RESEARCH ARTICLE

Characterization and Antimicrobial Effects of Titanium dioxide Nanoparticles Produced by Laser Ablation

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ABSTRACT

Colloidal solutions of Titanium dioxide nanoparticles were synthesized by laser ablation of TiO_2 pellet immersed in 3 ml distilled water (DW), and alcohol for antimicrobial tested. Absorption spectra and particle sizes are characterized by UV-VIS and Field Emission Scanning Electron Microscope (FE-SEM). X-ray diffraction shows the structure of TiO_2 NPs. FTIR characterization confirms the formation of Titanium dioxide nanoparticles. The shape and particle size have been confirmed by FE-SEM measurement, average size is around 36 nm. The antimicrobial activity was carried out against Staphylococcus aureus and Escherichia coli. The Titanium dioxide nanoparticles showed inhibitory activity in bacteria were the minimum inhabitation concentration (MIC) in the distilled water is (9.45 $mg.L^{-1}$) for E.coli and (18.91 $mg.L^{-1}$) for staph, while in alcohol the minimum inhabitation concentration is (4.72 $mg.L^{-1}$) for E.coli and (9.45 $mg.L^{-1}$) for staph.

Keyword: TiO_2 Nanoparticles, Metal Oxide, Laser Ablation in Liquid, Antimicrobial effect.

INTRODUCTION

Nanoparticles (NPs) are not just inescapable in our regular day to day existences they additionally make significant commitments to diagnostics and treatment in medicine [1]. Titanium dioxide for instance has gotten much enthusiasm for ecological applications including photocatalytic hydrogen age and poison expulsion, because of its solid optical assimilation, synthetic security, minimal effort and high reactivity [2] Titanium dioxide, otherwise called Titanium (IV) oxide or Titania is the normally happening oxide of Titanium, compound recipe TiO_2. At the point when utilized as a shade, it is called Titanium white, Pigment white 6 [3].

Titanium dioxide is a wide band semiconductor which exists in three crystallographic stages: one stable stage, rutile (tetragonal) and two metastable polymorph stages, brookite (orthorhombic) and anatase (tetragonal). Both metastable stages move toward becoming rutile (stable) while presenting the material at temperatures over 700 °C (in unadulterated state, when no added substances have been included) [4]. TiO_2 has vast band holes of 3.2, 3.02, and 2.96 eV for the anatase, rutile and brookite stages, individually [5].

Plasmonic conduct in the UV-area particularly with controlled morphology and molecule estimate makes TiO_2 nanoparticles an appealing prospect for use as a decent UV safeguard for pharmaceutical applications as well as in sun based cell applications with expanded ghostly range. Synthesis of high quality nanostructured materials is an extremely dynamic region as nanoparticles speak to an imperative class of material advancement field for novel gadgets that can be utilized as a part of numerous applications, for example, photothermal [6], therapy [7], surface-improved Raman spectroscopy [8], biochemical sensors [9], sun based cells [10], Nano prescription [11] and executing unsafe microscopic organisms [12].

Removal of material from the surface of a solid after laser irradiation is known as laser ablation [13]. Pulsed laser ablation in liquid media (PLAL) is a system for manufacturing metal and metal-oxide nanoparticles. It produces estimated controlled nanoparticles in colloidal and additionally powder stages, ordinarily in a one-advance top–down technique [14]. The primary favorable circumstances of this technique are straightforwardness, does not require expensive chambers and high vacuum pumping frameworks henceforth it is considered as a green strategy. In PLAL, the shape and size of the delivered nanoparticles can be controlled by utilizing an assortment sorts of fluid and a scope of laser parameters, for example, wavelength, removal time, beat vitality, and so on [15]. In the present work, considering the portrayal of colloid TiO_2 NPs were combination by PLAL and researched the antibacterial action of colloidal TiO_2 NPs against Gram negative microbes (*E. coli*) and Gram positive microorganisms *(Staph. aureus.)*

MATERIALS AND METHODS

Preparation of TiO_2 Nanoparticles

TiO_2 nanoparticle were produced by laser ablation of a TiO_2 target (diameter = 1.5 mm, thickness = 0.5 mm, 99.99% immersed in a vessel filled with 3 mL of distill water (DW). The target irradiated vertically by a Q-switched Nd- YAG laser (DIAMOND-288 pattern EPLS), with wavelength (λ = 1064 nm) duration time at 6 Hz. The laser beam was focused by a focal length 10cm), subsequently, we put the pure solution with the metal and re-irradiating by pulsed Nd-YAG laser, the spot diameter of the focused laser beam was 4 mm. The experimental setup is shown schematically in Figure 1.

Bacterial isolates and antimicrobial test

The antimicrobial activity of the TiO_2 NPs was tested using the standard microdilution method in 96-well plates, which determines the minimum inhibitory concentration (MIC) leading to the inhibition of bacterial growth. The strains tested were *Escherichia coli*, and *Staphylococcus aureus* was obtained from the Laboratory of Bacteriology, college of science, Wasit University. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standards (approximately 5x 10^8 CFU/ml). Two fold serial dilutions of the tested TiO_2 NPs were prepared. Firstly 199 µl of brain heart infusion broth was added to each well of the microtiter plate. Then, for TiO_2 NPS solution 200 µl was mixed with brain heart infusion broth in the first well and then the twofold dilution was followed. After that, 1 µl of bacterial suspension (0.5 x 10^8 CFU/ml) was added to each well to achieve a concentration of (5x10^5 CFU/ml). Each plate was wrapped loosely para film to avoid dehydration. Each plate contains two controls: one with all solutions with the exception of the bacterial solution and the second with all additions except the tested

material. The plates were incubated at 37 °C for 18-24 h. The presence (turbidity) or absence (clarity) of growth was then assessed visually. The absence of growth was recorded as positive. The lowest concentration, at which growth was absent, was taken as the MIC value.

The UV-Vis absorption spectra of colloids were recorded by a spectrometer (type –Metertech SP- 8001). Field Emission scanning Electron Microscope (FESEM) produce by MIRA3 model-TE-SCAN, (Dey Petronic Co. Tehran, Iran), provides topographical and element information at magnifications up to 200,000 times. The crystalline structure was examined by X-ray diffractions using (Philips PW) X-ray diffractometer system. For recording the FTIR spectrum, the (Testscan Shimadzu FTIR 8000 series) are performed over range between (400-4000) cm^{-1} for suspension to confirm the formation of Titanium dioxide nanoparticles.

RESULTS AND DISCUSSION

X-ray diffraction (XRD) analysis

The XRD pattern in Figure 2 shows a rutile quartzite phase of TiO_2 NPs, there is a matching between the orientations of the Miller Indices (hkl) to each peak for TiO_2 NPs with the JCPDS standard card no. (#96-900-9084), these planes (110), (101), (111), and (211) are at the position of (2θ=27.5546◦, 36.2047◦, 41.3591◦, and 54.4899◦), (110) direction represent the strongly peak (i.e the maximum intensity in the direction (110)). Table-1 shows structural parameters viz. Inter-planar spacing, crystalline size for the TiO_2 NPs.

FT-IR analysis

Figure 3 shows the FTIR spectrum TiO_2 nanoparticles colloidal solution prepared using laser ablation of titanium dioxide target with Nd:YAG at energy 200 mJ in distilled water. The spectrum was recorded in the range (400-4000cm^{-1}). This shows the peaks at (3431.98cm^{-1}, 1639.38cm^{-1}, and 450cm^{-1}). The intense and wide peak centered at (3431.98 cm^{-1}) assigned to O-H stretching bond, and the peak at 1639.38cm^{-1} corresponds to the H-O-H bending mode bond. The band at around (450cm^{-1}) was attributed to the O-Ti-O vibration.

Absorption spectra of TiO_2 NPs

Figure 4 demonstrate the UV– Vis retention spectra in the range (200 - 1100 nm) of colloidal TiO_2 NPs in refined water incorporated by removal utilizing 600 heartbeat Nd:YAG laser with (1064) nm wavelength with various laser energies (500, 600, and 700) mJ individually. It can be seen from this assume all movies have high absorbance at little wavelength then the absorbance diminishes with the expanding of wavelength (have low qualities in the obvious and close infrared area), this conduct can be clarified as takes after. At high wavelength the episode photons don't have enough vitality to collaborate with iotas, the photon will transmit. At the point when the wavelength diminishes, the cooperation between episode photon and material will happen, and after that the absorbance will increase [15]. Expanding laser vitality caused an extensively high absorbance, as appeared in Figure, which alludes to expanding NPs fixation.

Field Emission Scanning Electron Microscope (FE-SEM)

The Field Emission Scanning Electron Microscopy (FE-SEM) pictures were gotten for the example with a specific end goal to think about the size and state of the created particles. Figure (5) demonstrate the surface morphology of the TiO_2 nanoparticles arranged by beat laser removal in refined water saw from these pictures that the normal size of TiO_2 NPs is around 36 nm, and have circular shape.

Minimum Inhibitory Concentration (MIC)

The table.2 and table.3 shows the inhibitory concentrations in titanium dioxide nanoparticles (TiO_2) with energy (100) mJ, and (2000) number of pulse in distilled water (DW) and alcohol. Minimum inhabitation concentration is (9.45 $mg.L^{-1}$) for E.coli and (18.91 $mg.L^{-1}$) for staph, while in alcohol the minimum inhabitation concentration is (4.72 $mg.L^{-1}$) for E.coli and (9.45$mg.L^{-1}$) for staph. Figure 6 shows the MICs of TiO_2 nanoparticles against bacterial strains *Escherichia coli*, and *Staphylococcus aureus* in distilled water (DW) and alcohol.

Mechanism of Antibacterial Activity

The mechanism of inhibitory activity of titanium nanoparticles initiated by laser removal on microorganisms, however not unmistakably comprehended, could be by their bond to the cell layer and further infiltration inside or by cooperation with phosphorus containing mixes like DNA exasperating the replication procedure or ideally by their assault on the respiratory chain. The value of this clarification depends much on the atomic association of these particles. The particles may impact on cell division by adjusting the cell condition however actuate harm through an immediate activity on the cell divider and plasma film, which end up weaker areas which associated that with isolating cell for both gram-positive and gram-negative microscopic organisms. Another examination proposes that when E. coli microscopic organisms were treated with profoundly responsive metal oxide nanoparticles. A bacterial layer with this morphology shows a critical increment in porousness, leaving the bacterial cells unequipped for legitimately controlling transport through the plasma film and, at long last, causing cell passing [16].

CONCLUSIONS

The present work demonstrates that titanium dioxide nanoparticles can be effortlessly created by laser removal of titanium dioxide metal in water. Variety of size and state of nanoparticles has been found to rely upon laser vitality. FE-SEM pictures demonstrated the Nano estimate for these metal NPs with round shape. FTIR spectra confirm the bond between O-Ti-O at around (450) cm^{-1}. Antibacterial action tests performed on different microorganisms unmistakably exhibited the adequacy of TiO_2 nanoparticles against bacterial development because of littler molecule size and high grouping of this example, it was discovered that the impact of nanoparticles arranged by liquor on bacterial development was more viable than those readied utilizing refined water.

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Table 1: Structural Parameters for TiO_2 NPs.

2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	D (nm)	hkl	d_{hkl} Std.(Å)	Card No.
27.5546	0.17500	3.2345	46.74	(110)	3.2470	96-900-9084
36.2047	0.21760	2.4791	38.41	(101)	2.4870	96-900-9084
41.3591	0.18100	2.1812	46.91	(111)	2.1880	96-900-9084
54.4899	0.20850	1.6826	42.85	(211)	1.6874	96-900-9084

Table 2: MIC results for (TiO_2) with (100) mJ and (2000) pulses for both staph and E.coli bacteria in distilled water.

Con. (mg.L^{-1})	75.64	37.82	18.91	9.45	4.72	2.36	1.18	0.59	0.29	0.14
E.coli	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Staph.	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 3: MIC results for (TiO_2) with (100) mJ and (2000) pulses for both staph and E.coli bacteria in alcohol.

Con. (mg.L^{-1})	75.64	37.82	18.91	9.45	4.72	2.36	1.18	0.59	0.29	0.14
E.coli	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
Staph.	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve

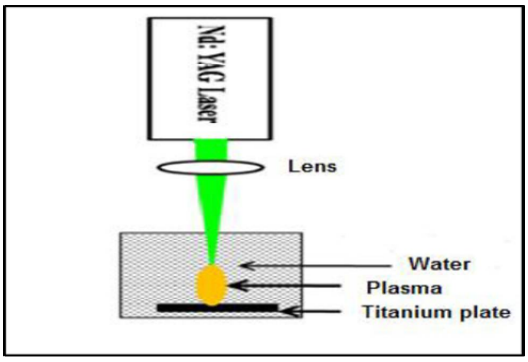

Figure.1: The schematic of the experimental setup

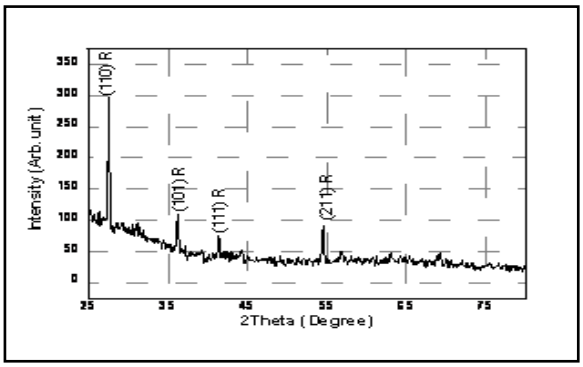

Figure 2: XRD pattern for TiO_2 NPs as function energy (500) mJ

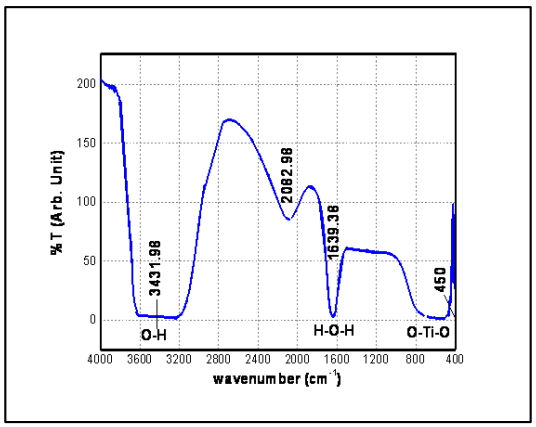

Figure 3: FTIR spectrum of Colloidal TiO_2 nanoparticles.

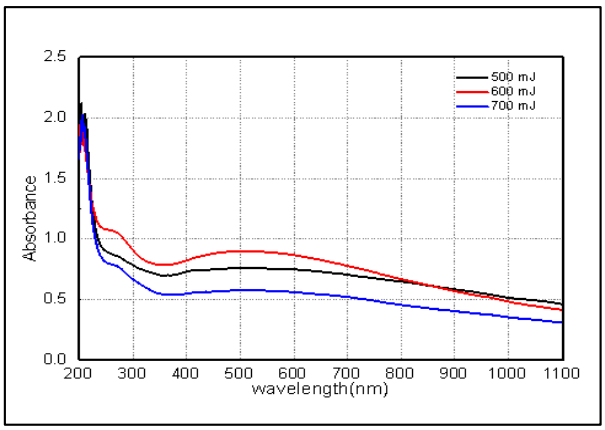

Figure 4: absorption spectra for (TiO_2) Nanoparticles

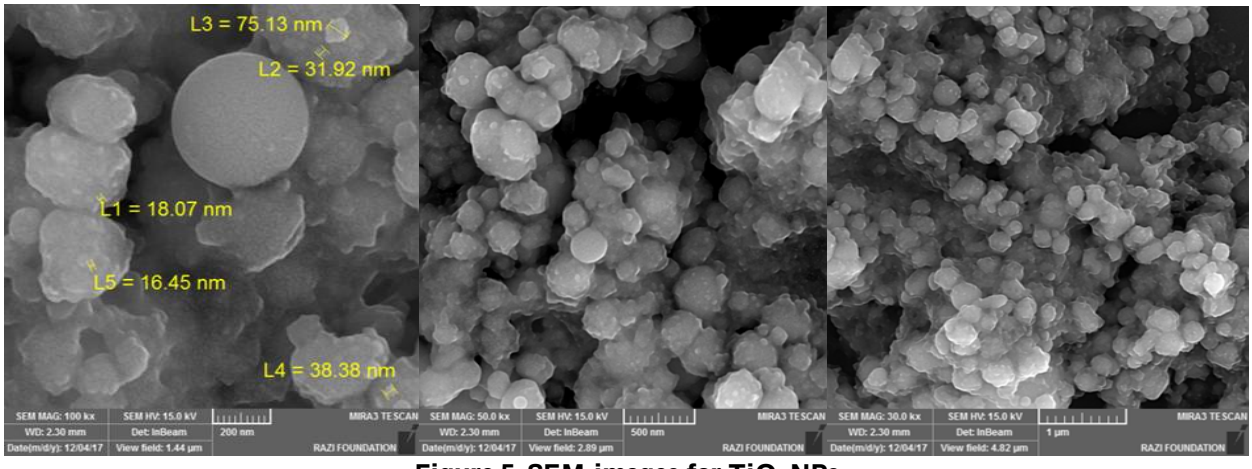

Figure 5: SEM images for TiO_2 NPs

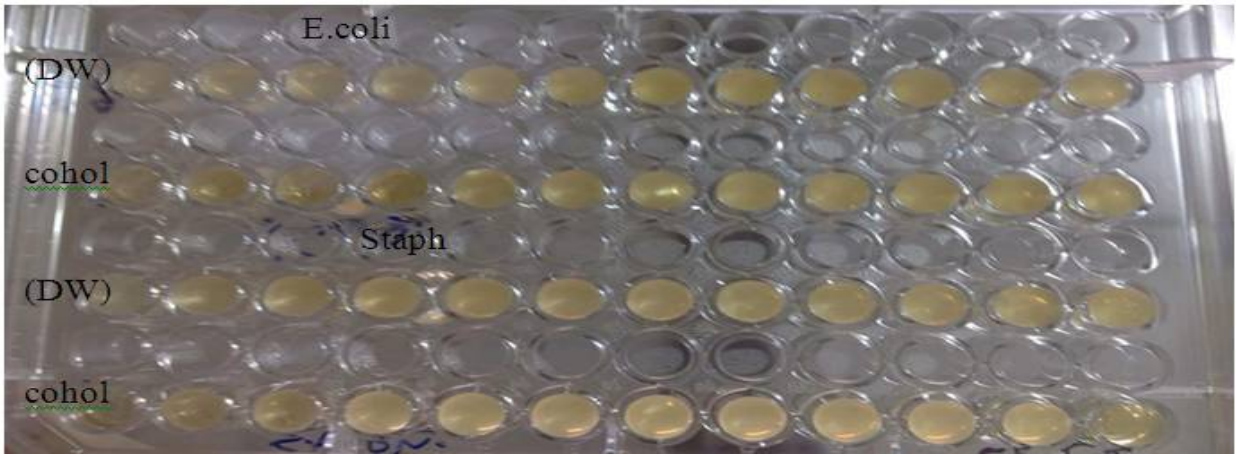

Figure 6: MICs of TiO_2 NPs against bacterial strains

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RESEARCH ARTICLE

Preparation and Characterization of Nanostructured Cadmium Oxide CdO Thin Film by Drop Casting

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ABSTRACT

In this work, the excellent properties were reported for nanostructure cadmium oxide CdO films. Thin film was deposited onto glass substrate by drop casting method. The nanostructure films were characterized by X-ray diffraction XRD, Atomic Force Microscope (AFM), Scanning Electron microscopy (SEM), UV-Vis spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). The XRD result showed that the deposited films have polycrystalline structure at 2θ= (32.9470, 38.2390, 55.1670, 65.9130 and 69.0030)^{o}. The morphological study by AFM showed that formation of uniform structure with average grain size of (101.02) nm. The band gap of the CdO has been calculated using UV visible spectroscopy and found to equal (2.5) eV. The FTIR spectroscopy the spectra give prominent and the distinct bonds.

Keyword: thin film, CdO Nanoparticles, Laser Ablation.

INTRODUCTION

Transparent conducting oxide (TCO) thin films are wide band gap conductive oxides (i.e.,>3.1 eV) which allows for applications in the visible and near ultraviolet spectral range, such as cadmium oxide (CdO), zinc oxide (ZnO), indium tin oxide (ITO) and nickel oxide (NiO) are widely used in semiconductor optoelectronic applications [1, 2, 3]. At the present time, transparent conductive oxides are broadly used as ohmic device in displays and electro-magnetic shielding or solar cells. Research on TCOs is still so active for the necessary understanding and optimization of known materials and the search for novel TCOs, counting materials with superior mobility for added economic solutions, indium-free materials or p-type materials. So semiconducting transparent oxides for diodes and transistors have acquired more attention the last decade due to flexible active electronics and applications in transparent. [4-6]. CdO is not a common TCO material because of its narrow optical band gap of (2.2-2.5) Ev, However, it has demonstrated unusually high electron mobility that is 5-10 times higher than commercially available TCOs [7]. Also, CdO is a n-type semiconductor with a crystal structure of a rock-salt crystal structure (fcc) such as NaCl [1]. Generally, these materials

indicate high optical transmission, high transparency in the visible region of the electromagnetic spectrum and low electrical resistivity. Recently, these materials have been intensively investigated as a potential candidate material for smart window, solar cells, conducting electrode, gas sensors, photodiode, IR detector, liquid crystal display and anti-reflection coatings [8-11]. Many methods have been used to synthesize CdO for different applications. Such methods include but not limited to magnetron sputtering ,ion beam sputtering , spray pyrolysis, thermal deposition, reactive pulsed laser deposition technique, SILAR, chemical bath deposition, metal organic chemical vapor deposition, drop casting etc[12]. In the present work undertaken, we use a widespread, simple and low cost method to fabricate CdO thin film namely drop casting [13,14]. Drop casting a simple process to prepared, low cost, no waste the material or solution but difficult to control the thickness [15].

MATERIALS AND MATHODS

Cadmium oxide used in this experiment as a Pellet with diameter 1cm and 0.2cm thick and it was powder then compressed under pressure 5T, it is deposited on the glass substrate, have width 2.5cm× length 2.5cm × thickness1.2 mm). The glasses were cleaned chemically and ultrasonically. By pulsed laser CdO was dissolved in ethanol, the laser Nd: YAG was used in this experiment with energy 600mJ and 1000 pulses shots. The preparation of the solutionis done by the Nd: YAG laser have a1064 nm and another parameters like the width of Pulse (10ns), frequency of laser (6 Hz), and A cycle of cold water. Thin films of CdO were fabricated using a drop casting technique at room temperature (300 K). The prepared films showed that the whole surface of the substrate is covered by a homogeneous layer of CdO film. In order to study the structural properties, the crystalline structure is analyzed with X-ray diffractometer (XRD, Shimadza-6000) using CuKα radiation. The morphology of the film was studied using atomic force microscope (AFM) (Digital Instruments, CSPM-AA3000), while the information about the sample's surface topography and chemical composition near the surface of the sample, high- resolution image-processing microscope (FE-SEM) (Hitachi (S-4160). The atomic bonds in CdO were analyzed with the Fourier transformed infrared spectroscope (FT-IR, Shimadzu IRAffinity-1). The optical transmittance of the films was measured using UV–vis spectrophotometer (Metertech) SP8001 in the spectral range 300–900 nm

RESULTS AND DISCUSSION

XRD Analysis

Figures (1) illustrate the XRD a spectrum of the film deposited by Drop casting at (300K) and is given data of crystalline information. it shows a scattering peaks in around 2θ of 32.9470°, 38.2390°, 55.1670 °, 65.9130 ° and 69.0030 ° referred to (111), (200), (220), (311) and (222) plane directions respectively [*3,*12,*16]. That indicate a well cubic Polycrystalline phase formation as compared with standard X-ray diffraction data file JCPDS (file No. 75-0594). While, the peaks observed at 2θ = 23.2110°, 30.2400° and 49.8340 ° corresponds, respectively, to the diffraction from (1 0 2), (1 0 4) and (108) crystal planes of the hexagonal polycrystalline phase of $CdCO_3$ due to presence of ethanol as confirmed from the ASTM card. The Scherrer relation, in X-ray diffraction is a formula that estimates the crystallite sizes.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where λ is the wavelength equal 1.54Å, K is a constant taken to be 0.9, θ is theangle between incident and reflected rays and β is full width half maximum (FWHM) of the preferential plane. The results of crystallite size of thin film obtained from XRD measurement shown in Table1

AFM Analysis

The morphological investigation of CdO thin film deposited on glass substrate at 300K was accomplished via using AFM. Figure (2) show a3-D image with granularity distribution of an AFM scan of the surface of nanostructure thin film. AFM results showed homogenous and having nodular structure CdO film. The average roughness, root mean square (RMS) and peak -peak for CdO estimated from AFM are equal (1nm, 1.18nm and 4.31nm) respectively. The granularity distribution chart of the CdO film is clear that the film has different GS (from 60 to 210 nm) and the average GS is approximately 101.02 nm.

SEM analysis

The three-dimensional surface morphological study of the CdO thin film using SEM image as shown in Figure (3). From the figure, it is clear that the sample is composed of the nanocrystal line grains along with some spongy clusters and uniform morphology Surface of the CdO with presence granular of average grain size (30.36) nm.

Optical studies

The optical properties of CdO film deposited on the glass substrate are determined from transmittance spectra recorded in the range of 300-1100 nm. Figure (4) displays the UV-Vis spectra of CdO thin film.The presence of absorption edge (496 nm) indicates that the film have a good degree of crystallinity. The maximum transmittance in the wavelength range of 900–1100 nm was found to be 60%. The band gap energy (Eg) of film was deduced from the intercept of the extrapolated linear part of the plot of $(\alpha h\upsilon)2$ versus the photon energy $h\upsilon$.Fig.5 show the plot of $(\alpha h\upsilon)$ 2 versus $h\upsilon$ for CdO thin film. It can see from this figure that the Eg equal 2.5eVand the transition is direct transition [*12].

FTIR analysis

FT-IR transmission spectrum of the CdO film prepared is shown in Figure (6). The bands at (435.88, 493.74, 709.43 and 727.11) cm-1are corresponding to Cd–O interaction. While , The peak at 1363.58 cm-1 is refer to wagging vibration of CdO. The peak assigned to C-O-H out of plane bending relation to carboxylic acide is showed at 912.65. the peak at 1066.56 cm-1 is refer to C-O stretching vibration band. The band at 1465.8 erfer to the C-H3 bending mode. The peak at 1623.96 cm-1 is associated with symmetric stretching mode of C=O and the peak at 2331.78cm-1 is refer to CO2 stretching vibration band. The peak at 2844.81and 2893.02 are associated with symmetric stretching of C-H and the last the peak at 3500.58 cm-1 to the formation of O-H in the adsorbed water molecule [9,10].

CONCLUSION

In this work, nanostructured CdO thin film was prepared via drop casting method. The formation of CdO was confirmed by XRD, AFM, SEM, UV-Vis and FTIR spectroscopy. XRD studies revealed that the CdO film deposited on glass substrate at 300K is cubic polycrystalline phase. AFM micrographs of the nanostructure film show that the homogenous of the thin film. SEM image showed that the CdO film is composed of the nanocrystalline grains along with some spongy clusters and average grain size (30.36) nm. The optical band gap of the CdO film equal 2.5 eV at the ultraviolet regain. Proper formation of the CdO film was established by FTIR measurement from during characteristic bond of it. The thin film was found appropriate to be used in solar cells applications.

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Table 1 Values of the grain size (GS) calculated from XRD

2θ (Deg.)	G.S (nm)	Phase	hkl	card No.
23.2110	19.0	Hexagonal $CdCO_3$	(102)	96-901-0219
30.2400	19.1	Hexagonal $CdCO_3$	(104)	96-901-0219
32.9470	25.8	Cubic CdO	(111)	96-900-8610
38.2390	37.8	Cubic CdO	(200)	96-900-8610
49.8340	18.8	Hexagonal $CdCO_3$	(108)	96-901-0219
55.1670	35.7	Cubic CdO	(220)	96-900-8610
65.9130	34.7	Cubic CdO	(311)	96-900-8610
69.0030	39.8	Cubic CdO	(222)	96-900-8610

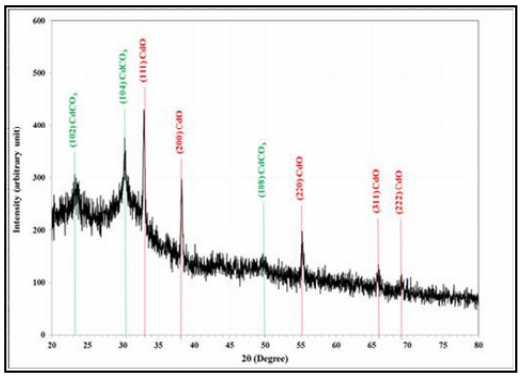

Figure 1. XRD pattern of CdO thin film.

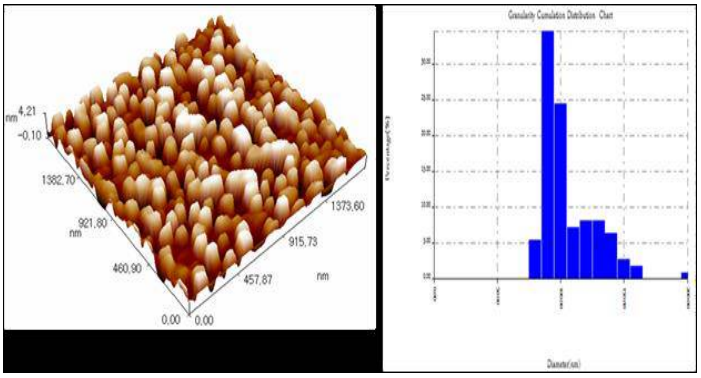

Figure 2. 3D image AFM and granularity distribution of nanostructured CdO film.

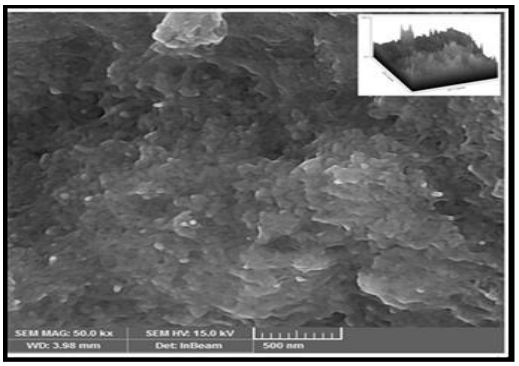

Figure 3. SEM image of CdO film, scale bar 500 nm.

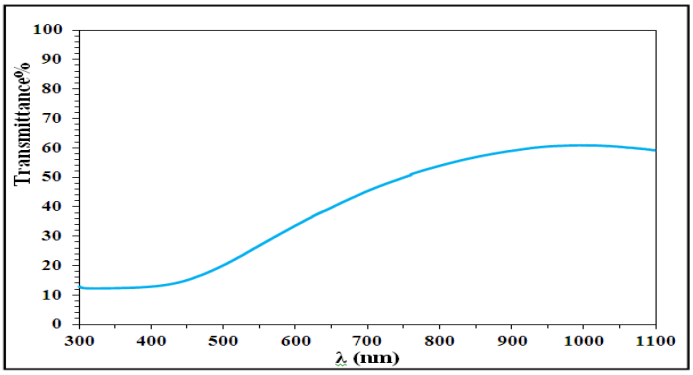

Figure 4. Transmittance spectra of the CdO thin film prepared at 300K.

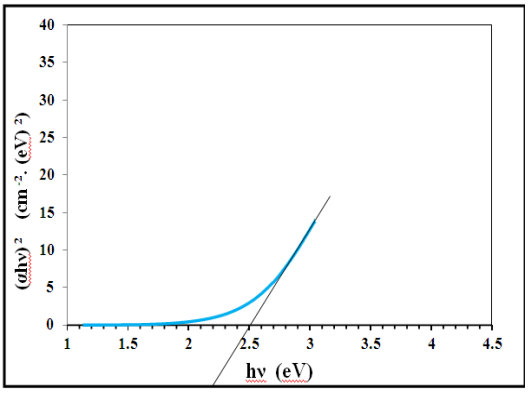

Figure 5. (αhν)2 versus (hν) plot. 300K

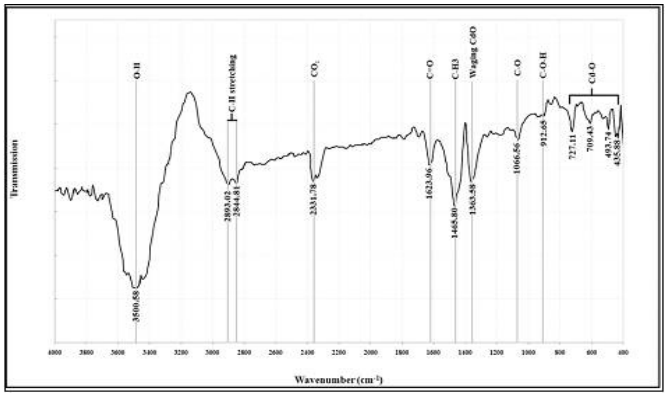

Figure 6. FT-IR spectrum of the CdO thin film synthesized at

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RESEARCH ARTICLE

Estimate Bacterial Contamination of Iraqi Paper Currency and Effectiveness of *Streptococcus* CFS and Some Hand Wash to Limit its Spread

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ABSTRACT

This study was conducted to identify the bacterial contamination in Iraqi currency notes and to evaluate the antibacterial activity to both of CFS of *Streptococcus* and some detergents (hand wash and hand Gel) during February to April 2014 .A total of 55 currency notes (1000, 5000, 10000, 25000) were obtained growth from workers, sellers, and students in Al-MustansiriyahUniversity .*Staphylococcusspp* .was the most frequently isolated, 29(55.7%). 14 (21.7%) of the 55 currency samples were identified as *Enterococcus spp.,* the tow isolate *Kocuria spp.* And *Streptococcus spp.* were had the same rate 5(8.5%) whereas *Listeria spp*. was the lowest rate in contamination 2(4.77%). by inhibition zone assay, the antibacterial activity of three agent were measured .The results of our study detected that very high antibacterial activity was obtained by *Streptococcus* CFS ranged (17-30) mm, Hand –wash was very good in its activity but not against all isolates and ranged (15-27) mm, the least activity ranged (11- 16) mm was given by Hand- Gel and also not against all isolates. So CFS is a natural active agents from lab especially *Streptococcus*, can be make as a disinfectant and alternative the chemical materials. The study suggested that Iraqi paper currency notes were highly contaminated with pathogenic bacteria and this may play a significant role in transmission of infectious disease. Hence CFS of Streptococcus must be takes as hand –wash while handling money during the preparation and handling of food to avoid cross contamination

Keyword: Paper currency, LAB *Streptococcus*, Hand wash

INTRODUCTION

Hand wash and hand gel are terms that are often used interchangeably in the general public and among health care workers (HCWs) (1).Globally, money is one of the items most frequently passed from hand to hand, during its passing, money can get contaminated and may thus plays a role in the transmission of microorganisms to other people (2).Money is used as medium for exchange for goods and services, settlement of debts and for deferred payments in economic activities, the contamination of notes could also be from several sources , it could be from the atmosphere, during storage ,usage ,handling or production (3). Lactic acid bacteria (LAB) are heterogeneous group of bacteria found widely in nature .LAB are also intentionally added to several probiotic products because of their potential health benefits (4).*Streptococcus* is considered to be one of the major LAB species and their antimicrobial activity had been reported in many studies(5).The aim of this study was to investigate the likelihood of bacterial contamination of Iraqi paper currency and estimate of effectiveness *Streptococcus* and some hand wash and gel against them and comparative between them, this is first of its kind study in Iraq.

MATERIALS AND METHODS

Collection of Samples

The study was conducted from February to April 2014. 108 Notes of different denominations paper currency notes of 1000, 5000, 10.000 and 25.000 IDs. were collected from various shops of fruit, vegetable selling and from workers in market place , students in universities and different peoples from Baghdad towns in Iraq, samples were randomly obtained by using large-denomination notes to smaller denominations by respective group. Each currency note was collected directly into a sterile plastic bag and transported to the laboratory of the department of science, AL-Mustansiriyah University, soon after collection and examination for bacterial contamination. The swab samples were dipped in 1% peptone water. The swab samples were carried to lab for further examination for microbiological analysis.

Isolation of Bacteria

Collected samples were processed by swab on nutrient agar, after incubated 24hrs. , colonies that were grown on nutrient agar were sub cultured on Blood agar, Mac Conkey agar and EMB agar for isolation of various bacteria. The plates were incubated for 24 hour at 37C° .Bacterial colonies that developed on the plates were then identified by colony morphology, microscopy of isolated bacteria and various biochemical tests (6), then sure from their identification by vitek2 system.

Source of Disinfectants

Two kinds of disinfectants were examined (hand sanitizer gel) and hand wash (liquid) higeen that commercially available in Baghdad markets. 2 hand gel as brand (ethanol 80%, iso-propanol 45% and mecetronium 0.2%) and hand liquid brand (ethanol 96%).

Streptococcus Isolate

Streptococcus was isolated from commercial dairy,0.1 ml of sample was added to 9ml of sterile 0.1% peptone water, All samples were serially diluted and 50ml of each dilution was spiral plated onto de man, Rogosa and sharp (MRS) agar .MRS plates were incubated at 37C°under anaerobic conditions for 24hr. (7) ,all gram positive ,catalase negative, isolates were purified and observed under a light microscope . All isolated were coded and stored in MRS.

Preparation of Cell- Free Supernatant from *Streptococcus* Isolate

Cell free supernatant (CFS) was prepared according to the method (8). *Streptococcus* was grown in MRS broth (pH 5.7) for 48 hrs. at 37C° in anaerobic condition . CFS was obtained by centrifuging the culture at 15000 rpm for 15 minutes at 4C° and then filtered through 0.45mm filters (Millipore, Bedford, and M.A).

Determination of Inhibitory Activity

Determination of inhibitory activity of CFS and two disinfectant hand liquid (HL) and hand gel (HG) against The present study revealed the extent and the level of contamination of Iraqi papers money with pathogenic bacteria.The cultures from the collected Iraqi paper money yielded 55 isolate representing selected 9 different types of bacterial species (with 5 genus) . Identification showed the active participation of these nine species in descending order of isolated bacteria was made by agar well diffusion assay, depending on this method, streaking of Muller Hinton agar medium by 0.1ml of bacterial inoculums. 4 wells were made by using cock poorer on each plates and filled with CFS, HL, HG, and control (media just), these plates incubated at 37C° for 18-24 hrs. The results were read by measuring the inhibition zone with mm (9).

RESULTS AND DISCUSSION

From the analysis of the 55 paper currency notes collected from different places of Baghdad town of Iraq , it was established that bacteria present on the notes ,see table (1). percentage as *Staphylococcus* (55%) , *Enterococcus* (21.7%) , *Kocuria* , *Streptococcus* (8.5%) and *listeria* (4.7%).The results indicated that both of the ID currency denomination (1000 and 5000) had microbial contamination and ID (10.000 and 25.000) had less contamination than other denomination.Theselower denominations paper money like (1000 and 5000) ID are used frequently for different normal daily activities. Higher denominations are not used as frequently as lower denomination, the second cause that smaller unit notes in size (1000 and 5000) ID appeared to be more highly contaminated than larger unit notes such as (10.000 and 25.000) ID, probably because of the smaller unit notes are most handled in petty, daily monetary transaction , these lower denominations money are often tattered and dirty .The study was in accordance with (10,11,12).The presence of pathogenic bacteria on the Iraqi currency samples is a case for great concern because paper money notes probably play a role in the transmission and spread of diseases because of each day people use money frequently for their daily activities , the study was also in accordance with similar pattern of microbial contamination as those obtained from the previous studies in Saudi Arabian, India ,Nigeria ,Sudan, Palestine,Ghana and Iran (13,14,15,16,17,18,19).

The presence of *Staphylococcus* species on paper money could have been due to rubbing off or may be surfing from a skin flake, pathogenic *Staphylococci*harbored either by an asymptomatic carriers or a person with a disease , can be spread by hands or expelled from the respiratory tract ,the Staphylococci are natural inhabitants of the animal body , which is the source of those found elsewhere .As Saprophytes, Staphylococciare ubiquitous , being found on normal skin and in the nose ,mouth and intestine as well as in the air, water ,milk and sewage and fomites, infections occur when Staphylococci enter the body through breaks ,cuts and abrasions in the skin(20,21,22).Also presence of *enterococcus* species on paper money could have been due to being the main causative agents for serious relevant infections like nosocomial ,urinary tract infections and bacteremia ,so enterococci have important impact to human health due to their natural presence among gut micro biota and conversely their deleterious role in spoilage process of fruit juices and meat products ,so it is too easy to transit from person to another and cause paper money contamination(23,24,25).In the same time ,we showed in our study that *Streptococcus*, *Listeria* and *Kocuria* had the lower percentage ,this was agree with other study that due to not washing hand after handling of money notes , allowing baby to handle money notes and using saliva during counting of paper money notes (26,27) because *Streptococcusthoraltensis* isolated from human oral cavity (28).The antibacterial activity of *Streptococcus* CFS and

(HL,HG) detergents showed the high response by *Enterococcus*spp .reached to 30mm to CFS effect ,While 24,22mm to HL,HG effect respectively (Table 2,figure 2). *Enterococci* have traditionally regarded as low grade pathogens (29).In spite of that CFS could inhibit all species of *Enterococcus* in our study but HG did not appear strong effectiveness,it could inhibit just 6 species with range 12-22 mm .HL was the lower agent in inhibition against *Enterococcus* sp. , out of 14 species 4 just inhibited by HL , the higher inhibitory was 24 mm against *E.faecium*. There are many resources about using liquid hand,Alcohol hand and hand gel (30,31,32) ,but there is no one about the study effectiveness the HG and HL against the pathogenic bacteria isolated from currency notes because this study it is the first .When we talking about the antibacterial activity of CFS, also we found many research confirm (33,34,35) .Table 3 and figure 3 showed the antibacterial activity of CFS, HL and HG against *Staphylococcus* ,CFS was the best agent to inhibit growth bacteria, HL stayed the second agent for inhibition while HG failed to inhibit growth of species except 2 species ranged 11-15 mm back to *S.lentus.*

In spite of Staphylococci considered one of the most prevalent causes of gastroenteritis worldwide (36), CFS of *Streptococcus* could inhibit all species of its , All L.A.B have the antibacterial activity (37) because of there are known probiotic potential and acid resistance and bile salt tolerance,in addition to that LAB produce various compounds such as organic acids, diacetyl, hydrogen peroxide ,and bacteriocins or bactericidal proteins (38,39,40). also many sources indicated the role of LAB in vitro ,they remember that LAB are capable of colonizing specific parts of the body ,e.g. the oral cavity and the gastrointestinal and uro-genital tract ,where they play on important role in the competitive exclusion of pathogen (41).Also, the antimicrobial activity of (LAB). Against bacterial pathogens emerges to be multi-factorial and to include the production of unknown heat-stable,non-lactic acid molecules (42,43,44).Table 4 and figure 4 appeared the inhibitory activity for 3 agent against the *Kocuria ,listeria ,Streptococcus* , CFS stilled the first agent in its high activity ranged between 20-25 mm but HL recorded activity ranged between 16-18mm while HG did not success in its viability ,it could not inhibit *Kocuria* or *Listeria* ,16mm just was the result of the inhibition of *Streptococcus* by HG .

There are many research about the anti-*listeria*, and *Streptococcus* activity of CFS, whatever the source of isolation (45,46,47) and in spite of the pathogenicity*Kocuria* in addition to *Listeria* and *Streptococcus*(48,49,50,51,52,53,54).CFS in our study could inhibit all of these pathogens that isolated from currency notes may that due to many mechanisms proposed for its activity is competition for nutrients ,adhesion in inhibition of pathogens to surface (55,56,57).The contamination of paper currency is spread in the world and it becomes carrier of pathogens (58,59),and there are another method to carry the pathogens like mobile , computer (60) and many detergents like soap ,liquid hand wash did not could to kill these pathogens isolated (61,62,63,64),this is agree with our current study ,So CFS is alone agent could do that and with highly action because of CFS have all active natural agent as remembered above (65,66).

CONCLUSION

Paper can serve as a vehicle for cross contamination of bacterial pathogens in different people if current recommendations on hand hygiene arenot meticulously followed, Presence of CFS produce by *Streptococcus* with potential the expression of pathogenic bacteria was reduced, hand gel and hand wash were less activity against the pathogenic bacteria.

Recommendations

Extraction and purification the natural active agent like CFS from LAB especially *Streptococcus* (study bacteria) and mix with detergents that carried by people like gel or liquid wash to prevent the contamination occurred by touching paper currency, phone, laptop or any other thing.

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Table 1. Percentage occurrence of bacteria per denomination of Iraqi paper currency.

	Listeria	Enterococus			Staphylococcus		Streptococcus	Kocuria
ID	ivanovii	E.faecium	E.faecalis	E.casseli flavus	S.lentus	S.xylosus	thoraltensis	kristinae
1000	1	4	3	1	9	5	3	2
5000	1	1	2	1	7	2	1	1
10.000	-	-	1	-	1	3	-	2
25.000	-	-	-	1	1	1	1	-
Total = 55D	**2**	**14**			**29**		**5**	5
Total = 99.99%	**4.77%**	**21.7%**			**55.7%**		**8.5%**	8.5%

Table 2.Antibacterial activity of *Streptococcus* CFS and HL, HG against *Enterococcus* spp.

rococcusEnte	CFS	HG	HL
(1) E.faecalis	23	_	_
(2)E.faecalis	27	_	_
(3)E.faecalis	20	_	20
(4)E.faecalis	22	12	_
(5)E.faecalis	29	19	_
(6) E.faecium	30	_	_
(7) E.faecium	25	17	24
(8)faecium E.	20	_	_
(9) faecium E.	23	-	_
(10)casseliflavus E .	17	21	_
(11)casseliflavus E .	20	22	18
(12)casseliflavus E .	18	_	_
(13)casseliflavus E .	20	_	_
(14)casseliflavus E .	18	17	12

Table 3. Antibacterial activity of CFS,HG and HL against *Staphylococcus* sp.

Staphylococcus	CFS	HL	HG
(1)lentus.S	26	26	_
(2)slentu.S	25	21	_
(3)lentus.S	20	_	_
(4)lentus.S	18	_	_
(5)lentus.S	18	_	_
(6)lentus.S	20	_	15
(7)lentus.S	26	18	_

(8)lentus.S	25	18	_
(9)lentus.S	18	25	_
(10)lentus.S	19	18	_
(11)lentus.S	22	18	_
(12)lentus.S	21	19	_
(13)lentus.S	13	17	_
(14)lentus.S	27	15	_
(15)lentus.S	20	20	_
(16)lentus.S	21	22	_
(17)lentus.S	25	21	_
(18)lentus.S	22	18	_
(19)lentus.S	28	19	_
(20)xylosus.S	19	20	-
(21)xylosus.S	23	19	11
(22)xylosus.S	18	20	_
(23)xylosus.S	22	17	_
(24)xylosus.S	20	16	_
(25)xylosus.S	27	25	_
(26)xylosus.S	23	18	_
(27)xylosus.S	26	20	_
(28)xylosus.S	26	17	_
(29)xylosus.S	29	16	_

Table 4.Antibacterial activity of CFS, HG and HL against *Kocuria* sp. *Streptococcus sp.* and *Listeria ivanovii*

Isolate	CFS	HL	HG
(1)Kocuria	20	20	_
(2)Kocuria	22	20	_
(3)Kocuria	20	18	_
(4)Kocuria	25	15	_
(5)Kocuria	24	20	_
Streptococcus	CFS	HL	HG
(1)*thoraltensis*	25	19	16
(2)*thoraltensis*	23	21	_
(3)*thoraltensis*	21	20	_
(4)*thoraltensis*	20	20	_
(5)*thoraltensis*	20	20	_
Listeria	CFS	HL	HG
(1)ivanovii	20	13	_
(2)ivanovii	24	17	_

( ): Number of species, CFS: Cell free supernatant, HL: Hand liquid HG: Hand gel.

(A) HL **(B) HG**

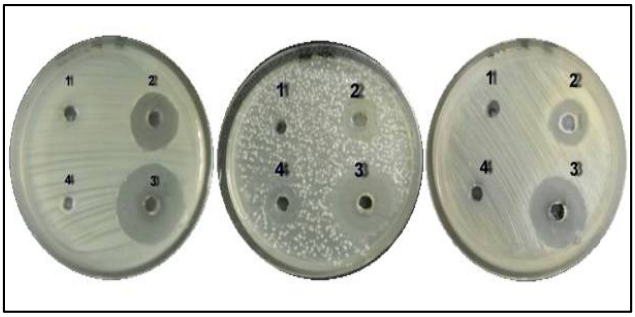
Figure 1. The used disinfectant in the study

A: *E. faecalis*(3) **B: *E. casseliflavus***

Figure 2.Antibacterial activity against *Enterococcus* spp.

A: *S. lentus* (2) **B: *S. xylosus* (21)**

Figure 3: Antibacterial activity against *Staphylococcus* spp.



S. thoraltensis(1) ***Kocuria* (2) *L. ivanovii (2)***

Figure 4. Antibacterial activity against *S. thoraltensis, Kocuria, L. ivanovii.*

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RESEARCH ARTICLE

Abomasal Nematodes in One-Humped Camel (*Camelus dromedarius*) in Al-Najaf Abattoir – Iraq

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ABSTRACT

Two hundred seventy abomasal samples were collected from slaughtered camels in Al-Najaf abattoir for abomasal nematodes investigation, from both sexes (230 male and 40 female) during the periodfromJan 2017 to the end of Oct 2017. Direct macroscopic and microscopic examination weredone for abomasal content to record the total infection rate 28.15% (76/270) with abomasal nematodes.Four species of nematodes were detected by the morphological examination under light microscope: *Haemonchus spp., Parabronemaskrjabini, Camelostrongylusmentulatus* and *Physocephaluscristatusand,* 11.11%, 25.56% , 9.63% and 0.74% respectively. The study showed significant different (P<0.05) between females and males infection rate, 37.5%, 26.52% respectively, and the study recorded highest infection rate in March 53.33% (16/30), while the lowest in July 13.33% (4/30), with significantly different (P<0.05).

Keyword: Camels, Abomasal, Nematodes, Infection, Iraq.

INTRODUCTION

Camels or “The ship of the desert” is an important animal in livestock in many countries of the world, for thousands of years, used by man as a multipurpose animal, in transportation methods as well as, considered as a source of meat, milk and wool production, they play significant roles in great economic and social development for people in numerous countries like in the desert of Arabia and Asia. (Richard, 1979; Solanki, *et.al.,* 2013). Due to its anatomically and behavioral peculiarity, it considers the most suitable domestic mammal which adapted for use in climatic extremes due to its physiological attributes intolerance of heat climate condition, which characterized by poor nutrition and occurs of pathogenic diseases. (Borji, *et.al.* 2010; Solanki, *et.al.* 2013).

Camels generally show strength and pliancy and generally can mask illnesses symptoms (parasitic, infectious and physical injuries), from other hands, the diagnosis of parasitic infection symptoms can be easy to recognize (Mohammed, *et.al.,* 2007) Parasitism is of the problems that effect on animals normal physiology mainly reduction in food consumption, decrease productivity and diarrhea.Although, helminthiasis has mostly occurred without clinical manifestation or asymptomatic in which animals seem normal but they performing at less their full potential. (Anvari, *et.al*., 2013). Few studies focused on gastrointestinal nematodes in camels due to its geographical distribution and samples collection difficulties due to the fact that majority rise by the Bedouin (Banaja and Ghandour 1994; Radfar and Gowhari,2013) and because of the lack of information about nematodes infection in camels in Iraq, this study was conducted.

MATERIALS AND METHODS

Abomasal fresh samples were collected directly from the animals' carcasses after slaughtering in Al-Najaf AL-Ashraf abattoir in the middle of Iraq, a total 270 abomasal samples from both sexes from the beginning of January 2017 till the end of September 2017. Abomasum was cut longitudinally by scissor and mucosal surface directly examined by a macroscopicalexamination for any adherence nematodes. The contents of the abomasum washed with tap water and sieved and examined macroscopically to find the parasites. The isolated adult worms were gleaned by forceps carefully to prevent its damage, washed with distilled water, and examined under the light microscope in a fresh state. They have then preserved 70% alcohol for further examination and identification. Few drops of Lactophenol were used for clarification of isolated nematodes. (Anvari, *et.al*., 2013)

RESULTS

Out of 270 dromedaries tested during this study, 76 were positive for nematode infection 28.15%(76/270) (table 1), and the study recorded several species of nematodes: *Haemonchus spp .Parabronemaskrjabini Camelot strongylus mentulatus* and *Physocephaluscristatus*(Fig:1,2,3,4,5).Species identification wasmade according to the diagnostic features described by Soulsby (1982). Females showed highest infection rate comparing to the males (37.5 %), (26.52%) respectively (table2). On other hand, months of the study revealed a significant difference (P<0.05), March recorded higher parasitized ration (53.33%) while July listed only (13.33%) (table3). From these 4 parasites: P. *skrjabini* recorded the higher infection rate followed by *Haemonchus spp., C. mentulatus*, and *Ph. Cristatus* with infection rates (25.56%),(11.11%),(9.63%)and (0.74%) respectively. (table4).

DISCUSSION

This study carried out on abattoir survey for 9 months in al-Najaf Al-Ashraf city–Iraq by direct examination to dromedary abomasum for nematodes as well as microscopic identification. There are a few epizootological studies carried out on adult gastrointestinal helminths in camels worldwide because of difficulties of samples collection , the first study in Iraqi camels infection was conducted by (Altaif,1974) and most of the researchers worldwide have investigated for eggs of these nematodes by dung examination.(Chhabra& Gupta, 2006) According to our results 76 ou of 270 were harboring abomasal nematodes during the study period with (28.15%) and this result un matched with result of : Shurrif et al.,(1998); Omer et al.,(2007) in Sudan ;Mohammed *et al.,(*2008) in Egypt and Al-khhatibe*et. al*.,(2012) in Kingdom of SaudiArabia, Radfar and Gowharin (2013) in Iran and Al-megrin (2015) in KSA they recorded the same identified parasites and other species with total infection rate 98 %, 71.4 %, 78.8 %, 50.55 %, 64% and 59.4% respectively, this variation may be related due to the fact that our study just focused on the Abomasal nematodes only as well as the age of the slaughtered animal and the methods of examination.Temperate month (March) recorded higher infection rate 16/30 while high temperature month (July) and this may related directly to the environmental conditions that effect on the pre-parasitic stages are found on pasture and the infective stage is reached in less than a week under favorable conditions. (EL-hassan*et. al.,* 2011) and theincreasing in the number of

flies and their larvae as they serve as vector for *Parabronema skrjabini* and this explain the high infection rate by this parasite during March. Four nematode species were recorded in the present study: *Haemonchus spp. Parabronemaskrjabini, Camelostrongylusmentulatus* and *Physocephaluscristatus* and match with the data that collected by (Borjiet *al*., 2010; EL-hassan*et. al.,* 2011; Taft*iet al.,*2013 and Alfatlawi, 2016). in the surrounding countries. Females showed highest infection rate in compare to males this may be related to the number of females that slaughtered during the period of study because females used for reproduction purposes and male mostly used for meat consumption these results go with (Swai*et al,* 2011) in Tanzania that recorded highest infection rate in females and differs from (Al-Megrin 2015) who referred that male infection was more than females to (122 males and 118 females) the difference may be related to the numbers of examined animals .

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Table 1: prevalence of nematodes in camels

Abomasal samples	No. of exam	No of positive	%
Total	270	76	28.15%

Table 2: prevalence of nematodes in camels according to sex

Sex	No.	+	%
Male	230	61	26.52%
Female	40	15	37.50%
Chi square Value	7.90		
P	0.004		

Table 3: prevalence of nematodes in camels according to months

Month	No. of exam	No of positive	No of negative	%
Jan	30	12	12	40.00%
Feb	30	10	20	33.33%
Mar	30	16	12	53.33%
Apr	30	11	19	36.67%
May	30	6	24	20.00%
Jun	30	6	24	20.00%
Jul	30	4	26	13.33%
Aug	30	6	23	20.00%
Sep	30	5	25	16.67%
Chi square Value	21.13			
P	0.006			

Table 4: Prevalence of total isolated nematodes species rate

Month	No.	*H. spp*	*P. skrjabini*	*C. mentulatus*	*Ph. Cristatus*
Jan	30	4	12	5	1
Feb	30	0	10	6	0
Mar	30	2	14	2	0
Apr	30	6	10	4	0
May	30	3	6	2	0
Jun	30	2	4	1	0
Jul	30	3	4	2	0
Aug	30	5	5	1	1
Sep	30	5	4	3	0
Chi square value	41.42				
P	<0.0001				

Fig (1)*Haemonchus spp .female.*

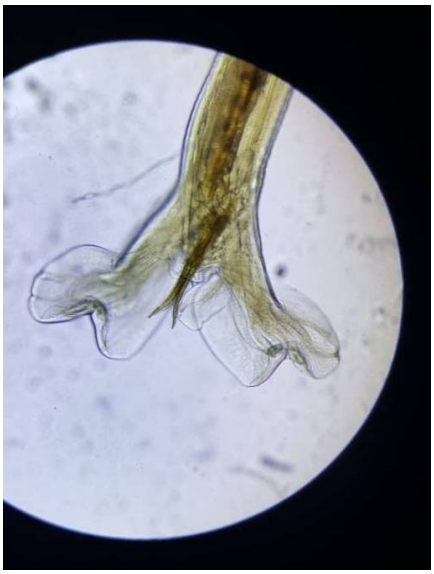

Fig (2)*Haemonchusspp.male*

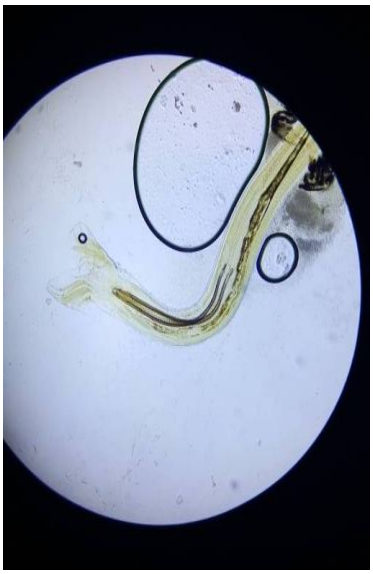

Fig3)*Camelo strongylus mentulatus*

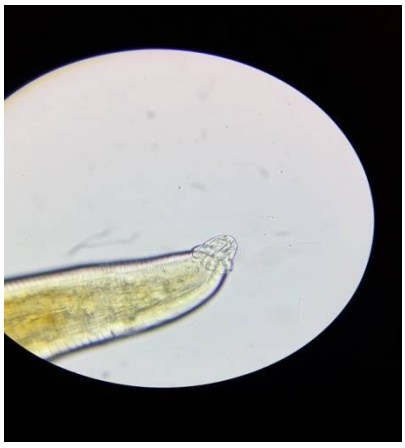

Fig (4) *Parabronemaskrjabini*

Fig (5) *Physocephalus cristatus*

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RESEARCH ARTICLE

Effect of Glass Fibers for Acrylic Elastomeric Water Proof Coating

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ABSTRACT

Chopped Glass fibers reinforced acrylic elastomeric composites water proofing compounds and are widely used for various applications .Glass fibers are excellent properties like high strength, stiffness, flexibility, and anti-fracture, insulating material, and resistance to chemical material. All the films exhibited antibacterial activity against E-coli. To prepare elastomeric Acrylic polymer and acrylic with glass fibers the volume fraction (v_0,v_1,v_2,v_3,v_4) (0, 2.34, 4.69,7.10, and 9.43)% , were added chopped glass fibers to acrylic and result solution was stirred by hand for 5 minutes, use the Hand-lay-up technique

Key word: glass fibers- acrylic elastomeric – mechanical properties – color stability - Anti Bacterial activity.

INTRODUCTION

Rubber Began To Be Used As Containers, Growth In Technical Developments And Applications In The 19th Century, Flexible Tubing, Elastic Bands And Waterproofing, Other Technological Advances Included Improved Compounding Techniques Which Enabled The Use Of Anti-Oxidants And Accelerators, And The Incorporation Of Carbon Black To Improve Strength. This Led to a Vast Increase in the Number of Applications, Which Included Seals, Belts, Flooring, Electrical Insulators, springs [1]. Composite Materials Are Considered As One Of The Most Potential Candidates For Aerospace Applications Owing To Their High Strengthto-Weight Ratio And Excellent Fatigue Resistance [2] . The Reinforcement Of Fiber Upon Polymeric Matrix Is Found To Bring About Significant Advancements In Mechanical Behaviors Of Polymeric Host With Added Advantages Of Light Weight, High Strength To Weight Ratio, Excellent Weathering Stabilities And Enhanced Dimensional Stabilities , In Addition To Low Maintenance Cost And Tailor Made Material Behaviors [2]. Glass Fibers Reinforced Polymers (GFRP) Is A Category Of Plastic Composite That Specifically Uses Glass Fiber Materials To Mechanically Improve The Strength And Stiffness Of Plastics, The Resin Provides Additional Protection To The Fiber Due To The Bounding Between Materials [3]. Tear Strength Is A Measure Of The Resistance Of An Elastomer To Tearing. Rubber Hardness Is An Indication Of

Its Rigidity Against Moderate Stress, As Those That Often Has To Bear In Service. The Most Popular Method Of Rubbers Hardness Is The Shore A In Which Is Performed The Measurement With An Instrument Called A Durometer, Based On The Penetration Of A Frusto-Conical Tip Against A Calibrated Metal Spring Reaction [4]. Most Acrylic Elastomeric Roof Coatings Are White Or Near-White In Color. The Whiteness Of The Coating Provides Two Very Important Features. First It Reduces The Temperature Of The Roof Surface And More Importantly To The Membrane To Which It Is Applied [5]. In 1976, The CIE (Commission Internationale De L´Eclairage) Introduced The CIE Color Space ("CIELAB" Or CIE 1976 L*A*B*). The CIELAB Color Space Is Based On Three Dimensional Coordinates With Numerical Values. L* Stands For The Lightness (Brightness) Of The Color, Where Absolute Black Is Given The Numerical Value 0 And White Is 100. The Chromaticity Coordinate A* Describes Redness Vs. Greenness: A High + A* Means Redness While A High - A* Means Greenness. The Chromaticity Coordinate B* Describes Yellowness Vs. Blueness: A High + B Means Yellowness, A High –B* Means Blueness [6]. The membranes for impact resistance. The method utilizes for different mass (gm) of steel balls dropped at various heights onto a roofing system test target. Water Vapor Transmission Rate (WVTR) Is Defined As The Steady Water Vapor Flow In Unit Of Time Through Unit Of Area Of A Body, Normal To Specific Parallel Surfaces, Under Specific Conditions Of Temperature And Humidity At Each Surface. The WVTR Was Calculated From The Steady-State Region Of The Water Losses Time Curves [7].

MATERIALS AND METHODS

Elastomeric material (LAMA Acrylic polymers) white elastomeric roof coating are liquid , tough, hard , and flexible Glass fiber (E-Class) of Surfacing mats composed of continuous glass filaments made of Chennai, india.

Physical Properties

Tear resistance, referencing ASTM D 624, a standard test to determine the tear properties of a cured material test sample to which a specific "cut" has been made to initiate the "tear". Tear is a good descriptor of how well the material might wear under physical demands. Again, because of the low crosslinking, cured gels exhibit very low tear strength [8]. Critical tearing energy (N/m) calculated the equation T c = 2F/ t [9] the F = tearing force and t = thickness.

Hardness test

The hardness of the cured material tested as penetration, Type "A". Penetration, the softest durometer, is tested on a penetrometer. The penetrometer allows a defined foot, or probe, to push into the cured sample at a defined force producing a measurement. Referencing ASTM D 2240, Type "A" durometer is measured by curing a sample at least 0.25" thick and placing it on a test stand with Type "A" indenter. The indenter is forced down into the material at a constant force and a measurement is obtained.

Impact resistance

These test methods cover the determination of the energy that causes plastic film to fail under specified conditions of impact of a free-falling dart. This energy is expressed in terms of the weight (mass) of the missile falling from a specified height which would result in 50 % failure of specimens tested [10]

Brightness and Reflectivity

Most acrylic elastomeric roof coatings are white or near-white in color. The whiteness of the coating provides two very important features. First it reduces the temperature of the roof surface and more importantly to the membrane to which it is applied. This reduced temperature coupled with the UV blocking properties of the coating reduces the rate of degradation and deterioration of the underlying roofing membrane. Second, the white color reflects as much as 95 percent of the heat portion of the sunlight, reducing the heat transferred into the building and thus reducing the air conditioning costs for that building. For most industrial applications, white acrylic elastomeric roof coatings are the perfect choice because most of these buildings have horizontal, or flat, roofs. The high brightness and reflectivity of the coatings is ideal for reflecting the sun's energy back into outer space[5].

Protable Colorimeter

The CIELAB color space is based on three dimensional coordinates with numerical values. L* stands for the lightness (brightness) of the color, where absolute black is given the numerical value 0 and white is 100. The chromaticity coordinate a* describes redness vs. greenness: a high + a* means redness while a high - a* means greenness. The chromaticity coordinate b* describes yellowness vs. blueness: a high + b means yellowness, a high –b* means

Preparation elastomeric and elastomeric with glass fibers composites

To prepare elastomeric Acrylic polymer and acrylic with glass fibers. To prepare the composites fibers with volume fraction (v_0,v_1,v_2,v_3,v_4) (0, 2.34, 4.69,7.10, and 9.43)% , were added chopped glass fibers to acrylic and result solution was stirred by hand for 5 minutes. Hand-lay-up technique was used to cast the samples in the mold plastic. The mixture was left 24 hours to dry. The cutted of samples according the ASTM of physical test.

RESULTS AND DISCUSSION

Tear resistance

Tear strength is defined as the resistance force which a rubber sample, modified by cutting or slitting, offers to the propagation of the tear. A multitude of test specimen configurations have been presented for tear test. The values for the tear strength of elastomeric materials with good tear properties are in the range 50-100 kN/m, and values over 100 kN/m are excellent. Table (1) and Figure (3) tear strength increase with increasing volume fraction of short fibers because the excellent strength of glass fibers [11].

Hardness test

Figure (4) the hardness values increase with increasing glass fibers because the strength of fibers and the bonding (interaction) between the matrix and fibers. The reinforcing fibers of advanced polymer composites are responsible for their high strength and stiffness. However, these can be fulfilled only if sufficient stress transfer from fiber to matrix and vice versa can take place by a proper bonding between the two constituents. This means that physical and to some extent chemical compatibility is required between fiber and matrix. Therefore, the structure and properties of the fiber–matrix interface play a major role in the mechanical and physical properties of composite materials [12].

Impact test

It is the ability of the material to resist the fracture under stress applied at high speed. The specimens are deformed within a short time and therefore exposed to high strain rates. Impact strength increase with increasing volume fraction of glass fibers due to the fibers improved the strength of matrix and good bonding between fibers and matrix figure (5) load values applied the samples when the rupture occurs of samples.

Water absorption

Water absorption is used to determine the amount of water absorbed under specified conditions. Factors affecting water absorption include: type of plastic, additives used, temperature and time Figure (6) water absorption as a function of times when the samples immersed in the water and for a period of time it was observed that the samples absorbed a small quantity of water and increased the amount of absorption with increased fiber ratio and the pure sample got her swollen due to water figure (7). When exposed to water samples of 50 °c , it was observed that the samples were removed from the absorbed water and returned to the first weight and shapes before immersion in water .

Brightness and Protable Colorimeter (color stability)

Most acrylic elastomeric roof coatings are white or near-white in color. The whiteness of the coating provides two very important features. First it reduces the temperature of the roof surface and more importantly to the membrane to which it is applied. This reduced temperature coupled with the UV blocking properties of the coating reduces the rate of degradation and deterioration of the underlying roofing membrane. Second, the white color reflects as much as 95 percent of the heat portion of the sunlight, reducing the heat transferred into the building and thus reducing the air conditioning costs for that building. When added glass fibers increase a little the lightness values (L) (140-142.89) and brightness (229.68-231.24) due to the color stability for composites materials .while a parameter reduced that mean The a parameter describes red/green chroma: a high positive numerical value of a means an intense red chroma and, respectively; a high negative value of a means an intense green chroma. Correspondingly, the b parameter stands for yellow/blue chroma: a high positive b represents yellow chroma and a high negative numerical value represents blue chroma [13]. Shows in table (2)

Anti Bacterial activity

The antibacterial activity of the films shown in table (3) and figure (8) The result showed that the elastomeric films and composites have activity a gainst E-coli. Lama acrylic polymer resist the bacterial type E-coil[14] due to the films good coating water proofing.

CONCLUSION

Excellent waterproofing capability , high strength and stiffness , resist the fracture under stress applied at high speed Elastomeric with excellent dirt pickup resistance which provide long lasting bright color , easy to clean , and resistance to bacterial .

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Table (1) tearing force and tear strength

Samples	Thickness (mm)	Tearing force (mN)	Tear strength(KN/m)
V0	0.8	43306.2	54.132
V1	1	56709	56.709
V2	1.02	65498	64.213
V3	1.01	69810	69.118
V4	1.02	74110	72.656

Table (2) color properties of acrylic elastomeric and composites

Samples	L	a	b	Brightness %
V0	140	-26.21	16.88	229.68
V1	140.82	-27.00	18.21	230.64
V2	141.1	27.23	18.43	230.87
V3	142.23	-27.89	19.13	231
V4	142.89	-27.9	19.00	231.24

Table (3) Antibacterial activity as the inhibition zone diameter (mm) of elastomeric film and composites

Samples	Inhibition zone (mm) E.coli
V0	8
V1	12
V2	15
V3	16
V4	16

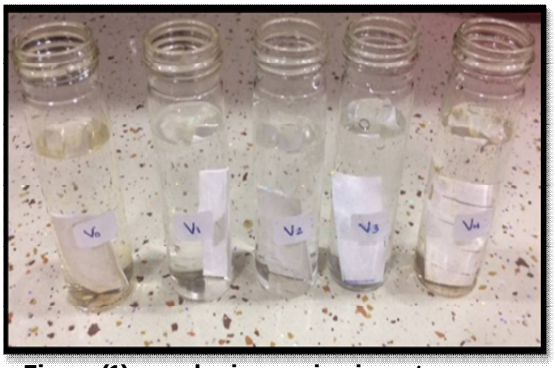

Figure (1) samples immersion in water

Figure (2) tear test and impact test for Elastomeric films Samples

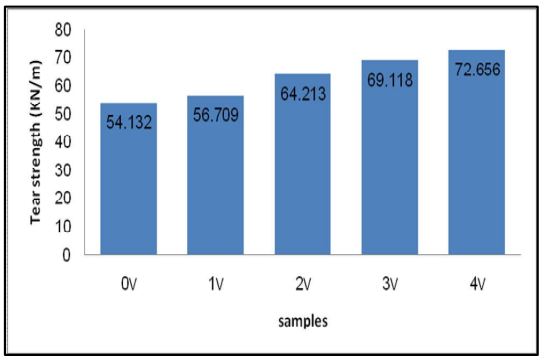

Figure (3) Tear strength as a function of samples

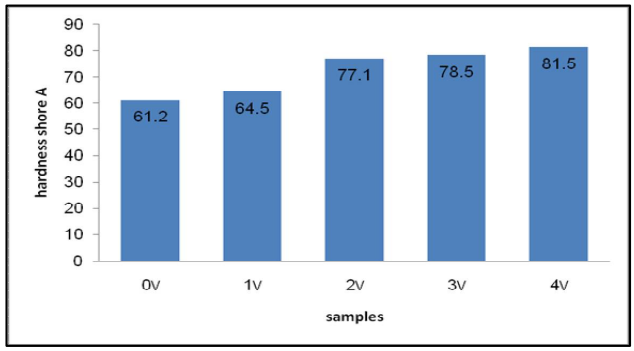

Figure (4) hardness values as a function of samples

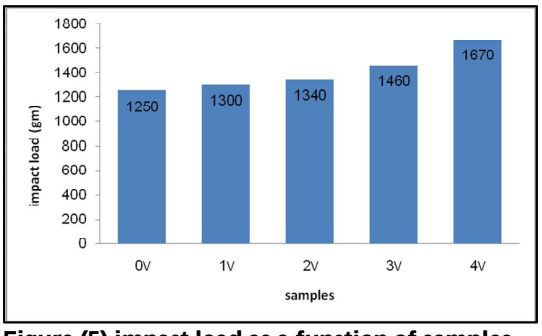

Figure (5) impact load as a function of samples

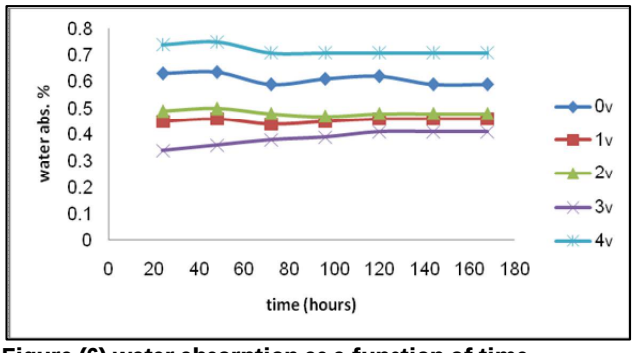

Figure (6) water absorption as a function of time

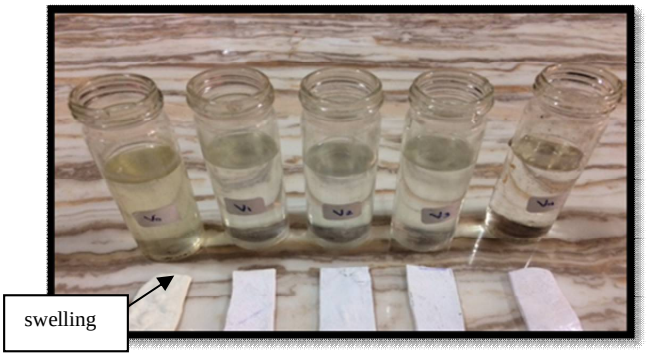

Figure (7a) samples after immersion water

Figure (7b) samples after drying 50 °C

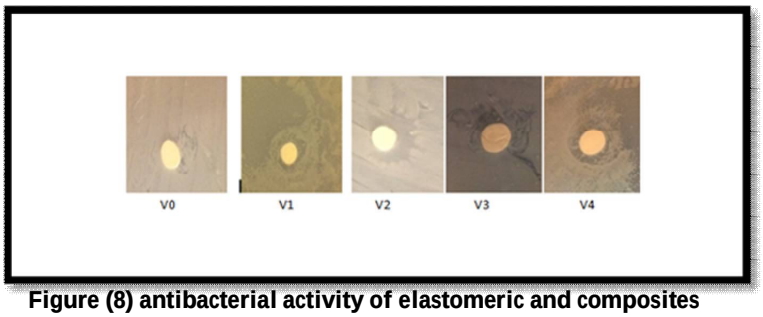

Figure (8) antibacterial activity of elastomeric and composites

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RESEARCH ARTICLE

Enhanced Mechanical, Electrical Properties of the Epoxy Polymer by Adding Hexagonal Boron Nitride Nanoparticles

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ABSTRACT

The goal of this work is to study the effect of addition hexagonal boron nitrite nanoparticle on the mechanical and electrical properties of thermosetting epoxy polymer at different weight fraction (0, 0.1, 0.3, 0.5, 0.7, 1, 1.5, 2, 3and5) such as tensile strength, young modulus, impact strength, hardness and dielectric strength, all samples were prepared by hand lay-up technique and mixing process. It was noticed from the results obtained that the tensile strength has most probably high values at a low weight fraction of h-BN nanoparticles, specifically at 0.5wt%, while a higher value of young modulus was obtained at 0.3 wt. %. The random behavior of changing in impact strength with the increase in the weight fraction of h-BN nanoparticles was observed but decreasing the values of impact strength of epoxy is very obvious., also it was found that the hardness values increased from (77.5) for the pure epoxy to (82.2) in addition of 1.5wt% h-BN nanoparticle and in general it was found well enhancement in the properties of the electrical insulation and mechanicals.

Keywords: nanoparticles, boron nitride, epoxy, nanocomposites, mechanical properties.

INTRODUCTION

Nanocomposites considered as solid structures are composed of Nano-sized particles (or nanoparticles) that are embedded in a matrix material. They can be designed to have mechanical, electrical, magnetic, optical, thermal, biological, and transport properties that are superior to conventional filler materials. Two factors account for these size-induced properties of nanoparticles are the increase in the ratio of particle surface area to volume and particles size [1].there are three primary properties that nanocomposites have over conventional composites as follows. Lighter weight because of low filler loading, low cost because of fewer amount of filler use and improved their properties at a very low loading of filler [2]. Polymer nanocomposites are commonly defined are the combination of organic/inorganic fillers that have at least one dimension in the nanometer range like (Nanotube), two-dimensional

like (Nanolayers), or three dimensional like (Nanoparticles) [3]. One of the simple methods to improve the thermal conductivity of the epoxy polymers is to introduce ceramic particles in its [4] .the hexagonal boron nitride (h-BN), also called "white graphite" a structural analog of graphene, possesses excellent mechanical properties, high thermal conductivity and chemical stability, electrical insulation and good lubrication properties. These unique characteristic make (h-BN) attractive as Nanofillers for the fabrication of polymer nanocomposite with enhanced performance. Among the polymeric resin in the composites industry, epoxy is the most commonly used as a thermosetting matrix resin [5]. Several reports have been carried out about the investigated such properties as in (2013) W. Zhou et al. studied the thermal, electrical, and mechanical properties of hexagonal boron nitride reinforced epoxy composites, it is clear that the higher values obtained for thermal conductivity and dielectric[6]. Q.Xiao et al. (2017) improved thermal conductivity of polyarylene ether nitrile (PEN) by the incorporation of boron nitride Nanosheet (BNNS) and when the content of BNNS increases to 2.0 wt%, the tensile strength reaches the maximal value (111.4 MPa), with an increment of 10% compared with that of PEN. But all results obtained still higher than of PEN at the BNNS content 5.0 wt %. For the tensile modulus, it increases with increasing content of BNNS and is as high as 2,925 MPa with 5.0 wt% of BNNS.[7]

MATERIALS AND METHODS

Materials used in this work to prepare the samples are:

Matrix Materials

Epoxy as a matrix (EUXIT 50 is primarily low viscosity (highly fluid) special mixture with high capillary action). Mix ratio (by weight) of the epoxy resin to the hardener was 3:1, viscosity 300 cps at20°C. The properties of the epoxy resin are specific density (gm/cm^3) at 20°C is 1.05, Compression (N/mm^2) is 85 and Tensile strength (N/mm^2) is 14.0889.

Reinforcing Particles

Nanoparticles (NPs) were used as reinforcing particles to improve the mechanical and dielectric properties of the epoxy polymer was hexagonal boron nitride .the nanoparticles (NPs) utilized in the present study have been procured from (US Research Nanomaterials, Inc.USA) (99.8+ % purity as per suppliers data),Atomic force microscopy (AFM) was used(CSPM scanning probe microscope) to measure the average particles size, surface roughness and root mean square (RMS) of BN nanoparticles as shown in Table (1), and Fig. (1) shows image (3D-AFM) of nanoparticles boron nitride and the particles size distribution.

Casting Method and Sample Preparation

Samples were prepared by hand lay-up technique and mixing process which can be summarized by the following steps

Preparation of Neat Epoxy polymer

The neat epoxy specimens were prepared by simple direct mixing of epoxy resin with the hardener, epoxy resin and hardener are weighted for suitable mixing ratio by using Electronic balance type (Sartorius BL 1500S/Germany) with sensitivity of 10-3g and with rang up to 1500g, and mixing in a container by mechanical stirring for 15 minutes to get good homogeneousness between epoxy resin and hardener before casting it in the mold. The casting was cured at room temperature for one day. Specimen put has in an oven for 1hour with temperature50□; it was left for two days before pulling out from the mold and then left at room temperature for 7 days before processing further.

Preparation of Nano composites

Nanocomposites with different weight percent (0.1, 0.3, 0.5, 0.7, 1, 1.5, 3, and 5 %) of BNNPs as reinforcement materials and epoxy resin as the matrix was prepared by the molding method. Since properties of composite depend upon the weight percent of reinforcement, mass for a given weight percent of BNNPs was determined by using equation (1).

$$\psi\% = \frac{w_f}{w_f + w_e} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: $\psi\%$ is the weight percent ,w_f is the weight of filler , w_e is the weight of the epoxy [8].

The nanocomposites were prepared in more complicated method such that, the nanoparticles were heated up in (120 for 2 h.) in order to get rid of absorbed moisture on their surface, to prepare homogeneous mixture of epoxy and the nanoparticles a weight ratio percent of BNNPs weighted with electronic balance of four digits type (Sartorius BL 210 S) then mixed with epoxy resin, the mixture put inside a container, an oil bath was used to heat up the mixture to desired (70^{O}C) temperature so the viscosity of epoxy base is depressed. Proper mechanical stirring (30 min) at this stage resulted in the better dispersion of nanoparticles, then the mixture was cooled to room temperature after that the hardener was added to the formulation being mixed by mechanical stirring (10 min). Specimens were left at room temperature for 24 hours for curing and then put in an oven for one hour at 50^{O}C for post curing.

Techniques

Tensile Test

The tensile test was used to construct a stress-strain curve for each composite specimen. This curve is used to get tensile properties such as tensile strength, Young's modulus, and Elongation percentage at the break as illustrate in Eq. (2, 3 and 4) the composites specimens tensile were prepared according to ASTM-D638-87. The tensile test was carried out at room temperature by utilizing the universal tensile instrument manufactured by (Laryee Company in China), type (WDW-50). the tensile load was applied gradually until the fracture of the sample occurs[9]

$$\sigma = \frac{F}{A} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: σ: Longitudinal stress for specimens (MPa).F: Applied load (N) and a: Original cross sectional area before testing (m^2).

The strain which is used in such stress –strain curve is a linear strain and canbe expressed as:-

$$e = \frac{\Delta l}{l} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where: e : strain

$$\Delta l = l - l$$

Where: l: The final length (m). l: The original length (m).

$$E = \frac{\Delta\sigma}{\Delta e} \quad \text{..... (4)}$$

Where: E. young modulus $\Delta\sigma$. Change stress Δe. Change strain [10].

Impact Test

The Charpy impact test instrument manufactured by (Test Machines Inc.) (AMITYVILLE, New York) Machine in the United States was used for measuring the energy required to fracture impact strength was calculated from the Eq.(5)[11].

$$I.S = \frac{U}{A} \quad \text{..... (5)}$$

Where: I.S. = impact strength (J/m^2), U = Energy of fracture in (J), A = Cross section area in (m^2)

Hardness test

The hardness of the material is indicative of its general mechanical behavior .Hardness is the characteristic of a solid material expressing surface resistance to scratching , cutting, wear ,indentation, penetration and workability from an applied force as sharp point and an indication of surface durability. The hardness of material relative to mechanical strength, therefore when the material has low hardness value have low yield strength, shore(D) using to measure the hardness of (TH210). Hardness test generally depends on the test load, dwell time, type of interatomic or intermolecular bonds, surface condition, and temperature. There are other several factors that influence on the hardness value for polymer composite materials such as particle size and volume fraction of particles reinforcement, chemical composition, and structure such as cross-link in the chains [12].

Dielectric Breakdown strength

Dielectric breakdown strength is defined as the highest voltage which samples can stand before they fail electrically, divided by sample thickness [13], or the magnitude of the electric field required to cause dielectric breakdown [14].when applied strong electric field on the insulator is higher than the value of the specific critical large numbers of electrons may suddenly be excited to energies within conduction band. As a result, the current through the dielectric by motion of these electrons increases dramatically, sometimes localized melting, burning, or vaporization produces irreversible degradation and perhaps even failure of the material [15,16]. So that the insulation properties will be lost for the insulator and becomes conductor [17]. So the voltage that occurs when the breakdown is called (Vbr), (Breakdown voltage) when divided by the thickness of the samples (h) [18]. We obtain (Ebr) dielectric strength in units (Kv/mm) or (V/m) as in Eq. (6), [19].

$$E_{br} = \frac{Vbr}{h} \quad \text{..... (6)}$$

RESULT AND DISCUSSION

Tensile strength

Fig. (2, 3) represented the curve of tensile strength and young modulus respectively for composite specimen reinforced by BNNPs. It can be noted from the figure (2) that the addition of the BNNPs with weight fraction (0.1wt% to 5 wt. %) Leads to increase the tensile strength of the nanocomposites which reaches a maximum value at

(0.5%) ratio of BNNPs compared with other ratios .but it was noticed that all the results obtained of tensile strength were higher than of epoxy. This result could be caused by that the BNNPs possessed excellent mechanical properties and large specific surface area, which would consume more energy during the tensile process. Howeverthe tensile properties of the composites could be adversely affected and the stress concentrations might have occurred if the usage amount of the as-prepared BNNP was excessive, such as 5 wt %. The values of tensile strength and young modulus for the pure epoxy and BNNPs composites materials for all samples that were fabricated in the current work are illustrated in table (2) Table (2) shows that the nanocomposites with weight fraction (0.3%, 0.5%, and 0.7%) of BNNPs have a higher modulus of elasticity and reaches to maximum values at (0.3%) ratio of these nanocomposites, as compared with another ratio as illustrated in fig. (3).

Impact strength

Fig. (4) Shows the effect of BNNPs content on the impact strength of epoxy of the prepared nanocomposites. It can be noticed that the value of impact strength increase when adding the h-BN nanoparticles leads to increase the impact strength. Epoxy/boron nitride nanocomposites to reach a maximum value at (0.5%) ratio of weight fraction as compared with pure epoxy and then decreased the impact strength with increase weight fraction until you reach the lowest value at (5%).

Hardness test

From the Fig. (5) it can be noticed the relationship between hardness value and the weight fraction of BNNPs for Epoxy/BNNPs nanocomposites which revealed that the values of hardness increased with increasing the weight fraction of BNNPs this is related to the high hardness and brittleness that have these particles. Therefore, the observed value of hardness ratio (1.5 wt %) composite are higher than values of hardness for all prepared specimens. This is due to the improvement of the mechanical properties that are associated with the addition BNNPs, thus the hardness values increased from (77.5) for the epoxy to (82.2) at1.5wt% for Epoxy/BNNPs composite.

Dielectric breakdown strength

From the Fig. (6) shows the result of the test of the strength of the insulation with the percentage of nanoparticles, the composites has good electrical insulation and increase this insulation by addition of nanoparticles, because the ceramic materials with high insulation compared with polymeric materials that the strength of insulation increases with the increase in the percentage of nanoparticles added ,This is due to the use of size particles 50nm resulted in the penetration of the polymer mixture, thus obtaining a high density of the particle that is distributed within the mixture, and the ratio of (5 wt. %) BNNPs have strength of the insulation less than epoxy pure [20]. The use of nanoparticles that the polymer mixture is high and the bond strength is high between the nanoparticles and the base material, which improves the properties of the electrical insulation well. The strength of the insulation for the nanocomposites in this test are summarized in table 5.

Samples were imaged after breakdown, by optical microscope Magnification power 160x The images showed in figures (7, 8, 9, 10, 11, 12, 13, 14 and 15) for the electric breakdown points by using optical microscopy, have shown that the sample coaled in the electric breakdown area as a result of destroying polymeric chains of epoxy, and the formation of micro cracks as a result of electric breakdown which extends directly to the point of electric breakdown. The optical microscope pictures show that there is micro cracks were extend and more sinuosity and distortion of the micro-samples, this is because the great convergence of nanoparticles, diffusing, dispersal, high homogeneity and high stable distribution in all directions, that nanoparticles enjoyed and the most important distinction of Nano-reinforcement

CONCLUSION

In this work, we studied fundamental mechanical properties of EP/BNNPs composites. It was observed from results obtained good improvement in the characteristics results of mechanical and dielectric of the specimens with the low weight fraction of BNNPs. Where the higher value of the tensile was 46.87 MPa obtained at the ratio 0.5 wt% and tensile modulus exceeding 920 MPa at the ratio 0.3 wt %.it can conclude that the fracture energy for Nanocomposite depended on the behavior of cracks propagation and plastic deformation and the value of hardness are depend on the distribution of Nanoparticles on the surface of the specimen.

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Table.1. Average Diameter, Surface Roughness and Root Mean Square values of nanoparticles boron nitride

Material	Average diameter(nm)	Surface Roughness(nm)	RMS (nm)	Peak-to-Peak(nm)
Boron nitride	52.91	2.83	3.27	11.3

Table2. Values of tensile strength and young modulus for the pure epoxy and its hBN nanocomposites materials

Sample	Tensile strength(MPa)	young modules (MPa)
Epoxy	14.0889	570
Ep +0.1 % $BNNP_s$	30.171	550
Ep +0.3 % $BNNP_s$	45.96	920
Ep+0.5 % $BNNP_s$	46.87	850
Ep +0.7 % $BNNP_s$	40.47	694.4
Ep+1 % $BNNP_s$	27	555
Ep+1.5% $BNNP_s$	18	522.8
Ep +2 % $BNNP_s$	17	370
Ep +3% $BNNP_s$	16.22	420
Ep +5% $BNNP_s$	15.34	330

Table .3. Values of impact strength for the pure epoxy and its hBN composites materials

Sample	Impact(KJ/m^2)
Epoxy	8.5
Ep +0.1 % $BNNP_s$	13.2
Ep +0.3 % $BNNP_s$	11.4271
Ep+0.5 % $BNNP_s$	11.655
Ep +0.7 % $BNNP_s$	6.7775
Ep+1 % $BNNP_s$	6.1427
Ep+1.5% $BNNP_s$	6.2169
Ep +2 % $BNNP_s$	3.65
Ep +3% $BNNP_s$	3
Ep +5% $BNNP_s$	1.57

Table 4.Values of hardness for the pure epoxy and its hBN composites materials

Sample	hardness
Neat epoxy	77.5
Ep +0.1 % $BNNP_s$	79.8
Ep +0.3 % $BNNP_s$	81.5
Ep+0.5 % $BNNP_s$	81.8
Ep +0.7 % $BNNP_s$	80.7
Ep+1 % $BNNP_s$	81.4
Ep+1.5% $BNNP_s$	82.2
Ep +2 % $BNNP_s$	73.6
Ep +3% $BNNP_s$	63.9
Ep +5% $BNNP_s$	56.06

Table 5. Values of dielectric strength for the pure epoxy and its hBN composites materials

Sample	Dielectric breakdown (Kv/mm for 5 Kv/s)
Epoxy	22
Ep +0.1 % BNNPs	24
Ep +0.3 % BNNPs	23.2
Ep+0.5 % BNNPs	22.4
Ep +0.7 % BNNPs	21.7
Ep+1 % BNNPs	21.3
Ep+1.5% BNNPs	21.1
Ep +2 % BNNPs	20.4
Ep +3% BNNPs	20.1
Ep +5% BNNPs	20

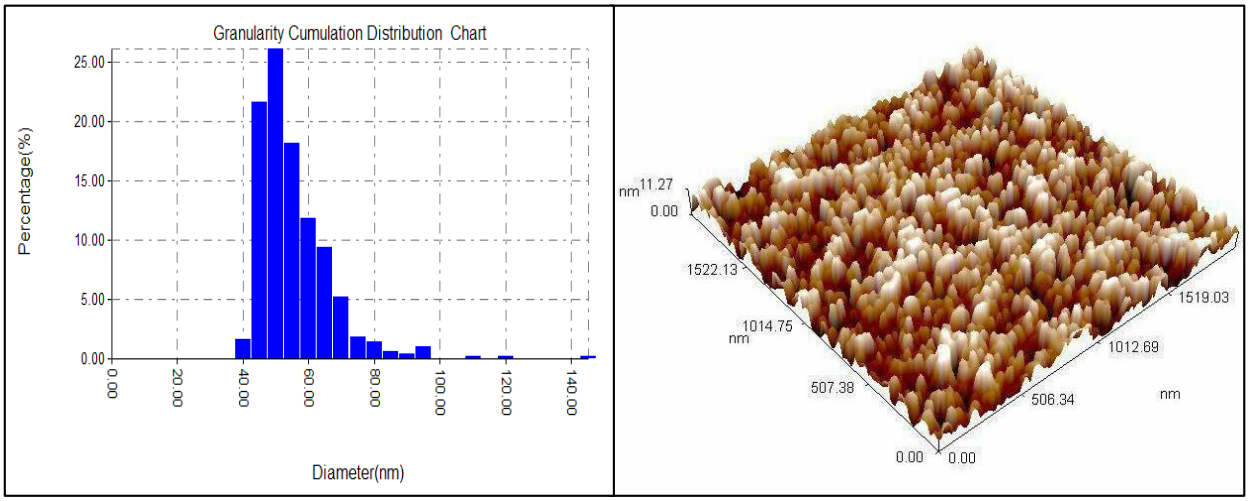

Figure (1): AFM of nanoparticles boron nitride

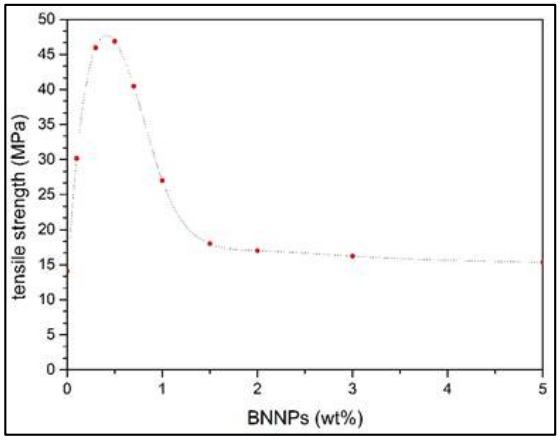

Fig.2tensile strength of EP/BNNPs composites

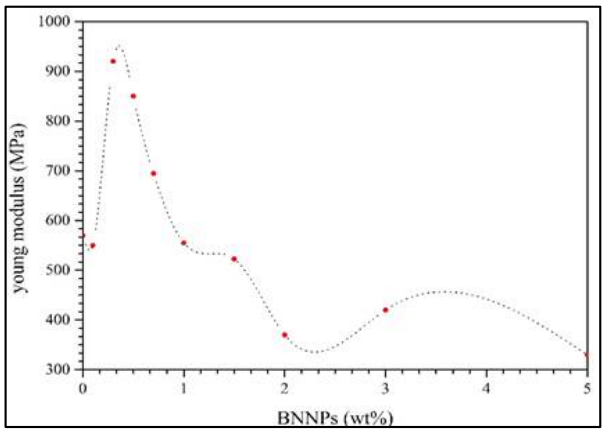

Fig.3young modulus of EP/BNNPs composites

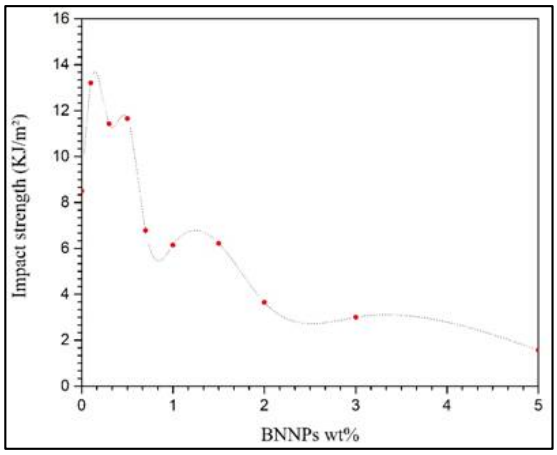

Fig.4 impact strength of the EP/BNNPs composite

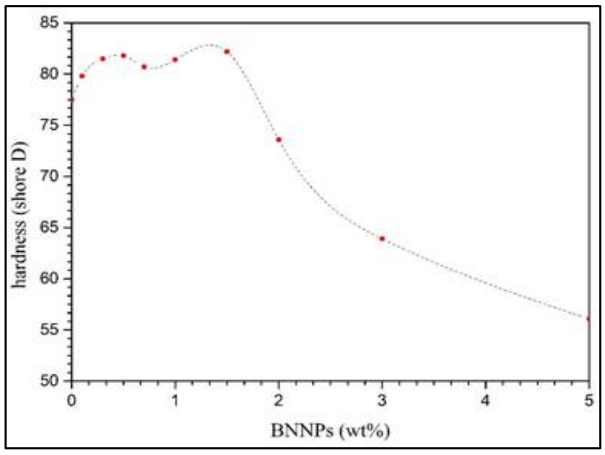

Fig.5hardness shore (D) of EP/BNNPs composite

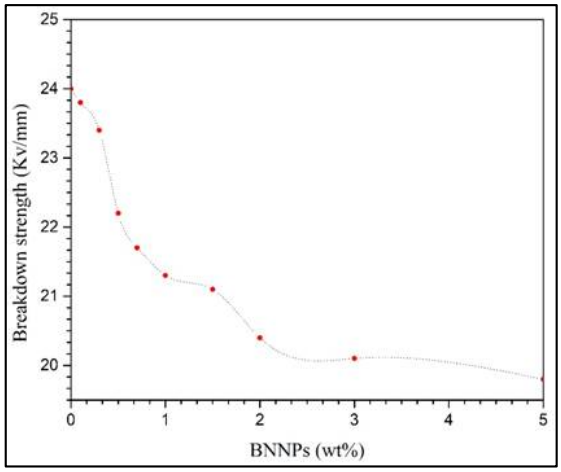

Fig.6 breakdown strength of EP/BNNPs composite

Figure (7) sample after breakdown (Epoxy)

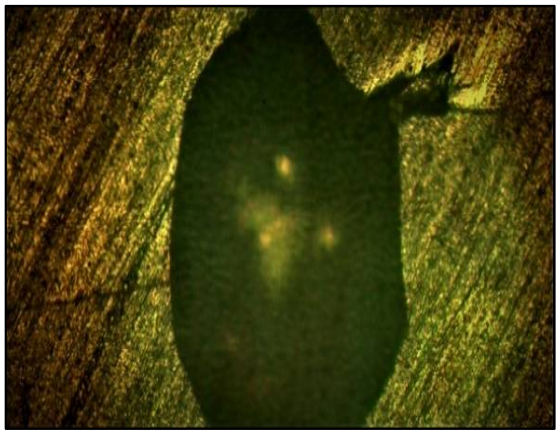

Figure (8) sample after breakdown (EP+0.1%BN)

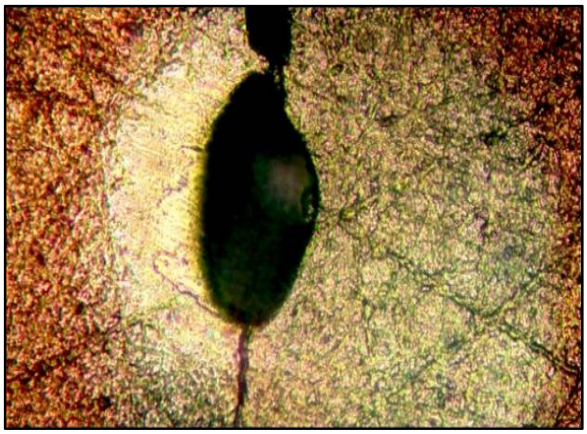

Figure (9) sample after breakdown (EP+0.3%BN)

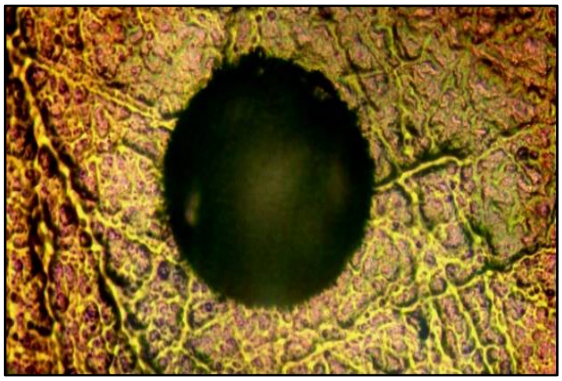

Figure (10) sample after breakdown (EP+0.5%BN)

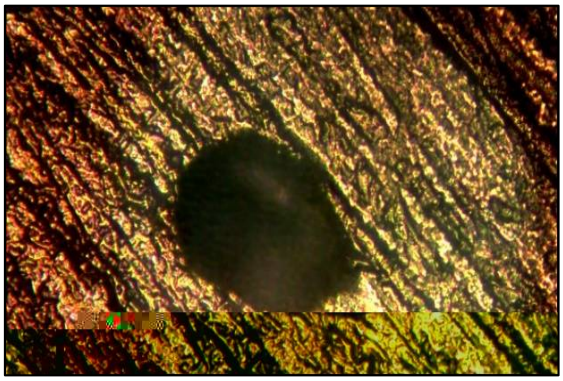

Figure (11) sample after breakdown (EP+0.7%BN)

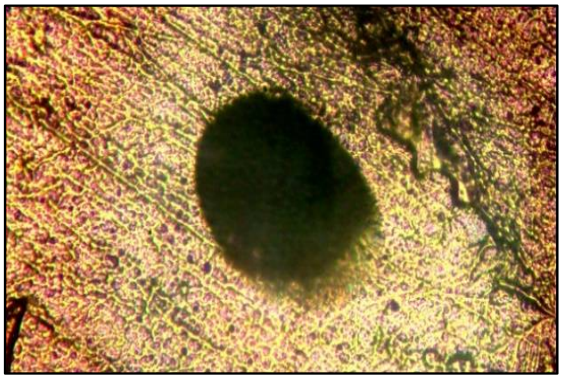

Figure (12) sample after breakdown (EP+1%BN)

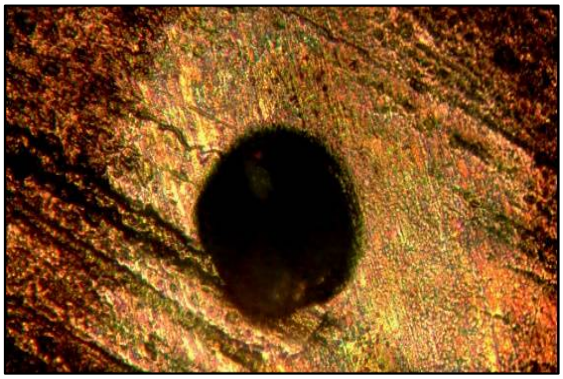

Figure (13) sample after breakdown (EP+2%BN)

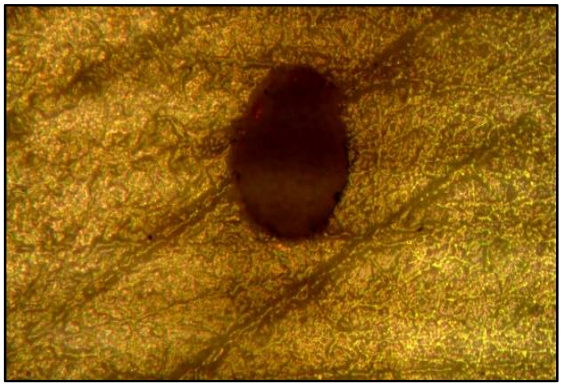

Figure (14) sample after breakdown (EP+3%BN)

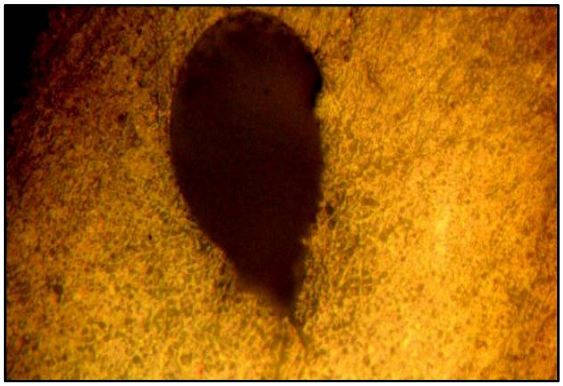

Figure (15) sample after breakdown (EP+5%BN)

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RESEARCH ARTICLE

Effect of Obesity on the Contralateral Breast Dose

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ABSTRACT

Contralateral breast cancer is one of the most common second cancers after breast radiotherapy. Obesity act as risk factor of breast cancer and could contribute with highly exposure to the contralateral breast. Our study performed to evaluate the effect of Body Mass Index (BMI), Planning Target Volume (PTV) and contralateral breast (CLB) volumefor the obese and none obese females on contralateral breast dose. Fifty females with breast cancer were involved in the current study. Semiconductor diode detectors were usedto measure the contralateral breast dose during treatment session. The CB volume and the PTV of each patient were measured from the CT simulator images for data analysis. Three points (**A, B, C**) were selected and determined on the contralateral breast for measuring the total dose, the first point **C** was selected on the nipple region and the other two points were determined at the level of nipple. A direct significant correlation was found between the BMI and the total dose of the CLB of the obese and none-obese patients. Increasing the volume of the CLB for both groups led to an increase in the total dose that was received by its volume. Increasing the PTV for obese patients causes an increase in the total dose of the CLB, while in non-obese patients a non-significant decrease in the total dose of the contralateral breast foundwith increasing thePTVof the ipsilateral breast. Increasing theBMI and the volume of the contralateral breast positively affect the contralateral breast dose of the obese and none-obese females that was received during the radiotherapy course. Increasing the PTV of the ipsilateral breast of the obese females contributed to a higher dose to the contralateral breast.

Keywords: BMI, Contralateral breast volume, Planning target volume, contralateral breast dose.

INTRODUCTION

Breast cancer comprises about 23% of all female cancer cases .The rate of mortality are at high level at age group of 40-50y [1]. Breast tissue considered as a highly radiosensitive tissue, this fact increases the incident of second malignancy especially at age of 45 years old.The risk of contralateral breast cancer has been determines to be 6.1% to 12% during 10-20 years after diagnosis [2]. Surgery, chemotherapy and radiotherapy are the standard treatment for breast cancer. During external beam radiotherapy of breast cancer, the contralateral breast is merely receives radiation due to many factors such as leakage from collimator, scatter from primary beam and patients movement [3].

Despite of reduction in local recurrence, breast radiotherapy leads to low dose scatter which increase the risk of contralateral breast cancer [4].Obesity is defined according to the national cancer institute as a condition that the individual has unhealthy amount or distribution of body fat. This medical condition affects the risk of developing a number of different cancers [5].Body Mass Index (BMI) is a useful tool refers to a general obesity of the individuals could be calculated by dividing a person's weight in (Kg) by his height squared in (m^2) [6]. Obesity has a significant effect for postmenopausal breast cancer. In contrast, it acts as protective factor for premenopausal breast cancer [7]. Recent studies has demonstrated that female at postmenopausal with body mass index of 33Kg/m^2 are at 1.3 times higher risk of breast cancer than with BMI less 21Kg/m^2 [8]. During breast radiotherapy obesitycould affects on the parameters that involved the concept of breast radiotherapy; this could increase the scattered dose that reached the opposite breast. In vivo dosimetry by using suitable detector is an important issue practically for obese patients who considered as risk group that may receive higher dose than none obese females. Our study evaluates the effects of obesity on the contralateral breast dose during breast radiotherapy as a function of volume.

MATERIALS AND METHODS

Fifty females were involved in the current study. They were diagnosed as having breast cancer. The patients were divided into two groups according to their BMI and classified as obese and non-obese subjects where each group included twenty five females. All patients were informed about the purpose of doing this research and all of them showed a verbal consent to be included in this study. The characteristics of patients are shown in table 1. Six Iba semiconductor diode detectors were used in the current study, they were of two different types where three of them of (EDP-10 3G) type were used with the energy (6) MV while the second three set of (EDP-20 3G) type were used with the (10) MV. They were differed in their sensitivity to detect the photon's energy. The two types have the same dimensions with (12*6.4*22.5) mm. The characteristics of each one is shown in table 2.

According to ESTRO booklets 2001[9] the diodes were calibrated against the ionization chamber in reference conditions for photons' beam energy.Two types of linear accelerators were used as sources of radiation with duel energy (6) and (10) MV, the Elekta Synergy11 Platform prototype linear accelerator and the infinity linear accelerator unit. The contralateral breast volume and the planning target volume of each patient were measured from the CT simulator images for data analysis. Figure 1. shows the planning target volume and the contralateral breast volume for the twogroups of patients. Three points (A, B, C) were selected and determined on the contralateral breast for measuring the dose, the first point **(C**) was selected on the nipple region and the other two points (A) and (B) were determined at the level of nipple. The diodes were placed on the breast skin surface at these three points during treatment session for fife fraction only. The average of the fife fraction readings is taken as one fraction dose for these three points, and then it was multiplied by the prescribed dose fraction to calculate the contralateral breast dose in all session. Statistical significance was considered at($P \leq 0.05$).

RESULTS

The data analysis of the current study showed that there was a direct significant correlation between the BMI and the total dose of the contralateral breast of the obese and none-obese patients (P=0.0001) increasing the BMI led to increase the total dose that was reached by the contralateral breast, The females who considered as obese according to their body mass index were received more dose than none- obese patients as shown in figure 2. Non-significant correlation found between the planning target volume of the ipsilateral breast and the total dose received by the contralateral breast of the obese patients, the increasing of the PTV cause an increase in the total dose of the contralateral breast (p=0.272), while the results of the none- obese patients showed a non-significant decrease in the total dose of the contralateral breast with increasing the target volume of the ipsilateral breast (p=0.568) as shown in figure 3. The results of this study showed that there was a non-significant direct correlation between the contralateral breast volume and the total dose that was received by its volume in the obese and non-obese patients, increasing the volume of the contralateral breast led to an increase in the total dose that was received by its volume (P=0.591, P=0.403) respectively as shown in figure 4.

DISCUSSION

In the current study, the significant impact of the BMI on the contralateral breast of the obese and none-obese patients was clear where the total dose that was received by the contralateral breast for the obese group was (145) cGy while for the none obese patients was (60) cGy. Many factors may contribute to this significant direct correlation during the process of breast radiotherapy, one of them may be related to the large irradiated field size for the obese patients. This finding is in accordance with Bhatnagar, et al., 2006 [10] who found a strong dependence on the primary breast size of the patients for the contralateral breast dose. Furthermore, the high photons' beam energy (10 MV) that was used for the treatment of the obese patient to provide high penetration could also influence the amount of the total dose that reached the contralateral breast as Boamah E, 2016[11] reported that the 15MV photon beam produced a higher doses to the contralateral breast as compared with the 6 MV by 6.42%. Wahba, et al., 2009[12] was also emphasis on these aspects as they mentioned that the large field separation in obese patients may require higher energies for adequate treatment which expect higher dose reached the contralateral breast. Increasing the contralateral breast dose of the obese females with increasing the PTV as noticed in the current work could be explained by some affecting factors like the BMI of the studied groups of patients and the tumor location in the ipsilateral breast which may affect the amount of the scattered dose that reached the contralateral breast. Moreover, the parallel increase in the total dose of the contralateral breast with increasing its volume for the both groups of patients is also confirmed by Tolia, et al., 2011 [13] who has measured and evaluated the mean, median, maximum dose of the contralateral breast depending on the DVH and they referred to the significant increase of these values with increasing the contralateral breast volume.

CONCLUSION

Increasing the planning target volume leads to an increase in the total dose that received by the contralateral breast for the obese females, while for the none obese females there was an reduction on the contralateral breast dose as planning target volume increased. The body mass index has a positive effect on the contralateral breast dose that received during the radiotherapy course. Both groups of patients affected at the same manner.

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Table 1. patients Age and BMI

	Obese group(n=25)			
	mean	**SD**	**Minimum**	**Maximum**
Age (y)	44.9	3.3	40	50
BMI (Kg/m²)	34.83	3.36	30.43	39.92
	None-obese group(n=25)			
Age (y)	44.7	3.5	40	50
BMI (Kg/m²)	27.04	2.33	22.03	29.90

Table 2. characteristics of Energy range

	1st type	2nd type
Code	EDP-10 3G	EDP-20 3G
Energy range (MV)	4 to 8	8 to 16
Built up region (mm)	10	20

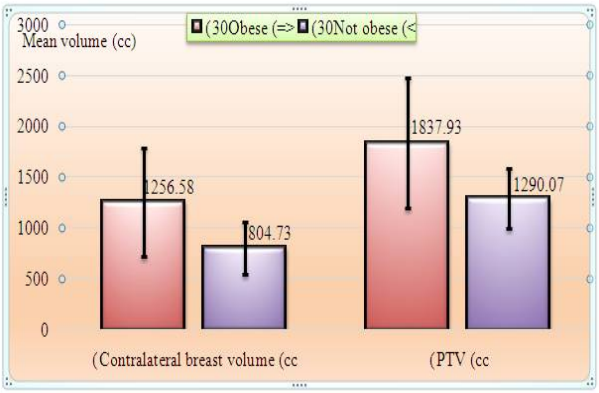

Figure 1: The planning target volume and the Contralateral breast volume for the obese and none bese patients.

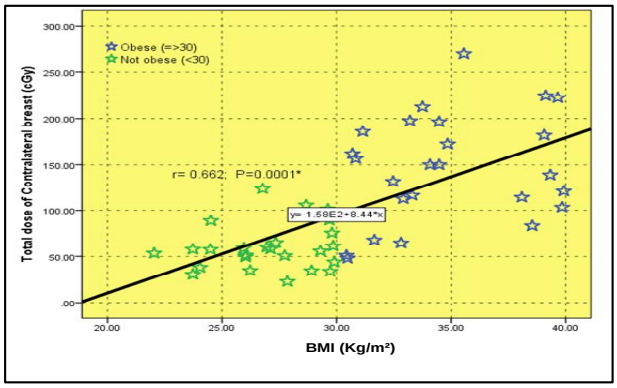

Figure 2: The correlation between the BMI and the total dose of the contralateral breast in obese and none-obese patients

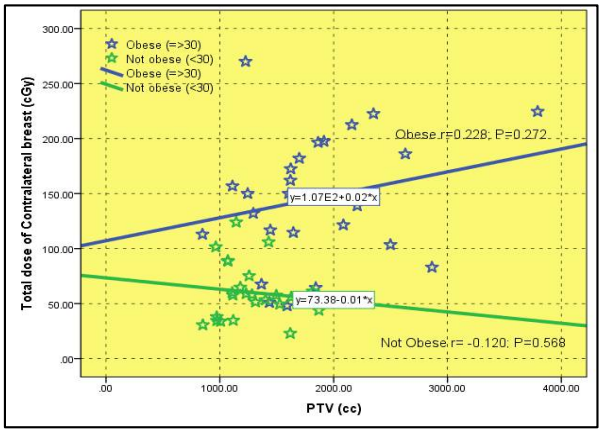

Figure 3: The correlation between the Planning Target Volume (PTV) of the ipsilateral breast and The total dose of the contralateral breast in the Obese and non-obese patients.

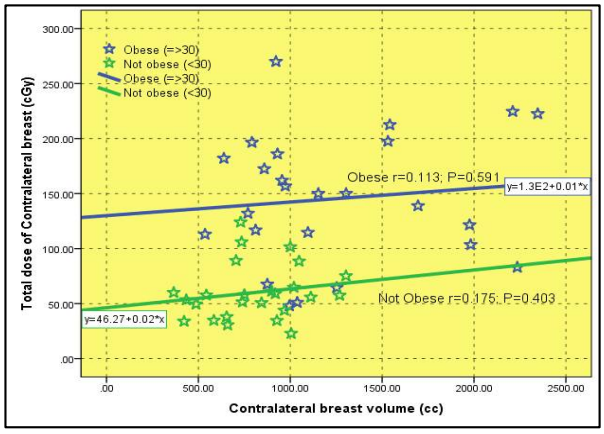

Figure 4: The correlation between the contralateral breast volume and the total dose received by its volume in the obese and non-obese patients.

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RESEARCH ARTICLE

Comparative Pathological Study of the Effect Crude Extracts of Oak Galls (*Quercus infectoria)* and Pomegranate Peels (*Punica granatum.* L) On Some Pathogenic Bacteria *In vitro* and *In vivo*

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Baghdad, Iraq.

ABSTRACT

This study was designed to see the Comparative Pathological study of the effect crude extracts of Oak galls (*Quercus infectoria)* and pomegranate peels (*Punica granatum.* L) on some pathogenic bacteria *in vitro* and *in vivo.* Crude extracts of Oak galls (*Quercus infectoria)* and pomegranate peels (*Punica granatum*. L) In *invitro* revealed against (*Salmonella typhimurium, E.coli, Listeria monocytogenus, Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*). Sensitivity test for three extracts measurement after (24, 48 &72) hours.The highest effect of Oak galls Extract during 24 hours was against *Staphylococcus aureus*, Pomegranate extract had the highest effect against *Pseudomonas aeroginosa*, while mix extract was the highest in equal effect on (*Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*).The results revealed that the effect of Pomegranate and mix extracts were increased for all bacteria after 48 &72 hr.s, except Oak galls extract showed that the effect was slightly reduced against *Staph aureus* & *E.coli* bacteria after 48 & 72 hr.s. *Invivo* as experimental study used Forty Balb C mice 6-7 weeks in age and both sexes weight from 25 grams were randomly divided into eight groups each one contain 5 animals as the following: first group as control group infected with *E.coli* bacteria for 24 hours with suspension ($1x10^8$) cfu /orally, second group as control group infected with *E.coli* bacteria for two weeks with suspension ($1x10^8$) cfu given orally. Third group treated with Oak galls (*Quercus infectoria)* that (100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks. forth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with Oak galls (*Quercus infectoria)* crude extract 0.2 cc obtained orally for each animal daily for two weeks, fifth group treated with pomegranate (*Punica granatum.* L) that 100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks**,** sixth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with pomegranate (*Punica granatum.* L) crude extract 0.2 cc obtained orally for each animal daily for two weeks**,** seventh

treated with group mix two extracts prepared as mixture that (100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks and eighth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with the mix two crude extracts 0.2 cc obtained orally for each animal daily for two weeks. Third, fifth and seventh groups shows no pathological change. In forth group presence of granules in the cytoplasm of hepatocytes in liver; sixth group appear in kidney congestion of blood vessel and infiltration of macrophage and neutrophiles, and in liver presence of granules in the cytoplasm of hepatocytes. The last group shows in intestine hyperplasia of goblet cells and infiltration of inflammatory cells in intestinal mucosa, and in liver infiltration of inflammatory cells in parenchyma. In our study results for the three extracts showed in *invitro* and *invivo* studies nearly similar results, that indicate the crude extracts of Oak galls (*Quercus infectoria)*, pomegranate peels (*Punica granatum.* L) And mix extracts have antibacterial activity against some pathogenic bacteria *invitro* and give agood treatment against *E.coli invivo*.

Keywords: *invitro, invivo,* treated, bacteria, extract

INTRODUCTION

A serious problem that has been worsening along the years is microbial resistance to drugs, affecting developed as well as developing countries. (1). In this context phytotherapy appears a search engine making it necessary to develop new therapeutic forms for the treatment of pathogenic microorganisms. (2).*Quercus infectoria* is an oak tree of the family Fagaceae in the Mediterranean area, especially in Greece, Syria, Iran, and Asia Minor (3). The main constituents of the galls are tannin (50–70%) with small amount of free gallic acid and starch (4). And Punica *granatum*, belongs to the family of Punicaceae, is commonly known as pomegranate, grenade, granats and punica apple (5). The constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties(6). Medicinal use of Quercus infectoria in the treatment of diarrhea, hemorrhage, and skin disease (7). Anti-inflammatory activities (8). Antioxidant (9) and larvacidal (10). *P. granatum* is reported to have antioxidant (11). Antibacterial (12), antiviral (13), and anti-atherosclerotic(14). Sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions (15). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (16).

MATERIALS AND METHODS

In vitro study

Preparation of extracts

Oak galls (*Quercus infectoria)* and pomegranate peels (*Punica granatum. L*) **-** according to (17).

Bacterial isolates

That include (*Salmonella typhimurium, E.coli, Listeria monocytogenus, Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*) were obtained from Zoonosis Unit/College of Veterinary Medicine/University of Baghdad and the biochemical properties were tested depending on the method of (18).

Sensitivity test

Muller Hinton were used, which wiped every three dishes for one type of bacteria four drops of bacterial suspension that prepared and calculated manner according to M cFarland tube (first tube), and after that dried the dishes, punctured dishes by using the drilling cork (four holes /one dish). All Petri dishs contain one of the pathogenic bacteria that used and injected the four holes by Oak galls (*Quercus infectoria)* and pomegranate (*Punica granatum.* L) Concentration 100 mg and mix extract from two mix of Oak galls (*Quercus infectoria)* and pomegranate (*Punica granatum. L*) 100mg and ethanol alcohol 70% as control. After that the dishes were incubated at 37° C for 24,48 and 72 hours.

In vivo study

Forty Balb C mice were used in this study 6-7 weeks in age and both sexes weight from 25 grams were randomly divided into eight groups each one contain 5 animals as the following:

a. First group as control group infected with *E.coli* bacteria for 24 hours with suspension ($1x10^8$) cfu /orally was prepared as the method in (18).
b. Second group as control group infected with *E.coli* bacteria for two weeks with suspension ($1x10^8$) cfu given orally.
c. Third group treated with Oak galls (*Quercus infectoria)* that (100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks.
d. forth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with Oak galls (*Quercus infectoria)* crude extract 0.2 cc obtained orally for each animal daily for two weeks.
e. Fifth group treated with pomegranate (*Punica granatum.* L) That (100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks.
f. Sixth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with pomegranate (*Punica granatum.* L) Crude extracts 0.2 cc obtained orally for each animal daily for two weeks.
g. Seventh treated with group mix two extracts prepared as mixture that (100mg/1cc) of crude extract, 0.2 cc obtained orally for each animal daily for two weeks.
h. eighth group infected by *E.coli* bacteria for 24 hours ($1x10^8$) cfu /orally, then treated with the mix two crude extracts 0.2 cc obtained orally for each animal daily for two weeks.

Histopathology

All animals killed after the end of experiment and the internal organs were taken for histopathological examination using 10% formalin as a fixative, and then processed routinely as (19).

RESULTS

In vitro study

The results showed in table (1) that the sensitivity test for the Oak galls Extract ,Pomegranate extract and mix of Oak galls and Pomegranate extracts against some of pathogenic bacteria measurement after (24,48 &72) hours which include (*Salmonella typhimurium, E.coli, Listeria monocytogenus, Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*). The highest effect of Oak galls Extract during 24 hours was against *Staphylococcus aureus*, Pomegranate extract had the highest effect against *Pseudomonas aeroginosa*, while mix extract was the highest in equal effect on (*Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*).The results revealed that the effect of Pomegranate

and mix extracts were increased for all bacteria after 48 &72 hr.s, except Oak galls extract showed that the effect was slightly reduced against *Staph aureus & E.coli* bacteria after 48 &72 hr.s. This effect of three extracts appears to be clear against pathogenic bacteria as shown in pictures (1,2&3).

***In vivo* study**

Control group after 24 hours shows in liver congestion of blood vessels and infiltration of inflammatory cells & vacculation the cytoplasm of hepayocytes (Fig.1), and in intestine revealed infiltration of inflammatory cells of mucosa sub mucosa and hyperplasia of goblet cells (Fig.2). While the control group infected with *E.coli* for two weeks shows in kidney infiltration of inflammatory cells, hyperplasia of renal tubules epithelia and hemorrhage in renal parenchyma (Fig.3), lung congestion of blood vessels with thickening of alveolar septa (Fig.4), appearance of granules in the cytoplasm of hepatocytes, kupffer cells and presence of hemosidren pigment in the parenchyma in liver (Fig.5), necrosis with Inflammatory cells infiltration presence of hemosidren pigment in the parenchyma of spleen (Fig.6), and in heart control group shows infiltration of inflammatory cells with blood between muscle fibers(Fig.7).

Group that treated with crude extraction of Oak galls (*Quercus infectoria)* for two weeks shows no pathological change in kidney(Fig.8) and presence of granules in the cytoplasm of hepatocytes in liver (Fig.9);while group treated with crude extraction of Oak galls(*Quercus infectoria)*for two weeks and infected with *E.coli* shows in kidney congestion of blood vessel and infiltration of macrophage and neutrophiles (Fig.10), and in liver presence of granules in the cytoplasm of hepatocytes (Fig.11). Group treated with crude extraction of pomegranate (*Punica granatum. L*)for two weeks shows no pathological changes in kidney (Fig.12) and other organs, and when infected with *E.coli* bacteria for two weeks after the treatment shows in liver congestion of blood vessels and few inflammatory cells(Fig.13), no pathological changes in kidney (Fig.14). The last group which treated with crude extraction of mix extracts for two weeks and infected with *E.coli* shows in intestine hyperplasia of goblet cells and infiltration of inflammatory cells in intestinal mucosa (Fig.15), and in liver infiltration of inflammatory cells in parenchyma (Fig.16).

DISCUSSION

Pomegranate extract and mix of Oak galls and Pomegranate extracts against some of pathogenic bacteria which include (*Salmonella typhimurium, E.coli, Listeria monocytogenus, Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*). The highest effect of Oak galls Extract during 24 hours was against *Staphylococcus aureus*, Pomegranate extract had the highest effect against *Pseudomonas aeroginosa*, while mix extract was the highest in equal effect on (*Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*).as in table and this study agree with (20) who revealed that *Quercus infectoria* aqueous extracts are shown to exhibit widespread antimicrobial activity agains Gram positive bacterial strains studied were inhibited by galls of *Quercus infectoria* extracts, with same degrees of inhibition *Staphylococcus aureus*, and *Bacillus cereus.* The galls of *Quercus infectoria* have been pharmacologically documented to possess astringent, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory properties (21)

Tannins are phenolic compounds that are known to possess antimicrobial property. The antibacterial property of gall extract of *Quercus infectoria* can thus be attributed to the high concentration of tannins present in it (22). Pomegranate extract had the highest effect against *Pseudomonas aeroginosa*, as in table(1),also the inhibitory effect against other bacteria like *E.coli* and *Staphylococcus aureus* and this result agree with (23) that revealed *P. granatum* peel extracts have antibacterial activity against *Escherichia coli* O157 and methicillin-resistant *Staphylococcus aureus* and *Salmonella* bacteria.The present of phytocompounds is related to antimicrobial activity of pomegranate fruit peel, predominantly alkaloids and tannins, in addition to that punicalagin compound, ellagitannin with proven antimicrobial activity (24), and the inhibitory potential of polyphenols and flavonoids also been reported (25). May be indicative of the presence

of several metabolic toxins or broad-spectrum antibiotics. Several metabolites from herb species, including alkaloids, tannins and sterols, have previously been associated with antimicrobial activity (26). Mix extract was the highest in equal effect on (*Staph aureus, Pseudomonas aeroginosa* and *Streptococcus pneumonae*) table (1). Synergistic interaction between plant extracts and antibiotics against infectious disease had been reported (27). Natural plant and its derivatives are developed as pharmaceutical agents for the treatment of various infections (28). Other studies showed synergistic interaction between natural products and antibiotics against infectious disease (29). That agrees with our results.

In vivo study control groupthat infected with *E.coli* bacteria showed pathological changes in some internal organs like kidney, liver, intestine and spleen. *E.coli* infection triggers physiological changes in the intestinal epithelium, including altered ion transport (30), E. coli is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. *E. coli* produce alpha- and beta-hemolysins, which cause lyses of urinary tract cells (31).Group treated with crude extraction of pomegranate (*Punica granatum.* L) For two weeks shows no pathological changes in kidney, and other organs.(32) Said that Pomegranate juice, oil or powdered extracts appeared no adverse effects on renal or liver function.

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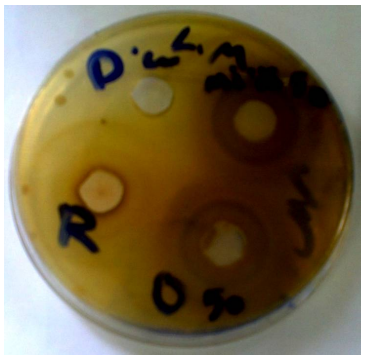
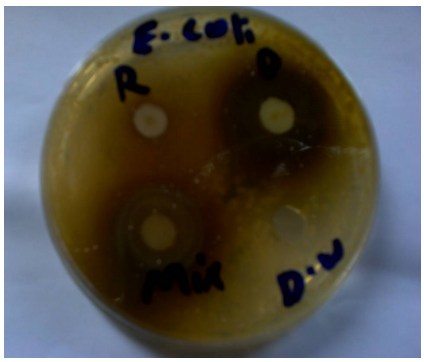
Table 1: The sensitivity test results of Oak galls Extract ,Pomegranate extract and mix of Oak galls and Pomegranate extracts against some of pathogenic bacteria measurement after (24,48 &72) hours

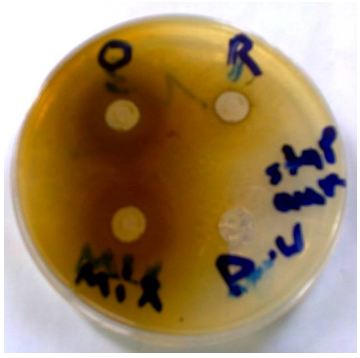
Bacteria	Oak galls Extract (mm)			Pomegranate Extract (mm)			Mix two extracts (mm)		
	24 hr.	48hr.	72hr.	24 hr.	48hr.	72hr.	24 hr.	48hr.	72hr.
Salmonella typhimurium	26	29	30	19	31	32	24	29	32
Staphylococcus aureus	32	30	30	21	29	30	26	29	31
Listeria monocytogenus	17	21	23	21	24	24	19	26	28
Pseudomonas aeroginosa	23	24	24	28	30	31	26	29	33
Streptococcus pneumonae	29	34	36	20	34	34	26	31	33
E.coli	24	22	22	22	32	34	22	30	33



Picture 1: showed sensitivity test of the three extracts against *Listeria monocytogenus* bacteria as *invitro* study



Picture 2: showed sensitivity test of the three extracts against *E.coli* bacteria



Picture 3: showed sensitivity test of the three extracts against *Staphylococcus aureus* bacteria

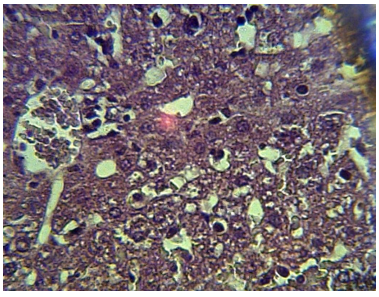


Fig.1:Histopathological changes liver of one animal of control group for 24 hours shows congestion of blood vessels and infiltration of inflammatory cells & vacculation the cytoplasm of hepayocytes (H&E stain, X400)

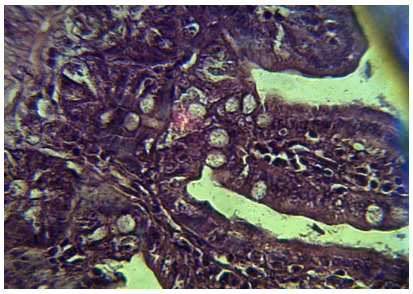


Fig.2:Histopathological changes intestine of one animal of control group for 24 hours shows infiltration of inflammatory cells of mucosa sub mucosa and hyperplasia of goblet cells (H&E stain, X400)

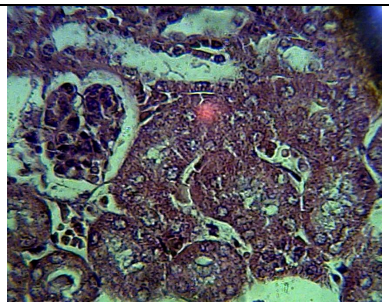


Fig.3:Histopathological changes in kidney of one animal of control group infected for two weeks shows infiltration of inflammatory cells, hyperplasia of renal tubules epithelia and hemorrhage in renal parenchyma (H&E stain, X400)

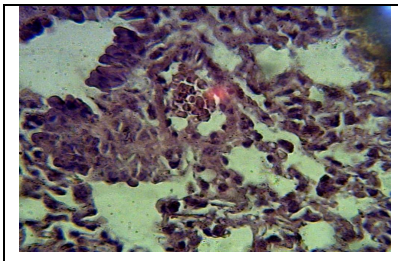


Fig.4:Histopathological changes in lung of one animal of control group infected for two weeks shows congestion of blood vessels with thickening of alveolar septa (H&E stain, X400)

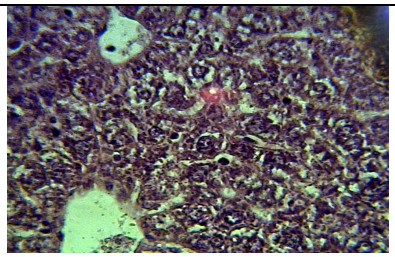


Fig.5:Histopathological changes in liver of one animal of control group infected for two weeks shows granules in the cytoplasm of hepatocytes, kupffer cells and presence of hemosidren pigment in the parenchyma (H&E stain, X400)

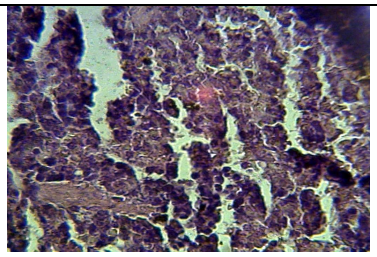


Fig.6:Histopathological changes in spleen of one animal of control group infected for two weeks shows necrosis with Inflammatory cells infiltration presence of hemosidren pigment in the parenchyma (H&E stain, X400)

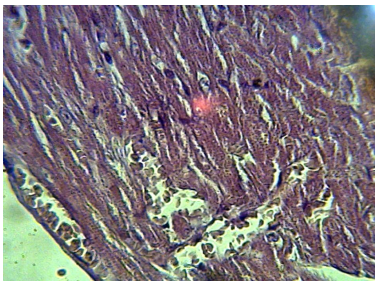


Fig.7:Histopathological changes in heart of one animal of control group infected for two weeks shows infiltration of inflammatory cells with blood between muscle fibers (H&E stain, X400)

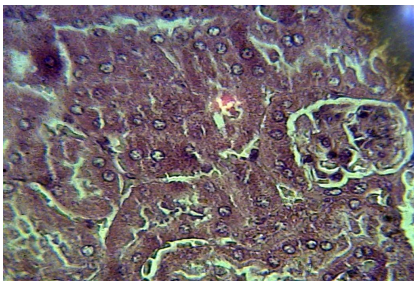


Fig.8 :Histopathological changes kidney of one animal treated with crude extraction of Oak galls(*Quercus infectoria)*for two weeks shows no pathological changes (H&E stain, X400)

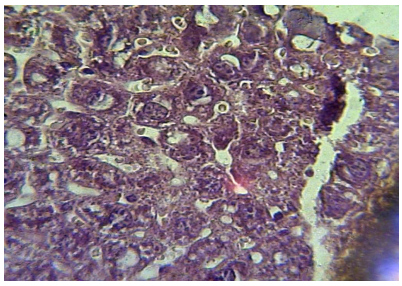


Fig.9 :Histopathological changes liver of one animal treated with crude extraction of Oak galls(*Quercus infectoria)*for two weeks shows presence of granules in the cytoplasm of hepatocytes (H&E stain, X400)

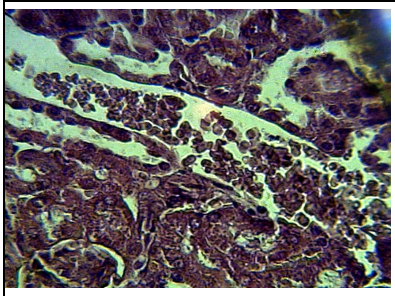


Fig10. :Histopathological changes kidney of one animal treated with crude extraction of Oak galls(*Quercus infectoria)*for two weeks and infected with *E.coli* shows congestion of blood vessel and infiltration of macrophage and neutrophiles (H&E stain, X400)

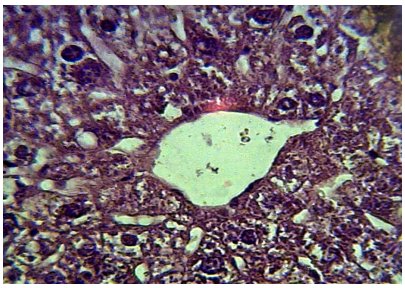


Fig.11 :Histopathological changes liver of one animal treated with crude extraction of Oak galls(*Quercus infectoria)*for two weeks and infected with *E.coli* shows presence of granules in the cytoplasm of hepatocytes (H&E stain, X400).

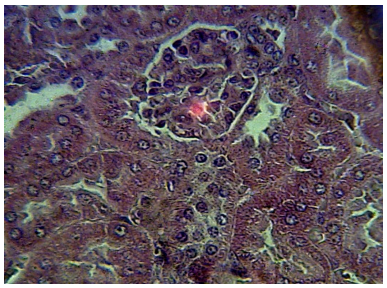


Fig.12 :Histopathological changes kidney of one animal treated with crude extraction of pomegranate (*Punica granatum. L*)for two weeks shows no pathological changes (H&E stain, X400).

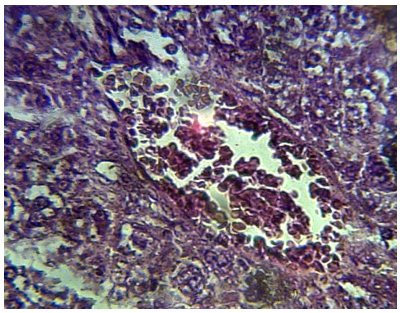


Fig.13: Histopathological changes liver of one animal treated with crude extraction of pomegranate (*Punica granatum. L*)for two weeks and infected with *E.coli* shows congestion of blood vessels and few inflammatory cells (H&E stain, X400).

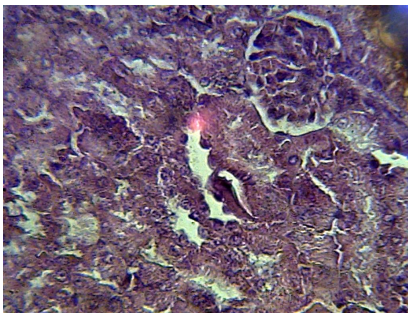


Fig.14: Histopathological changes kidney of one animal treated with crude extraction of pomegranate (*Punica granatum. L*)for two weeks and infected with *E.coli* shows no pathological changes (H&E stain, X400).

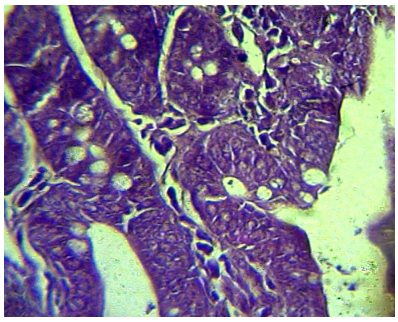


Fig.15 :Histopathological changes intestine of one animal treated with crude extraction of mix extracts for two weeks and infected with *E.coli* shows hyperplasia of goblet cells and infiltration of inflammatory cells in intestinal mucosa (H&E stain, X400)

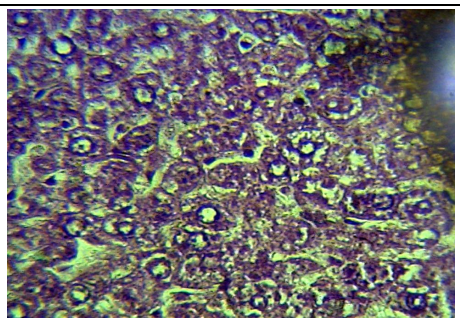


Fig.16 :Histopathological changes liver of one animal treated with crude extraction of mix extracts for two weeks and infected with *E.coli* shows infiltration of inflammatory cells in parenchyma (H&E stain, X400).

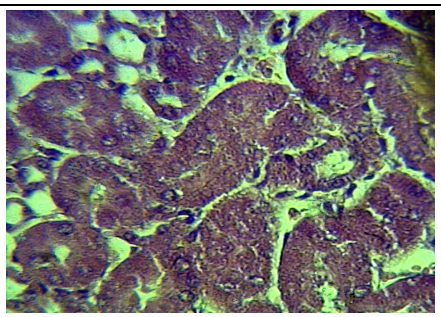


Fig.17 :Histopathological changes kidney of one animal treated with crude extraction of mix extracts for two weeks and infected with *E.coli* shows infiltration of inflammatory cells in parenchyma (H&E stain, X400).

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RESEARCH ARTICLE

Application of Reference Value Advisor to Estimate Some Serum Biochemical Parameters in Commercial Ross 308 Broilers

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ABSTRACT

In the current study some serum biochemical parameters were estimated using reference value advisor. A total of 50 birds (25 males and 25 females) with age of 35 day were used. The biochemical parameters included aspartate aminotransferase (AST), low density lipoprotein (LDL), triglyceride (TG), albumin, cholesterol and total protein. The results revealed that the differences between males and females were not significant for all parameters. Results showed that some of biochemical parameters did not normally distribute. Also results indicated that the reference values of these parameters were overlapped with standard reference values. It was concluded that the establishment of reference values is important not for commercial Ross but also for indigenous birds.

Keywords: Serum biochemical criteria, Ross, reference value

INTRODUCTION

Serum biochemical parameters have been used in numerous species of domestic livestock to monitor the health status and to identify the subclinical disease. The application of these parameters in commercially poultry has been limited as there are no estimations of the reference values. The determination of blood parameters is very important procedure to assist the breeders for the diagnoses of various poultry diseases and disorders.The biochemical parameters reflect the level of management, stress and diet(Kohne et al., 1975; Smithand Hattingh, 1979). To increase the production potential of poultry, it is very important to make the body functions in good balance (homeostasis). Hence, the estimation of the levels of some biochemical parameters provides very important information about the metabolic processes requirements to maintain the body balance status (Rezende et al., 2017).

Numerous researches were carried out to investigate the effect of different supplements on the biochemical parameters (Sadeghi and Pourreza, 2007; Abudabos et al., 2016; Lala et al., 2016). However there are some researches performed to establishing reference values of some indigenous animals such as sheep (Al-Jboryand Al-Samarai2016)

and goats (Al-Samarai1 and Mohammad, 2017) but there is a lack of data concerning the reference values of the biochemical parameters of the Ross broilers in Iraq. Rezende et al.,(2017) studied some biochemical parameters in Cobb broilers and the results obtained from this study confirmed the sex-specific biochemical patterns for most biochemical parameters. Moreover, the biochemical values were different from many studies with broilers.The current study aimed to determine some serum biochemical parameters in the Ross 308 broilers to assist the breeders for disease diagnoses and to observe the health status of broilers.

MATERIALS AND METHODS

Blood Collection

A total of 50 birds (25 male and 25 female) at age of 35 day were purchased from the local market (Kerbala south of Baghdad) on 6 February 2018. Blood samples (5mL/bird) were collected from all birds and stored at -20^{o} C then transferred into universal bottles without anticoagulant for serum analysis.

Biochemical Parameters

Total serum protein was estimated according to bromocresol purple method (Varley et al., 1980).Aspartate aminotransferase (AST) was estimated by using Randox test kits (Randox_ Laboratories, Antrim, UK). Low-density lipoprotein cholesterol (LDLC) was calculated using the below equation:

LDLC mgdL g = Cholesterol - high-density lipoprotein cholesterol (HDLC)- TG/5

Statistical Analysis

The data were subjected to analysis using SAS software (SAS, 2010) to compare the significant differences in biochemical parameters between males and females. The differences were assisted using unpaired t test. The data also subjected to analysis to determine the reference values of the studied biochemical parameters using reference value advisor which was created by Geffré et al., (2011) and represents a new freeware set of macroinstructions connected with Microsoft Excel. The freeware set included five methods: the standard, robust, transformed standard, transformed robust, and nonparametric (percentile). Unfortunately, most of software such as SAS, Minitab, and Graph pad Prism did not include these methods. However, SPSS software includes the nonparametric method only while the MedCalc software includes three methods. The freeware set includes the test of normality (Anderson-Darling), outlier (Dixon-Reed) and symmetry test for robust. The distribution of valuesdisplays in three forms dot plots, histograms and constructs Q-Q plots. A nonparametric method can be used when the sample size ≥ 40. When all the four methods were not suitable due the lake in normality and symmetry the nonparametric method should be the last choice.

RESULTS AND DISCUSSION

The results of the present study showed that the differences in biochemical parameters due to sex were not significant (Table 1). The mean values of cholesterol, TG, LDL, TP, and AST were 111.68 mg/dl, 85.28 mg/dl, 66.81 U/L, 5.24 g/dl, and 81.45mg/dl respectively while the corresponding means in females were 115.76 mg/dl. 84.08 mg/dl, 66.69 U/L, 5.37 g/dl, and 79.84 mg/dl. Similar results were obtained by Abdi-Hachesoo et al., (2011) in the Ross 308 who reported that the male mean values of AST, total protein, TG, and cholesterol did not differ from the females. Also in other study conducted on local chickens in Saudi Arabia by Albokkhadaim et al., (2011) results revealed that the differences in some biochemical parameters (total protein, cholesterol, TG, and AST) were not significant between males and females in young and adult birds.The non-significant effect of sex could reflect the

high homogeneity of the Ross 308 broiler as the variation in the body weight is a low.The overall means of the biochemical parameters are presented in the Table (2). The mean of LDL, AST, cholesterol, TP, and TG was 66.75 U/L, 80.65 U/L, 113.71 mg/dl, 5.32 g/dl, and 84.70 mg/dl respectively.The means obtained from the current study are closed to results of Hachesoo et al., (2011) who reported that the means of some biochemical parameters in Ross such as AST (198.40 U/L), TP (3.53 g/dl), TG (89.90 mg/dl), and cholesterol (74.50 mg/dl) in the males whereas the corresponding means in the females were 119.0 U/L, 4.96 g/dl, 71.10 mg/dl, 181.50 mg/dl.

Concerning with reference values results showed that LDL, AST, and TP were normally distributed according to Anderson-Darling test and Q-Q plotswhile the TG and cholesterol were not normally distributed and their distribution become normal after the transformation of data by Box-Cox (Fig. 1, 2). The reference interval of each of LDL (56.68 – 76.82 U/L)and AST (64.60 – 96.70 U/L) were within the general reference intervals which were 58.6-100 U/L and 10-106 U/L, while the cholesterol reference interval (101.40 – 123.29 mg/dl) was lowest than that of the general reference interval (129-297 mg/dl). On the other hand, the reference interval of TP (4.74-5.91 g/dl) was overlapped with that of the general reference interval (3.0-4.9 g/dl). The result of the TG showed that the reference interval has higher width compared with that of the general reference interval.

CONCLUSION

Based on our results, we can conclude that the effect of age was not significant on the biochemical parameters. The reference values of the most chemical parameters are within general reference interval. Some parameters showed overlap in their reference intervals. Therefore, establishing reference values is very important under the local condition of the count

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Table 1: Mean±SE of some biochemical parameters according to sex in the Ross 308 broiler

Group	Choles	TG	LDL	TP	Albumin	AST
Male	111.68±1.05	85.28±0.81	66.81±1.10	5.24±0.04	3.17±0.03	81.45±1.72
Female	115.76±0.91	84.08±0.96	66.69±0.88	5.37±0.06	3.26±0.06	79.84±1.44

Table 2: The reference intervals of some biochemical parameters in the Ross 308 broiler

Parameter	Unit	Mean	SD	Median	Lower and Upper limit RI	90% CI of lower limit	90% CI of upper limit	Method	GRI
LDL	U/L	66.75	4.96	66.75	56.68 – 76.82	54.67 – 58.79	74.57 – 78.93	S	58.6-100
AST	U/L	80.65	7.91	850	64.60 – 96.70	61.44 – 67.66	93.53 – 100.04	S	10-106
Cholesterol	mg/dl	113.72	5.32	114.50	101.40 – 123.29	98.26 – 104.28	121.38 – 124.72	SBC	129-297
TP	g/dl	5.32	0.28	5.30	4.74-5.91	4.63-4.85	5.79-6.02	S	3.0-4.9
TG	mg/dl	84.70	4.5	85.00	74.90-93.00	72.5-77.2	91.3-94.3	SBC	29-67

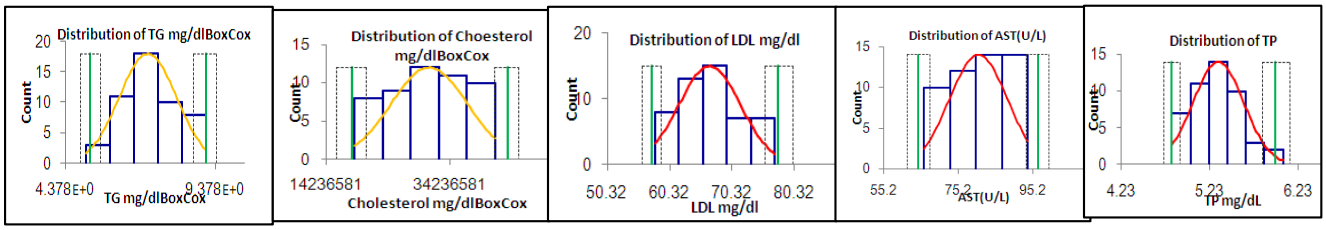

Fig 1.The distributions (on the right) of observed values (blue boxes) and fitted curves (red) in normally distribute parameters and yellow curves in the parameters normally distribute after transformation of the biochemical parameters in the Ross 308. Green vertical lines are the reference limits with corresponding 90%confidence intervals as the dotted line.

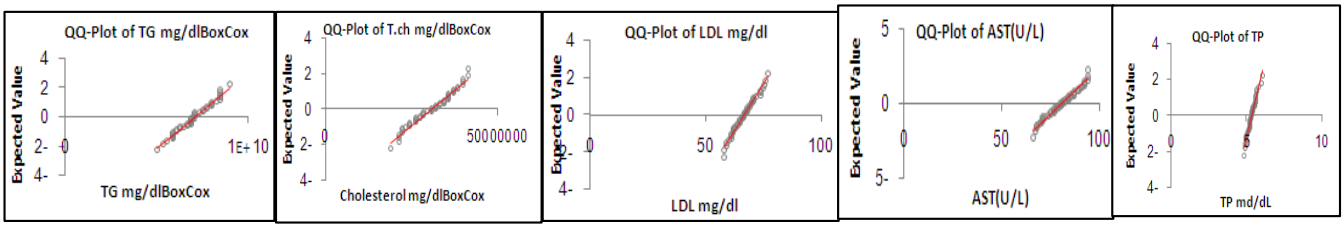

Fig. 2 The quantile-quantile plot (Q-Q plot) on the left illustrates the distribution of some homological parameters of the Ross 308 broiler.

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RESEARCH ARTICLE

All Optical Properties and Optical Constants of Copper Oxide for Optoelectronic Application

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ABSTRACT

A High quality high transparent conductive copper dioxide (Cu_2O) thin film was prepared by reactive pulsed laser deposition method. The prepared samples were analyzed and measured using the UV-visible and the photoluminescence. The Optical properties shows a high transparency reached to about (62 and 66) % and decreases sharply with the decreasing of annealing. The values of the band gab for the deposited film at the optimum preparation condition is about 2.62-2.67 eV.

Key words: Copper Oxide; thin film; optical properties; Optical constants; PLD technique.

INTRODUCTION

Copper oxides (CuO, Cu_2O, CuO_2) are the semiconductors which has been studied for several reasons such as natural abundance of the copper metal (Cu); [1, 2]. It has a cubic crystal structure with a lattice parameter are a= 4.6837A°, b= 3.4226A°, c= 5.1288Aand this material is suitable for solar cell applications [3, 4]. As a solar cell material, it has low cost advantages and great availability. They are highly attractive as the light materials due to the high values of the absorption coefficient in a visible areas, non-toxicity, available starting materials (Cu) on earth, low cost of production, theoretical energy conversion efficiency in order of 20% [5, 6]. The easy of preparation and deposition by oxidation of the copper. Non-toxic natures of electrical and optical properties are reasonably good by Cu_2O [7].The use of several methods of techniques of precipitation for the preparation of the films of the copper oxide, such as copper oxidation Anodizing through the process of simple electrolysis [11], plasma evaporation [19],thermal evaporation [20], electro deposition [21], pulsed laser deposition [22],dip coating [23], simple electrolysis based oxidation of metals[24], molecular beam epitaxy[25], chemical bath deposited[26] Electrolytic Method[27] activated reactive evaporation[28] reactive and conventional evaporation[29] reactive magnetron sputtering[30] thermal

evaporation and electroplating[31].In this manuscript, we report on the growth and the optical investigations of the high purity-CuO_2 films on glass substrates by the reactive PLD method. High purity cupper target and very simble and cheep Nd-Yag Laser tatto removal was used to deposite the CuOx films. The research continued to study and analyze the effect of Laser fluincy on the growth mechanism of the material and, thereafter, transmission, reflection, bandgap and refractive index are presented and discussed deeply.

EXPERIMENTAL METHODOLOGY

The undoped CuO_2 deposited films on the high quality glass slides as a substrate by Nd: YAG laser tattoo removal. The Laser pulse duration is 7 ns (FWHM) with wavelength= 1.064 nm, the focal length of the laser beam was focused through a lens =10 cm spotted on a target of the high purity cooper metal (provided by Fluka company). The targets spin at a rate of one cycle per minute. The density of the energy for the pulsed laser at the selected target was preserved within range of 500-1000 mJ/cm^2. All prepared thin films were created by 50 shots of laser at the temperature of the substrate is 150 C^o. The optical properties such as the transmittance spectrum of the prepared CuO films were tested at spectral values range (300–1000) nm by using the UV-VIS double beam spectrophotometer from Shimatzu-japan.

The value of the incident energy of photon was studded as a function of (λ) depending on the formula [42-48].

$$E_g(eV)=1240/\lambda(nm) \quad (1)$$

where the λ is wavelength. The value of the optical energy band gaps are dependence on the value of the absorption coefficient (α) and the excitation of It can be described as the transition, depending on the formulas of the Tauc [48-53].

$$(\alpha h\nu)=B\,(h\nu-E_g)r \quad (2)$$

where the *B* is the inversely proportional constant to amorphous, the *r* is the constant = 2, 3, etc… depending on the raw used materials and the type of the optical transition are $\alpha h\nu$. If the straight part of the plot from $(\alpha h\nu)^{1/r}$ against ($h\nu$) is extrapolated to $(\alpha h\nu)^{1/r}$ =0, the intercept gives the energy gap value. The value of the (α) for all wavelengths was examined using the formula [54-57].

$$\alpha=2.303(A/t) \quad (3)$$

where *A* is absorbance and *t* is the thickness of nanophotonic films.

RESULTS AND DISCUSSION

The transmission of the deposited thin film and prepared under different laser energy conditions are present in Figure (1). In the general, a slight decrease in the value of optical transmission as a function of energy of laser can be identified with the wavelength increasing with laser energy due to the increase in laser ablation efficiency and amount of ablated material. These value of the transmission peaks have as lightly red shift as the laser energy will be increased (increase in particle size), this result agrees with other work [58], implying that the deposited films at the low value of laser energy have a much smaller size. The spectral characteristic of the semiconductor has been shown to vary with the effects of quantization.

The values of the energy band gap (ΔE) vary with the radius values of the particles (d).

$$\Delta E = \left(\frac{h}{2m_e^*}\right)\left(\frac{\pi^2}{d^2}\right) \quad (4)$$

where ΔE (energy shift or optical band gap shift with respects to bulk band gap value (3.35 ev) and *d* is the size of the particle, *h* the constant of Planck's and *me** is the electron reduced mass thus with the decrease in the value of the particle size.

Indeed the increase in the laser energy means transfer more of the laser energy and indicate to ablating the larger amount of the raw materials. It was observed that the increase of the energy of the used laser produce the plume of the plasma become denser and could become more intense and this gives an indication that the large particles will be produced due to the two fact, the first fact is due to the longer time of growth and the other fact as a result of the high probability of clustering. The alteration of the $(\alpha h\nu)^2$ value with the values of the photon energy ($h\nu$) are present in figure 2, the value of the optical band gap (E_g) of Cu_2O are calculated from the extrapolating of linear part of $(\alpha h\nu)^2$ as a function of photon energy ($h\nu$) plot on x-axis. The values of the optical band gap (Eg) are found to be varied from (2.62-2.66) eV with the effect of laser energy.

CONCLUSION

Cu_2O Films have been deposited using PLD technique. It was found an approximate match between the value of the energy band gap that calculated by UV. The maximum value of the transmission was found to be about 65%. The results give indications to appropriate for optoelectronic applications.

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Table 1: The energy band gaps, refractive index and optical dielectric constant corresponding to molarity concentration of $LiNbO_3$ nanostructures.

Laser Energy (mj)	E_g measured (eV)	E_g (eV) exp.[a]
900	2.62	1.66- 3.6
1000	2.66	

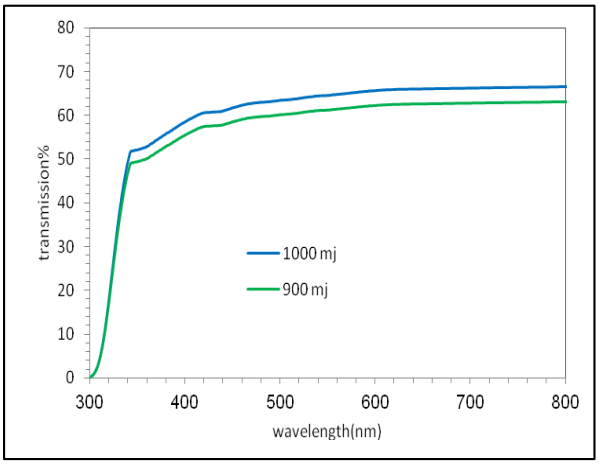

Figure 1. Optical transmission spectra of Cu_2O at two different laser energy

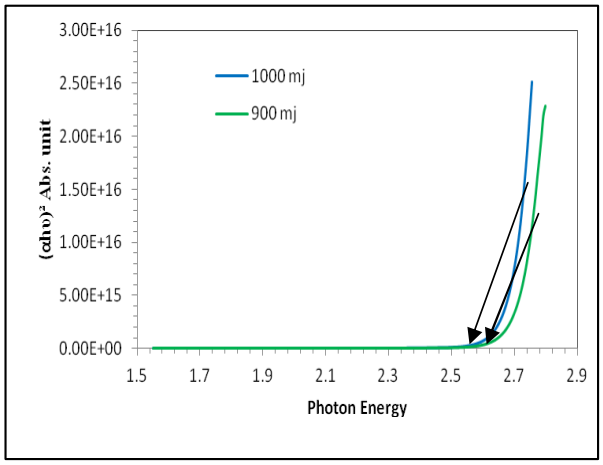

Figure 2. The optical band gap (E_g) of Cu_2O

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RESEARCH ARTICLE

The Formation, Structure, and Electronic Properties of TiO_2 Nanoparticles Drug Delivery Conjugated with Curcumin (Theoretical and Experimental Study)

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ABSTRACT

Curcumin is recognized as an important natural biomaterial which has a wide range of biological importance, unfortunately it lacks in bioavailability predominantly due to its poor aqueous solubility. The intention of the present investigation was to develop a novel nanocomposite of curcumin with TiO_2 nanoparticle in order to improve its aqueous-phase solubility and develop its efficiency on cancer cells. Therefore, we have constructed an aqueous solvable curcumin/ TiO_2 nanocomposite from the insoluble commercial curcumin, consequently enhancing its biological importance. The synthesized TiO_2 nanoparticles, nanocurcumin and the nanocomposite were analyzed SEM, AFM and XRD analysis. All the observed results declared that it has great potential for anticancer applications. The observed results of this investigation demonstrate that the present nano-conjugate can effectively deliver the anticancer drug curcumin towards the targeted bio molecules. The present Theoretical study using PM3 (semi empirical molecules orbital calculations) also DFT calculations. The energies of HOMO and LUMO orbitals have been computed for curcumin and TiO_2 and predicted UV spectra and IR vibration and optimization structure for curcumin and TiO_2.

Key words: TiO_2 nanoparticles; Curcumin; Hela cell; PM3 (semi empirical molecules orbital calculations); DFT calculations; HOMO and LUMO orbitals.

INTRODUCTION

TiO_2 nanomaterial's show potential biomedical applications, such as drug carriers. As one of the typical semiconductor materials with potential photocatalytic properties, nano-TiO_2 is widely studied and believed to be one

of the most promising nanocomposites in the field of materials science, energy science and also biology and life science (1-4).Curcumin is polyphenolic dye which has wide range of potent medicinal activities including antitumor, anticancer, antioxidant, anti-inflammatory, and remedy for Alzheimer disease(5-8). curcumin has been found to be very efficacious against many different types of cancer cells(8). Generally, cancer with its diverse origins has several molecular markers involved in its onset and progression. Curcumin is capable of interfering with several biochemical pathways involved in the proliferation and survival of cancer cells by directly and indirectly binding to different targets. Curcumin has been shown to interact with various targets including transcription factors, growth factors, DNA, RNA, and several proteins that are involved in cell signal transduction pathways (9,10). There are several features of curcumin's chemical structure that make it a favourable and versatile binding partner for a wide variety of molecular targets. For example, curcumin possesses two hydrophobic phenyl groups connected by a relatively flexible linker. This allows the molecule to assume different conformations that can maximize p-p and van der Waals interactions with aromatic and other hydrophobic amino acid residues of proteins. The phenolic hydroxyl and methoxy groups, as well as the ketone and enol groups present on the ends and in the middle of the molecule respectively can participate in strong and directed hydrogen-bonding interactions.

The possibility for keto-enol tautomerism introduces additional functionality, with the possibility to arrange donor and acceptor groups for hydrogen bonding in multiple ways. Furthermore, Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent having therapeutic impact. Curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin. In addition to its use as a spice and pigment, turmeric has been used in India for medicinal purposes for centuries (11,12). Major disadvantage associated with the use of curcumin is its low systemic bioavailability when administered orally due to its poor aqueous solubility (13-18). Hela cell, is a cell type in an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line (19). The line was derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, a patient who died of her cancer on October 4, 1951. The cell line was found to be remarkably durable and prolific which warrants its extensive use in scientific research (20). Alternative to co-crystals, which simply enhance the dissolution rate of the drug, methods have been developed to transport curcumin throughout the body to the desired sites for therapeutic applications. A variety of nano-vehicles including liposomes, exosomes, micelles, nanoparticles, and dendrimers have been used to encapsulate and deliver curcumin, resulting in enhanced water solubility, stability and bioactivity. Nanoparticle vehicles for the encapsulation and transportation of curcumin have also been developed. For example, in the formulation THERACURMINÒ, curcumin powder and glycerin was added to a solution of polysaccharides from ghatti trees, then the mixture was processed by wet grinding and high-pressure homogenization to produce a stable colloidal dispersion of nanoparticles with diameters of 190 nm.109 In clinical trials, the area under the blood concentration-time curve was found to be 27-fold higher for this formulation than for curcumin powder (21).

MATERIALS AND METHODS

Materials

Titanium tertiary isopropoxide 97%, was purchased from Aldrich. Sodium hydroxide, Curcumin, Ethanol, PVA (Molecular weight ≈125,000), chloroform from Merck.

Synthesis of TiO2 nanoparticles by co precipitation route

TiO_2 were synthesized using co precipitation method. In typical synthesis of TiO_2 nanoparticles, 5 ml. of TIIP (Titanium tertiary isopropoxide) placed in 100 ml. beaker and kept stirring on magnetic stirrer further 10 ml. ethanol and 2 ml. NH_4OH added successively till precipitation of hydroxide. Then total precipitate washed three times with

25 ml of DD water and dumped in Petri plates to dry in oven at 90 C°. The TiO_2 nanoparticles further ignited at 1000C° in muffle furnace to get dry anatase phase.

Synthesis of nanocurcumin

Curcumin dissolved in chloroform was added drop wise in to hot water maintained at 70°C and stirred vigorously for one hour. Appropriate amount of PVA in distilled water was added in to the above reaction solution, the temperature was gradually reduced to below 50°C and stirring was continued for another 2 hr. This final solution was kept aside to separate the organic layer for one day at -14°C. The aqueous layer was removed from the reaction solution and it was washed with distilled water for several times to completely remove PVA. The obtained final organic layer which contains curcumin was kept in water bath at 60°C to absolutely evaporate the CH3Cl, which gave nanocurcumin as fine powder.

Preparation of nanocurcumin/ TiO_2 nanocomposite

The nanocomposite has been synthesized for the first time as follows, the nanocurcumin in ethanol was added drop wisely to the TiO_2 NPs in DD water under constant sonication and the sonication was continued for another 4 hr. The obtained solid materials was separated from the solution by repeated centrifuge and dried it to give the fine powder of curcumin/TiO_2 nanocomposite.The anticancer effect of curcumin/TiO_2NPs, were evaluated in the hela cell lines.

RESULTS AND DISCUSSION

Atomic Force Microscope

The AFM analysis provides the measurements of average grain size the figures (1) shows typical surface AFM image (in three and tow dimensional) and the granularity cumulating distribution for curcumin/TiO_2NPs. The average diameter is 68.24 nm.

XRD

The X-ray diffraction pattern of the synthesized TiO_2nanoparticles is shown in Fig.2 reports that absence of spurious diffractions indicates the crystallographic purity. The experimental XRD pattern agrees with the JCPDS card no. 21-1272 (anatase TiO_2). The 2Θ at peak 25.4° confirms the TiO_2 anatase structure. Strong diffraction peaks at 25° and 48° indicating TiO_2 in the anatase phase. There is no any spurious diffraction peak found in the sample. The 2 Θ peaks at 25.27° and 48.01° confirm its anatase structure. The intensity of XRD peaks of the sample reflects that the formed nanoparticles are crystalline and broad diffraction peaks indicate very small size crystallite.

IR analysis

TiO_2 nanoparticles the TiO_2 absorb between 500 and 656 cm^{-1} and 850-950 cm^{-1} region of IR spectra. The peak at 531 and 661 cm^{-1} suggest the Ti-O stretch band (Broad) in anatase phase. The broad stretching hydroxyl peak between 3500 and 3000 cm^{-1} for curcumin, the characteristic sharp peak of C=O,C=C stretching at 1628 $cm^{-1}$the peak at 1603 cm^{-1} of symmetric aromatic C=C, enolic C-OH peaks of curcumin at 1429 and 1376 cm^{-1}.The peaks at 1628 1663,1429 ,and 1376 cm^{-1} were diminished, and a strong peak at 1654 cm^{-1} appeared, representing a conjugated C=O bond. The O-H peak broadened and shifted to 3422, meaning that more hydroxyl bonds formed in the conjugated product, while the enolic (C-OH) peaks were not found, consistent with the C=O

Cytoxicity assay

In this study, a cytotoxicity assays of curcumin/TiO_2NPs ware assessed on cancer cell lines (HeLa). The results indicated that curcumin/TiO_2NPs had an inhibition effect on HeLa.When the concentration of curcumin/TiO_2NPs was reduced by serial dilution, the effects on HeLa cell-lines were also increased.The calculated inhibition rate indicated that the curcumin/TiO_2NPs was more cytotoxic to HeLa cell lines.

$$\text{Inhibition Rate\%} = \frac{\text{Absorbance of negative control- Absorbance of test}}{\text{Absorbance of negative control}}$$

Theoretical study

Theoretical study using PM3 (semi empirical molecules orbital calculations) also DFT calculations. The energies of HOMO (heights orbital occupied and LUMO lowest unoccupied orbitals have been computed for curcumin predicted UV spectra and IR vibration and optimization structure for curcumin.

Geometry structure of curcumin

To study theoretical of curcumin show optimization structure have minimum energy figure 7.

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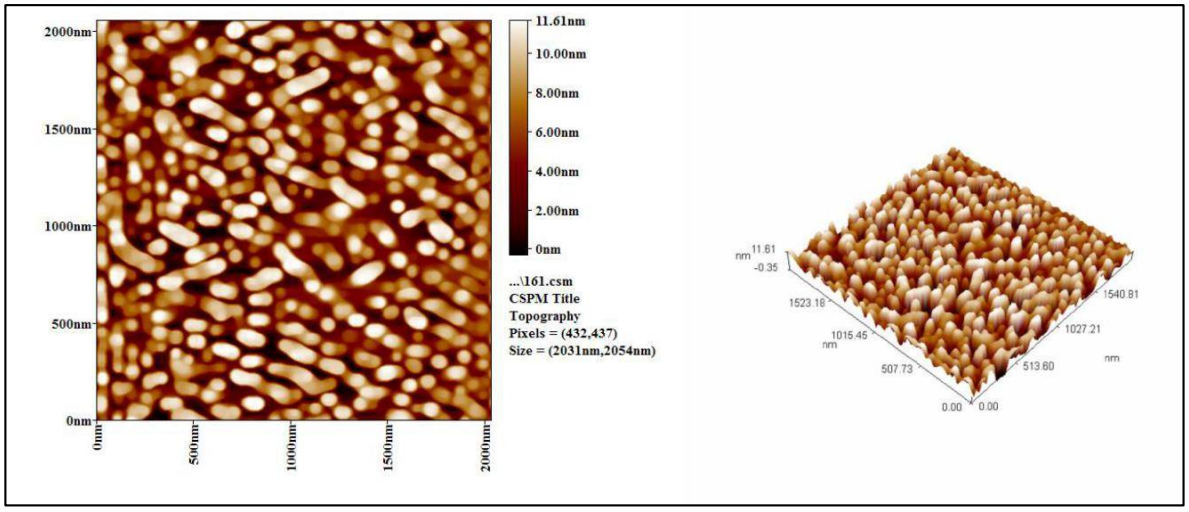

Figure 1.View of AFM image of curcumin/TiO_2NPs

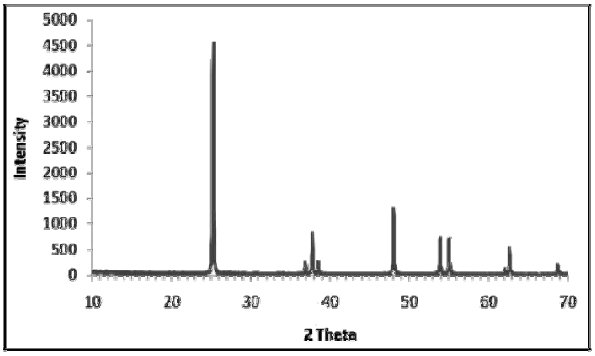

Figure 2. XRD for of TiO_2NPs

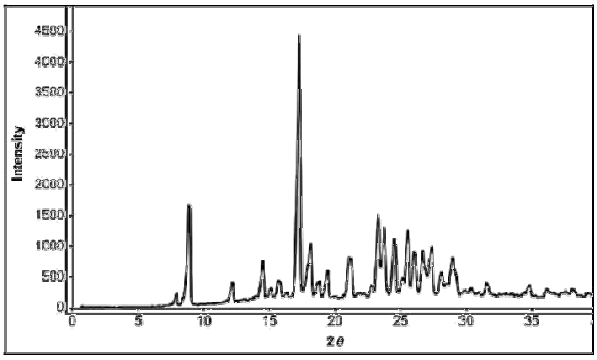

Figure 3.XRD for of curcumin NPs

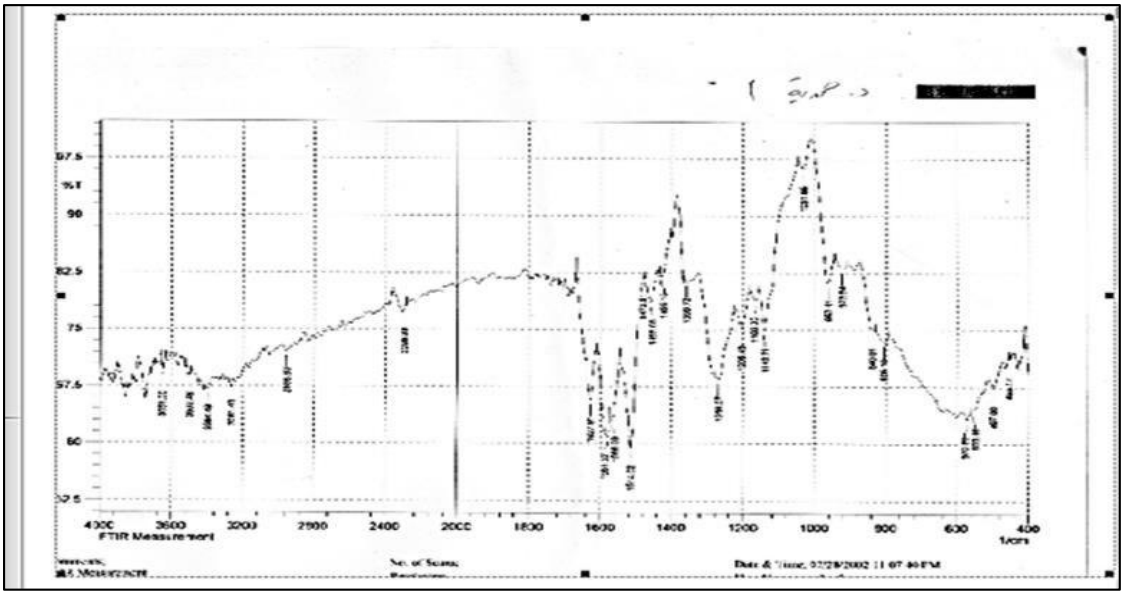

Figure 4. IR of curcumin/TiO_2NPs

Table 1. (I.R. %) of curcumin/TiO_2NPs

Concentration µg/ml	(I.R. %)	Viability %
5	52.15	57.85
1.5	55	55
2.15	55	55
5.515	55	55
5.1215	55	55

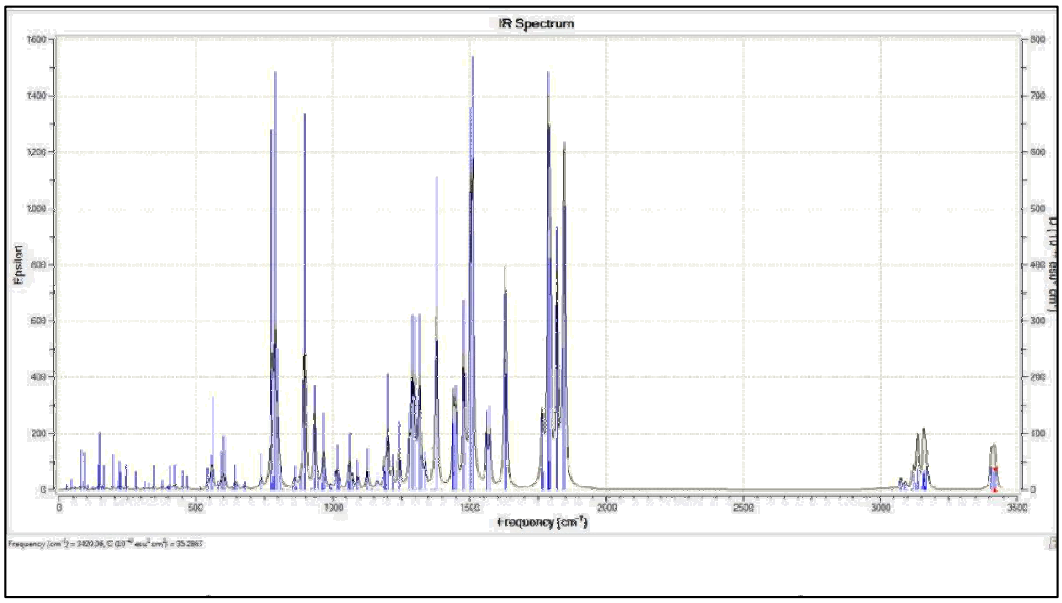

Figure 5. IR of curcumin theoretical

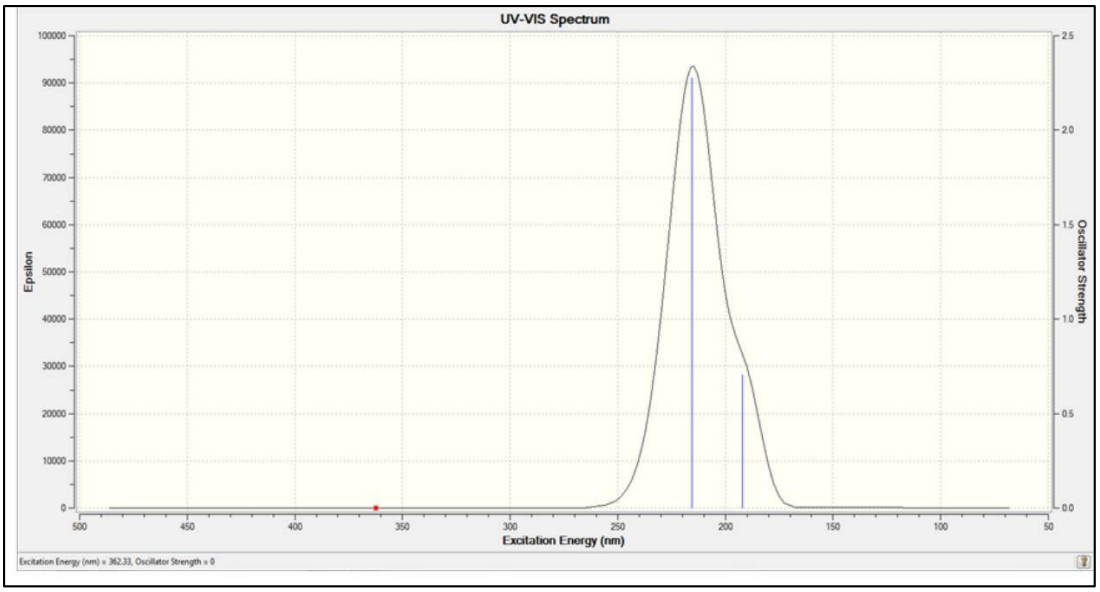

Figure 6. UV- spectrum of curcumin theoretical

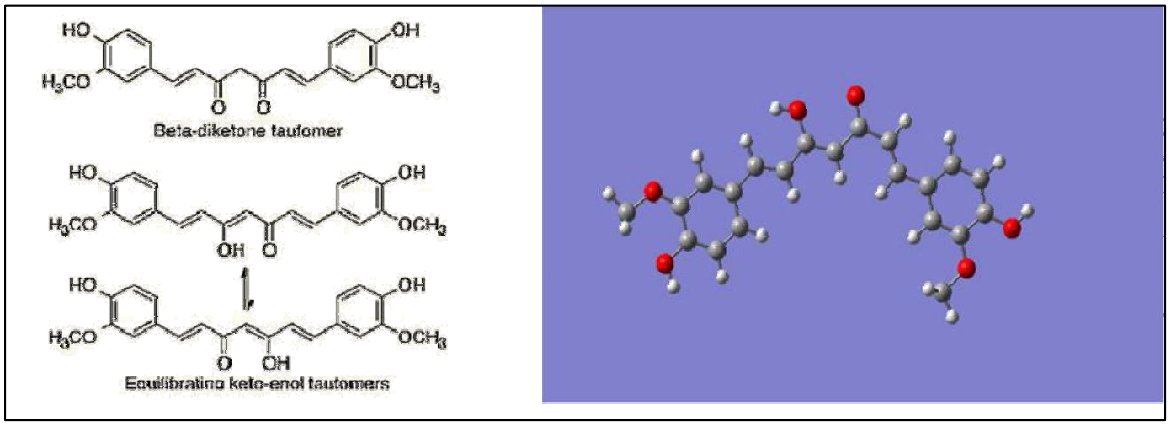

Figure 7. Optimization structure of curcumin theoretical

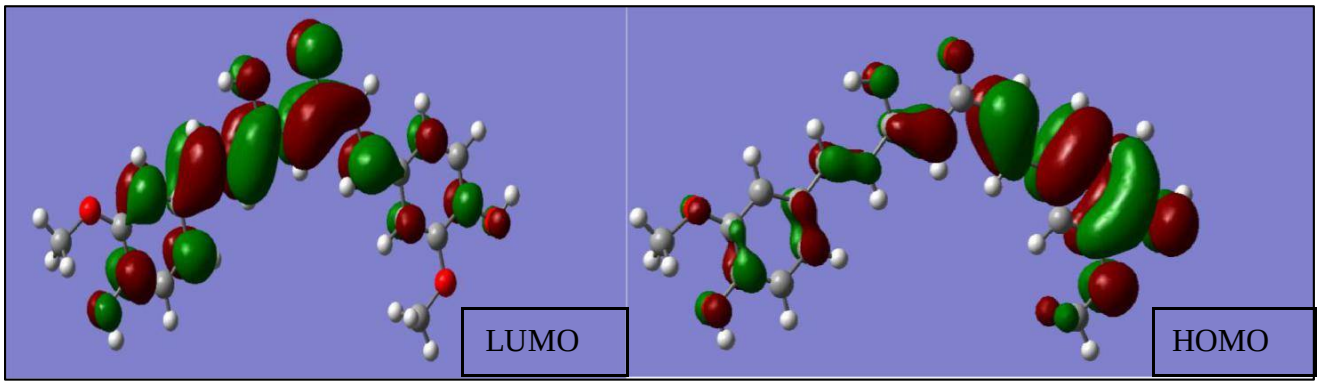

Figure 8.HOMO and LUMO orbitals for curcumin

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RESEARCH ARTICLE

Effect of Pomegranate Seed Oil on Glycemic Index in Diabetic Rabbits

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ABSTRACT

This study aimed to investigate the effect of oral administration of pomegranate seed oil on diabetic in male rabbits. A total of 32 adult male rabbits.6 months old weighting 1-2kg were divided in to four equal groups. G- Control (negative) Rabbits were received olive oil, G2- (control positive) Rabbits were received (30 mg/kg B.W/daily of the Pomegranate seed oil orally and daily for 45 day. G3-Rabbits were received (150mg/kg of Alloxan injected Intraperitonealy, G4-Rabbits were received the same dose orally of the Pomegranate seed oil four 45 days. There is significant increase (Insulin, Insulin resistance) and decreased glucose in group received Pomegranate seed oil (G4) as compared with G3 (diabetic group). On conclusion, the administration of Pomegranate seed oil at dose (30mg/kg B.W/daily) for 45day act as anti-diabetic by reducing hyperglycemia.

Key words: Pomegranate Seed Oil, Diabetic, Glycemic index

INTRODUCTION

Diabetes mellitus is one of the most important public health challenges with an enormous economic burden worldwide. The estimated number of diabetic patients worldwide was 366 million in 2014, and it is projected to rise to 552 million by 2030 [1]. In recent decades, lifestyle interventions, including dietary micronutrients or functional food supplementation have generally been used to improve glycemic levels and have been incorporated into guidelines for the prevention and treatment of diabetes [2].The pomegranate is one of the first cultivated fruits for its beneficial properties and it has been described as a paradise fruit. Recently, pomegranate seed oil (PSO) has received considerable dietary attention. The oil's possible beneficial effects have been attributed to its main bioactive component, punicic acid (*cis9, trans11, cis13CLnA*; conjugated linolenic acid), which constitutes64-83% of PSO [3, 4]. studied widely for its therapeutic potential. An in vitro study showed punicic acid ameliorate tumor necrosis factor-α

(TNF-α) induced protein dysfunction therefore glucose uptake and insulin resistance may improve after administration of the punicic acid [5]. There is vidence indicates that pomegranate fractions from different parts of the fruit have been used to prevent and treat a wide range of diseases, including CVD, hypertension, obesity, and diabetes [6].PSO may reduce the risk of type 2 diabetes by ameliorating high fat diet-induced obesity and insulin resistance [7,8]. PSO reduces body weight, leptin and insulin levels, enhances glucose tolerance, improves peripheral insulin sensitivity, increases carbohydrate oxidative capacity, and inhibits the progression of type2 diabetes [9] .This study was designed to investigate the anti-diabetic effect of pomegranate seed oil on diabetes mellitus experimentally in rabbits.

MATERIALS AND METHODS

The current study has been conducted on 32 male Rabbit , their weights between (1-2)kg, and their ages abought 6 months .they were allocated after acclimatization for 45 days in the animal house of the college of science ,Baghdad University and Animal were randomly divided into 4 equal groups , (8/group) . They were treated daily for 45 days as follows:

1-Group 1(G1): Animals were administrated olive oil (negative control)

2-Group 2(G2): Animals given PSO (30mg/kg) orally administrations through stomach tube (positive control)

3-Group3 (G3): Animals were received single dose of alloxan monohydrate 150mg /kg BW intraperitonealy for diabetic induction [10].

4-Alloxan - PSO Treated (G4): They were received single dose of alloxan monohydrate 150mg/kg BW intraperitonealy ,after 7days they were received 30mg/kg BW of PSO for 45 days [11] diabetes was induces in diabetic and diabetic treated groups by a single intraperitonealy (IP) injection of alloxan monohydrate (150mg/kg of body weight), (Sigma chemical Co., USA).The rabbits were fasted 12 h before and 12 h after alloxan injection then, after 7 days from alloxan received. Pomegranate was administrated orally for 45 days. After that, the blood was uptake via cardiac puncture technique [12]. This serum were used for biochemical determination including (glucose, insulin, insulin resistance)..Data were subjected to one – way ANOVA and LSD was applied to assess to significant differences among mean SAS (2012).

RESULTS

Effect of pomegranate seed oil (PSO) on glycemic index glucose (mg/dl), insulin (µIU/ml) and insulin resistance (IR)

The mean value of serum glucose and insulin concentration in different treated and control groups were clarified in table (1-1) in addition to insulin resistance .It has been found that there was significant ($P<0.05$) decrease in glucose concentration in the diabetic group receiving PSO (G4) (127.00±9.63) as compared with diabetic group receiving alloxan (G3)(484.00±26.91). At the same time there was no significant ($P \leq 0.05$) difference between control group (G1) (131.00±79) and G2 and G4 group (128.0±6.4) (127.0±0.63) respectively as show in figure(1). Serum insulin of the diabetic rabbits receiving PSO (G4) was significantly ($P<0.05$) increase (10.97±0.72) than that of diabetic group (G3) (6.11±0.26). Meanwhile, there was no significant ($P<0.05$) differences observed in control (G1), (G2) and (G4) groups comparing with (G3) group as show in figure(2).

The result in table (1-1) also showed that serum insulin resistance (IR) of diabetic group (G3) (7.28±1.04) increased significantly ($P<0.05$) as compared with diabetic group receiving PSO (G4) (3.13±1.57) and control group G1

(3.55±0.83), within the time non-significant (P<0.05) differences of IR was observed in G2 group (3.97±1.94) as compared with G4 and control group as show in figure (3).

DISCUSSION

The findings of the present study indicate that administration of PSO characterized by decreased serum glucose, increased insulin as well as with decreased insulin resistance. The mechanism of a PSO induced increase in serum insulin is not clear. However, it might be related to the up regulation of peroxisome proliferators activated receptor – □ (PPAR-□) genes (13) Also, PSO ameliorate tumor necrosis factor –α (TNF-α) induced protein dysfunction (TNF-α increased insulin resistance) therefore, glucose up take and insulin resistance may improve after administration of PSO [14]. Thus, it is expected that the use of Pomegranate seed oil (PSO) in diabetic condition or insulin resistance, impaired glucose, lipid metabolisms and increased of oxidative stress [15]. Recently investigations suggest that PSO may reduce the risk of diabetes by ameliorating high fat induced obesity and insulin resistance [8] . The levels of fasting blood glucose were only decreased by punicic acid, methanolic seed extract, and pomegranate peel extract. Known components in pomegranate (punicalagin and ellagic, gallic, oleanolic, ursolic, and uallic acids) were found to have anti-diabetic effect [16]. Parmar and Kar (2007) reported that the administration of 200 mg/kg of pomegranate peel extract normalized all the adverse changes induced by alloxan, a widely used compound for inducing diabetes mellitus since it increases the serum levels in mice [17]. Das and others (2001) investigated the hypoglycemic activity of pomegranate seed extract in rats made diabetic by streptozotocin. The seed extract (300 and 600 mg/kg, orally) caused a significant reduction of blood glucose levels in induced diabetic rats of 47% and 52%, respectively, after 12 h [18].

The main compounds that present antidiabetic properties are polyphenols, which may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut. Several in vitro studies in cultured cells have shown that polyphenols may increase glucose uptake by peripheral tissues,which would diminish glycemia [19].The mechanisms include inhibition of gluconeogenesis [20], adrenergic stimulation of glucose uptake [21], and stimulation of insulin release by pancreatic β-cells [22].

CONCLUSION

Aloxan monohydrate with 150mg/kg B.W caused diabetes. Intubation the animals with pomegranate seed oil 30mg/kg B.W act as anti-diabetic by reducing hyperglycemia and oxidative stress which result from diabetes due to its antioxidant properties. Natural products as an alternative source of medicinal compounds are interested in the researchers' view point due to their safety, low adverse effects, easy to earn and popularity, therefor, Pomegranate or any of its extracts can be medically recommended for the management of type 2 diabetes [23].

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Table1-Effect of pomegranate seed oil (PSO) on glycemic index. Glucose (mg/dl), insulin (µIU/ml) and Insulin resistance (IR).

LSD	G4	G3	G2	G1	Group / Para meter
48.713	127.00±9.63 B	484.00±26.91 A	128.00±6.43 B	131.00±7.94 B	**Glucose**
4.271	10.97±0.72 A	6.11±0.26 B	12.00±2.09 A	11.00±1.8 A	**Insulin**
3.973	3.13±1.07 B	7.28±1.04 A	3.79±1.94 B	3.55±0.83 B	**Insulin resistance**
*** (P<0.05)** **Means having with the different letters in same column differed significantly**					

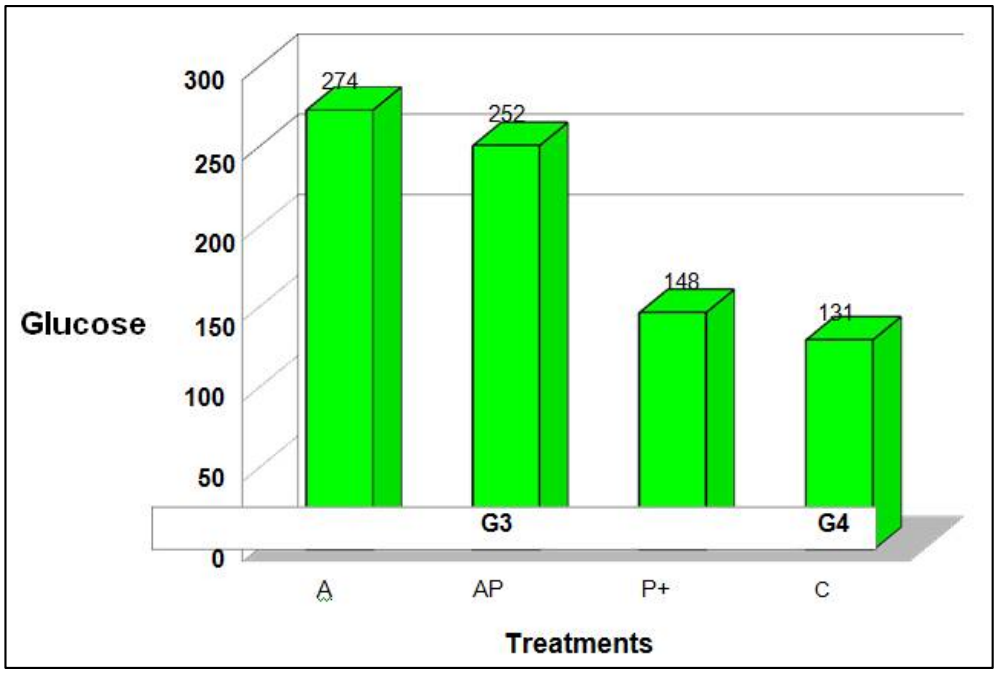

Figure 1. The effect of different treatment among animals groups in Glucose

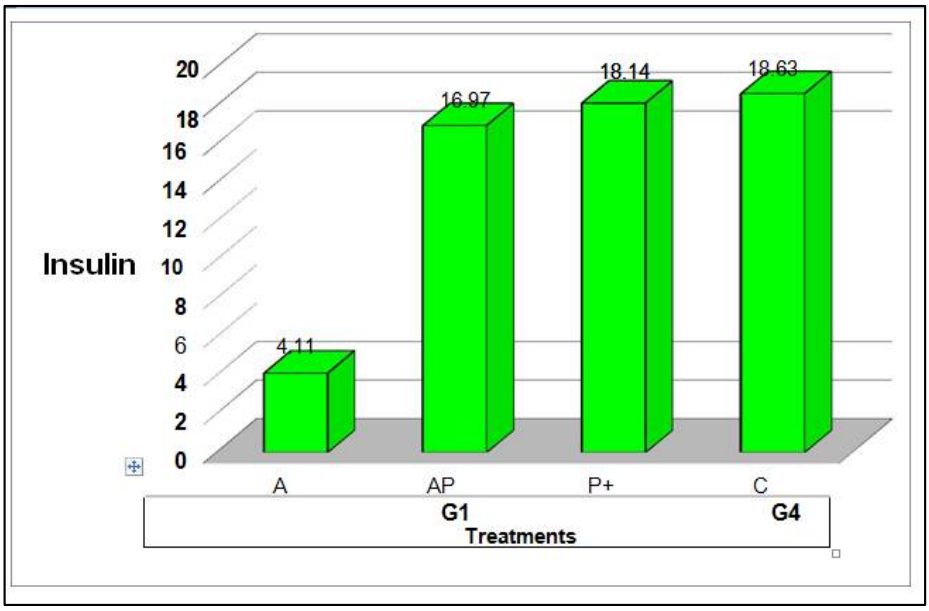

Figure 2. The effect of different treatment among animals groups in insulin

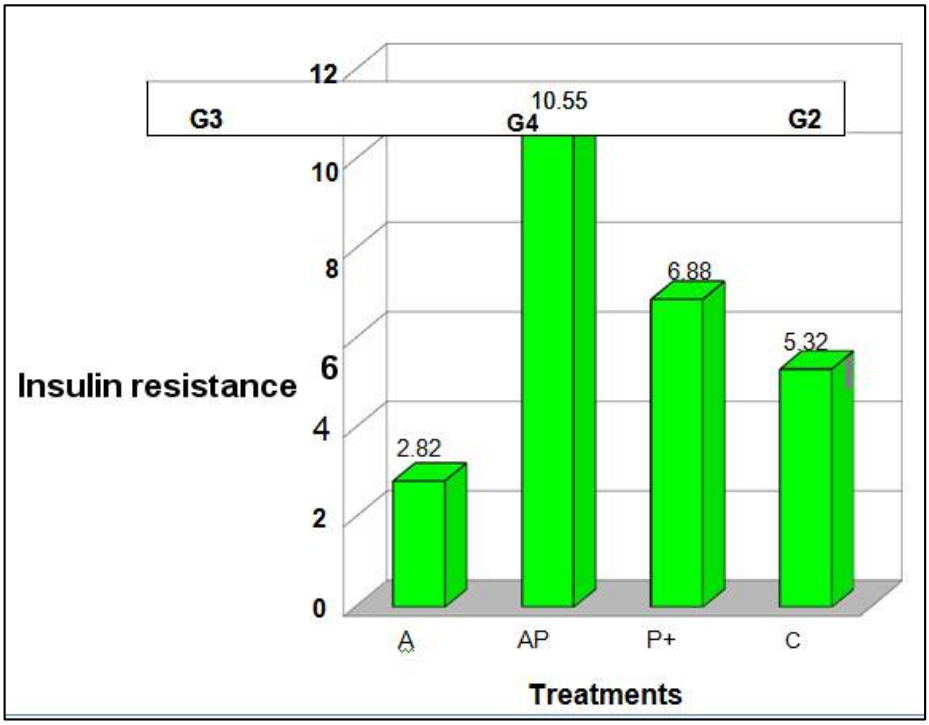

Figure 3. The effect of different treatment among animals groups in sulin resistance

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RESEARCH ARTICLE

Peri Hatching Development of the Lens in ISA Brown Chicken (*Gallus gallus domesticus*)

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ABSTRACT

The present work wasinvestigated the developmental events of the lens in Isa brownchicken.A total 120 fertilized egg samples of Isa Brown chicken were allocated into 29 periods involved (20, 24, 28, 33, 38, 43, 48, 53, 58, 63, 65, 69, 72 , 77, 82, 87, 92 and 96 hours) (5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17 days), (Three eggs for each).The tissue sections were stained with H&E stain.The development of the eye was divided into undifferentiated and differentiatedperiods; the undifferentiated period was started by period of 33 hour at which the eyes primordium was seen as narrow grooves of neuroectoderm of the forebrain. During the 38 to 44 hours of incubation period the grooves transferred into primary optic vesicle.The age of 61 hours showed development of the optic cup and lens placode. The 65 hours showed lens placode invagenate the optic cavity. The period of 69 hours showed formation of lens vesicle. The 3rd to 4th day's embryosdisplaying increased in size of the optic cup and lens placode. The differential periods have started at the 5th day at whichthe proliferating and migrating cells turned into lens fibers at central region of lens.At period of 7 days the lens showed development of lenticular capsule. During 12-16 days embryos the lensanterior surface and lateral parts are covered with epithelium and proliferation of lenticular cellswas continuing.At periods of 18-19 days embryos the lens has complete its development.

Key words:- lens, eye development, peri hatching,chicken.

INTRODUCTION

The eyes in birds differed from those in mammalian, the main differences are restricted by the shape, sized,presence of ossicles oculi and the sclerotic ring cartilage that surrounding the eye [1], [2], [3]. Birds have fixed and immovable eyes and there for the birds has single occipital condyle making them to rotate the head in 360 to increase the vision

area [4].The eye of bird as in other vertebrates consists of fibrous, vascular and nervous tunica in addition to eye lens [5].The lens is located between the posterior chamber and vitreous body, it is surrounded by a capsule followed by the annular pad area, it attaches to the wallet at the zonular fibers, this device is important inaccommodation, allows focusing on close and distant objects by changing the lens curvature, the lens covers bylow simple cuboidal epithelium, the lens's equator, the posterior part of the lens consists of long and flat fibers in the form of ribbed circular cells with thick, pointed end [3], [5].The birds have a softer lenscan which altering their focal lengths to allow accommodation also the lens shape varies from spherical to ellipsoid [1], [6]and the refractive index may be graduated or uniform to provide a wide range of optical capabilities [7]. In vertebrate lens growth taking place in a unique and ubiquitous mechanism when newly formed epithelial cells in the anterior and marginal zone then migrate through the equatorial zone and differentiate into fiber cells [8]. The aim of thestudy is to determine the onset time of lens evolution and time at which completion in chick.

MATERIALS AND METHODS

A total 120 fertilized egg samples of Isa brown chickenwere brought from Al-Mutasem Company for eggs production in Baghdad governorate. Egg samples were allocated into 29 periods involved (20, 24, 28, 33, 38, 43, 48, 53, 58, 63, 65, 69, 72 , 77, 82, 87, 92 and 96 hours) (5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17 days), (Three eggs for each).To ensure a high hatching percentage and hygiene the egg samples have washed up with warm water then dry and sterilized by fumigation with formalin steam, then the samples have introduced to the hatchery and eggs were extracted successively according to the required ages. For histological study, in early embryonic stages (20 hours up to six days), the whole embryos has dehydrate by passing them for 30 minutes in a series of ascending up grades of ethanol (30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), then clear in xylen for 15 minutes. After that the embryos have embed in paraffin wax for one hour. Finally blocks have sectioned serially at 5-6 µm thickness. In embryonic stages (Seven days and up) the whole embryos has dehydrate by passing them for two hours in a series of ascending up grades of ethanol (50%, 60%, 70%, 80%, 90% and 100%), then clear in xylen for 30-60 minutes[9],[10]. After that the embryos have embed in paraffin wax for one –two hours. Blocks have sectioned serially at 5-7µm thickness and the sections will be stain with Harris Hematoxylin and Eosin stain [11].

RESULTS

Undifferentiated periods: The results of undifferentiated periods have started at 33 hour embryos at which the eye primordium was seen as pair of narrow grooves on either sides of the forebrain and composed of neuroectoderm cells and at periods of 38 to 44 hours embryos, the ectoderm cells developed into two bilateral outpocketings which representing the evolution of the primary optic vesicle that surrounded by cephalic mesoderm (mesenchymal tissue) and anteriorly covered by superficial ectoderm (fig.1&2). At 61 hours embryos the head region showed development of the optic cup and lens placode which evoluted at a thickening of superficial ectoderm cells,the optic cup was composed of inner thick sensitive layer and outer thin pigmented layer and the two layer were separated by a lumen (intra retinal space)(fig.3).At 65 hours embryos the lens placode was invaginated as a cellular mass toward the cavity of optic cup (fig.4). The period of 69hours embryos showed formation of lens vesicle which displaying highly proliferative cellshad a clear cavity andat this period the lens vesicle detached completely from superficial ectoderm (fig.5 & 6). During periods of the 3rd and 4th days embryos the evolution showed increased in size of the optic cup and lens vesicle which occupied most space of cup cavity (fig.7 & 8).Differentiated periods:At periods of 5 to 6 days embryos the lens has composed of outer layer which showing mass of proliferating and migrating cells those turned into primary lenticular fiber at central region of lens in order to fill the cavity of lens vesicle (fig.9&10).At 7 days embryo the lens showed development of lenticular capsule which appeared thin at the anterior and posterior surfaces (fig.11). During 12 to 16 days embryos the lens appeared as biconvex disc and its posterior surface and lateral parts were covered with the stratified epithelium "sub capsular epithelium". The proliferation of lenticular cellswas continuing at the level of the equatorial zone and atthe back of the lens the lenticular fiber showed elongated nuclei (fig.12).At 18 to 19 days embryos the lens showed complete its development by arrangement of the lenticular

fibers in parallel pattern and their nuclei have positioned at the center of lens and epithelium turned into low simple cuboidal epithelium (fig.13).

DISSCUSION

The eye of bird is more complex organ [12], [13], in chick as in other vertebrate the eye primordium has arises from the neuroectoderm of the fore brain and superficial ectoderm& [5], [14],[15],[16]&[17]such development is under the effect of the growth factors released by fore brain which leads to development of retina, iris and optic nerve, meanwhile the superficial ectodermal responsible for evolution of the lens[18].The current results revealed two periods of eye development, this result compatible with result of [12].Present study showed thatthat in chick the eye evolution was at 33 hours ofincubation period, this result dissimilar that recorded in mallard duckby [5] such variation was due to the long period of hatching in duck (28 days).Current result revealed that the period of 61 hours chick embryo manifested by evolution of the optic cup and lens placode, this result parallel with results of[14]. [15]&[16]. In compare with results of other studiesthe evolution time of the lenticular disk in vertebratesis varies and depending on the animals species, in chickens was recorded at 53 hoursof incubation [19], in Japanese quail was at 50 hour incubation [20]., in mallard duck was at 52-hour [5]. During 69 hours the lens placode has a cavity and detached completely from superficial ectoderm, such observation has mentioned by[5],[21]or remains in some vertebrates as mammals[22]. Rresultsrevealed that the at the ending of undifferentiated periods (4th days)indicates an increased in size of the optic cup and lens due to cells proliferation such result agree with result of [5].&[23] thissuggest that the differentiated periods required an optimal cell masses of eye in order to develop their tunicae.The current result revealed that the periods of 5 days chick embryo was the time at which the lensmass displaying development of primary groups of lenticular fibers at central region of lens, such observation has mentioned by [5], [14],[16]&[18], also [24] has stated that the at the posterior wall of the lens vesicle the cells begin to lengthen to form the primary lens fibers which fill the cavity of the vesicle.The periodof 7th days embryosshowedappear the lenticular capsule with marked elongation of lenticular fibers such result also has recorded by [5] at 6-8 days mallard embryo,also [25]observed that the lens vesicle is consists of an outer wall that represents epithelium and an inner wall consisting of elongated cells known as the lens cells.The current study showed that the mitotic division of lenticular cells is continued until 12-16 daysembryos that agree with result of [24]who refereed that the lens growth will not stop at this stage and secondary lens fibers are added to the center of the lens continuously,the lens epithelium was developed such observation has recorded by [5] in mallard duck and [17], also [6]&[7] whom showed that the growth of the lens occurby proliferationof the epithelial cells and differentiate into fiber cells that build up blocks for constructing a refractive index gradient by packing mature fiber cells into the central nuclear area through the removal of water, such process of proliferation was recorded by [26]. At periods of 18-19 days embryos the lens complete its development by arrangement of the lenticular fibers in parallel pattern and nuclei have positioned at the center of lensand the growth of lens was in theresting, this result compatible with [27] & [28] who mentioned that the differentiation of the lens placode into a transparent tissue capable for directing the light on the retina involves changes in the shape and composition of the cells as well as the synthesis of the lens protein known as "Crystalline".The current study, concluded that lens growth took place through two stages: Growth and development, the first one was early and fast while the second was slowoccur during differential period and the proliferation of the lens fibers has stopped nearly in 1-2 days peri hating .

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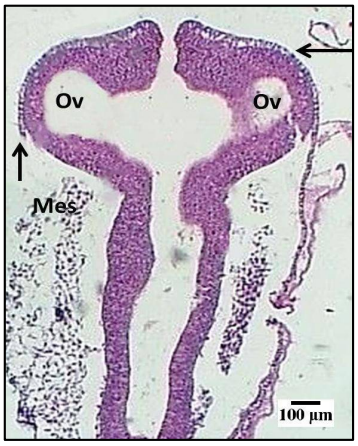


Figure 1: Section at the cephalic end of chick embryo (38 H) shows: Bilateral optic vesicle (Ov), cephalic mesoderm (Mes) & superficial ectoderm (arrows). H&E stain

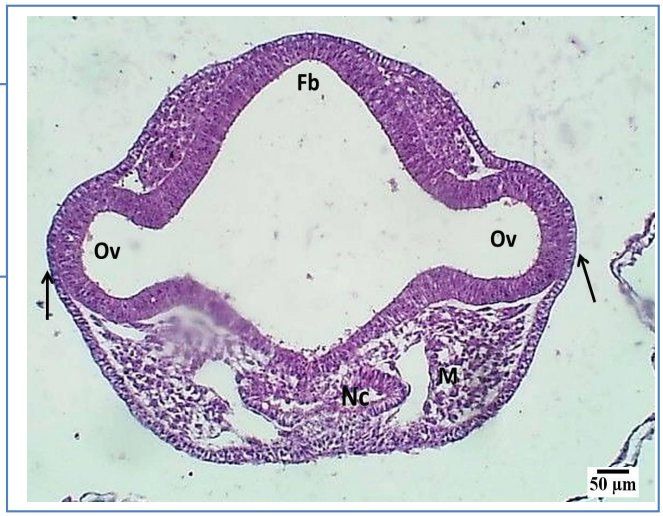


Figure2: Transverse section at cephalic end of chick embryo (44 H) shows: optic vesicle (Ov), cephalic mesoderm (Mes), notochord (Nc) & superficial ectoderm (arrows). H&E stain

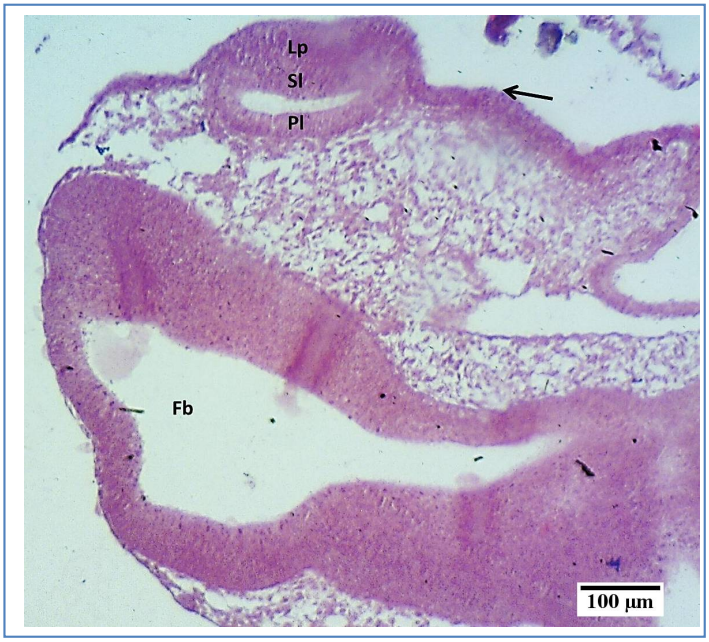


Figure 3: sagittal section at cephalic end of chick embryo (61 H) shows: sensitive layer of optic cup (Sl), pigment layer of optic cup (Pl), lens placode (Lp) &superficial ectoderm (arrows). H&E stain

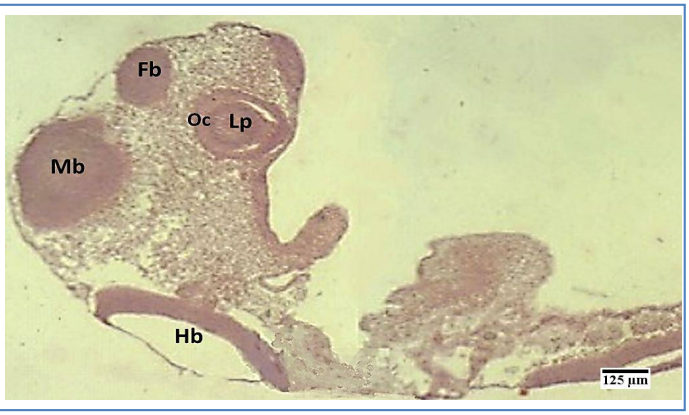


Figure 4: sagittal section at cephalic end of chick embryo (65 H) shows: optic cup (Oc), lens placode (Lp) & fore brain (Fb), mid brain (Mb) & hind brain (Hb). H&E stain

Figure 5: Sagittal section at cephalic end of chick embryo (69 H) shows: Optic cup (Oc), lens vesicle (Lv), fore brain (Fb), heart (H) & cephalic mesoderm (Mes). H&E stain

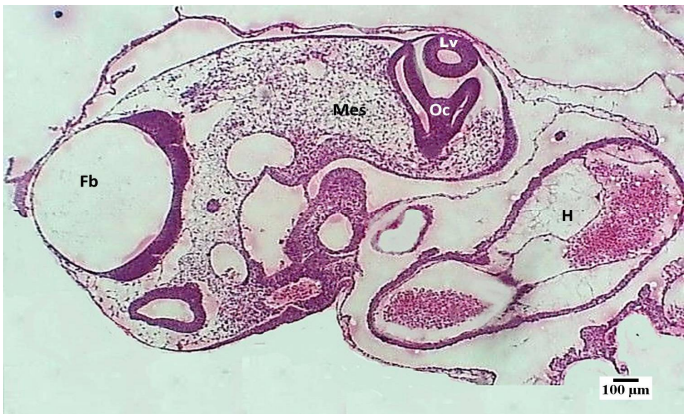


Figure 6: Magnified section of optic cup of chick embryo (69H) shows: optic cup with sensitive layer (Sl), pigmented layer (Pl), lens vesicle (Lv), optic cavity (Oc), cephalic mesoderm (Mes), superficial ectoderm (Se) &

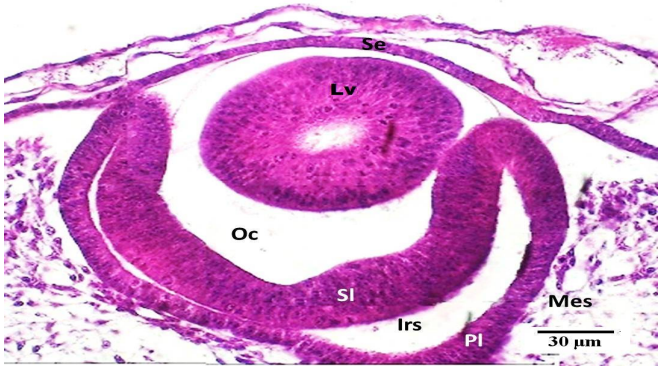


Figure 7: Sagittal section at head region of chick embryo (4 Days) shows: Optic cup (Oc), lens (L) & cephalic mesoderm (Mes). H&E stain.

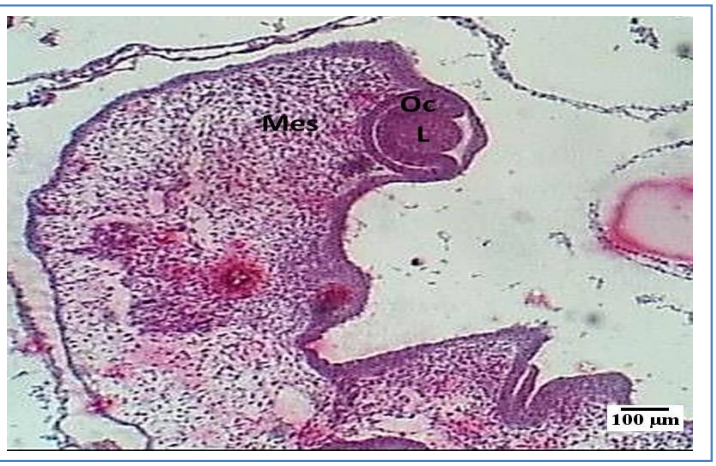


Figure 8: Magnified section of the eye of chick embryo (4 Days) shows: Lens (L), sensitive layer (Sl), pigmented layer (Pl), cephalic mesoderm (Mes), superficial ectoderm (se) and intra retinal space (arrow). H&E stain

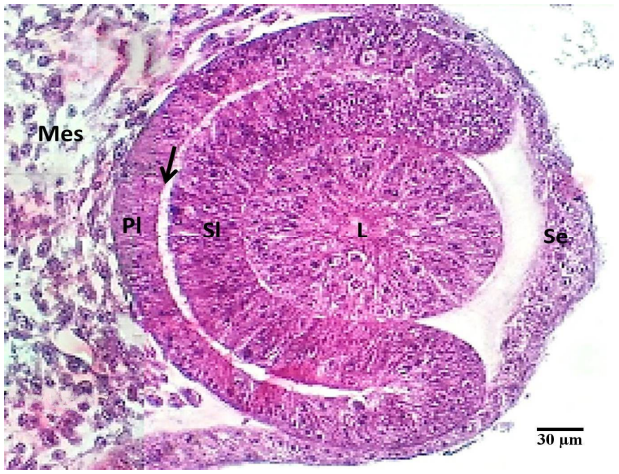


Figure 9: Superficial section of chick embryo (5 Days) shows: eye cavity (Ec), lens (L), brain (B), Heart (H), primitive digestive canal (Dc) & spinal cord. H&E stain

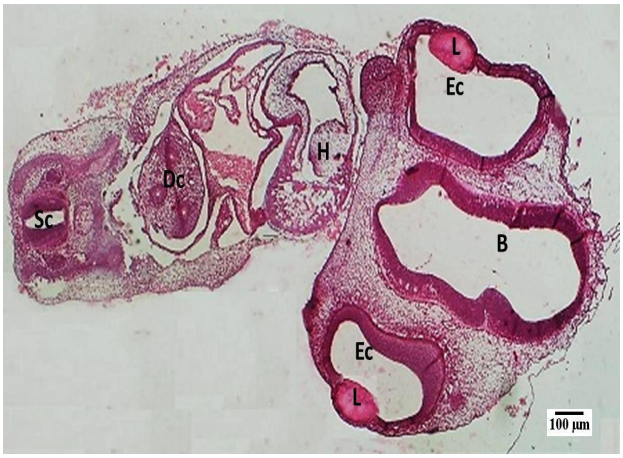


Figure10: Magnified section of lens of chick embryo (6 Days) shows: remnant of the lens vesicle cavity (C), corneal primordium (Black arrow), nuclei of lenticular fibers (Yellow arrow), dividing and migrating lens cells (Red arrows). H&E stain.

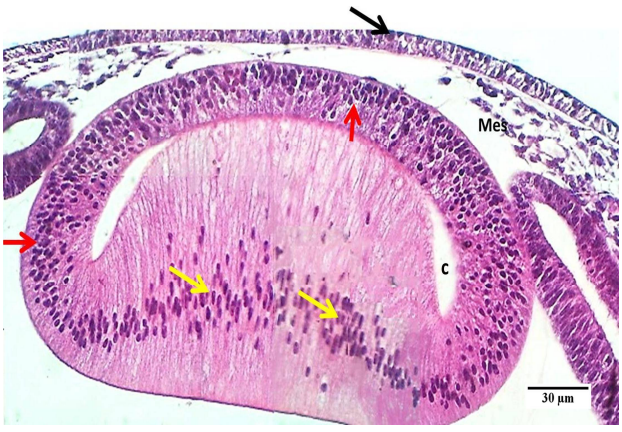


Figure 11: Magnified section of the lens chick embryo at (7 Days) shows: eye primordium of cornea (Co), capsule (Red arrows), lenticular fibers (Black arrows) & equatorial line of lens (Eq) .H&E stain

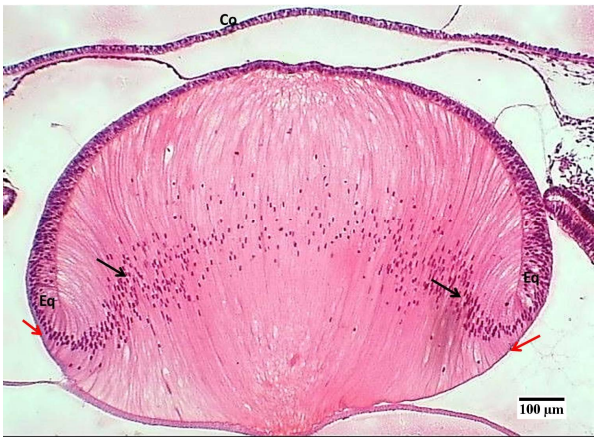


Figure 12:Magnified section at pecten oculi (16 dayschick embryo) shows: posterior epithelium (Pe), equatorial line (El) & nuclei of migrating lenticular fiber (arrows) H&E stain

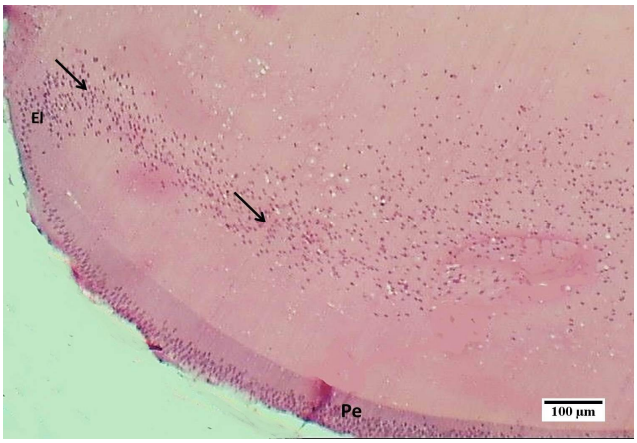
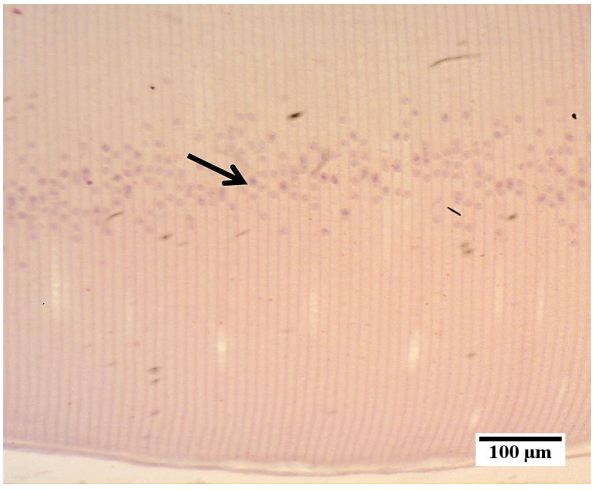


Figure 13: Magnified section lens oculi (19 dayschick embryo) shows: nuclei of lenticular fibres (arrows) H&E stain



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RESEARCH ARTICLE

Impact Strength of Dewaxed and Bleached Natural Fiber Composite

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ABSTRACT

This research was carried out to study the effect of different chemical treatments of coconut coir fiber on impact strength of masturbated poly ester composites. These treatments including dewaxed and bleached fiber. The weight fraction of coconut coir of all composites is constant (20% wt).the results were compared with row fibers. The results showed that the bleaching and dewaxing treatment improved the impact strength by about 43.3 % and 39% respectively compared with row fiber.

Keywords: coconut coir fiber, bleaching, dewaxing .impact strength.

INTRODUCTION

Composites materials are now being more important in todays products, they are used in many industries such as aerospace, construction, auto motive is recreational [1]. In recent years coir fiber composites attracted a huge concern in the composts material research community as well as in industry. Wide ranges of research has recently been carried out on fiber reinforced polymer composites[2]. Because coir fibers have a range of potential advantages such as low specific weight, producible , friendly processing , good thermal and acoustic insulating properties , wide range of researches has been done of fiber reinforced polymer composited [3] . If the hydrophilic fibers and hydrophobic polymers are not compatible, it is necessary to add another material with intermediate properties between the fibers and polymers. Actually, this method can progress the properties of the fiber such as strength, surface, amount of impurities and matrix fiber interaction. Chemical modification methods can help to promote the interfacial adhesion between fibers and polymers Resulting in better overall mechanical properties and minimized water absorption .In order to exceed the water absorption of fiber , using hydro phobic aliphatic and cyclic structure for treating fiber have been developed . These cyclic structures consist of reactive groups having the possibility to make new bonds with reactive groups on the polymer. Therefore, the goal of chemical treatment of natural fiber is to make the fibers more hydrophobic causing stronger adhesion between matrix and the fiber in composite [4, 5].

Commercially, several chemical treatments like dewaxing , bleaching and acetylation are applied to enhance the mechanical performance by surface properties modifications .Usually , dewaxing is achieved by using benzene or alcohol then treatment with NaoH and finally ,drying at ambient temperature . In bleaching, numerous agents like hydrogen, peroxide sodium, hydrochloric and alkaline calcium are frequently used but this treatment leads to loss of tensile strength and weight. This loss is mainly associated with the action of alkali or alkaline on hemicellulos or lignin [6].A lots of researches are carried out on this field. Samia and her group used polypropylene and polyethylene composites reinforced with coir fiber and study the effect of some chemical treatments on the properties of these composites [7]. Nur etal. Prepared biodegradable kenaf fiber/poly(lactic acid) composites and investigated the Influence of hydrogen peroxide on the mechanical properties of them[8] . Lix. etal studded the chemical treatment of natural fiber for use in natural fiber reinforced composites . [9]. Salam and his group examined the effect of bleaching onto sulfonated jute fiber using hydrogen peroxide [10].

The main aim of this work is to study the effect of different chemical treatments of coconut coir fiber on the impact strength of the unsaturated polyester composts.

EXPERIMENTAL WORK

The unsaturated polyester (up) resin and its hardener methyl ethyl ketone peroxide (MEKP) 2 volume percent was used, The catalyst cobalt naphenate about 1% (volume percent) was added to accelerate the process of hardening. The curing of (up) was done at room temperature (25c°).The coir fiber were obtained from coconut plants which naturally grown in Thailand. The chemical composition and microfibirllar angle of the coir fiber are showed in table 1.[11]. These fibers were washed with distilled water and dried at(80c°) for several hours to overcome the problem of moisture. To achieve a good adhesive between the fibers of matrix, the chemical treatments were applied on the fibers.

For dewaxing, the coir fibers are immersed in a mixture of ethanol and benzene in ratio 1:2 and cooked with these chemical 12 hours under gradual increase and decrease of temperature of the bath from 30-55.5 c°. This process of heating of cooling was done per 2 hours for a period 12 hour. Finally these fibers are removed from the mixture at 30 c° and washed and dried in oven at a temperature 80 c°.In the case of bleaching treatment, the fibers were treated with solution containing (30%, w%w) hydrogen peroxide and sodium hydroxide. The fibers were heated in the solution at 80 c° for 1 hour .During this process temperature was changed alterntray for 30 c° to 85 c°. This process was done for period of 1 hour. Finally, these fibers are removed from the solution at a temperature of 30 c°. Again, they were washed and dried at 80 c°.Charpy impact test was performed using (5Jules) pendulum impact testing machine. The measurements dimensions of spacimen were: length 55mm width and 10mm according to ISO – 179.

RESULTS OF DISCUSSION

Fourier Transform Infrared (FTIR) Analysis

To study the characteristics of the coconut coir fiber before and after chemical treatments, FTIR analysis was used. In the case of bleaching, the hydrogen peroxide was conducted to oxidize the hydroxyl groups from cellulose to carboxyl groups potential. Figure 1. show that the broad peak at 3398 cm-1 in row fiber is due to O-H group for hydrogen –hydroxyl group present in polysaccharide , this peak increases in the intensity in both bleached and dewaxed coir fiber. Peak at 2924 cm-1 (C-H) group increase in dewaxed fiber and disappear in bleached fiber . This absence of peak may be due to the removal of lignin and hemicellulose. The broad peak at 1730 cm-1 in row fiber (Aldehyde C=O stretch) , this peak gets narrower in dewaxed fiber and disappear in bleached in bleached fiber , indicating that most of the lignin has been removal . Because of the presence of hemicelluloses which associated with the C=O group , the weakness in peak occurs at 1384 cm-1 in row fiber , but it is increased significantly after both chemical treatments

Impact strength

Figure 2. show the impact strength of row fiber, dewaxed and bleached fibers. The results show that the bleached fibers had value of impact strength highre than dewaxed and row fibers, while row fiber had the lest value. Also, it can be seen that bleached and dewaxed fibers composites improved the impact strength of row fibers by approximately 43.3% and 39% respectively .As believed earlier, the mechanism of absorbing energy depends on the interfacial interaction between fiber and matrix. Therefore, the increase is due to the improvement in fiber- matrix adhesion of bleached fiber composites. While in the case of row fiber composites the poor adhesion formed a crack in the inner part of the composite which required less energy to break it [12,13].

CONCLUSIONS

The following conclusions could be drown :

1-Impact strength of bleached fiber composites improved by about 43.3% due to the improvement in fiber –matrix adhesion after treatment with hydrogen peroxide. . Whereas dewaxed fiber composites had lower value of impact strength about 39% compared with row fiber composite.

2-From FTIR analysis it can be seen that the peaks at 2924 cm-1 and 1730 cm-1 were disappeared in bleached fiber due to the removal of lignin of hemicelluloses as compared with row fiber . While these peaks in dewaxed fiber get increase and narrower respectively

3-The weak peak occurs at 1384 cm-1 in row fiber because of the presence of hemicelluloses which associated with c=o group , but it is increased significantly after treatments .

4-Bleaching is procedure of one treatment in which improvement in both mechanical properties and physical appearance (discoloration) can be achieved. Therefore bleached fibers can be extensively used in textile industry [13].

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Table 1.Chemical composition and microfibrillar angle of the coir fiber [11].

Chemical composition and microfibrillar angle of the coir fiber	Unit (%)
Cellulose	32-43
Lignin	40-45
Pectin	3-4
Hemicellulose	32-43
Ash	2
Water soluble	5
Micro angle(degree)	30-49

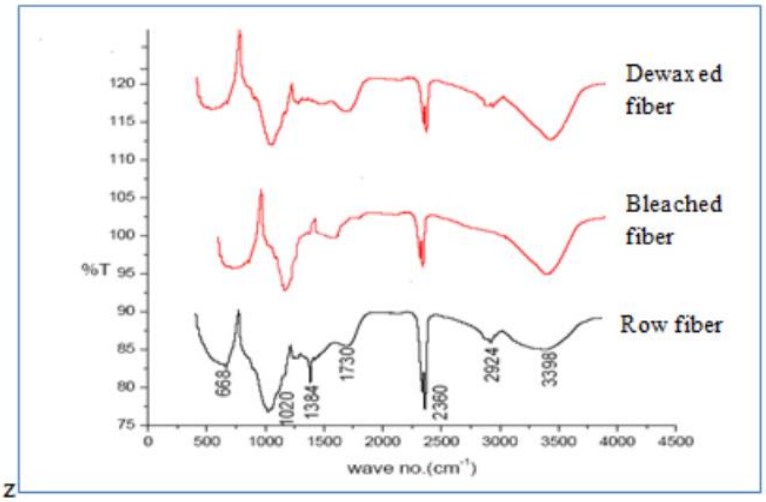

Figure 1.IR spectra for the row and various treated coconut coir fiber composites.

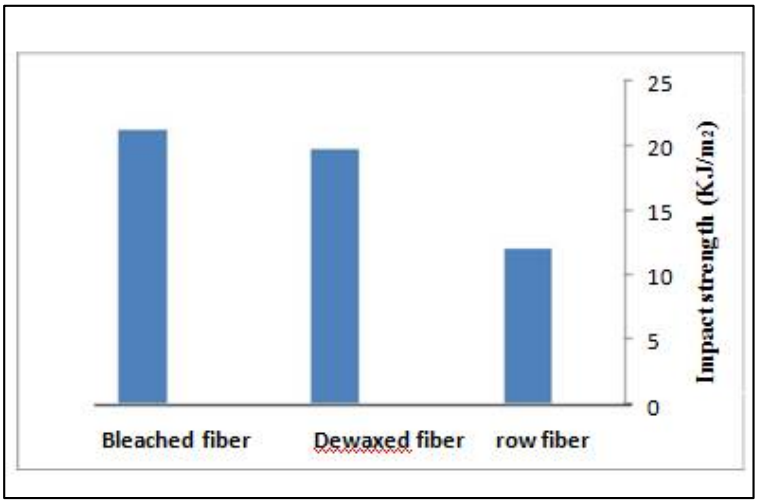

Figure 2: Impact strength graph for the row and various treated coconut coir fiber composites.

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RESEARCH ARTICLE

Preparation and Study of the Effect of Y^{3+} Substitution on the Structural and Electrical Properties for $Tl_2Ba_{n-x}Y_xCa_nCu_{n+1}O_{10+\delta}$ Superconductor System

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ABSTRACT

In the present work, the solid-state reaction technique was used to prepare a high-temperature superconductor of bulk polycrystalline samples type Tl-2223 by substituting Ba^{2+} with Y^{3+} in $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ superconductor compound. The sample at x=0.2 gave the highest value of T_c (137.5K). The x-ray diffraction data confirmed that all superconducting samples had a tetragonal crystal structure with a high ratio of the superconductor phase, Tl-2223.

Key words: - Tl-2223 high, Electrical Resistivity, Superconducting, Critical temperature, Lattice parameters.

INTRODUCTION

High-temperature superconductors have been widely investigated because of their considerable importance in various technological applications [1]. The formula of $MCa_{n-1}Cu_nO_{2n}$, where M refers to $HgBa_2$[2,3], Bi_2Ba_2[4], Bi_2Sr_2[5], $HgSr_2$[6], and Tl_2Ba_2[7], generally describes the superconductor compounds which consist of multilayered cuprates.
The most important group of homologues among all the known high-temperature superconductors is the thallium-based homologue in the form of $TlBa_2Ca_{n-1}Cu_nOy$ [8]. The (Tl-2223) Tl bilayer phase is considered one of the superconducting cuprates of high-temperature which has potential for practical applications, particularly at temperatures approaching 120 K [9].Most researchers have concluded that it is considerably difficult to synthesize the Tl-2223 phase purely because it is often accompanied by the Tl-2212 phase and achieved with small quantities of Tl-1223, which may be attributed to the kinetics, temperature, and the transformation mechanism of the compound

Tl- 2212 to Tl- 2223 [10]. In general, the lanthanum and yttrium superconductor compounds take the chemical formula of $Tl_2Ba_2Ca_nCu_{n+1}O_{6+2n}$ with n= 0,1,2 [11].

Many researchers have reported the superconducting phase to be orthorhombic, but it can be changed to tetragonal phase by substitution with certain rare earth elements. It was found that the phase transformation was related to the redistribution of oxygen atoms in the structure [12] and Tl-2223 has a tetragonal structure with unit parameters, a=b=3.848Å, c=35.58Å, (S.G.I4/mmm139), JCPDS no. (41-1334).The transition temperature (Tc) has been found to be as high as 128K in the bulk form of superconducting cuprate [9,13] and up to 122 K in superconducting thin film form [9,14].

In this work, a single-phase superconducting of thalliated superconductor has been synthesized according the chemical formula $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ (where x= 0, 0.05, 0.1, 0.15, and 0.2) and investigated the effect of substituting the barium ion with the Yttrium ion on the superconducting and structural properties.

MATERIALS AND METHODS

The samples of thalliated superconductor $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ compound (where x= 0, 5, 10, 15, and 20%) were prepared by solid-state reaction method, employing proper weights of powders of high purity (99-99.5%) materials as shown in Table (1). All chemical materials were purchased from Merck, India without any purification.

The equation for the preparation of the thalliated superconductor is given below:

$$2Tl_2O_3+xY_2O_3+2\text{-}xBaO+2CaO+3CuO \longrightarrow Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$$

The raw materials were weighed, mixed thoroughly and grinded for about 12 hours in a vortex mixer. The process was repeated several times to make the mixture more homogenous and to obtain a fine powder.The resulting powders were placed in a drying oven at 80°C for 12 hours continuously. The powders were pressed into pellets (15mm in diameter and 25 mm in thickness) using a hydraulic press at a pressure of nearly 8 ton / cm^2. Finally, the pellets were sintered in an oven at 800° C for 24 hours at a heating rate of 5º C / minute. This was done to make the pellets more cohesive, increase hardness and to reduce the pore size. The oven was turned off and the pellets were left inside until they cooled at R.T.

The lattice parameters determined by using X-ray diffraction (Shimadzu XRD-6000, Japan) with $CuK\alpha_1$ radiation = 1.5406Å.On the other hand the electrical resistivity of all samples was measured by using the common standard four-probe technique.

RESULTS AND DISCUSSION

The structural properties of the superconductor samples were characterized using an X-ray diffractometer. It can be observed from Figure (1) that all patterns of X-ray confirm the existence of the polycrystalline Tl-2223 phase. There are also two phases in all samples of the Tl-base systems, high-T_c phase thalliated superconductor (Tl-2223) as the majority phase and low-T_c phase (2212) as the minority phase. Figure (1) represents the patterns of X-ray of the (Tl-2223 system have found to exhibit a tetragonal structure with the space group (S.G.I4/mmm (139)) related to JCPDS no. (41-1334) which they in a good agreement with the literature [15, 16]. The appearance of another phase related to the tetragonal structure with JCPDS no. 45-0518.The related peaks of X-ray patterns of all samples after indexing confirm that all the patterns have reflections of the high-Tc phase; these reflections singed as (H) peaks and low -Tc phase reflections as (L) peaks.

Rietveld refinement was performed using the FullProf software [17] and the lattice parameters were calculated. The results are shown in Table (2). The content of oxygen (δ) was calculated using the chemical iodometric titration method.

As shown in table (2), it can observe that (a) parameter was fluctuated with the increasing of Y content while (c) parameter increases with the increasing of Y content. It is known that the lattice constants are controlled by the length of the Cu-O bond inside the lattice [18]. The length of the bond may shrink or widen as the electrons change to the opposite orbit. In addition, during additional heating, the excess liquid phase (dissolved grains) is likely to cause more stress and produce an increase in the growth of lattice parameters [19,20]. Another interpretation is that the increase in oxygen content in CuO chains causes the elongation in lattice parameters. This may be attributed to the yttrium doping which may increase the combination of O_2 in the structure and thus the occupancy of the O^{2-} site; consequently, a dilation in lattice parameters occurs [21]. In addition, the unit cell of thalliated superconductor Tl-2223 contains three layers of Cu-O as shown in Figure 2A and this has been confirmed by many researchers [22] (Figure 2A was sketched using VESTA ver. 3.4.4.). The relationship between the length of the c axis and c/a with Y content is shown in Figure (2B).

Figure 3 shows the relationship between the temperature and electrical resistivity of $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ samples (where x=0, 5, 10, 15, and 20%).The specimen x=0 shows a performance characteristic of undoped thalliated superconductor Tl-2223: a critical transition temperature (Tc) at 125.5K and zero resistance at (Tcoff)117K. The Y doped specimens have higher resistivities than the undoped specimens. The specimen with x= 0.2 shows a higher transition temperature (Tc=137.5K) and the results are shown in Table (3).

From Figure (3) it can be observed that substitution of Ba^{+2} by Y^{+3} increases the Tc from 125.5K to 137.5K because increasing the c-axis leads to an increase in the CuO layer. These results were almost identical to those reported in reference [23]. The effect of Y doping in the thalliated superconductor Tl-2223 were also investigated and it was found that Tc increases with increasing Y content in $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ compound. This may be attributed to the variation in the Cu-O bonds and c parameter which causes an increase in oxygen content (δ), thereby leading to an increase in CuO2 layer thickness [24].

The stability of the Tl-2223 doped by an yttrium has been discussed as follows; in pure thalliated superconductor Tl-2223 with ideal stoichiometry all the atoms of Cu would be in the 2^+ state, which imparts insulating properties at room temperature. The higher Cu valence state, which is essential for a cuprate to be superconducting, is formed through the exchange of Ba^{2+} with Y^{3+} leaving Ca3+ vacancies at cation sites, and the internal redox reaction is:

$$Tl^{3+} + Cu^{2+} \longrightarrow Tl^{(3-\delta)+} + Cu^{(2+\delta)+}$$

These complicated internal mechanisms are difficult to manage through construction. Incomplete substitution of Ba^{2+} by Y^{3+} in $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ compound gives a stable Tl-2223 phase with the highest possible Tc and increases the effective valence of copper. This creates a perfectly doped system and increases the creation of the thalliated superconductor Tl-2223 phase [16,24].

CONCLUSION

We successfully synthesized and studied the effect of the substitution barium by yttrium in the thalliated superconductor samples $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ on the structural and superconducting properties. The pattern of X-ray diffraction shows that all the samples have a tetragonal crystal structure in polycrystalline nature. It was also found that the increase in yttrium content of all samples produced a significant change on the T_c, the lattice constants a and c, volume fraction V_{phase}, c/a ratio and the oxygen content δ. The optimum T_c of 137.5 K was observed for the composition Y=0.2 in the $Tl_2Ba_{1.8}Y_{0.2}Ca_2Cu_3O_{10+\delta}$ superconductor.

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Table 1: Percentage of raw materials used for sample preparation

Raw Materials	Molecular Weight Ratio (g/mol)
Tl_2O_3	2(456.76)
Y_2O_3	x(225.81)
BaO	2-x(153.33)
CaO	2(56.0774)
CuO	3(79.545)

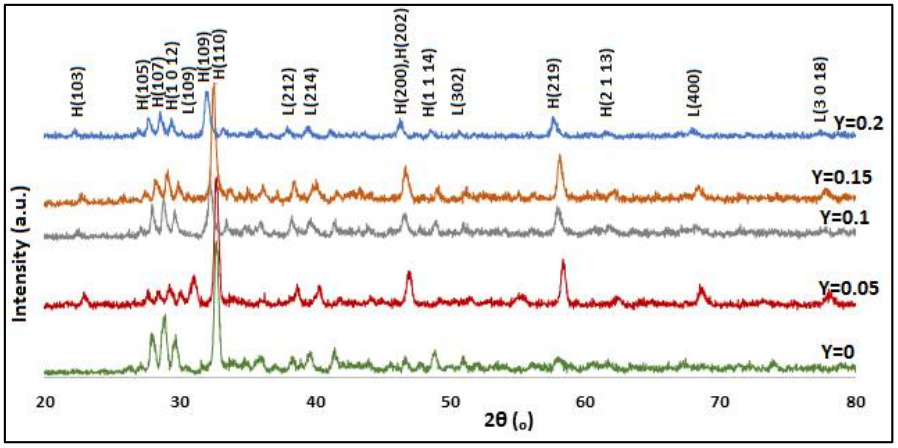

Figure 1.XRD of superconductor patterns $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$

Table 2: The unit cell constant, c/a, ϱ, δ, volume fraction of Tl-2223 phase data.

x	a=b (Å)	c (Å)	c/a	V (Å^3)	ϱ (g/cm^3)	δ (O_2)	Volume fraction (%)	
							Tl-2223	Tl-2212
0	3.844939	34.8328	9.059391	514.953	7.183537	0.17	81.39	18.61
0.05	3.85532	35.1244	9.117715	521.258	7.081222	0.18	91.08	8.92
0.1	3.855045	35.3375	9.166567	525.164	7.013245	0.19	81.86	18.14
0.15	3.855455	35.5596	9.223198	528.577	6.95275	0.22	86.75	13.25
0.2	3.848908	35.5832	9.245012	527.133	6.956543	0.24	82.23	17.77

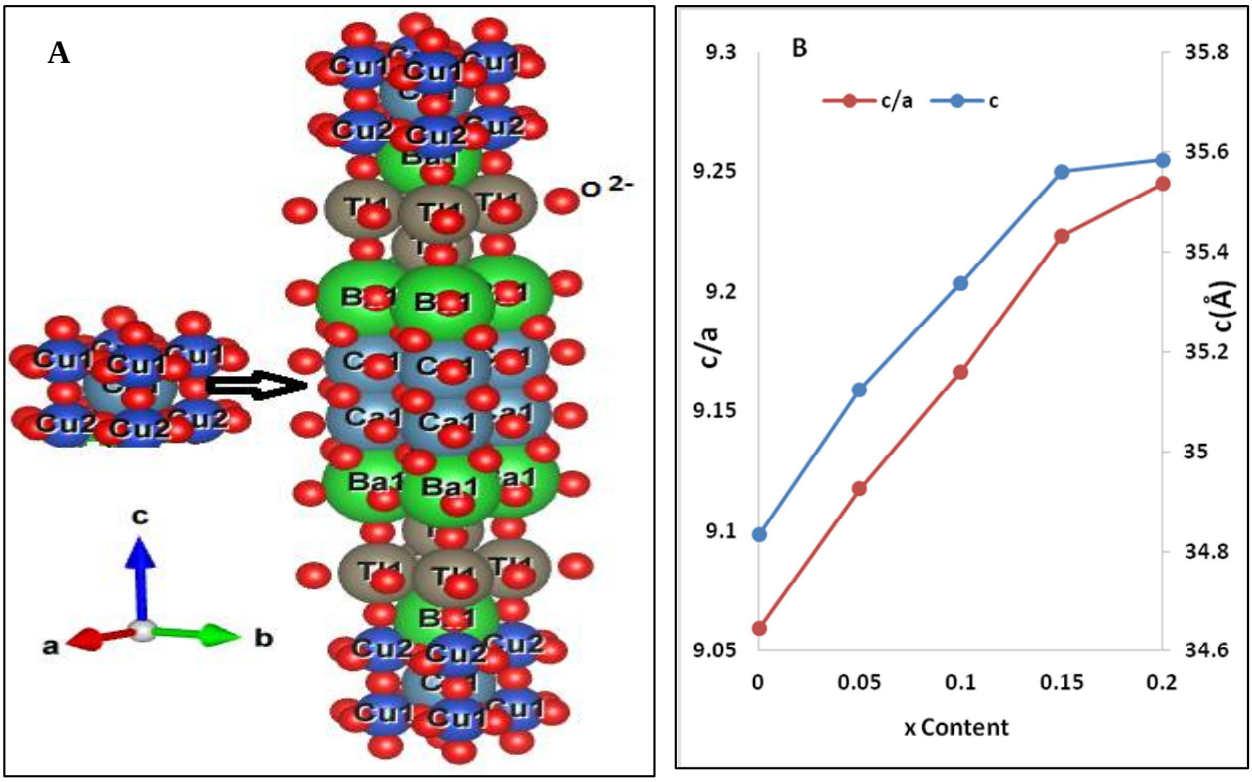

Figure (2)(a) Crystal structure of $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ showing the layers of Cu and atoms positions. (b) The variation of c and c/a with x Content.

Table 3.Variation in Tc values, for different samples of$Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$.

X content	T_{onset}(K)	T_{offset}(K)	Tc(K)
0	135	118	125.5
0.05	131	121	126
0.1	130	123	126.5
0.15	135	132	133.5
0.2	141	134	137.5

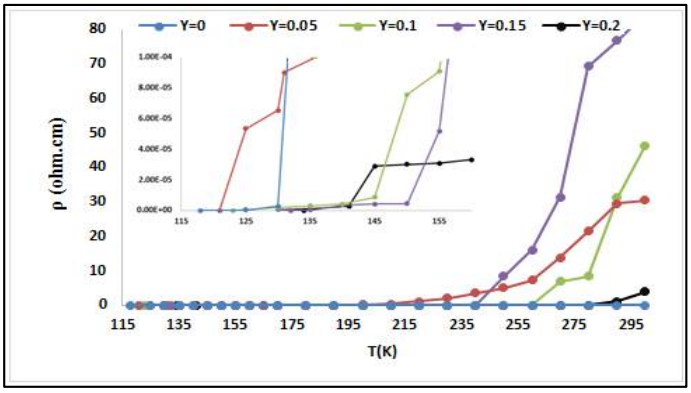

Figure 3: The electrical resistivity versus temperature for $Tl_2Ba_{2-x}Y_xCa_2Cu_3O_{10+\delta}$ compound

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RESEARCH ARTICLE

In vitro Evaluation of Ayurvedic Pharmaceutical Byproducts as an Alternate Feed Source

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ABSTRACT

The study was conducted to evaluate the effect of inclusion of ayurvedic pharmaceutical byproducts as cattle feed ingredients. Four TMRs were prepared by using ayurvedic pharmaceutical byproducts such as dhanwantharam thailam residue, ksherabala residue, cooked barley and spent grapes replacing the conventional ingredients and *in vitro* gas production technique (IVGPT) for 24 hour was done. The concentrate to roughage ration of the ration was maintained 50:50. The results indicated that the *in vitro* dry matter digestibility, organic matter digestibility, metabolisable energy and microbial bio mass production was maximum in TMR1 and TMR3 which contain ksheerabala residue and dhanwantharam thailam residue respectively. Methane production was seen minimum in TMR-3. Hence it was concluded that Dhanwantharam thailam residues and ksheerabala residues can be incorporated in the cattle ration with improved rumen fermentation potential. Among the four TMRs tested TMR-3 containing dhanwantharam thailam residue produced best results and hence recommended.

Keywords: TMR, IVGPT, *in vitro* dry matter and organic matter digestibility, microbial bio mass production, methane production, dhanwantharam thailam residue

INTRODUCTION

The availability of nutritionally balanced feed and high cost incurred in feeding are the two major constraints for attaining the optimum production and growth in our livestock. The shrinking size of cultivable land is the limiting factor for availing quality fodder and bulging human population limits the availability of cereals, pulses and beans for feeding cattle Jeong *et al* (2016). The feeding cost increased to about 80 per cent on last decade due to the shortage of feed grains. In Kerala there is a huge gap between feed and fodder requirement and availability Economic Review (2017). These deficiencies also hindered our attempts to utilize maximum production from our genetically superior herd. This tempted the scientist to tryout newer low cost but nutritionally rich feed resources as alternate feeds.

Many ayurvedic pharmaceutical byproducts which are available in large quantities can be used in total mixed ration (TMR) as ingredients for livestock feeding, which otherwise will be wasted and causing pollution. Evaluating nutritive value of unconventional feed ingredients has to be done prior to incorporation in animal feeds to study the rumen fermentation changes. *In vitro* gas production technique can be used as a perfect tool for this Mould *et al* (2005). The assumption that *in vitro* results will mimic the fermentation characteristics like substrate digestion, volatile fatty acid production, nutrient digestibility and microbial protein production as *in vivo* conditions Keiser and Weniger (1994). This method will be helpful when large number of samples has to be evaluated. This will also provide opportunity for cost effective evaluation of feed resources since the animal trials are much laborious and huge money investment has to be done. However the influence of ayurvedic pharmaceutical byproducts such as Dhanwantharam thailam residue, Ksherabala residue, cooked barley and spent grapes are not much studied in crossbred cows. Hence the study has been taken up with following objectives.

1.Evaluation of total mixed ration containing various unconventional feed ingredients by *in vitro* gas production technique.

2. To suggest suitable total mixed ration for crossbred cattle for efficient nutrient utilization.

MATERIALS AND METHODS

Sample collection and preparation

The ayurvedic pharmaceutical byproducts such as Dhanwantharam thailam residue, Ksherabala residue, cooked barley and spent grapes were brought from Oushadhi, Thrissur and SNA Oushadasala Pvt. Ltd.Thrissur. The samples were dried and ground to pass through a 1mm screen and their proximate analysis was done AOAC (2012) (Table-2). Four iso caloric and iso nitrogenous total mixed rations containing 13- 14 per cent crude protein and 65-70 per cent total digestible nutrient were prepared by using these unconventional feed ingredients replacing conventional ingredients from control concentrate mixture. The proximate analysis of all the four TMRs were done as per standard procedure AOAC (2012) (Table 3). The ingredient composition of four TMRs prepared given in Table-1

In Vitro Gas Production Technique

The four TMR's were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988). Rumen liquor was collected from six crossbred cows maintained in standard farm ration as TMR containing concentrate and green grass 50:50 using a stomach tube before morning feeding. The rumen liquor was transferred into a pre warmed thermos flask and strained through a four layered muslin cloth and pooled together which was used as inoculums source for conducting *in vitro* trial to estimate various rumen fermentation parameters.

Total Gas Production

Gas produced (ml/ 200 mg substrate) by fermentation of substrate feed during 24 hour was measured after correcting corresponding blank values Menke and Steingass, (1988).

In vitro True DM AND OM Digestibility

Goering and Van Soest (1970) method was followed for the determination of true DM and OM digestibility of TMR used as substrate

$$TDMD\% = \frac{(\text{DM taken for incubation} - \text{NDF residue})}{(\text{DM taken for incubation})} \times 100$$

$$TOMD\% = \frac{(\text{OM taken for incubation} - \text{residual OM})}{(\text{OM taken for incubation})} \times 100$$

Microbial Biomass Production (MBP)

Microbial biomass production (MBP) of the TMR tested was calculated from TDOM using equation

MBP (mg) = TDOM (mg) – (Corrected gas production for 24 hrs×2.20)

Where 2.20 is the stoichiometric factor for roughages (Blummel *et al*., 1997) and for mixed diets (Blummel and Lebzien 2001).

Metabolizable Energy (ME)

ME of target TMR was calculated by the method of Menke and Stienglass *et al*. (1989)

ME (KJ/kg DM) = 1.24+ 0.146 × gas (ml/200mg DM) + 0.007× CP + 0.0224× EE

Where, CP - Crude protein, EE - Ether extract

TA- Total Ash, GP- corrected gas production for 24 hours.

Methane Estimation

Methane production capacity of the TMR was determined by using methane sensor fabricated analyzer developed in Kerala Veterinary and Animal Sciences University. The data from the experiment were analyzed statistically as per Snedecor and Cochran (1994).

RESULTS

The analysis of chemical composition of ksheerabala residue, cooked barley, dhanwantharam thailam residue and spent grapes revealed that the dry matter was 7.50, 13.55, 15.48 and 9.75 per cent respectively. The crude protein values were 29.52, 9.23, 11.99 and 6.35 per cent, ether extract values were 13.26, 3.43, 17.70 and 2.54 per cent, crude fiber values were 6.39, 6.28, 25.20 and 8.53 per cent, total ash values were 8.42, 2.14, 5.89 and 3.10 per cent and nitrogen free extract were 59.00, 76.33, 41.90 and 80.22 per cent respectively for ksheerabala residue, cooked barley,

dhanwantharam thailam residue and spent grapes Tabe-2.Chemical composition of ksheerabala residues, dhanwantharam thailam residues, cooked barley and spent grapes reveal that these ingredients can be included in cattle ration replacing conventional energy, protein and fibre sources and this observations were in accordance with the findings of Seethal *et al*(2016) they found that ksheerabala residues can be included up to 40 per cent in calf ration without any adverse effect on digestibility. The chemical composition of four TMR prepared were in Table-3.

Among the four TMRs tested using IVGPT the gas production was maximum in TMR-2 i.e. 21.8±0.12ml/200mg compared to TMR-4(19.5±0.29) and lowest in TMR-2 & 3 i.e. 14.1±0.06. The maximum IVDMD per cent was seen in case of TMR-3 (79.5±0.29) it was 79±0.29 in TMR-1, 76±0.58 in TMR-4 and lowest in TMR-2 i.e. 71±0.29. The organic matter digestibility values for TMR-1 was 80.74±0.25, TMR-2 (69.45±0.25), TMR-3(78.64±0.06) and for TMR 4 it was (73.61±0.58). Metabolisable energy values were 5.5±0.01, 5.55±0.03, 4.62±0.02 and 4.62±0.02 respectively for TMR-1, TMR-2, TMR-3 and TMR-4. The maximum microbial biomass production was seen in TMR-3 i.e. 48.6±0.01 and it were 25.59±0.01, 21.52±0.01and 30.7±0.03 respectively for TMR-1, TMR-2 and TMR-4. The minimum methane production was seen inTMR-3 & 4 i.e. 7.92±0.01 and in TMR-1& 2 it were 8.50±0.01 and 8.8±0.03 respectively.Table-4. The higher dry matter digestibility and organic matter digestibility in TMR-1 and 3 attributed to the increased concentration of nutrients available for the microbes to act. Our findings was in accordance with the findings of and Wahayuni *et al* (2009) and Reddy *et al* (2016). The higher microbial bio mass production in TMR-3 indicates that the combination of feed in the ration resulted in highest nutrient rich supplementation which otherwise promote maximum microbial activity.

From the above parameters the TMRs showing maximum TDMD%, TOMD%, MBP(mg), ME and minimum methane production were TMR-1 & 3, but TMR-3 showed high microbial biomass production with high dry matter and organic matter degradability with minimum methane production hence TMR-3 containing dhanwantharam thailam residue can be recommended among above four TMRs as a cattle feed ingredient.

CONCLUSION

Based on the results it was concluded that ayurvedic pharmaceutical byproducts such as Dhanwantharam thailam residues and ksheerabala residues can be incorporated in the cattle ration with improved rumen fermentation potential. Among the four TMRs tested TMR-3 containing Dhanwantharam thailam residue produced best results hence recommended.

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Conflict of Interest

The authors declare no conflict of interest

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Table-1: TMR-1

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	5
Ksheerabala residue	5
Soya bean meal	20
Wheat bran	21
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-2

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	5
Cooked barley	5
Soya bean meal	22
Wheat bran	20
De oiled rice bran	13.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-3

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	5
Dhanwantharam residues	5
Soya bean meal	23
Wheat bran	17
De oiled rice bran	15.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-4

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	5
Spent grapes	5
Soya bean meal	23
Wheat bran	17
De oiled rice bran	15.5
Mineral mixture	3
Salt	1.5
Total	100

Table-2: Chemical composition of unconventional feeds on % DM basis

Un conventional feed	Moisture*	Crude protein*	Ether extract*	Crude fiber*	Total ash*	Nitrogen free extract*
Ksheerabala residues	7.50	29.52	13.26	6.39	8.42	59.00
Cooked barley	13.55	9.23	3.43	6.28	2.14	76.33
Dhanwantharam thailam residues	15.48	11.99	17.70	25.2	5.89	41.90
Spent grapes	9.75	6.35	2.54	8.53	3.10	80.22

*Average of six values

Table-3: Chemical composition of TMRs on % DM basis

Ingredient	Dry matter*	Crude protein*	Ether Extract*	Crude fiber*	NDF*	ADF*	Total ash*	Acid insoluble ash*	NFE*	OM*
TMR-1	29.20	15.02	2.60	18.20	45.38	28.00	9.28	4.10	54.90	90.72
TMR-2	30.40	15.20	1.80	17.60	44.28	27.50	9.52	3.90	55.88	90.48
TMR-3	30.00	14.70	2.00	16.56	51.31	30.15	9.38	3.95	57.36	90.62
TMR-4	29.4	15.00	4.7	17.2	42.93	26.5	9.4	4.00	53.7	90.6

*Average of six values,

Table-4: Rumen fermentation parameters of TMRs using IVGPT

Parameters	TMR-1	TMR-2	TMR-3	TMR-4
Gas production (ml/200mg)	14.1±0.06	21.8±0.12	14.1±0.06	19.5±0.29
IVDMD%	79±0.29	71±0.29	79.5±0.29	76±0.58
IVOMD%	80.74±0.25	69.45±0.25	78.64±0.06	73.61±0.58
ME(kJ/kgDM)	5.5±0.01	5.55±0.03	4.62±0.02	4.62±0.02
MBP (mg)	25.59±0.01	21.52±0.01	48.6±0.01	30.7±0.03
Methane %	8.50±0.01	8.8±0.03	7.92±0.01	7.92±0.02

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RESEARCH ARTICLE

Traditional and Molecular Study for Prevalence of Coccidiosis in Sheep in Wasit-Iraq

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ABSTRACT

This study was designed to know the prevalence of *Eimeria* species in Wasit-Iraq by two techniques (microscopy and PCR), conducted from July to December 2017. 120 feces sample of sheep was examined from different areas Alkut, Al-Numaniyah, Al hay and Badra) in Wasite governorate – Iraq by microscopy and PCR techniques, showed the PCR more sensitive by recorded (70%) positive sample while the microscopic (50%).The female have a high infection rate by using PCR (73.81%) but in microscopy (47.36%) than male. According to the months, the high infection rate was recorded in November in both techniques, no significant differences were recorded in areas, in microscopy method showed the seven species of coccidia infection (*E.ahsata, E.ovina*, *E.crandalis*, *E.intricata*, *E.weybridgensis*, *E.ovinoidalis* and *E.parva). E.ovina* showed high infection rate than other spp.

Keywords: Wasit (Iraq), Sheep, *Eimeria*, Prevalence

INTRODUCTION

Coccidiosis is a specific infectious parasitic disease caused by a protozoan parasite *Eimeria* species. Which affect the intestine of sheep [1, 2]. Coccidiosis has a worldwide distribution and causes a huge economic loss. Coccidia (*Eimeria*) highly hosts specific and the disease is usually caused by sporulated oocysts [3, 4]. The disease was mostly found in young animals but in the severe case, the adult animals were also infection [5,6]. Ingestion of infected food and water are the main source of spreading the parasite, so the symptoms of *Eimeria* infection begin with diarrhea; sometimes containing (blood or mucus) and then affecting the animal's health as weight loss, loss of appetite, anemia, wool breaking, fatigue and death (10-40% morbidity and 10% mortality) [7].

Fifteen *Eimeria* species considered to have the capability of infecting sheep are *E. crandallis, E. ahsata, E. bakuensis, E. faurei*, *E.gonzalezi, E.granulosa, E. gilruthi, E. marsica, E. intricata, E. ovinoidalis, E. parva, E. weybridgensis, E. pallida, E.punctata* and *E. gilruthi* (8). 14 species infect the sheep intestine and in one species (*E. gilruthi*) the abomasums is target tissue (9). *E .bakuensis ,E. ovinoidalis* and *E. ahsata* are the most pathogenic species in ruminants (9).The taxonomy of *Eimeria* genus has been based primarily on the morphology of the sporulated oocysts and the identity of the host from which the oocysts have been recovered **(**10). This procedure is not only very subjective and time-consuming, but is also unreliable since the different species have overlapping properties **(**11**).** Furthermore, morphological observations combined with fecal examination are very laboratory intensive and require skillful technique. Recently we need an essential to develop a more rapid, convenient, and accurate diagnostic method. Thus, molecular tools have been proven useful for the species identification or classification of this genus to overcome the limitations of traditional methods and have furthermore demonstrated the phylogenetic position of each *Eimeria* spp (12,13).

MATERIALS AND METHODS

Sample collection

A total of 120 fecal samples (44 males, 76 females) were collected directly from the rectum of sheep, in a period of (1-7 to 31-12-2017) and stored, until the examination. Sheep were selected randomly from the stockholders in four cities of Wasit provinces; 30 from Alkut, 30 from Al-Numaniyah, 30 from Al hay and 30 from Badra. Than divided into 4 different age groups; 0-6 months, 6-12months,12-24 months and 24-36 months.

Parasitological examination

Faecal samples (3-5 g) transported to a Department of Parasitology-Veterinary Medicine College-Baghdad University-Baghdad –Iraq were analyzed using floatation technique with saturated NaCl for coccidian oocysts. *Eimeria* species were identified following sporulation of feces in 2.5% potassium dichromate. Identification of *Eimeria* species was based on the morphological features of the oocysts (size, shape, color, presence or absence of micropyle and its cap) (14 and 15).

Feces DNA extraction

DNA from feces samples were extracted (16) by using commercially available kit AccuPrep® Stool DNA Extraction Kit,Bioneer, Korea, and done according to company instructions.

PCR master mix preparation

Multiplex PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix was done according to company instructions by add DNA template (5µL), Forward primer (10pmol) (GCAAAAGTC GTAACACGGTTTCC)(1µL), Reverse primer(10pmol)(CTGCAATTCACAATGCGTATCGC) (1µL), PCR water(39 µL).standard AccuPower PCR PreMix Kit that containing all other components which needed to PCR reaction such as Taq DNA polymerase, dNTPs, Tris.HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye, Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (THECHNE.USA).

PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis and under UV light.

Images were captured on a computer and printed.

Statistical analysis

The data were analyzed statistically using the Chi-Square test (SPSS for Windows, Version 12).

RESULTS

One hundred and twenty sheep feces examined in Wasit - Iraq, by two methods (microscopy and PCR) 60 (50%) was found to harbor the oocysts of *Eimeria* spp in the microscopic method when 84 (70%) in PCR fecal samples from sheep (Table 1). According to the gender and age, the result of male sheep infection in microscopy and PCR 24 (54.54) and 29 (65.9%) while in female sheep infection was 36(47.36%) and 55(73.81%)respectively and recorded all ages that have an infection (Table 2).

During the period of the study, the overall prevalence of *Eimeria* parasites in sheep was, according to months of the year, the present study recorded the high prevalence of *Eimeria* parasite infection in November PCR (75%) and microscopy (65%) but lower infection in Julia (Table 3). Analysis of the data on the basis of the area to study showed no significant differences (P<0.05) in the overall prevalence of *Eimeria* parasites in Badra (70%) when in Kut (40%) by microscopy method, While in PCR Badra (66.67%) when in Kut (73.34%)(Table 4).

Seven *Eimeria* species were identified from infected sheep, namely, *E. ahsata, E. weybridgensis, E. bakuensis (syn: E. ovina), E. ovinoidalis, E. crandallis, , E. intricata,* and *E. parva,* (figure 1). The most common *Eimeria* species were *E.bakuensis* (*ovina*) (24.16%) *E.parva(20.8%), E. ovinoidalis (18.3%), E. crandallis (9.16%) E. ahsata* (7.5%),, *E.weybridgensis*(5.83%) and *E.intricata* (0.83%) of samples (Table 5). The study recorded the high infection of *E.ovina* and *E.parva* in October, November, and December (30%).(Table 6).All infection detected by microscopic examination, of *Eimeria*, was confirmed by PCR using a primer that is general to all *Eimeria* species in sheep based 18S small-subunit (SSU) rRNA gene, Agarose gel electrophoresis showing the PCR product analysis of 18S rRNA gene in *Eimeria* spp positive samples at (~650 bp) PCR product as shown in figure (2).

DISCUSSION

The prevalence of *Eimeria* species oocysts is influenced by the examination methods, showing a direct relationship between the technification level and infection intensity (17). Previous studies reported 92.7% prevalence of *Eimeria* sp.oocysts (18), 78.3% (19), and 25.3% and 58.9% (20,21). Limiting factors related to the environment (climate and management) or to the animal (genetic and immunologic status) may promote dissemination and increase the prevalence of coccidia (22). The animal category was a significant factor influencing oocysts excretion, because young animals were more affected than adults, regardless of the season. The high susceptibility of young animals is related to immunological aspects once the species-specific immunity against *Eimeria* sp. occurs after the initial infection (23).although our study showed the adult more infected than younger.Sex was also a factor influencing eimeriosis prevalence, especially in the rainy season. The ram's susceptibility to infection by *Eimeria* sp. can be attributed to immunosuppression caused by elevated plasma levels of androgens, mainly testosterone, throughout the breeding season (24).

During the rainy season, physical exhaustion from the intense reproductive activity certainly contributed to the increased susceptibility of the male to eimeriosis. Climate-related aspects, especially the moisture caused by rain in places where drainage is difficult, may influence the prevalence of *Eimeria* sp. A warm and moist environment provides ideal conditions for oocyst sporulation and thereby increases the potential for infection (25). The effect of weather on oocyst sporulation can be potentiated by extreme variations in pluviometry and temperature that occur

throughout the year (9). This fact was observed in the area where the present study was carried out, where maximum and minimum monthly values of temperature and pluviometry during the dry and rainy season were 15-30 °C and 15-140 mm, respectively. Recognizing that an environment with high moisture and mild temperature leads to oocyst sporulation, promoting its higher elimination (25), the rainy season in the semiarid region presents favorable environmental conditions for oocysts sporulation of *Eimeria*, suggesting the necessity of management targeted toward its control during this critical period of the infection. The quantity of excreted oocysts can vary depending on the infecting dose of oocysts (26,27) and the animal's immune status (28).

However, the diagnosis of eimeriosis cannot be excluded when the OPG is low or non-existent. Adult animals that excrete small amounts of oocysts are important in the epidemiology of eimeriosis; the oocysts released by these animals are usually the cause of infection in young animals (29). Regarding the prevalence of *Eimeria* sp., there have been 15 species identified that parasitize sheep (18). Previous studies suggest that species and their respective prevalences vary according to the region, probably due to the influence of climate (9) and husbandry systems (17). Eight species of *Eimeria* were identified, from which *E. bakuensis, E. ovinoidalis, E. parva,* and *E. faurei* were the most prevalent (20). and in the Rio Grande do Sul State, *E. parva, E. ashata, E. punctata,* and *E. granulosa* (8) were the most prevalent species. The analysis of all this information shows that the survey of the species present in a determined region, especially the pathogenic ones, has great importance in facilitating our understanding of the epidemiology (30) of eimeriosis and contributes to defining strategies for its control in a herd. Among the seven species identified in the present study, four exhibited a micropylar cap (*E. intricata, E. bakuensis, E. ahsata,* , and *E. crandallis*). The presence or absence of a micropylar cap, the oocyst and sporocyst diameters, and the shape of the oocysts are reliable criteria to differentiate *Eimeria* species (31). Although the morphometric method has limitations in differentiating the species due to the overlap of some parameters (32).

CONCLUSION

The present survey revealed that prevalence of coccidial infection in Wasit Province-Iraq is significantly high. Knowledge of the prevalence of coccidiosis and current *Eimeria* species will help to minimize the economic losses in the sheep industry, evaluate infection potential and control programs, especially for lambs. These results also provide relevant "base-line" data for assessing the effectiveness of future control strategies against coccidiosis in sheep.

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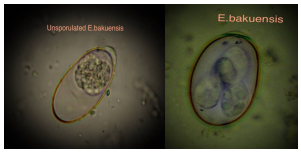
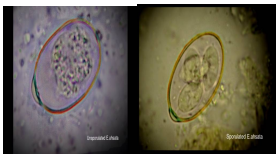
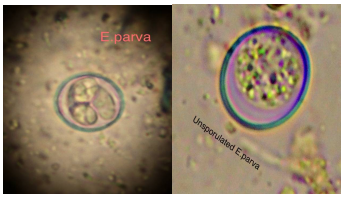


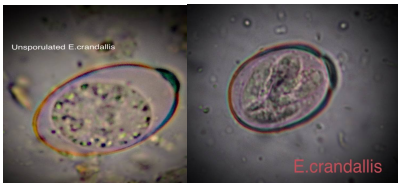
Sporulated and unsporulated *E.ovina*



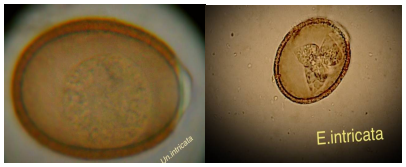
Sporulated and unsporulated *E.ahsata*



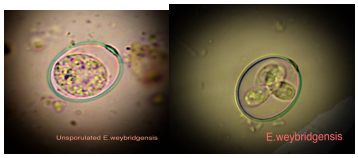
Sporulated and unsporulated *E.parva*



Sporulated and unsporulated *E.crandallis*



Sporulated and unsporulated *E.intricata*



Sporulated and unsporulated *E.weybridgensis*

Sporulated and unsporulated *E.ovinoidalis*

Fig.1 : *Eimeria* sp. of sheep sporulated and nonsporulated (X100)

Table 1.Infection rate of *Eimeria* sp.

Samples	Positive Microscopic	Positive PCR
120	60 (50%) ^{B}	84(70%) ^{A}

The capital and small later refer to significant differences P< 0.05

Table 2.Infection rate of sheep according to the age and gender

	Male			Female		
Ages / Months	No. of sample	Positive Microscopic	Positive PCR	No. of sample	Positive Microscopic	Positive PCR
0-6	15	4 (26.66%) $^{B\ c}$	10(66.66%) ^{A}	25	6 (24%) ^{B}	18 (72%) ^{A}
6-12	20	11 (55%) ^{b}	11(55%) ^{b}	39	18 (46.15%)^{B}	29(74.35%) ^{A}
12-24	9	9 (100%) ^{a}	8 (88.88%)	6	6 (100%) ^{A}	4 (66.66%)^{B}
24-36	-	-	-	6	6 (100%)	4 (66.66%)
Total	44	24(54.54%)	29(65.9%)	76	36(47.36%)	55(73.81%) ^{A}

- The capital and small later refer to significant differences P< 0.05

Table 3.Infection rate of *Eimeria* spp. in during the months of study

Months	Examined samples	Positive Microscopic	Positive PCR
July	20	4(20%)	14(63.64%)
August	20	6(30%)	14(63.64%)
September	20	11(55%)	14(63.64%)
October	20	13(65%)	14(63.64%)
November	20	13(65%)	15(75%)
December	20	13(65%)	13(65%)

- The capital and small later refer to significant differences P< 0.05

Table 4.The infection rate of *Eimeria* sp. In Wasit Province, according to the areas of the study.

Areas	Samples Examined	Positive Microscopic	Positive PCR
Kut	30	12(40%)	22(73.34%)
Al-Numaniyah	30	13(43.34%)	21(70%)
Al-Hayy	30	14(46.66%)	21(70%)
Badra	30	21(70%)	20(66.67%)

Table 5.*Eimeria* spp .in sheep in Wasit Province (Microscopic)

Species of *Eimeria*	No. of Samples positive	Percentage (%)
E.ahsata	9	7.5% C
E.ovina	29	24.16%A
E.crandalis	11	9.16%C
E.intricata	1	0.83%
E.weybridgensis	7	5.83% C
E.ovinoidalis	22	18.33% B
E.parva	25	20.8%A

- The capital and small later refer to significant differences P< 0.05
-

Table 6 : Infection rates of *Eimeria* sp. according to months of the study.

Eimeria spp. / Month	July	August	September	October	November	December
E.ahsata	2(10%)A	1(5%)	0	2(10%) A	2(10%) A	2(10%) A
E.weybridgensis	0	0	1(5%)	2(10%)	2(10%)	2(10%)
E.crandalis	1(5%)	2(10%)	2(10%)	2(10%)	2(10%)	2(10%)
E.ovinoidalis	2(10%)	2(10%)	5(25%)	5(25%)	5(25%)	3(15%)
E.ovina	2(10%)	3(15%)	6(30%)	6(30%)	6(30%)	6(30%)
E.intricata	1(5%)	0	0	0	0	0
E.parva	1(5%)	2(10%)	4(20%)	6(30%)	6(30%)	6(30%)

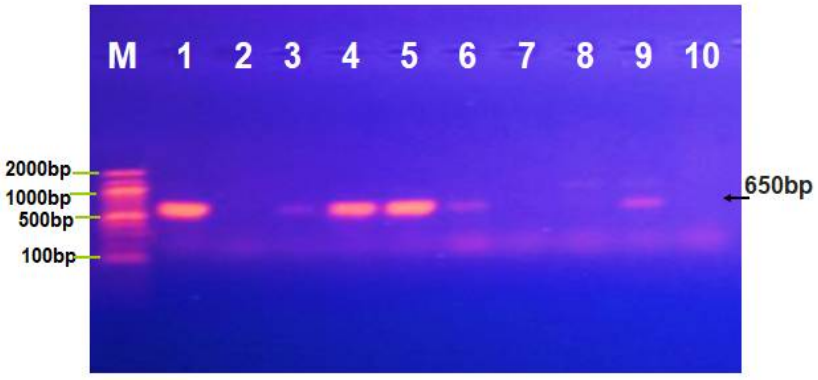


Fig.2 :- Agarose gel electrophoresis image that shows the PCR product analysis of 18S rRNA gene in *Eimeria spp* positive samples. Where M: marker (2000-100bp), lane (1-10) stool positive samples at (~650bp) PCR product.

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RESEARCH ARTICLE

Protective Role of *Nigella sativa* Oil on Thyroid and Parathyroid Glands Function in Adult Male Rats Treated with Zinc sulphate

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ABSTRACT

This study was designed to investigate the prophylactic effect of *Nigella sativa* oil on some physiological parameter related with thyroid and parathyroid function in male rats treated with zinc sulphate. Twenty four male adult rats were divided randomly into four M equal groups and were treated for 56 days as following. First group (C) received normal water orally as a control group; the second group (T1) received (0.1 ml/100 gm B.W) *Nigella sativa* oil, while rats of the third group (T2) received (15 mg/kg B.W) Zinc sulphate ; animals in the fourth group (T3) received *Nigella sativa* oil (0.1 ml/gm B.W) and Zinc sulphate (15 mg/kg B.W) orally. Fasting blood sample were collected at 14, 28, 42 and 56 days of experiment to study the following parameters serum parathyroid hormone concentration, serum calcitonin concentration and calcium ion concentration as bio parathyroid body weight was assessment before and after treatment for all groups, also section of parathyroid was assessed for histological studies. The result revealed that treating rats with *Nigella sativa* oil caused significant increases in serum calcitonin and parathyroid hormone concentration during the last period as compared with control group; While T2 that showed increase of calcitonin at all periods except first one of parathyroid hormone the same effect at the same period. On the other hand treatment of animals with combination of Zinc sulphate and *Nigella sativa* oil also caused significant increase in the two hormones at all periods except 2nd week as compared with T1 and control group. Within group, there were significant increases of calcitonin and parathyroid hormone concentration in T2 and T3 group especially at 6th and 8th week as compared with the 2nd week. Treatment of rats with zinc sulphate (T2 group) led to significant decreases in Ca^{++} ion concentration, while T1 and T3 group showed non-significant differences in comparison to control group. Histological study of zinc sulphate treated rats (T2 group) indicated histopathological changes in the parathyroid gland, while *Nigella sativa* treated rats (T1 group) revealed possibility of decrease these effects and improving of histological picture of this gland. In conclusion, it seems likely that dosage of rats with (15

mg/kg B.W) of Zinc sulphate caused harmful effects on the function of parathyroid and Ca^{++} metabolism ;and the protective dose (0.1ml/100gm B.W) of *Nigella sativa* oil showed improvement of these parameters.

Kew words: *Nigella sativa*, thyroid and parathyroid, Zinc sulphate, metabolism.

INTRODUCTION

Zinc has wide variety of role in mammalian system [1]. Physiological role of zinc in the growth and calcification of bone tissue, has been suggested in zinc deficient rats [2] Hsieh *et al* .,1980). McDonald and Keen (1988) have reported that it is important to know the relationship between interaction diet Zinc and other elements for the health sports person and his performance. It has been postulated that Zinc has asignificant effect on elements distribution in the body [3]. High dose of Zinc exerted as porotic effect on the rat bone [4],tended to inhibit the bone collagen synthesis in young animals [5], and caused bone resorption related to abnormality of calcium metabolism [6]. According to ,[7] it was reported that there is a dose related effect of zinc on the rat bone metabolism and it was comparatively low dose of zinc may have stimulatory effect on the bone growth and calcification in early stage while prolong administration of zinc may induce bone resorption related to hypocalcaemia . Parathyroid hormones are centrally regulator calcium homeostasis which act as protein that remodels the bone , reclaims filtered calcium in the kidney and facilitates absorption of calcium from gastrointestinal tract by its action of stimulating the renal production of 1,22-dihydroxy-vitamin D [8]. Therapeutic potential of *Nigella sativa* seed has been studied by many researchers .Who have used the seed in traditional medicine for treating many disorders [9]. *Nigella sativa* has many pharmacological properties including hypotensive, uricosuric, antinociceptive, chpleretic, antiinfertility, antidiabetic, antioxidant, anti-inflammatory, antimicrobial, antitumor and immunomodulatory effect [10] The pharmacological activities of *Nigella sativa* are attributed to the presence of thymoquinone as active ingredient [11]. Thymoquinone has antioxidant effects by enhancing the oxidant scavenger system and it is potent as anti-inflammatory mediators prostaglandins and leukotriens [10][12]. There is potential beneficial effect of Nigella sative in improving thyroid status. Thymoquinone differentially modulates thyroid hormone and improves thyroid status in rats [13]. The effect of oral administration of Zinc does not inhibit intestinal absorption of calcium; but the hypocalcaemia effect by Zinc is mainly based on an increase in gastric calcium secretion [14]. The mechanism of hypocalcaemia effect by Zinc on calcium metabolism in mammals remain to be elucidated, so that the present study was designated to investigate the effect of oral administration of Zinc sulphate on calcium metabolism and its effect on thyroid and parathyroid function.

MATERIALS AND METHODS

Chemical Reagents and Apparatus

Zinc sulphate oral tablet 20 mg was purchased from Lincolin Company (India). *Nigella sativa* oil was purchased from AL-Emad company(Iraq), Ketamin and Xylazin and other reagents were obtained commercially.

Experimental animal

The animals were housed in the college of veterinary medicine, University of Baghdad, department of physiology and biochemistry and pharmacology. Twenty four adult male rats 180-300 gram were incubated in studn , the animal were adapted for ten days before experiment beginning and kept in plastic cages under uniform environmental conditions, at temperature between 21-25 c, with controlled lightening using automatic electrical timer

which provided daily light of twelve hours (7.00 to 19.00) and twelve hours night cycle. Animals had free access to water, and standard pellet diet along the experimental period.

EXPERIMENTAL DESIGN

Twenty four adult male rats were divided randomly into four groups (6 rats/group) and handled follows for 6 weeks:
1-Control group: Animal of this group received (2ml/kg B.W) of ordinary tap water
2-Zinc treated group (T1): Animal of this group were received zinc sulphat (15mg/kg B.W) [15] orally twice weekly.
3-*Nigella sativa* oil treated group (T2): Animal of this group received (0.1ml/100gm B.W) of *Nigella sativa* oil[16] orally once daily.
4-Zinc sulphat-*Nigella sativa* treated group (T3): Animal of this group received *Nigella sativa* oil (0.1ml/100 gm B.W) orally once daily and zinc sulphat (15mg /kg B.W) orally twice weekly.

Blood collection

At 14, 28, 42, 56 days of experiment blood sample were collected from retro orbital artery and cardiac puncture while animal anesthetized by I.M injection of (Xylazin 40mg/kg B.W and ketamine 90mg /kg B.W). Samples were centrifuged at 2500(rpm) or 500 minutes and then serum samples were stored in freezer at (-18c) till use.

Estimation of serum Calcium concentration

Calcium concentration was measured by optical method[17] with a complex composition (O-CRESOLPHTHALIEN) to get rid from interaction of magnesium ion deposition of (8-hydroxyquinolone).

Estimation of serum parathyroid concentration

The essential reagents required for a solid phase enzyme immunoenzymometric assay [18]. Include immobilized antibody, enzyme – antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme –antigen conjugate and serum containing the native antigen, competition reaction results between the native antigen and the enzyme – antigen conjugate for limited number of insolubilized binding site.

Estimation of serum calcitonin concentration

The essential reagent required for an immunoeenzymatic assay according to[19] include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition , in excess, and native antigen .In this procedure , the immobilization take place during the assay at the surface of micro plate through the interaction of streptavidin coated in the well and exogenously added biotinylated monoclonal anti-calcitonin antibody .Upon mixing monoclonal biotinylated antibody , the enzyme – labeled antibody and a serum containing the native antigen , reaction result between the native antigen and antibody without competition , to form a solution sandwich complex .

Serum Calcium Concentration

The data showed a significant ($p<0.05$) decrease in Ca concentration (mg/dl)(in group T2) which treated with Zinc sulphate compared with control and T1 groups at all periods Table (1), while N.S oil with Zinc (T3 group) showed non-significant ($p \square 0.05$)differences as compared with control group. During the different periods of experiment, there were significant differences within T2 group between the second week and other periods and between first and last period with others in T3 group.

Serum Parathyroid hormone concentration

The effect of N.S oil and Zinc sulphate in serum Parathyroid hormone (Pg/ml) showed that treatment of rats with N.S oil caused non-significant (p□0.05) increase in hormone concentration except at last period as compared with control group. While treatment of rats with Zinc sulphate (T2 group) and N.S oil plus Zinc sulphate (T3 group) caused a significant ($p<0.05$) increase of PTH level as compared with control and T1 group except at the first period Table (2). On the other hand, there were significant increases in PTH concentration within all three treated group at all periods compared with the first period (second week).

Serum Calcitonin concentration

The data pertaining to calcitonin hormone concentration of control and three treated groups were detected in Table (3) Statistical analysis indicated that the mean of calcitonin concentration (Pg/ml) significantly ($p<0.05$) increase in T1 group (N.S oil treated group) at last period with non significantly (p□0.05) increase at (6 weeks) as compared with control group. The zinc sulphate also had the same effect at the same periods. While the hormone concentration showed significant ($p<0.05$) increase in T3 group except at second week as compared with control, T1 and T2 groups.On the other hand, there were significant increases of hormone concentration within group in T2 and T3 group at all periods compared with the first period (second week) of treatment period.

DUSCUSSION

Our data in Table (1) showed that Ca^{++} ion concentration non significantly increase in T1 group compared with control group and this result reflexes the effect of *Nigella sativa* oil component on calcitonin hormone . *Nigella sativa* seed oil also revealed higher degree of unsaturation and the major unsaturated fatty acids which were linoleic followed by oleic acid which helped maintain bone health and increase Ca^{++} level [20]These unsaturated fatty acid promoted the absorption of Ca^{++} as well[21]. On the other hand [22] reported that oleic acid may decrease bone loss by enhancing Ca^{++} absorption [23]. In addition the enhancement of Ca++ level after *Nigella sativa* oil administration in rats has occurred because *Nigella sativa* seeds contain useful quantities of Calcium which make them a natural source of calcium supplementation, which might partially explain raised Ca++ levels [24]. While Zinc sulphate caused significant hypocalcaemia (T2 group). These data suggested that Zinc sulphate has a wide variety of roles in mammalian system [25] such as growth and calcification of bone tissue so that the high dose of Zinc use in this study (15 mg /kg B.W) was exerted. Furthermore the decrease of Ca++ ion concentration in Zinc sulphate group may be suggested that Zinc accumulated in the bone cells may activate DNA polymerase, since the enzyme might be a zinc-enzyme [26]. So that DNA content in the bone tissue increased by Zinc administration and stimulated the bone calcification, growth and this process requires Ca++ ions and Ca++ deposit in bone with significant decrease in blood [27]. The finding data showed that administration of *Nigella sativa* oil to the rats at dose (0.1 ml/100gm B.W) for 8 weeks tended to non-significant increase of parathyroid hormone (T1 group). This result may be due to response of parathyroid gland to Ca^{++} increment, and this hormone is a central regulator of calcium homeostasis, functions as a protein that remodel the bone reclaim filtered calcium in the kidney and facilitate absorption of calcium from gastrointestinal tract by the action of Vitamin-D [28]. The antioxidant effect of *Nigella sativa* oil may also be caused improvement of parathyroid function and attributed to increase Ca^{++} ion concentration.On the other hand, the increment of calcitonin level in T1 group and calcitonin hormone play important role in increase of Ca^{++} level in blood [29]. The result in Table (2) showed that Zinc sulphate may lead to increase of parathyroid hormone and this may be due to decrease of Ca^{++} ion in rats treated with Zinc sulphate. Thus the Ca^{++} ion is a major regulator of this hormone, so the hypocalcaemia caused activation of parathyroid gland to secretion large amount of hormone [28]. On the other hand, combination of *Nigella sativa* oil and Zinc sulphate tended to marked effect on parathyroid concentration and this significant increment may be due to synergistic effect between *Nigella sativa* and Zinc sulphate on parathyroid function as antioxidant and calcitonin release [30].The synergistic effect of *Nigella sativa* oil caused significant increase

of parathyroid hormone due to effect of estrogen which accelerated bone loss rats and increase calcium absorption [31]. So that parathyroid hormone increased and this secondary increment was associated with its responsible in ensuing bone loss [32] and came with concomitant increase of calcium [33][34] reported that *Nigella sativa* supplementation caused significant decrement of PTH level and this result was desirable as an agent intended to replace convening estrogenic effect of *Nigella sativa* which must mimic the role of estrogen in preventing excessive bone resorption and this might be an indication that *Nigella sativa* exhibits the effect by time –dependent and dose dependent manner. The serum calcitonin concentration increment at the last periods of treatment in *Nigella sativa* oil group and Zinc sulphate group. This effect may be due to the role of calcitonin in hemostasis of calcium, the decrement of Ca^{++} ion concentration although normal serum calcium level largely attributed to the action of parathyroid hormone, however calcitonin has been shown in to have a hypocalcaemic effect [35] by increasing calcium compartment sizes and increasing flow and from these compartments [36] so that the increase of calcitonin may appear as a result to increment of Ca^{++} ion concentration.The estrogenic effect of *Nigella sativa* oil component also caused increase of intestinal Ca++ absorption that could be attributed to increment of plasma 1,25 dihydroxy Vitamin D levels [37]. This effect may lead to increase of calcitonin concentration as a result of high level of calcium [37]while Zinc sulphate decremented Ca++ concentration as a result of reduced of Vitamin D and impaired intestinal Ca++ absorption.

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Table 1: Effect of *Nigella sativa* oil and Zinc sulphate on serum Calcium concentration (mg/dl) in adult male rats.

Groups / Periods	Control Group	T1 Group *Nigella sativa* oil	T2 Group Zinc sulphate	T3 Group N.S&Zinc sulphate
2 weeks	8.75±0.47 A a	8.45±0.47 A a	7.25±0.47 B a	8.32±0.58 AB a
4 weeks	7.52±0.51 A a	7.70±0.45 A a	5.52±0.23 B b	6.57±0.32 AB bc
6 weeks	7.82±0.49 A a	8.00±0.40 A a	3.50±0.64 C c	7.25±0.47 AB b
8 weeks	8.00±0.40 A a	8.52±0.20 A a	3.40±0.37 B c	8.62±0.37 A a

L.S.D=1.235,Values are expressed as Mean ±SE. (n=6/group),Capital letters denote between groups differences (p<0.05).Small letters denote within group differences (p<0.05)

Table 2: Effect of *Nigella sativa* oil and zinc sulphate on serum parathyroid hormone concentration (Pg/ml) in adult male rats.

Groups / Periods	Control Group	T1 Group *Nigella sativa*	T2 Group Zinc sulphate	T3 Group N.S &Zinc sulphate
2 weeks	2.10±0.22 A a	2.13±0.12 A b	2.52±0.08 A b	3.54±0.13 A b
4 weeks	2.77±0.27 B a	4.05±0.56 B a	6.10±0.74 A a	7.50±1.38 A a
6 weeks	2.77±0.27 B a	4.47±0.4 B a	6.60±0.45 A a	7.32±1.16 A a
8 weeks	2.85±0.37 C a	5.19±0.79 B a	7.30±0.34 A a	7.07±1.02 A a

L.S.D=1.77,Values are expressed as Mean ±SE. (n=6/group),Capital letters denote between groups differences (p<0.05).Small letters denote within group differences (p<0.05).

Table 3: Effect of *Nigella sativa* oil and zinc sulphate on serum calcitonin concentration (Pg/ml) in adult male rats.

Groups / Periods	Control Group	T1 Group *Nigella sativa*	T2 Group Zinc sulphate	T3 Group N.S&Zinc sulphate
2 weeks	2.57±0.22 A a	2.16±0.24 A c	2.10±0/20 A b	2.78±0.27 A b
4 weeks	2.80±0.11 B a	2.17±0.35 B bc	3.03±0.7 B ab	6.27±0.69 A a
6 weeks	2.83±0.11 B a	3.27±0.42 B ab	3.27±0.42 B a	6.60±0.8 A a
8 weeks	2.03±0.35 C a	4.05±0.39 B a	4.05±0.39 B a	6.27±1.02 A a

L.S.D=1.386,Values are expressed as Mean ±SE. (n=6/group)**,**Capital letters denote between groups differences ($p<0.05$).Small letters denote within group differences ($p<0.05$).

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REVIEW ARTICLE

Melatonin the Hormone of Darkness: A Review

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ABSTRACT

Melatonin(*N*-acetyl-5-methoxy tryptamine) is also known as the *hormone of darkness,* Melatonin is produced naturally from the amino acid tryptophan, secreted into the blood stream and carried by the circulation from the brain to all parts of the body. Its production is influenced by the detection of light and dark by the retina of the eye. Melatonin helps to regulate biological rhythms such as sleep and wake cycles, providing circadian and seasonal signal to the body, in addition it has anti-inflammatory ,antioxidant ,anti-coagulopathic properties, antiaging, endothelial protective effects, immune-stimulant also It stimulates synthesis of collagen fibers and bone formation. Clinically, significant effects of melatonin treatment for patients with for example circadian rhythm-related sleep disorders, jet lag and shift work, insomnia , poor sleep quality, nocturnal hypertension and Alzheimer's disease.

Keywords: - Melatonin, Circadian Rhythm, Pineal gland , Sleeping disorders.

INTRODUCTION

Melatonin (*N*-acetyl-5-methoxy tryptamine) is also known as the *hormone of darkness*, secreted by the pineal gland, which is a tiny endocrine gland that is deeply seated in the brain between the right and left hemispheres. Once produced, it is secreted into the blood stream and cerebrospinal fluid and carries signals to distant organs. Melatonin is carried by the circulation from the brain to all parts of the body. It helps to regulate biological rhythms such as sleep and wake cycles, providing circadian and seasonal signal to the body[1].

Its production is influenced by the detection of light and dark by the retina of the eye. Which is stimulated in the absence of light and inhibited in the presence of it. Circulating melatonin have a peak levels at night, at least 10-fold higher than daytime concentrations[2].Different studies indicate that melatonin as an immunomodulatory compound, and acts as immunostimulant[3], in addition to the anti-inflammatory properties[4]. Melatonin production gradually declines with age[5], has antiaging properties by acting as antioxidant throught control and

regulation of several important antioxidant enzymes for example the inhibition of inducible nitric oxide synthase[6]. melatonin may have a role in the treatment of cancer, It presents many oncostatic properties in a different types of tumors, because its circadian rhythm-regulating properties are essential for arranging designs for hormone secretion, the imbalance of which is associated in a wide range of hormone-dependent cancers[7]. It can modulate the immune response on distinct levels with a significant effect on inflammation. Melatonin stimulates the production of natural killer NK cells and CD4+ cells and inhibits CD8+ cells[8]. In addition, production and release of various cytokines from NK cells and T-helper lymphocytes also are enhanced by melatonin. Melatonin probably regulates immune function by acting on the immune system , through affecting G protein-cAMP signal pathway and by regulating intracellular glutathione levels[9]. Because pineal melatonin production occurs during the dark phase and reduced at light, and because melatonin is quickly cleared from the circulation following the end of its production, the time, and duration of the melatonin peak reflect the environmental night period . Melatonin exhibits its physiological functions with high levels at night peak concentrations of plasma melatonin between 02:00 and 04:00 am. Longer nights result in the longer duration of melatonin secretion and this related with the winter months leading to seasonal Affective Disorder which is a type of depression as a result of the lack of sunlight and a higher production of melatonin[10]. Normal plasma M LT levels range between 14 and 60 pg/mL. MLT has its highest levels in plasma during nighttime and early mornings (60–200 pg/mL) peaking between 12 AM and 4 AM and is lowest during the day (between 12 PM and 2 PM [11].

Biosynthesis

Melatonin is synthesized mainly in the pineal gland in addition to the other areas of the body such as gastrointestinal tract, skin, bone marrow, retina and in lymphocytes, from which it may control other physiological functions through paracrine signaling. It is produced naturally by pinealocytes from the amino acid tryptophan. Dietary tryptophan is absorbed into the bloodstream and circulated through the body. In the pineal gland,through a sequence of enzymatic reactions it is hydroxylated by Tryptophan-5-Hydroxylase (TPOH) to 5-hydroxy-Trp in the mitochondria, then decarboxylated by 5-Hydroxy-L-Tryptophan Decarboxylase (Aromatic Amino Acid Decarboxylase AADC) in the pineal cytosol. This intermediate is called serotonin, another important neurotransmitter. The 5-HT is acetylated on its free amine by Arylalkylamine-*N*-acetyltransferase (AANAT), then O-methylated on the hydroxyl group by Acetyl-serotonin O-methyltransferase (ASMT) to form the final product of melatonin[12], once it formed is not stored in the pineal gland but it released into the blood or cerebrospinal fluid, about 70% of it is bound to the albumin of the blood. Recent studies have shown that it is also synthesized in various other parts of the body including salivary glands[13].

Exogenous melatonin

In animal foods, eggs and fish contain higher concentrations of melatonin than found in meat[14]. Also melatonin presents in Cereals as corns and rice[15], fruits, like Grapes, cherries and strawberries[16]. Melatonin exists in lots of common vegetables,Tomatoes and peppers with relatively high melatonin concentrations in the vegetable group[17].

Metabolism and excretion of melatonin

Both endogenous as well as exogenous MLT is metabolized by cytochrome *P* 450 mono-oxygenase enzymes, , in the liver, melatonin is first hydroxylated to 6-hydroxymelatonin (by cytochrome P_{450} mono-oxygenases) and conjugated with sulfate.The half-life of MLTis 35 to 50 minutes and is mainly excreted through the Kidneys[18]. 89% as sulphated and glucoronide conjugates of 6-hydroxymelatonin and 2% is excreted as melatonin (unchanged active substance)

Receptors of melatonin

Numerous physiological functions of melatonin are mediated via activation of two G-protein-coupled receptors, MT1 and MT2. MLT is a nontoxic, highly lipophilic indole and hence can penetrate through cell membranes and its compartments[19]. Melatonin receptors are found in the suprachiasmatic nucleus SCN in hypothalamus and the pituitary gland of the brain, as well as in the ovaries, blood vessels, and intestinal tract.There is a high concentration of receptors in the SCN because this is where melatonin mediates the majority of its effects on circadian rhythm.

Physiologic Functions of Melatonin

Melatonin molecule is one of the most useful and multipurpose, play a role in a wide variety of physiological responses, many biological effects of melatonin are produced through the activation of melatonin receptors whereas others are due to its role as a powerful antioxidant. Clinically, significant effects of melatonin treatment for patients with for example circadian rhythm-related sleep disorders[20], jet lag and shift work, insomnia , poor sleep quality[21], nocturnal hypertension and Alzheimer's disease[22]. Melatonin is a safe therapeutic agent[23]. It is endogenously produced, nontoxic, Diffuses rapidly into all cells and body fluids, penetrates all subcellular compartments, stimulates a number of antioxidant enzymes[24]. In addition to regulation of circadian rhythm of melatonin a variety of other physiological effects such as hypnotic, antidepressant, antiepileptic, oncostatic, immunomodulatory, antiosteoporotic.

In cardiovascular disease, neuromodulatory and cerebral ischaemic condition have been reported, also antioxidant properties have been widely reported[25].Recently several publications have reported the evidence for cardioprotective effects of melatonin via its direct free radical scavenger and its indirect antioxidant activity [26]. Melatonin interacts with reactive oxygen and nitrogen species and it also upregulates antioxidant enzymes and downregulates pro-oxidant enzymes. These findings implicate the protective effects of melatonin in cardiac diseases induced by oxidative stress [27]. The oncostatic action of Melatonin by *protective effect* as reversible cellular injury through neurohormone regulation or by *antiproliferative effects*. Moderation of cellular cGMP and cAMP ratios regulates cellular metabolic processes that control the production of antioxidants in the cell. Melatonin deficiency results in uncontrolled cAMP synthesis, leading to unregulated oxidative processes and subsequent free radical damage [28].High concentrations of melatonin in cancer cells, as a result of alterations of the intracellular redox state which effects the reducing conditions that related with a decrease in cell proliferation and oxidative conditions with apoptosis[29]. Melatonin, has also been used for treatment of sleep problems related to perturbations of the circadian time keeping system like those caused by jet lag and shift-work disorder[30]. That associated with a loss of daily rhythms and increasingly fragmented sleep[31].

Previous researchs study the effect of melatonin on sleep disorder in shift worker to rebalance the sleep-wake cycle and promotion of sleep[32]. In addition to this, several studies reported that melatonin is used to counteract jet lag, associated with travel results due to changes in time zone in both adult and children.For the gastrointestinal tract clinical studies reported that melatonin capable of protecting gastrointestinal mucosa against damage through stimulating the immune system. Melatonin has been involved in the regulation of both cellular and humoral immunity,it is not only stimulates the production of natural killer cells, monocytes and leukocytes, but also alters the balance of T helper (Th)-1 and Th-2 cells mainly towards Th-1 responses and increases the production of related cytokines such as interleukin (IL)-2, IL-6, IL-12 and interferon-gamma. The regulatory function of melatonin on immune mechanisms is seasonally depend. Moreover, its play an important role in regulation of epithelial functions as well as significant anti-inflammatory and anti-apoptotic effects. The mechanism of melatonin may be due to its ability to reduce bacterial translocation and its anti-apoptotic effect and therefore it can reduce the extent of mucosal damage, and therefore melatonin exert a beneficial role in human inflammatory bowel disease (IBD) through reduction of the tumor necrosis factor-a (TNF-a) levels[33]. Also melatonin can decrease free radical levels by

stimulating the activities of enzymes involved in antioxidative defence[34]. In addition melatonin shows a regulatory role in bone metabolism[35]. Melatonin may affect bone metabolism through bone anabolic as well as antiresorptive effects. Bones are structures under a continuous process of remodelating by the coupled activity of cells with resorptive functions (osteoclasts) and cells responsible for the formation of new bone (osteoblasts). Nocturnal plasma melatonin levels significantly decline after the age of 50 in both genders[36], the reduction of melatonin production affecting bone metabolism leads to the progression of bone deterioration.

Many studies shows the role of the biological clock in the development of depression as it drives 24hrhythms in physiology and behaviour, and associates endogenous rhythms to the external solar day in a close temporal relationship. Disturbance of sleep and circadian rhythms is a prominent feature of depression. disrupted melatonin secretion is considered as a link between circadian rhythm and major depression, that is indicate the antidepressant activity of melatonin[37].Numerous scientific reports on the therapeutic potential of melatonin in treatment of asthma, respiratory diseases for infections, chronic obstructive pulmonary disease, lung cancer, pleural cavity diseases, as well as vascular pulmonary disease. melatonin relieves lung infection-induced oxidative stress and lung inflammation via decreasing of the levels of malondialdehyde, NO, and OH·, as well as increasing of the glutathione and superoxide dismutase activity. They also found that melatonin inhibits the pro-inflammatory cytokines production such as TNFα production[38].

CONCLUSION

All the previously mentioned properties of melatonin with potent multifunctional biological and pharmacological effects, applicable clinically in which appropriate concentrations should be used to give optimal activity and avoid side effects. Therefore it is an important matter of future research to investigate the clinical efficacy and safety of melatonin in detail, under different pathological conditions with different body organs.

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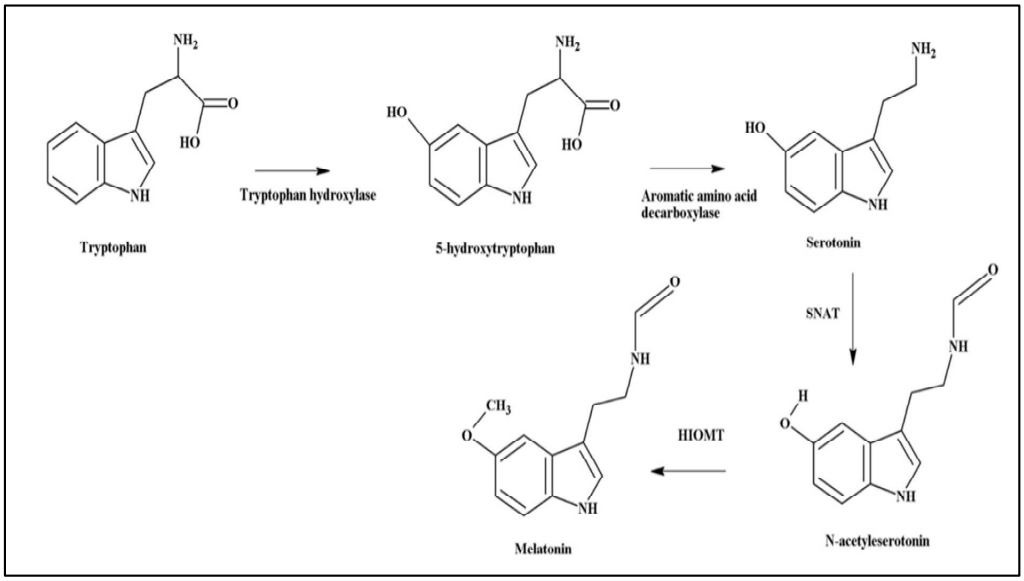

Fig.1. Biosynthesis of Melatonin

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REVIEW ARTICLE

A Review on the Bacteria and Osmotic stress

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ABSTRACT

The presence of bacteria is found in industrial foods because of their ability tolerates high degrees of an osmotic pressure which are due to the multiplicity of resistance mechanisms. This paper reviews previous studies an understanding the role of cellular parts (protein membranes) and the role of the Mechanosenser channel, which are very sensitive to osmotic changes, so the importance of transporters that are spread in many bacterial species and which have the property of higher sensing of the osmosis shocks with the importance osmosenser such as KdpF, EnvZ , explains the activity of cell cytoplasm. This study suggests adding different treatments during industrial processes and not relying on increase or decrease the level of osmotic pressure to eliminate the effects of bacteria because it has mechanisms to resist the various stresses.

Keywords: Mechanosenser Channel, Transporters, Osmosensor.

INTRODUCTION

Osmosis, water crosses permeable membranes and goes from dilute solutions to more concentrated solutions in dissolved elements. The cell membrane of the bacteria has a high permeability to water, because of the existence of the aquaporins on its surface. The water leaves the cells when the concentration of solutes in the medium increases (osmotic upshift) and returns when the medium is diluted (osmotic downshift). The cellular hydration is therefore rapidly altered after a change in osmotic pressure. The cellular hydration is rapidly altered after a variation in osmotic pressure. The higher concentration dissolved in the medium, the less free water there is available for the reactions, these results in a decrease a water activity or WA. The water activity represents the water vapour pressure a moist product divided by the saturated vapour pressure p0 at the same temperature. It is much used in the food industry because it is a food conservation factor (salting, candying, and dehydration) was conducted by O'Byrne &Booth(2002), Heermann *et al*,(2004).

Membranes: Sensors (/regulator)

The membrane is a key point in the feeling and regulation of osmotic stress via trans membrane proteins mainly . These proteins in a particular environment are lipid bilayers of the plasma membrane.

Role of membrane lipids and the envelope

The lipid bilayer is directly involved in its thickness and composition in lipid and protein acids. The nature of lipids (length of acyl chains, nature of polar heads, the composition in AGI/AGS or their cis or trans configuration) are directly responsible for the different interactions in the membrane: steric congestion, Hydration, electrostatic charges, proton fixation (H +).

* Overall proteins have an affinity advantage for anionic lipids

The **envelope is involved the composition of techniques** acid for Gram+ and LPS and Brawn lipoproteins in Gram-. Finally, it is also suggested that appendages have a role in modulating the length and flexibility of flagella (for "relieve" osmotic pressure) and regulating chemotaxis (for direct the cell to less stressful environments) López C., *et al*, (2000).

Mechanosensors Channels: Hypo-osmotic shock relief valves

The 3 main channels found in E. coli are MSc L, MSc S, and MSc M with respective conductances of 3ns, 1ns, and 0.3 ns, which imply that MScL (the most effective) is activated as a last resort during a very important stress. The structure of the MSc L channel in *Mycobacterium tuberculosis* is relatively well described with 10 transmembrane propellers whose 5 internals allow the opening or closing of the channel (30- 40 angstroms) , Romantsov, *et al*.(2009).

These channels discriminate against molecules only according to their size and feel the lateral pressure of the membrane which depends on the size of the acyl chains of the lipids, their polar heads (**EX: electrostatic** interactions), and Steric congestion. While the osmotic and electrostatic pressures are modified during stress, the channel will respond to these environmental changes , Boris M., (2011).

Transporter

They allow accumulating compatible dissolved (osmoprotective) up to the order of the molar. They are more or less specific to the osmoprotective or are more or less affine for the molecules (Km). The response can be immediate when they are already present in the membrane or delayed the time to produce more. The necessary activation energy is provided by an ion symporter or by ATP. Studies show that, in reality, they do not feel the osmotic "shock" but feel other parameters and to each its method for detecting changes in osmotic pressures.

Ex1: ProP in *Escherichia coli*

This transporter, composed of 12 transmembrane propellers, allows the transport of proline, glycine, betaine (GB) and ectoine through Proton symporter. Its activation dependeds on its terminal C end (term C) which allows the homodimerization of Pro P forming coiled-coil structure (anti-parallel alpha-helical) and the stabilization of this confirmation by electrostatic interactions. The term C also allows locating Pro P to the cell pole. Finally, the activation of ProP also depends on a soluble protein: ProQ (Basic and hydrophilic) which is suspected to interact with the acids of the term C of ProP. If this protein is mutated or deleted, the transport speed decreases and the activation threshold is higher, in other words, the transporter is less sensitive and efficient.

In reality, ProP is rich in hydration and the proposed mechanism is the following: change Osmolarity, changes the prop conformation which will then be a dimer (Prop-prop) through the C term and then this structure is then

stabilized by Pro Q forming an active ternary complex. The transport would be by fixation of the substrate and then a change of conformation following the arrival of the "facilitator" (H +) causing the translocation and then release into the cytoplasm. The hydration would be due to a mechanism of competition between the moisturizing water molecules ProP and the H + ions, when the osmolarity increases, the water molecules are removed, giving way to the H + ions that can activate the carrier study by all (Francius *et al*,(2011b) , Janet M (2006) , Francius *et al*,(2011a) , Wood *et al*, (2001) .

Ex2: Opu A in *Lactococcus lactis*

ABC Carrier (ATP- binding cassette) with a CBS sensor domain (cystathionine beta-synthase) in the Cytoplasm, 4 transmembrane domains will translocate and an intracellular AT Pase domain. It is GB specific (1GB transported for 2ATP consumed) , unidirectional . Its activation depends on the nature of the polar heads of lipids, this means, and electrostatic interactions. The more anionic lipid fraction increases the activation threshold increases and since proteins have more affinity with anionic lipids, the presence of H + destabilizes these interactions. In conclusion, Opu A feels the ionic strength, has been studied by Bouvierj *et al*,(2000) , Janet M (2006) , Wood *et al*, (2001) .

Ex 3: Bet P in *Corynebacterium glutamicum*

It is also composed of 12 transmembrane propellers and is part of the BCCT family (betaine, carnitine, Choline transporters). It transports the GB with a Sodium symporter (Na +) to feel of the osmolarity implies its cytoplasmic hydrophilic terminal N and C ends, Krämer *et al* (2004).

Ex4: *Staphylococcus aureus a* halotolerant food bacterium

This species has a KdpFABC transport system that also acts when the K + concentrations are low. Another carrier (KTR) was shown to play an important role in K + tolerance, (Price-W *et al*, (2011) , Janet M (2006), Wood *et al*, (2001)) .Halophilic and halotolerant bacteria appear to have higher capacities to accumulate compatible solutes (Trehalose, GB, Ectoin, etc...), Hengge-A *et al*,1991 , and appear faster to modulate their response over time and as a function of growth phases

Impact in the cytoplasm

The Osmosenseurs

To make the transition between membrane response to osmotic stress and impact in the cytoplasm, attention will be focused on the membrane osmosenseurss whose activity occurs in the cytoplasm. These are the Histidines kinases **KdpD** and **EnvZ** , Wood,(2007) , Wood *et al*, (2001)

KdpD

In *E. coli*, KdpD is a dimer with each monomer consisting of 4 transmembrane segments and large N-and C-terminal hydrophilic extensions (400 amino acid residues), located in the cytoplasm. Whereas for osmosenseurs carriers, transmembrane segments are indispensable, in the case of KdpD, they are not essential for the detection of variations but appear to be important for the relative positioning of the N-extensions and C-terminal. An ATP binding pouch in the N-terminal domain stabilizes interactions with the KdpE related response controller.

KdpD and KdpE are a typical sensor kinase/response regulator system. During osmotic stress the concentration of solutes in the cytoplasm increases and the volume of the cell decrease modifying the membrane properties , Jung, K *et al,* (2002) .

EnvZ

The *E. coli* EnvZ sensor kinase belongs to the P-type sensors (periplasmic signal-transducing) with a linkage domain called HAMP (linker domain in Histidine kinases, Adenylyl cyclases, Methyl binding proteins and phosphatases) between the domain Transmembrane and the transmission domain.EnvZ is capable of detecting changes in K + concentration in the cytoplasm, thanks to its hydrophilic part (not integrated into the membrane). When the concentration increases, EnvZ is subjected to autophosphorylation on a highly conserved His-243 residue. The Phosphoryl group is then transferred to the preserved ASP-55 residue of OmpR, a response regulator. After phosphorylation, OmpR-P plays the role of transcription factor promoting the expression of the major genes of the outer membrane, OmpC and OmpF. OmpC and OmpF form channels in the outer membrane, called porins allowing the passive diffusion of small hydrophilic molecules of smaller size than 650 Da.

EnvZ also has a phosphatase activity directed against OmpR-P in order to dephosphorylate. Osmotic stress increases the ratio of kinase activity to EnvZ phosphatase activity to increase the cell level of OmpR-P, favouring transcription of OmpC and OmpF. Conversely, when osmotic pressure decreases, EnvZ phosphate activity is stimulated, decreasing the amount of OmpR-P in the cell, and by the same, the number of porins in the membrane, . Cay *et al*,(2002) .

Accumulation of Solutes

In an osmotic stress situation, the cell has no choice but to accumulate solutes in the cytoplasm, by capture or synthesis, in order to limit the outward movement of water. The accumulated solutes are usually molecules of small weight molecules, because holding better water, and preferentially unloaded. These are often derivatives of amino acids or sugars. The reaction of the cell is cut in two phases:

"Primary Response" consisting of accumulating K + potassium ions and glutamate as a counter-ion.

“Secondary Response" triggered by the increase or maintenance of the concentration of potassium glutamate in the cell, meaning that the stress increases again or extends over time. The cell then accumulates so-called compatible solutes, or osmoprotecteurs, because they do not disturb the cellular functions.

The **osmoprotecteur** most used in bacteria developing at low salinities (particularly pathogens) is trehalose. Although derived from sugars, it is not a routine molecule like glycogen, but it remains soluble and neutral at higher concentrations. The increase in cell content decreases the concentration of potassium glutamate but not totally, as it serves as a signal for the synthesis of trehalose. Its accumulation is in fact only by synthesis, the trehalose captured from the outside being first degraded to glucose and glucose-6-phosphate to reform the Trehalose in the cell. The Trehalose also protects bacteria from cold and desiccation , Hengge-A *et al*,(1991).

In other microorganisms that develop at higher salinity, we will find:

Ectoine, derived from the biosynthesis of the connected amino acids.
Glycerol in yeasts.

Proline in Bacillus, up to 0.4 M and then synthesis of Trehalose. *E. coli* also use proline if it is present in the medium and can absorb it but does not synthesize it, . Hengge-A *et al*,1991.

Glycine betaine, produced by algae and plants by photosynthesis, can be recovered by bacteria.

But all these osmoprotecteurs do not have the same properties as shown by the classification of Hofmeister : The Hofmeister series classifies molecules (primary ions) according to their influence on the behaviour of many "aqueous" mechanisms, from colloidal assemblage to protein folding. Although it has long been thought that this influence of ions depends on their ability to bind water and modify the structure of open water, new models show that this is not the main reason. The influence of ions would, in fact, depend on their direct interactions with macromolecules (DNA, RNA, proteins...), as well as with their first layer of hydration.

Thus the first members of the series increase the surface tension of the water and decrease the solubility of the apolar molecules, reinforcing hydrophobic interactions. They are called Chaotropic Agent because they destroy the three-dimensional structure of macromolecules and distort them Conversely, the last elements of the series reduce the surface tension of the water and increase the solubility of the apolar molecules, so they are called cosmotropes.

Disruption of cellular functions (Fig.8)

A. Modification of protein/DNA interactions
B. Modulation of protein folding

Impact on gene regulation

A. At the transcript level
In an osmotic stress situation, the σS factor promotes the expression of 18 genes, including:
-ProP and ***ProU*** and more the osmosenseurs carriers
-***OsmB*** and ***OsmE*** encoding external membrane lipoproteins of unknown functions
-***OtsA***, ***OtsB*** and ***TreA*** involved in the synthesis of Trehalose :
-***OtsA***: Trehalose-6-phosphate synthase
-***OtsB***: Trehalose-6-phosphate phosphatase
-***TreA***: Trehalase
- ***CFA*** responsible for the synthesis of Cyclopropane fatty acids in Gram –
- The ***RcsCDB*** system, essential in stress situations, activated by alterations of the membrane, responsible for the induction:
- ***CPS*** genes, involved in the synthesis of the capsule (in the bacteria concerned)
- ***OsmC***: Organic Hydro-péroxidase
- The ***Bdm-Sra*** *(*biofilm-dependent modulation-stationary phase-inducible ribosome-associated protein) operon

Regulation of the σS factor

Transcriptional Fusion Of ***Lacz*** On ***Rpos***
Western Blot Analysis
Electrophoresis 2-D On O'Farrell Gel

The σS factor is responsible for the general response to stress and stationary phase in bacteria. As presented at the level of transcription. It promotes the expression of certain genes in case of osmotic stress. For this, the ***RpoS*** gene must be expressed itself more. It appears in fact that osmotic stress essentially influences the post-transcriptional regulation of ***RpoS***, in different ways has been studied by Barth *et al*(1995) , Loewen, P *et al*, (1998), Hengge-A *et al*, (1991), Hengge-A, *et al* (1993) :

By stimulation of translation: ***RpoS*** mRNA naturally presents a secondary structure that prevents its translation. The ***HfQ*** protein is capable of destabilizing this secondary structure but is itself inhibited by NAP H-NS. When

osmotic pressure increases, it appears that the amount of H-NS in the cell decreases, thus allowing ***HfQ*** to attack the secondary structure of ***RpoS*** mRNA and thus promote its transcription.

By modulating the stability of the σS factor: In the case of osmotic stress, it appears that the amount of RssB decreases in the cell, whereas its role is usually to promote degradation of σS. The σS factor is, therefore, more stable.

By promoting its association with the RNA polymerase Apo-enzyme: the accumulation of Trehalose and glutamate in the cell due to osmotic stress, promotes the association of σS with the RNAP, thus promoting the expression of its surgery. Finally, NAP Fis promotes the expression of certain genes dependent on the σS factor.

Other influenced mechanisms: an example of breathing

It is known that oxidative phosphorylation via aerobic respiration of organisms and a more effective way for the production of ATP than the pathway of glycolysis. In general, NaCl stress in non-halophilic bacteria such as E. coli results in a reduction in respiratory activity, although ATP productivity is independent of the ***NaCl*** concentration in the medium. But this is not the only metabolic function disturbed by osmotic stress. Absorption and catabolism of carbonaceous substrates, as well as cell growth, are also affected. The study by Nagata (2002) was designed to compare the effects of different osmoprotecteurs on these functions.

Thus, the inhibition of cell respiration by high osmolarity was reversible by the addition of osmoprotecteurs, especially proline. The activities of transporting carbohydrates through the cell membrane also increase despite the high salinity. Finally, although this requires a longer incubation time, cell growth also re-increases in the presence of osmoprotecteurs. In all three cases, proline is the most effective. A correlation of these three functions was also demonstrated, showing that cell growth is related to biosynthesis processes that can only be initiated after substrate and solute accumulation.

A bit of methodology

The study of a membrane protein can be done in vivo but its specific role will be difficult to study due to the presence of many other proteins with similar roles in the membrane. It's in vivo overexpression is difficult to conceive since the membrane will be destabilized leading to a deleterious phenotype. In general, these studies are therefore made in membrane vesicles (a bacterial membrane lacking its cytoplasm) or in proteoliposomes (in vitro method where the composition of the lipid bilayer is fully controlled and verified by different methods described.

The study of the mechanosensors channels requires a specific method: the patch clamping which allows measuring the electric current passing through a pore (here: a channel) thanks to a micropipette filled with a solution of electrolytes which will transmit the Variations of currents via an electrode connected to an amplifier. Depending on whether the effect of intracellular or extracellular variations is to be measured, two configurations exist: inside-out (inward-facing channel) and outside-out (outward-facing channel), Blount, *et al*.(1999) , Booth ,*et al*. (2007).

To check the function of a channel, an original method is based on the premise that if the channel is opened, there will be cell death.This is done by combining osmotic stress with acid stress (PH < 3.6 in E. coli) (described at p. 55 by Booth *et al*, 2007) to identify a carrier, several methods are possible. The study of the confirmation or the folding of a protein can be done in different ways.

CONCLUSION

The bacteria are able to withstand more or fewer variations of osmotic pressure depending on the efficiency, the number and the diversity of the resistance mechanisms. Some have very effective mechanisms and are then problematic in the food industry (ex: *Staphylococcus aureus*).

In addition, osmotic stress activates the Sigma stress factor (Sigma S in E. coli), hence increased resistance of bacteria to other stresses (thermal, oxidative, etc...) When osmotic stress is generated.It is therefore essential to combine different treatments in the food industry and not to use the AW as the only criterion.

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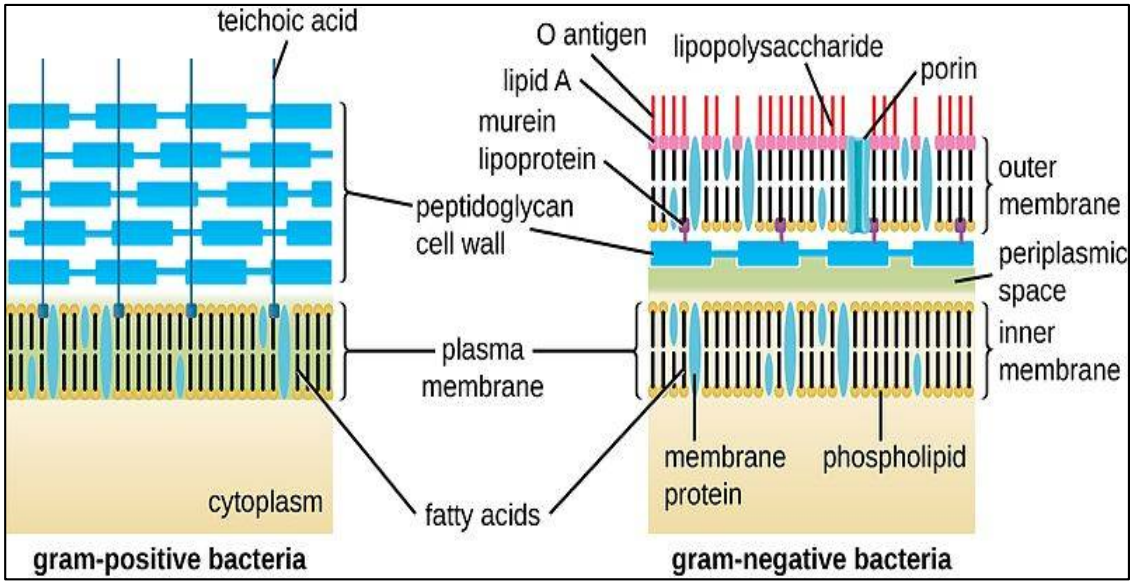


Fig.1Simplifld schematic of cell wall in Gram-Negative and Gram-positive

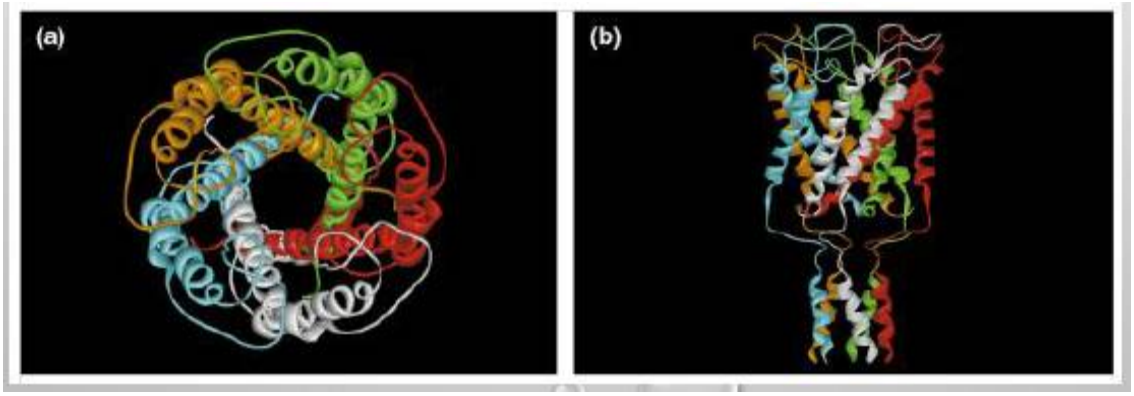


Fig.2. MscL channel in *Mycobacterium,* Romantsov, *et al.*(2009), Boris M. (2011).

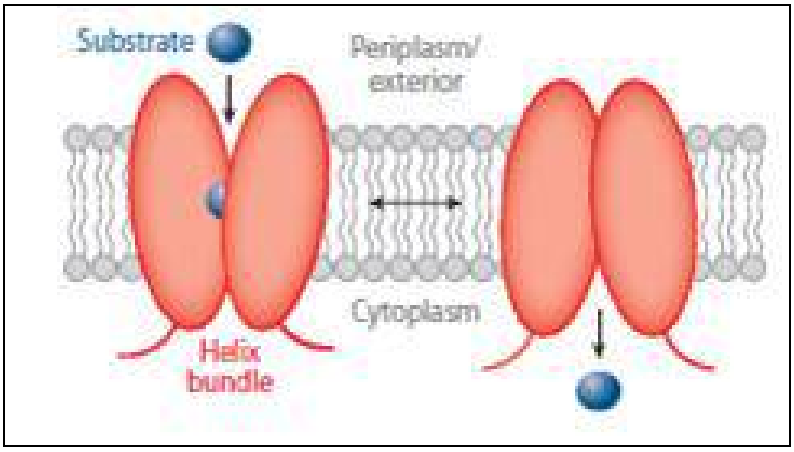
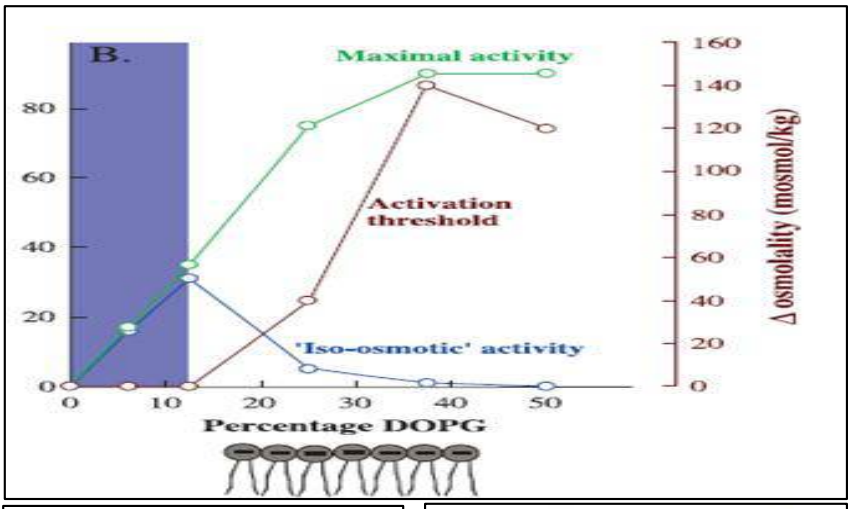
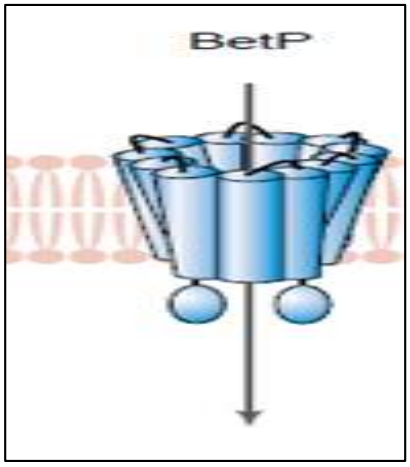


Fig.3.ProP in *Escherichia coli* Francius *et al*,(2011b)





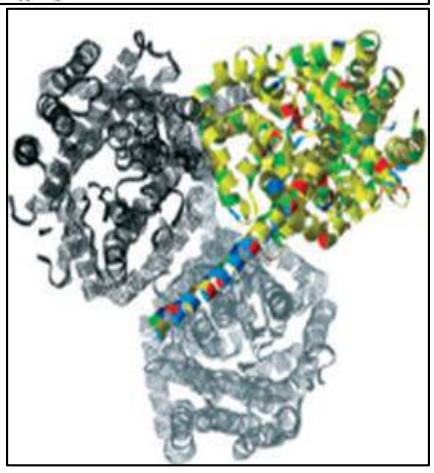


Fig.4. BetP in *Corynebacterium glutamicum* , Heermann *et al*,(2004).

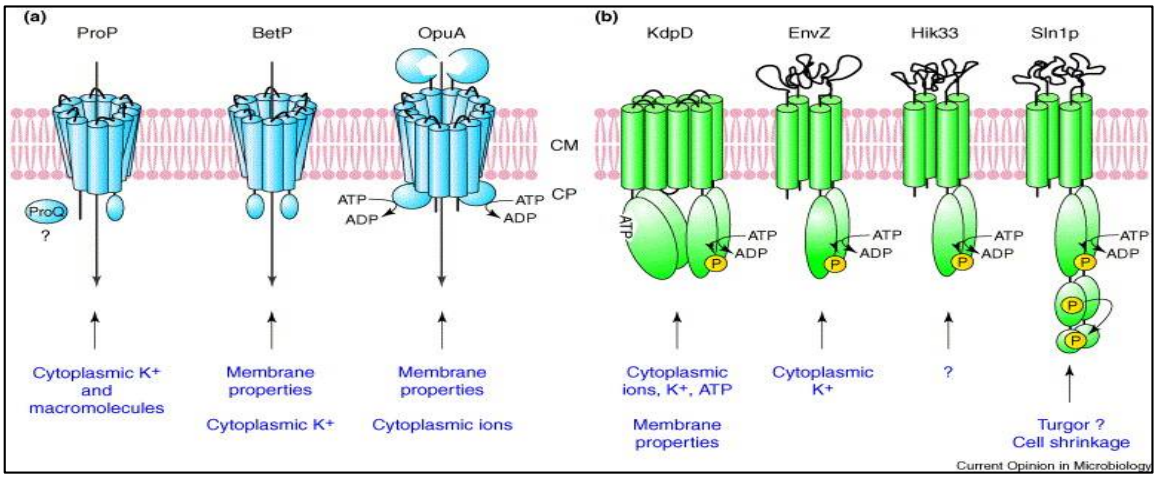


Fig.5.Structural features and mechanisms for sensing high osmolarity in microorganisms .Heermann *et al*,(2004).

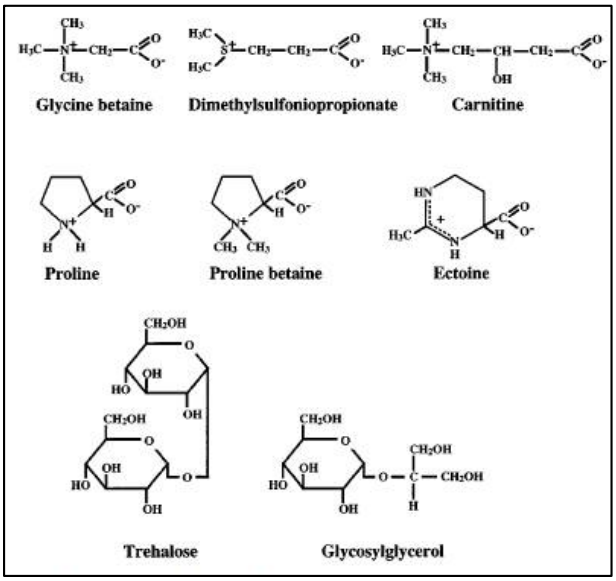


Fig.6. structures of selected osmoprotectants

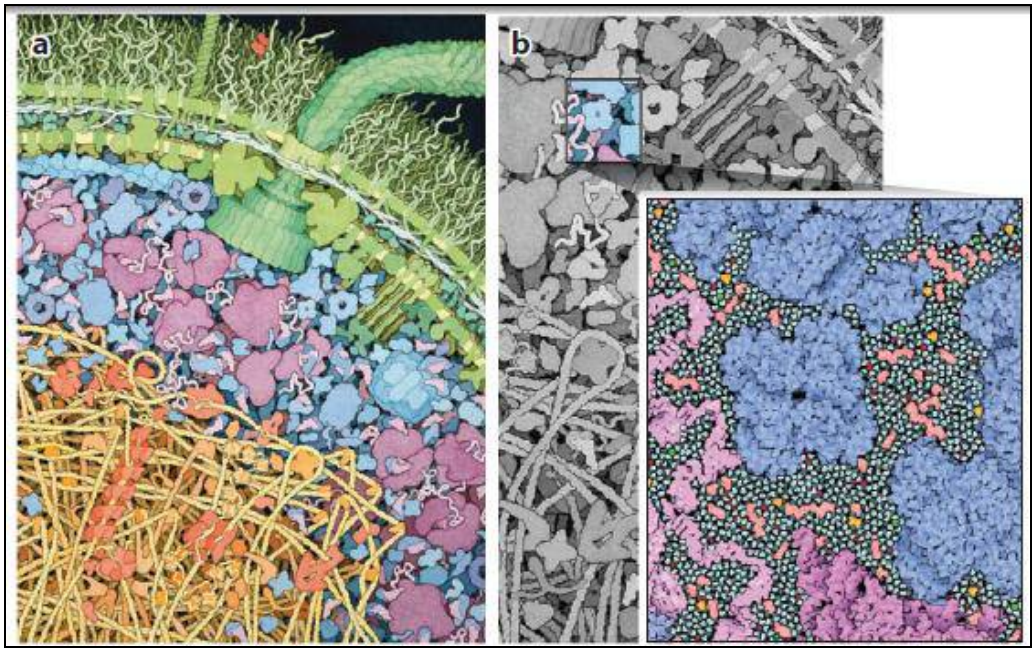
$$F^- \approx SO_4^{2-} > HPO_4^{2-} > \text{acetate} > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$

$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium}$$

Fig.7. Classification of Hofmeister, Hebert *et al* (2009)

Disruption of cellular functions

A. Modification of protein/DNA interactions



B. Modulation of protein folding

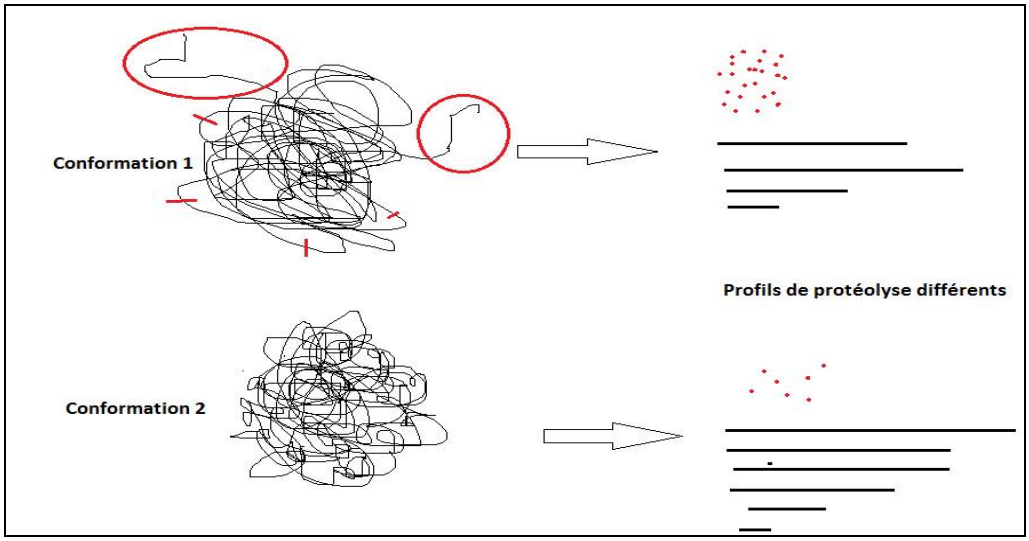


Fig.8.Profiles of differences proteolysis

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REVIEW ARTICLE

Role of Phytoestrogens in Augmenting Reproductive Performance in Poultry

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ABSTRACT

Phytoestrogens are Estrogen-like compounds have the ability to alter growth, development, and function of estrogen-dependent target tissues. They have estrogen like biological activity which influence the growth and functioning of female and male reproductive tissues. Mechanistically these compounds have been shown to bind of estrogen receptors. Isoflavones, a group of natural phytoestrogen, are present in plant foods as glycoside conjugates and have molecular mass and structure similar to endogenous sex steroids. Apart from reproductive performances phytoestrogens also influences skeletal and central nervous system, provide cardio protective effects in cardiovascular system and prevent against tumorogenesis and ageing of skin. In birds estrogen is proposed to be a pivotal factor involved in the development of sexual differentiation, female secondary sexual characteristics and vitellogenesis.

Key words: Poultry, Reproduction, Phytoestrogen, Isoflavone

INTRODUCTION

The global production, consumption, and trade of poultry meat have grown faster than that of any other meat in recent decades. This growth is expected to continue because poultry meat and egg are cheaper, more versatile, and provides more health benefits, than do other animal husbandry products. Decrease in reproductive performance of birds causes major economic impact in terms of lower egg production. The current researches are focusing on supplementation of phytoestrogens in feed so as to augment reproductive performances in poultry industries. Phytoestrogens are estrogen-like compounds have the ability to alter growth, development, and function of estrogen-dependent target tissues. Phytoestrogens are group of plant derived polyphenolic, nonsteroidal

compounds that have estrogen like biological activity *i.e.* they mimic the action of estrogen in humans as well as in animals, therefore they are considered to play an important role in reproductive physiology and in prevention of some physiological and pathological conditions like cancer, heart disease, prostate cancer, osteoporosis (1, 2, 3).

Estrogen influence the growth and functioning of female and male reproductive tissue, maintain the skeletal and central nervous system, provide cardio protective effects in cardiovascular system and prevent against tumorogenesis and ageing of skin (4, 5). In avian species, phytoestrogen (daidzein) up regulates m-RNA expression of gonadotropin receptors to improve follicular development in chicken developing follicles and laying performance after peak laying period (6). Isoflavones, a group of natural phytoestrogen, are present in plant foods as glycoside conjugates and have molecular mass and structure similar to endogenous sex steroids. It had been demonstrated in many studies that isoflavones exerted estrogenic activities in several *in vitro* test systems (7, 8). Different cellular and biochemical properties have been attributed to the various isoflavones besides their affinity for the estrogen receptors (9).In poultry factors that are known to influence this reproductive behaviour are hormonal level, light, temperature, protein and energy content of the diet. However, research reports highlighting the influence of dietary phytoestrogens on production and reproduction aspects in guinea fowls are limited. In this regard we are reviewing the all aspects of phytoestrogen that have scope to improvise the research ideas in augmenting reproductive performances.

Phytoestrogen

Estrogen-like compounds have the ability to alter growth, development, and function of estrogen-dependent target tissues. Phytoestrogens are group of plant derived polyphenolic, nonsteroidal compounds that have estrogen like biological activity *i.e.* they mimic the action of estrogen in humans as well as in animals, therefore they are considered to play an important role in reproductive physiology and in prevention of some physiological and pathological conditions like cancer, heart disease, prostate cancer, osteoporosis (1, 3). Phytoestrogen defined functionally are substances that promote estrogenic action in biological system and structurally are similar to mammalian estrogen 17-β estradiol (E2) (10, 11). Phytoestrogen can bind to the estrogen receptors to induce estrogen like effect in animals, humans and in cultured cells (7, 12). Estrogen influence the growth and functioning of female and male reproductive tissue, maintain the skeletal and central nervous system, provide cardio protective effects in cardiovascular system and prevent against tumoregenesis and ageing of skin (4, 5). In avian species, phytoestrogen (daidzein) up regulates m-RNA expression of gonadotropin receptors to improve follicular development in chicken developing follicles and laying performance after peak laying period (6). Isoflavones, a group of natural phytoestrogen, are present in plant foods as glycoside conjugates and have molecular mass and structure similar to endogenous sex steroids. It had been demonstrated in many studies that isoflavones exerted estrogenic activities in several *in vitro* test systems (7, 8). Isoflavones are noted for their putative ability to prevent cancer, menopausal symptoms, cardiovascular disease, atherosclerosis, osteoporosis, and reduce cholesterol levels in the blood (13, 14, 15). Different cellular and biochemical properties have been attributed to the various isoflavones besides their affinity for the estrogen receptors α and β (9).

Estrogen, sexual maturity and production

In birds estrogen is proposed to be a pivotal factor involved in the development of sexual differentiation, female secondary sexual characteristics and vitellogenesis. As hens mature sexually, estrogen concentration in plasma gradually increase, with more marked increase occurring from 16 to 20 weeks of age (16). Concentration remains high for the next several weeks with daily surges occurring approximately 4 to 6 hrs prior to ovulation and coincident with the daily surges of LH and progesterone (16, 17). The exact time course of the secretion pattern of estrogen after hens reach peak production is not clear; few studies have monitored plasma estrogen systematically over the entire production period and those that have done so have not taken samples often enough to give a clear picture. It is generally accepted that estrogen declines over the production year (18), drops very low during molt (19),

and increases again as hens are brought back into production (18). These changes underlie the egg production pattern of commercial layers, in which there is gradual decline in egg numbers from the peak, reached shortly after sexual maturity. Through in vitro studies, it is also observed that the sensitivity of LH for ovulatory response is lower in poor layer as compared to good layer and further this response reduces with the increase in age (20).

Oviduct responses to sex steroids

The functions of the oviduct are sustained by the synergistic action of estrogen, progesterone and androgens. The relative importance and dynamic interactions of these hormones in normal laying females have not been clarified; they are obviously complex.Estrogen is a prerequisite for differentiation and development of the oviduct of female birds but excessive early exposure to estrogen can cause oviduct abnormalities and impair egg laying ability (21). In the upper oviduct where the egg is moving quickly the major concentration of adrenergic receptors are α-excitatory and hence significant contraction of the infundibulum occurs upon stimulation. Whereas, in the lower oviduct adrenergic receptors are predominantly β-inhibitory, and the relaxation of the uterus is the observed effect (22). In mammals, the degree of adrenoreceptor stimulation depends upon the levels of circulating estrogen and progesterone. Estrogen increases the α-receptor sensitivity while progesterone enhances the β-sensitivity (23). The nor adrenaline content of the avian oviduct is increased significantly during the active laying phase and falls to a low level when the oviduct becomes inactive as in nonlayers.

Mechanism of action

Phytoestrogens can bind to the estrogen receptors to induce estrogen like effects in animals, humans and cultured cells (7, 24). Mechanistically these compounds have been shown to bind 2 types of estrogen receptors: ER-α, which was cloned in 1986 and ER-β cloned in rats (25) and in humans (26). The two receptors differ in their tissue distribution and affinity to ligands, yet there is some overlap. In rats ER-α and ER-β both are clearly expressed in ovary and uterus tissue (25). ER-β has been shown to have ligand specificity towards phytoestrogens and is distributed in humans in ovary, spleen, testes and thymus tissue (26) and in rats in bladder, brain, lungs, ovary, prostate, testes and uterine tissue (9). Phytoestrogens show a lower binding affinity than E2 and some show a high binding affinity for ER-β than for ER-α, which may suggest different pathway for their action and explain tissue specific variability of phytoestrogen action (9, 1).

Effect of phytoestrogen on Growth

Supplementing soybean genistein (200 mg/Kg) and daidzein (200 or 400 mg/Kg) in feed could improve growth in virally challenged pigs (27, 28). Supplementing isoflavone into the diet at 1% and 5% did not influence the growth performance of Japanese quail (29) however isoflavone levels in excess of those in a corn soybean meal diet decreased gain: feed ratio but did not affect average daily gain and average feed intake in broilers (31). Adding 10 or 20 mg of isoflavone per Kg to the diet significantly increases final body weight, weight gain and feed intake in birds; feed: gain ratio of birds significantly decreased by supplementation of 10 mg/ Kg isoflavone during 43 to 63 days experimental period in male broilers (31). Likewise, daidzein treatment significantly increased feed efficiency by 6.44% in shaoxing duck breeders (32).

Effect of phytoestrogen on reproductive performance
Organ weight

Daidzein stimulated germ cell proliferation in embryonic chicken through estrogenic and antioxidant actions (24). Laboratory studies have shown that female rats fed a diet high in the soy isoflavone genistein and daidzein had increased uterine weight and earlier 1st estrus (33). In birds genistein has been shown to induce oviduct growth in

broilers and zebra finches (34). Administration of individual isoflavone (coumestrol & Biochanin-A) to captive scales quail and found slight induction of oviduct growth (35).

Follicle numbers

The growth and development of ovarian follicles undergo a series of complex biochemical and physiological changes, which include gonadotropin receptor expression, steroid biosynthesis, cell proliferation and differentiation. Among these changes the expression of gonadotropin receptors (FSHR and LHR) play very important role in inducing follicular development (36). Daidzein treatment markedly increased the numbers of small yellow follicles (SYF) and large white follicles (LWF) compared with control in the ISA hens after peak laying. Isoflavone supplementation improved egg production in laying Japanese quail reared under heat stressed conditions (34°C) during the late laying period (37). Daidzein supplementation improved post peak egg production of Shaoxing ducks in a dose dependent manner (32). Feeding daidzein to white silky fowls significantly improved the laying performance (12). Controversial positive and negative effects of phytoestrogen on reproduction have been reported, earlier studies suggest that phytoestrogen may cause follicular abnormalities, infertility in animals and decline in egg laying. However, daidzein stimulated germ cell proliferation in chicken embryonic ovary, and daidzein increased laying performance, follicle development and m-RNA expression of FSHR and LHR in laying hens (12). Daidzein has been shown to increased egg laying in ducks; however egg composition was altered (increased albumin and decreased yolk volume) (36).

Egg quality

The proportion of defective eggs can be a serious problem affecting the profitability of laying poultry. Recent studies have suggested that higher isoflavone intake is associated with increased bone mineral content and reduced markers of bone resorption (38). Calcium (Ca) plays an important role in the formation of egg shell and bone. Increasing Ca absorption might be responsible for increased egg shell strength or decreased occurrence of bone fracture; there is a direct relationship between soy isoflavone and Ca (39). Soy isoflavone have been shown to decreased intracellular Ca concentration in osteoclasts implying an increasing amount would be available for bone and egg shell formation. Daidzein supplementation improved in egg shell quality and bone mass in chicken. Soy isoflavone supplementation improved egg quality (namely, shell thickness, shell weight and Haugh unit) in laying Japanese quail reared under heat stressed conditions (34°C) during the late laying period (37). The improvement in egg quality was probably due to increase in Ca which was increased by supplemental isoflavone; this is in agreement with the result of previous studies on genistein in which the amount of Ca, P, Mg, Mn, Zn, Fe and Cu in the excreta decreased while Ca, P, Mg concentration in serum increased in birds with genistein supplementation (40).

CONCLUSION

Reproductive tract irregularity is one of the major cause which have economic impact in terms of lower egg production. Estrogen-like compounds have the ability to alter growth, development, and function of estrogen-dependent target tissues. Phytoestrogens are group of plant derived polyphenolic, nonsteroidal compounds that have estrogen like biological activity i.e. they mimic the action of estrogen. Phytoestrogens can improve the reproductive efficiency (egg number, egg size, age of sexual maturity, fertility and hatchability) by alleviating reproductive anomalies (atresia, internal ovulation and abnormal hierarchy) through the modulation of neuroendocrine events at molecular level.

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RESEARCH ARTICLE

Assessment of Shallow Aquifer Groundwater for Different Uses, Southern Iraq

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ABSTRACT

Water quality has become an important requirement in recent years, assumed the enormous pressure on water resources. As a result of the rapid population growth and climate change. Seventeen wells were chosen around the river and water sampled were collected. After analyzing the water samples, it was found that pH values range from (7.1 to 7.5). The values of total dissolved ions ranged from (1024-1610) mg/l. Total hardness range between (472.50 to 721.40 mg/l) for the study periods and all the samples are considered very hard. Groundwater samples were compared with what Langmuir put it from determinants, It was found that the pH rate, TDS and major ions exceeded those determinants of natural water .The water content of magnesium hazard ranges from (51.1 to 53.7), it is within the impermissible limits for watering purposes. With respect to the RSC value, all groundwater are safe for irrigation purposes. Evaporation and ionic exchange were studied to evaluate hydrogeochemical processes where ionic exchange processes is dominant

Keywords: - Groundwater, water quality, SAR, Kellys Ratio, Permeability Index

INTRODUCTION

In several arid and semi-arid countries, water is becoming more insufficient resource and enforced planners to suppose any sources of water which might be use cheaply and successfully to encourage further development. The provision of drinking and irrigation water faces major challenges, including the shortage of water and poor quality (Al-Ansari, 2013), for the purpose of drinking or irrigating agricultural land to cope with food shortages, as a result of population growth. Groundwater are one of the most polluted water sources, because most carrying municipal, industrial, sewage waste as well as the agricultural land drainage channel (Al-Khafaj, 1985). Therefore, the problem requires particular research to evaluate the hydrochemical and mechanical pollution processes to control it.

According to spatial and temporal changes in the water quality of the groundwater, the situation requires development of an integrated program to monitor the physic- chemical variables in the area in order to draw a clear map of the groundwater quality (Kenneth, 2003).

Location and Geology

The study area occupies the areas between Tigris and Euphrates rivers among the provinces of Kut and Dhi-Qar.It locates between the Latitude (32°31'21.40" N), Longitude (45°49'17.38"E) and Latitude (31°05'41.60" N), Longitude (46°29'22.05"E). There are no clear and important studies about groundwater (aquifer size, quantity and depth of groundwater) in the area, and only some shallow wells around Gharraf River, it is the only fresh water source in the area. The Mesopotamia plain comprises a lake, marsh complex which covered with Quaternary deposit. The thickness of the Quaternary deposit exceeds 250 m. The Quaternary deposit distributed to Pleistocene and Holocene deposits (Jassim and Goff, 2006):

Pleistocene deposits

Pleistocene deposits cover all parts of the study area and the upper limit of this sediment could be up to (1.5 m) below the surface of the ground and up to a thickness of (174 m) and consists of sand, silt, clay inter bedded with each other (Jassim and Goff, 2006).

Holocene deposits

The upper part of the sequence, most of the Holocene period comprises fluviatile flood silts and aeolian silts. It is alluvial plain deposit which comprises from rivers deposit, a deposit of Shallow.Depression and Marshes deposits **.**The Quaternary sediments are unconsolidated and usually finer grained than the underlying formations (Jassim and Goff, 2006).

MATERIAL AND METHODS

During the year 2017, specifically for wet periods seventeen water samples were collected, through seventeen wells selected around Gharraf River (Fig. 1). The water samples were collected according to Fitter method (Fetter, 1980).For valuation of water quality for human consumption and irrigation, the following parameters were being studied:

Magnesium ions hazards

The concentrations of magnesium ions hazards are measured to estimate the water validity for irrigation purpose. If the concentration of magnesium hazard is more than 50, water is as harmful and unsuitable (Szaboles and Darab, 1964). Magnesium hazard can be calculated using (Eq.1), while the concentration of ions is in meq/l.

$$\text{Magnesium Hazard} = \frac{[Mg]}{[Ca]+[Mg]} \times 100 \quad \text{---------- (Eq.1)}$$

Remaining sodium carbonate

An extra concentration of CO3 and HCO3 in water have too impacts on the quality of water for irrigation purpose. Extreme remaining sodium carbonate (RSC) will deterioration the soil structure and restrict the air and water movement through the soil (Swarna, and Nageswara, 2012).

Harmonizing to the U.S. Salinity Laboratory (US Salinity Laboratory Staff, 1954). Where RSC value <1.25 meq/l is considered 'safe water' for irrigation; The RSC value is proposed by (Eq.2), where ionic concentrations are stated in meq/l:

$$RSC = (HCO^{-}_{3}) - (Ca^{2+} + Mg^{2+}) \text{ ---------- (Eq.2)}$$

Kellys Ratio

Kelly's ratio, that is equal to 1 or <1, it indicates good quality of water for irrigation, and if the Kelly's ratio is > 1, the groundwater is not suitable for agricultural purposes owing to the extra values of Na+ in the water (Kelley, 1951). Kelly's ratio (KR) can be calculated by (Eq.3) and the concentration of ions is in meq/l:

$$KR = \frac{Na\ ion}{(Ca+Mg)\ IONS} \text{ ---------- (Eq.3)}$$

Permeability Index

Doneen suggests an equation to assess the validity of irrigation water established on a permeability index (PI) (Doneen, 1964). The PI value is calculated by (Eq.4) and the concentration of ions is in meq/l:

$$PI = \frac{Na+\sqrt{HCO3}}{Ca+Mg+Na} \text{ ---------- (Eq.4)}$$

Where PI is used to assess sodium risk on irrigation water, and therefore, specify its appropriateness for irrigation purposes. Water can be classified into three orders, Class I and Class II waters are categorized as 'good' for irrigation purposes, with 75% or more of permeability, whereas Class III waters are 'unsuitable' for irrigation purposes, with only 25% of maximum permeability.

Evaporation and ion exchange

Evaporation is an important climate factor, which has a direct and negative impact on the water sources in the area of the study, and this importance's comes from the hot and dry climate prevailing in the region. The evaporation process is not only a common phenomenon in surface water, but also in shallow groundwater systems. Na/Cl ratio can be used to recognize the evaporation process in groundwater (Subramani et al., 2009). Evaporation will raise the concentration of TDS in groundwater. Ion exchange is one of the essential processes responsible for the concentration of ions in groundwater. Chloro alkaline indices 1 and 2 (CAI 1 and CAI 2) were calculated for the groundwater samples of the study area, it is a powerfully advise for the occurrence of ion exchange process (Subramani et al., 2009).

$$CAI\ 1 = C1^{-} - (Na^{+} + K^{+}) / C1^{-} \text{ ---------- (Eq.5)}$$

$$CAI\ 2 = C1^{-} - (Na^{+} + K^{+}) / SO_4^{2-} + HCO_3^{-} + CO_3^{2-} + NO_3^{-} \text{-------- (Eq. 6)}$$

(All values are expressed in meq/l).

RESULTS AND DISCUSSION

The hydrochemical variables were studied and the values of the groundwater constituents are explained in ppm expect EC in µS/cm (Table.1).

Water suitability according to global and local determinant

The minimum, maximum and average physic-chemical parameters of groundwater were cited in Table (1) for comparative with/and according to Langmuir (Langmuir, 1997), the mean results of analyzes for the seventeen wells were compared with what Langmuir put it from determinants. It was found that the pH rate, TDS, major ions exceeded those determinants of natural water (Table.2).

There are several standards defining the suitability of water for drinking such as (WHO, 2006, 2007) and Iraqi standards (IQS, 2009). According to these two standards, all groundwater in the study area were un suitable for drinking purpose (Table 6 and 8), TDS is out of permissible limits (Table.3).

According to (Him , 1970), all the water samples studied were occupied with slightly saline categories water (Table 4).Total hardness range between (472.50 to 721.40 mg/l) for the study periods. All the samples are considered very hard and the high concentrations of hardness are due to the ionic exchange and evaporation processes (Tables 5 and 6).

The groundwater had been evaluated for livestock uses depending on the classification proposed by (Altoviski , 1962). This classification is based on some of the major cations and anions as shown in Table (1). According to (Altoviski ,1962) classification, all the groundwater samples were very good for livestock uses (Table 7). The suitability of water samples for building purposes is based on (Altoviski ,1962) classification; all water samples of the groundwater are suitable for building purposes (Table 8).

Magnesium Hazard

The concentration of Magnesium Ions Hazards is calculated to evaluate the groundwater validity for irrigation purpose. The value of magnesium hazard more than 50, water is as harmful and unsuitable (Szaboles and Darab, 1964). All groundwater well samples water are in the range of magnesium hazard, which were ranged between (51.1 to 53.7) for all samples, and more than 50 percent. So, it is unsuitable for irrigation purpose (Table 9). But they are worthy of attention, because the magnesium ion concentration is close to the permissible limit of the ratio which form a significant risk on soil and plant.

Residual Sodium Carbonate in groundwater

An extra of CO3 and HCO3 in water are too impacts the quality of water for irrigation purpose. Extreme remaining sodium carbonate (RSC) will decline the soil structure and confine the air and water movement through the soil (Swarna and Nageswara, 2012). Agreeing to the U.S. Salinity Laboratory (U.S. Salinity Laboratory Staff, 1954), RSC value <1.25 meq/l is considered 'safe' for irrigation; The RSC value is proposed as below, where ionic concentrations are stated in meq/l.The RSC value in groundwater samples varied from -12.46 to -8.09 meq/l. U.S. Salinity Laboratory (1954) specified that an RSC value <1.25 meq/l is considered safe for irrigation; a value between 1.25 and 2.50 meq/l is of moderate quality and a value >2.50 meq/l is unsuitable for irrigation. Approximately 100% of the samples show negative values, which indicated that dissolved Ca^{2+} and Mg^{2+} concentrations were higher than HCO3- content. However, with respect to the RSC value, all groundwater are safe for irrigation purposes, where the RSC values were less than 1 meq/l (Table 10).

Kelly's Ratio

Kelly's ratio of 1 or <1 indicates good quality of water for irrigation. If the Kelly's ratio is > 1, the water is not suitable for agricultural purposes due to the extra level of Na^{+} in the water (Kelly, 1951)

The Kelly's ratio of groundwater, which is less than 1 was specified the water is appropriate for agricultural purposes (Kelley, 1951). It is observed that almost 100% of the groundwater samples were equal to 0.7. All groundwater are safe for irrigation purposes (Table 11).

Permeability Index

Doneen (Doneen, 1964) suggests an equation to assess the validity of irrigation water established on a permeability index (PI). Where PI is used to assess sodium risk on irrigation water, and, therefore, specify its appropriateness for irrigation purposes. Water can be classified into three orders. Class I and Class II, waters are categorized as 'good' for irrigation purposes, with 75% or more of permeability, whereas Class III waters are 'unsuitable' for irrigation purposes, with only 25% of maximum permeability.

Through using Doneen diagram, it was found that, all groundwater samples were in the first class of the diagram. This means that water is good for watering purposes (Table 12 and Fig. 2). All samples were failed in the low class, which is indicated low concentration of sodium and bicarbonate. These classes indicate low to moderate salinity water. It can be exploited for irrigation (Richards, 1954

Evaporation and Ion exchange

Perhaps it is important to study the impact of evaporation and ionic exchange, and its effect on the geochemistry of groundwater, and this effect is possible to know through the geochemical factors.

Evaporation

Evaporation is an important climate factor, which has a direct and negative impact on the water sources in the area of the study and its quality (Ewaid and Abed, 2017). This importance comes from the hot and dry climate prevailing in the region.

The evaporation process is not only a common phenomenon in surface water, but also in groundwater systems. Na/Cl ratio can be used to recognize the evaporation process in groundwater (Subramani et al., 2009). Evaporation will raise the concentration of TDS in groundwater, and the Na/Cl ratio remnants alike, and it is one of the good revealing factors of evaporation. If evaporation is the prevailing process, the Na/Cl ratio should be constant when EC increases (Jankowski and Acworth., 1997). The EC versus Na/Cl scatter diagram of the groundwater samples of the area, demonstrate that the trend line is inclined, and Na/Cl ratio decreases with increasing salinity (EC) which seems to be removal of sodium by ion exchange reaction or dilution process as a result of compensations for evaporated water from the river adjacent to those wells (Table 13). This observation indicates that evaporation may not be the major geophysical-chemical process controlling the chemistry of groundwater in this study area or ion exchange reaction controlling over evaporation (Figure 3).

Ion exchange

Ion exchange is one of the essential processes responsible for the concentration of ions in groundwater. Chloro alkaline indices 1 and 2 (CAI 1 and CAI 2) calculated for the groundwater samples of the study area, they are powerfully propose the occurrence of ion exchange process (Subramani et al., 2009).

$CAI\ 1 = Cl^- - (Na^+ + K^+) / Cl^-$ ---------- (Eq. 4.3)

$CAI\ 2 = Cl^- - (Na^+ + K^+) / SO_4^{2-} + HCO_3^- + CO_3^{2-} + NO_3^-$--------- (Eq. 4.4)

(All values are expressed in meq/l)

There is an exchange between Ca or Mg in the groundwater with Na and K in the aquifer material bearing water, both the above indices are negative, and if there is a reverse ion exchange, then both these indices will be positive (Schoeller 1965, 1967). CAI 1 and CAI 2 values of the study area are negative in most of the wells, and only well 2 show positive values, and normal ion exchange is noticed in one well during the study period . This notice indicates that ion exchange is the predominant process in the groundwater (Figures 4 and 5).Through the relationships that supported evaporation, as a non-key factor in influencing groundwater chemistry, it turns out that ion exchange is an important factor.

CONCLUSION

According to WHO and Iraqi standards, groundwater is unsuitable for human consumption. This is as a result of the ionic exchange and evaporation processes, also accumulation of fertilizers and agricultural pesticides, which are percolated directly to subsurface. The groundwater is suitable for watering purposes. According to Kelly ratio and Permeability Index, all groundwater are safe for irrigation purposes. Evaporation, as a non-key factor in influencing groundwater chemistry, it turns out that ion exchange is the important factor which is controlling groundwater geochemistry.

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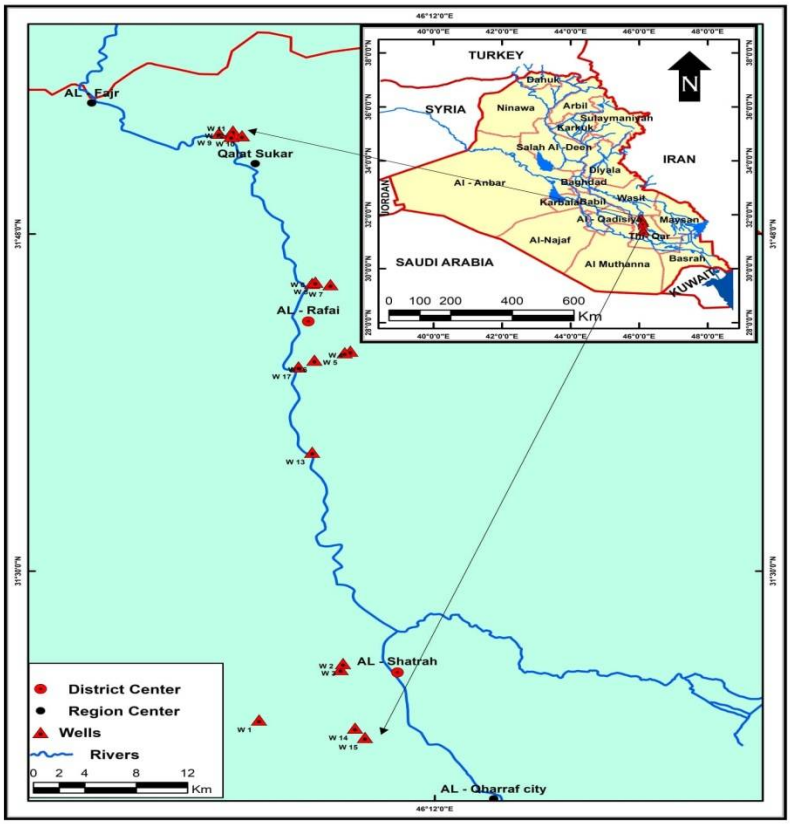

Figure 1: The study area, showing location of the sampling wells

Table 1: Hydrochemical parameters of groundwater water

Well. No	pH	EC	TDS	K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-
W1	7.5	2048	1434	25	220	83	119	346	533.4	107	1.6
W2	7.2	2300	1610	28	247	94	134	389	599	120	1.8
W3	7.1	1915	1341	23	206.	78	112	324.8	499.52	100.8	1.5
W4	7.9	1681	1177	22	197.8	67	106	295	397	95	1.3
W5	7.7	1675	1177	28	246	93	134	388	597.95	120	1.8
W6	7.1	2104	1473	25.8	226	86	123	356.7	548.58	110	1.7
W7	7.3	2225	1558	27	239	91	130	377	579.8	117	1.8
W8	7.2	2032	1426	24	218	83	119	345	530	107	1.6
W9	7.4	1692	1185	20	182	69	99	287	441.15	89	1.3
W10	7.5	1881	1317	23	202	77	110	319	490.6	99	1.5
W11	7.1	1594	1116	20	185	63.7	99.36	277	372.6	89	1.29
W12	7.3	1845	1293	22	198.7	75	108	313	481.6	97	1.1
W13	7.2	1865	1306	22.8	200	76	109	316	486	98	1.2
W14	7.2	1611	1128	19	178	59	92.9	259	348.45	83.8	1.2
W15	7.2	1641	1149	20	176	67	96	278	428	86	1.3
W16	7.1	1462	1024	19	172	59	92	257	345	83	1.2
W17	7.1	1711	1198	21	184	70	100	290	446	90	1.4

Table 2: The maximum, minimum and average physic-chemical parameters of the groundwater with (Langmuir, 1997) mean.

Para.	Unit	Min.	Max.	Mean	Mean*
pH	-	7.1	7.9	7.3	ND
EC	µS/cm	1462	2300	1840	ND
TDS	mg/l	1024	1610	1288	120
Ca^{2+}	ppm	92	134	100	15
Mg^{2+}	ppm	59	94	78	41
Na^+	ppm	172	247	204	63
K^+	ppm	19	28	23	23
HCO_3^-	ppm	83	120	90	58.4
Cl^-	ppm	257	389	290	7.8
SO_4^{2-}	ppm	345	599	446	11.2
NO_3^-	ppm	1.1	1.8	1.4	1
TH	ppm	472	721	589	ND

* (Mean of natural water worldwide After (Langmuir, 1997), ND= Not detected.)

Table 3: Groundwater Samples comparing with (WHO 2006, 2007) and (IQS, 2009) standards for Drinking Water Suitability.

Parameter	IQS 2009	WHO 2007		
			Current study	**Exceeding limits**
TDS	1000	1000	1024-1610	Exceed
PH	6.5-8.5	6.5-8.5	7.1-7.9	Not exceed
TH	500	500	472.50 to 721.40	Exceed
Ca	150	75	92-134	Exceed
Mg	100	125	59-94	exceed
Na	200	200	172-247	exceed
K	-	12	19-28	Exceed
Cl	350	250	257--389	Exceed
SO_4	400	250	345-599	Exceed
NO_3	50	50	1.1-1.8	Not exceed

Table 4: Classification of the groundwater according to total dissolved solid (TDS) after (Him, 1970).

Water type	**TDS**	**Groundwater**
Very fresh	< 300	
Fresh	300-1000	
Slightly Saline	1000-3000	All the groundwater (W1-W17)
Moderately saline	3000-10000	
Very saline	10000-35000	
Brine	>35000	

Table 5: Total Hardness in groundwater

Well. No	TH	Well No.	TH
W1	638.68	W10	591.52
W2	721.40	W11	510.22
W3	600.63	W12	578.30
W4	540.38	W13	584.91
W5	717.29	W14	474.75
W6	661.02	W15	515.41
W7	699.07	W16	472.50
W8	638.68	W17	537.75
W9	531.13		

Table 6: Classification of groundwater samples according to their Total Hardness concentration, after (Boyd, 2000).

Water type	**TH(mg/l)**	**groundwater**
Soft	< 50	(472.50 to 721.40 mg/l)
Medium hard	50-150	
Hard	150-300	
Very hard	>300	

Table 7: Specifications of waters for livestock consumption purposes.

Element	Very good water(ppm)	Good water(ppm)	Permi(ppm)	Can be use	Threshold
Na	800	1500	2000	2500	4000
Ca	350	700	800	900	1000
Mg	150	350	500	600	700
Cl	900	2000	3000	4000	6000
SO_4	1000	2500	3000	4000	6000
TDS	3000	5000	7000	10000	15000
TH	1500	3200	4000	4700	54000

Table 8: Water quality Guide for Building Uses [27].

Parameters	Na^+	Ca^{+2}	Mg^{+2}	Cl-	SO^{-2}_4	HCO^-_3
Permissible limit	1160	437	271	2187	1460	150

Table 9: Magnesium Hazard ratio for shallow groundwater (%).

Well	Mg^{2+} percent	Well	Mg^{2+} percent
W1	53.4	W10	53.5
W2	53.6	W11	51.3
W3	53.7	W12	53.3
W4	51.0	W13	53.5
W5	53.3	W14	51.1
W6	53.5	W15	53.4
W7	53.5	W16	51.4
W8	53.4	W17	53.5
W9	53.4	W10	53.5

Table 10: RSC for shallow groundwater.

Well	RSC (meq/l)	Well	RSC(meq/l)
W1	-11.02	W10	-10.21
W2	-12.46	W11	-8.74
W3	-12.44	W12	-9.97
W4	-9.24	W13	-10.09
W5	-12.37	W14	-8.13
W6	-11.42	W15	-8.89
W7	-12.06	W16	-8.09
W8	-11.02	W17	-9.27
W9	-9.16	W10	-10.21

Table 11: Kelly's Ratio for shallow groundwater.

Well	KR (meq/l)	Well	KR(meq/l)
W1	0.7	W10	0.7
W2	0.4	W11	0.7
W3	0.7	W12	0.7
W4	0.7	W13	0.7
W5	0.7	W14	0.7
W6	0.7	W15	0.7
W7	0.7	W16	0.7
W8	0.7	W17	0.7
W9	0.7	W10	0.7

Table 12: Permeability Index Ratio for Groundwater.

Well No.	□ Ion(epm)	PI %	Well No.	□ Ion(epm)	PI %
W1	45.78	49	W10	42	48
W2	47.34	38	W11	35.78	50
W3	51.32	48	W12	41.19	49
W4	38.1	50	W13	41.61	48
W5	52	48	W14	33.3	50
W6	46.93	48	W15	36.6	49
W7	49.78	48	W16	33.2	51
W8	45.35	48	W17	38.2	49
W9	37.77	49			

Table 13: The groundwater hydrochemical parameters (The unit in epm).

Well	Ca/Mg	SO4/Cl	Ca +Mg	SO4+ HCO3	Cl	Na+K	Na/Cl	Na	Cl/Cl+ HCO3	Na/ Na+Ca
W1	0.8	1.138	12.77	12.85	9.75	10.389	1	9.75	0.8	0.6
W2	0.8	1.136	14.43	14.47	11	7.416	0.609	6.7	0.8	0.5
W3	0.8	1.136	14.41	14.47	11	11.416	0.972	10.7	0.8	0.6
W4	0.9	0.992	10.8	9.81	8.31	9.163	1.034	8.6	0.841	0.6
W5	0.8	1.137	14.34	14.37	10.9	11.416	0.981	10.7	0.846	0.6
W6	0.8	1.14	13.22	13.2	10	10.49	0.983	9.83	0.84	0.6
W7	0.8	1.1	13.98	14.02	10.6	11.091	0.981	10.4	0.846	0.6
W8	0.8	1.13	12.77	12.75	9.72	10.094	0.975	9.48	0.847	0.6
W9	0.86	1.1	10.62	10.63	8.08	8.422	0.97	7.91	0.84	0.6
W10	0.8	1.1	11.83	11.82	8.99	9.368	0.976	8.78	0.847	0.6
W11	0.94	0.9	10.2	9.21	7.8	8.552	1.03	8.04	0.842	0.6
W12	0.87	1.1	11.56	11.59	8.82	9.203	0.979	8.64	0.847	0.6
W13	0.86	1.1	11.7	11.71	8.9	9.283	0.97	8.7	0.846	0.6
W14	0.95	0.9	9.5	8.61	7.3	7.876	1.012	7.39	0.841	0.6
W15	0.86	1.1	10.3	10.31	7.83	8.162	0.97	7.65	0.847	0.6
W16	0.94	0.9	9.45	8.53	7.24	7.966	1.03	7.48	0.841	0.6
W17	0.86	1.13	10.75	10.75	8.17	8.534	0.97	8	0.846	0.6

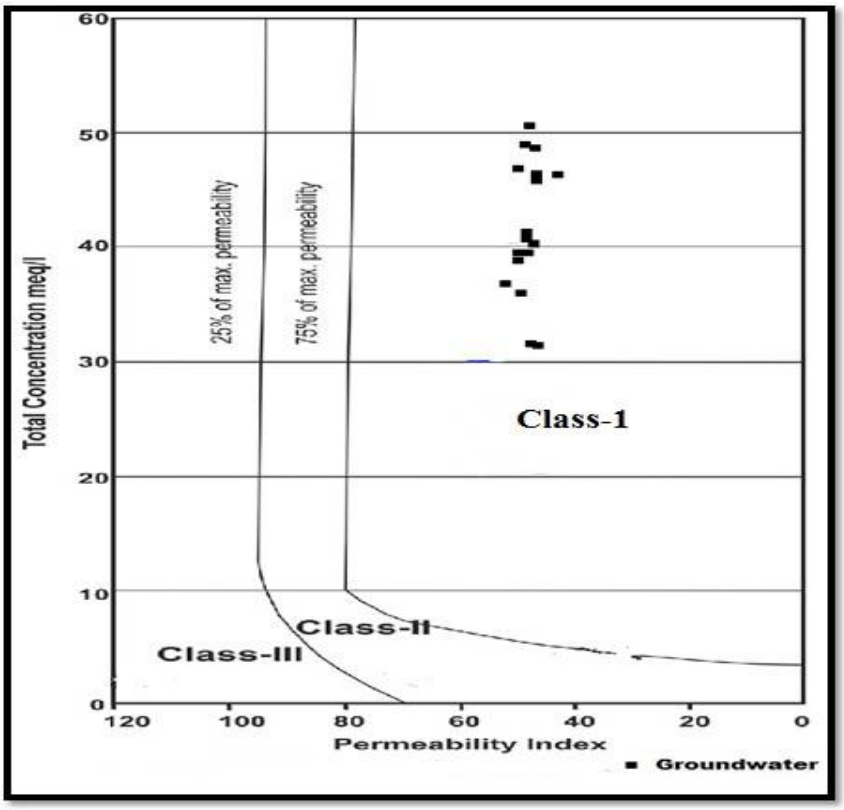

Figure 2: Doneen Diagram for validity of irrigation water.

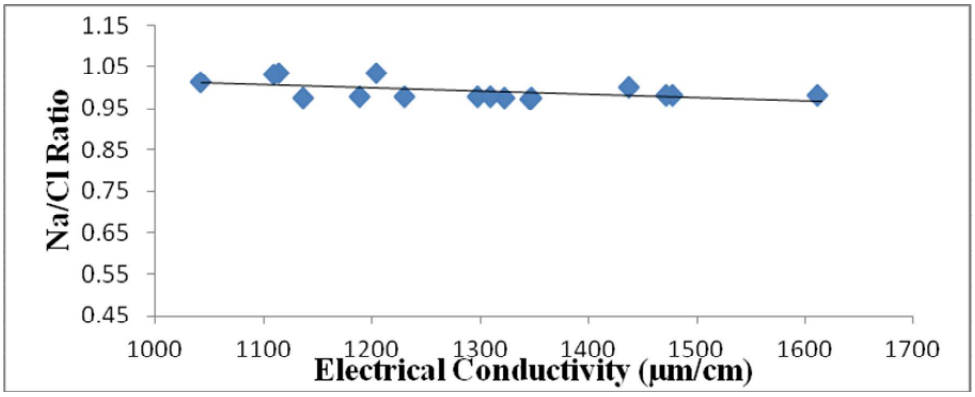

Figure 3: Relation between EC and Na/Cl in groundwater.

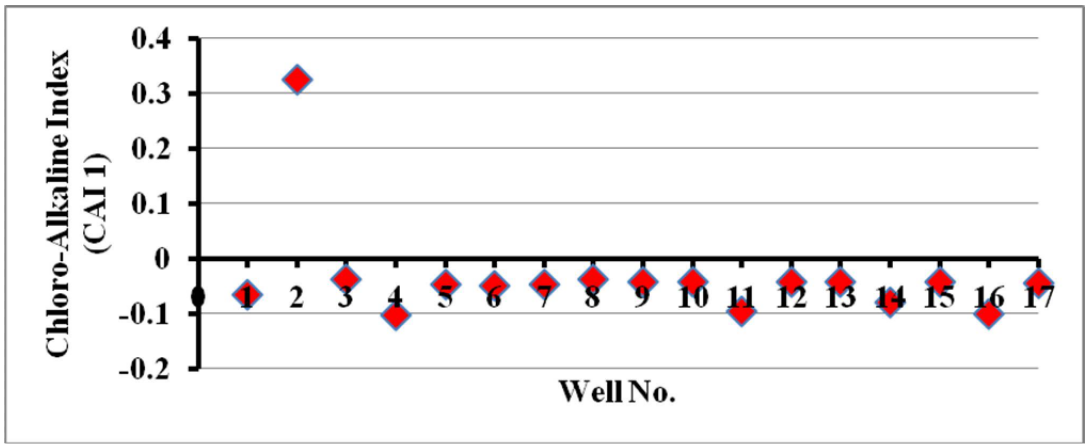

Figure 4: Chloro-alkaline indices 1 and 2 (CAI 1 and CAI 2) indicating ion exchange procedure.

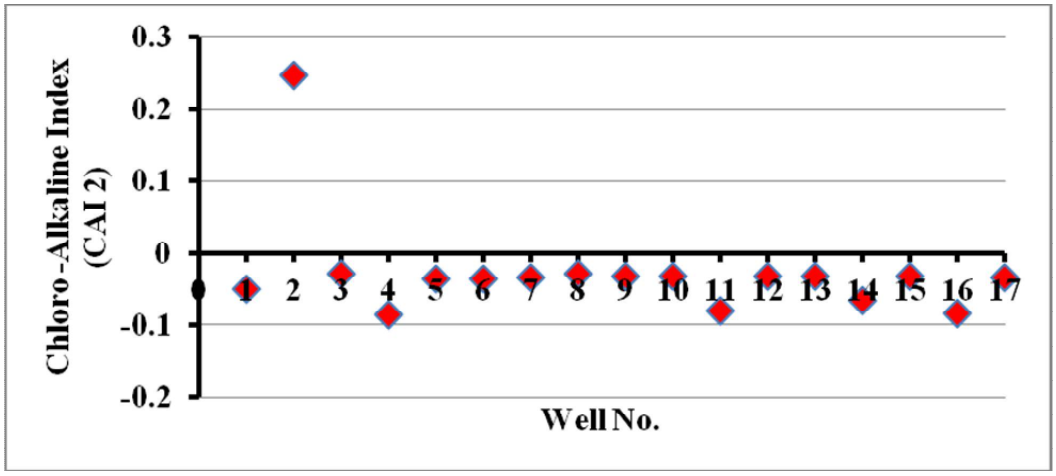

Figure 5: Chloro-alkaline indices 1 and 2 (CAI 1 and CAI 2) indicating ion exchange procedure.

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RESEARCH ARTICLE

Effect of Constant Inflow Discharge on the Development of Matric Suction, Volumetric Water Content and Vertical Erosion Process for Dike Soil during Overtopping Test

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ABSTRACT

This research paper presents an experimental test to determine the responses of negative pore water pressure (matric suction), volumetric water content and vertical erosion process through an embankment dike of sand soil during 2-D spatial overtopping failure. An Experiment test was conducted in Hydraulic Geotechnical laboratories at the Universiti Sains of Malaysia. Dike embankment was installed in small flume channel to observe the development of pore pressure during spatial overtopping test. Twelve electronic sensors comprising a tensiometer-transducer probe assembly model and a Time Domain Reflectometer (TDR), were installed at several locations below the downstream and upstream slopes of dike. Responses of the negative pore matric suction and volumetric water content were measured throughout the erosion process of sand dike under constant inflow discharge of 20 l/min. The vertical erosion process is measured through side-view camera installed in front of PVC flume channel. Results show that the matric suction inside soil particles decreased, and thus, the volumetric water content increased for all sensors groups during the overtopping failure. Inflow discharge rate was effected widely in groups near the upstream slope because of the longer saturation time in dike soil, while the behaviors of groups near downstream slope are significantly changed after overtopping failure. The rate of erosion process inside breach channel is increased after overtopping failure due to collapses dike's side slope.

Key Words: Dike, Vertical erosion process, Tensiometer, TDR, Volumetric water content, Matric suction

INTRODUCTION

The embankment dike is constructed of earth or other suitable materials. It helps to prevent or reduce the effects of water damage on people and property, as well as controls the flow in conjunction with floodway [1]. Breach in dikes occurs as a result of water overtopping above the crest of the structure in which materials along the downstream face of the dike are continuously eroded due to erosion process [2]. Breaches may be influenced by several geotechnical and hydraulic factors such as dike materials, dike dimensions and field parameters [3-6]. One of earlier breach embankment tests were conducted in 1959 and 1978 for evaluating the wash rate and breach channel development during overtopping tests for different grain sizes, specific gravity and geometric configurations [7-8]. Delft University of Technology (TUD) has discussed the evolution of erosion process for three large-scale experiments using two grain sizes with different upstream slopes inclinations. Increasing the sizes of soil particles and inclination of upstream slope lead to increase the erosion rate of sand embankment [9].

Simmler and Samet [10] have studied the influence of compaction on homogeneous and composite dikes during the erosion process of breach channel. The compaction parameter has no effect on the development of erosion, while the extracted side-view pictures for material eroded in breach channel was difficult in non-cohesive earthfill dike due to the presence of impervious element. Visser et al. [11] described field overtopping test in the Zwin Channel for observing four stages of erosion process- later developed into five stages - inside sand dike in which a pilot channel was cut on top of dike crest to initiate the spatial test.

A silty sand dikes were constructed by the U.S. Department of Agriculture (USDA) Agricultural Research Service Hydraulic Engineering Research Unit (ARS-HERU) inside series of flume channels in 1990 [12]. These tests are included two types of vegetated and non-vegetated grass under two constant inflow discharges. The erosion rate for the non-vegetated grass is faster than that for vegetated one. Tingsanchali and Chinnarasri [13] have studied the mechanism of breach channel for non-cohesive materials inside large flume channel at the Asian Institute of Technology in Khlong Nueng, Thailand. Twelve experimental tests with six wave gauges are installed inside dike embankment for measuring the water surface level.

The purpose of the test was to develop a numerical model for breach erosion. Additional nine tests are constructed by Chinnarasri et al. [14] at the Asian Institute of Technology for the breach mechanism of mixtures soil. A rectangular pilot channel is cut in the dike crest to initiate and develop erosion processes inside dike construction. They stated that the mixture of (70%) sand and (30%) clay are considered as cohesive materials with hydraulic jump occurred in the downstream slope. The studying of phases II and III for erosion process are analyzed through conducting series of overtopping tests, by the USDA ARSHERU in 2003, under different water contents. Initial pilot channel is cut in sand dike to initiate breach channel inside dike crest centerline [15]. The overtopping failure in the downstream slope resulted in huge materials overhang similar to Mohamed et al. [16] and Coleman et al. [17].

The breach mechanism in cohesive materials is also studied through large embankments field's tests in the Dawa Reservoir, Chuzhou, Anhui province, China J. [18]. The study included the measurement of velocities in the breach channel and tailwater conditions. The observation results indicated four types of breach failure formed as headcut erosion processes in stratified material, helix flow' in upstream and downstream slopes and side-slope failure mechanisms in the breach channel. In this study, a 2-D spatial overtopping test was conducted to evaluate the responses of matric suction, volumetric water content and vertical erosion process for homogeneous sand dike, in Hydraulic Geotechnical laboratories at the Universiti Sains of Malaysia, inside PVC flume channel through using tensiometer, TDR sensors and digital camera, respectively.

MATERIALS AND METHODS

A spatial overtopping test was conducted at the Hydraulic Geotechnical Laboratories of Universiti Sains of Malaysia to observe the response of matric suction, volumetric water content and vertical erosion process during overtopping failure. A homogeneous sand dike is constructed at the end of the PVC flume channel under dry condition. The sandy soil is classified as poorly graded coarse sand (SP) as shown in Table 1 according to BSCS. The flume dimensions are 4.5, 0.5, and 0.6m for length, width, and height, respectively. The length of the dry sand dike was 175 cm length, width was 50 cm, and crest height was 30cm. The dimensions aimed to eliminate the effect of tailwater condition in the erosion process. The length of the dry sand dike was 1.75m length, width was 0.5cm, and crest height was 0.3cm. One digital camera was installed in front of flume channel to capture the evolution of water levels and vertical breach channel as shown in (Fig. 1). The type of soil used in dike construction is poorly graded coarse sand (SP). A trapezoidal pilot channel, with dimensions of 4cm width and 3cm height, was initiated at the dike crest length along the side-wall of flume channel to initiate the spatial breach channel as shown in (Fig.2). The 2100F Tensiometer and Trime-pico32 TDR sensors are developed by Soil moisture Equipment Corporation and IMKO Micromodultechnik GmbH, respectively. These instruments were installed on the side wall of the flume channel with different locations and depths to conduct the pressure suction and volumetric water content, respectively. Tensiometer contains porous ceramic round bottom with a dimension of 6mm in diameter and 25mm in length while TDR probe body has dimensions of 155mm and 32mm in length and diameter, respectively.

They were connected to data logger and computer (PC) to analyze the test results. The two sensors are distributed into six groups named as: A, B, C, D, E and F, along with the downstream and upstream slopes. Each group contains one tensiometer and one TDR sensors. The coarse sand is oven dried under 105 ^{o}C in order to become in dry condition. The sand has been constructed with the compactions of 10 cm soil height till the last soil layers in the dike crest of 30 cm height in order to get a constant bulk (dry) density of 1.8g/cm^{3}. For each layer, with the known of bulk density and soil volume values, the weight of soil layers was calculated and applied into the flume channel. A pilot channel is initiated in dike crest to begin overtopping failure in the downstream slope. Constant inflow discharges of 20 l/min were applied during the transition water flow from upstream into downstream slopes. The responses of matric suction and volumetric water content are measured during the overtopping test for all groups in which results completed at the end of material erosion around each group. The vertical erosion process is captured by the digital camera during the initiation of breach channel until the erosion process for the whole dike construction is stabilized.

RESULTS AND DISCUSSION

The effect of reservoir water on the stability of dike embankment is dependent mainly on the relation between the reservoir water flows in the upstream slope and the flow in the breach channel after overtopping failure and thus a precise inflow water in the reservoir water has not existed in nature. Nevertheless, the effect of a constant inflow discharges is applied in the experimental test for practical use. Groups F and E locate in the upstream slope while group D locates below dike crest. Groups A and B locate in the downstream slope and group C locates in the transition area between upstream and downstream slopes. The location of these groups indicated the saturation path as well as the erosion stages inside dike embankment. (Fig 3) shows the responses of matric suction and volumetric water content for each group during overtopping test. The Y-axis represented the matric suction, measured in kPa, and volumetric water content in percentage against the time of overtopping test under constant inflow discharges of 20 l/min. The purposes of placement one tensiometer and one TDR sensors in each group is to measure the quantity of negative pore water pressure and water saturation, respectively inside soil voids. The matric suctions are increased before the beginning of experimental tests while the measuring results of volumetric water content are closed to zero. The installations of tensiometer and TDR sensors have disturb soil composition and thus increasing amount of air content in soil particles while the water content is almost non-existed due to lack penetration of water infiltration.

The behavior responses of matric suction and volumetric water content are changed after t = 7 minutes. The sharp decreasing and increasing of matric suction and volumetric water content, respectively are occurred due to the infiltration of phreatic water level inside soil particles. The meniscus curve, forms between the air and water phases inside particles, is decreased gradually and thus reduces the matric suction due to the increase of the percentage water content during the dike saturation and vice versa in de-saturation process. The meniscus curve is still adjacent to outer soil boundary and cannot penetrate more inside other voids due to decrease negative pore water pressure. The moisture content is changed gradually from dry condition (water content = zero) into the partially saturated soil. It is almost difficult to get fully saturated condition due to the presence of some air bubbles inside the particles. The continuity of water saturation occurs in the later minutes while the matric suction and volumetric water content are 3.72 kPa and 30% at t = 9 minutes. The responses of matric suction is less decreased at t = 10 minutes, compared with that at the previous time, and reduced to 2.65 kPa with a volumetric water content of 31%. This is due to the gradual water fill inside voids and thus the permeability of soil to water infiltration is reduced in which the permeability of the sandy soil is dependent on the amount of water content inside particles. The results ended at t = 13 minutes due to the ended of erosion process for dike materials surrounding group F. For group E, the noticeable responses of decreasing and increasing matric suction and volumetric water content, respectively are occurred at t = 8 minutes with tensiometer and TDR records of 12.3 kPa and 12 %, respectively. The increasing amount of water content is basically dependent on the development of negative pore water pressure (matric suction) inside soil particles. The decreasing of matric suction is decreased the shear strength of dike soil while the phreatic water level is raised from partially saturated soil near the toe of upstream slope into unsaturated one near group E. The matric suction and volumetric water content are 6.6 kPa and 29.05 %, respectively at t = 10 minutes while they are 5.57 kPa and 29.05%, respectively at t= 11 minutes. The small declination of matric suction for the later time is indicated the rate of infiltration water level during the overtopping test. The infiltration water rate is high at the beginning of soil saturation and then decreased gradually depending on the soil infiltration capacity.

For group E, the erosion process is ended at t = 12 minutes in which the soil capacity for water infiltration is reached nearly steady state, due to increase volumetric water content; consequently, the factor of safety for slope reduced dramatically. The saturation process in group D represents the beginning of shear soil strength reduction for soil below dike crest. The overtopping failure occurred when water flows cross above crest at t = 9 minutes. The huge increasing of volumetric water content is occurred at t= 11 minutes due to the quick saturation of soil layers below crest with a volumetric water content of 22%. The gravity assists to accelerate the water infiltration rate into soil voids of group D. For groups B and A, the responses of matric suction and volumetric water content occur at t = 10 and 11 minutes due to the transportation of overtopping water into the downstream slope during the evolution of breach channel inside dike crest. The water pressure is higher than the air pressure and thus gradually destroyed the interface curve that formed between air and water pressure during progressive increasing of volumetric water content. The saturation process in group C is represented the penetration of infiltration water inside the transition area between the upstream and downstream slopes while the responses of matric suction and volumetric water content have occurred at t = 11 minutes. The decreasing and increasing of matric suction and volumetric water content are 13.87 kPa and 7%, respectively. (Fig 4& 5) show the behavior of matric suctions and volumetric water content for all groups during overtopping test. Groups F and E are first responded to infiltration water in term of decreasing and increasing matric suction and volumetric water content, respectively. This is due to their positions in the toe and middle of upstream slope, respectively.

The reduction and increasing of matric suction and volumetric water content, respectively in group F is earlier than that in group E. This is because of faster infiltration rate near the toe of upstream slope while it is decreased gradually near area group B in which the velocity of water infiltration is reduced. Increasing of volumetric water content in group D is occurred during the overtopping failure, at t = 9 minutes, due to the contact of phreatic water level near the top of upstream slope. The small inflow rate of 20 l/min is deaccelerated the velocity of water infiltration near the top of upstream slope and thus delay the decreasing and increasing of matric suction and volumetric water content, respectively before overtopping failure. The reductions of matric suction is higher in group A than in group D at t =

10 minutes. This is due to the end of erosion process for materials surrounding group D faster than that in group A. The development of breach channel resulted in decreasing and increasing the matric suctions and volumetric water contents for groups A and B after overtopping failure. Increasing the volumetric water content is occurred faster in group B than that in group A at t = 10 and 11 minutes, respectively. This is due to the acceleration water velocity in the middle of downstream slope in which huge amount of water content is transferred during the erosion of breach channel. The rate of matric suction reduction in group C is considered longest, besides group F, compared with other groups while the development of erosion process reached nearly the bed of dike embankment. At t = 13 minutes, the responses of matric suction and volumetric water content are 11.79 and 30.68%, respectively. The water content of group F is higher compared with that in group C at the end of erosion process. This is due to continuous saturation process in soil voids near group F in which more air bubbles are contacted with infiltration water near the toe of upstream slope.

(Fig 6) shows the transition of infiltration water from the toe of upstream slope until dike crest. At t = 10 seconds, the water flow started to fill soil voids in the upstream slope in the vertical direction and the horizontal direction while the saturation process is small at the beginning of infiltration water. This is due to the higher soil density in the toe of the upstream slope that prevent the faster saturation. The phreatic water level reached the middle of upstream slope nearly at t = 280 seconds. The lower inflow discharges of 20 lmin resulted in decreasing the rate of infiltration flow during the saturation process due to lower flow velocity while most of water are occupied soil voids in the horizontal direction. The rising water level is gradually decreased after 420 seconds. The soil particles, near the face of the upstream slope, is partially saturated with a high percentage of water content and thus delay the velocity of phreatic water level near dike crest. The water level attached the dike crest at t= 590 seconds while the middle of transition area between the upstream and downstream slope has been saturated. (Fig 7) shows the distribution of vertical and horizontal water levels during the transition of water level in the upstream slope. The vertical water level is measured from the base of upstream slope until dike crest while the measurement of horizontal water level is recorded along the horizontal basement of dike length. The horizontal water level is faster than the vertical water level during the saturation of upstream slope until dike crest. At t =0.166 minutes, the vertical water level and horizontal water level are 1.1 and 3.53 cm, respectively while they are 2.4 and 8.4 cm, respectively at t = 0.5 minutes. This is due to the effect of earth gravity that prevents the rising of vertical water level and thus deaccelerate the velocity of infiltration water. The rates of vertical water level and horizontal water levels are accelerated after t = 1min, whereas the soil water pressure transferred the phreatic water level from lower tension zones into higher tension zones inside soil particles and thus increase the percentage of water content.

The distribution of horizontal water level along the horizontal distance of dike length is increased significantly at later times because of the negligible effect of earth gravity against the movement of water flow. The horizontal water levels are 53.5, 61.6 and 73.5 cm at t = 4.3, 5 and 6 minutes, respectively. The vertical water rate is gradually decreased near dike crest after t = 8 minutes. This is due to the water saturation for soil voids in the transition area between upstream and downstream slopes while most of water content has occupied the unsaturated zones. The vertical water levels attached dike crest at t =9 minutes in which the breach channel failure is initiated in the dike crest.

The failure of pilot channel is occurred when the flow water cross over the dike crest. (Fig 8) show the 2-D dike breach profile in the dike crest after the overtopping failure and then transmitted into upstream and downstream slopes. The lines, initiated from dike crest until the dike bed under certain times in seconds, indicated the 2-D vertical erosion process due to the development of breach channel inside dike crest at t = zero second. The layers below dike crest is saturated with high percentage of water content inside soil particles and thus lead to decrease significantly the matric suction. At t = 10 seconds, the eroded martials is small because of the high quantity of soil layers, that prevents the faster reduction of dike height, in the crest as well as the small rate of inflow discharges. The majority of dike height is not influenced by overtopping flow in which it still 27 cm while very small amount of materials is began to erode as debris flow in downstream slope. The reduction of dike height of 26.6 is started at t = 20 seconds while vertical erosion process took place in the top of downstream slope. Decreasing the negative pore water

pressure resulted in lowering the factor of safety for dike soil, consequently side slope instability occurred. The noticeable reduction of dike height is occurred at later seconds at t = 80, 100 and 120 seconds with heights of 22.8, 21.59 and 20.5, respectively. Almost half of dike height is eroded due to the continuous increasing of water velocity above the downstream stream.

The water flow condition is changed from transitional in dike crest into supercritical in the downstream slope and thus accelerated the vertical erosion process inside dike. The rate of flow water is high enough to overcome the soil stresses and thus the side slope is saturated and failed with huge amount of soil materials transported along the base of flume channel into sediment box. The rate of the vertical erosion process is slow after t = 240 seconds while most of dike height is eroded, consequently the water velocity inside layers is decreased. The dike breach shape is reached near the bed of flume channel while the vertical erosion process is stabilized at t = 300 with dike height of 13.7 cm.

CONCLUSION

A constant inflow discharge of 20 l/min was applied during overtopping test to evaluate the responses of matric suction, volumetric water content and vertical erosion process. The test was conducted in Hydraulic Geotechnical laboratories at the Universiti Sains of Malaysia for sand dike. Twelve sensors of tensiometer and TDR were used to measure the matric suction and volumetric water content, respectively as well as digital camera for calculating the vertical erosion process. The results show that the infiltration water increased the volumetric water content inside soil particles and thus reduced the matric suction. Consequently, the shear strength of dike soil is decreased gradually during the transition of water level in upstream slope. The vertical erosion process occurred when water flow cross over the dike crest. The resistance of dike crest against the overtopping failure is higher during the primary initiation of breach failure compared with the development of erosion processes in the downstream and upstream slopes. Increasing the water velocity have caused huge amount of materials eroded near the toe of downstream slope. Most of dike body were collapsed in the transition area between downstream and upstream slopes while the vertical erosion process transferred into upstream slope. The velocity of water flow inside breach channel is reduced gradually near the toe of upstream slope and thus the effect of evolution breach channel on lowering dike height is negligible.

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Table 1. Sieve analysis test for dike soil

Dike components (%)		Parameters (mm)	(C_u)	(C_c)
Sand	80.59	D_{60}= 1 D_{30}= 0.58 D_{10}= 0.33	3.03	1.019
Gravel	19.1			
Silt &Clay	0.31			

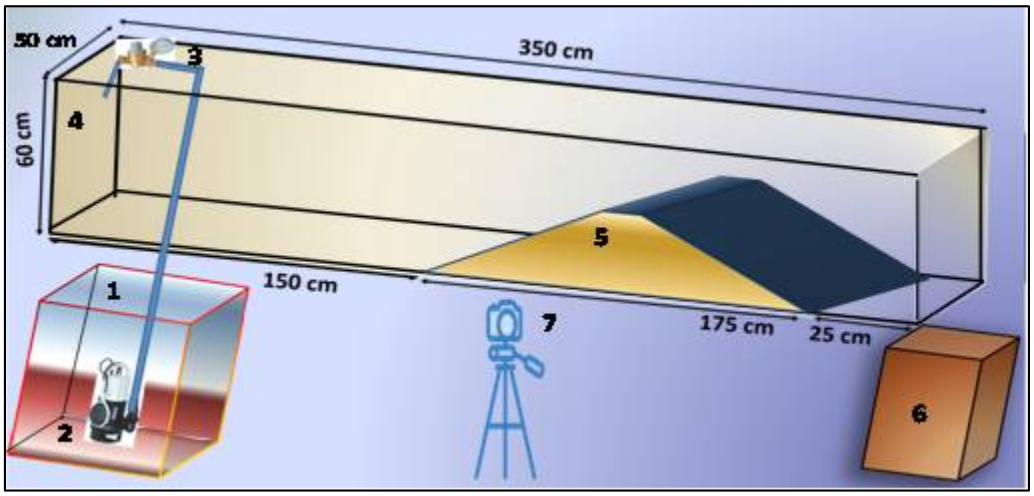

Fig 1. Components of overtopping channel test: 1) Water tank, 2) Discharge pump, 3) Flowmeter, 4) Flume channel, 5) Dike construction, 6) Sediment tank,7) Camera

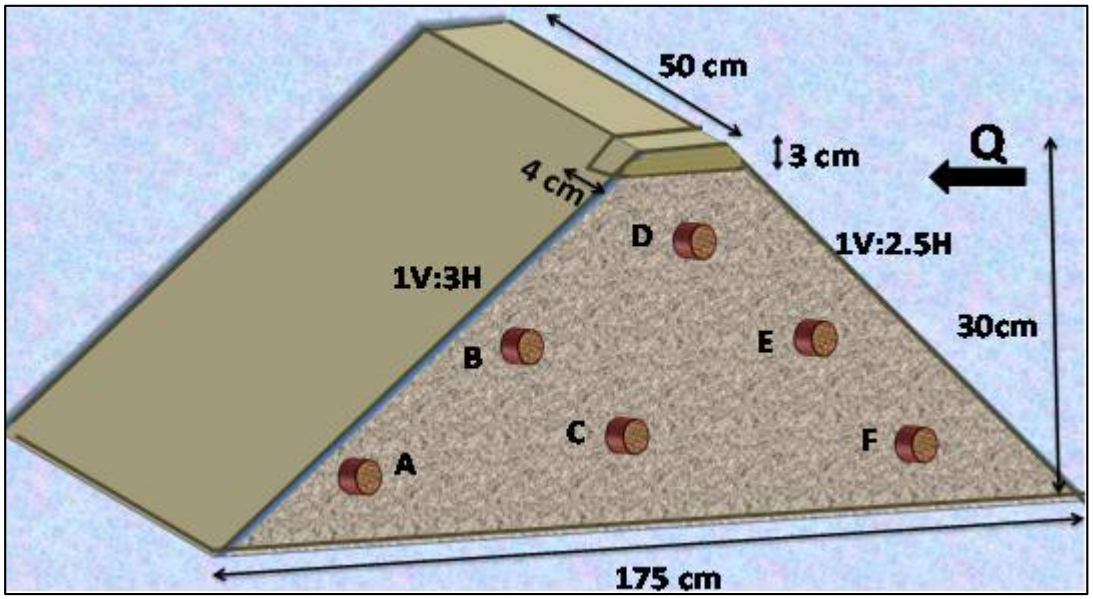

Fig 2. Location of group's sensors and pilot channel below dike crest

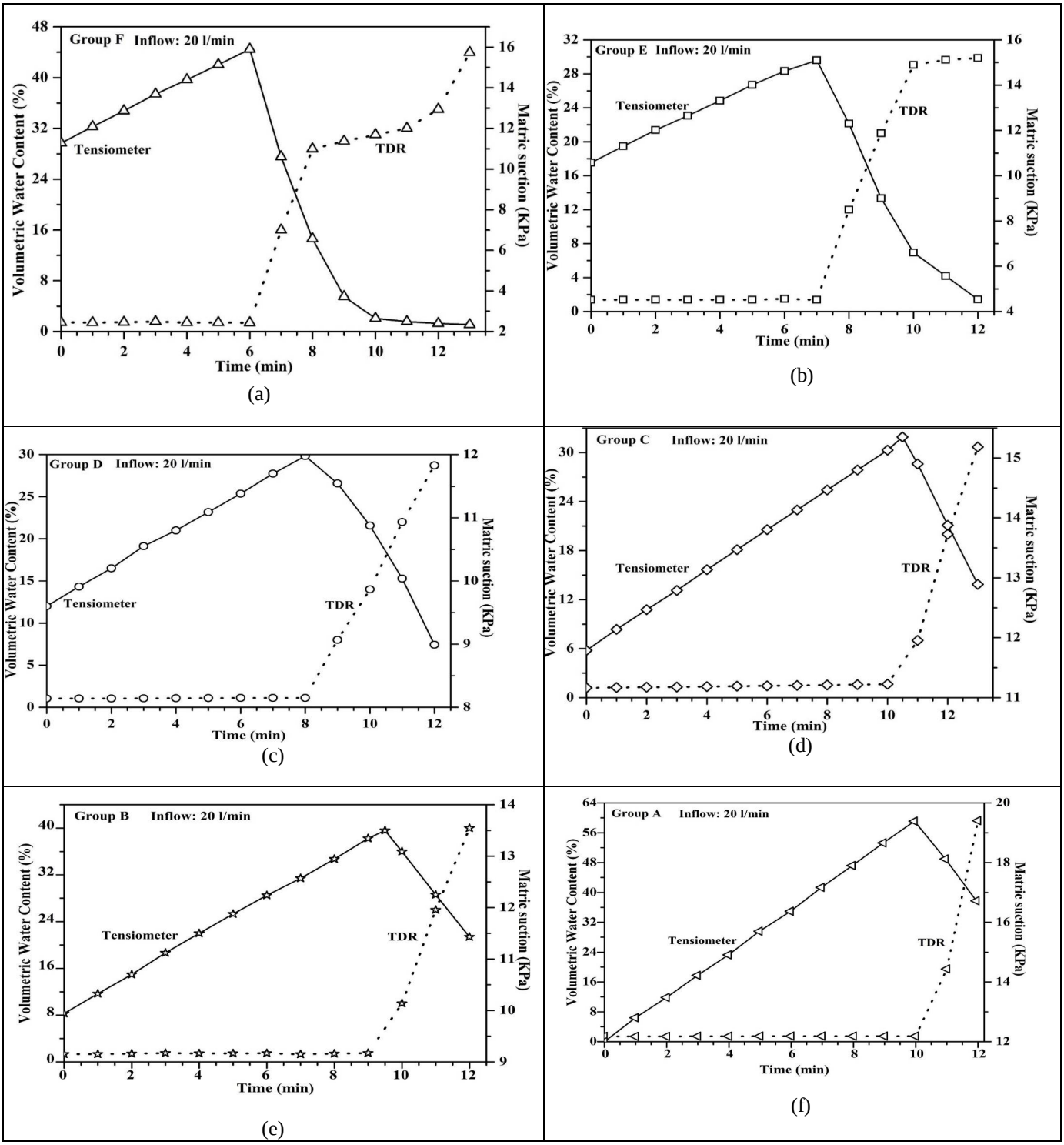

Fig 3. Responses of matric suction and volumetric water content for groups: : a) F, b) E, c) D, d) C, e) B, f) A, respectively.

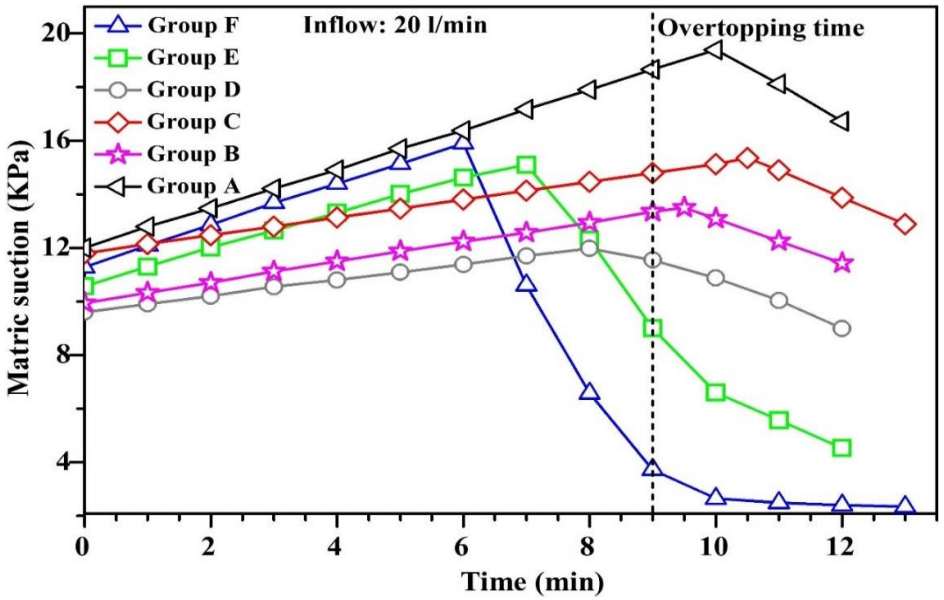

Fig 4. Behavior of matric suctions for all groups

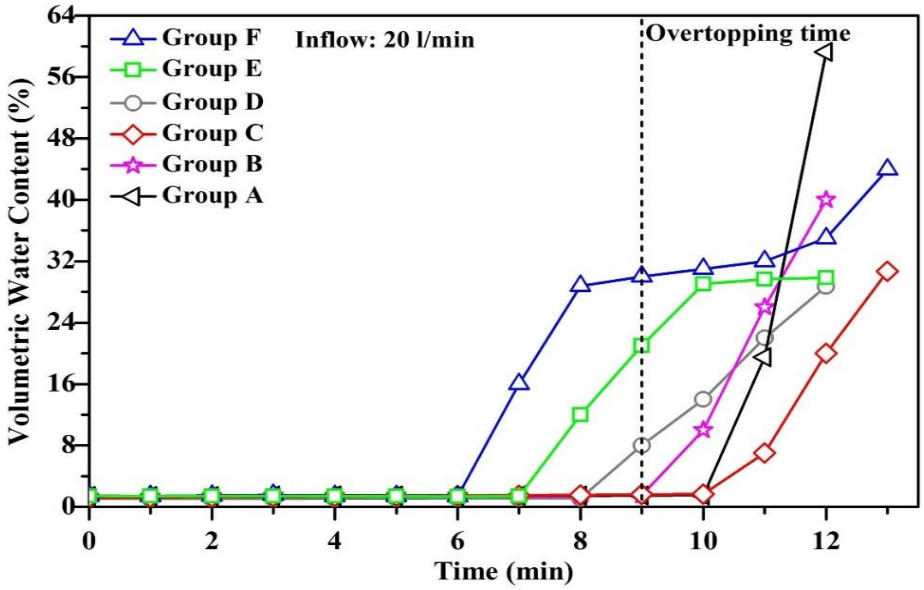

Fig 5. Behavior of volumetric water content for all groups

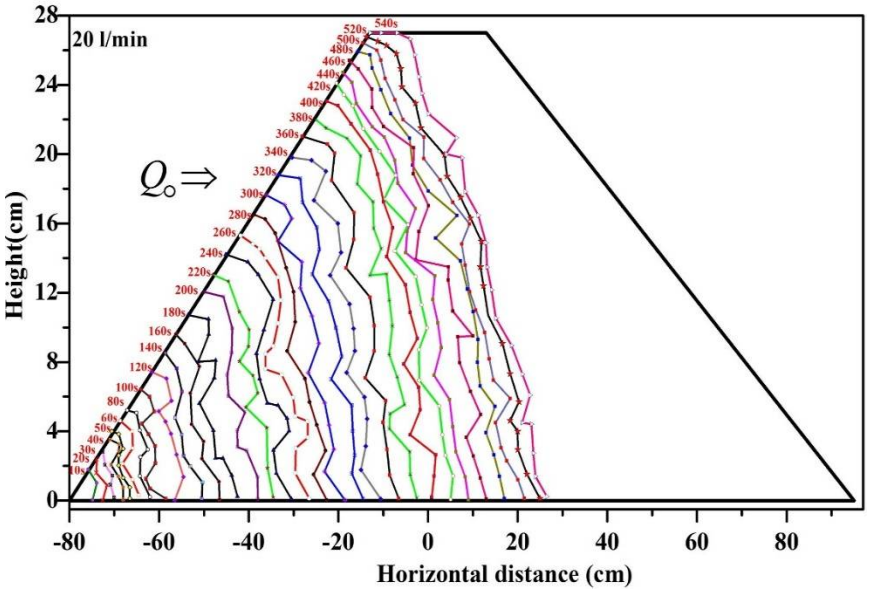

Fig 6. Infiltration of water level inside dike embankment

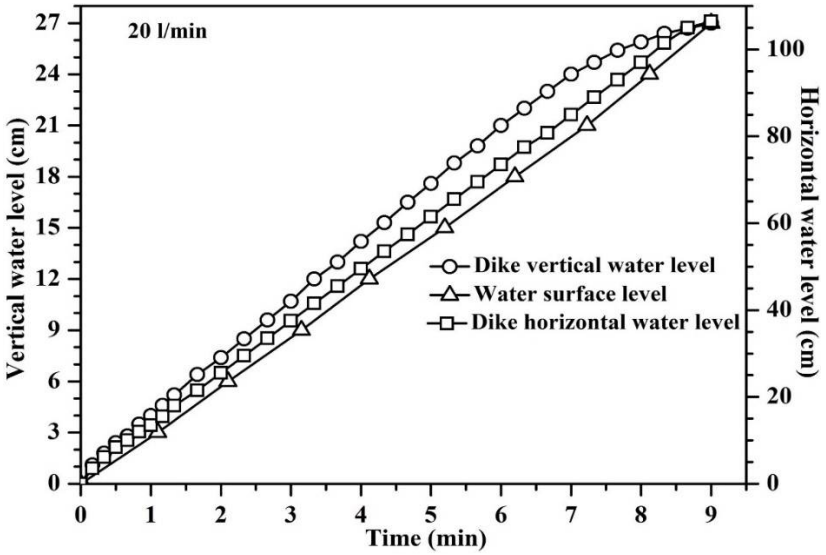

Fig 7. Distribution of vertical and horizontal water levels

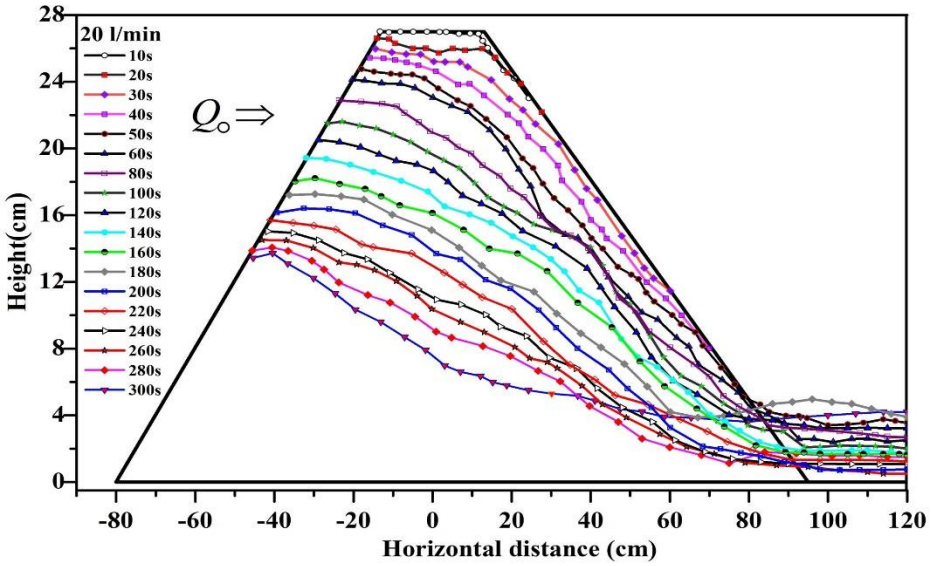

Fig 8. Dike breach profile during vertical erosion process

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RESEARCH ARTICLE

Qualitative and Quantitative Analysis of Phyto-Chemicals in Cashew Apple Waste

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ABSTRACT

The current research was conducted to study presence of phyto chemicals in cashew apple waste (CAW).The study material is one of the by-product from the cashew industry where cashew apple fruits are wasted as such in the field after nuts are removed. The chemical composition of CAW analysed in this study contains 87.10 ± 0.19 per cent of dry matter; 96.20 ± 0.12 per cent of organic matter; 20.68 ± 0.27 per cent of crude protein; 2.90 ± 0.40 per cent of ether extract; 13.90 ± 0.11 per cent of crude fibre; 3.80 ± 0.09 per cent of total ash; 58.72 ± 0.47 per cent of nitrogen free extract; 1.72 ± 0.14 per cent of acid insoluble ash; 0.76 ± 0.08 per cent of calcium and 0.40 ± 0.11 per cent of phosphorus. The qualitative analysis of phyto-chemicals confirmed presence of tannins, alkaloids, flavonoids, phenolic compounds and phyto sterols in CAW. The estimated condensed tannin (as Proantho cyanidins) was 0.150 ± 0.02 per cent.

Key words: cashew apple waste, chemical composition, condensed tannins, phyto-chemicals

INTRODUCTION

Cashew apple is one of the seasonally available fruit in south India. Cashew apple waste (CAW) is one of the by-products of cashew processing industry. In states like Kerala and Goa, livestock farmers are using CAW such as after juice extraction; however it was estimated that 41,734 metric tonnes of cashew fruits were wasted as such in kerala (Murugan *et al*., 2015). Even though, CAW contains good crude protein (19.02 per cent) and less per cent of crude fibre (12.58 per cent) (Sreekutty *et al*., 2017), but inclusion of these materials are limited by presence of phyto chemicals. Usually poultry nutritionists consider these tannins (hydrolysable and condensed) as anti-nutritional factors because in poultry, it affect feed intake, reduces crude protein digestibility and consequently growth performance (Longstaff and McNab, 2007). However, anti-nutritional effects of tannins are mostly based on concentrations of tannins in feed, or plant with excess of tannins like tannic acid from sorghum grains. Meanwhile, it is now known that their beneficial or detrimental properties of phyto-chemicals are depend upon their chemical structure; have multiple biological activities, including antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, and antibacterial properties. The qualitative and quantitative estimation of phyto-chemicals was carried out to ascertain use of cashew apple waste as one of the ingredient in poultry diet.

MATERIALS AND METHODS

Sample collection and chemical composition estimation

The sun dried study material was collected after juice extraction of cashew apple from M/s. Plantation Corporation of Kerala (Government of Kerala Undertaking, Kottayam). About 1000 g of sample was dried in a hot air oven at 60^0C for 48 hours and ground to pass through 1mm sieve and preserved in air tight containers for chemical and phyto-chemical analysis. The samples were analyzed for proximate principles as per AOAC (2016) and acid insoluble ash content was analyzed as per BIS (IS: 14826; 2000).The calcium and phosphorus were estimated following procedure of Talapatra *et al*. (1940).

Qualitative analysis of phyto- chemical properties

The phyto chemical properties are qualitatively estimated according to the (Geetha *et al*., 2014). The cashew apple waste extract was prepared in acetone (70 per cent and concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analyses.

Quantitative estimation of condensed tannins

The condensed tannins are determined as proantho cyanidins as per the procedure given by Porter *et al* (1986).

RESULTS

The chemical composition of CAW indicates this material could be considered concentrate because it contains 20.68 per cent crude protein and 13.90 per cent crude fibre. The chemical composition, condensed values are presented in Table 1 and Qualitative analyses of phytochemicals are presented in Table 2. However, phyto-chemical analysis is important in identifying presence of anti-nutritional factors. The presence of phyto-chemicals is recorded as +, ++, +++ indicates intensity of coloration/foam/other physical observations which determines abundance of the compound present. The qualitative tests for phyto-chemicals in acetone extracted CAW indicates presence of alkaloids, flavonoids, glycosides, phenolics and tannins.

DISCUSSION

The estimated values of CAW for crude protein slightly differ with the reported values of other authors 19.60 and 19.02 per cent Bhamare *et al.* (2016) and Sreekutty *et al*. (2017) respectively. However, very low value of 11.5 percent crude protein, 8.5 per cent crude fibre was reported by Swain and Barbuddhe (2007). Further, Murugan *et al*., (2015) revealed that crude protein content of CAW of 8 different varieties ranges between 9.81 to 14.74 per cent. The difference in the chemical composition of CAW might be due to variety of plant grown, location and sun drying process. Crude fibre content determines the use of these type of feed stuffs in poultry feed. The values of crude fibre content indicated by different authors are within the range of 8.5 to 14.64 per cent (Bhamare *et al.,* 2016; Sreekutty *et al*., 2017). The restricted use of CAW in poultry diets by local farmers may be due to presence of tannins which is due to astringency of cashew apple fruit juice. The astringency is due to interaction of tannins and salivary proteins that lubricate the mouth, leading to a drying and puckering sensation (Fontoin *et al*., 2008). However, estimated value of condensed tannins as proantho cyanidins in the present study was in the range of 0.0621-0.877 per cent as reported by Okpanchi *et al*. (2016) in red and yellow variety of CAW.

CONCLUSION

The chemical composition and phyto-chemical composition of cashew apple waste indicates it could be considered as one of the feedstuff for poultry.

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Table -1 Chemical and condensed tannin content of cashew apple waste (on DM basis)

Parameters	Percent
Dry matter	87.10 ± 0.19
Organic matter	96.20 ±0.12
Crude protein	20.68 ± 0.27
Ether extract	02.90 ± 0.40
Crude fibre	13.90 ± 0.11
Total ash	03.80 ±0.09
Acid insoluble ash	01.72±0.14
Nitrogen free extract (NFE)	58.72±0.47
Calcium	00.76±0.08
Phosphorus	00.40±0.11
Condensed Tannin (as proantho cyanidins)	0.150 ± 0.02

Table-2.Qualitative analysis of Phyto-chemicals in CAW

Parameters	Phyto-chemicals (acetone extract)
Alkaloids	
1.Hagers test	+++
2.Dragondrafs test	+++
Flavonoids	
1.Ferric chloride Test (FeCl3) 10 percent	+++
2.Lead acetate test	+++
Phenolic compounds	
1.Gelatin test	+
2. Lead acetate test	+++
3. $FECL_3$	+++
Tannins	
1.Gelatin test	+
2. Lead acetate test	+++
3 Ferric chloride Test (FeCl3)	+++
Glycosides	
1.Legals test	++
Saponins	
1.Foam test	+
Carbohydrate	
1.Benedicts	+++
2. Starch	+++
Terpenoids	
1.Salkowiski test	+
2.Copper sulphate test	+++
Phytosterols	+
1.Salkowski's test	
Test for amino acids and proteins	
1.Xanthotrophic	+++
2.Biuret	+++

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RESEARCH ARTICLE

Feministic Perspective in Margaret Atwood's Surfacing Feministic Perspective Cat's Eye

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ABSTRACT

This is an attempt to analyze the suppression of women as portrayed in Margaret Atwood's Surfacing. Atwood analyses the inner conflict of the protagonist and their quest for survival. Remarkably a good writer of Canada, Margaret Atwood is the most prominent figure in Canadian Literature. She not only reflects society but also aims to reform it.Feminism, a theme in many of Atwood's novels, is explored through the perspective of the female narrative, exposing the ways women are marginalized in their professional and private lives. Margaret Atwood's second novel, Surfacing (1972) pursues and develops further the feminist themes of The Edible Women- the protest against the female sex role and the predatory and aggressive attitude and behaviour of men towards women anti-capitalist, anti-American and ecological concerns continue to be part of the author's radical, perhaps revolutionary message of these early novels. The theme of the heroine's dilemma as an artist/writer is also ever present. In Surfacing she involves herself in a search for, among other things the roots of her creativity, burried within her and relating to her past and childhood. Surfacing predates the environmentalist movement, but the narrator's reverence for the Canadian wilderness is a proenvironmentalist one. Thus these environmental concerns still resonate today given continuing trends toward over consumption and the prevalence of technology that relies upon natural resources. Copyright of Language in India is the property of Language in India and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use. This abstract may be abridged. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material for the full abstract. This research paper examines Margaret Atwood's Cat's Eye through the lens of feminist theory.

It traces the issue of identity quest and explores movingly the painful psychic effects of adolescent cruelty and bullying, switching between the narrative and the protagonist's childhood.This research not only focuses on the evolution of the fragmented identity of the female protagonist, taking into account the different influences that shaped the formation of her selfhood, but also dramatizes the limitations and unreliability of human memory and the effects of time upon the human subject. Throughout this dissertation, I have attempted to highlight the importance of past memories and the need to deal with them in order to cope with present reality.

Key words: Feminism, Margaret, human, narrator's

INTRODUCTION

Margaret Atwood 's 'Surfacing'1972 deals with the major theme of the novel feminism, Identity of selfhood and Identity of womanhood. "Surfacing is a novel of self realization, but it also possesses an element of feminism. At the end of the novel there is sanity, a real understanding of reality". Heroine of the novel 'Surfacing' is unnamed. She is an unsuccessful artist in the city of 'Toronto' who has left her husband and family long ago. During her city life, she entered a empty marriage and emotion numbing abortion and divorce. After that she returns to her home with her three companion Anna, narrator's friend and a model also, David, Anna's husband and a flim maker also, Joe, David's friend, a camera man and narrator's lover also. The world from which narrator returns, she express in such words: "It is not perfect, not heaven but neither is it the hell of madness." Purpose of narrator's returns to her home ground is to find out her missing father.

She wants to find out what has happened with her father. He was alive or dead. Narrator was worshiped her father as a logical and scientific man. She was very devoted to her father .Narrator remarks her parents' attitude toward her. Narrator remembers that she had always depended on her father's rational explanations. Her mother's silence had been a mystery to her. At the end of part one the memory of her brother's drawing."After she'd told the story I asked our mother where he would have gone if she hadn't saved him. She said she didn't know. My father explained everything but my mother never did, which only convinced me that she had the answer but wouldn't tell." Mr.Paul, whose' her father's best friend, informed narrator that her father is reported to have vanished mysteriously Narrator has to enquire about her father. She likes and trusts her friends, but they hadn't accompanied her on this errand of finding her father, "I like them, I trust them, I can't think of anyone else I like better, but right now I wish they were not here. Though they're necessary: David's and Anna's car was the only way…….But my reason for being here embarrasses them, they don't understand it. They all disowned their parents long ago, the way you are supposed to: Joe never mentions his mother and father."

Narrator's friend treats the trip as a break from city life and holidays while narrator is very serious about her missing father and will discover something. Narrator is physically mutilated rather she is half dead. The journey revised the memory of her unhappy past. Narrator thinks about her father in chapter three and said: "If he's safe I don't want to see him. There is no point, they never forgave me, they didn't understand the divorce; I don't think they even understand the marriage and leaving my husband and child, that was the unpardoned able sin; I admit I was stupid, stupidity is the same as evil if you judge by the results and I didn't have any excuses." The narrator is convinced that her parents never forgave her for either her divorce or leaving her child. The mystery of her father's disappearance is becoming a 'tangled maze' for her. Narrator wouldn't want to be alone that place. While in the company of her friends, the narrator reflects on her own brief marriage, which was not actually a marriage because it destroyed their relationship. Narrator's group departs on a search of a trail. Narrator thinks that: "I seen now the impossibility of searching the island for him, and even then they could miss him, dead or alive, accident or suicide or murder, or if for some unfathomable reason he's chosen this absence and is hiding, they'd never find him."

The physical journey now turns into a physical search for her father. The narrator continues to look for clues to her father's where abouts and she thinks of other possibilities, strangest of all is, he might be hiding somewhere in the island. The search on the trail becomes difficult and the abandon it. Narrator's fear of her father is still evident and her fear seems to be directed not only at her father but for herself. In the chapter ten suddenly Joe propose narrator to marry him. Narrator is shocked and cries not to laugh at the suddenness of the proposal and refuses to his proposal after that Narrator begins to tell him about her marriage and her child. The memory of her wedding that follows is very strange. When the ceremony was over, her husband behaved as if she were invalid, not a bride. Art teacher asked narrator if she was feeling better and she could hardly stand because her legs were shaking. He said: I know it's tough, out it's better this way. At night she goes into one of the bedrooms of the cabin and discovers something that she felt were out of place in that remote area: a photo album, some unused wedding presents of a childhood rhym sums up narrator's view of herself as a victim; "Nobody loves me Everybody hates me I'm going to the garden to eat worms."

We find that Mr. Paul, her father's friend, seemed certain that her father was dead. Thinks of this she doubts her theory about his hiding in the forest. Narrator thinks perhaps the CIA had done away with him to get the land. Narrator becomes sure that her father must have gone totally mad. Narrator has proof of her father's sanity and therefore of his probable death. She is sure now that he is not a madman lurking in the woods."Crazy people can come back, from wherever they go to take refuse, but dead people can't, they are prohibited."After rejecting the Joe's proposal, Joe and Narrator discuss in detail something regarding their uncertain future. All of a sudden, she offers Joe, to move out of the city apartment and said;"He did not love me, it was an idea of himself he loves and he wanted me to join him……..I didn't matter, so I didn't have to care."Narrator believe that Joe does love her, and that is the reason he wants to marry her. Narrator's comments on Joe's response to her refusal of his proposal and the discussion of their relationship is revealing. Joe is very unhappy to rejecting his marriage proposal. Narrator saw Anna without make up and says 'May be he {David} won't notice it', At this Anna says, 'he will notice…. he wants me to look like a young chick all the time'.

Anna is extremely upset over it. She reveals to the narrator sordid details about their marriage. David has a set of rules if she breaks them she is punished. So narrator suggests a divorce, Anna explains that she loves him, even through she thinks he'd like her to die. The narrator still hasn't found out what happened to her father. As narrator goes down to the shore, she overhears an argument between David and Anna. She looks better and also happy but at this moment, David is trying to talk her into taking her bathing suit off so that Joe can take some shots of naked her for movie, they are making a movie called 'Random sample' David was telling her, "Come on, take it off…..It won't hurt you, we need a naked lady…..you'll go in beside the dead bird, it is your chance for stardom, you've always wanted fame."This was the pose which was taken by Joe, against Anna's desire and this is the lost Ann's identity. The narrator wants to stop the fight but she doesn't. Both Anna and David become very angry, David threatens to throw her into the lake if she doesn't cooperate. Finally, Anna gives to takes off her bathing suit and dives into the water while Joe films her. Anna jumped into the lake. Narrator is thinks that David is like her. "We are the ones that don't know how to love, there is something missing in us, we were born that way."

Narrator asked David why he forced Anna to humiliate herself {that too in front of Joe}, David tells her that Anna is devious and unfaithful. She is stupid, according to David,"She goes with other man, she thinks she can get away with it, but she is too dumb, every time I find out; I can smell it on her…..God knows that I'm not jealous….But she is devious, I can't stand that."Narrator has had an abortion; it is a sock for the readers. Earlier she has told that she was married, had a child and left the child with her husband when they divorced Narrator comes to the realization that I could have said, no to the abortion, but I'm a killer,"I thought, whatever it is part of myself or a separate creature, I killed it. It was not a child but it could have been one, I didn't allow it."Narrator wanted to escape the moral responsibility for the death of her child. It was easies for the narrator to present herself as the victim of a broken marriage and killer of her child. Narrator think that she could not allow it. In chapter seventeenth David and Anna tell her that her father has been found by some Americans fisherman.

His body was unrecognizable, but Mr.Paul identified the cloths as her father's. The narrator is suspicious of the information, when she asks them where they found the body they tell her it was near the cliff where she was diving. The narrator doesn't believe them. Even though her friend avoids her because of the news of her father's death, the narrator is convinced that, "Nothing has died, everything is alive, everything is waiting to become alive."

Narrator wants to make contact in some way with her parents. She burns everything connected with civilization, drawing, photographs, maps etc. she baptizes herself in water like fire. Her quest is clearly a visionary one, and she must search for the power of the gods, the ancients Indian gods of nature, through a ritual of personal purification. All alone on the island, the narrator is awoken suddenly the next day by the sound of power boat. She runs into the woods to hide. She thinks it might be the police or possibly tourists, she even wonders if they are American invaders. "They cannot be trusted. They'll mistakes me for a human being, a naked woman wrapped in a blanket…. They won't be able to tell what I really am. But if they guesses my true form, identity, they will shoot me… and hang me up by the feet from a tree.'

Unnamed narrator thinks that her mother transformed into a bird, and she recognized that her father has been transformed in to a fish. Narrator witnessed her mother turning into a bird and she sees her father turning into a fish. Both of her parents have become embodiments of nature, taking on shapes of animals and birds. The end of her visionary quest is signaled by the narrator discovering that the foot prints she thoughts of her father's turn out to be her own. Narrator dreams of her parents that they have been gone and never comeback. The next morning when she wakes, she realizes, "I know they have gone finally, back into the earth, the air, the water, wherever they were when I summoned them. I am the only one left alive on this island." Narrator is pretty sure that her parents will never appear to her again. she cannot stay there on the island forever. She pretended to be a victim of a failed marriage. "The above all, to refuse to be a victim, unless I can do that can do nothing. I have to recant, give up the old belief that I am powerless and because of it nothing I can do will ever hurt anyone….with drawing is no longer possible and the alternative is death."

Narrator gets ready to re-enter life by putting her clothes on. She wonders about the baby she is carrying. If she is pregnant. She feels it is her duty to feed and take care of herself so that she will be able to deliver a healthy baby. Lost in thought she sees a boat arrive with Paul and Joe in it Joe gets out of the boat and he calls her. He has returned {leaving David and Ann} especially for her. He won't wait much longer. But right now he waits. The lake is quit; the trees surround me, asking and giving 'nothing'. The novel ends without an ending what the narrator will actually do afterwards.Literature has an intertwined relationship with society, culture and history. Its quality as well as its content, style and form involve and reflect on social facts and cultural ideals. My attempt in this first chapter is to explore the social, cultural and historical events that marked the period in which Margaret Atwood's novel Cat's Eye was written and to illustrate the movements by which her works were influenced. Though published in 1988, Cat's Eye spreads out the time period that extends from World War II to the late 1980s. Margaret Atwood began writing her novel in 1964 but set it aside till the late 1980s, drawing her ideals from the movement of feminism that emerged in Canada and depicting the role of women in Canadian society.

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RESEARCH ARTICLE

Feministic Aspects in Shashi Despande's Roots and Shadows and that Long Silence…

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ABSTRACT

That Long Silence, which won Shashi Deshpande the Sahitya Akademi Award for 1990, tells the story of an Indian housewife, who maintains silence throughout her life. The novel ends with her resolve to speak, to break her long silence. The novel is a protest against the limitation of Women's lives. The issues and problems of contemporary middle class woman have always been the subject matter of Shashi Deshpande's writings. This paper seeks to study the feminist perspective in Shashi Deshpande's novel That Long Silence. Although many women writers tried their hand at expressing this long silence that had turned woman into non-entities, they could only provide psychological depths to their characters. They either created unreal sentimental romances or finally succumbed to the temptation of mouthing feminist ideology. But Shashi Deshpande's success lies in her representation of real life experience. She realistically depicts the inner conflicts of Jaya and her quest for the self or identity. She has woven the tragic tales of Jaya's relations and her acquaintances into the texture of the novel, and so the novel inevitably takes on a feminist character. The novel is about gross gender discrimination and inequality prevalent in society. Present paper deals with feminist perspective in Shashi Deshpande's novel roots and shadows.

Keywords: that long silence, shashi deshpande, Feminist, Shashi Deshpande, roots and shadows.

INTRODUCTION

Feminism is quite late in the development of the twentieth century English literature. Women are always oppressed, suppressed and marginalized by men. They have been ill-treated and exploited in all walks of life. After independence, many Indian women novelists have raised their voice against the exploitation of women. Among these women writers appear the names of Sahgal, Anita Desai, Kamala Markandaya, Arundhati Roy, Shashi Deshpande and so on.Shashi Deshpande occupies a distinctive place in the postcolonial Indian women writers in English. She was influenced by her father Adya Rangachar who was the distinguished Kannada writer. She was also influenced by the literary works of Jane Austen, Charles Dickens and George Bernard Shaw.Her novels are women oriented like Jane Austen and deals with "Women's struggle, in the context of contemporary Indian society, to find and preserve her identity as a wife, mother, and most of all as human being" [1]. As a contemporary author, Deshpande presents the realistic picture of the male-dominated middle class society of India. Her protagonists are caught between the tradition and modernity but they try to strike a balance between the two.

Deshpande is very realistic in the sense that suggests marriages are not based on love but convenience. This paper is an attempt to analyze the novel Roots and Shadows from the feminist perspective. It discovers the pain and suffocation of the protagonist Indu in the male-dominated society. She tries to escape from this to find her real 'self', but every time she is deceived. After a long time and much introspection her journey ends with the realization that she has been chasing shadows, leaving her roots behind. Indu lives in a joint family with her Kaka's (Uncle) and Atya's. She is brought to this house when she was only fifteen days old child. In this house 'Akka' her father's Atya is a dominant person. She rules over the house. Akka came to this house as a childless widow with her property and old uncle. As a girl child in their joint family, Indu always taught to be obedient, submissive, meek and unquestioning. Indu is an educated modem Indian woman who has her own way of living.Feminist movement advocates the equal rights and equal opportunities for women. The true spirit of feminism is into look at women and men as human beings. There should not be a gender bias or discrimination in familial and social life. Establishing gender justice and gender equity is the key aspects of feminist movement. In India, women writers have come forward to voice their feminist approach to life and the patriarchal family set up. They believe that the very concept of gender is not merely biological phenomenon but it has a social construction. Shashi Deshpande is a renowned novelist of Indian writing in English. She has the credit of writing well known novels namely; The Dark Holds No Terrors; Roots and Shadows; and That Long Silence. Her first novel The Dark Holds No Terrors was translated into German and Russain languages.

That Long Silence (1988) was her fifth novel which was recognized with 'Sahitya Akademi Award' in 1990. Her works primarily deals with the problems of women in the present social context. Deshpande's quest for identity and freedom has become dominant themes in literature. She unfolds the problems of women in the patriarchal society in a very positive way.According to her, woman has every right to live her life, to develop her qualities, to take her decisions, to be independent and to take charge of her destiny.Shashi Deshpande has presented in her novels modern Indian women's search for the definition about the self and the society; and the relationship that are central to women. Her novels highlight the image of middle class women squeezed in between tradition and modernity. She portrays her heroines in a realistic manner. She deals with a woman's psyche which is made to feel inferior and a burden on the family. Her heroines are courageous enough to revolt against the marginalization of women by men and society as revealed in That Long Silence and The Dark Holds No Terrors.In That Long Silence, the protagonist Jaya journeys towards self-actualization. The novel embarks on with the receptive appearance of the solitude of a woman and the poignant question of the eventual purpose of her life in the milieu of her familial bond. The novel is an individual's journey in search of one's true self who confronts the gender oriented tradition. It depicts the plight of a wife who suffers silently in the name of family. Marriage is still a social necessity, where women seek security and men respectability. In her early married life, Jaya had yielded her decisions to her husband. The forced isolated stay in Dadar flat facilitates her to reconsider her life built around the needs of a husband only.

Jaya's creativity provides her an outlet for her dissatisfaction. The novel ends with a renewal of faith. Jaya's decision to wipe out the silence and have a balanced contented life is a decision in the right direction.Selfactualization is possible if a woman decides to be herself, to reveal the genuine value of her free and inborn individuality in its entirety. Deshpande's major apprehension in That Long Silence is to search deeply into the psyche of a woman who is made to face all kinds of mental tortures. The question what a woman does is never asked, but "who she belongs to" is always considered important. She never has an identity of her own. Her name changes as per the wishes of others. In That Long Silence, the writer has presented this fact through the character Jaya, who is recognized by two names: Jaya and Suhasini. Jaya is the name given by her father when she was born which has the meaning "victory"; and Suhasini is the name given after her marriage which means a "soft, smiling, placid, motherly woman". Both the names stand for the persona of her individuality.

The earlier one symbolizes revolt whereas the final one symbolizes submission. To make the story a reliable one, Deshpande has made use of first person narrative to represent the psyche of the modern middle-class learned woman.To appeal to the readers, she uses flashback technique. The first chapter deals with the present, but the remaining chapters are more in reminiscence with the final chapter ending in the present.The narrator in this novel is Jaya, the protagonist herself. While narrating her heartbreaking experiences, her mind wavers and she unfurls her whole life – from her childhood days to her father's death.She is a typical modern woman who has her roots in tradition, while her husband Mohan, a traditionalist has his roots in customs. Their outlook is different and they fail to understand each other. Due to differences in their attitude, their marital life grows shaky and gloomy. It becomes more of a compromise than love based on social fear rather than mutual need of each other.

The choice may be rooted in their choice of a partner. For example, from the very beginning, Mohan wanted a wife who was well-educated and cultured and never a loving one. To Mohan, a woman sitting before fire, waiting for her husband to come home and eat her food is the real strength of a woman, but to Jaya it is nothing more than despair. He wanted his rice fresh and hot, from a vessel that was untouched. She had just finished cooking this second cooking and was waiting, hoping, perhaps that he would not be too late, for it wouldn't do to allow and as for lighting the fire again, that was unthinkable.Her Ajji along with silence had taught her to "wait" the waiting game. For a man waiting brings in restlessness but for woman the game of waiting starts quite early in her childhood wait until you get married, wait until your husband comes, wait until you go to your in law's home, wait until you have kids. Yes, ever since I got married I had done nothing but wait. Women are blamed unfeminine and unnatural if they break the social system and so they are enforced to adhere to be termed feminine.

Deshpande reveals the consciousness of Jaya through an account of her mind in the process of thinking, feeling and reacting to the stimuli of the moment and situation. In doing so, she goes on to assert the feminine psyche of the protagonist, to break away from the strong hold of a social framework rooted in patriarchy which repels as it attracts. Jaya is a modern predicament and the flood of consciousness that ensure out of it is a silent stream of thoughts and feelings. She knows pretty well that in order to get by in a relationship one has to learn a lot of tricks and silence is one of them. Jaya surrenders Mohan without revolting. She never refuses or complains about anything. Her identity, personality is totally crushed which leads her to total confusion along with loss of self identity. We get a glimpse of Hinduism in the numerous fasts observed by women for the well being of husbands, sons or brothers. "Generally, a woman's identity is defined in terms of her relationship with man as a daughter, a wife and a mother It means virtually a woman doesn't have an identity of her own" says Indira Kulkshreshtha. The narrator Jaya, an upper-middle-class housewife with two teenage children, is forced to take care of her life when her husband is suspected of fraud. They shift to a small flat in a poorer locality of Bombay, leaving their luxurious house.

The narrative reveals the futility of modern Indian life, where success is considered only with the upwardly mobile husband along with their children studying in "good" schools. The daily chores of normal life of a woman with material comforts is significantly represented in the following lines -the glassware that had to sparkle, the furniture and curious that had to be kept spotless and dustfree, and those clothes, God, all those never-ending piles of clothes

that had to be washed and ironed, so that they could be worn and washed and ironed once again. Jaya's creativity is muted by strong social and family pressures and holds all creative activities in submission to her role as a homemaker. Though she is a writer, Jaya has not achieved true self expression. There is something almost overpowering about the restriction of the narrator's life. The story is unfolded by Jaya, ironically again symbolizing victory, while in the actual life situation, she is supposed to lead a traditional, passive life like; "Sita following her husband into exile, Savithri, dogging death to reclaim her husband, Draupadi stoically sharing her husband's travails….." (11).

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R.Kala is a research scholar at Prist University, Thanjavur, TamilNadu and she is continuing her research work under the supervisor of Dr. R.Visalakshi, M.A., M.Phil., Ph.D, Assistant Professor in the English Department

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RESEARCH ARTICLE

A Study on Social Realism in Jane Austen's Emma and Pride and Prejudice

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ABSTRACT

Jane Austen Born in 1875 she was the seventh of eight children born to the rector of steventon Hampshire. Austen began writing at the age of eleven. Her notebook containing novels were passed around her family for their entertainment.Austen and her family were as she said, great Novel readers and not ashamed of being so. Some of her works include pride and prejudice. The most obvious influence occurs in the change of genre in pride and prejudice. In their original forms.

Key words: Jane Austen, pride and prejudice, entertainment.

According to Austen three or four families in country village is the very thing to work upon. She concentrated on the limited part of English society. Austen herself resisted the temptation to stray from familiar ground even when nudged by royalty. When the domestic chaplain to the Prince of Wales suggested that in her next novel. She might delineate the character of a clergyman. Austen's privacy even after death. For it is important to realize how fiercely Austen's and her family regarded her privacy. All her novels were published anonymously and their authorship was a well kept secret.One of the most popular Austen's novels pride and prejudice introduces many of the stylistic devices commonly associated with austen's work: witty, cutting dialogue between couples strong willed heroines without a strong role model settings that reveal the underlying character of the male protagonist and an ironic undertone, often established in the opening lines of the novel.

This marks a slight diversion from the pattern established in Austen's earlier novels for here the heroine is an orphan adoped into her rrich uncle's family. Despite being condescendingly treated as a poor relation fanny's honesty and modest disposition gradually make her an indispensable part of the household, particularly when her uncle is away on business for an extended period and the familys sense of discipline is relaxed.In this novel it is the male characters, particularly Edumund Bertram and Bertram and Henry Crawford, who represet contrasting personalities

and it is Edmund who is self deluded and eventually comes to see fanny's virtues. To many critics Mansfield park is one of austen's lesser novels, perhaps because of her reversal of her usual male and female portrayals, a reversal she did not repeat.In contrast the novels, which followed emma are considered by many to be her best works. The heroine Emma is again virtually alone in the world her mother is dead her father is absentminded and ineffectual, and her governess companion has left to be married. But unlike fanny in Mansfield Park Emma is not especially wise. In this novel Austen again deals with the theme of feminine self delusion but the focus on a strong willed character makes the impact stronger than in the previous works.

In persuasion, Austen returns to the contrasting of sisters but focuses primarily on the second of the three Anne is pretty, intelligent, amiable, but also malleable.Lady Russell, a trusted friend, persuaded here to break off a long standing engagement, despite her feelings for her lover.During the resulting confusion both lovers become entangled in other relationships but eventually realize that their affection for each other still exists.In this Austen's last complete work the satire and ridicule take a milder form, the tone is graver and tenderer, the interest lies in a more subtle interplay of characters. Austen herself apparently recognized the difference in tone for she wrote of anna she is almost too good for me.Although well received, Austen was not immediately successful few of her works reached a second edition during her lifetime.In fact, the collected edition of 1833 supplied the market until 1882. Attention began to rise during the 1890 as indicated by the appearance of biographies and critical pieces. Today almost 175 years later all of her books are in print and Austen is one of the top selling authors. No doubt much of her popularity is due to a desire to escape to a better place and time.

Yet, because much of her work depends on character analysis, many readers still identify with much of her work.Other are captivated by her style her careful construction and use of dramatic method where characters are introduced through dialogue before putting in an appearance.To some she is one of the greatest ironists who ever lived. And to most she is a challenge.As asten herself put in I do not write for dull elves who cannot think for themselves.The publication of Jane Austen's six novels between 1811 and 1817 marked a turning point in the development of English fiction. To her contemporary audience they revealed that the novel was capable of unsuspected power that it was not to be dismissed as a mere pastime but was not to be taken seriously as a form of literature, on a level with poetry and drama. In the words of George Moore, Jane Austen turned the washtub into the vase. In effect, she transformed the eighteenth century novel which was clumsy and primitive, uncertain in its technique, into a work of art. She gave elegance and form to its shaping, style to its writing and narrative skill to the presentation of the story.

She invented her own special mode of fiction the domestic comedy of middle class manners. Her account of this world is limited and highly selective.Her focus is upon the experiences of young women on the path of marriage. The modesty of fictional world is caught in her remark to a novel writing niece that 3 or 4 families in a country village is the very thing to work upon and her famous comment to a novel writing nephew about the little bit of Ivory on which I work with so fine a brush which produces little effect after much labour.Jane austen presents an account of society from the womans point of view the womans experience of men of other women, of their families the social circles to which they were confined and ultimately their experiences. Fot the first time in English literature since Shakespeare the reader comes across heroines who are credible, with mind with the capacity to think for themselves, with ambition and wit, with an interior life independent of men and the will to challenge them emotionally and intellectually with the energy to shape their relationships.The six novels are repeated dramatizations of this theme. Each of these heroines travels the path of self discovery and growth.

Jane austen leaves it an open question open to us as readers to decide how far they win and how far they fail.Jane austen's attitude towards things is not extraordinary.She has no exciting messages and no doctrines for womens liberation. Her view is coldly realistic. The novels confirm that life is a comedy to those who think. A thinking novelist she has created thinking heroines. But the other side most powerful tension arises from Jane Austen's struggle to maintain a hold over experiences that threaten the comic surface of the novels with the feeling tones of

tragedy.A study on social realism in Jane Austen's Emma and pride and prejudice is my research topic. Jane Austen's was a great famous novelist writer. Her concept described to social realism. The Emma and pride and prejudice are a great social realism novel. It is true from my research end of the results.

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R.Priya is a research scholar at Prist University, Thanjavur, TamilNadu and he is continuing his research work under the Supervision of Dr. R.Visalakshi, M.A., M.Phil., Ph.D., Assistant Professor in the English Department.

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RESEARCH ARTICLE

An Analysis of Main Concept in Margaret Atwoods Novel in the Hand Maidtale and the Edible Women

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ABSTRACT

Margaret Eleanor Atwood is a Canadian poet, novelist, short story writer, critic and author of children's books. She explores the relationship between humanity and nature, the dark side of human behavior, and power as it pertains to gender and politics.Atwood was born in Ottawa and grew up in suburban Toronto.As a child, she spent her summers at her family's cottage in the wilderness of northern. She first began to write while in high school, contributing poetry, short stories, and cartoons to the school newspaper.As an undergraduate, at the University of Toronto.

Key words: novelist, humanity and nature, human behavior.

Atwood published her first volume of poetry, Double Persephone, in 1961. In 1962, Atwood completed her Master's degree at Radcliffe College of Harvard University. She taught English at the University of British Columbia for a year and completed her first novel, The Edible Woman.Teaching Victorian and American literature at Sir George Williams University in Montreal in 1967, Atwood began teaching creative writing at the University of Alberta, while continuing to write and publish poetry.Her poetry collection The Circle Game won the 1967 Governor General's Award, Canada's highest literary honour husband, Graeme Gibson. Atwood received the Governor Generals Award again in 1986 for her novel The Handmaids Tale, was published that same year.

She continues to be a prominent voice in Canada's cultural and political life. Since 1961, Atwood has produced a highly acclaimed body of work includes fiction, poetry and literary criticism.Atwowd further developed this dichotomy in power politics in which she explores the relationship between sexual roles and power structures by focusing on personal relationships.Atwoods novels explore the relationship between personal behaviours and political issued as well. The Handmaids Tale, a dystopian novel concerning an oppressive future society. Criticism of Atwoods work has tended t o emphasise her political and social views. Many critics identify her use of grotesque,

shocking imagery and heavy irony as hallmarks of her style.Because her poetry and fiction often portray physical and psychological violence in relationships between men and women.The Handmaids Tale for example has been favorably compared with George Orwells the Edible woman the Handmaids tale. Marian MacApin, the first person narrator of the first and third sections of the edible woman and the central character in the second section, is a normal, average young woman she becomes engaged to Peter. She cannot eat red meat her behavior becomes erratic in other ways as well manipulative student, Duncan. She finds her job with a market research company less and less bearable.Marians decision to marry peter is based on his suitability.He is a conventional young man destined. Marian in order to marry him believes that she is willing to be something like a servant doing what peter wishes fetching for him and meeting his sexual needs.

As time goes on however she feels more and more uncomfortable with this role and that discomfort is the cause of her inability to eat.Marian's restlessness causes her to continue relationship with Duncan whom she first meets in a Laundromat. Marian finds herself drawn to duncans total rejection of responsibility.The next day Marian learns that their sexual encounter has meant no more to him than anything else in his affection less life. Marian returns to her apartment and bakes a cake which she arranges in the shape of a woman. When peter comes to remonstrate with her she offers him the cake.He leaves, offended and Marian happily eats the cake sharing it with Duncan who drops in casually.Surfacing is the problem of the woman who is unable to accept the roles provided for her by a male dominated society. Atwood does not provide alternative possibilities.At the end of the novel there is no suggestion of what kind of life she may begin to lead. The important question for Atwood is always whether her protagonists can assert their individuality and begin the process of discovering who they are.

Atwood is pessimistic about social change. Nothing in her novels suggests that society is recognizing the need for women for self realization. Her novels are clear demands for such change. Atwood is among the most powerful and successful of the women writers who have presented the central conterns of feminism.The Edible woman her first novel is very much a product of the late 1960s. The men in the company occupy executive positions and work on the floors above the women on the lower floors can have no hope of advancement and can only hope to be rescued from their meaningless jobs by marriage.At the end of part Marian bakes and serves peter a cake edible woman and rejects him as marriage partner. The third part witnesses the emergence of Marian from the third person anorectic space her ability to eat and speak for herself. The novel begins with paranoia that a lead to decomposition.Which is only five pages long, sets the decomposition at pace.Marian's description of her company Seymour surveys is revealing. The company is layered like an ice cream sandwich with three floors, the upper crust, the lower crust and our department the gooey layer in the middle.On the floor above are the executives and the psychologists referred to as the men upstairs, since they are all men below us are the machines mimeo machines.LBM machines for counting and sorting and tabulating the information. Our department is the link between the two: we are supposed to take care of the human element.

The interviewers themselves reflect the social reality of a certain class of female workers I the early 1960 especially the reality of the secretarial proletariat.Becoming a woman in such conditions meant going beyond identity and subjectivity. In the edible woman Marian mcalpins breakdowns can be seen as breakthroughs. Margaret Atwood and her contribution to the Canadian literature of Feminism is generally considered an umbrella term for a range of views about injustices against women.Nonetheless, the best way to define Feminism is to identify it in terms of a set of ideas or beliefs rather than as participation in any political movement.Feminism is based on the as participation in any political movement. Feminism is based on the belief that women are oppressed or disadvantaged in comparison to belief that women are oppressed or disadvantaged in comparison to men and that their oppression is in some way illegitimate or unjustified.The Feminist theory and practice have brought into notice the discursive construction of gender sexuality, social and domestic relations. Feminism is interested in the problems related to marginalization and suppression of women. It is both an intellectual commitment and a political movement that seeks justice for women and the end of sexism in all forms.By sexism, the emphasis on physical differences between the sexes is implied.This harping on externals, which culminated in the burning of the bra movement has fortunately transformed into what is

today called gender that analyzes male and female characteristics in terms of behavior, demeanor and psychology. Feminism is an area where in the most natural organic way subjectivity and politics have comes together.It has resulted in protest against the exclusion of women from literary canon. It focuses upon the personal and makes a political argument.This movement has aligned itself with other movements and has redefined literary theory and language itself. Some argue that Feminism is not just another interesting critical approach but it represents one of the most important social, economic and aesthetic revolutions of modern times.We also touched upon the various dimensions of feminism including biological feminism, psychoanalytic feminism, linguistic feminism, Marxist feminism, post structuralist feminism, post colonial feminism, cultural studies, ethnic and race studies, black feminism, lesbian and gay studies and gender studies.We then introduced two celebrated authors in feminism. Margaret Eleanor At moods born on November 18, 1939.She born in Ottawa and raised is tronto and she spent the larger part of her youth in Canada.She is a renowned novelist and poet.The Handmaids tale a dystopian novel concerning an oppressive future society atwood received the Governor Generals Award again in 1986 for her novel the handmaid's tale which was published that same year.

Margaret Eleanor Atwoods was struggle against male chacrvenism and religions extremism for the women.Her works have been translated to an array of different language.This novel was adapted into Television mini services for Hulu. She was writing the novel to start the age of six and she was writing Morality plays, poems, comic books and novels. It is one of the famous novels written by Margaret atmoods.Margaret Atwoods the Handmaids Tale in a get award novl is the society. This thesis "An Analysis of main concept of Margaret Atwoods novel in the Handmaids tale and the edible woman"Atmoods told the main concept of "feminism" and "Male chauvinism" in this novel. I research this thesis the power structure of Gilead and also critique the feminine roles in this society.Here explained the way of author critique views that means functions expression, of the disunity of women.The author Margare Atwoods the Handmaids tale from the point of view of feminism and male chauvinism.The point of view of intranarrational unreliability.A first person narrator she does not have access to the thoughts, feelings and motivations of other characters. Offered tells the story she needs to tell.The essential honesty of needing to tell the story is what makes her reliable.She is telling the story she needs to tell and not telling a story that focuses on scientific accuracy.The Handmaids tale becomes a proptetic call to action.This novel described social structure in the republic of Gilead.It explores the mechanics of fear in the novel. The paper published on different way of women and their passion in the over all society Atwoods novel compares the sexual repression and religions fundamentalism important fundamentalism given by atwoods novel.

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V.Subash is a research scholar at Prist University, Thanjavur, TamilNadu and he is continuing his research work under the guide advisor of K.Jayapriya Assistant Professor in the English Department.

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RESEARCH ARTICLE

A Comparative Evaluation of Fatty Acid Profiles of Rendered Buffalo Fat and Soybean Oil

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ABSTRACT

Six samples each of soybean oil and rendered buffalo fat were evaluated for their fatty acid profiles. Both showed its own unique fatty acid profile with significant variation within each individual fatty acid. Soybean oil exhibited the highest total polyunsaturated fatty acids (63.13±0.12 percent) while the highest total saturated fatty acids was noticed for rendered buffalo fat (49.13±0.06 percent). Significantly higher level of linoleic and linolenic acids (55.39±0.18 and 7.75±0) were observed in soybean oil. Soybean oil showed total polyunsaturated/total saturated fatty acids ratio 4.03±0.00 and n-6/n-3 ratio 7.16±0.08 closer to WHO recommendations.

Key words:- Rendered buffalo fat, Soybean oil, Fatty acid profile

INTRODUCTION

Fats and oils are recognized as essential nutrients in both human and animal diets. They provide the most concentrated source of energy of any foodstuff, supply essential fatty acids (which are precursors for important hormones, the prostaglandins), contribute greatly to the feeling of satiety after eating, are carriers for fat soluble vitamins, and serve to make foods more palatable. Fats and oils are present in varying amounts in many foods. Dietary fats can be derived from plant and animal sources. Fats that are used as such at the table or during cooking (vegetable oils, vanaspati, butter and ghee) are termed as "visible" fats. Fats that are present as an integral component of various foods are referred to as "invisible" fat. Fats in processed and ready to eat foods are known as hidden fats (Johnson and Saikia, 2009).

Vegetable oils are the main sources of essential fatty acids for the body. Essential fatty acids (EFA) are those fatty acids which the body cannot synthesize and need to be supplied through diet. It also helps in raising High density lipoproteins (HDL), the so-called good cholesterol. Low-fat diets can result in reduction of HDL cholesterol. Fat in the diet imparts certain textural qualities, taste and palatability to the food (Dorni *et al.,* 2018).

Saturated fatty acids are known to increase serum total and LDL-cholesterol levels, reduce insulin sensitivity, enhance thrombogenicity and increase cardio-vascular risk. Therefore, SFA intake should not exceed 8-10% of total energy. Poly unsaturated fatty acids are essential components of cell membranes. While n-6 PUFAs are predominant in all cells, the nerve tissue has high levels of long chain n-3 PUFA. An appropriate balance of these two classes of PUFAs, namely, linoleic and alpha-linolenic acids in the diets, is essential for the functioning of vascular, immune, nervous and renal systems and for early human development. The intake of PUFA should be 8-10% of energy intake. The remaining 8-10% of fat calories can be derived from mono-unsaturated fatty acids, which also help in maintaining plasma cholesterol. On the basis of these recommendations, there are basically three parameters to adjudge any oil as healthy oil - ratio of saturated/ monounsaturated/ polyunsaturated fatty acid, ratio of essential fatty acids (n-6/n-3) and presence of natural antioxidants. It is now widely accepted that the human body needs a ratio varying from 5:1 to 10: 1 of n-6 to n-3. The PUFA/SFA ratio should be 0.8 to 1.0 (ICMR, 2011).

Edible rendered fat from buffalo, bovine and ovine carcasses is termed tallow which is solid at room temperature. A significant use of tallow is for the production of shortening. Tallow is sometimes used in deep frying in place of other oils. Use of edible tallow might impart an undesirable oily mouth coating to processed meat products (Pati *et al.,* 1992). The American Heart Association's report states that consumption of large quantities of animal fat is linked with heart disease as excess animal fat consumption results in the supply of large amount of saturated fat. The dietary guidelines point out that the consumption of unsaturated fats appears to have no adverse health impacts (Hendricks *et al.,* 1991).

Soybean oil is a vegetable oil extracted from the seeds of the soybean (*Glycine max*). It is one of the most widely consumed cooking oils. Of the total global vegetable oil production, soybean oil contributes 27.4 per cent; whereas of the total vegetable oil consumption soybean oil accounts to 27.7 per cent. Fatty acid profile of soybean oil had linoleic acid (54.17 per cent) in the highest proportion followed by oleic (24.77 per cent) and palmitic (11.67 per cent) acids accounting for almost 91 per cent of the total fat. Small but significant amount of α-linolenic acid (5.16 per cent) was also found in soybean oil (Dorni *et al*., 2018). With this background, the current study was undertaken to compare the fatty acid profiles of rendered buffalo fat and soybean oil from a human health perspective.

MATERIALS AND METHODS

Soybean oil

Fortune Soya Health refined soybean oil, Adani and Wilmer Ltd., Ahmedabad, India, was purchased from local market.

Preparation of rendered buffalo fat

Six female Murrah buffaloes from Kerala Veterinary and Animal Sciences University Buffalo Farm, Mannuthy, were utilized in this study. All the animals were in the age group of four to six years. They were reared intensively with occasional periods of grazing. After scientific slaughter of the animals at the Meat Technology Unit, KVASU, Thrissur, perirenal fat was collected from buffaloe carcasses. The fat tissue was minced through a 4 mm plate in a meat mincer (MADO primus Model MEW 613, Germany). The minced adipose tissue was added with sufficient quantity of water and subjected to wet rendering in a domestic pressure cooker (PRESTIGE Model SIGNATURE, India). Rendering of perirenal adipose tissue for about 90 minutes yielded liquid edible fat which was filtered using a strainer to remove solid particles. It was then allowed to stand for about 30 min in a glass cylinder at 60^{o}C for separation of aqueous and fatty layers. The top fat layer was carefully decanted into glass containers.

Fatty acid profile analysis

Fatty acid profiles of rendered buffalo fat and soybean oil were determined as per O'Fallon *et al*. (2007) at the Animal Feed Analysis and Quality Assurance Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Namakkal. Fatty acids were converted into fatty acid methyl esters (FAME). The fatty acid contents were expressed as percent of the total ether extract.

RESULTS

Analysis of the fatty acid profile of rendered buffalo fat and soybean oil revealed significantly higher ($p<0.01$) myristic, palmitic, stearic and oleic acids in rendered buffalo fat than soybean oil. Rendered buffalo fat also had significantly higher (<0.01) amount of total saturated fatty acids (SFA, 49.13±0.06 and 15.67±0.04 percent for rendered buffalo fat and soybean oil, respectively), total mono unsaturated fatty acids (MUFA, 48.70±0.02 and 21.16±0.16) and SFA/UFA ratio (0.97±0.00 and 0.19±0.00) than soybean oil. Soybean oil contained significantly higher ($p<0.01$) amount of total polyunsaturated (PUFA, 63.13±0.12 and 2.1±0.08 per cent, for soybean oil and rendered buffalo fat, respectively) and total unsaturated (84.29±0.04 and 50.84±0.06) fatty acids contents. The soybean oil had a significantly higher PUFA/SFA ratio than rendered buffalo fat (4.03±0.00 and 0.05±0.00, respectively). Moreover, soybean oil contained significantly higher ($p<0.01$) amount of n-3 (linolenic acid, 7.75±0.16 and 0.44±0.13 per cent, for soybean oil and rendered buffalo fat, respectively) and n-6 (linoleic acid, 55.39±0.18 and 1.70±0.10) fatty acids, and a correspondingly higher n-6/n-3 ratio (7.16±0.08 and 3.92±0.340 for soybean oil and rendered buffalo fat, respectively) as compared to rendered buffalo fat.

DISCUSSION

Fatty acid profile of rendered buffalo fat and soybean oil revealed that rendered buffalo fat contained significantly higher amount of total saturated fatty acids (49.13±0.06 and 15.67±0.04 percent of ether extract, for rendered buffalo fat and soybean oil, respectively). These values were similar to the values reported by Dorni *et al.* (2018) for rendered buffalo fat and Johnson and Saikia (2009) for soybean oil.

The total mono unsaturated fatty acid (MUFA) content of rendered buffalo fat (48.70±0.02 percent) was significantly higher than that of soybean oil (21.16±0.16, per cent) and SFA/UFA ratio was significantly higher for rendered buffalo fat (0.97±0.00 and 0.19±0.00).

Soybean oil contained significantly higher amount of total polyunsaturated fatty acids (PUFA) than rendered buffalo fat (63.13±0.12 and 2.1±0.08 per cent, respectively). Consequently, a higher PUFA/SFA ratio was observed for soybean oil (4.03±0.00). Rendered buffalo fat had an extremely low PUFA/SFA ratio of 0.05±0.00. Previous authors have recommended a dietary PUFA/SFA ratio between 0.4 and 1.0 (Enser, 2000). Soybean oil contained significantly higher amount of n-3 and n-6 fatty acids, and a correspondingly higher n-6/n-3 ratio (7.16±0.08 and 3.92±0.34, for soybean oil and rendered buffalo fat, respectively). Dorni *et al.* (2018) also reported n-6/n-3 ratio of 10.50 for soybean oil. Masterjohn (2014) reported the n-6/n-3 ratio of tallow from grass-fed cattle as 1.5:1 and that of grain-fed cattle as 16.8:1. Though Wood *et al.* (2003) recommended that the n-6/n-3 PUFA ratio should not exceed 4, WHO (2008), in its report of an expert consultation on fats and fatty acids in human nutrition, has stated that there was no rational for a specific recommendation for n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lay within the recommendations.

The ICMR recommends PUFA/SFA ratio of 0.8 to 1.0 and linoleic acid (n-6) /alpha linolenic acid (n-3) ratio of 5-10 in the diet (ICMR, 2011). The soybean oil had a PUFA/SFA ratio of 4.03±0.00 which was significantly higher than that of rendered buffalo fat. The n-6/n-3 ratio of soybean oil was 7.16±0.08 which was within the ICMR recommended range. Hence, compared to rendered buffalo fat, soybean oil could be a better choice as shortening or cooking medium from a health perspective.

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Table 1: Fatty acid profile of rendered buffalo fat and soybean oil (Mean±S.E.) #

Parameters	Rendered buffalo fat	Soybean oil
Myristic acid	2.40±0.05^{b**}	0.07±0.03^{a}
Palmitic acid	26.11±0.08^{b**}	11.48±0.05^{a}
Stearic acid	20.63±0.02^{b**}	4.13±0.12^{a}
Oleic acid	45.56±0.04^{b**}	21.08±0.12^{a}
Linoleic acid	1.70±0.10^{a}	55.39±0.18^{b**}
Linolenic acid	0.44±0.01^{a}	7.75±0.06^{b**}
Palmitoleic acid	3.15±0.06^{b**}	0.09±0.04^{a}
Total Saturated FA	49.13±0.06^{b**}	15.67±0.04^{a}
Total Monounsaturated FA	48.70±0.02^{b**}	21.16±0.16^{a}
Total Polyunsaturated FA	2.14±0.08^{a}	63.13±0.12^{b**}
Total Unsaturated FA	50.84±0.06^{a}	84.29±0.04^{b**}
ω-3	0.44 ± 0.13^{a}	7.75±0.16^{b**}
ω-6	1.70±0.10^{a}	55.39±0.18^{b**}
ω-6 / ω-3	3.92±0.34^{a}	7.16±0.08^{b**}
PUFA/SFA	0.05±0.00^{a}	4.03±0.00^{b**}
SFA/UFA	0.97±0.00^{b**}	0.19±0.00^{a}

#Mean±S.E. with different superscripts differ significantly (P<0.05); n = 6 for each treatment * Significant (P<0.05);** Highly significant (P<0.01)

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RESEARCH ARTICLE

Isolation and Diagnosis of Some Contaminated Bacteria that Isolated From Different Halls in the Faculty of Science, University of Al-Qadisiyah, Iraq

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ABSTRACT

This study was conducted in order to take an idea about some bacteria that presented in some halls of the college of Science, University of Al-Qadisiyah. 60 samples of the halls of the Departments of Biology, Chemistry and Environment. The Isolates of *Escherichia coli* were highest in the in the department of Biology (14%), followed by *Pseudomonas aeruginosa* (10%) in the environmental sciences department, followed by *Staphylococcus aureus*(6%) in both the Chemistry and Biology department and only one isolates of*Enterobacteria*in the halls of Biology and also one isolates *of Streptococci*ssp in the Department of Chemistry.

Key words: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococci*, Contaminated, Iraq.

INTRODUCTION

*Streptococci ssp, Escherichia.coli Pseudomonas aeruginosa*considered as opportunistic pathogens, some of which are aerobic or aerobic, cause disease in healthy but highly virulent people in patients with mild bacteremia or eye injury Ear infection, skin infection, wound injury, central nervous system infection, heart attack, and joint infections [1][2]. Therefore, contamination in some hall areas due to these pathogens has an impact on transmission of the infection From some places contaminated with bacterial pathogens where infections are considered These pollutants are

common in some places, where many diseases and local invasions caused by the disease, which leads to the reduction of the defenses of the body where the disease has a reciprocal relationship between the situation and microbial contaminants and it is a common causes of the infections acquired by some students due to lack of hygiene and the presence of microbes in some places, especially when there is moisture and spread from person to person or by the staff at the college.

Pseudomonas aeruginosa, Streptococci ssp possesses a number of different enzymes such as Lipase enzymes, Coagulase, Elastass, Alkaline protease, Al kaline phosphatase – Danes, gelatinase – hemolysin, Leukocidin. Lecithenase as well as iron carriers and intestinal flares [3][4].

There are several changes behind the emergence of kidney infection due to lack of cleanness or transmission of bacteria by people infected by air or high density of the proportion of students in the same stage[5][6].
Some microorganisms are able to move from person to person by passing through the air by inhaling those particles of these organisms (bacteria, fungus, viruses)[7][8].

In the absence of previous studies on this subject, the aim of this study is to take indoor swabs from the ground, walls, ventilation and culture the examination samples to investigate the existence of microbiology and diagnosis [9][10][11].

MATERIALS AND METHODS

Samples Collection

The Samples were collected during the November 2017 till February 2018 and the samples randomly took about 50 samples from the halls of the Faculty of Science, including Biology, Environment and Chemistry, in University of Al-Qadisiyah from different places (walls, floors, chairs, air fresheners, and blackboard eraser).

Samples Cultured

The samples were cultured on the Macconkey agar, Blood agar and Mannitol agar. We plotted the sample taken from the site by a sterile swab on the culture media near the burning of the benzne lamp. This swab was then spoiled and the sample was incubated in the incubator for 24 hours and 37 ° C Inverted and the growth was monitored.Non-growth dishes were incubated for another 24 hours before being treated as a negative result [12][13].

Diagnosis of Bacterial Isolates

The isolates were diagnosis through the following:

Morphological and Cultural Characteristic

The morphological characteristics of the growth colonies in their shapes and colors, the surface of the colonies, their strength and transparency, were observed as a pattern of glycolysis and fermentation of sugars in the middle of the triple sugar icon.

Microscopical Characteristics

Wipes from the pure colonies were made on glass slides and dyed Gram and examined under the microscope by force of the central optical microscope. The forms of the cells, their type of composition, and their response to the negative or positive gram stain.

Biochemical Tests

Gram Negative Bacteria

Pepton Water was fertilized with bacteria to be tested and incubated at 37 ° C for 48 hours. Add 0.5 kovaus reagent to the fertilized tube and shacked, the appearance of a red ring indicates the positive of the test [13] [14].

Methyl Red Test

The center of MR-VP was incubated with bacteria and incubated at 37 ° C. For 48 hours, 5 drops of red reagent were added to the medium and change to the red indicated complete decomposition of sugar and acid production. If the color changed to yellow, this indicates a negative test result.

Motility Test

The sterilized loop is taken by one drop of water and placed in the lid of the glass slide. The carrier loop is then sterilized by a benzene lamp and cooled. Very few bacteria are taken in the samples. The bacteria to be tested are mixed with distilled water and carefully placed on the sterile motion test strip. If the motion is observed, the test is positive. If the motion is not observed, it is a negative test.

Gram Positive Bacteria

Mannitol Salt Agar

Gram positive samples were recultured on the salted Mantle media (M.S.A) to distinguish between *staphylococcus aureus* and fermented mantles from those non-fermented; they grow without any change in the media.

Catalase Test

A 24-hour bacterial cultured was set up by a sterile agricultural carrier on a clean glass slide near a benzene lamp and a drop of hydrogen peroxide was placed. 30% the appearance of the gas bubbles indicates the positive result of the test.

Slide Coagulant Test

A drop of the package on a clean glass slide placed a drop of distilled water on the other end of the slice as a control and by a carrier that took a number of colonies and mixed with each drop after the reaction was positive if there was coagulation within twenty seconds. This test is used to detect the coagulation enzyme (Ceagulasex).

Hemolysis

Vaccination between the blood agarcultured with pure bacteria and incubation at 37 ° C for 24 hours transparent areas around the colonies emerging bacteria demonstrate the susceptibility of bacteria to the secretion of hemolysin [11].

RESULTS AND DISCUSSION

The results of this study in the table (1) show the distribution of isolates according to the source of isolation. Hall No. (1) of the Biology Department contained (5) isolates of *Escherichia coli*(10%), which is the highest recorded percentage. While *Staphylococcus aureus* had 2 in the percentage of (4%).

The table below shows the distribution of isolates according to the source of isolation. It was found that Hall No. (4) of the biology department contained two isolation and 4% the highest percentage recorded in *Escherichia coli*, and the *Staphylococcus* bacteria contains only one isolates with 2%, while the *Enterobacteriaiceae* also contain the same isolated with the same percentage of the previous one Table (3) shows the distribution of isolates according to the source of isolation. It shows that Hall No. (1) of the halls of the Department of Chemistry contains 3 isolates (6%) of *Staphylococcus aureus* bacteria. *Pseudomonas aeruginosa*contain one isolate with (2%) and *Streptococci* sspwith the one isolate by (2%).

Table (4) shows the distribution of isolates according to the source of the isolation, where Hall No. (2) of the Chemistry Department contained one isolate by 2% of the *Escherichia coli* bacteria and *Pseudomonas aeruginosa* contains one isolate 2% in equal proportions. Table (5) shows the distribution of isolates according to the source of isolation, where Hall No. (1) in the Environment science Department contained 3 isolates (6%) of *Pseudomonas aeruginosa*, the highest percentage of bacteria and *Escherichicoli*, one isolate with % 2. Table (6) shows the distribution of isolates according to the source of the isolation, where Hall No. (2) in the Environment department contained two isolates (4%) of *Pseudomonas aeruginosa*, the highest percentage of bacteria and *Staphylococcus aureus*, one isolates with (% 2).

60 samples of the halls of the Faculty of Sciences in all departments (environment - Biology and chemistry), where the growth of a number of bacterial species were observed, according to the tables shown earlier in the research,table No.1 and 2 shows the percentages of bacteria that isolated from the halls of the Faculty of Science in the department of Biology and showed *E-coli*with (14%), the highest rates in all sections and the Department of Chemistry in table (3) and (4) *Staphylococcus*with (6%), the highest proportion in the Department of Chemistry and Table (5) And (6) in the halls of environmental department *Pseudomonas aeruginosa*with (6%), which is the highest percentage in the Department of Environmental Sciences. Table (1) and (2) refer to the distribution of isolates according to the sources of isolation and show that the halls of the Faculty of Biology constituted the highest percentage.

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Table 1. Isolated Bacteria form Hall No. (1) of the Department of Biology

Total	Ventilation	Wall	stage	Ground	Chair	Isolates Location / Bacteria types
(%10)(5)	(%2)1	(%2)1	(%2)1	(%2)1	(%2)1	*Escherichia coli*
(%4) (2)	-----	-----	-----	(%2)1	(%2)1	*Staphylococcusaureus*

Table 2. Isolated Bacteria from Hall No. (4) of the Department of Biology

Total	Ventilation	Wall	Stage	Ground	Chair	Isolates Location / Bacteria types
(%4)(2)	------	-----	(%2)1	(%2)1	-----	*Escherichia coli*
(%2) (1)	-----	-----	----	-----	(%2)1	*Staphylococcus aureus*
(%2) (1)	-----	-----	-----	(%2)1	-----	*Enterobacteriaiceae*

Table 3. Isolated Bacteria from Hall No. 1 in the Chemistry Department.

Total	Ground	Stage	Chair	Eraser	walls	Isolates Location / Bacteria types
(%6) (3)	-----	(%2)1	(%2)1	(%2)1	-----	*Staphylococcus aureus*
(%2) (1)	-----	-----	(%2)1	-----	-----	*Pseudomonas aeruginosa*
(%2) (1)	----	-----	-----	(%2)1	-----	*Streptococci ssp*

Table 4. Isolated Bacteria from Hall No. 2 of the Chemistry Department

Total	**Chairs**	**Ground**	**Wall**	**Isolates Location / Bacteria types**
(%2)1	----	(%2)1	----	*Escherichia.coli*
(%2)1	(%2)1	-----	----	*Pseudomonas aeruginosa*

Table 5. Isolated Bacteria from Hall No. (1) of the Department of Environment

Total	**Stage**	**Walls**	**Ground**	**Chair**	**Isolates Location / Bacteria types**
(%6)3	(%2)1	----	------	(%4)2	*Pseudomonas aeruginosa*
(%2)1	-----	----	(%2)1	----	*Escherichia.coli*

Table 6. Isolated Bacteria from Hall No. (2) of the Department of Environment

Total	**Stage**	**Ground**	**Chair**	**Isolates Location / Bacteria types**
(%4)2	(%2)1	------	(%2)1	*Pseudomonas aeruginosa*
(%2)1	-----	----	(%2)1	*Staphylococcus uergines*

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RESEARCH ARTICLE

Spatial and Seasonal Variation of Atmospheric Particulate Matter Heavy Metals in Al-Diwaniyah City, Iraq.

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ABSTRACT

The current study was conducted to estimate heavy metals pollution in the atmospheric total suspended particles (TSP) in Al- Diwaniyah city. To achieve this, seven sites were identified in different directions within the city center. The results showed that the seasonal mean of Pb, Cr, Cd, Cu and Zn concentrations ranged between 0.1-3.19, 0.07-0.33, 0.018-0.059, 0.73-5.25 and 0.74-2.34 $\mu g.m^{-3}$ respectively. The findings showed that the annual mean of the lead and cadmium at all sites exceeded the WHO guideline criteria of air pollutants. The results also showed local variation depending on the differences of human activities and seasonal variation according to the metrological variables.

Keyword: Total suspended particles, heavy metals, air pollution, toxic.

INTRODUCTION

Air is an important environment that plays a major role in the transmission of many pollutants whether in gas or particles state, which contributes to their spread and distribution among different environments (Saghatelyan *et al*., 2013). The air pollutants have a very wide effect. In humans, absorption and sedimentation within the lung have direct effects on the health and life of the human, while indirect effect on public health is through the deposition of these pollutants into the environment and taking by animals and plants through the food chain or drinking water, resulting in additional sources of human exposure to these pollutants. (WHO, 2000). The air pollutants have a wide impact on the plants in the forests or agricultural crops, causing great losses and also affect the wild and domestic animals directly or indirectly through the food chain causing the death in large numbers (Vallero, 2008).

Heavy metals are one of the most important environmental pollutants and their emission into the atmosphere represent the greatest threat to the environment and finely to human health, due to their large quantities and widespread, resulting in many ways of exposure to these toxic pollutants (Dinis and Fiuza, 2011). The heavy metals among the various chemical compounds, whether essential or non-essential to organisms, are particularly important in environmental toxicology because they are highly stable, bioaccumulative, carcinogenic, mutagenic, teratogenic and all of which have the potential to be toxic to organisms (Storeli *et al*., 2005; Agrawal, 2012).

Heavy metals are released into the atmosphere from natural and anthropogenic sources; natural sources include volcanic eruptions, rock weathering, soil processes, forest fires and ocean evaporation (Cyranlak and Bolzan, 2014). The anthropogenic sources include combustion of fossil fuels and coal for power generation, industry, transportation and various household purposes; various mining and smelting processes; industrial activities such as cement, glass and tile industry; burning of municipal solid waste and organic matter burning processes (Wood and residues of agricultural processes) and cars tires and brakes wear (Tian *et al*., 2015). Re-suspension of Earth's surface dust contributes significantly to increased concentration of heavy metals in the atmosphere (Yang *et al*., 2015).

Heavy metals are the most important components of airborne particles and thus play a major role in exacerbating the problem of air pollution, especially in cities, causing toxic and carcinogenic effects of humans (Hassan *et al*., 2013). It is well known that suspended particles contain high concentration of many toxic metals such as cadmium, chromium, copper, iron, manganese, nickel, lead, zinc (Radulescu *et al*., 2015), and heavy metals attached to the particles suspended in the air can be enter to the human body through direct inhalation or by ingestion, especially in children or through absorption through the skin, which leads to health damage to humans (Wan *et al*., 2016). After entering the body, heavy metals may causes various effects such as neurological, renal, hepatic, and immunological toxicity; and congenital malformations; and may directly affect human behavior, especially children; and may cause brain and nervous system dysfunction and leads to attention deficit syndrome (Saghatelyan *et al*., 2013).

MATERIALS AND METHODS

Study area

The current study was achieved in Al- Diwaniyah city south of Iraq. Seven sites were identified in the different directions of the city center for samples collection, as shown in Table (1) and Figure (1).

Sampling

Total suspended particles TSP were collected from September 2016 to August 2017 by air sampler model HI-Q (D-AFC-50), in the elevation of 1.5 meters above ground, using Knowing weight cellulose filters 0.45μm, then placed in clean polyethylene containers and stored until heavy metals are extracted (Gioda *et al*., 2007).

Analysis

The heavy metals are extracted using the hot acid extraction method. By add10 ml of 1:3 nitric and hydrochloric acid mixture to the filters containing the suspended particles in a 100 ml volume beaker with ensuring that the entire filter is covered with acid, heating to 80 °C for 30 minutes after covering the beaker, then diluted to 25 ml with deionized distill water (USEPA, 1999d).

The samples were measured using the Flame Atomic Absorption Spectrophotometer (Shimadzo AA-6300), after a series of standard solutions for Pb, Cr, Cd, Cu and Zn were prepared. The concentration of elements in the air samples was calculated in micrograms per cubic meter (USEPA, 1999b).

RESULTS AND DISCUSSION

The table (2) showed that the highest values of Pb and Zn concentrations appeared in the sixth site during the summer, while the lowest values were recorded in the third site in the fall, this is due to the power plant emission (diesel plant of East Diwaniyah) in this site, it works by heavy oil which is a source of the atmospheric heavy metals (Wang *et al*., 2010). Also the site is located near the highway linking Al-Diwaniyah city from the east, which adds another source of heavy metals pollution, that the transportation are a major source of heavy metals to be released into the atmosphere from exhaust or from corrosion in different parts such as tires, brakes and asphalt surfaces (Vianna *et al*., 2010).

Increasing concentrations during summer and fall are due to the increase in fuel combustion by the power generators of houses and residential districts because electricity cut off during the summer (Al-Duhaidahawi, 2015), the results showed a positive correlation r = 0.624 between Pb and temperature. While the observed increase in wind speed during the summer table (3) may lead to an increase in the re-suspension of lead-adsorbed surface dust especially in both sides of the roads to the atmosphere again (Krzeminska-Flowers *et al*., 2006), and the results showed a positive correlation r = 0.273 and r = 0.063 between the wind speed and Pb and Zn respectively. The observed decrease in concentrations during the fall may be attributed to the low energy consumption because of low temperature as well as the increased sedimentation of the suspended particles due to the increase in relative humidity. The results showed an inverse correlation r = -0.176 between TSP and relative humidity.

The results in table (2) showed that the chromium, cadmium and copper concentration recorded highest value in site four during autumn. The high concentrations of these elements are due to exhaust emissions, also the construction and demolition activates contribute the increase in the concentrations of heavy metals in the particles suspended in the air (Eneji *et al*., 2015), and the increased in fuel combustion use in domestic heating (Vaio *et al*., 2018), the results recorded an inverse correlation r = -0.512 , r = -0.491 and r = -0.558 between the temperature and chromium, cadmium and copper respectively. Also the decrease in wind speed recorded during the winter contributes to reducing the dispersion of contaminants (Li *et al*., 2015), especially in residential areas and areas containing high buildings as the lack of surface homogeneity reduces the horizontal mixing and this leads to a concentration of pollutants in surface air layer (Owoade *et al*., 2012). The results showed an inverse correlation r = -0.197, r = -0.103 and r = -0.195 between wind speed and concentrations of chromium, cadmium and copper respectively.

CONCLUSION

The study showed an increase in heavy metals concentration as a result to the human activities primarily transportation, energy production, construction and demolition processes and heating activities. The concentration of heavy metals various according to the site and season due to the type of activity and metrological factors. The annual mean of Pb and Cd were exceeded the WHO guide line (Pb=0.5, Cd=0.005 $\mu g.m^{-3}$) (WHO, 2000).

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Table 1. Shows the locations, specifications and activity of study sites.

Site	location	Activity	East	North
First	Al-Mawakeb street	Traffic and commercial	55° 44' 37.69"	59° 31' 11.89"
Second	Doctors street	Traffic and industrial	55° 44' 07.62"	58° 31' 54.41"
Third	Al-Wahdah district	Residential	56° 44' 19.23"	59° 31' 24.79"
Fourth	Al-Jamieh district	Residential	52° 44' 36.91"	59° 31'43.24"
Fifth	North Al-Diwaniyah power plant	Energy production and Residential	54 ° 44'03.75 "	01 ° 45'47.45"
Sixth	East Al-Diwaniyah power plant	Energy production and traffic	58° 44' 24.15"	59° 31' 39.91"
Seventh	Reference site	Agricultural	51° 44' 16.11"	58° 31' 38.16"

Table 2. Heavy metals concentration (µg.m^{-3}) (mean±SD).

Site No.	season	Pb	Cr	Cd	Cu	Zn
1	Fall	0.47±0.57	0.13±0.094	0.031±0.009	1.65±1.11	1.09±0.76
	Autumn	0.28±0.20	0.15±0.049	0.037±0.001	3.07±0.37	1.83±0.90
	Spring	0.87±0.14	0.12±0.007	0.034±0.004	2.12±2.37	1.31±0.23
	Summer	2.44±0.68	0.10±0.015	0.038±0.006	0.89±0.64	1.98±0.69
	Annual mean	1.014	0.128	0.035	1.930	1.552
2	Fall	0.42±0.37	0.07±0.048	0.027±0.020	1.31±1.18	1.09±0.76
	Autumn	0.59±0.22	0.32±0.129	0.047±0.006	4.23±1.51	1.83±0.90
	Spring	1.14±0.25	0.15±0.030	0.037±0.007	1.06±0.35	1.31±0.23
	Summer	2.69±0.90	0.10±0.011	0.035±0.009	1.27±0.94	1.98±0.69
	Annual mean	1.209	0.158	0.037	1.969	1.552
3	Fall	0.10±0.05	0.07±0.045	0.018±0.003	1.29±1.30	0.74±0.47
	Autumn	0.42±0.22	0.23±0.114	0.046±0.023	4.38±2.41	1.91±0.74
	Spring	1.08±0.41	0.14±0.020	0.036±0.003	2.98±1.01	1.78±0.36
	Summer	2.67±0.45	0.10±0.011	0.036±0.003	2.17±0.39	1.98±0.42
	Annual mean	1.068	0.135	0.034	2.705	1.604
4	Fall	0.42±0.30	0.08±0.071	0.027±0.012	1.24±0.90	0.94±1.10
	Autumn	0.80±0.45	0.33±0.191	0.059±0.023	5.25±2.45	1.98±0.82
	Spring	0.95±0.48	0.13±0.008	0.035±0.006	4.92±0.61	1.11±0.12
	Summer	3.02±0.72	0.12±0.001	0.041±0.004	3.36±0.25	2.23±0.72
	Annual mean	1.294	0.164	0.040	3.692	1.566
5	Fall	0.28±0.27	0.09±0.068	0.031±0.017	1.69±1.14	1.87±0.90
	Autumn	0.63±0.46	0.15±0.072	0.036±0.004	3.42±0.06	1.30±0.09

	Spring	1.09±0.56	0.13±0.025	0.031±0.003	1.29±1.36	1.63±0.26
	Summer	2.86±0.56	0.13±0.008	0.033±0.008	1.59±0.79	2.21±0.60
	Annual mean	1.216	0.125	0.033	1.996	1.751
6	Fall	0.42±0.51	0.08±0.069	0.030±0.015	1.97±1.52	1.09±0.75
	Autumn	0.73±0.28	0.15±0.022	0.038±0.009	3.61±1.29	1.71±0.23
	Spring	1.29±0.48	0.13±0.003	0.034±0.006	1.46±0.96	2.27±0.88
	Summer	3.19±0.75	0.13±0.005	0.038±0.009	2.74±0.54	2.34±0.39
	Annual mean	1.408	0.122	0.035	2.447	1.852
7	Fall	0.28±0.24	0.09±0.059	0.038±0.026	2.31±1.92	0.76±0.63
	Autumn	0.47±0.17	0.18±0.030	0.046±0.003	4.72±1.10	1.83±0.57
	Spring	1.16±0.38	0.14±0.033	0.031±0.003	0.73±0.86	1.23±0.18
	Summer	2.33±0.55	0.10±0.014	0.027±0.004	1.22±0.40	1.65±0.38
	Annual mean	1.061	0.125	0.036	2.245	1.367

Table 3. Metrological parameters during the study time.

Season	Tem. °c	R.H. %	L.I. lux	W.S. m/s
Fall	27.3	22.6	61464.0	1.4
Autumn	15.5	45.9	45566.7	1.1
Spring	32.7	23.2	58498.1	1.7
Summer	42.2	12.1	91849.6	2.2
Annual mean	29.4	25.9	64344.6	1.6

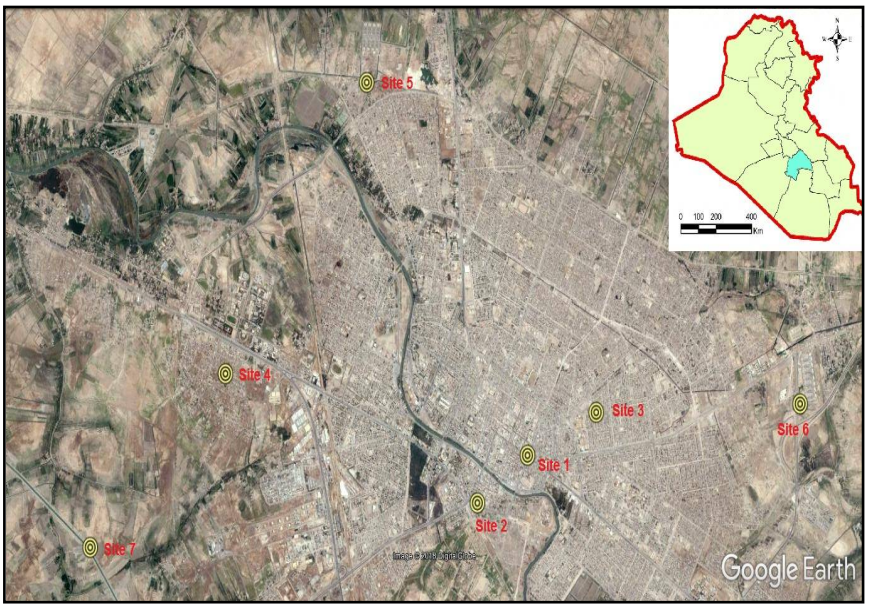

Figure 1. Study Area

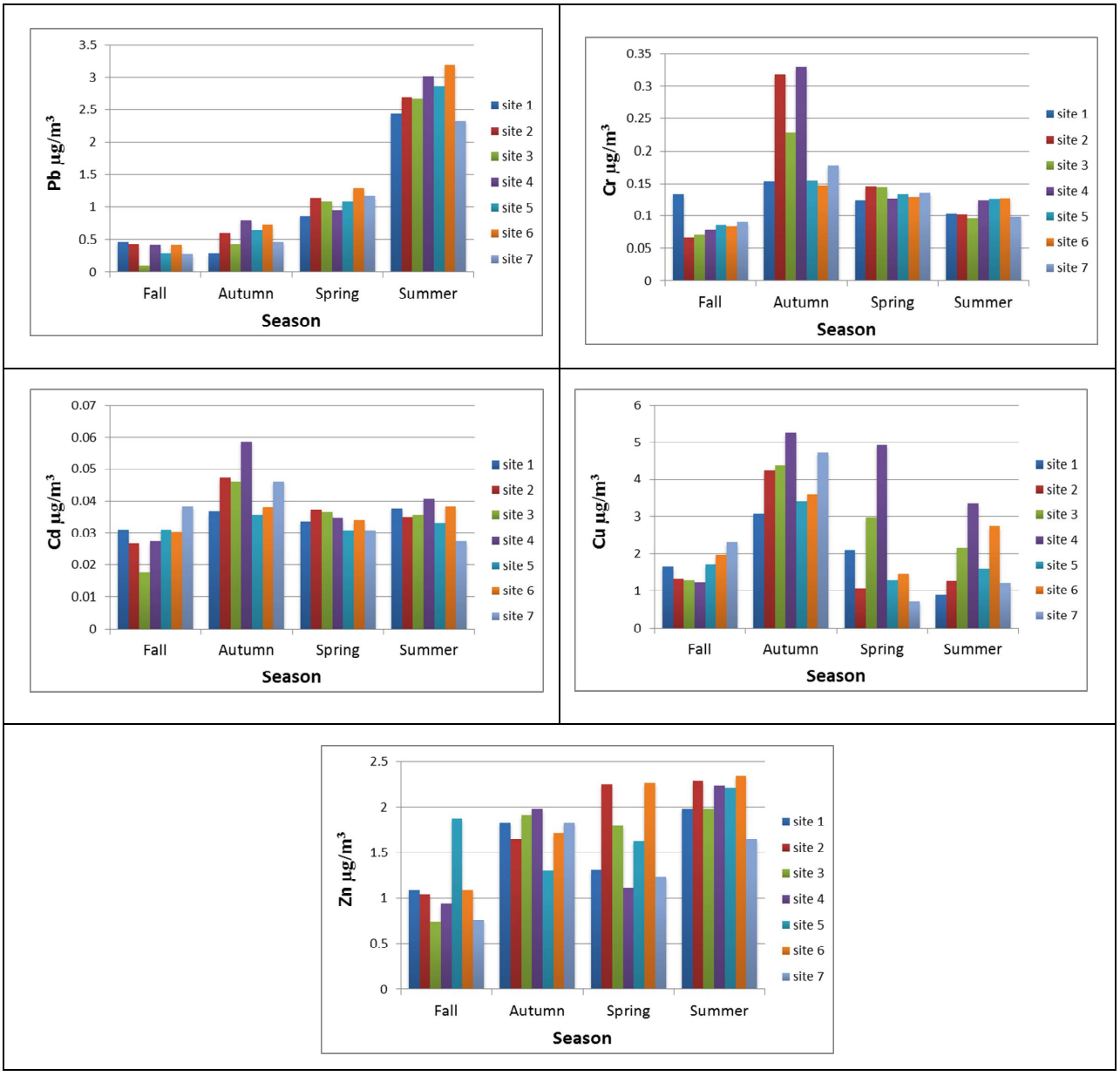

Figure 2. Heavy Metals Concentrations

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RESEARCH ARTICLE

Detection of Isolated Bacteria from Students' Water Cycle in the College of Science, Babylon University, Iraq

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ABSTRACT

Samples of male and female students' toilets were collected from department of Physics and Biology in May to August 2018. The results shows the *Escherichia coli* was isolated by the highest percentage (11%) in the male baths of the Department of Biology, followed by *Streptococci ssp* and *Staphylococcus ssp* with the highest percentage in female baths for the Department of Physics, while *Pseudomonas aeruginosa* was highest in male and female baths (7%).

Keywords: *Streptococci ssp*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus ssp*.

INTRODUCTION

There are many microbes scattered in the environment surrounding the human and there are many types of pathogenic and the universities are one of the sources of external microbes from the bathrooms, halls, laboratories and others [1].All types of bacteria do not cause injuries in normal cases, but some occur once they are located in a place called the pathogenic bacteria and sometimes the infection occurs if the body's defenses, called opportunistic bacteria. Where bacterial contamination in universities is one of the main problems experienced by workers in this field [2].Many bacteria are considered (*Pseudomonas aeruginosa - Streptococci ssp – Escherichia coli* and *Staphylococcus spp*) One of these pollutants and the most dangerous because it has the natural resistance to many disinfectants as well as the characteristics of spread in bathrooms and may be the cause of this is the most intrusive places (workers, students, professors), which is the transition from outside to inside and from the inside out. It is therefore necessary to sterilize and sterilize periodically because of the presence of these germs [3].

Infection acquired by students in universities is one of the most serious infections, especially if the disease is chronic or a disease that causes immunity. This type of infection also affects university workers, service workers and

laboratory technicians directly or indirectly. Many people are unaware of the nature of this infection, how it is transmitted and how to prevent it [4].The infection acquired in universities is one of the most serious problems that result from the contamination of university baths with different types of microbes that may affect students, teachers and workers, especially the elderly and those with limited immunity [5]. The negligence in following the medical basics in the application of treatment techniques are important factors that may help to identify students, staff or professors to infection from within the university, which opens the way to other types of microbes are often more dangerous [6]. First bacteria that founded during this survey is E. coli or Escherichia coli is a type of Gram-negative bacteria found in the human intestines naturally without causing any symptoms or health problems, but if these species turned into strains carrying genes that can penetrate or destroy cells or secretion of toxins within the body Health problems begin to appear. The infection occurs as a result of eating foods or drinks contaminated especially vegetables and non-cooked meat. Such conditions of the pathology and the nature of symptoms experienced by the patient are diagnosed, and the diagnosis is confirmed by a procedure for the implantation of faeces to confirm the presence of these types of bacteria [7]. Prevention of *E. Coli* can be done by many methods like treatment of pasteurized milk, juices and sterilized beverages is one of the most important points that must be met and emphasized, since it is of utmost importance in preventing the transmission of these pathogenic bacteria and reduce their spread. It is worth mentioning is the modification of lifestyle and domestic relations within the family and in society at large , The ideal health education in the home, school and workplace is important to prevent the risk of infection, so people are accustomed to constantly washing hands before and after eating and attention to clean kitchens in homes and restaurants and fast food stores because they have a wide spread in the Popular markets and regions, as well as washing and cooking utensils always wash raw foods carefully [8].

The second bacteria that investigated in this study are *Pseudomonas aeruginosa* is defined as a type of aerobic and gram-negative bacteria that can grow in difficult environments. These bacteria are abundant in soils and swampy water and can be transmitted to plants and humans. In general, pseudo-pals are not strong enough for healthy people, and they only have opportunities to attack vulnerable people, such as hospital patients, pregnant women, preterm infants, infants born underweight, older infants and children with poor bowel defenses [9]. While the last bacteria that founded in this study is *Streptococcus spp.* are the main bacterial cause of pharyngitis and cellular tissue. They are also the catalyst for immunosuppressive diseases: rheumatic fever, acute hepatitis and kidney disease. *Streptococcus spp.* are positive spherical forms of chromium forming in chains or pairs. All swimming pools are negative for catalysis. Non-aerated, pneumatic (optional pneumatic) and no air-conditioning, not assembled for spurts [10]. These bacteria return to the group of lactic acid, which has been described as environmentally friendly, called biobiotic probiotics. These bacteria help in the preparation and recycling of nutrients and the destruction of organic matter, which in turn makes the environment clean and free of contaminants. *Streptococcus ssp.* contains bacteria It is widely used in this field and has a long history in its safe use in the yoghurt industry. Bio-enhancers are known as microbial cells and their derivatives, which have a beneficial effect on host health. They produce lactic acid bacteria, including *Streptococcus ssp.* Many substances inhibitory microbiological growth and the effect of the activation of the growth of pathogenic microbes and those that cause food damage and those substances lactic acid and acetic acid and formic acid and ethanol and hydrogen peroxide and dill acid and bacteriocene and fatty acid. These bacteria are effective in preventing and treating many diseases [11,12].

MATERIALS AND METHODS

Collection of Samples

Samples randomly collected about 20 samples of the faculty of science - Babylon University in Hilla governorate from different places (ie, from the Faculty of Science, Physics Department and Biology Department) and from both sexes, male and female.

Cultured of Samples

The samples were cultured on the center of MacCon keg and Blood agar, Mannitol agar. We plotted the sample taken from the site by a sterile swab on the center of the plant near the burning of the benzene lamp. This swab was then damaged and the sample was incubated in the incubator for 24 hours and 37 ° C o Inverted and monitoring growth [13][14].

Diagnosis of Bacterial Isolates

Isolations are diagnosed by Morphological and Cultural Characteristic: The morphological characteristics of the developing colonies in their shapes and colors, the surface of the colonies, their strength and transparency, were observed as a pattern of glycolysis and fermentation of sugars in the middle of the triple sugar icon.

Microscopic Properties

Wipes from the pure colonies were made on glass slides and dyed Gram and examined under the microscope with the greatest force in the central optical microscope. The forms of the cells were observed, their type of composition was observed and their response to the negative or positive chromium was observed.

RESULTS

The result of this study shows that table.1 shows the distribution of isolates according to the source of isolation [15]. The male salinity of the Biology department contained 7 isolates (11%), the highest percentage recorded for *Escherichia coli*. *Streptococci ssp.* contains 3 (6%) while the *Pseudomonas aeruginosa* contains 2 (4%) and *Staphylococcus ssp.* contains 2 (4%). Table. 2 show the distribution of isolates according to the source of the isolation. The female baths for the Biology department contained 5 isolates (10%, the highest percentage recorded by *Escherichia coli*). *Streptococcus ssp.* contains 2% (4%). *Pseudomonas aeruginosa* contains (1%) (2%). Table.3 shows the distribution of isolates according to the source of isolation. The male baths of the Physics Department included (4 isolates) (8%), the highest percentage recorded by the bacteria *Escherichia coli*. And *Streptococcus ssp.* (3%) (6%). and *Pseudomonas aeruginosa* (1) by (2%). Table. 4 show the distribution of isolates according to the source of the isolation. The female baths of the Department of Physics included 4 isolates (8%) for *Escherichia coli. Streptococci ssp.* had 3% (6%). *Pseudomonas aeruginosa* contained (3%) (6%).

DISCUSSION

Escherichia coli, Bacteria are present naturally in our bodies, but if increased in quantity they are harmful and cause some gastrointestinal disorders such as diarrhea. These bacteria are transmitted to humans by eating contaminated foods such as meat products, meat and dairy products and can grow at temperatures between (50 - 17 m) and ideal (37 m). These bacteria cause diarrhea, abdominal pain, fever and can lead to intestinal diarrhea, dryness or renal failure. *Streptococci ssp and Staphylococcus spp,* It is a highly contagious bacteria transmitted from one person to another through the air carrying bacteria disease, coughing and sneezing infected person or surfaces contaminated with bacteria and then put the hands on the nose and mouth Kitchen tools and bathrooms are common means of transmission Similarly infection can be infected with Streptococcus bacteria at any time of the year. These bacteria are not considered dangerous but the risk comes from complications that follow the injury if not treated. These complications are inflammation of the tonsils, sinuses, kidney inflammation and others. *Pseudomonas aeruginosa,* It is an organic bacterium that is commonly found in water and has the ability to produce dyes colored in yellowish or reddish color that have no special growth requirements or ideal growth temperature (37 m) and are resistant to antibiotics [15].

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Table 1. Species Bacteria isolated from male baths for the Department of Biology

Total	Ground	Tap	Wall	Washbasin	Isolates location / Bacteria Sp
(%10) (5)	(%4) 2	-----		(%2) 1	*Escherichia.coli*
(%4) (2)	-----	-----	(%4) 2	-----	*Streptococci ssp*
(%2) (1)	-----	-----	-----	(%2) 1	*Pseudomonas aeruginosa*
(2%) (1)	-----	2 (4%)	-----	2 (4%).	*Staphylococcus ssp*

Table 2. Species Bacteria isolated from female baths for the Department of Biology

Total	Ground	Tap	Wall	Washbasin	Isolates location / Bacteria Sp
(%10)(5)	(%2)1	-----	-----	(%4)2	*Escherichia.coli*
(%4) (2)	-----	(%4)2	-----	-----	*Streptococci ssp*
(%2) (1)	-----	(%2)1	-----	-----	*Pseudomonas aeruginosa*
(2%) (1)	-----	1 (2%)	-----	-----	*Staphylococcus ssp*

Table 3. Species Bacteria isolated from male baths for the Department of Biology

Total	Ground	Tap	Wall	Washbasin	Isolates location / Bacteria Sp
(%8) (4)	(%4)2	-----	-----	-----	*Escherichia.coli*
(%2) (1)	-----	(%2)1	-----	-----	*Streptococci ssp*
(%6) (3)	(%4)2	-----	(%2)1	-----	*Pseudomonas aeruginosa*
(2%) (1)	-----	-----	1 (2%)	-----	*Staphylococcus ssp*

Table 4. Species Bacteria isolated from female baths for the Department of Physics

Total	Ground	Tap	Wall	Washbasin	Isolates location / Bacteria Sp
(%8) (4)	(%4)2	-----	-----	(%2)1	*Escherichia.coli*
(%6) (3)	-----	-----	(%4)2	(%2)1	*Streptococci ssp*
(%6) (3)	-----	(%2)1	-----	(%4)2	*Pseudomonas aeruginosa*
(2%) (1)	-----	-----	1 (2%)	-----	*Staphylococcus ssp*