enterotoxin genes in cheese and meat products.Braz. J. Microbiol.,2009; 40:145-148.
17. Veras J. F,Carmo L. S, Tong L. C,Shupp J. W, Cummings C., Santos D. A. Astudy of the enterotoxigenicity of coagulase negative and coagulase –positive Staphylococcal isolates from food poisoning outbreaks in Minas Gerais,Brazil. International Journal of Infectious Diseases.2008; 12: 410-415
18. Bianchi D.M,GallinaS,BellioA,ChiesaF,Civera T, Decastelli L. Enterotoxin gene profiles of Staphylococcus aureus isolated from milk and dairy products in Italy. Letters in Applied Microbiology.2014; 58:190-196.
19. Akineden O, Hassan A.A,Schneider E, Usleber E..Acoagulase-negative variant of Staphylococcus aureus from bovine mastitis milk. J. Dairy Res.2011; 78:38-42.
20. .Katsande S. , Matope G. , Ndengu M., Pfukenyi D. M. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. The Onderstepoort J. Vet. Res.,2013; 80:523.
21. Peles F. M.,WagnerL,Varga I, Hein P. ,RieckK,GutserP,Gkardos I.,Turcsanyi B. ,SzaboA.Characterization of Staphylococcus aureus strains isolated from bovine milk in Hungary. Int. J. Food Microbiol.,2007; 118: 186-193.
22. Cretenet M, Even S, Loir Y.L. Unveiling Staphylococcus aureus enterotoxin production in dairy products: a review of recent advances to face new challenge. Dairy Sci. Technol.,2011; 91:127-150
23. KamilSh.O.Theprevalance and source of staphylococcus aureusin the raw milk and locally produced soft cheese in Diyala province (M.sc thesis).Collage ofVeterinaryMedicine,BaghdadUniversity,2017
24. Mahmood R.M. The Efficacy of ozone Treatment on the Microbiological , Physical and Chemical Quality of Raw Milk at Different Storage Temperatures. Collage of Veterinary Medicine,Baghdad university, 2017.
25. FarhanM,Salk S. Evaluation of Bacteriological contamination in raw (unprocessed) milk sold in different regions of Lahore (Pakistan). Journal of Agriculture & Social Sciences.,2007; 3: 1813-2235.
26. EkiciK,Bozkurt H, Isleyici O. Isolation of some pathogens from raw milk of different milch animals. PakistanJournal of Nutrition,3(3):161-162
27. ZelalemY, Bernard F. Handling and Microbial Load of Cow's Milk and Irgo-Fermented Milk Collected from Different Shops and Producers in Central Highlands of Ethiopia, Eth. J. Anim. Prod.,2006; 6:67-82Journal of Nutrition . 3: 161-162
28. Pourhassan M, TaravatN.A.The spatial distributionof bacterial pathogens in raw milk consumption on Malaye City, Iran.,2011;12:2-10

29. Bergonier D., De Crémoux R., Rupp R, Lagriffoul G, Berthelot X. Mastitis of dairy small ruminants. Veterinary research.,2003; 34(5): 689-716.
30. Mühlherr J. E, Zweifel C, Corti S, Blanco J. E, Stephan R. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. Journal of Dairy Science,2003; 86(12):3849-3856.
31. Jakobsen R. A, HeggebøR,Sunde E. B, Skjervheim M. Staphylococcus aureus and Listeria monocytogenes in Norwegian raw milk cheese production. Food Microbiology,2011; 28(3): 492-496.
32. Fagundes H, Oliveira C.A.F.Infecçõesintramamáriascausadaspor S. aureus e suasimplicaçõesemSaúdePública. Ciência Rural.,2004; 34: 1315-1320
33. Morris Jr J. G. How safe is our food?. Emerging Infectious Diseases,2011; 17(1):126.
34. Jin T, He Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. Journal of Nanoparticle Research, 2011;13(12): 6877-6885.
35. Kumar A, Kumar J. On the synthesis and optical absorption studies of nano-size magnesium oxide powder. Journal of Physics and Chemistry of Solids, 2008;69(11): 2764-2772
36. Li Y,LeungP,Yao L, Song Q. W, Newton E. Antimicrobial effect of surgical masks coated with nanoparticles. Journal of Hospital Infection,2006; 62(1): 58-63.
37. Huang L, Li D, Lin Y,Evans D. G,Duan X. Influence of nano-MgO particle size on bactericidal action againstBacillussubtilis var. niger. Chinese Science Bulletin,2005; 50(6): 514-519.
38. Zhang K, An Y, Wang F, Lin L, Guo H. Experimental investigation on water treatment by the combined nanoMgO–nanofiltration technique. Water Sci. and Technol.,2011; 63(11):2542-2546.
39. Pal S, Tak Y. K,Song J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and Environmental Microbiology,2007; 73(6): 1712-1720
40. Yamamoto O, Ohira T, Alvarez K, Fukuda M. Antibacterial characteristics of CaCO3–MgO composites. Materials Science and Engineering: B, 2001;173(1-3): 208-212.
41. Stoimenov P. K, Klinger R. L, Marchin G. L, Klabunde K. J. Metal oxide nanoparticles as bactericidal agents. Langmuir,2002; 18(17): 6679-6686.
42. Sawai J, Shiga H, Kojima H. Kinetic analysis of death of bacteria in CaO powder slurry. International Biodeterioration&Biodegradation, 1997;47(1):23-26.
43. Merisko-LiversidgeE,Liversidge G. G,Cooper E. R. Nanosizing: a formulation approach for poorly-water-soluble compounds. European Journal of Pharmaceutical Sciences,2003; 18(2): 113-120

Table, 1: Recovery percentages of S. aureus from total Cow's and Ewe's raw milk Samples

Milk Sample	No. of samples	No. of Isolates	Isolation %
Cow's	25	13	52%
Ewe's	25	12	48%
Total	50	25	50%
Chi square value	8.08		
P	0.77 NS		

Table, 2: The mean values of *Staphyloccocusaureus* count (log cfu/ml) in the locally produced Cow's raw milk samples before and after adding of different concentrations of MgO NP 30-40 nm using the Baird-Parker agar, (mean ±SE).

Staph. aureus count Cfu/ml	Control	1st Conc. 100µg MgO NP/ 1ml raw milk	2nd Conc. 250µg MgO NP/ 1ml raw milk	3rd Conc. 500µg MgO NP/ 1ml raw milk
after 6 hrs	A b 4.31±0.21 2×104	A b 4.19±0.20 1.2×104	B a 3.48±0.15 1.5×103	C a 0.93±0.14 2.45

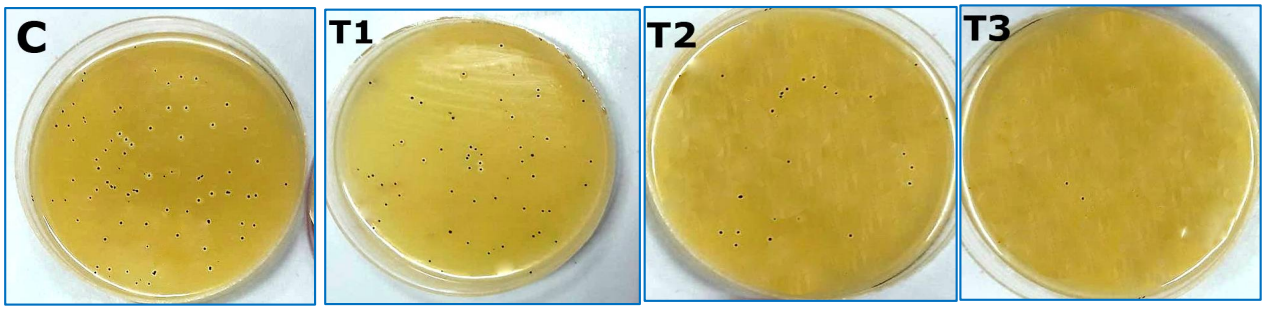
after 24hrs	A a 5.47±0.23 2.9×105	A a 5.40±0.23 2.5×105	B a 4.52±0.23 a 3.3×104	Ca 1.62±0.42 41.6
LSD	0.709			

Means with different small letter in the same column significantly different (P<0.05)
Means with different capital letter in the same row significantly different (P<0.05)

Table, 3: The mean values of Staph.aureus count (log cfu/ml) in the locally produced Ewe's raw milk samples before and after adding of different concentrations of MgO NP 30-40 nm using the Baird-Parker agar, (mean ±SE).

Staph.aureus count Cfu/ml	**Control**	**1st Conc. 100µg MgO NP/ 1ml raw milk**	**2nd Conc. 250µg MgO NP/ 1ml raw milk**	**3rd Conc. 500µg MgO NP/ 1ml raw milk**
after 6 hrs	A b 3.24±0.19 1.7×103	Ab 3.17±0.17 1.4×103	B a 2.29±0.24 1.9×102	C a 0.77±0.11 5.8
after 24hrs	A a 4.42±0.31 2.6×104	A a 4.37±0.31 2.3×104	B a 3.06±0.24 1.1×103	C a 1.36±0.20 22.9
LSD	**0.6771**			

Means with different small letter in the same column significantly different (P<0.05)
Means with different capital letter in the same rowsignificantly different (P<0.05)



Figure, 1:Effect of defferent concentration of MgO NP inhibition on *Staphylococcus aureus* bacteria after 24 hr. incubation of raw milk by using Baird-Parker Agar.

C= Raw milk without adding MgO NP (Control)
T1= 100µg MgO NP/ 1ml raw milk.
T2= 250µg MgO NP/ 1ml raw milk.
T3= 500µg MgO NP/ 1ml raw milk.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Anti Tumor Orient Experiment – ATOX

Deepak Bhattacharya[1]* and Unnati Bhattacharya[2]

[1]Fight Cancer-Metastasis at Home, C/o Kedar Gouri road, Bhubaneswar, India.
[2]MSc-II[nd] Yr.,Dept.of Microbiology, SBRR College,University of Mysore, Karnataka,India

Received: 18 Mar 2018 Revised: 21 Apr 2018 Accepted: 05 May 2018

[*]**Address for correspondence**
Deepak Bhattacharya,
Fight Cancer-Metastasis at Home,
C/o Sri Radha Krishna, Kedar Gouri road,
Bhubaneswar, India.
E-mail: fightcancermetastasisathome@gmail.com.

ABSTRACT

ATOX *et.al*., are comprised of herbs. It seems to serve the enticing objective of anti-mitosis + anti-angiogenesis + anti-tissue (compounded therapy against solid cancers). These be CSAMs (complementing & supplementing alternative medicaments) synergic with allopathy.Also potent as solo. CSAMs embolden the case for use of nano-tech in natural extract based drug making (isolated & purified fractions as the APIs). The immediate objective is to '*Fight Cancer At Home*'. Drug designers, makers and all are encouraged to consider the opportunities. There are No patents. Open for all purposes.

Keywords: Fight Cancer At Home; *ATOX, KND, VAC, ATAMCOX, Dyospyrous Melanoxylon; S. Xanthocarpum (Suratt; Barelia Prionits; Anonna Squamosa ; Hemidesmus indicus; Punica granatum; CSAM;* Anti-metastasis; Anti-cancer.

INTRODUCTION

New approaches in therapeutics (specially vis-à-vis cancer & metabolic diseases) are taking roost [1].This has reference to our previous communication [2]. Therein, we had presented details of a new & novel anti-metastasis drug. We had also hinted in part about anti-tumor activity. However, before any metastasis can happen the native first experiences solid tumor genesis and carcinoma. Circulating lysozomes as dormant-potent oncogens (why & how) are an enigma and any prophylaxis\therapy is afar. Hence, there is a need. In this communication we provide the details as to how to home make & hand make an effective Anti-Tumor ATOX (*Anti Tumor Orient Experiment*). ATOX indicates specific and general application is non blood cancer types. Our idea is 'combat commences at home' [3]. This author since 2003 has been working among the needy & the afflicted with case specific & patient centric efforts, namely ATAMCOX/VAC [4], etc. as part of translational medicine at MRC [5]. Now, constituents of all such efforts can be admixed in whole and or in parts to make ATOX\ATAMCOX\VAC to serve fixed dose therapeutic objectives as in Table 1. However, merely causing necrosis (kill) of the benign and/or the cancerous cells does not lead to cure as because the gone wrong mitochondria and other constituents of the lysed cells get released into the blood (lymph also) and metastasis gets up-regulated. Blood borne lysozomes (oncogens) settle in cells lines such as

liver, lungs and bones as because the forcings of the hemodynamics are in a state of down regulation in such locuses (non turbulent\low Reynolds i.e., precipitating type mechanics); also due ion affinity, receptors & cytokines. Oncogens are ionic. They are released into the blood due to insult by any type of biopsy; surgery and EBRT (*external beam radio therapy* {Proton bean included}). Process metastasis is insurmountable (too varied & dynamic) and has remained as the killer. Moreover, the full of the neoplastic mass is never smited (only part killing\removal happens). Neoplastics wrap and infiltrate organs; have sturdy blood vessel matrix. Therefore, a novel and effective anti-mitosis component(s) is the need (Table-1, activity column). These prime neo components can also be given as a mono therapy. The various combinations as stand alone therapies invariably arrests carcinomas (even omental). Along with other concurrent therapies it is indicating enviable results (has salutary effect too). ATOX et.al., are a new tool in the hands of the family physician and\or the treating clinician.

MATERIALS AND METHODS

Process

Cut, gather, clean, wash, dry in desiccated room (shade), slow grind (dry) to fine powder, sieve, weigh, mix, fill. Wet grinding also ok.

Sterilisation

Gamma irradiation @ 20-25Kilo Gray. Excepting *Dioscorea* rest of the ingredients have excellent keeping property at room temperature even in the torrid, humid tropical agro-met conditions.

Fixed dose

500mg per cap in 00 sizes of shells. 1-2 cap per day depending on body wt. Best if therapy commences 7 days post Chemotherapy and also as stand alone.

Period

10 – 30 days therapy arrests almost any internal tumor (non blood). 60-120 days = significant retraction. No hair loss.

Unique Aspect

Classical Ayurveda and or the subsequently developed Sino classical medicine do not have anything or even something alike. ATOX is original mint. No hair loss.

Drug Holiday (wash downs)

Cancer pathophysiology and anti-cancer therapy create ketons & metabolites. Therefore, occasional drug holiday in the order of 3-10days (wash down of ketons & metabolites) should be part of any anti-cancer regimen (even for herbals). Drug holidays because wash downs. The physiological systems thence remain more receptive to medicaments. With ayoorvedic\natural medicines the systems receives a gamut of compounds. Periodic wash down enhances therapeutic index and averts resistance. And, safe-smooth-successful cancer treatment requires case basis (patient centric) periodic plan alterations. During Chemo therapy the cross section of the gut lumen reduces and in sections even collapse. The saliva glands under perform; the pancreases produces excess of Chromogenin; whole liver goes slack while insulin destruction in the portal vein gets reduced = extra insulin available i.e., acute metabolic

aberration (supporting info; read with Ref. No. 20). This results in peristalsis failure, recalcitrant hypotension, diarrhea, appétit loss, spasm, ion-salt-fluid swings (off balance), listlessness, sleeplessness, severe-obstinate urticaria, idiopathic refractile responses, etc., (mouth soars apart). This is reduced with stepped down ATOX + slimy glutonic food + oral restoratives ORS + non phosphate hematinics + mini vol. wine + wash down periods. Stepped up ATOX (MRC) modality assists. Indicatively, our candidates have lot much to offer. They are loaded with opportunities.

Nano Tech & CSAMs

Novel approaches in cancer treatment [6,7] is the hallmark of current research. Let us consider ATOX. In a capsule of 500mg the gross of all the natural active pharmaceutical ingredients (API) as are in the Xanthocarpum may hover around 50mg only. Xanthocarpum has numerous glycosides which have anti-tissue (anti neoplasia) and also immunomodulator property. In the absence of tumor\cancer Xanthocarpum's immunomodulator moiety can selectively be used in asthma – with good response. In cancer\ATOX such moieties need to be packed into the same tab/cap and yet be kept apart, for either confabulate the other. *Barelia Prionitis* has a long blood life; is mildly toxic yet has excellent scope of use in patients suffering from epistaxis; aviation medicine; vasculitis & also in thrombocytopenia (post engineering of the API). *D. Melanoxylon's* API is light sensitive and also to containers\rooms that de-gas. *Anonna Squa.,* is potent vis-à-vis cancers & cysts in female mammary and fallopian tube & vaginal genic neoplasias\malignancies. Peerless in reversing stage I-II filariasis caused baggy limbs. *Anonna* (apart having scavenging & anti-viral property) also yeomanly helps in firming the feminine mammary (post lactation; chemo and or otherwise). To tap & collate all these diverse APIs calls for nano engineering.

If all the API moieties of all the ingredients are considered they sum up to around 60-90. Many of them are products of a cascade. As mono have varied roles. Yet, in collated (ATOX\MRC) from solely address the caption objective. The bulk density varies significantly.Inadequate homogenization leads to internal patch or nodule\granule formation apart tablet punching\ slug problems, etc. Yet, slugging becauses clumps. In-vivo, the well homogenized mass degenerate efficiently. Therefore, the classical formularies of ayoorveda e.g., 'Nighantu' (formulary) or the 'Salagram…' (shiest pestle) talk only of finger rolled pills and or use of fresh suspensions (no pounding pressure) [8]. At a column pressure of 2kg per square inch leads to coalescing and auto formation of neo compounds (pure herbal compounds are load sensitive). In purified state the natural APIs from the isolated fractions are very reactive and have to be embedded in neutral conditions and also be shielded from one another. Extract based herbal formulations suffer xenobiotics; short shelf life & formation of new compounds i.e., fail too often. The purified fractions also indicate very short blood life with toxicity ranging between high to very high (viro & MRC 5 cells ~ unacceptable). These APIs therefore, need to be slow released. Need to be released in pico and nano quantities; in various sections of the gut (= lovely results & smooth recovery). Finally, of the 90 compounds some have opposite property (i.e., pro cancer and or teratogenic).

They too be in nano quantities and eulate on a time scale basis. They need to be either isolated and screened and not merely be neutralized. All these can only be done by adoting forward looking engineering of nano-pico concepts. Therefore, we have conceptualised a Nano Tablet [9]. Our decadal experience arises from the observation of 'n' number of cases of total obstruction of the epiglottis\fossa; esophagus; fundus (fluid or guided probes failing to pass when forced pushed) slow open up due to retraction of the obstructing neoplasia even if ATOX combined therapy (MRC modality) is licked taken in fractional doses (by the patient as day long effort). It elicits sterling response. Patient gets to be up & about and fit for chemo modality. ATOX et.al., + Chemo = virtual defeat of cancer. Substitutes chemo well in under weight, reactive & refractile patients (collateral info). The requirement of chemo also reduces drastically. And, all this in the remote of the rural clinical setting. Again, nutraceuticals (like ATOX et.al., etc.) provide inspiration windows and leads for the drug discovery personnel has to fjord a long way. Furthermore, such CASMs (complementing, supplementing alternative medicaments) permit the patient-clinic nexus to keep moving forward in the instant moment. It is a bottom up model that can also part finance the research for chirals with feed back concurrently from clinical & nursing staff. Quality control in natural medicines are difficult to

maintain and also not that very essential (for they be near food items). Nutraceuticals also mean large doses and often long course therapy. Miniaturized nutraceuticals evens out such shortcomings.CASMs are physiologically more compatible and employment enabling. Therefore, nano engineering is more/urgently relevant for CASMs.

DISUSSION

Ayurveda is India's National School of Medicine. Written text from hoary past are available in Sanskrit lingua scripted in Devnagari (India's national lingua & script, respectively) engraved with iron stylus on palm leafs. They are dated to *c.* 400-500 A.D. [10,11]. Versions and derivatives also abound in every (Indian) regional vernaculars. The pan india cumulative volume is a historically unsurpassed tome [12]; mankind's heritage also in anatomy based sciences [13].The nearest historically comparative is the Sino school of herbal medicine [14]. It too is of great antiquity [15]. However, it is not as exhaustive nor as scientific as is the Ayoorveda. The Occidental school of medicine transpired out of the Hippocrates's testaments (Greeco-Latin school). Furthermore, modern medicine & surgery has more arisen off the 'Barber Surgeon Associations' of Europe [16-17], while the covenance of the Ayoorveda is ascribed to the erudite ('pandit' in indo lingua). Modern period scientists of 'History of Medicine' have indicated that while the Greek scholars had visited India to learn & imbibe health care sciences & practices, the Indians (sanatans) are said not to have done anything alike [18]. Now, whatever may have been the historical process past in relation to our caption any of these schools, members, texts, et.al., do not make any mention of anything that we now know and associate clinically with the term 'cancer' and or 'metastasis'. The constituents as in Table-I (including sister species) also were never used for addressing any clinical symptom that can with much liberal intuitive stretching and \or indulgent academic gymnastics be associated with 'cancer' and or 'metastasis'.

With ATOX et.al., MRC modality effective necrosis, anti-angiogenesis and above all near almost complete halt to mitosis in/of neoplastic masses is being observed for the long period (own data; deferred). Progression free status extends (significantly). Quality of life improves (drastically). Excellent, broad spectrum complementing and supplementing role with modern therapies are the hallmarks. In breast cancers, metastasis is swift. This happens due to efficient lymph drainage & a direct vein-lymph connect. Some drugs such as the ones that have the element fluorine upregulate wildly **[***own data***].** ATOX et.al., modality are proving effective in reversing metastasis, reducing primary masses and even in averting mastectomy. In the developing world patients often report at a stage when chemotherapy is very hard as a prescription (deleterious, often resulting in rejection \non compliance). Often the patients (at 1st presentation) are too run down for chemo tolerance. Again, in cancers involving mouth, elementary canal, and in post radio-therapy radiation changes are marked by (near-complete) food ingestion difficulty and or peristalsis failure and or gut lumen collapse. Even the intubation tube and or the endoscope probe do not pass (once taken out; or even during initial probe). Equipment & resource person wise hospitals are not enabled (even large referral centers are rudimentary compared to the western world) in the developing world.

Therefore, first aid, preparatory, and or stop gap oral medication becomes necessary. And, there are non available even to the down-town clinician. And whereas, ATOX et.al., modality is proving as the regimen to initiate such (good) spade work. It is positing as a Quick Acting First Aid – QAFA. From such perspective, ATOX et.al., modality possibly is the sole in the domain of 'cancer' and 'metastasis'. This indeed is helping the modern medicine practitioner (specially the Rural clinician and the Family physician in urban centres). ATOX is limitedly contra indicated only in psoriasis. ATOX et.al., are excellently CASMs therapeutics (food based). It is broad spectrum and synergistic with modern medicaments. ATOX et.al., does not seek to replace modern therapies. ATOX et.al., modality was initiated around 1982 (as palliative; such aspect continues even now). From 2004 it was used only in deep brain tumor cases. From 2009 onwards wider applications emerged [see Ref. 4 & 5]. Thereafter, it has been in regular use as part of family welfare-patient centric care and specially for reversing metastasis form bones, liver & brain (in particular, apart others). And whereas, anti-metastasis drugs thus far are unavailable [19,20]. Being anti-mitosis; high safety factor; easy to make and good to use; with assured results [see Ref. 2&5] ATOX et.al., modality

offers a paradigm shift; with an never before opportunity to *Fight Cancer Unitedly & Holistically*; and specially *Fight Cancer& Metastasis at Home*. ATOX et.al., also fails\thwarts pregnancy i.e., indicates herbal contraceptive application property. The indicated anti-mitosis property down turns the side effects of modern contraceptive pills (collateral information). Thus, ATOX et.al., offers myriads of branching opportunities to the drug discovery community. A vital aspect of successful cancer combat is nursing [21]. Even the nurses and or the primary health workers are advocating such methods & models of fighting cancer unitedly and deploying every safe means. MRC modality (atox\atamcox\avir\ata\dk\vac\knd\R-sol) are proving to be vital tools for the family physicians. Hence, patent shall not be filed by this author (inventor\discoverer). All are welcome to participate and take benefit from MRC modality which all (includes ATOX) are bench-to-bed Translational Medicine that has withstood continuous use test for over a decade. We have attempted a heuristic model of narration to encourage ease of replication. And, cancer is the name of any natural process(s) that has gone wrong, uncontrollably. Hence, world over there is a new urge towards natural medicines [22].

Therapeutic Limitations

ATOX et.al., are not as much useful in leukemias. Are synergic and are not contra indicated. Contra indicated in Psoriasis and in all sub-types of necrolyting/migrating dermal pathologies. Other constituents of the MRC modality have numerous usages and synergic roles. VAC is useful for blood cancer and is contra indicated in congestive heart etc., related cases.

CONCLUSION

The immediate objective is to '***Fight Cancer At Home***'. MRC modality i.e., ATOX *et.al*., can be hand made at home. In other words, they are compoundable. Safe-sure-economic cancer combat calls for individual patient centric compounded multi drug therapy. It also means greater employment. Jointly or severally ATOX et.al., have been indicating excellent clinical response vis-à-vis solid tumors & cancers, extending over a decade period in very difficult clinical & nursing conditions. Excellent also in all types of distant metastasis. Anti-mitosis + anti-angiogenesis + anti-tissue (enrolled into one) makes these presents stand out. Difficult objective seem to have been addressed (rudimentary; in parts). At evolution stage ATOX et.al., were introduced as 'end stage palliatives' for patients suffering from end stage cancers and or intractable tumors.

They are currently in slow flow (non commercial) social service motion and sample can be availed from the author/finder (no propaganda; no advertisement). ATOX et.al., modality have passed the long period validation (free, palliative) social service use as CASMs (patient centric care; convenience of family members). It is now time to open the same information for world wide examination. ATOX et.al., modality offers excellent synergic results with chemos and post oncological surgery (no metastasis nor any complications post needle & or true cut biopsy included). The constituent herbs are common in the tropo-equatorial belts of the globe. These CSAMs embolden the case for use of nano-tech in natural extract based drug making (isolated & purified fractions as the APIs) & superb QC for ayoorveda\poly or mixed herbo-chem drugs. We have mentioned the making process in a fail safe copy method so that low technology societies & stake holders (all fields of health care) can adopt with ease (duplication & wider use or research permitted).

ACKNOWLEDGMENTS

Author (social worker) is grateful to the kind long period assistance to Prof. P.C.Dey, Director of Clinics; Prof. Medicine; Prof & HOD Surgery (he decorated 3 post from time to time & at times all three) of Veterinary Sc College; Orissa University of Agriculture & Technology. Erudite & immensely capable vet surgeon (one man institution). He

even participated in the field use of ATOX on animals. Provided impetus and encouragement between 1993-2003. Without his continuous unconditional all out support this work could not have been done. One of the finest persons (God's own make). Hence, ATOX is dedicated to the public in his name. Prof. Jeffrey White, MD, HIH CAM, Cancer div., also provided fillip from around 2003-04. ATOX is indeed saving lives (proving very helpful in cancers). Nothing happened overnight nor sans support & encouragement. Moreover, Pat Gen of India overtly supports IPR Grant on the maxim of ~ by the Govt; of the Govt; for the Govt. Next in priority be their siblings & protégées; thence, the indo corporates and it (variously & variedly) fails the unaided native individuals (specially the Gandhians and the social workers). MRC modality & ATOX in particular are products of a life long effort with missionary zeal. So many to thank.Hence, additionally open. Got delayed due to (life threatening) conspiracy hatched by Pradeep-Basanti-Arundhoti. Thanks to IJONS for assisting this dedication.

REFERENCES

1. Irache Juan M. , et.al., 2011. Nanomedicine : Novel approaches in human and veterinary therapeutics, *Veterinary Parasitology,* Vol. 180 (1–2), pp. 47-71.
2. Bhattacharya Deepak, A Novel Anti-Metastasis and Chemotherapy's Side Effect Reducer, *Translational Medicine*, (Sunnyvale), 2016, 6:3. http://dx.doi.org/10.4172/2161-1025.1000178
3. Bhattacharya Deepak, 2017. Fight Malaria at Home: Ghare Maro Malaria Social Service to Drug Discovery – Bottom Up Model: A Review, *Air Water Borne Diseases*, 2017, Vol.6:1 DOI: 10.4172/2167-7719.1000135
4. www, ATAMCOX-Vedic Anti Cancer – VAC.
5. www, Metastasis Reversal Centre, Bhubanesar, Odisa, india.
6. Kizilbash Nadeem & Jamal Alruwaili, 2017. Novel Approaches for Treatment of Breast Cancer Tumors, *Novel Approaches In Drug Designing & Approaches*, Vol.2 (2).
7. Utku N, 2011. New Approaches to Treat Cancer – What They Can and Cannot Do, *Biotechnology Healthcare,* 8(4), pp. 25–27.
8. Bhattacharya Deepak, et.al., 2013. Transmission Blocking of Resistant Malaria with OMARIA, *British Jor of Pharmaceutical Research*; 3(1) : 54-77.
9. Bhattacharya Deepak, 2017. Nano Tech Tablet: A Concept, *Novel Approaches In Drug Designing & Approaches*, Vol.2 (2).
10. *Atharva Veda Samhita*, (any issue), Medicine Chapter.
11. *Charak Samhita* (Jivananda Ed.).
12. Bhattacharya Deepak, 2014. Select Palm Leaf Manuscripts On Health Care, Indian National Science Academy, *Indian Journal of History of Science,* Vol. 49.3, pp. 294-97.
13. Bhattacharya, Deepak, Depiction Of Human Anatomy In Indian Archaeology : A Report, *Indian Journal Of History Of Sciences,* INSA, Vol.44.2 (2009), pp.313-22.
14. Cidian ZD (2005) *Grand Dictionary Of Chinese Traditional Medicine* (2nd Ed). Shanghai Science & Technology Press, Shanghai.
15. Read Bernard E., (1936). Chinese Medical Plants from the Pen Ts'ao Kang Mu, *c.*1596 A.D., Peaking, Natural History Bulletin. See also 3rd. Edition of a Botanical, Chemical and Pharmacological Reference List. Reproduced in Chinese Medicine Series, Taipei : Southern Materials Center. 1977 Ed.
16. Wikipedia, *Surgeon Barber Association*, https://en.wikipedia.org/wiki/Barber_surgeon
17. http://www.abc.net.au/radionational/programs/bodysphere/when-barbers-were-surgeons-and-surgeons-were-barbers/6391468
18. Hoernale Rudolf, C.I.E., "Studies in The Medicine of Ancient India, Part-I, Osteology of Bones of the Human Body, OXFORD, Clarendon, 1907 (Full Book).
19. Steeg P. S., 2016. Targeting Metastasis, *Nature Review, Cancer.* Vol. 16(4), pp. 201-18. doi: 10.1038/nrc.2016.25.
20. Stock A.M, et.al., 2013. Targets for anti-metastatic drug development, *Current Pharmaceutical Design*, Vol. 19(28), pp. 5127-34.

21. Bhattacharya Deepak, 2017. Nursing Defeats Cancer ?, *Journal of Complementary Medical & Alternative Healthcare,* Short Communication, Vol. 5 (3) On Line, Juniper Publications, DOI: 10.19080/JOJNHC.2017.05.555662
22. Kesari A, Susant Kumar Padhi, 2018. Alchemic (*Spagyric*) Method of Solid Fixed Dose Therapy for Electro-Homeopathy: Natural Medicine, *Indian Journal of Natural Sciences, Vol.8 (47), pp. 13652-55*

Table 1. Composition of ATOX

Sl No.	Botanical Name	Part	w/w/ or v/v Range	Activity	Figures	Opposite Or Anti Action	Label
1	*Dyospyrous Melanoxylon (KND)*	Whole Fruit (sans seed)	15-30%	Anti-mitosis	1 to 3	Seeds	During 2003-2017 all constituents have been used as mono and in 'n' number of combinations to make Tab\Cap having broad spectrum and or specific activity under ATOX; ATAMCOX; VAC;KND;ATA;R-Sol;AVIR\Azad;DK; etc., names during the long period bottom up development phase.
2	*S. Xanthocarpum / Violecenum Virginium*	Ariel	20-25%;	Anti-Tissue	4a & 4b	Seeds	
3	*Barelia Prionitis (DK)*	-do-	10-20%	Anti-Angiogenesis. Broad spectrum high efficacy anti-cancer.	5	Flower	
4	*Artimissia Vulgaris*	-do-	-do-	Anti-mitosis & anti-lymphadinopathy	Not Provided	Flower & Pollen	
5	*Hemidesmus indicus (sariba\Ananta moola)*	Carp of stem	10%.	Blood & Tissue Perforation Force	6	Central stem	
6	*Curcumo Longa*	Rhizome	10%	Anti-Lysozomes; etc	Not provided	Un matured	
7	*Anonna Squamosa* (a.t.a.)	Unripe fruit's aril (seed less)	10-30%	Anti-KB, HeLa, etc., cells	7	Seeds	
8	Indian Country Yam *(Dioscorea)* & Jhaaoon phala\pine *(Pinus; coastal or mountain); Punica Granatum.*	Rhizome & Carp	Anti-mitosis (stand alone) AND boosters, mass enhancers, bulking, binding, anti-allergen, Smooth, systemic, broad spectrum, blood life enhancer, potentiate, synergy inducers for Anti-mitotic therapies; gut lumen, anti-parasites, also excellent for skin tone & psoriasis, etc. 5-15%		-Do -	Carp of yam & Seed of pinus & punica	
9	*P Granatum* classical Ayurvedic dalimba (AVIR)	Carp of fruit (sun dried)	Potent anti-virus (that causes & up-regulates cancer); broad spectrum anti HIV; also anti-inflammatory high efficacy.		Not provided	Seeds	
i - Dyospyrous Melanoxylon & Punica granatum are anti-lysozome, anti-viral and also process scavenger, etc. *Ii - Anonna Squamosa alone is very effective in lymphy draining (elephantiasis) & tightens\firms mammary*. **Author**.							

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.8 / Issue 48 / June /2018 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Measuring of Nuclear Radiation and CO_2 Gas Concentration in Pure Water Stations of Baghdad.

NaseerA.Ahmed and Asia H. Al-Mashhadani *

Department of Physics, College of Science, University of Baghdad. Iraq.

Received: 13 Mar 2018 Revised: 15 Apr 2018 Accepted: 07 May 2018

***Address for correspondence**
Asia H. Al-Mashhadani
Department of Physics,
College of Science,
University of Baghdad. Iraq.
E:mail:assia19662006@yahoo.com

ABSTRACT

In this work the large nuclear radiation and environmental measurement has been achievedin Baghdad city, hundredsof readings have been collected in many pure water stations. The huge data was processed by new method of data visualization which makes all the data appeared on the map by using software named Google Earth Pro. The lowest average exposure of ionizing radiation in air for workersin is 1.314mSv/y that forAl-Kadhimiya and Al-Sadr pure water stations, while the highest one is 1.8396mSv/h in the project water of East,the average of radiation exposure in the air for workers is 1.438mSv/y.

Key words: Nuclear radiation, Environmental measurement, Google Earth Pro.

INTRODUCTION

Ionizing radiation is a type of energy emitted by atoms in the form of electromagnetic waves or particles [1]. People are exposed to natural foundations of ionizing radiation as in soil, water, and vegetation as wellas to the human-made sources. Ionizing radiation is released by radioactive elements and by equipment such as x-ray and radiation therapy machines [2,3]. Low doses of ionizing radiation may growth the risk of long term effects as a cancer [4]. Ionizing radiation is a kind of energy emitted by atoms that transferred in the form of electromagnetic waves gamma and X-rays or particles like neutrons, beta and alpha [5].The spontaneous disintegration of atoms is called radioactivity, and the additional energy released is a form of ionizing radiation. Ionizing radiation has plentiful energy to release electrons from an atom, thereby exit the atom charged, while non-ionizing radiation, like radio waves, visible light or ultra-violet radiation, does not [6].The Sievert Sv is the unit of the effective dose that considers into account the type of radiation and sensitivity of tissues and organs. It is a method to know ionizing radiation in terms of the potential for causing harm [7].

MATERIALS AND METHODS

Baghdad depend on many water stations of pure water. They take row water from Tigris river directly like Al-Karkh water project and the big water of Al-Ruasafa project or they take the water non-directly, like Al-Baladiyat project which receive the row water from Abu-Now'aas station which pumps row water.The water is process in all these stations by many stages, the first is deposition in large water basins with different shapes rectangular or circular. The water transport from basin to basin and that late assist in more deposition.All that achieved by new modern device GMC-520. The water transport to second stage, the basins of nomination. These basins depend on many layers of special sand so as to filter the row water with acceptable rates of Iraqi standard for purified water. That is happened by benefiting from the gravity feature.

Al-Rusafa project and Al-Baladiyat project

It is a large project in Baghdad it takes the row water from Tigris river directly and transport it to the large basins that circular shapes. The samples of water were pumped from these basins to the laboratory in order to analysis it before process, after that the process of water is started in next stages as shown in Figure (1). The readings of nuclear radiation for gamma and beta in many locations as in above the basins and among them that has been changed between 0.06uSv/h for minimum detects and 0.15uSv/h for maximum detect. The levels of CO_2 emanation in the air start from 453ppm as the minimum level and reach to 491ppm for maximum reading. In spite of checking the hall of pumps, there is no discover in data more than that levels. Also the area between halls of pumps and the upper of the large tanker of pure water was scanned in the final stage as shown in Table (1).

Al-Baladiyat water station is smaller than Al-Rusafa water station, it takes the row water non directly from Tigris river so the Abu-Nwa'as station pumped the row water for it as shown in Figure (2). The smallest reading of gamma and beta by GMC-520 is 0.06uSv/h in the stages of water deposition and other departments, while the largest reading is 0.14uSv/h in the same places. The CO_2 levels were checked in this project among pumps of pure water in the final stage, stage of Chloride addition and between other buildings. The readings changed from the smallest one 480ppm to the largest reading 515ppm in these places.

Al-Jadriyah Water Station

This project locates beside university of Baghdad, the altitude of the tanks of water and pumps is about 31 m.The readings of ionizing radiation altered from 0.05uSv/h as a low detect to 0.18uSv/h as a high detect for different positions in this project as shown in Figure (3). While the CO_2 levels reaches to 519ppm as a high number and 449ppm for the low number as shown in Table (2).

Water Project of East Tigris

It is the third large station of water in Baghdad city. About ten basins of row water circular shape, it takes the water directly from the Tigris River, as shown in figure (4). The readings of ionizing radiation among these basins is about 0.05uSv/h for smallest detect while the largest one is 0.21uSv/h. The readings in the hall of aluminum sulfate (AL_2SO_4) was checked and the halls of pumps of pure water, too. The carbon dioxide CO_2 readings altered between 452ppm as a small data and 571ppm for the high data for this place. the upper of the large basin of pure water was scanned. This basin of pure water is underground.

Project Water of AL-Sadr City

This project consists of two parts; the first is a large tank of pure water which receives this pure water from Al-Rusafa project and the second receives row water non-directly from the project of East Tigris as shown in Figure (5).The detect of nuclear radiation is about 0.05uSv/h which is the smallest of data in this project and 0.15uSv/h is the largest one.The CO_2 readings changed from the low one 450ppm to high readings 554ppm in different locations in these two departments like the above basins of row water in the water deposition stage.

Al-Wathba Water Station

It is the oldest pure water station in Baghdad city and all over Iraq, it has small square basins and rectangular shaping. The high reading of nuclear radiation is 0.18uSv/h while the smallest detect was 0.06uSv/h. The readings of CO_2 beside the old basins and new basins are within 525ppm and the low one reaches to the 453ppm.

Water project of Al-kadhimiya

The location of Al-kadhimiya project for pure water is in the middle of residential area. This new project was completed after 2010. the nuclear radiation detection for Gamma and beta within 0.04uSv/h to 0.15uSv/h. In this place the readings of CO_2 levels among many circular basins of depositions the row water is found that the emanation of CO_2 reached to 574ppm and in the low level 435ppm. Also this scanning compromised the hall of adding the chlorine.

Al-Karamah Water Station

It takes the row water from the Tigris and it contains two kinds of water deposition basins circular and rectangular shapes. The levels of CO_2 in all stages is about 449ppm to 525ppm. The measuring of gamma and beta among basins, inside the hall of the pure water pumps and other stages altered from 0.16uSv/h to the low detect 0.06uSv/h.

Project of the pure water for the north of Al-Karkh

It is a large tank of pure water, no processes of deposition and filtering in this large station, because it receives the pure water from another water station in north of Baghdad. After that the process of adding the chlorine is repeated again so as to ensure the biological sterilization for the pure water before pumping it to the people by using the underground net. The reading of nuclear radiation in the large hall of pumps has been changed from 0.05uSv/h to 0.15uSv/h. The CO2 levels beside the large tank of pure water are about 448ppm to 556ppm.

The project water of Al-Dora

It consists only from two circular basins for deposition the row water. This new project takes the water directly from Tigris river. All records of nuclear radiation are about 0.05uSv/h to 0.16uSv/h inside the hall of pumping the pure water and outside of them between the buildings. The CO_2 levels altered within 451ppm to 560ppm in different locations in Al-Dora water project as in near the river and in the halls of the process of the water.

The project Water of AL-Wihda

It is an ancient project before about 35years. The readings of nuclear radiation are about 0.05uSv/h to 0.17uSv/h among the basins of water depositions and the hall of the pumps of the pure water.The CO_2 levels between buildings

and inside the halls of the pumps of the pure water is about 467ppm to 524ppm and the same is in different sites of this station.

The project water of AL-Rasheed

It is also old and started its work before more than 40years. This water station consists of two circular basins and a hall of many pumps of pure water. The reading of nuclear radiation above the basins of water deposition and the hall of pumps is from 0.05uSv/h to 0.15uSv/h. In the same time CO_2 concentration among all stages changed from 442ppm to 557ppm.

The project Water of Qadisiyah city

It takes row water directly from Tigris river. The readings of gamma and beta is between 0.06uSv/h to 0.16uSv/h in different sites inside this water project.There are many gardens in the Qadisiyah project. The readings of CO_2 changes from 421ppm to 461ppm in the hall of aluminum sulfate, among basins of deposition water and the hall of the pure water pumps.

RESULTS AND DISCUSSION

Big Data analytics plays a key role through decreasing the data size and complexity in Big Data applications. Visualization is chief the approach to help Big Data to obtain a complete view of data and determine data values [8]. The first step of visualization process is known a mapping. Mapping means how to visualize information or how to encode information into visual form. In mapping data or information transform into graphical form under assumption of visual features [9,10]. The average of radiation exposure in the air for workers is about 1.314mSv/y as a smallest in the Al-Kadhimiya and Al-Sadr projects while the higher one is 1.8396mSv/y in the project water of East Tigris, as shown in the Table (3).

The sixth column number and the fourth one are the lowest 1.314mSv/y, they represent projects of Al-Kadhimiya and Al-Sadr cities, third column is the highest one 1.8396mSv/h in the project water of East Tigris, all the blue columns belong to the pure water stations while the orange one represent the average of radiation exposure in the air for workers 1.438mSv/y, as shown in Figure (6).

CONCLUSION

1. The average of ionizing radiation exposure in the air for the workers in the pure water stations is between 1.314mSv/y to the 1.839mSv/y, whereas the average of exposure for all water stations is 1.438mSv/y.
2. The reading of carbon dioxide in the different sites of pure water stations is about 421ppm in the project water of Qadisiyah city as a low measuring to 574ppm in the water project of Al-kadhimiya as a high measuring.

REFERENCES

1. Merck Manual for Health Care Professionals, Radiation Exposure and Contamination.Seiler, F.A. & Alvarez, J.L. 1994, The Scientific Method in Risk Assessment, Technology Journal of Franklin Inst 331A.
2. United Nations Environment Programme. 2016. Radiation Effects and Sources.
3. James E. Turner. 2007 WILEY-VCH Verlag GmbH & Co. KGaA, WeinheimAtoms, Radiation, and Radiation Protection.

4. International Atomic Energy Agency (2007). IAEA Safety Glossary: Terminology Used in Nuclear Safety and Radiation Protection (PDF). Vienna: IAEA.
5. KhandokerAsaduzzaman. Natural Radioactivity and Heavy Metal Pollutants In Staple Foodstuff And Human Teeth Collected From Selected Areas In Peninsular Malaysia. Faculty of Science University of Malaya Kuala Lumpur. 2017.
6. International Atomic Energy Agency Vienna. Nuclear Technology Review. 2009.
7. E. M. James, "Physics for Radiation Protection", 2nd edition, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2006).
8. V. Sucharitha, S.R. Subash and P. Prakash, Visualization of Big Data: Its Tools and Challenges, International Journal of Applied Engineering Research, 9(18), 2014.
9. Intel IT Center, "Big Data Visualization: Turning Big Data In to Big Insights," White Paper, March 2013.
10. Jin X, Wah BW, Cheng X, and Wang Y, "Significance and challenges of big data research," Big Data Research, 2015 Jun 30.

Table 1: Data of checking Al-Rusafa project

Date	CPM	ACPM	uSv/h	Latitude	Longitude	Altitude	Air Pressu	CO2	Temperat	Humidity
9/12/2017 14:41	17	16.22	0.11	33.41794	44.38141	34	1003	494	43.14	10
9/12/2017 14:40	19	16.22	0.12	33.42131	44.38062	36	1002	491	42.82	8
9/12/2017 14:39	20	16.22	0.13	33.42423	44.38011	38	1003	486	42.54	8
9/12/2017 14:37	25	16.22	0.16	33.42833	44.3801	38	1003	487	43.38	8
9/12/2017 14:36	22	16.22	0.14	33.43248	44.38001	33	1003	480	43.28	6
9/12/2017 14:35	16	16.22	0.1	33.43585	44.37996	28	1003	479	43.47	6
9/12/2017 14:34	8	16.22	0.05	33.44066	44.37996	17	1003	472	42.91	6
9/12/2017 14:33	11	16.22	0.07	33.44661	44.37983	12	1003	467	43.4	6
9/12/2017 14:32	16	16.22	0.1	33.44718	44.37797	18	1003	486	43.73	6
9/12/2017 14:29	19	16.22	0.12	33.4476	44.37437	27	1003	457	43.21	6
9/12/2017 14:28	20	16.22	0.13	33.44798	44.37416	26	1003	461	42.88	9
9/12/2017 14:27	14	16.22	0.09	33.44809	44.37388	27	1003	473	42.61	8
9/12/2017 14:26	19	16.22	0.12	33.44793	44.37336	26	1002	470	41.97	7
9/12/2017 14:25	15	16.22	0.1	33.44825	44.37397	21	1003	482	41.23	12
9/12/2017 14:21	17	16.22	0.11	33.30408	44.29765	0	1003	476	38.83	11
9/12/2017 14:20	17	16.22	0.11	33.30408	44.29765	0	1002	482	39.5	10
9/12/2017 14:19	17	16.22	0.11	33.30408	44.29765	0	1003	473	39.73	10
9/12/2017 14:18	15	16.22	0.1	33.44685	44.37121	37	1003	463	40.66	10
9/12/2017 14:17	22	16.22	0.14	33.44686	44.37119	39	1003	459	42.12	9
9/12/2017 14:16	16	16.22	0.1	33.44655	44.37122	36	1003	462	43.26	9
9/12/2017 14:15	23	16.22	0.15	33.44635	44.37179	30	1003	450	43.53	7
9/12/2017 14:14	14	16.22	0.09	33.44726	44.37441	38	1003	457	43.83	6
9/12/2017 14:13	18	16.22	0.12	33.44972	44.37253	26	1003	464	43.78	6
9/12/2017 14:12	20	16.22	0.13	33.44975	44.37335	34	1003	464	43.88	5
9/12/2017 14:09	15	16.22	0.1	33.44913	44.37278	40	1002	461	43.99	6
9/12/2017 14:08	15	16.22	0.1	33.44895	44.37275	33	1002	460	43.82	7
9/12/2017 14:07	19	16.22	0.12	33.44911	44.37277	33	1002	460	43.7	7
9/12/2017 14:06	16	16.22	0.1	33.44893	44.373	37	1002	459	43.89	7
9/12/2017 14:05	14	16.22	0.09	33.44905	44.37314	36	1002	461	44.25	9
9/12/2017 14:04	17	16.22	0.11	33.44905	44.37327	33	1002	463	43.97	7
9/12/2017 14:03	11	16.22	0.07	33.44936	44.37328	34	1002	465	43.83	6
9/12/2017 14:02	10	16.22	0.06	33.44951	44.37331	32	1002	467	43.85	7
9/12/2017 14:01	19	16.22	0.12	33.44981	44.3733	32	1003	473	43.74	7
9/12/2017 14:00	23	16.22	0.15	33.44981	44.37324	30	1003	485	43.67	7
9/12/2017 13:59	17	16.22	0.11	33.4515	44.3732	35	1003	453	43.18	7
9/12/2017 13:58	17	16.22	0.11	33.45158	44.37258	30	1003	455	42.8	6
9/12/2017 13:57	17	16.22	0.11	33.45135	44.37233	36	1003	461	42.64	8
9/12/2017 13:56	17	16.22	0.11	33.45158	44.37205	46	1003	468	42.26	6
9/12/2017 13:55	18	16.22	0.12	33.452	44.37238	48	1003	476	42.32	9

Table 2: Data of measuring of Al-Jadriyah project

9/7/2017 11:23	16	16.18	0.1	33.27224	44.39059	36	1000	464	39.49	15
9/7/2017 11:22	16	16.18	0.1	33.27208	44.39093	32	1000	448	38.5	14
9/7/2017 11:20	20	16.18	0.13	33.27242	44.39074	33	1000	446	38.65	16
9/7/2017 11:19	11	16.18	0.07	33.27287	44.39071	28	1000	446	38.12	15
9/7/2017 11:18	11	16.18	0.07	33.27279	44.39062	25	1000	448	38.25	17
9/7/2017 11:17	14	16.18	0.09	33.27237	44.39018	27	1000	455	38.22	15
9/7/2017 11:16	18	16.18	0.12	33.2722	44.39019	32	1000	462	38.46	14
9/7/2017 11:15	18	16.18	0.12	33.27214	44.3903	34	1000	458	38.62	14
9/7/2017 11:14	18	16.18	0.12	33.27213	44.39033	34	1000	453	38.77	14
9/7/2017 11:13	17	16.18	0.11	33.272	44.39037	33	1000	459	39.07	13
9/7/2017 11:12	16	16.18	0.1	33.27193	44.3905	35	1000	462	39.33	14
9/7/2017 11:11	18	16.18	0.12	33.27197	44.39087	36	1000	449	39.22	13
9/7/2017 11:10	21	16.18	0.14	33.27158	44.39096	34	1000	457	39.3	13
9/7/2017 11:09	15	16.18	0.1	33.27149	44.3908	32	1000	458	39.26	14
9/7/2017 11:08	20	16.18	0.13	33.27147	44.39079	31	1000	464	39.05	13
9/7/2017 11:07	21	16.18	0.14	33.27169	44.39121	31	1000	470	38.89	13
9/7/2017 11:06	18	16.18	0.12	33.27164	44.39155	28	1000	476	38.5	13
9/7/2017 11:05	18	16.18	0.12	33.27145	44.39142	29	1000	495	38.36	14
9/7/2017 11:04	27	16.18	0.18	33.2713	44.3912	28	1001	466	38.41	14
9/7/2017 11:03	16	16.18	0.1	33.27125	44.39122	30	1000	468	38.37	15
9/7/2017 11:02	17	16.18	0.11	33.27097	44.39129	29	1001	467	38.34	16
9/7/2017 11:01	18	16.18	0.12	33.271	44.39087	29	1001	478	38.09	16
9/7/2017 11:00	17	16.18	0.11	33.27095	44.39077	29	1001	483	37.63	15
9/7/2017 10:59	19	16.18	0.12	33.27126	44.39082	31	1001	460	37.92	17
9/7/2017 10:58	11	16.18	0.07	33.27139	44.391	32	1000	461	37.58	17
9/7/2017 10:57	11	16.18	0.07	33.27152	44.39115	29	1000	455	37.3	17
9/7/2017 10:56	20	16.18	0.13	33.27188	44.39098	31	1000	469	36.82	16
9/7/2017 10:52	15	16.18	0.1	33.272	44.39082	25	1000	470	41.63	17
9/7/2017 10:51	19	16.18	0.12	33.27234	44.39023	21	1000	480	43.49	12
9/7/2017 10:50	17	16.18	0.11	33.27242	44.39024	18	1000	469	43.5	12
9/7/2017 10:49	15	16.18	0.1	33.2727	44.3901	17	1000	486	43.77	11
9/7/2017 10:46	15	16.18	0.1	33.27297	44.38881	29	1000	477	43.34	13
9/7/2017 10:44	17	16.18	0.11	33.27289	44.38872	28	1000	468	43.4	11

Table 3: The average of radiation exposure in pure water stations

Pure Water Stations of Baghdad	The largest reading in uSv/h unit	Average exposure of workers to ionizing radiation in air per a year uSv/y	Average exposure of workers to ionizing radiation in air per a year mSv/yr.
Al-Rusafa project and Al-Baladiyat projec	0.15	1314	1.314
Al-Jadriyah Water Station.	0.18	1576.8	1.5768
Water Project of East Tigris.	0.21	1839.6	1.8396
Project Water of AL-Sadr City.	0.15	1314	1.314
Al-Wathba Water Station	0.18	1576.8	1.5768
Water project of Al-kadhimiya	0.15	1314	1.314
Al-Karamah Water Station	0.16	1401.6	1.4016
Project water of the north of Al-Karkh	0.15	1314	1.314
The project water of Al-Dora	0.16	1401.6	1.4016
The project Water of AL-Wihda	0.17	1489.2	1.4892
The project water of AL-Rasheed	0.15	1314	1.314
The project Water of Qadisiyah city	0.16	1401.6	1.4016
Average			1.4381

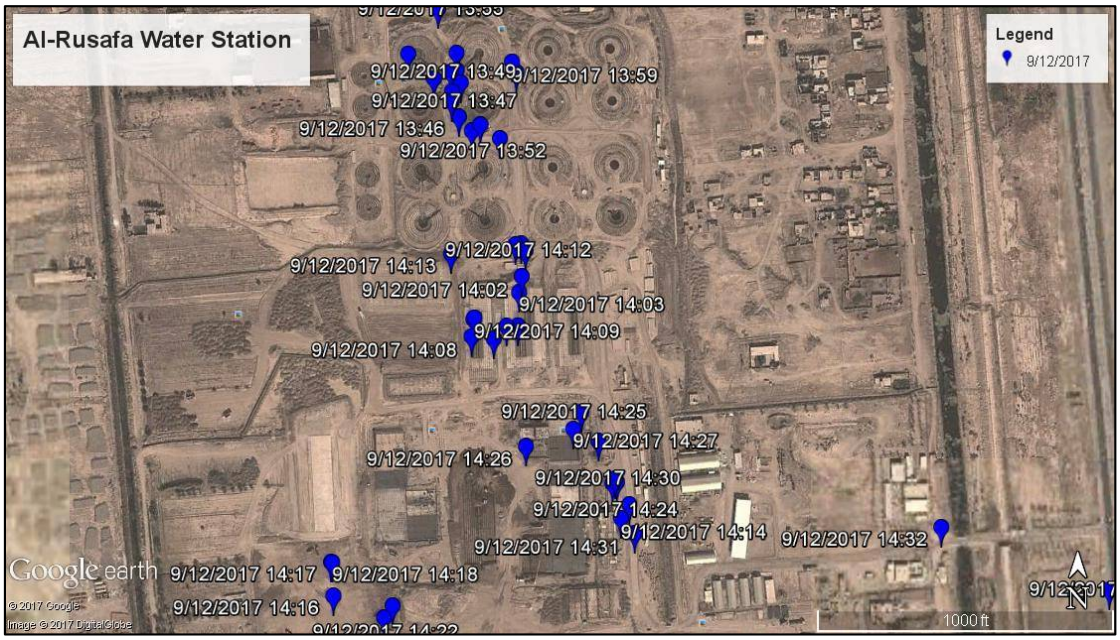


Figure 1: Locations of measuring for Al-Rusafa

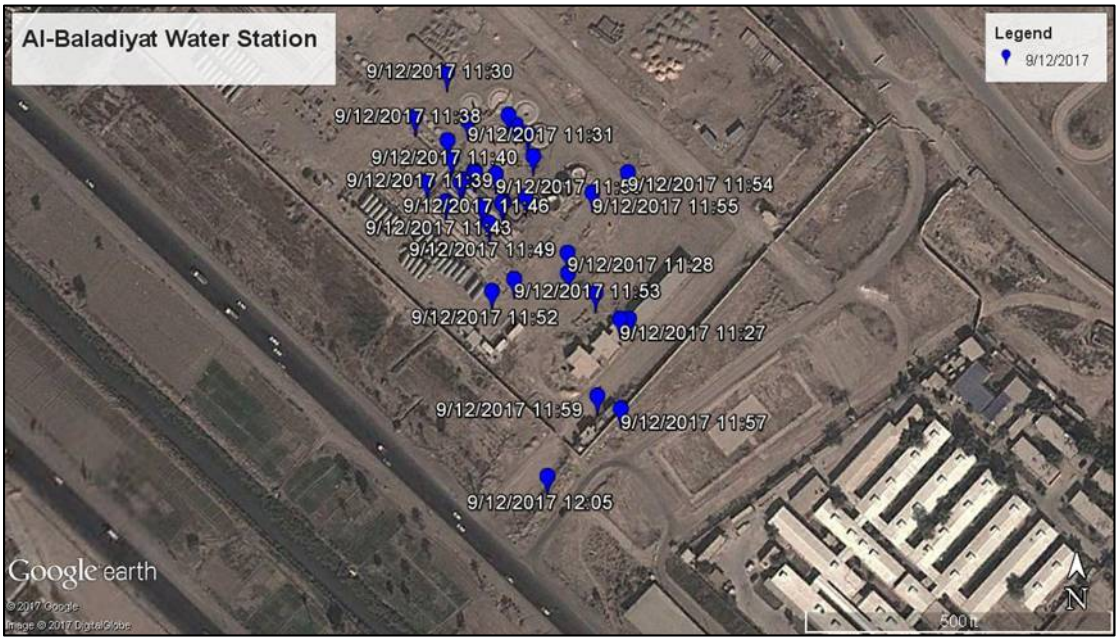


Figure 2: Locations of measuring for Al-Baladiyat

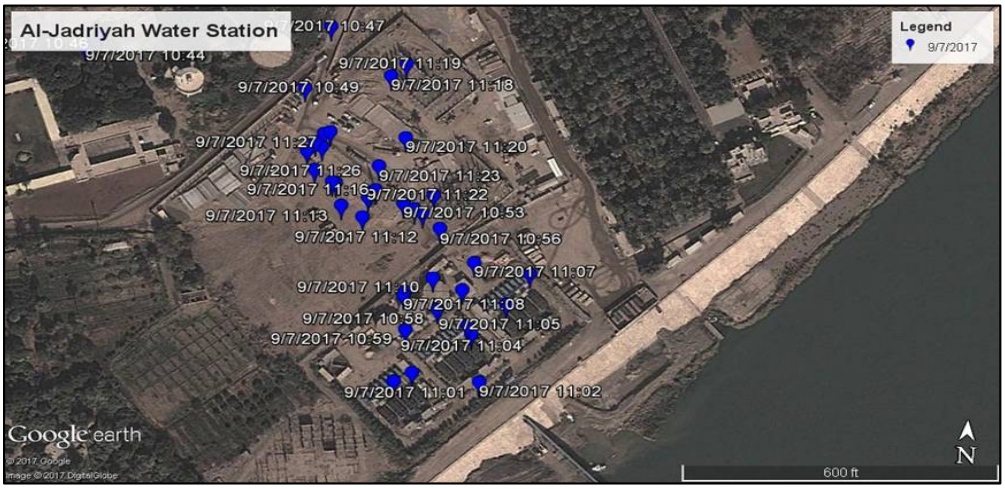


Figure 3: The locations of measuring to Al-Jadriyah project

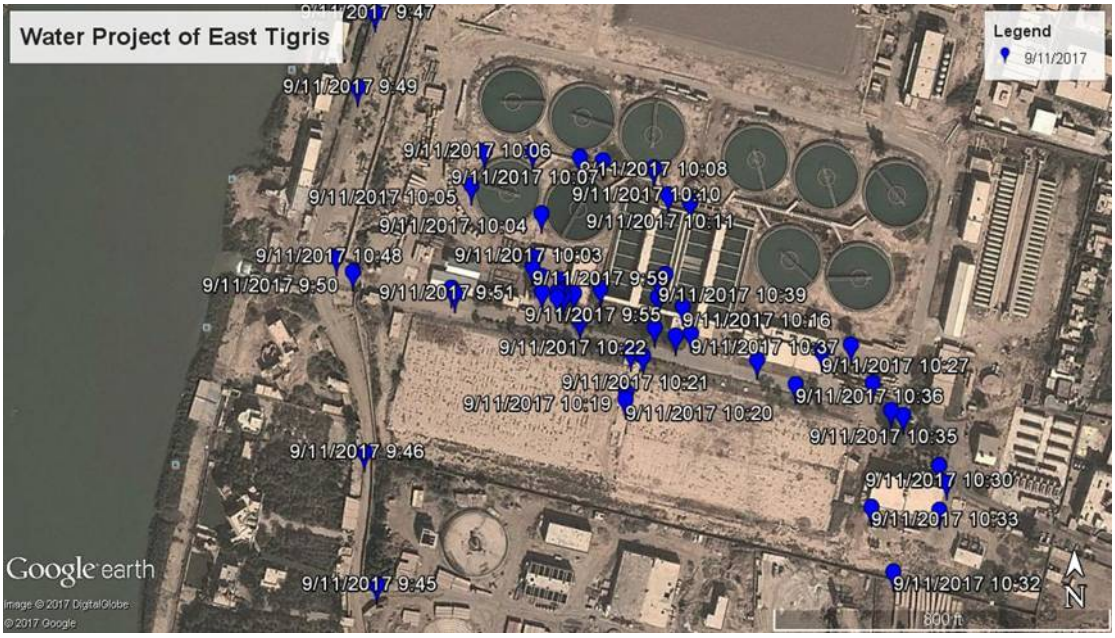


Figure 4: The locations of measuring to East Tigris project

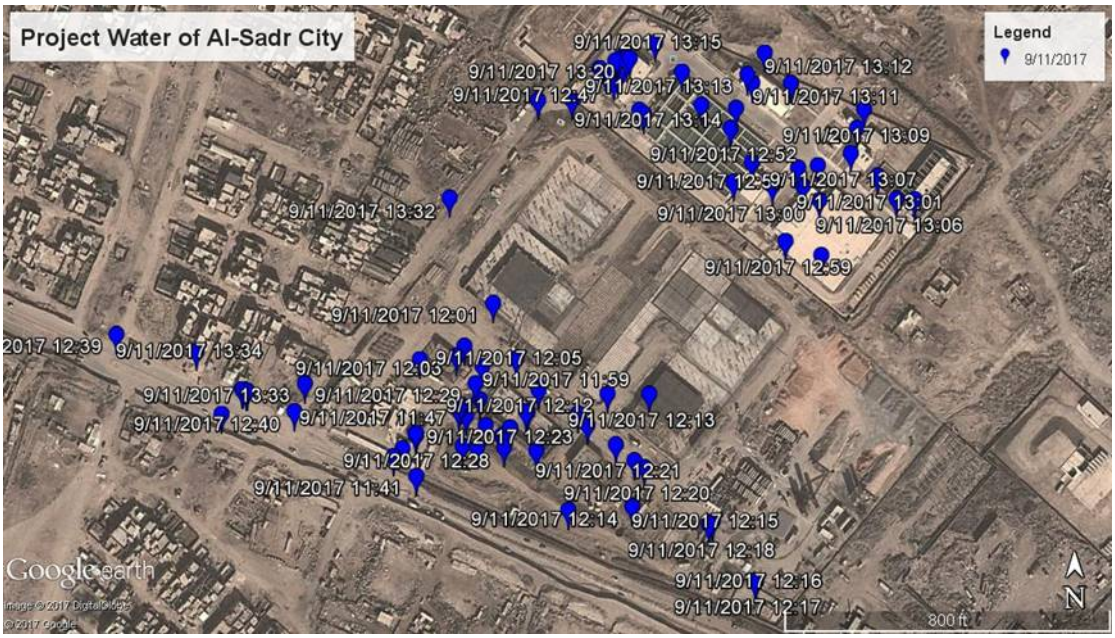


Figure 5: Locations of measuring to Al-Sadr City project

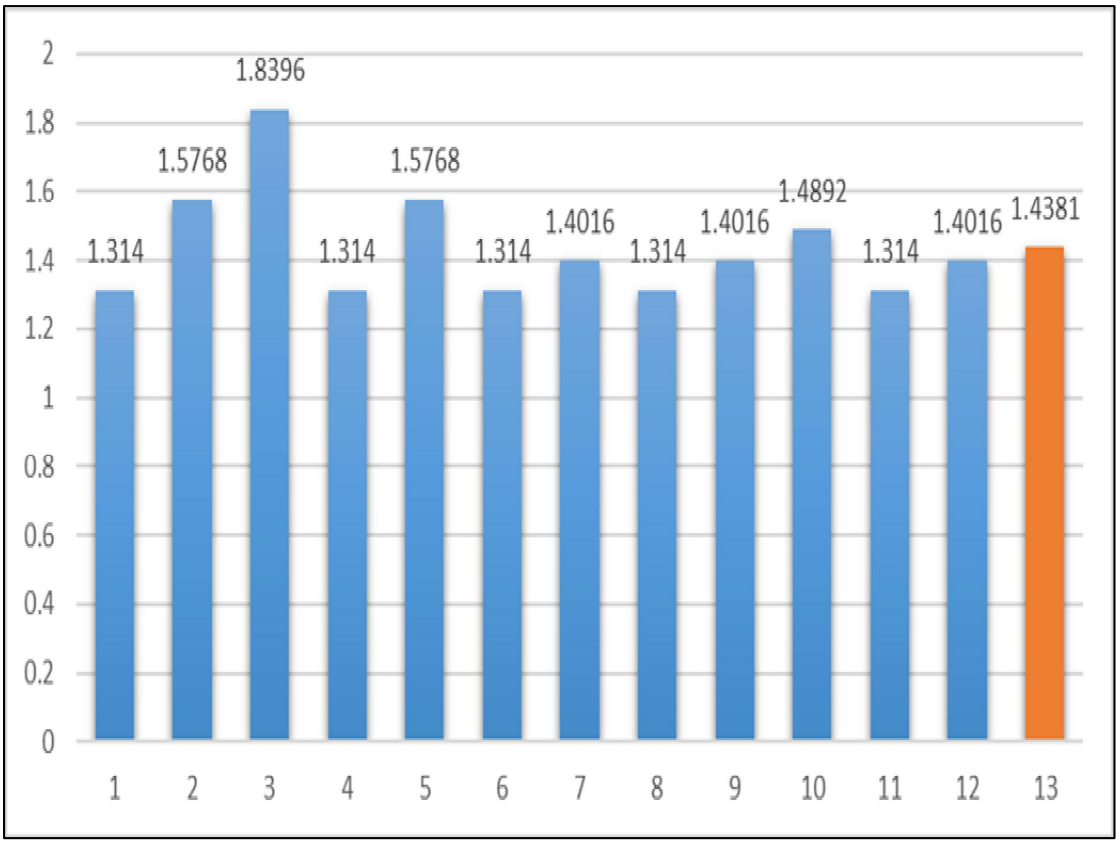


Figure 6: The distribution of the average radiation exposure for pure water stations