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RESEARCH ARTICLE

Diversity of Aquatic Algae in the Nakhon Nayok Canal of Thailand

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ABSTRACT

The present study investigates the diversity of aquatic algae in the Klong 14 canal of Nakhon Nayok, located in the North Eastern part of Bangkok, Thailand, with the geographical coordinates of 14.2069° N, 101.2131° E. The water samples were collected randomly from the 10 selected sites in the Klong 14 canal of Nakhon Nayok during March 2018. Physicochemical parameters of the water samples were also analysed to evaluate the algal diversity. A total of 128 species were found, of which 63 species belong to Cyanophyta, 48 species belong to Chlorophyta and 17 species belong to Bacillariophyta. 11 microalgal genera such as *Actinastrum*, *Anaebaena*, *Calothrix*, *Craticula*, *Gomphonema*, *Lyngbya*, *Merismopedia*, *Microcystis*, *Oscillatoria*, and *Spirogyra* were found to be predominant in the canal due to the suitable trophic condition. It was found that the dominance of algae and the formation of bloom was mainly due to the eutorphic nature of the canal.

Keywords: Aquatic algae, Micro algae, Phyco diversity, Physico chemical parameter, trophic condition

INTRODUCTION

Microalgae represent the largest, yet one of the most poorly understood groups of microorganisms on earth1. Microalgae are diverse groups of prokaryotic and eukaryotic photosynthetic microorganisms that can grow rapidly due to their simple structures. They are microscopic unicellular organisms capable of converting solar energy to chemical energy via photosynthesis2, 3, 4.. Microalgae are of great economic importance as they are commercially used to produce plethora of biotechnological and industrial products5[, 6, 7, 8, 9, 10]. In fresh water aquatic ecosystems, microalgae act as the major primary producers and stands as a dominant group of organisms. Microalgae require sufficient sunlight, nutrients such as nitrogen, phosphorous, silicon, carbon and other micro nutrients. One or more species of microalgae are known to form algal bloom in eutrophic aquatic habitat under favorable conditions[11, 12, 13, 14, 15, 16].

The Nakhon Nayok is one of the provinces located in the central part of Thailand surrounded by Saraburi, Nakhon Tatchasima, Prachinburi, Chachoengsao, and Pathum Thani. It is popular for its refreshing waterfalls, "The Nakhon Nayok River", and the famous "Khun Dan" "Prakan Chon Dam", the biggest dam in the country. All these bodies of water flow down to the central part of the province which is a flat river plain with abundant rice fields irrigated by different Klongs or canals sustaining the people to have three crops a year. These Klongs do not only irrigate the rice fields but are also sources of water for domestic as well as agricultural purposes. Moreover, these Klongs have abundant water organisms like fish, micro and macro algae. This abundancy had promped the researchers to identify the diversity of micro algae in the water system of one of the Klongs i.e. Klong 14. The water of this Klong is sustained by flowing from Rangsit, where the first Klong commences. Other water tributaries make the water bigger and stronger, as it flows downward from one Klong to the other Klong and eventually meets the water flowing to Ban Na district which is located in the western part of Nakhon Nayok Province. The Klong 14 is approximately 2500 meters in length and 12 - 15 meters in width with abundant life forms such as, toads, frogs, variety of fresh water fish, shells, snails, weeds, varied green plants, micro and macro algae.

A few reports have been made on aquatic microalgae of Thailand including the benthic diatoms of different lakes of Thailand[17], benthic diatoms of Mekong River and its tributaries in northern and north-eastern Thailand[18] and Phytoplankaton diversity in the Rama IX lake of Pathum Thani Province[19] but no reports were available with respect to the microalgal diversity of the Nakhon Nayok canal. The present study investigated the analysis of water quality and also the diversity of microalgae in the Klong 14 canal of Nakhon Nayok, Thailand by random sampling of the water at ten different sampling sites during March 2018. Microalgae require sufficient sunlight, nutrients such as nitrogen, phosphorous, silicon, carbon and other micro nutrients. One or more species of microalgae are known to form algal bloom in the eutrophic aquatic habitat under favorable conditions.

MATERIALS AND METHODS

Sampling area

The water samples were collected during the hot season of Thailand i.e. (March 2018) from ten different sites at Klong 14 of Nakhon Nayok canal. The water samples were collected by following the standard procedure of American Public Health Association[20]. Each water samples were collected separately in sterile 100 mL plastic water bottles with proper labeling mentioning the date, time and spot of collection. The collected samples were kept in ice box and immediately brought to the laboratory for further analysis[21]. The selection of the sampling site was such considering the intense microalgal population of the spot.

Physico-chemical parameters

The various physical parameters namely temperature, pH, photon light intensity ($\mu mol\ m^{-2}\ sec^{-1}$) using quantum meter (Spectrum Technologies, USA) and light illumination (Lux) using illuminometer (Kyoritsu, Japan) were collected at the sampling sites. The different chemical parameters viz., Dissolved oxygen, Biological demand oxygen (BOD), Chemical demand oxygen (COD), Calcium, Chloride, Carbonate, Bicarbonate (Alkalinity), Magnesium, Nitrate, Nitrite, and Sulfate were determined by following the standard protocol[22]. All the parameters were determined thrice to get the reproducibility of the result and to rule out the errors and the bias. The average values of the each parameter were recorded.

Microscopic identification of the Microalgae

The collected water samples were centrifuged at 4000 rpm for 10 min and then both the residue and supernatant were used for the identification of microalgae by microscopic observation. Identifications of the microalgae were done through microscopic slide preparation by morphological and structural observations based on the standard monographs and published reports[23, 24, 25, 26 27, 28, 29, 30].

RESULTS

Physico – chemical parameters

The results of the physical parameters analysis of the water samples collected at ten different sampling sites (S1-S10) were shown in the Table1. The time of sample collection were 6.30, 7.15, 7.50, 8.20, 8.55, 9.30, 10.05, 10.40, 11.25 and 12.00 for the sampling sites S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 respectively. It was observed that, except the parameter pH, the values obtained for all other parameters (Illumination light intensity Photon light intensity and temperatures) studied were in the increasing order as the time of sample collection also increased. The illumination light intensity (Lux) of each of the ten different sampling sites were S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 were recorded to be 8000, 8300, 8400, 8650, 9250, 9410, 9610, 9700, 9890 and >10000 respectively. The photon light intensity (µmol m^{-2} Sec^{-1}) values at the different sampling sites S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 were found to be 860, 930, 1450, 1580, 1630, 1690, 1740, 1860, 1990 and >1999 respectively. The temperature values determined for the sampling site in the sequence from S1 – S10 were 26.9, 27.3, 27.7, 28.6, 29.8, 30.1, 30.5, 31.2, 32.4, and 32.8 respectively. The pH values obtained were 7.3, 7.5, 7.1, 7.2, 6.9, 6.9, 7.4, 7.0, 7.1 and 7.0 respectively. The results of the analysis of the chemical parameters of the water samples collected at each sampling sites are shown in Table 2.

The dissolved oxygen (DO), Chemical oxygen demand (COD) and Biological oxygen demand (BOD) varied greatly between 3.9 ± 2 to 7.6 ±2, 11.12 to 20.98 and 0.10 to 0.19 respectively. The Calcium content was in the range of 41.75 ± 0.2 to 93.74±0.2. The Carbonate and Bicarbonate were in the ranges of 22.22 ± 0.8 to 32.16 ± 1.4 and 3.98 ± 0.8 to 10.26 ±0.5 respectively. The Chloride and Magnesium were found to be 263.30± 28 to 362.20 ± 50 and 13.08 ±0.4 to 28.61 ±0.4 respectively. Similarly, the values obtained for the Nitrate and Nitrite were in the ranges of 22.6 ± 0.3 to 33.1 ± 0.5 and 15.90 ±0.3 to 25.10 ± 0.5 respectively. The sulphate content of the water samples determined was in the range of 15.64 ±0.2 to 38.10±1.0. Table 3 represents the distribution and diversity of the microalgae in the different sampling sites of Nakhon Nayok canal, Khlong 14. The micro algal population found in the water samples belongs to 3 different Phylum namely, Bacillariophyta, Chlorophyta and Cyanophyta. A total of 17 Bacillariophyta, 48 Chlorophyta and 63 Cyanophyta were identified. The Cyanophytes were found to be dominant in the Nakhon Nayok Klong 14 canal; Chlorophytes were the second largest group of aquatic microalgae found in the Canal and Bacillariophytes were known to be least in the Canal. Table 4 represents the diversity and distribution of Bacillariophyta in the Klong 14 canal of Nakhon Nayok. A total of 17 different Bacillariophytes were identified from the water samples collected at the 10 different sampling sites.

Among all the 17 different Bacillariophytes identified, Craticula was found to be the dominant one with rich species diversity. All the other genera of Bacillariophytes such as *Amphipleura*, *Cymbella*, *Diatoma*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Gyrosigma*, *Navicula*, *Pinnularia*, *Pleurosigma*, *Stephanodiscus*, *Synedra* and *Tabellaria* were found to be the natural inhabitant with sparse distribution.Table 5 represents the diversity and distribution of aquatic Chlorophytes in Klong 14, Nakhon Nayok canal. A total of 48 Chlorophytes were microscopically identified from the water samples collected at the 10 different sampling sites. The identified 48 Chlorophycean members belong to 25 different genera and *Spirogyra* were found to be the predominant one with high species diversity. The genus *Actinastrum* was also found to be dominant with great species diversity. The six genera of the Chlorophytes *Carteria*, *Cosmarium*, *Oedagonium*, *Staurastrum*, *Tetraedron* and *Tetrastrum* were found to be moderate with respect to their distribution and

species diversity. The other genera of the Chlorophytes such as *Chlamydomonas*, *Oocystis* and *Pediastrum* were also known to be natural inhabitants of the canal. The genera of Chlorophytes such as *Chlorella*, *Chlorogonium*, *Chlorosarinopsis*, *Cyanophora*, *Crucigenia*, *Cylinocystis*, *Haematococcus*, *Klebsormdium*, *Micractinium*, *Monoraphidium*, *Mesotaenium*, *Pandorina*, *Scenedesmus* and *Volvox* were found to be very sparsely distributed with least species diversity. Table 6 represents the diversity and distribution of Cyanophytes in the Nakhon Nayok canal, Khlong 14. In total, 63 different Cyanophycean members belong to 24 different genera.. Among all the 24 genera of Cyanophytes identified, *Microcystis* was found to be very dominant, and next to *Microcystis* were the *Anabeana*, *Gomphonema*, *Merismopedia* and *Oscillatoria* with rich species diversity. The genus *Calothrix* and *Lyngbya* were found to be good in distribution as well as in species diversity. The four different genera of *Cyanophyceae*, viz., *Blennothrix*, *Cylindrospermum*, *Dermocarpa*, and *Nodularia* were also found to be moderate in distribution and also in terms of species diversity. The Cyanophycean genera such as *Chlorobotrys*, *Cylindrospermiopsis*, *Dermocarpella* and *Gloeotrichia* were also found less in number with less species diversity. The genera of the Cyanobacteria such as *Acaryochloris*, *Gloeobacter*, *Gloeocapsa, Phormidium*, *Prochloron*, *Spirulina*, *Synechococcus*, *Tolypothrix* and *Trichodesmium* were found to be the least with poor species diversity in the water samples tested.

In total, the 128 species belong to 63 genera of Cyanophyta, 48 genera of Chlorophyta and 17 genera of Bacillariophyta. The results clearly reveal that the Cyanophytes were dominant in the canal, next to the Cyanophytes were the Chlorophytes which are close in number to the Cyanophytes. The Bacillariophytes standing least in terms of their population. Craticula was found to be dominant among the other Bacillariophytes with 5 different species. *Spiyrogyra* was found to be the dominant Chlorophytes with 7 different species. Among the Cyanophytes identified, Microcystis was found to be more in number with 6 different species.

DISCUSSION

The various chemical parameters of the water samples analyzed depicted the high level of the nutrients in this fresh water system, which caused the eutrophic condition of the canal due to the drainage of rain water and agricultural wastes etc., The pH value ranged from near neutral (6.9) neutral (7.0) to slightly above neutral (7.5) which favored the dominance of microalgae in such freshwater systems. The temperature around 32°C and high light intensity obtained reflected the high photosynthetic condition. The Dissolved Oxygen value was in the range of 3.9 – 7.6 mg/L and Chloride was in the range of 263.30 – 362 mg/L. These values were found to favor the population of microalgae[31]. The nitrate and nitrite contents were found almost similar as reported by Vijayan *et al.*[31] and were literally higher than the reports of Mohideen *et al*,[32].The obtained results revealed the diverse distribution pattern of the microalgae in the ten different sampling areas. The canal water was known to be rich with high number of Cyanophycean species followed by Cholorphycean and Bacillariophyceans. But in the context of generic diversity and species diversity of the microalgae, it was the Chlorophytes which was dominant than the Cyanophytes and the Bacillariophytes. It was known that the Chlorophyta and Cyanophyta acted as the primary producers in this fresh water aquatic ecosystem. "Eutrophication often succeeded in rich aquatic ecosystem with weeds and algal blooms"[33].The diversity pattern obtained was almost similar to the other reports [31, 34, 35, 36].

Overall, the rich biodiversity of the microalgae in the fresh water aquatic system of Klong 14 canal of the Nakhon Nayok province was explored. However, more extensive seasonal studies are required to reveal the algal diversity, distribution pattern, factors responsible for the growth of the algae and bloom formation.

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Table 1. Physical parameters of the water samples at different sampling sites of Nakhon Nayok canal, Klong 14

Sl. No	Parameter	Sampling Sites									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
	Time of sample collection (h)	6:30	7:15	7:50	8:20	8:55	9:30	10:05	10:40	11:25	12:00
01	Illumination Light Intensity (Lux)	8000	8300	8400	8650	9250	9410	9610	9700	9890	>10000
02	Photon Light Intensity (µmol m^{-2} Sec^{-1})	860	930	1450	1580	1630	1690	1740	1860	1990	>1999
03	pH	7.3	7.5	7.1	7.2	6.9	6.9	7.4	7.0	7.1	7.0
04	Temperature (°C)	26.9	27.3	27.7	28.6	29.8	30.1	30.5	31.2	32.4	32.8

Table 2. Chemical parameters of the water samples at different sampling sites of Nakhon Nayok canal, Klong 14

Sl. No	Parameter (mg/L)	Sampling Sites									
		1	2	3	4	5	6	7	8	9	10
01	DO	3.9± 0.2	4.1± 0.1	4.7± 0.2	5.3± 0.3	4.4± 0.1	4.0± 0.2	6.8± 0.3	7.6± 0.2	6.5± 0.2	7.2± 0.1
02	COD	15.62	17..37	11.12	20.98	19.97	18.34	12.74	13.25	14.61	16.49
03	BOD	0.13	0.10	0.16	0.18	0.15	0.19	0.17	0.14	0.12	0.19
04	Calcium	41.75± 1.2	56.18± 1.5	64.96± 0.8±	79.13± 1.4	59.43± 1.2	88.67± 0.8	61.52± 1.4±	48.49± 0.8	93.74± 1.2	90.82± 1.5
05	Carbonate	28.32± 0.8	30.54± 1.2	34.18± 1.2	25.83± 1.4	22.22± 0.8	24.69± 1.0	32.16± 1.4	31.71± 1.2	27.58± 1.0	26.43± 0.8
06	Bicarbonat e	5.13± 0.5	8.10± 0.8	4.27± 0.8	9.64± 0.4	7.82± 0.4	3.98± 0.8	10.26± 0.5	8.43± 0.5	5.33± 0.8	4.66± 0.4
07	Chloride	263.30± 28	297.70± 50±	311.10± 50	326.60± 28	345.50± 50	328.80± 50	334.40± 50	359.90± 28	362.20± 50	286.60± 50
08	Magnesiu m	13.08± 0.4	22.19± 0.2	18.24± 0.1	28.61± 0.4	15.38± 0.5	19.73± 0.3	26.41± 0.1	16.16± 0.4	23.57± 0.5	20.98± 0.2
09	Nitrate	22.6± 0.3	26.8± 0.2	24.5± 0.3	29.2± 0.1	33.1± 0.5	30.4± 0.1	28.7± 0.2	23.4± 0.3	27.3± 0.1	32.9± 0.4
10	Nitrite	17.30± 0.2	21.80± 0.1	24.70± 0.1	15.90± 0.3	19.60± 0.3	22.40± 0.5	16.10± 0.2	20.20± 0.4	18.30± 0.1	25.10± 0.5
11	Sulphate	15.64± 0.2	38.10± 1.0	18.68± 0.8	24.82± 0.4	29.64±1. 0	16.28± 0.2	20.38± 0.8	27.14± 0.4	34.66± 0.6	32.12± 0.2

Table 3. Microalgal diversity and distribution in terms of its phylum at different sampling sites of Nakhon Nayok canal, Klong 14

SL. No	Identified Phylum of Microalgae	Sampling Sites (SS)										Total microalgal number
		1	2	3	4	5	6	7	8	9	10	
01	Bacillariophyta	01	02	03	01	02	01	01	03	02	01	17
02	Chlorophyta	08	01	07	02	05	01	09	07	03	05	48
03	Cyanophyta	3	13	06	11	04	7	02	05	04	08	63
Grand Total number of microalgae obtained												128

Table 4. Diversity and distribution of Bacillariophyta in different sampling sites of Nakhon Nayok canal, Klong 14

Identified Algae of Phylum Bacillariophyta			Sampling Sites (SS)									
Sl. No	Genera	Species	1	2	3	4	5	6	7	8	9	10
01	*Amphipleura*	*Pellucid*	+			+			+		+	
02	*Craticula*	*Citrus*	+	+	+			+		+		
03	*Craticula*	*Dissociate*	+	+	+		+	+				
04	*Craticula*	*Procera*		+		+	+		+	+		
05	*Craticula*	*Subminuscula*			+	+		+	+	+	+	
06	*Craticula*	*Zizix*	+	+			+	+		+		
07	*Cymbella*	*Yabe*				+						+
08	*Diatoma*	*Vulgaris*	+	+		+		+	+		+	
09	*Eunotia*	*Rivularis*	+				+					+
10	*Fragilaria*	*Pelta*		+			+			+		+
11	*Gomphonema*	*Acuminatum*			+			+	+			
12	*Gyrosigma*	*Balticum*		+			+			+	+	
13	*Navicula*	*Tripunctata*	+	+	+	+			+		+	+
14	*Pinnularia*	*Viridis*				+		+		+		+
14	*Pleurosigma*	*Elongatum*			+			+			+	+
15	*Stepahnodiscus*	*Minutulus*	+				+		+		+	
16	*Synedra*	*Ulna*			+	+					+	+
17	*Tabellaria*	*Flocculosa*					+	+	+	+		

Table 5. Diversity and distribution of Chlorophyta in different sampling sites of Nakhon Nayok canal, Klong 14

Identified Algae of Phylum Chlorophyta			Sampling Sites (SS)									
Sl. No	Genera	Species	1	2	3	4	5	6	7	8	9	10
01	*Actinastrum*	*aciculare*				+		+			+	+
02	*Actinastrum*	*guttula*			+		+			+		+
03	*Actinastrum*	*indicum*			+	+		+	+			
04	*Actinastrum*	*mixtum*		+		+		+		+		
05	*Carteria*	*crucifera*				+	+	+	+			+
06	*Carteria*	*inversa*		+	+					+	+	
07	*Carteria*	*obtusa*	+						+	+		+
08	*Chlamydomonas*	*moewusii*	+	+		+	+		+			
09	*Chlamydomonas*	*reinhardtii*	+		+			+			+	
10	*Chlorella*	*vulgaris*		+		+	+		+	+		
11	*Chlorogonium*	*elongatum*			+		+			+		+
12	*Chlorosarsinopsis*	*gelatinosa*	+	+	+	+						
13	*Cosmarium*	*botrytis*		+		+		+		+		
14	*Cosmarium*	*dilatatum*							+	+	+	+
15	*Cosmarium*	*quadrum*				+	+	+	+			
16	*Cyanophora*	*paradoxa*		+	+				+	+	+	

17	*Crucigenia*	*tetrapedia*	+			+	+	+			+	
18	*Cylindrocystis*	*gracilis*					+	+	+	+		
19	*Haematococcus*	*pluvialis*	+	+	+	+						
20	*Klebsormdium*	*bilatum*		+	+							
21	*Micractinium*	*pusilum*					+	+	+			
22	*Monoraphidium*	*braunii*				+	+					
23	*Mesotaenium*	*caldariorum*					+	+	+			+
24	*Oedagonium*	*fragile*		+	+	+						
25	*Oedagonium*	*nodulosum*				+		+		+		+
26	*Oedagonium*	*aquaticum*	+				+		+		+	
27	*Oocystis*	*apiculata*				+		+		+		+
28	*Oocystis*	*solitaria*			+		+		+		+	
29	*Pandorina*	*morum*	+	+	+	+						
30	*Pediastrum*	*duplex*					+	+				
31	*Pediastrum*	*simplex*							+	+		
31	*Scenedesmus*	*acuminatus*					+	+	+		+	+
32	*Spirogyra*	*acanthopora*	+	+	+				+			
33	*Spirogyra*	*adnata*					+	+	+	+		
34	*Spirogyra*	*microspora*							+	+	+	+
35	*Spirogyra*	*minor*	+	+	+	+	+					
36	*Spirogyra*	*neglecta*				+	+	+	+	+		
37	*Spirogyra*	*oblate*				+			+		+	+
38	*Spirogyra*	*papulata*	+		+	+		+		+		+
39	*Staurastrum*	*avicula*		+		+		+	+		+	
40	*Staurastrum*	*dispar*			+	+	+		+	+		+
41	*Staurastrum*	*muticum*		+			+	+		+	+	
42	*Tetraedron*	*incus*			+	+	+	+	+			
43	*Tetraedron*	*striatum*				+		+	+	+	+	
44	*Tetraedron*	*vulgare*	+	+	+		+					+
45	*Tetrastrum*	*asymmetricum*	+		+	+			+			
46	*Tetrastrum*	*glabrum*					+			+	+	+
47	*Tetrastrum*	*triangulare*				+		+	+			
48	*Volvox*	*aureus*								+	+	+

Table 6. Diversity and distribution of Cyanophyta in different sampling sites of Nakhon Nayok canal, Klong 14

Identified Algae of Phylum Cyanophyta			Sampling Sites (SS)									
Sl. No	Genera	Species	1	2	3	4	5	6	7	8	9	10
01	*Acaryochlrois*	*marina*									+	+
02	*Anabaena*	*azollae*		+	+		+		+			
03	*Anabaena*	*affinis*	+			+		+		+	+	
04	*Anabaena*	*cylindrical*	+	+	+	+						
05	*Anabaena*	*flos aquae*			+	+	+	+				
06	*Anabaena*	*spiroids*						+	+	+	+	+
07	*Blennothrix*	*elegans*				+	+					
08	*Blennothrix*	*major*		+		+						

09	*Blennothrix*	*vermicularis*	+	+	+	+	+					
10	*Calothrix*	*crustacean*							+	+	+	
11	*Calothrix*	*fusca*				+	+	+	+			
12	*Calothrix*	*pilosa*							+	+	+	+
13	*Calothrix*	*scopulorum*	+	+		+		+		+		
14	*Chlorobotrys*	*bacillaris*	+		+		+		+	+		
15	*Chlorobotrys*	*regularis*		+		+		+				+
16	*Cylindrospermum*	*badium*			+		+	+	+			
17	*Cylindrospermum*	*majus*	+	+	+		+	+				
18	*Cylindrospermum*	*siamensis*			+	+	+	+	+			
19	*Cylindrospermiopsis*	*philippinensis*			+		+	+			+	+
20	*Cylindrospermiopsis*	*raciborskii*				+	+	+	+	+		
21	*Dermocarpa*	*pacifica*	+		+		+	+	+			
22	*Dermocarpa*	*enteromorphae*		+		+		+		+	+	
23	*Dermocarpa*	*violacea*					+		+	+	+	+
24	*Dermocarpella*	*gardneri*	+			+			+			
25	*Dermocarpella*	*hemisphaerica*		+	+				+			
26	*Gloeobacter*	*violaceus*				+	+	+	+	+		
27	*Gloeocapsa*	*magma*		+	+	+	+	+				
28	*Gloeotrichia*	*echinulata*	+	+	+	+	+					
29	*Gloeotrichia*	*ghosei*		+	+	+	+	+				
30	*Gomphonema*	*acuminatum*						+	+	+	+	+
31	*Gomphonema*	*clavatum*			+	+	+	+	+			
32	*Gomphonema*	*minutum*	+	+	+	+	+					
33	*Gomphonema*	*parvulum*						+	+	+	+	+
34	*Gomphonema*	*pumilum*			+	+	+	+	+			
35	*Lyngbya*	*birgei*	+		+	+	+					
36	*Lyngbya*	*majuscule*		+		+		+	+			
37	*Lyngbya*	*polychroa*				+		+	+		+	
38	*Lyngbya*	*semiplena*			+		+		+	+		
39	*Merismopedia*	*convolute*	+			+			+			+
40	*Merismopedia*	*elegans*		+	+		+	+		+		
41	*Merismopedia*	*minima*	+			+			+		+	+
42	*Merismopedia*	*punctata*		+	+		+	+	+			
43	*Merismopedia*	*sinica*				+		+	+		+	+
44	*Microcystis*	*aeruginosa*	+	+		+	+		+			
45	*Microcystis*	*botrys*			+	+	+	+	+			
46	*Microcystis*	*marina*	+		+		+	+	+			
47	*Microcystis*	*pallid*		+	+		+			+	+	
48	*Microcystis*	*salina*				+		+	+		+	+
49	*Microcystis*	*viridis*		+	+		+	+		+		
50	*Nodularia*	*armorica*			+	+			+		+	
51	*Nodularia*	*spumigena*					+	+		+		+
52	*Nodularia*	*quadrata*	+	+	+				+	+		
53	*Oscillatoria*	*amoena*	+									
54	*Oscillatoria*	*crassa*					+					
55	*Oscillatoria*	*limosa*	+	+			+		+		+	

56	*Oscillatoria*	*major*				+		+		+		+
57	*Oscillatoria*	*sancta*			+	+	+				+	+
58	*Phormidium*	*autumnale*						+				
59	*Prochloron*	*didemni*	+		+	+			+	+		
60	*Spirulina*	*maxima*			+		+	+	+			+
61	*Synechococcus*	*elongates*	+			+			+		+	
62	*Tolypothrix*	*tenuis*		+		+	+	+				
63	*Trichodesmium*	*lacustre*	+					+		+		+

RESEARCH ARTICLE

Virulence Factors Detection for some Dermatophytes Isolated from Human Skin Infections

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ABSTRACT

Five Dermatophyte isolates were obtained from fifty specimen samples collected from patients with hair, nail and skin infections. These isolates were *Microsporumcanis, Trichophyton rubrum, T.mentagrophytes, T.tonsurance,* and *Epidermatophyton flocossum.* The identification of these isolates was achieved according to cultural and microscopic characteristics. The ability of these isolates to produce enzymatic and non-enzymatic virulence factors was investigated using plate assay. All isolates showed lipase, phospholipase, gelatinase, andProtease activity except gelatinase activity from *T. rubrum* andprotease from *T. tonsurance*, and hemolytic activity was obtain only from *M. canis*and *T. mentagrophyte*s.

Keyword: Dermatophytes, Virulence Factors, Plate assay.

INTRODUCTION

Dermatohytes are a group of closely related fungi that have the capacity to invade keratinized tissues of human and other animals to produce an infection. Dermatophyte refers to three main genera of *Epidermophyton*, *Microsporum* and *Trichophyton* which are distributed around the world. Therefore, the infection of Tinea (dermatophytosis) is one of the most important superficial infections worldwide. Dermatophytes may lead to acute or chronic diseases with high morbidity but not mortality [1], commonly referred to as 'ringworm [2]. Dermatophytes are grouped according to their natural habitat as anthropophiles (human associated), zoophiles (animal associated) and geophiles (soil dwelling) [1]. Dermatophytes produce virulence factors such as keratinases, lipase, phosopholipase, protease and gelatinase to penetrate stratum corneum of host tissues and produce disease, The first step of dermatophytes infection involves contact and adherence of the infectious elements from dermatophytes. The transmission of

dermatophytoses may occur by direct contact with infected animals and humans or by indirect contact with contaminated fomites [3]. After adherence, the spores germinate and penetrate to the stratum corneum cells for the strong attachment sites a, during which the dermatophytes produces a variety of virulence factors which includes both enzymes and non enzymes [4]. The present work aimed to isolation and identification of some dermatophytes from human skin infection and detect the ability of these isolates to produce enzymatic and non-enzymatic virulence factors using plate assay.

MATERIALS AND METHODS

Sample Collection

A total of 50 clinical specimens (hair, nails and skin scrapings) were collected from patients who attended admitted to Dermatology Department of Al-yarmuk Hospital and from Al-Kadhimiya Teaching Hospital, during the period of beginning of November 2017 till end of September 2018, Traditional mycological diagnostics involve specimen direct microscopy, and culture medium,

Direct Microscopic Observation

Direct examine of fungi in clinical samples is obviously a cheap and short-time diagnostic method [5]the evidence of infection based on demonstration of fungal elements (branching septate hyphae and spore by direct microscopically examinationSpecimen were placed on a microscope slide, and few drops of 10% KOH were added on the specimen, and then cover slip was applied and warming over a small flame just before boiling because that leads to precipitates KOH crystals. The slide was Examined under the low power and high dry objectives to detect fungi [6].

Samples Culturing

The specimens were inoculated on Sabouraud dextrose agar (SDA; Oxoid, Basingstoke, UK) containing cycloheximide (0.5 g l-') and chloramphenicol (0.05 g 1-') The distinguishing features of dermatophyates are usually produced within 10-20 days, Most dermatophytes lose their distinctive cultural and microscopically features when kept for a long time in culture. Identification of the growth depends on the following [7].

1- colony observation (consistency, color and topography).
2- colony reverses (color, significant pigment).
3- Microscopic morphology (macro conidia and micro conidia; their size, shape, arrangement, and hyphal structures) examination was made by examination of many preparations from different areas of fungal growth mounted with lacto phenol cotton blue stain to reveal spores which include large sepate macro conidia and small, single-celled microconidia

Detection of Virulence Factors

For virulence factor test, an inoculum of 5 mm disc of each fungal culture was transferred on to plates containing the test medium. Enzyme activity was expressed by the diameter of clear or precipitate zones around the fungal colonies.

Phospholipase activity

The phospholipase medium contained peptone 1%, dextrose 2%, sodium chloride 5.73%, calcium chloride 0.05% and agar 2%. The medium was autoclaved and allowed to cool at 50°C. The egg yolk was separated and poured to a sterile flask containing glass beads. The egg yolk was completely mixed by vigorous shaking. A 5% egg yolk was

added to the molten Sabouraud Dextrose Agar (SDA) at approximately 50°C. The test medium was inoculated and incubated at 28°C. A clear halo zone of clearance around the colony indicated phospholipase production [8].

Lipase activity

The lipase medium contained peptone 1%, sodium chloride 5%, calcium chloride 0.01% and agar 2%. The medium was autoclaved and 1% tween 80 was added to the molten medium at approximately 50°C. The enriched medium was mixed thoroughly and poured in sterile petridish. The test medium was inoculated and incubated at 28°C. A clear halo zone of precipitation around the colony indicated lipase production [9].

Protease activity

The medium contained dextrose 2%, potassium dihydrogen phosphate 0.1%, magnesium phosphate 0.05% and agar 2%. The medium was allowed to cool and 1% bovine serum albumin was added to the molten medium at approximately 50°C. The enriched medium was mixed thoroughly and poured in sterile petridish. The test medium was inoculated and incubated at 28°Cl. A clear halo zone of clearance around the colony indicated protease production [10].

Gelatinase activity

For the detection of gelatin hydrolysis, nutrient gelatin stab method was performed. The nutrient gelatin medium contained peptone (5 g/L), beef extract (3 g/L) and gelatin (120 g/L). The medium was autoclaved and allow to cool 50°C. A total volume of 5 ml was added in each test tube and allowed to cool in an upright position. The test organisms were stab inoculated into gelatin tubes and the un-inoculated tubes were incubated at 28°C for a week and reviewed for gelatin liquefaction at regular intervals. Gelatin usually liquefies at 28°C and above and therefore to confirm gelatin liquefaction due to gelatinase production, the test and the un-inoculated gelatin tubes were refrigerated at 4°C for 30 minutes. Later, the tubes were tilted to observe for gelatin liquefaction. The hydrolyzed gelatin resulted in liquid state even after exposure to cold temperature demonstrated gelatinase production [11].

Hemolytic activity

Blood agar base supplemented with sheep blood was utilized for the detection of hemolytic activity. The sheep blood (5%) was added to the molten blood agar base at approximately 50°C. The medium was mixed properly and poured in sterile petridish. The test medium was inoculated and incubated at 28°C. A transparent zone of clearing around the colony indicated complete hemolysis [12].

RESULTS AND DISCUSSION

Isolation and Identification of Dermatophytes

Out of the fifty cases of dermatophytoses only 35 (70%) cases were positive by direct KOH mount smear as dermatophytes and 37(74%) cases were negative after six week incubation although the direct KOH mount examination was positive. The remaining 13 (26%) cases gave a positive culture. The possible reason for negative culture from microscopically positive specimens was the increased use of topical antifungal agent for short period of time on skin lesion of unknown etiology. The other possible reason was the highly contamination specimens over grown by fast growing saprophytic species which prevent the growth of dermatophytes even on medium with cyclohexamide [13].

Macroscopic and microscopic findings of the dermatophytes

Five dermatophyte isolates were obtained from 13 specimen samples which gave positive in culture on SDA medium. These isolates classified within three genera; Tichophyton, Microsporum and Epidermatophyton. The identification of these isolates at species level was achieved according to cultural and microscopic characteristics mentioned in [14] and [15] as described in table (1).Out of 63 isolates of dermatophytes *T. rubrum* was the most common followed by *T. mentagrophytes, T. tonsurance*and *M. gypseum* [13]. In various studies conducted by [14], [15], [16], and [17] *T. rubrum* was the commonest isolate with incidence ranging from 42.3% to 66.2 %.

Detection of virulence factors

The ability of dermatophyte isolates for production both enzymatic and non-enzymatic virulence factors was detected using plate assay. The results in table (2) showed that, the five deramtophyte isolates have ability to produce, lipase, phospholipase gelatinase, and protease as enzymatic virulence factorsFigure(1), (2), (3) and (4), except gelatinase from *T. rubrum* and protease from *T. tonsurance* while the hemolytic activity as non –enzymatic virulence factor was appears only from *M. canis*and *T. mentagrophytes* Figure (5).The solid media used in this study allowed the assessment of exoenzyme activity raised by fungal isolates. The major role of enzyme activities produced by pathogenic fungi is to break-down the fatty, protein and substances present in human skin tissues. The present data demonstrated that the examined dermatophytes were able to form different enzymes they are apparently capable of damaging the keratinized structure of the skin hair and nail [13]. The high enzymes activity of *M.canis* and *T.mentagrophyte* in comparison with other related fungi may explain their ability to invade skin, hair and nail break down keratin. The protease can divided in to endoprotase and exoprotase, endoprotase cleaves peptide bonds within a polypeptide. Exoprotase Cleaves peptide bonds only at the C-or N-terminus of polypeptides [16]. The gelatinase hydrolyze gelatin into sub-components such as polypeptides, peptides and amino acids [17]. Hydrolyzes phospholipids into fatty acids and other lipophilic substances, The medium enriched with specific substrates showed rapid growth of dermatophytes within7-14days of incubation showing that they have the ability to breakdown the substrate present in the skin of patients with dermatophytosis for their growth..The dermatophytes initially break the lipid surface layer during the first phase of growth and subsequently, colonize the stratum corneum of the skin , lipase enzyme plays a vital role during dermatophytic infections [18], and [19].

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Table 1. Identification of dermatophytes after 2 weeks of incubation at 28°C on SDAmedium

Isolate No,	Source	Cultural characteristics	Microscopic characteristics	Species
1	**Skin**	Colonies were growing as white, and cotton to velvety appeared on this medium and diameter reaching 7.5 cm	This organism produced very small microconidia which oval and born along the sides of hyphae	*T.rubrum*
2	**Skin**	The colonies were growing with diameter 6.5 cm and characterized as yellow granular of diffused, and flat and powdery characters, and color of colony is pale yellow in reverse	Microconidia were more in number, and spherical and accumulate in the form of cluster, macroconidia were rare	*T. Mentagrophytes*
3	**Skin**	Fast-growing colonies with various colors from off-white to grey, with dark pigments that may diffuse into the medium. and surface textures, flat, powdery, and yellow and develops into a flatter, creamy disk with raised edges	Pear-shaped microconidia, borne on matchstick-like stalks. It also forms fewer macroconidia that are 4-6 cells long, with thick cell walls	*T. tonsourans*
4	**Skin**	A white, coarsely fluffy spreading colony with a distinctive "hairy" or "feathery" texture with deep yellow pigment develops in reverse	Form undeveloped macroconidia.Cultivation on polished rice.	*M. canis*

5	**Skin**	Colonies are usually slow growing, greenish-brown or olive- colored with a suede-like surface, raised and folded in center, with a flat periphery and submerged fringe of growth. A deep yellowish-brown reverse pigment is usually present.	Microscopic morphology shows characteristic smooth, thin-walled macroconidia which are often produced in cultures growth directly from the hyphae.	*E. flocossum*

Table 2: Production of enzymatic and non- enzymatic virulence factors

Species	Lipase	phosophlipase	Gelatinase	Protease	Hemolytic Activity
T. rubrum	+	+	-	+	-
T. mentagrophytes	+	+	+	+	+
T. tonsurance	+	+	+	-	-
M. canis	+	+	+	+	+
E. flocossum	+	+	+	+	-

+: Production, **-**:non- Production

Figure 1. Production of Phospholipase from *M. canis*on egg yolk mediumafter incubation at28°C for 2 weeks.

Figure 2. Production of lipase from *M. canis*on tween 80 medium afterincubation at 28 °C for 2 weeks

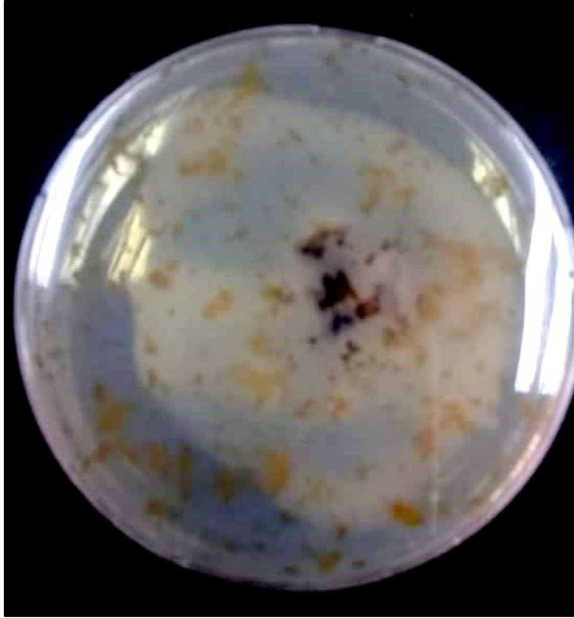

Figure 3. Protease production from *M.canis*activity on BovineSerum Albumin (BSA) medium incubated at 28°C for 2 weeks

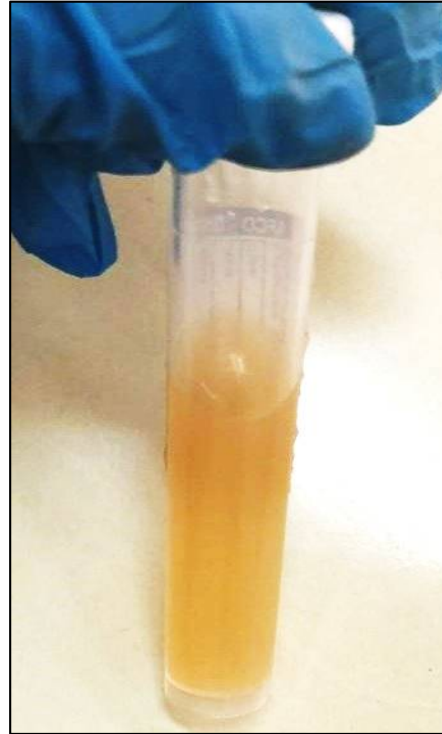

Figure 4. Gelatinase activity from *M. canis* on nutrient gelatin medium afterincubation at 28°Cfor 2 weeks

Figure 5. Hemolytic activity from *M. canis*on blood agar base with sheep blood after incubation at 28°C for 2 week

RESEARCH ARTICLE

Morphometrical Study of Small Intestine in the Adult Guinea Fowl (*Numidia meleagris*)

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ABSTRACT

The present study aims to investigate the morphological andmorphometricalstudy of the different parts ofthe small intestine of Adult Guinea Fowl.The present study was carried out on 10guinea fowl of both sex to investage the morphological and morphometricalstudy of small intestine of adult guinea fowl. The present study investigate that the small intestine consist of three segment named duodenum, jejunum and ileum. The duodenum was the first segment and formedas a 'U' shaped loop. The jejunum was arranged in the form of garland land like shape suspended by mesentery. Ileum was observed as a straight portion of small intestine located in between two caeca and was the shortest of the twoother parts. Also the present study showed that the gross anatomy of the small intestine in both sexes of guinea fowl was similar to that observed in other avian species and no major differences between them.

Keywords: anatomy, guinea fowl, Small intestine, duodenum, jejunum and ileum.

INTRODUCTION

Small intestine is the principle organ for digestion and absorption, its contents are mixed with the pancreatic and bile juice, and the mucosal secretions of the mucosa (1). The small intestine begins at the pylorus proventriculus and ends at the ileocaecal junction. As in mammal animals the small intestine composed of unequal three segments named duodenum, jejunum and ileum.There is no demarcation between them(2). But, in carnivorous birds, the small intestine is shorter than that in herbivorous (3). Anatomicallyand functionally of the small intestine are more variable than the anterior digestive organs probably due to the diverse physical nature of different foods (4). The initiation of thesmall intestine was from the pyloric end of stomachto the junction with the large intestine, caeca and colon and

generally it is long and made of a coiled tube forming a number of loops within the abdominal cavity (5).The present study was planned toexplorethe morphometric of small Intestine of the adult guinea fowl (*Numidia meleagris*).

MATERIALS AND METHODS

The current study was carried out on ten birds of male and female. After recording the live body weight, the birds were sacrificed and the topography and relationship of small intestine was recorded. The gross parameters of small intestine such as weight, volume, length and diameter of each segment were recorded listed in table. All numerical values were expressed was Mean ±SE and the statistical analysis was carried out using't' test for the methods suggested of comparison of parametric variances of the small intestine between male and female and the significance level was at $P<0.05$ by (6).

RESULTS

Gross anatomy

The small intestine appeared as a thin coiled tube like structure after exposing the abdominal cavity. Duodenum was clearly visible towards the right side of abdominal cavity(Fig.1), whereas the other two parts jejunum and ileum were partially covered by duodenal loop (Fig.2). Ventrally, the duodenum appeared as a bulged part of small intestine and itorganized in a U-shaped structure which held together by fold of mesenteryoccupied by pancreas (Fig.3a,b&4) the distinct duodenal loop consisting of a descending and an ascending limbs. It started from the antero-dorsal side of the gizzard and ended at the terminal point of ascending limb towards the anterior aspect of the abdominal cavity(Fig 3a,b).its left side was related to the gizzard right side and dorsally it covered jejunum,ileumand caecum (Fig. 2, 3a,b). whereas, on the right side it was in attached liver right lobe and lateral body wall(Fig 3a,b). Two bile and two pancreatic ducts were opened in ascending limb of duodenumopposite the cranial part of the gizzard (Fig.5).Its displayed into three main parts that were duodenum, jejunum and ileum. There was no clear demarcation between the first and the second parts and between the second and the first parts (jejunum and ileum)(Fig.6a&b). Generally the three parts were uniform in diameter and may be only the duodenum was distinguish.

The jejunum appeared as the longest part of the small intestine arranged in the form of coils which were suspended by mesentery towards the dorsal part of abdominal cavity(Fig6b&7a,b). As observed in other avian species the jejunum begins from the termination of the duodenal loop and organized in the jejunal loops which occupies most of the space of the coelomic cavity (Fig 7A&B) and it grossly structured in a number of short garland like coil (Fig.6a,b) and it in contact with duodenum and was related to the some organs such as stomach, spleen, ovary in female and testes in male (Fig.7B 8,&9). The ileum was followed the jejunum without clear demarcation observed as aa straight portion of the small intestine and located in between two caeca, but darker than the jejunum and its terminal at ileocecal junction and there was on both side for most its length a right and left ceca(Fig. 6b,8,10) .No vitelline diverticulum was observed between the jejunum and ileum in studied birds (Fig.6b,8).As showed in Table(1)There were a significant, difference in the body weight, total small intestine weight and total small length and volume between male and female at $P<0.05$. The mean body weight washigher in female than in male studied birds with a significant differences $P\leq 0.05$ between them(Table.1).The total small intestine weight in female was more higher than in male, there was a Significant difference between them at p0.0≤0.05(Table.1), there was appositive correlation between the total length of the small intestine and its weight. The present study noticed that the relative small intestine weight to body weight in female was higher than in male (Table.1). The mean length of small intestine in female was longer than that in male guinea fowl, there was a significant differences between them at $p\leq 0.05$(table.1). The mean volume of small intestine in female guinea fowl reach a higher value than in male, there was a significant difference between male and female(Table.1), the present study showed appositive correlation between small intestine weight and its volume.

There were a significant differences between two sexes in weight of all segments of small intestine, in female is heigher than that in the male. The current investigate that the jejunum is the longest segment in the small intestine, these may be related to increase in whole intestine weight in female than in male due to a differences in food intake amounts. The mean volume of the duodenum, jejunum and ileum in male was less than in female. There is a correlation between weight and volume of each segment of small intestine, there are a considerable anatomical differences between male and female due to sex of birds. The width of duodenum was maximum and slightly higher than the jejunum and least in ileum(Table 2).

Dissecting Microscopically Observation

At adult of guinea fowl the duodenum, jejunum and ileum in both sex showed clear long finger like shape of villiin cross section. Whereaslongitudinal section at the both sex they have clear villus appear as longitudinal folds(Fig. 11, 12,13) as follow.

DISCUSSION

Gross anatomy

The small intestine appeared as a thin coiled tube like structure after exposing the abdominal cavity Its displayed into three main parts that were duodenum, jejunum and ileumarranged in relatively long coiled tube formed as series of loops exited from the pylorus of the gizzard and ends at the ileocecal junction and located within the abdominal cavity, this findings were observed in other avian species such as fowl (7), ostrich (8), chicken (9) and in fowl (5) and in quail(10; 11). The duodenum was organized in a U-shaped structure as observed perviously in cattle ergert (*Bublucus ibis*) birds (12, 13). The present study was disagree with (14) and (15) on black winged kite and black shouldered kite in that the small intestine was differentiated in two parts, duodenal loop and ileum. While in this study its consist of three parts named duodenum, jejunum and ileum. In this study as a U shaped structure the duodenum distinct in the right side of the abdominal cavity and it covered partially the jejunum and ileum as found in Japanese quail by (10).The current study revealed that there aretwo bile ducts and two pancreatic ducts opened towards the ascending limb of duodenum opposite to the cranial part of the gizzard as reported in quailby 10), and agreement with (16) on pied crow *Coryusalbus*. And disagreement with (17) on the Turkey and(18) in moorhen Gallinula (*CoryusChoropas*). Also the present studies in disagreement with (19) in geese and with (20, 22) on local ducks, (21) on Rock dove, they mention that the pancreas and the gall bladder empty their secretions through the bile and pancreatic ducts in the duodenum.The jejunum arranged in the form of coils as observed in other avian species and it begins from the termination of the duodenal loop and organized in the jejunal loops which occupied most of the space of the coelomic cavity and grossly itsstructured in a number of short garland like coil which was related to the stomach, spleen, ovary in female and testes in male similar Findings (23) (24), (25) in mallard and (26) in barn owl.

Whereas the Ileumis third and shortest part of the small intestine followed the jejunum without clear demarcation except darker than the jejunum. It's a straight part and lies between the caeca, this observation was confirmed the observation of (10) on *coturnixcoturnix*, but in contrary to(27) on yellow and blue macawa in which the ileum was continuous with the colorect and and no aceca were present.No meckels diverticulum was observed between the jejunum and ileum in studied birdsimilar finding was noticed in Captive bustard by (28) and disagree with (26) in Barn owlin which was a presence of vitelline diverticulum limits the end of the jejunum and the beginning of ileum. A short blind remnant of in the formed meckel׳s diverticulum was observed in poultry by (29). The gross anatomy, location and the relationship of the ileum to other organs was similar to the previously noticed by (10) in quail. The mean body weight were higher in female than in male, this results have been disagreement with (30) in Indigenous ducks and (31) in common pheasant.

The small intestine total weight in female was more higher than in male, there is appositive correlation between the total length of the small intestine and its weight, as observed by(32) on the broiler chickens. The mean length of small intestine in female was longer than that in male guinea fowl, there was a significant differences between them as showed by (33) that the sex had a significant effect on the length digestive tract. The mean volume of small intestine in female guinea fowl reach a higher value than in male, this results was in parallel with (34) they mentioned that these features are connected with the amount and type of food, sex and species characteristics, housing type and also the current investigation showed that the jejunum was the longest segment in the small intestine,this finding was agreement with (34). The weight of each region of small intestine in male were less than in female, these may be related to increase in whole intestine weight in female than in male due to a differences in food intake amounts (35). The width of duodenum was maximum and slightly higher than the jejunum and least in ileum as reported in (10)in Japanese quail.

Dissecting Microscopically Observation

At adult of both sexes guinea fowl the duodenum, jejunum and ileum in both sex showed clear long finger like shape of villiin cross section at the both sex they have clear villus,this finding agreement with (36) in white leghorn and broiler chicken, by (37).

CONCLUSION

This study revealed that general the gross anatomy in the studied bird in both sexes and the relationships of the small intestine was similar to that observed in other avian species and no major differences wasobserved between them.

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Table. 1. difference in the body weight, total small intestine weight and total small length and volume between male and female

Gross measurements	Male	Female
Body weight(gm)	**1465±0.032 B**	**1532±0.020 A**
Total small intestine weight(mg)	**25.755±0.499 B**	**32.604±0.562 A**
Relative weight	**0.017±0.398**	**0.091±0.488**
Percentage%	**1.7%**	**2.1%**
Total small intestine length(mm)	**85.36± 0.598 B**	**87.5±0.650 A**
Total small intestine volume(ml)	**31.44±0.483 B**	**37.94±0.4 A**

Table. 2. The width of duodenum was maximum and slightly higher than the jejunum and least in ileum

Gross Measurements		**Small intestine Mean ±SE**		
		Duodenum	**jejunum**	**ileum**
Weight (gm)	Male	10.231±0.271 B	13.102± 0.288 B	2.422 ±0.139 B
	Female	13.676±0.367 A	15.343±0.652 A	3.585±0.241 A
Relative weight	Male	0.0069±0.016	0.0089±0.032	0.0016±0.0001
	Female	0.0089±0.012	0.010±0.015	0.0023±0.0001
Length(cm)	Male	21.360±0.601 A	51.000±1.553 A	13.000±0.262 A
	Female	23.100±0.809 A	51.000±1.553 A	13.400±0.263 A
Diameter (mm)	Male	9.234±0.1966 A	6.588±0.145 A	5.268±0.092 A
	Female	8.636±0.160 B	5.284±0.118 B	4.036±0.239 B
Volume (mm^3)	Male	11.200±0.515 B	16.980±0.706 B	3.260±0.399 B
	Female	14.400±1.352 A	19.200±1.577 A	4.340±0.505 A

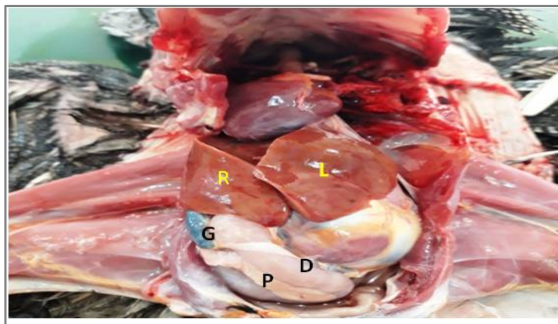


Fig. 1. Photograph viscera in abdominal cavity of adult male guinea fowl show the duodenum(D), Pancreas (P), right and left liver(R&L) and gallbladder(G).

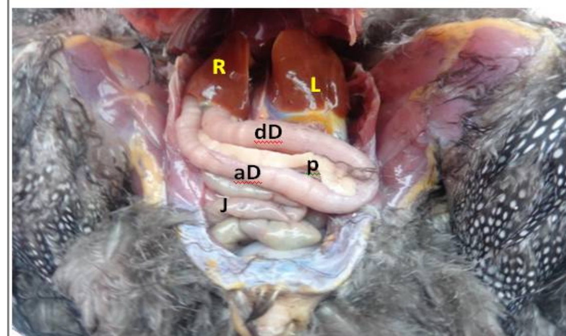
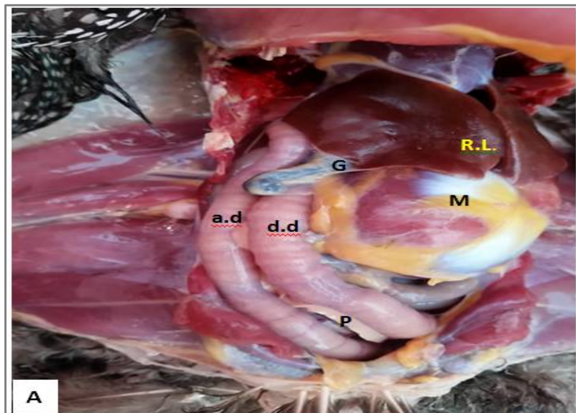


Fig. 2. Photograph viscera inabdominal cavity of adult female guinea fowl show the jejunum and ileum(J) , duodenal loop the descending and ascending duodenum(dD&aD), Pancreas (P), right and left lobes of liver(R&L).



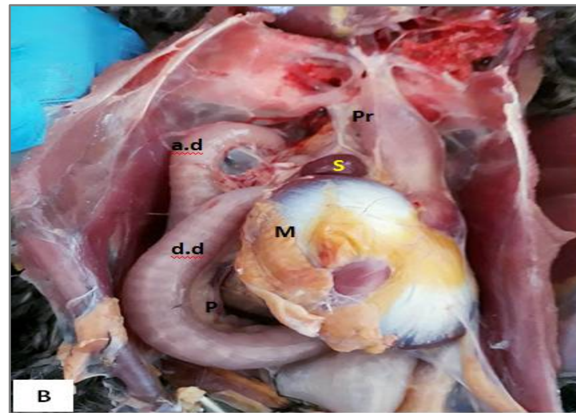


Fig. 3. A&B Photographs viscera of adult female guinea fowl show ascending and descending duodenum (ad&d.d), gizzard (M), Pancreas (P), Proventriculus (Pr), gallbladder (G),spleen (S) and liver (L).

Fig. 4. Photograph viscera of adult male guinea fowl show the jejunum(J) duodenum (d) , cecal tonsil(C), gizzard (M), Proventriculus (Pr) and pancreas (A)

Fig.5. Photograph parts of digestive tract of adult male guinea fowl show Two pancreatic (black row) and Two bile ducts (red row),ascending and descending duodenum(ad&d.d) gizzard (M), proventuculs (Pr), gallbladder(G) and pancreas (P),

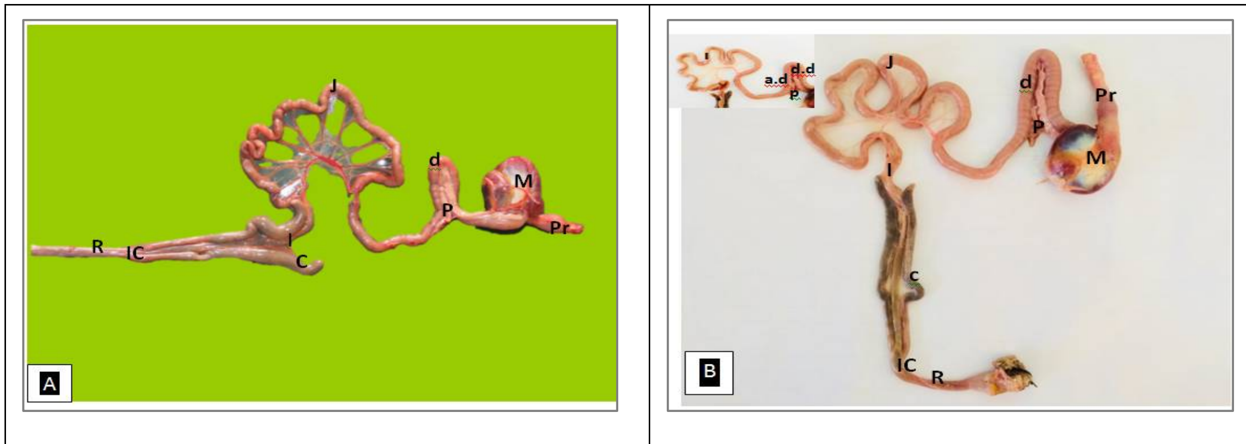


Fig.6. Photograph gastrointestinal tract of female(A) and male(B) adult guinea fowl show the jejunum(J) duodenum (d),Ileocecal junction(IC), cecal tonsil(C), gizzard (M), proventuculs (Pr) and pancreas (p)

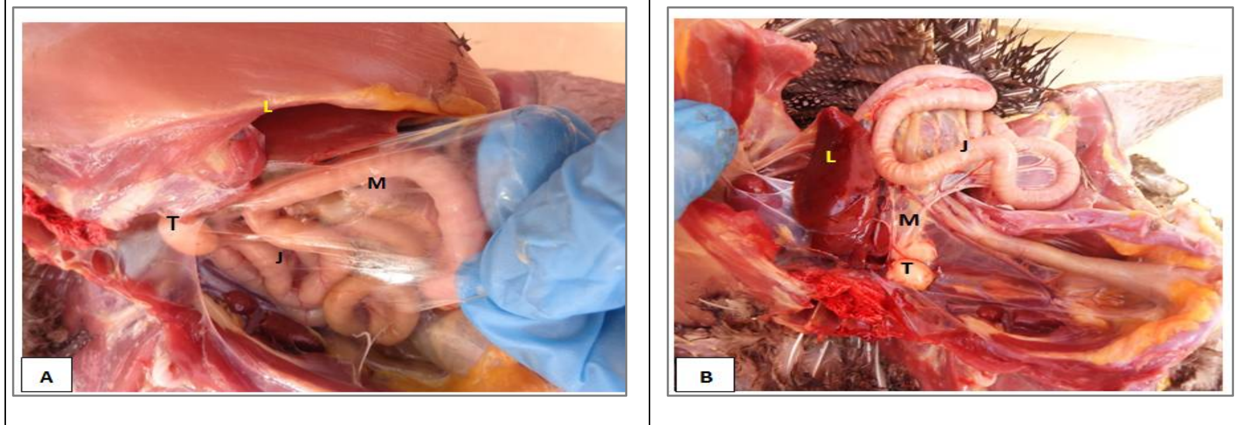


Fig. 7. Photographs of viscera adult maleguinea fowl show the jejunum(J) Test (T)and Mesentery (M): A&B in male

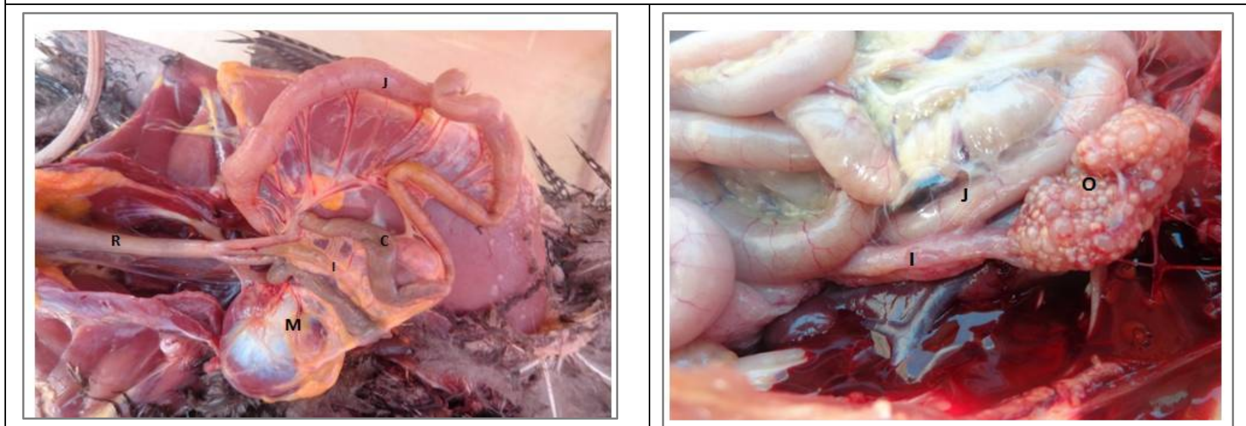


Fig. 8. Photograph of viscera inabdominal cavity of adult male guinea fowl, jejunum(J) and ileum(I). cecal tonsil (C) and rectum(R).

Fig. 9. Photograph of viscera inabdominal cavity of adult female guinea fowl, jejunum(J) , infundibulum (I) and ovary (O).

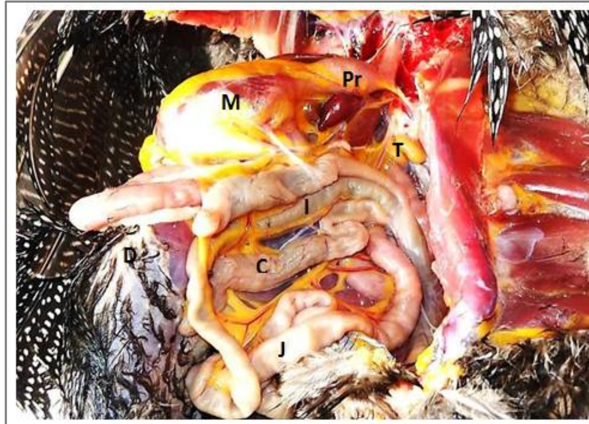


Fig. 10. Photograph of digestive tract abdominal cavity of adult male guinea fowl, jejunum(J), ileum(I), gizzard (M), proventuculs (Pr), cecal tonsil (C) and rectum(R).

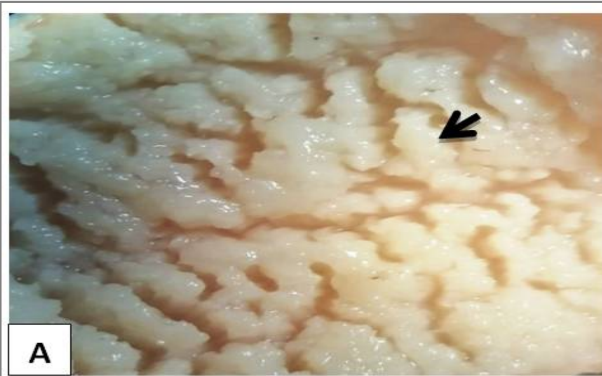
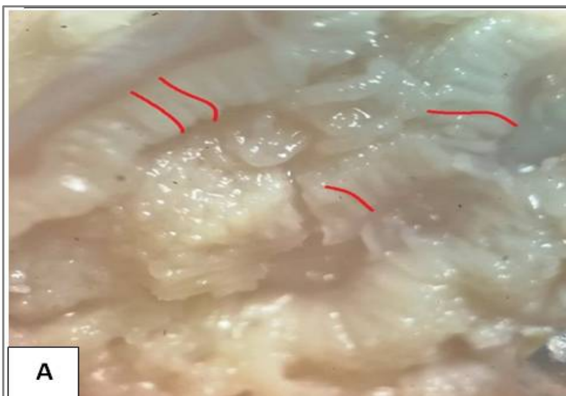


Fig.11. A. longitudinal section of duodenum in both sex showed clear long Velvet shape of villi. B: cross secti duodenum appeared as finger like projection.



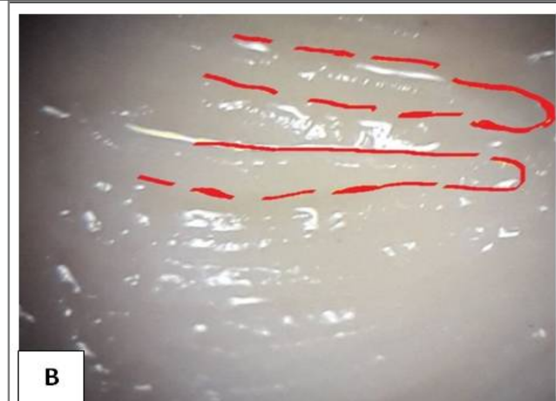


Fig.12. A longitudinal section of jejunum in both sex appear as longitudinal folds. B: cross section of jejunum appeared as finger like projection.

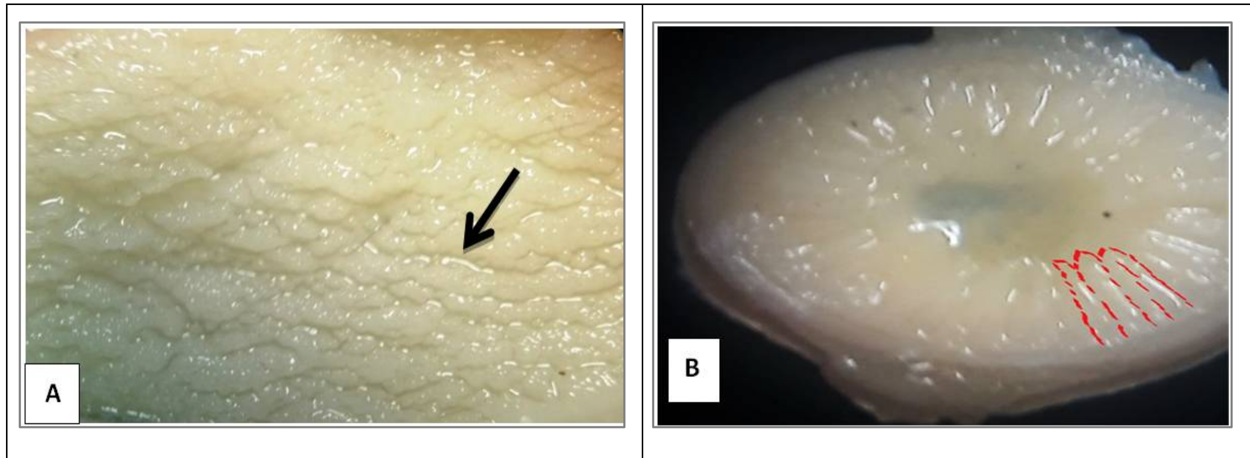


Fig.13. A.longitudinal section of ileum in both sex showed appear as longitudinal folds: B: finger like villus in cross section

RESEARCH ARTICLE

Studies on Root Morphology in Bhendi and Cluster Bean on Onetime Application of Postmethanated Distillery Spentwash

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ABSTRACT

Eco-friendly utilization of distillery effluent in agriculture may serve as one of the nutrient management practices for enhancing crop yields besides reducing the cost involved in fertilizers. But, it has to be used judiciously and cautiously on a limited scale, because of very high organic and chemical load. So, a field experiment was conducted at Amaravathy sugars, Udumelpet to study the impact of spentwash on the root parameters of Cluster bean and Bhendi crop. Results revealed that onetime application of postmethanated distillery spentwash @ 40 kL ac^{-1} increased the root parameters in both cluster bean var. PusaNavabhar and Bhendi hybrid Co1 crops in field and pot conditions. The enormous quantities of plant nutrients present in postmethanated distillery spentwash offers an excellent opportunity to use it as a liquid fertilizer along with irrigation water, thus enabling the farmers to save cost incurred on fertilizers and at the same time achieve higher yields of crops.

Key words: Distillery effluent, spentwash, Bhendi, Cluster bean, Fertilizers.

INTRODUCTION

Spentwash is an agro-industrial waste generated during alcohol production in distilleries. At present in India, there are 319 distilleries with the capacity of producing 3.29 billion litres of alcohol which in turn generates 49.35 billion litres of wastewater (Kumar and Chopra, 2013).Utilization of industrial effluent in agriculture either for irrigation or cultivation of the crop with the nutrients in spentwash has been increased in recent times (Tharakeshwari and Jagannath, 2011). Most of the crops showed higher yield with respect to different concentration of effluent application. Since spentwash generated contains organic and inorganic nutrients exceeding the normal level; proper treatment of the wastes is emphasized before using it for either irrigation or composting. Hence the effluent undergoes biomethanation process to reduce the high bio-chemical oxygen demand and chemical oxygen demand and the resulting product is called as post-methanated distillery effluent which can be further utilized in agriculture. Recent research results suggested that one time application of spentwash is safe and eco-friendly (Selvakumar, 2006).

When the applications are repeated in the small area, it leads to salinization of land and surface and ground water contamination. Spentwash application to soil at low concentration was reported beneficial and increased sugar cane growth attributing parameters (Haroon and Bose, 2004). Mahimairaja and Bolan (2004) concluded that non-judicious use of spentwash adversely affected crop growth and soil properties by increasing soil salinity. On the other hand, Extensive use of inorganic fertilizer not only degrades the soil quality but also affects the productivity of the crop. The application of post-methanated effluent for crop production reduces the environmental impact of inorganic fertilizer on soil properties which in turn reduces the need of inorganic fertilizer. This research was carried out to study the effect of post-methanated effluent application on crop growth, yield and quality.

METHODS

A field study was conducted at research and development farm, M/s Amaravathi Co-operative Sugars Mills Ltd., Krishnapuram, Udumalpet, Tirupur District, Tamil Naduwith cluster bean var. Pusa Navabhar and bhendi hybrid Co1 to study the effect of PMDSW on root morphology of cluster bean which includes control and PMDSW application of 40 kL ac-1. Pot culture study was also conducted at the same period and observations were made. Calculated quantity of PMDSW was uniformly applied in the plots. It was mixed with soil by thorough ploughing in order to provide better soil aeration and consequent reduction of BOD level in the soil system. On 25th day of PMDSW application, the plots were ploughed. Subsequently ridges and furrows were formed by adopting a spacing of 45 cm between the two ridges. Bhendi and clusterbean seeds were sown by adopting a spacing of 45 cm between the two ridges and 30 cm apart. All the cultural practices including gap filling, thinning, weeding and plant protection measures were carried out as per the crop production guide.

Treatment Details

T1 – Control (Recommended NPK)
T2 – PMDSW application 40 KL ac-1

RESULTS AND DISCUSSION

Pot experiment to study the effect of PMDSW on root parameters of the crop

Consistent growth and yield was observed between the different dosages of spentwash application. In this context, a field experiment was conducted and the results of the study are discussed below. Application of PMDSW favoured the yield attributes of Bhendi by enhancing the availability of nutrients. The per cent increase over the control was 15 and 28 per cent, respectively. In case of dry matter production, treatment PMDSW @ 20.83 KL/ha showed an increase of 19% from control which is almost similar to the findings of Gahlot et al., 2011, which showed an increased yield in Red gram due to spentwash application. Pot experiment was conducted with cluster bean var. PusaNavabhar and Bhendi hybrid Co1 to study the effect of PMDSW on root morphology of cluster bean which includes control and PMDSW application 40 KL ac-1. PMDSW was applied to the pots except the control and after 15 days of PMDSW application, sowing was carried out. The parameters commonly used to express root growth are root length, root fresh weight, root dry weight, number of lateral roots per plant and number of root nodules.

Impact of PMDSW application on the root morphology of the crops

PMDSW application @ 40 KL ac-1influenced the root growth by increasing the root length (cm) up to 7.78 cm in cluster bean and 13 in bhendi (Table 1). Nodulation was influenced by the PMDSW application @ 40 KL ac-1and the number of nodules was 1.75 whereas in case of control, it was 1.5. However, sensitivity of nodules to PMDSW application has to be studied further.

Field experiment

The results revealed that the mean shoot length of Bhendi in spentwash applied field (40 kL ac-1) was more than 15 cms over the control. The number of primary, secondary and tertiary roots was also more in the plants in spentwash applied field than control. The same result was also observed in cluster bean crop. Supporting to this study, a study conducted in sugarcane crop by continuous application of spentwash for 5 to 10 years registered significantly higher growth, yield and quality parameters of sugarcane like millable cane height, diameter of cane, number of internodes, intermodal length, number of millable canes, single cane weight, dry matter, yield, brix per cent, pol per cent and purity per cent (Kamble and Hebbara, 2015).

CONCLUSIONS

This study reveals the efficiency of utilizing post Methanated distillery spentwash for Bhendi and cluster bean cultivation. The number of primary, secondary and tertiary roots in both the crops Bhendi and cluster bean was also more in the plants in spentwash applied field than control. The treatment (40 kL ac-1) through PMDSW showed prominent growth, yield and quality.

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Table 1. Impact of PMDSW application on root parameters in Bhendi and Cluster bean at field (mean of 3 plants)

S.No.	Shoot length (cm)		Root length (cm)			
			Primary		No. of Secondary roots	
1.	Bhendi					
	Control	Spentwash applied	Control	Spentwash applied	Control (no's)	Spentwash applied(no's)
	66	83	30	39	6	4
	105	120	31	31	3	4
2.	Cluster bean					
	145	142	13	15	4	4
	120	125	21	20	4	4

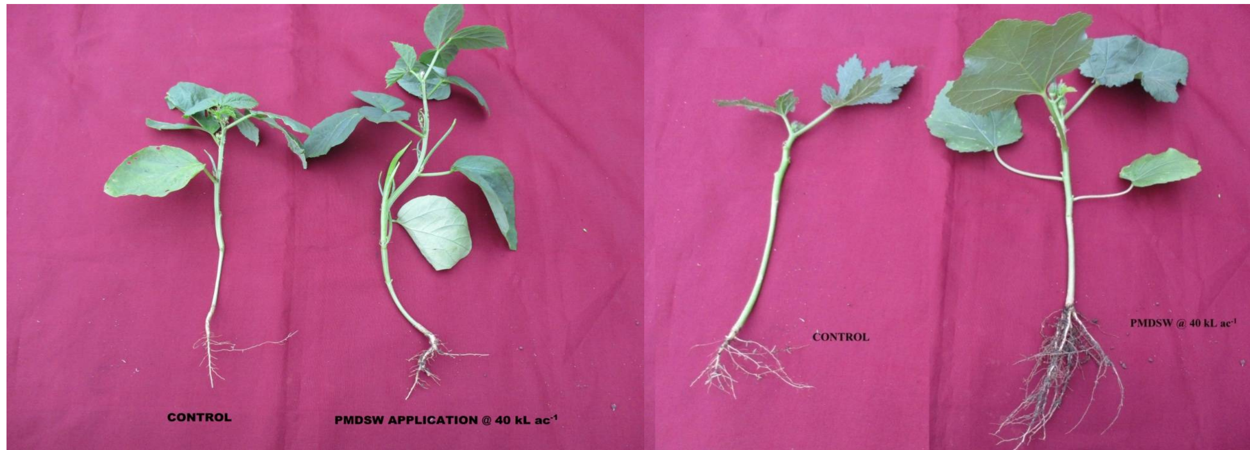

Fig. 1. A view of root growth parameters of cluster bean and bhendi

Fig. 2. A view of root growth parameters of cluster bean and bhendi

RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Determination of Methyl and Propyl Paraben in Bulk and Marketed Sterile Dosage Forms

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ABSTRACT

A simple, precise and specific High Performance Liquid Chromatographic method was developed and validated for the determination of preservatives in sterile dosage form. The method employed LC-10 AT VP Shimadzu Liquid Chromatograph HPLC with Hyper ODS 2 C18 size 4.5x250mm column as a stationary phase. The solvent system consists of Acetonitrile (HPLC Grade) :1 % w/v ammonium chloride (1:1)at flow rate1 ml/min.The analysis of Methyl Paraben and Propyl Paraben was carried out at254nm.The Calibration curve for Methyl Paraben was linear from 18-90µg/ml, for Propyl Paraben 2-10µg/ml. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Methyl and Propyl Paraben in marketed sterile dosage forms. The LOD and LOQ for Methyl paraben was found to be 0.016 µg/mL and 0.049 µg/mL, for Propyl paraben, 0.047 µg/mL and 0.142 µg/mL respectively. The accuracy of the proposed method was evaluated by performing recovery studies and the % RSD and % recovery were within the acceptable limits in all 3 levels and % recovery was found to be 100.52 % for Methyl paraben, 98.65 % for Propyl paraben, and reproducibility were found to be satisfactory.

Keywords: Methyl Paraben, Propyl Paraben, HPLC Method, Validation.

INTRODUCTION

Parenteral preparations are sterile products those are administered by injection into the body. Parenteral preparations which are packaged in multiple dose containers may contains suitable antimicrobial preservatives in the appropriate concentration to inhibit the microbial growth in the containers. Methyl Paraben (Methyl 4-hydroxy benzoate) is Colorless crystal or white crystalline powder, freely soluble in ethanol (95%), ether, and methanol and very slightly soluble in water. Propyl Paraben (Propyl-4-hydroxy benzoate) is white crystalline powder, freely soluble in ethanol (95%), ether, acetone, and methanol and very slightly soluble in water. It was found that some analytical methods such as UPLC, HPLC other methods were reported for analysis of preservatives. The objective of the present study is to develop simple and accurate HPLC method for the determination of Methyl Paraben and Propyl Paraben in sterile dosage form.

MATERIALS AND METHODS

Chemicals and Materials

All solvents were of HPLC grade. All other materials were purchased of analytical grade E-Merck, Qualigens, and Rankem etc. Distilled water and Whatman filter paper Grade-I were used throughout the experimental work. A Standard Methyl and Propyl Paraben were procured as a gift sample from Unijules life Sciences. Pvt. Ltd.Nagpur. The marketed formulations were purchased from the local market.

HPLC Instrumentation

LC-10 AT VP Shimadzu Liquid Chromatograph HPLC with Hyper ODS 2 C18 (size 4.6mmx250mm, particle size 5µm) column was used for the study. SPD-10 A VP Shimadzu UV-Visible is used as a detector and Shimadzu PU 2080 plus was the solvent delivery system. The system control and data processing were performed by ANALCROM software.

Mobile Phase

The Mobile Phase consists of Acetonitrile (HPLC Grade) : 1 % w/v ammonium chloride (1:1)at flow rate 1 ml/min.

Preparation of standard drug solution

Preparation of Methyl Paraben standard solution

From the freshly prepared standard stock solution (1000µg/ml), 0.5 ml of stock solution was pipetted out in 10.0 ml of volumetric flask and volume was made upto 10 ml with mobile phase to get final concentration 50µg/ml.

Preparation of Propyl Paraben standard solution

From the freshly prepared standard stock solution (1000µg/ml), 0.05 ml of stock solution was pipetted out in 10.0 ml of volumetric flask and volume was made upto 10 ml with mobile phase to get final concentration of5µg/ml.

Calibration curve of methyl Paraben

Serial standard five different concentration levels of Methyl paraben (18-90μg/ml) were prepared. For HPLC analysis, a20 μL sample volume was injected 5 times. The chromatograms were monitored by UV at 254nm. The peak area of UV chromatograms were plotted versus the concentration and the calibration curve was constructed using a leastsquare regression equation for the calculation of slope, intercept, and square of correlation coefficient.

Calibration curve of Propyl Paraben

Serial standard five different concentration levels of Propyl Paraben (2-10μg/ml) were prepared. For HPLC analysis, a20 μL sample volume was injected 5 times. The chromatograms were monitored by UV at 254nm. The peak area ofUV chromatograms were plotted versus the concentration and the calibration curve was constructed using a leastsquare regression equation for the calculation of slope, intercept, and square of correlation coefficient.

Analysis of Preservatives in Marketed sterile Dosage Form

a) Preparation of sample solution of methyl Paraben and Propyl Paraben

Accurately 1 ml of marketed sample solution (label claim-MP-0.18% w/v, PP-0.02% w/v) was transferred in 10 ml volumetric flask and dissolved in methanol (1800μg/ml). The solution was filtered through 0.45 u membrane filter and volume was made to the mark with mobile phase. Further 5 ml of solution were pipette out dilutions were made with the mobile phase to get final concentration of 90 μg/ml of sample solution.

Procedure

Equal volumes (20μl) of standard and sample solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and response i.e., peak area of major peaks were measured. The content of MP and PP was calculated by comparing a sample peak area with that of standard peak area.

Validation of method

Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out five independent assays of test sample preparation and the % RSD of assay was calculated.

Recovery studies

The accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The analysed samples were spiked with80, 100 and 120% of the standard Methyl and Propyl Paraben and the mixtures were reanalyzed by proposed method. The experiment was conducted three times. This was done to check for the recovery of the drug at different levels in the formulation.

Stability of Methyl and Propyl Paraben in standard and test solutions

A standard solution of Methyl and Propyl Paraben was initially prepared (std.no.1) and held under refrigerated condition (4^0 – 8^0c) for up to 30 days and tested against freshly prepared standard solution (std.no.2).

A test solution of one injection brand was initially prepared (Test 1) and held under refrigerated condition (4^0 – 8^0 c) for up to 30 days and tested against freshly prepared test solution (Test 2).

RESULTS AND DISCUSSION

Development of the optimum mobile phase. Each mobile phase was filtered through 0.45 μ membrane filter. The mobile phase was allowed to equilibrate until steady baseline was obtained. The standard solutions containing Methyl and Propyl Paraben was run and different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing Acetonitrile (HPLC Grade) : 1 % w/v ammonium chloride (1:1)was selected as it shows sharp peak with symmetry and significant reproducible retention time for Methyl and Propyl Paraben. Chromatogram ofMethyl andropyl Parabenstandard drug is shown in Figure 5.

Validation of the method

Precision

The repeatability of sample injection and measurement of peak area were expressed in terms of %RSD and results are depicted in Table No. 3, which revealed intra and inter-day variation of Methyl and Propyl Paraben.

Recovery studies

The proposed method when used for extraction and subsequent estimation of Methyl Paraben and Propyl Paraben from marketed sterile formulations after spiking with 80,100 and 120% of additional drug afforded recovery of 99.01-101.45% and 98.11-98.94% respectively as listed in table 4.

Stability of Methyl Paraben and Propyl Parabenin standard and test solutions

Under refrigerated condition (4^0 – 8^0 c) standard and test solutions remains stable for up to 30 days (Table 5, 6).

Analysis of Methyl Paraben and Propyl Paraben in marketed formulations

The Methyl Paraben and Propyl Paraben content in marketed formulation for marketed sterile formulations, % drug estimation was calculated and % RSD were found to be 0.0024 for MP, 0.058 for PP respectively. The % RSD were within limit.It may therefore be inferred that the marketed formulations can be analysed using this new and simple method. The low %RSD value indicated the suitability of this method for routine analysis of Methyl Paraben and Propyl Paraben in sterile dosage forms.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed HPLC method. The LOD is the smallest concentration of the analyte that gives ameasurable response (signal to noise ratio of 3). The LOD for Methyl Paraben and Propyl Paraben was found to be 0.016 and 0.047 respectively. TheLOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ for Methyl Paraben and Propyl Paraben was found to be0.049 and 0.142. It was concluded that the developedmethod is sensitive.

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generateresults of acceptable accuracy and precision. The requirements for system suitability are usuallydeveloped after method development and

validation have been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates, Resolution (R), Tailing factor (T),LOD (mg/ml), LOQ (mg/ml) were calculated and compared with standard values to ascertain whether theproposed HPLC method for the estimation of Methyl Paraben and Propyl Paraben in sterile pharmaceutical formulations was validated or not. The results are recorded inTable-7.

From the optical characteristics of the proposed method it was found that the drug obeys linearity range within the concentration of 18-90 µg/ml for methyl Paraben and 2-10µg/ml for propyl paraben. From the results shown in precision it was foundthat the percent RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy it was fond that the percent recovery values of pure drugfrom the preanalysed solutions of sterile formulations were in between 99.01-101.45% and 98.11-98.94% for Methyl Paraben and Propyl Paraben respectively which indicates that the method is accurate. The system suitability parameters are within the specified limits andwhich refers the commonly used excipients and additives present in the sterile pharmaceuticalformulations did not interfere in the proposed method. The proposed method was found to besimple, precise, accurate and rapid for determination of Methyl Paraben and Propyl Paraben from pure and sterile dosage forms. The mobile phase is simple and economical.

CONCLUSION

A convenient and rapid HPLC method has been developed for estimation of Methyl Paraben and Propyl Paraben in bulk drug and sterile dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and interday % RSD coupled with excellent recoveries. Hence, this method can beeasily and conveniently adopted for routine analysis of Methyl Paraben and Propyl Parabenin pure form and its dosageforms and can also be used for dissolution or similar studies.

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Table. 1. Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	LC-10 AT VP Shimadzu Liquid Chromatograph- HPLC with Hyper ODS 2 C18 (size 4.6mmx250mm, particle size 5µm) column
Mobile Phase	Acetonitrile(HPLC Grade) : 1 % w/v ammonium chloride (1:1)
Flow rate (ml/min)	01 ml/min.
Run time (minutes)	10
Volume of injection loop (µl)	20
Detection wavelength (nm)	254
Drug RT (min)	3.5(Methyl Paraben), 5.2(Propyl Paraben)

Table 2. Linear regression data for the calibration curve

Parameters	Results	
	MP	PP
Linearity range (µg/ml)	18-90	2-10
Correlation coefficient	0.999	0.998
Slope ± S.D.	75.74	70.26
Y intercept	57.86	15.99

Table 3. Intra and Interday precision of HPLC method

Sr. No.	Sample No.	% Drug Estimation			
		Interday		Intraday	
		Methyl Paraben	Propyl Paraben	Methyl Paraben	Propyl Paraben
1.	I	101.20	98.98	101.20	98.94
2.	II	101.22	99.20	101.22	98.99
3.	III	101.24	99.49	101.21	98.98
	Mean	101.22	99.22	101.21	98.97
	±S.D.	0.01613	0.2083	0.008165	0.0216
	R.S.D.	0.01613	0.2104	0.00806	0.2180

Table 4. Recovery studies of mixture of Methyl Paraben and Propyl Paraben

Sr. No.	Conc.(µg/ml) MP PP	Amount of Pure drug added (µg/ml) MP PP	Peak area of MP(mv)	Peak area of PP(mv)	% Recovery* MP PP
1	90 10	72 8	12390.85	1290.85	101.45 98.92
2	90 10	90 10	13890.35	1428.35	101.11 98.11
3	90 10	108 12	14970.28	1590.68	99.01 98.94
				Mean	100.52 98.65
				±S.D.	0.9852 0.3866
				R.S.D.	0.9801 0.3981
* Each value is the mean of five observations					

Table 5. Results of Std. solution stability

Standard no.	1		2	
	MP	PP	MP	PP
Concentration(µg/ml)	90	10	90	10
Preparation date	04/02/2017		05/03/2017	
Mean area (mv)	6840.2853	690.80	6841.58689.80	
RSD	0.0024	0.058	0.00620.0754	

Table 6. Results of sample solution stability

Test no.	MP	PP	MP	PP
Concentration	90 mcg/ml	10mcg/ml	90mcg/ml	10 mcg/ml
Preparation date	06/02/2017	06/02/2017	09/03/2017	09/03/2017
Mean Assay	100.00	100.06	100.05	100.15
RSD	0.0024	0.058	0.0024	0.058

Table. 7. System Suitability Parameters

Sr.No.	Parameters	Obtained Value	
		MP	PP
1	Theoretical plates (N)	5037.35035.8	
2	Resolution (R)	-	2.311
3	Tailing factor (T)	1.4	0.9
4	LOD (mcg/ml)	0.0160.047	
5	LOQ (mcg/ml)	0.0490.142	

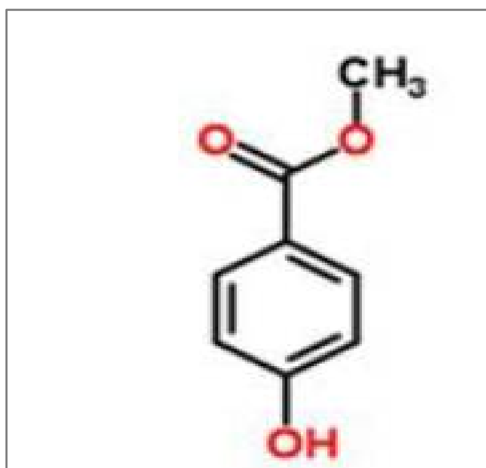

Fig. 1- Structure of Methyl Paraben

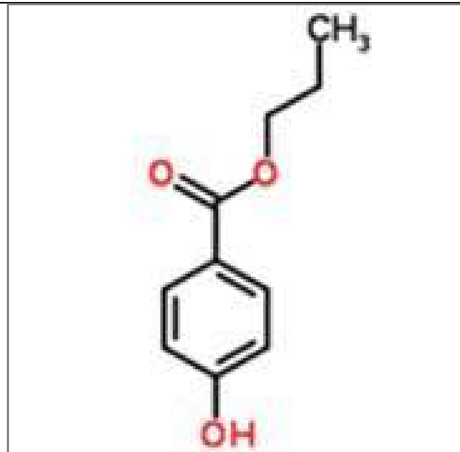

Fig.2. Structure of Propyl Paraben

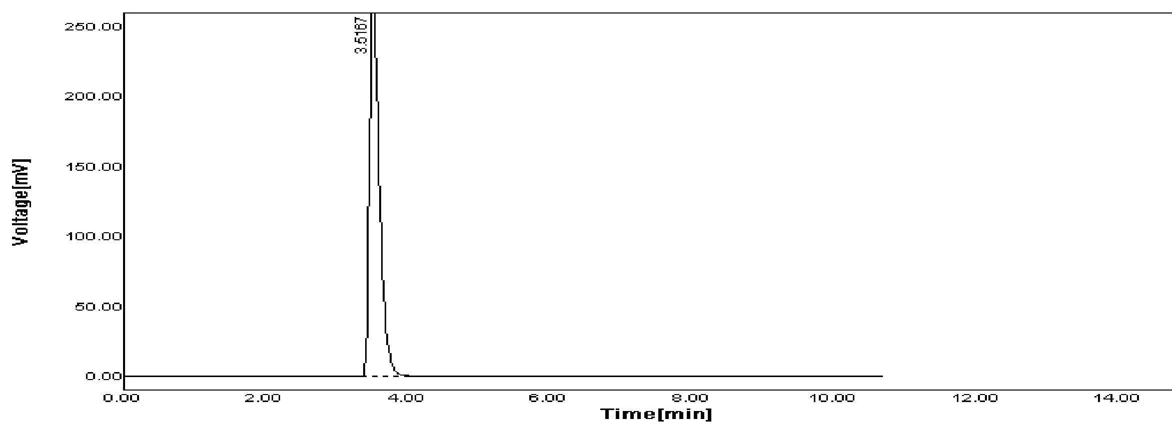

Fig.3 Chromatogram of Methyl Paraben standard (50µg/ml)

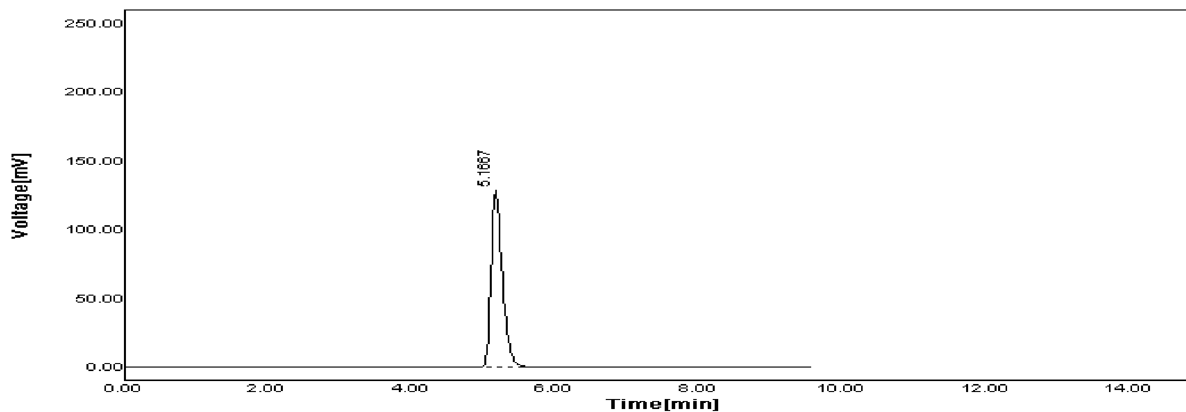

Fig. 4 Chromatogram of Propyl Paraben standard (5µg/ml)

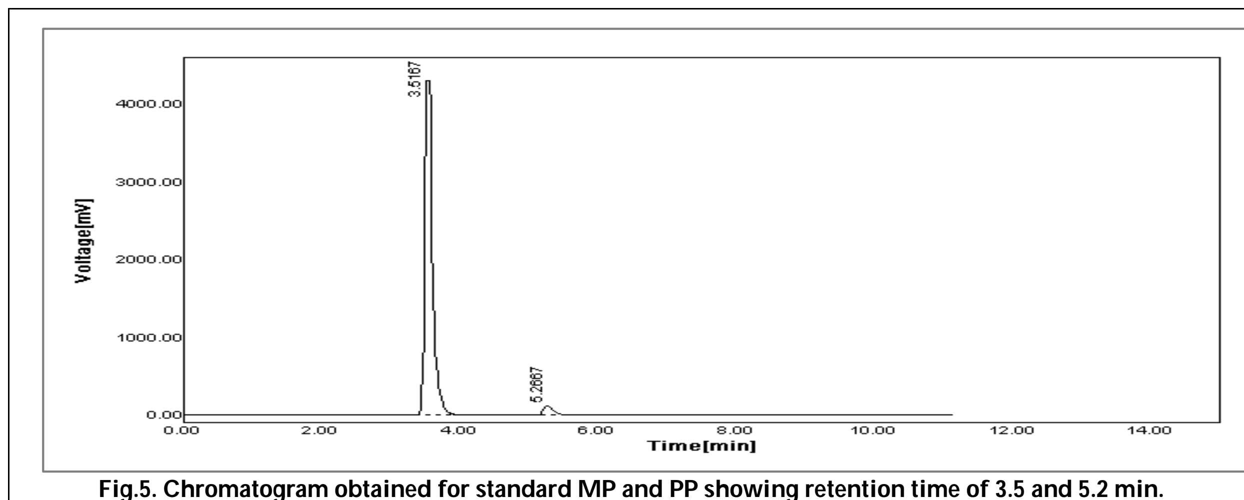

Fig.5. Chromatogram obtained for standard MP and PP showing retention time of 3.5 and 5.2 min.

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RESEARCH ARTICLE

An Inventory Control Model for Deteriorating Items and Break-Ability Consideration without Shortage Cost

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ABSTRACT

This is paper investigates optimal ordering policy in an inventory control system under the effect of time value of money and inflation on inventory at store. We proposed an economic order quantity model to manage deteriorating and break-ability rates for items which have both characteristics at the same time. Here we assumed that the finite horizon planning under which back ordering and delay in payment. The demand ,deterioration and break-ability rates are constant .The present value of total cost during the planning horizon in this inventory system is modelled first ,then a three phases solution procedure is proposed to derive the optimal order ,the number of replenishment during the planning horizon .Finally analysis is reported to find some managerial insights. For simplicity purpose we have used regression technique to fit the model for purposed study.

Keywords: Inventory-deterioration-break-ability-total cost function (TC).

INTRODUCTION

In real world problem, there are many items having deterioration and break-ability characteristics at same time as the eggs and blub and each items packed in can made from glass as medicines and milk, some fruits as water melon and nosier, olive ,olive oil, these types of items require advanced model to manage the items have close validity as deterioration characteristic to sale at a lower price and same types of items but with break-ability characteristic .In general, deterioration is defined as the decay, damage, spoilage, evaporation and obsolescence of stored items and it results in decreasing usefulness, break-ability is defined as the broken items cannot use it under any circumstances the items in this case may have deterioration rate or break-ability or both at same time. So that the management and

holding of inventories of perishable items becomes an important problem for inventory managers' .In the same time delayed payment in an important form of financing for business in aboard range of industries and economies that well developed and are being used in financial markets. All above mentioned issues (deteriorating items, break-ability items, delayed payment, inflation and time value of money) are separately regarded in some inventory models .It can show there are fewer researches which considered topics are mentioned together. Also there is no research in which all of them had been considered that deterioration rate with break-ability rates in same models [1] .For instance Ghare and Schrader [2] were the pioneers to establish an inventory model for deteriorating items.Goyal [3] firstly explored a single items EOQ model under permissible delay in payment and Chung [4] simplified the search of the optimal solution for the problem explored by Goyal.

Ray and Chaudhuri [5] developed a finite time horizon EOQ with backordering where the varying demand rate whereas the effects of inflation and time value of money are taken into account. Covert and Philips [6] and Misra [7] have used a variable deterioration rate of two parameters Weibull distribution to formulated the model with assumptions of a constant demand rate and no shortages allowed.Elsayed and Teresi [8] extended their works by allowing shortages and using a time varying demand rate. Time varying demand patterns are commonly used to reflect sales in different phases of product life cycle. Donaldson [9] the inventory model with linear trend in demand was initially developed by him.Buzacott [10] initiated a pioneer research in this direction. Bier-man and Thomas [11] investigated the effects of inflationary trends and time discounting on an EOQ model with the aim of minimizing the present value of future costs.Chandra and Bahner [12] studied the effect of time discounting and inflation on decision variables of the order level system with shortages and EOQ with a finite replenishment rate.Hariga [13] assumed model with constant deterioration rate, demand rate is time proportional, shortages are allowed.

Ouying and Yang [14] developed an inventory model for non-instantaneous deteriorating items with permissible delay in payment. When suppliers provide price break for bulk purchases or products are seasonal. Teng and Yang [15] generalized the partial backlogging EOQ model to allow for time –varying purchase cost.Rong and Mahaparta [16] developed an optimization inventory policy for a deteriorating item with imprecise lead time, partially or fully backlogged shortages and price dependent demand under two warehouse systems.Goyal and Giri [17] stated an excellent survey on recent trend in modelling of deteriorating inventory.Mandal and Maiti [18] developed an inventory model of breakable items with stock dependent demand and break-ability. Balkhi [19] developed an optimal solution of a general lot size inventory model with deteriorated and imperfect products, taking into account inflation and time value of money. Guria and Mondal& Maiti [20] are represent model for inventory policy for an item with inflation induced purchasing price and demand with immediate part payment. Liang and Zhou [21] developed model for a two warehouse inventory model for deteriorating items under conditionally permissible delay in payment. Dye [22] developed model for Joint Pricing and Ordering Policy for a Deteriorating Inventory with Partial Backlogging. In next sections, sub-sequent assumptions and notations are stated proposed model is derived &finally feasibility of the model is checked numerically for the efficiency of the model.

Assumptions and notions

Assumptions

The mathematical model in this paper is developed with the following assumptions
Planning horizon is finite.

1) Single item inventory control is mentioned.
2) Demand and deterioration and break-ability rates are constant.
3) Deterioration and break-ability occur as soon as the items are received into inventory.
4) There is no replacement or repair of breakable items during the period under consideration.
5) Shortage is not allowed.

6) Delayed payment strategy is offered by the seller.
7) Inflation rate is constant and time value of money is considered.
8) The lead time is zero.
9) The inventory level at the end of planning horizon will be zero.
10) The cost factors are deterministic.
11) The number of replenishments is restricted to one integer.
12) The total relevant cost consists of fixed ordering, purchasing, holding interest payable, interest earned from sales revenue during the permissible period.
13) The last order is only being placed to satisfy the shortage of last period.
14) The goal of the model is to determine the decision variables under which the net present value of total cost during the horizon planning is minimized.

Under the above assumptions following notations are used to develop the model where (j=0, 1, 2... N).

Notations

C_s = The present value of shortage cost during first replenishment cycle.
C_p = The present value of holding cost during first replenishment cycle.
IC = The interest payable during the first replenishment cycle.
IE = The interest earned during the first replenishment cycle.
Q = The order quantity in the each replenishment.
TC_A =The total fixed ordering cost during (0, b).
TC_h =The total holding cost during (0, b)
TC_p = The total purchasing cost during (0, b).
TIC =The total interest cost during (0, b).
TIE =The total interest earned during (0, b).
TC = The total relevant cost during (0, b).

Parameters

A = The fixed ordering cost per replenishment, $\order.
C =The unit purchasing price at time zero, $\order.
$C(t)$ = The unit purchasing price at time t, $C(t)=Ce^{-Rt}$.
D = The constant demand rate per unit time.
b =The length of the finite planning horizon.
i =The constant inflation rate.
$I(t)$ =The inventory level at time t.
I_C = The interest charged per $per year by the supplier.
I_e = The interest earned per $per year by the purchaser.
I_h = The holding cost rate per unit time excluding interest charges.
M = The permissible delay in setting account.
r = The discount rate representing the time value of money.
$R = r - 1$, representing the net constant discount rate of inflation.
T = The length of each replenishment cycle.
T_j = The total time that elapsed up to, including interest charges.
t_j = The time at which the inventory level in the jth .
V = The unit selling price at time t.
$V(t)$ =The selling price per unit at time t, $V(t)=Ve^{-RT}$
θ =The constant deterioration rate, units/unit time.
ϕ = The constant break-ability rate, units/unit time.

Mathematical model

Let $I(t)$ is the inventory level at any time t, $0 \le t \le t_1$, Depletion due to demand and deterioration, break-ability will occur simultaneously. The first order differential equation that describes the instantaneous state of $I(t)$ over the open interval (0, b) is given by.

$$\frac{dI(t)}{dt} + \theta I(t) + \phi I(t) = -D, \quad 0 \le t \le t_1 \tag{1}$$

$0 \le \theta \le 1, 0 \le \phi \le 1$

$\frac{dI(t)}{dt} = -D, t_1 \le t \le T$, Where $I(t_1) = 0$ for equation of number

$$I(t) = I_0(t) \int_t^{t1} De^{(\theta+\phi)u} du = \left(\frac{D}{\theta+\phi}\right)\left(e^{(\theta+\phi)(t1-t)} - 1\right), I_0(t) = e^{-\theta t}$$

$$I(t) = \left(\frac{D}{\theta+\phi}\right)\left(e^{(\theta+\phi)(t1-t)} - 1\right) \tag{2}$$

According to equation (2) the maximum inventory quantity at the begin each period is given as

$$Q = \left(\frac{D}{\theta+\phi}\right)\left(e^{(\theta+\phi)t1} - 1\right) \qquad t_1 = \frac{Fb}{N} \tag{3}$$

Then, we obtained the following
Special phases are

i) If $\theta = 0$ then $I(0) = \frac{D}{\theta}\left(e^{\theta t1} - 1\right)$

ii) If $\phi = 0$ then $Q = \frac{D}{\theta}(e^{\theta t1} - 1)$

iii) If $\theta = \phi = 0$ then to find value of Q as the following

$$Q = \lim_{(\theta,\phi)\to(0,0)} \left(\frac{D}{\theta+\phi}\right)\left(e^{(\theta+\phi)(\frac{Fb}{N})} - 1\right) = D(\frac{Fb}{N})$$

Fixed ordering cost

We assumed the number of replenishment is N so that the fixed ordering cost over the planning horizon under the inflation consideration is:

$$TCA = \sum_{j=0}^{N} A\,jT = \sum_{j=0}^{N} Ae^{-jRT} = A\left[\frac{e^{-(N+1)RT} - 1}{e^{-RT} - 1}\right], \quad T = \frac{b}{N} \tag{5}$$

Then

$$TC_A = \sum_{j=0}^{N} A_{jT} = \sum_{j=0}^{N} Ae^{-jRT} = A\left[\frac{e^{-(N+1)\frac{Rb}{N}}-1}{e^{-\frac{Rb}{N}}-1}\right] \quad (6)$$

Special case for total fixed ordering cost

Following are the cases for total fixed ordering cost we obtained when
i) Number of replenishment is zero I.e. there is no replenishment

$$N \to 0, TC_A = Ae^{-Rb}$$

ii) Number of replenishment is infinite i.e.

$$N \to \infty, TC_A = Ae^{-Rb}$$

Holding cost excluding interest cost

We find the average inventory quantity to obtain holding cost

$$\bar{I} = \int_0^{t1} I(t)dt = \int_0^{t1} \frac{D}{\theta+\phi}\left[e^{(\theta+\phi)(t_1-t)}-1\right]dt = \frac{D}{(\theta+\phi)^2}\left[e^{(\theta+\phi)t1}-(\theta+\phi)t_1-1\right] \quad (7)$$

By using equation number of (7) we have obtained holding cost is as follows

$$TC_h = \sum_{j=0}^{N-1} I_hC_j\bar{I} = \sum_{j=0}^{N-1} I_hC_e^{-RjT} = \frac{I_hCD}{(\theta+\phi)^2}\left[e^{(\theta+\phi)t1}-(\theta+\phi)_{t1}-1\right]\left[\frac{e^{-NRT}-1}{e^{-RT}-1}\right] \quad (8)$$

Since $T = \frac{b}{N}$ then equation number (9) it will be as

$$TC_h = \frac{I_hCD}{(\theta+\phi)^2}\left[e^{(\theta+\phi)t1}-(\theta+\phi)_{t1}-1\right]\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right] \quad (9)$$

Purchasing cost

According to figure (1) of inventory level the purchasing cost of jth cycle is calculated as

$$C_P(j) = C_jI_m = C_j\left[\frac{D}{\theta+\phi}\left(e^{(\theta+\phi)\frac{Fb}{N}}-1\right)\right], j = 1,2,3,\ldots\ldots,N \quad (10)$$

The total purchasing cost over the planning horizon can be obtained as

Special case for total purchasing cost when

$$TC_p = \sum_{j=0}^{N-1} C_P(j) = \frac{CD}{\theta+\phi}\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right], T=\frac{b}{N} \quad (11)$$

i) Rb=0 then R=0,b ≠ 0 then

$$TC_p = \frac{NCD}{\theta+\phi}$$

ii) $Rb \to \infty$ then $R \to \infty, b < \infty$

$$TC_p = \frac{CD}{\theta+\phi}$$

Interest charged and earned

To obtain interest charged and earned we have assumed two possible cases $M \le t1$, $M > t1$ which are described as

Case 1 $M \le t1$

Interest earned

For the items are sold and before the replenishment settled count, the sales revenue is used to earn interest .The backordered quantity should be replenished first and the maximum accumulated sold until M will be $\int_0^M Dt_1 dt_1$ in this case the interest earned for the first cycle based in figure(2) which is obtained as

$$IE1 = I_e V(t)\int_0^M Dt_1 dt_1 = V(t) I_e\left[\frac{DM^2}{2}\right] \quad (12)$$

So that total interest earned under assumption $T=\frac{b}{N}$ is given as

$$TIE1 = \sum_{j=0}^{N-1} IE1(j) = \sum_{j=0}^{N-1} IE1 e^{-jRT} = VI_e\left[\frac{DM^2}{2}\right]\left[\frac{e^{-Rb}-1}{e^{-\frac{Rb}{N}}-1}\right] \quad (13)$$

Calculating interested charged and earned when the following are

$TC1 = TC\,A + TC\,h + TC\,p - TIE1$

Then,

$$TC1 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\begin{array}{l}\frac{CD}{\theta+\phi}(e^{(\theta+\phi)t_1}-1)+\\ \frac{CD\,I\,h}{(\theta+\phi)^2}(e^{\frac{(\theta+\phi)Fb}{N}}-\frac{(\theta+\phi)Fb}{N}-1)\\ +\frac{CD}{\theta+\phi}(e^{\frac{(\theta+\phi)Fb}{N}}-1)\\ +\frac{CD\,I\,c}{(\theta+\phi)}(\frac{e^{(\theta+\phi)(t_1-M)}}{(\theta+\phi)}\\ +M-\frac{1}{(\theta+\phi)}-t_1)-VI\,e(\frac{DM^2}{2})\end{array}\right]\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right] \tag{14}$$

Case 2 $M > t_1$

Interest earned

Similar to the case (1), because t the shortage is not allowed so that the backordered quantity is zero, its interest earned will be zero. Then the maximum accumulated sold up to M is equal to $\int_0^{t_1} Dt_1 dt_1$ while the interest earned will be $\left[(M-t_1)\,Dt_1+\frac{Dt_1^{\,2}}{2}\right]I_eV$

Based in figure (3) the interest earned for the first cycle be:

$$IE_2 = \left[(M-t_1)\,Dt1+\frac{Dt_1^{\,2}}{2}+\int_0^{t_1} Dt_1dt_1\right]I_eV = I_eV\left[(M-\frac{Fb}{N})\frac{DFb}{N}+\frac{DF^2b^2}{2N^2}\right] \tag{15}$$

The total interest earned for second case over horizon planning where $T=\frac{b}{N}$ is

$$TIE_2=\sum_{j=0}^{N-1}IE(j)=\sum_{j=0}^{N-1}IE_2e^{-jRT}=I_eV\left[(M-\frac{Fb}{N})D\frac{Fb}{N}+\frac{DF^2b^2}{2N^2}\right]\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right] \tag{16}$$

Interest charged

In this case the number of items are in stock be zero when the replenishment account is settled so that the total cost function is obtained as

$$TC2 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\begin{array}{l}\frac{CDIh}{2(\theta+\phi)}(e^{\frac{(\theta+\phi)Fb}{N}}-\frac{(\theta+\phi)Fb}{N}-1)\\ +\frac{CD}{\theta+\phi}(e^{\frac{(\theta+\phi)Fb}{N}}-1)-\frac{DVbI_e}{N}(M(1-F)+\\ (M-\frac{Fb}{2N})F)\end{array}\right]\left[\frac{e^{\frac{-Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]$$

Economic order quantity

Economic order quantity when $M \le t_1$

To find EOQ by minimizing the total cost function as the following

$$TC1 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\begin{array}{l}CQ\\ +\frac{CDIh}{(\theta+\phi)^2}(e^{\frac{(\theta+\phi)Fb}{N}}-\frac{(\theta+\phi)Fb}{N}-1)+\frac{CD}{\theta+\phi}(e^{\frac{(\theta+\phi)Fb}{N}}-1)\\ +\frac{CDIc}{(\theta+\phi)}(\frac{e^{(\theta+\phi)(t_1-M)}}{(\theta+\phi)}\\ +M-\frac{1}{(\theta+\phi)}-t_1)-VIe(\frac{DM^2}{2})\end{array}\right]\left[\frac{e^{\frac{-Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]$$

Since $t1=\frac{Fb}{N}$

$Q=\left(\frac{D}{\theta+\phi}\right)\left(e^{(\theta+\phi)t1}-1\right), by$ Utilizing a truncated Taylor series for the

$e^{(\theta+\phi)t_1}, about$ $t_1=0$, then

$e^{(\theta+\phi)t_1}=1+(\theta+\phi)t_1+\frac{(\theta+\phi)^2t^2}{2}$, after simplifying we can write Q as

$$Q = e^{(\theta+\phi)t_1} = \frac{D}{\theta+\phi}\left[\left[(\theta+\phi)t_1 + \frac{(\theta+\phi)^2 t_1^2}{2}\right] = \left[Dt_1 + \frac{D}{2}(\theta+\phi)t_1^2\right]\right]$$

$$Q = \frac{DFb(\theta+\phi)}{N}\left[\frac{Fb(\theta+\phi)}{2N}+1\right] \tag{18}$$

$$TC1 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right] + \left[\begin{array}{l} CQ \\ +\frac{CD_{Ih}}{2(\theta+\phi)^2}t_1^2 + \frac{CD}{\theta+\phi}(e^{\frac{(\theta+\phi)Fb}{N}}-1) + \\ CD_{Ic}\frac{(t_1-M)^2}{2} - VI_e(\frac{DM^2}{2}) \end{array}\right]\left[\frac{e^{\frac{-Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right] \tag{19}$$

By substituting the equations (18) and (19) in the equation (14), then it can be rewritten as

$$TC1 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right] + \left[\begin{array}{l} CQ + \frac{CD_{Ih}}{2(\theta+\phi)^2}\left(\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{1}{2}}-1)\right)^2 \\ +\frac{CDI_c}{2}\left(\begin{array}{l}\frac{1}{\theta+\phi}\left(\frac{2(\theta+\phi)Q}{D}+1)^{\frac{1}{2}}-1)-M\right)^2 \\ -VI_e(\frac{DM^2}{2}\end{array}\right)\end{array}\right]\left[\frac{e^{\frac{-Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right] \tag{20}$$

By taking derivate the equation (20) w.r.t Q to find out the minimum value of total cost function then

$$\frac{dTC_1}{dQ} = 1 + \frac{I_h + I_C}{(\theta+\phi)} - \left[\frac{I_h+I_C}{(\theta+\phi)} + MI_c\right]\left(\frac{2Q}{D}+1\right)^{\frac{-1}{2}} = 0$$

$$Q^* = \frac{D}{2(\theta+\phi)}\left[\left[\frac{I_h + I_c + M(\theta+\phi)I_c}{I_h + I_c + (\theta+\phi)}\right]^2 - 1\right] \tag{21}$$

$$t_1^* = \frac{1}{\theta+\phi}\left[\left(\frac{2(\theta+\phi)Q^*}{D}+1\right)^{\frac{1}{2}} - 1\right] = \frac{1}{\theta+\phi}\left(\left[\frac{I_h + I_c + M(\theta+\phi)I_c}{I_h + I_c + (\theta+\phi)}\right] - 1\right) \tag{22}$$

To test the type of critical point at second derivative for total cost with respect to Q as

$$\left.\frac{d^2TC_1}{dQ^2}\right|_{Q=Q^*}=\frac{1}{2}\left[\frac{I_h+I_c+M(\theta+\phi)I_c}{(\theta+\phi)}\right]\left[\frac{2(\theta+\phi)Q}{D}+1\right]^{\frac{-3}{2}}\geq 0$$

So that the total cost function has minimum value at the point Q^*.

The period of first run

$$t_1^*=\frac{1}{\theta+\phi}\left[\left(\frac{2(\theta+\phi)Q^*}{D}+1\right)^{\frac{1}{2}}-1\right]=\frac{1}{\theta+\phi}\left(\left[\frac{I_h+I_c+M(\theta+\phi)I_c}{I_h+I_c+(\theta+\phi)}\right]-1\right) \tag{23}$$

Economic order quantity when $M>t_1$

Similarly to case (2) when $M>t1$

Since $Q=\frac{DFb(\theta+\phi)}{N}\left[\frac{Fb(\theta+\phi)}{2N}+1\right]$

$$t_1=\frac{1}{\theta+\phi}\left[\left(\frac{2Q}{D}+1\right)^{\frac{-1}{2}}-1\right]$$

$$TC2=A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\begin{array}{l}\frac{\frac{CDIh}{2}}{(\theta+\phi)}\left(e^{\frac{(\theta+\phi)Fb}{N}}-\frac{(\theta+\phi)Fb}{N}-1\right)\\+\frac{CD}{\theta+\phi}\left(e^{\frac{(\theta+\phi)Fb}{N}}-1\right)\\-\frac{DVbI_e}{N}\left[M(1-F)+\left(M-\frac{Fb}{2N}\right)F\right]\end{array}\right]\left[\frac{e^{\frac{-Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]$$

Then

$$TC_2=A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\frac{CDI_ht_1^2}{2}+CQ-VI_e\left[\frac{MDt_1}{F}-MDt_1^2+\frac{Dt_1^2}{2}\right]\right]\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right]$$

The equation can be written as

$$TC_2 = A\left[\frac{e^{\frac{-(N+1)Rb}{N}}-1}{e^{\frac{-Rb}{N}}-1}\right]+\left[\begin{array}{l}\frac{CDI_h}{2(\theta+\phi)^2}\left[\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{1}{2}}-1\right]\\ +CQ-VI_e\left[\begin{array}{l}\frac{MD}{(\theta+\phi)F}\left(\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{1}{2}}-1\right)\\ +D(\frac{1-2M}{2})\left(\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{1}{2}}-1\right)^2\end{array}\right]\end{array}\right]\left[\frac{e^{-Rb}-1}{e^{\frac{-Rb}{N}}-1}\right]$$

By derivation the equation (22)

$$\frac{dTC_2}{dQ}=\frac{CI_h}{(\theta+\phi)}\left[1-\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{-1}{2}}\right]+Q-\frac{VI_eM}{F}\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{-1}{2}}-\frac{VI_e}{(\theta+\phi)}(1-2M)\left[1-\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{-1}{2}}\right]=0$$

Then we obtained extreme value for total cost function at second case

$$Q^*=\left[\frac{\frac{1}{(\theta+\phi)}(CI_h-VI_e(1-2M))+\frac{VMI_e}{F}}{\frac{1}{(\theta+\phi)}(CI_h-VI_e(1-2M))+C}\right]^2-1 \qquad (24)$$

If we test the extreme value Q*in the second derivative, then we find out it is minimum value for total cost function as

$$\left.\frac{d^2TC_2}{dQ^2}\right|_{Q=Q^*}=\frac{(\theta+\phi)}{D}\left(\frac{1}{(\theta+\phi)}(CI_h-VI_e(1-2M)+\frac{VMI_e}{F})\right)\left(\frac{2(\theta+\phi)Q}{D}+1\right)^{\frac{-3}{2}}>0$$

So that

$$Q^*=\left[\frac{\frac{1}{(\theta+\phi)}(CI_h-VI_e(1-2M))+\frac{VMI_e}{F}}{\frac{1}{(\theta+\phi)}(CI_h-VI_e(1-2M))+C}\right]^2-1$$

Is the minimum value for total cost function.

And we obtained period of first run time t_1^*

$$t_1^* = \frac{1}{(\theta+\phi)}[(\frac{2(\theta+\phi)Q^*}{D}+1)^{\frac{1}{2}}-1]$$

$$= \frac{1}{(\theta+\phi)}\left[\frac{2(\theta+\phi)}{D}\left[\frac{\frac{1}{(\theta+\phi)}(CI_h - VI_e(1-2M)) + \frac{VMI_e}{F}}{\frac{1}{(\theta+\phi)}(CI_h - VI_e(1-2M)) + C}\right]^2 - \frac{2(\theta+\phi)}{D}+1)^{\frac{1}{2}}-1\right] \quad (25)$$

Numerical examples

There are two data tables the first data table explains the sensitivity analysis for first case3.4.1. And the second data table explains the sensitivity analysis for the second case3.4.2. As the following

Example (1)

$D = 2000, I_c = 7, I_h = 20, R = 0.05, b = 2, V = 5, A = 100, I_e = 0.06, M = 0.167, C = 3$

Table 1.The sensitivity analysis 3.4.1

Example (2)

$A = 100, D = 2000, I_h = 20, R = 0.05, b = 5, N = 4, V = 5, A = 100, I_e = 0.89, M = 0.95, C = 3, F = 0.7$

Table .2. The sensitivity analysis 3.4.2

CONCLUSION

In the above assumed mathematical model we found some realistic features .The deterioration rate and break-ability rate are affected in inventory control system at the same time. We have developed the mathematical model to manage the inventory control system problems for items have the following characteristics deterioration and break-ability as decreasing its quality in this case we can used them with lower quality and breakable items in this case we can used that items when the shortage is not permitted and delay payment is allowed for supplier. We assumed the deterioration rate and break-ability rate and demand are constant this inventory control system with considering two obtained cases for the period of first run. According to two cases there are two tables and two examples to illustrate the sensitivity analysis for the performance of the model parameters. At the first case 3.4.1. The interest charged and the holding cost rates are more than one to obtain the feasible value for the actual quantity, the relationship between the optimal total cost function and deterioration rate is cubic but between the total cost function and optimal quantity is quadratic. At second case 3.4.2. The interest charged and the holding cost rates are less than one to obtain feasible value for quantity, the relationship between the optimal total cost function and deterioration rate is cubic and between optimal total cost and optimal quantity also. In sensitivity analysis 3.4.1, 3.4.2 the optimal quantity increases when deterioration and break-ability rate increase with each other at the same time.

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Example (1)

$$D = 2000, I_c = 7, I_h = 20, R = 0.05, b = 2, V = 5, A = 100, I_e = 0.06, M = 0.167, C = 3$$

Table 1. The sensitivity analysis for 3.4.1.

θ	ϕ	M	D	I_c	I_h	N	Q*	TC1*
0.005	0.005	0.167	2000	7	20	3	0.001251	5307.401
0.05	0.05	0.167	2000	7	20	3	0.124762	3794.689
0.1	0.1	0.167	2000	7	20	4	0.497368	-1040.15
0.2	0.2	0.167	2000	7	20	4	1.976157	-25747.1
0.3	0.3	0.167	2000	7	20	3	4.416794	-49684.5
0.4	0.4	0.167	2000	7	20	4	7.800216	-124574
0.5	0.5	0.167	2000	7	20	4	12.10786	-198694
0.6	0.6	0.167	2000	7	20	4	17.32164	-289284
0.8	0.8	0.167	2000	7	20	3	30.39757	-385832
0.9	0.9	0.167	2000	7	20	3	38.22582	-489730
1	1	0.167	2000	7	20	3	46.89238	-605851
0.5	0.5	0.167	2000	7	20	3	12.10786	-147474
0.55	0.55	0.167	2000	7	20	3	14.60259	-179561
0.555	0.555	0.167	2000	7	20	3	14.86444	-182937
0.6	0.6	0.167	2000	7	20	3	17.32164	-214703
1	1	0.167	2000	7	20	3	46.89238	-605851
0	0	0.167	2000	7	20	3	0	5322.681
0.005	0.005	0.167	2000	7	20	3	0.001251	5307.401
0.05	0.05	0.167	2000	7	20	3	0.124762	3794.689
0.1	0.1	0.167	2000	7	20	3	0.497368	-789.276
0.2	0.2	0.167	2000	7	20	4	1.976157	-25747.1
0.3	0.3	0.167	2000	7	20	4	4.416794	-66925.2
0.4	0.4	0.167	2000	7	20	3	7.800216	-92467.4
0.5	0.5	0.167	2000	7	20	4	12.10786	-198694

Example (2)

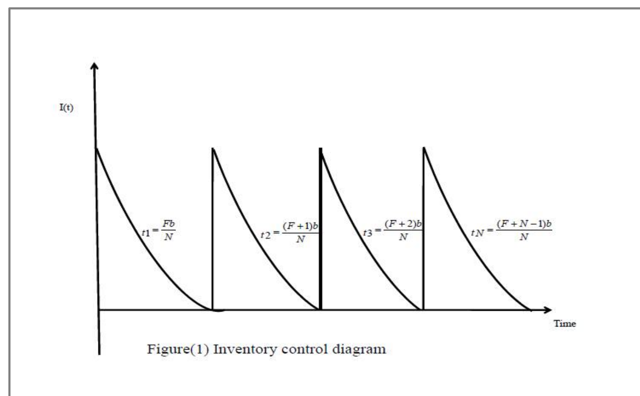
$$A = 100, D = 2000, I_h = 20, R = 0.05, b = 5, N = 4, V = 5, A = 100, I_e = 0.89, M = 0.95, C = 3, F = 0.7$$

Table 2. The sensitivity analysis for 3.4.2.

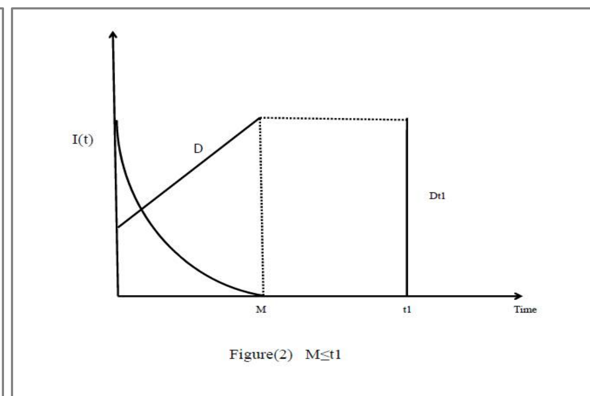
θ	ϕ	M	D	I_e	I_h	N	Q*	TC2*
0.005	0.005	0.167	2000	0.89	20	4	6.769507	133.7908
0.05	0.05	0.167	2000	0.89	20	4	70.22168	1354.375
0.1	0.1	0.167	2000	0.89	20	4	145.9424	2747.954
0.2	0.2	0.167	2000	0.89	20	4	313.1772	5662.64
0.3	0.3	0.167	2000	0.89	20	4	500.3619	8790.215
0.4	0.4	0.167	2000	0.89	20	4	706.2528	12333.99

0.5	0.5	0.167	2000	0.89	20	4	670.5063	11402.44
0.6	0.6	0.167	2000	0.89	20	4	604.5631	10281.31
0.8	0.8	0.167	2000	0.89	20	4	871.904	19601.14
0.9	0.9	0.167	2000	0.89	20	4	1015.595	28056.39
1	1	0.167	2000	0.89	20	4	1165.416	40453.86
0.5	0.5	0.167	2000	0.89	20	4	481.83	7757.836
0.55	0.55	0.167	2000	0.89	20	4	542.2337	8910.162
0.555	0.555	0.167	2000	0.89	20	4	548.381	9036.323
0.6	0.6	0.167	2000	0.89	20	4	604.5631	10281.31
1	1	0.167	2000	0.89	20	4	2275.552	100706.1
0	0	0.167	2000	0.89	20	4	0	infinite
0.005	0.005	0.167	2000	0.89	20	4	6.769507	133.7908
0.05	0.05	0.167	2000	0.89	20	4	70.22168	1354.375
0.1	0.1	0.167	2000	0.89	20	4	145.9424	2747.954
0.2	0.2	0.167	2000	0.89	20	4	313.1772	5662.64
0.3	0.3	0.167	2000	0.89	20	4	500.3619	8790.215
0.4	0.4	0.167	2000	0.89	20	4	706.2528	12333.99
0.5	0.5	0.167	2000	0.89	20	4	929.6986	16814.09

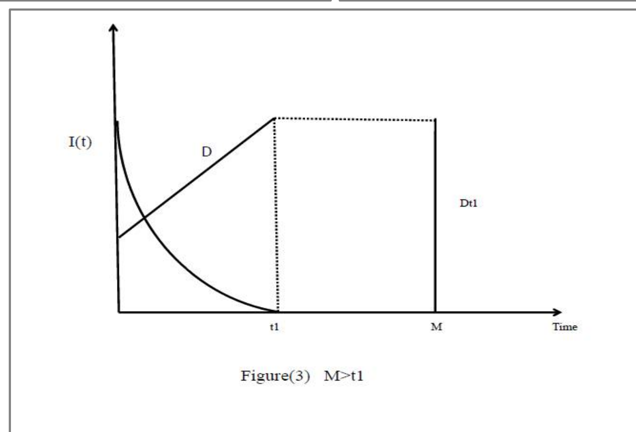
Based in table (1) the relationship between Q* and TC1 as figure (1) and θ and TC1 shown below as figure (2)



Figure(1) Inventory control diagram



Figure(2) M≤t1



Figure(3) M>t1

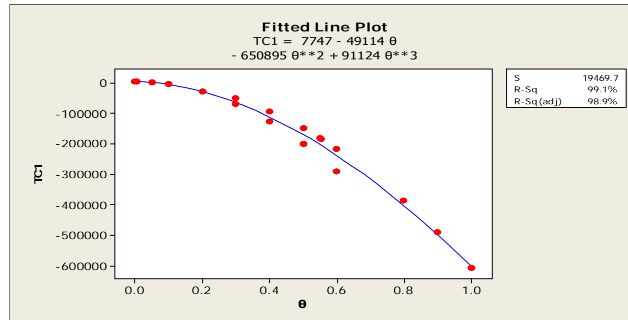


Fig.4 Fitted curve for TC1 and deterioration rate

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	7.63998E+11	254.70	0.000
Quadratic	1	5.83245E+10	159.75	0.000
Cubic	1	8.55441E+07	0.23	0.640

Based in table (2) the relationship between θ and TC2 and Q* and TC2 shown below as fig. (3) And as fig. (4)

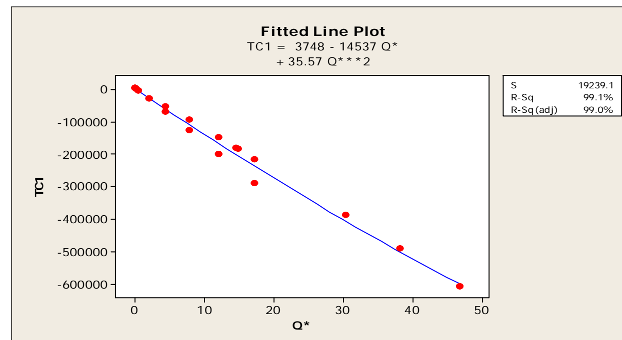


Fig.5 Fitted curve for TC1 economic order quantity

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	8.21060E+11	2022.97	0.000
Quadratic	1	1.15615E+09	3.12	0.092

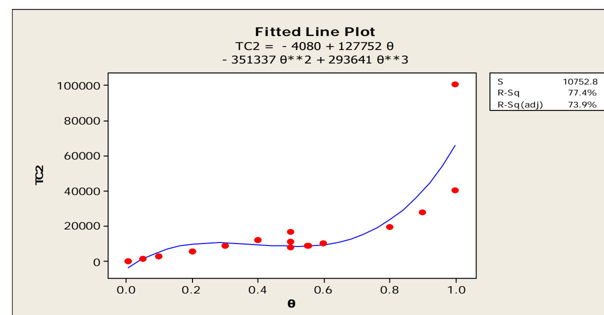


Fig.6. Fitted curve for TC2 and deterioration rate

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	5152264186	23.61	0.000
Quadratic	1	1567277397	10.40	0.004
Cubic	1	818124024	7.08	0.015

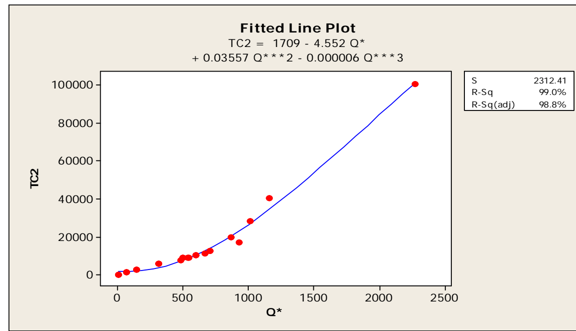


Fig.7. Fitted curve for TC2 economic order quantity

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	8616217853	161.80	0.000
Quadratic	1	990255191	154.71	0.000
Cubic	1	26419624	4.94	0.039

RESEARCH ARTICLE

Effect of ZrO_2 on Mechanical Strength, Antibacterial, and Anti Fungal of Epoxy Adhesive

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ABSTRACT

The aim of the research studying the effect of ZrO_2 additives on epoxy Use epoxy resins and added ZrO_2 by weight ratio (0,1,3,5,7, and 9)% for two methods in situ and solvent for Preparation nano composites. To make the mechanical strength (bending, hardness, andCompressive) , antibacterial , and anti fungal. The results of the mechanical tests showed that all of the above tests improved the specifications of epoxy resin with the addition of zirconium. The strength of the overlapping material was increased by increasing the percentage of zirconium to 5% and then the strength and stiffness of the material decreased by increasing the ratio (7, 9%) due to the phenomenon of aggregation of nanoparticles . whene added acetone with solvent method improves the mechanical strength of epoxy nano composites compared with in situ method .

Keywords : bending , hardness, compressive , anti bacterial , anti fungal.

INTRODUCTION

Polymer-matrix composites are very popular among the various industrial applications such as mechanical, electrical, automotive, semiconductor, and aerospace. This is because of the reason that the composite materials show a significant improvement in the properties such as chemical, electrical, mechanical, and coefficient of thermal expansion as compared to their original phase. Additives and fillers are widely used in the synthesizing process to enhanced the mechanical and thermal properties of polymers [1] Nanocomposite consist softwoor more components, one of them being matrix or continuous phase in which nanosized particles are dispersed. These nanoparticles, or nano fillers, constitute the second phase [2].Thermo setting epoxy polymers are widely used as adhesives, they are amorphous and highly cross linked, and the micro structure of these materials has many interesting properties for engineering application such as light weight, high failure strength, low creep, and interesting adhesive properties [3].

Zirconia shows good chemical resistance, excellent wear resistance and high hardness properties and also exhibits low thermal conductivity, high modulus, good strength and good fracture toughness [4].Nanoparticle uniformity and agglomeration: Based on their chemistry and electro-magnetic properties, nanoparticles can exist as dispersed aerosols, as suspensions/ colloids, or in an agglomerate state for example, magnetic nanoparticles tend to cluster, forming an agglomerate state, unless their surfaces are coated with a non-magnetic material. In an agglomerate state, nanoparticles may behave as larger particles, depending on the size of the agglomerate. Hence, it is evident that nanoparticle agglomeration, size and surface reactivity, along with shape and size, must be taken into account when deciding considering health and environmental regulation of new materials [5].

Preparation of polymer/Nano filler

Various approaches are available for each of the three synthesis methods, namely solution- blending, melt-blending and in-situ polymerization. An important task in the preparation of polymer/nanocomposite is to achieve a uniform dispersion of nanomaterial in the polymer matrix. The solution-blending technique often yields favorable dispersion in the polymer matrix, in comparison with melt blending, due to its low viscosity and high agitation power. On the other hand, melt blending is considered industrially viable and ecofriendly, with high economic potential [6].

Melt blending

Compared with other methods, melt blending is more attractive for the preparation of commercial products because it is convenient and environmentally friendly without using organic solvents [7]. For this method, researchers can mix polymer and nanocomposite directly. Polymers, such as polyurethane, polypropylene, poly (ethylene terephthalate), polystyrene, poly (ether ketone), styrene ethylene/ butylene-styrene triblock copolymer [8].

Solution blending

is the most extensive method for preparation of polymer/nanocomposites, especially in laboratory. There are three steps in solution blending. Firstly, disperse nanomaterial in a suitable organic solvent under sonication. Secondly, mix polymer in the solvent and make it dissolved. Thirdly, remove the solvent. A variety of nanocomposites, such as polytetrafluoroethylene composites [9].

In-Situ Polymerization

The melt-blending method often leads to insufficient filler dispersion, which causes aggregation and intercalation, particularly at high filler contents [10].in-situ polymerization is more effective in the formation of composites. Moreover, in-situ polymerization allows versatile molecular designs of the polymer matrix; it deliversan effective approach to the synthesis of different polymer/ Nanomaterial with an expanded property range and enables the design of the interface between the Nano clays and the polymers byflexible tuning of the matrix composition and structure [11].

MATERIALS AND METHODS

Materials

Epoxy as a matrix (Nitofill, EPLV with Nitofill EPLV hardener from Fosroc Company). Made in Jordan . The mixingratioforresinandhardeneris 3:1.Zirconuim oxide (ZrO_2) :- zirconia nanoparticles were purchased from Sigma Aldrich Germany , density 0.5 gm/ cm^3 ..Acetone: -BHD Chemical Ltd Poole, England, the purity 98 %

Atomic force microscopy analysis

The surface morphology of ZrO_2 powder nanoparticles was observed with AFM micrographs as shown in Figures(1).It has emerged that average diameter for ZrO_2 particles is 56.9 nm

Preparation samples of epoxy and epoxy / ZrO_2 nanocomposites by situ method

The solution of epoxy and hardener was formed with a ratio of 3:1.the hardener liquid was added slowly to the epoxy resin at room temperature, this mixture was stirred manually for 5 min , and the composition was left at room temperature for 24 hours today. To prepare epoxy/ ZrO_2 nanocomposites using situ polymerization method,ZrO_2 powder of percent 1,3,5,7,9% were added to the epoxy resin, then the resulting solution was put in a glass tube on a magnetic stirrer at 60 °C for one hour. After that, the composite was left at room temperature for 24 hours after mixing with hardener for 5 minutes.

Preparation of epoxy and epoxy / ZrO_2 nanocomposites with the solvent method

Epoxy resin was diluted by adding acetone and stirred for one hour the ratio of the epoxy/ acetone (10gm epoxy /1 ml acetone). After that, the composition was left at room temperature for 24 hours after mixing with hardener for 5 minutes.To prepare epoxy/ZrO_2 nanocomposites with solvent method, ZrO_2Nano powder of percent 1,3,5,7,9% were added to the epoxy solution was put in a glass tube on magnetic stirrer at 50°C for one hour. After that, the composition was left at room temperature for 24 hours after mixing with hardener for 5 minutes.

Mechanical testing

Flexural test

The flexural strength of the composite was evaluated performing three –point bending tests on the composite. the test was performed at room temperature, at a crosshead speed of 5 mm/min. on according with ASTM d790 standard procedure. [12]Make was Shimadzu-japan. A range of machine was 1-100KN.The flexural strength of composites was found out using the following equation and maximum shear stress are calculated according to the equation. [13] Figure (2) shows the samples epoxy and Nano composites

F. S= 3PL/2bd^2 ……. 2-2
t=3P/4bd ………. …….2-3

where :- F. S=flexural strength(MPa) , P=force at fracture(N), L=length of the sample between predicate (mm) , b=thickness(mm) ,d=width(mm) , t=maximum shear stress (Mpa)

Hardness test

Hardness test maybe defined as a material resistance to permanent indentation. Durometer (shore D), like much other hardness tests ASTM D2240 , measure the depth of an indentation in the material created by a given force on a standardized press foot. This depth is dependent on the hardness of the material.

Compressive test

One of the most interesting mechanical tests for materials is the diametrical compression of a solid disk. This test was performed on disk using a compression test device Intron, the sample was fixed between the upper and lower platens of the device to start compressing at a rate (cross-head speed =0.5 mm/min) until fracture occurs. Figure (3) shows epoxy and Nano composites

Evolution of antibacterial and antifungal activity

The antibacterial activity of the samples was evaluated using disc diffusion method with Muller–Hinton agar and sample membranes were cut into disc shapes of 6 mm diameter the discs were placed on the bacterial culture, and a determination of inhibition zone in millimeters(mm).The films were scrutinized against Gram-negative bacteria *Escherichia coli* (E.coli) and Gram-positive *Staphylococcus aureus* (*S.aureus*) in 37°C for 6 hours for pure epoxy and epoxy with ZrO_2 nanocomposites and the films was scrutinized against Anti-fungal *Penicillin* and *Aspergillus* in 37°C for 5-7 days.

RESULTS AND DISCUSSION

Flexural Strength

Flexural strength indicates the ability of the material to withstand bending forces applied perpendicularly to its longitudinal axis. From load-deflection or stress-strain curves in flexural test we can get the flexural strength, a flexural modulus and energy fracture for epoxy pure and EP/ZrO_2 nano composite. Show the values of flexural strength for the epoxy resin rein forced with ZrO_2 . It can be noticed that the values of flexural strength increased with increasing of the weight ratio of ZrO_2. This is due to the ability of these particles to hinder the crack propagation inside matrix according to the strengthening mechanism additionally to the strong bonding between the epoxy matrix and these particles. Furthermore, this increase may be due to the factthe flexural strength of ZrO_2 particles are much higher than epoxy matrix, The flexural strength of the composites increases as the filler addition increases up to 5 wt% for both methods and starts degrading beyond 5wt% this maybe due to the excess filler addition and which leads topoorbondingin the fillers shown in figure (4). More addition of ZrO_2 nanoparticles lead to reduce in space distance (decrease free space distance) between epoxy chains when the addition of ZrO_2 nanoparticles which are polarparticles [14]. Figure (5) shows the flexural modulus with increasing of ZrO_2 nanoparticles concentration of EP/ZrO_2 nanocomposites. Flexural modulus has increase beyond 1% wt. in situ method and 3% wt. solvent method, modulus reach maximum increase, in general all the value obtained after the addition of ZrO2 nanoparticles to epoxy were higher than that of epoxy especially at low concentration of ZrO2 nanoparticles, This behavior of EP/ZrO2 nanocomposite singood agreement with the behaviour obtained by K.S.Harishan et al. [15]. The increase of the concentration of ZrO_2 nanoparticles lead to rising constraint of epoxy chains when chains deflections and mobility will decrease, so all the results of flexural modulus were higher than that of epoxy.

Hardness test

The hardness gives us a good idea about the strength of the material. The results of the hardness test in figure (6) show the influence of ZrO_2 weight ratio the epoxy matrix. the hardness values increase with the increasing weight ratio of ZrO_2 up 5% in situ method and 3% in solvent method, the samples with higher concentration of nanoparticles showed higher resistance to the indentation force, therefore, exhibiting higher shore D values and this has helped to increase the area of contact between the components of the composites materials ,and zirconium dioxides high strength, high hardness and high toughness [16] .this could be attributed to the inherent hardness of the ZrO_2 particles compared with neat epoxy, as well as the interface effect between the matrix and the filler, also the

high adhesion between them . Which agree with [17]. Increase the values of hardness by adding but as soon as the loading of nanofiller increased beyond 5% in situ method, and 3% solvent method hardness value decreased. The hardness values increase by adding acetone because The addition of solvents to thermoset resins is a possible route to decrease resin viscosity, allowing a better distribution of fillers [18], including the acetone gave decrease the density of the cross-link in the polymer leading thus helps spread the nanoparticles between the epoxy bonds and to the increase of the values of the hardness.

Compressive strength

Compression strength increase in the epoxy matrix with increasing the ratio of ZrO_2 in 5%wt. both two methods(in situ and solvent).The Compressive strength of the epoxy pure sample was 7Mpa in situ method and 13 Mpa in the solvent method , which increased to a maximum value of 14 and 36 Mpa respectively .Increase in wt% beyond 5% caused a decrease in compressive strength trend is shown in figure (7)Increase in compressive strength with increase up to 5 wt % is a result of high strength of ZrO_2 which is transmitted uniformly to the host matrix due to very high interfacial area between resin matrix and nanoparticles assists in transfer of physical stress. Nanoparticles are uniformly dispersed in the matrix and occupy spaces between polymer chains decreasing mobility of the chains and increasing resistance of matrix to deformation and crack growth. The Possibility of air bubbles reduces by addition of ZrO_2 thus preventing crack initiation due to void spaces in the matrix. The decrease in compressive strength on increasing wt% beyond 5% was observed. This decrease can be due to agglomeration of nanoparticles and lack of resin material to accommodate the high content of ZrO_2. Cracks can initiate in such regions with stress concentrations present due to poorly dispersed reinforcement or lack of resin material to bond high content of reinforcement. Which agree with [17]. The formation of such H – bonds at the interface does not only affect the polymer, mechanical properties, but also the filler dispersion , limiting the aggregation that typically occurs with ZrO_2 .[18]

Antibactrial and Antifungal activity

Resin coat epoxy antibacterial and antifungal coating has been independently tested (iso 22196) and given according to biomaster. Our antibacterial and antifungal epoxy coating, reduction against the initial loading of bacteria for E-coil and S. aureus [19]. Epoxy and nano composites with ZrO_2 nanoparticles are tested for its antibacterial activity and antifungal against the bacterial pathogens, *S.aureus* (gram positive) and *E.coli* (gram negative) by Agar diffusion method, antifungal *Penicillium* and *Aspergillus*. Zone of inhibition values determined for epoxy and nanocomposites is shown in figure (8 a) .ZrO_2 nanoparticles pronounced significant growth inhibitory effect against both bacteria due to their large surface area by their nanosize .However ZrO_2 nanoparticles superior antibacterial activity against *E.coli* bacteria than with *S. aureus* bacteria which are clearly visualized in the anti bacterial photographs. This difference in antibacterial performance may be due to the following assumptions: active oxygen species generated from the ZrO_2- nanoparticles actively inhibits the growth of S.aureuscells by accumulation or deposition on the surface of S.aureuscells [20] . Antifungal images of Epoxy and epoxy with ZrO_2 nanoparticles in Fig. (8 b) clearly predict its antifungal activity by actively inhibiting the growth of both Penicillium and Aspergillusstrains. Due to its high surface area, ZrO_2 nanoparticles almost show similar and significant in hibition effect against both fungal strains.

CONCLUSIONS

ZrO_2 nano enhance mechanical strength (bending, hardness, compressive) ,Antibacterial and anti fungal of epoxy resin with different weight ratio of ZrO_2 for two method (in situ and solvent), the best value of addition of Zro_2 which is used to improvement of mechanical strength , anti bacterial , and anti fungal at 5% weight ratio. When addition acetone with solvent method improving mechanical strength compared with in situ method

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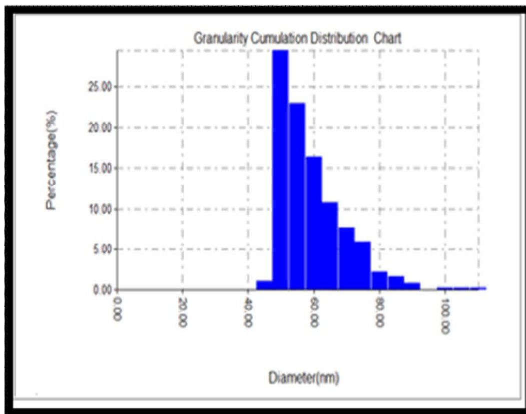

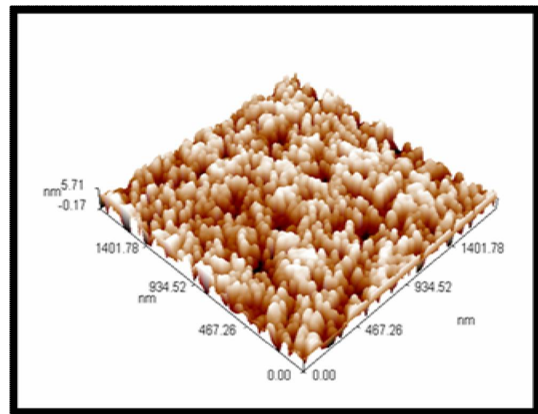

Figure1. AFM analysis images of ZrO_2 nanoparticles used in the work

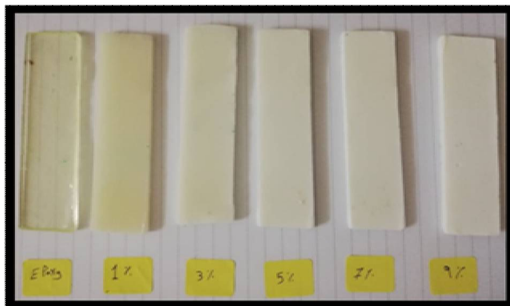

Figure 2. Samples of epoxy and Nano composites

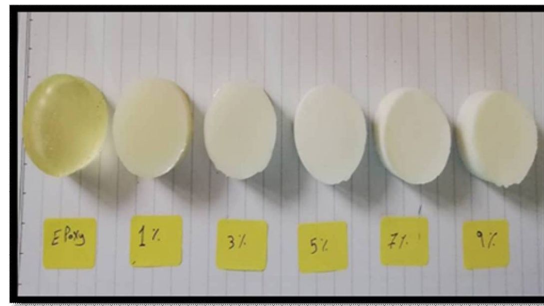

Figure 3. Shows epoxy and Nano composites

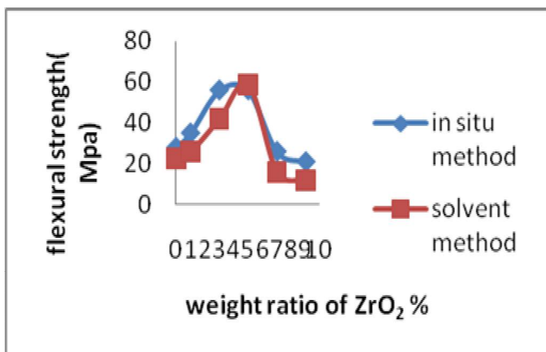

Figure 4. Flexural strength as a function

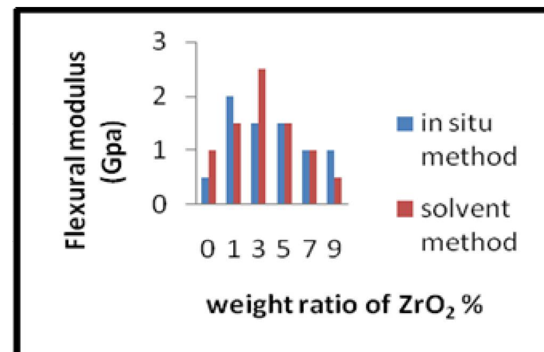

Figure 5. Flexural modulus as a function of weight ratio

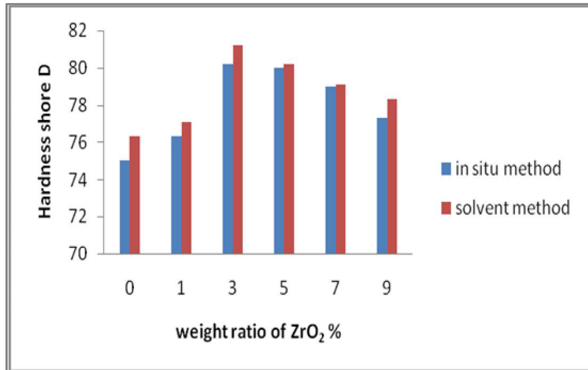

Figure 6. Hardness values as a function of ZrO_2 nano particles

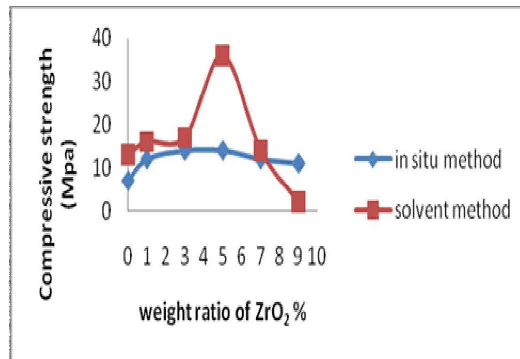

Figure 7. compressive strength as a function of ZrO_2

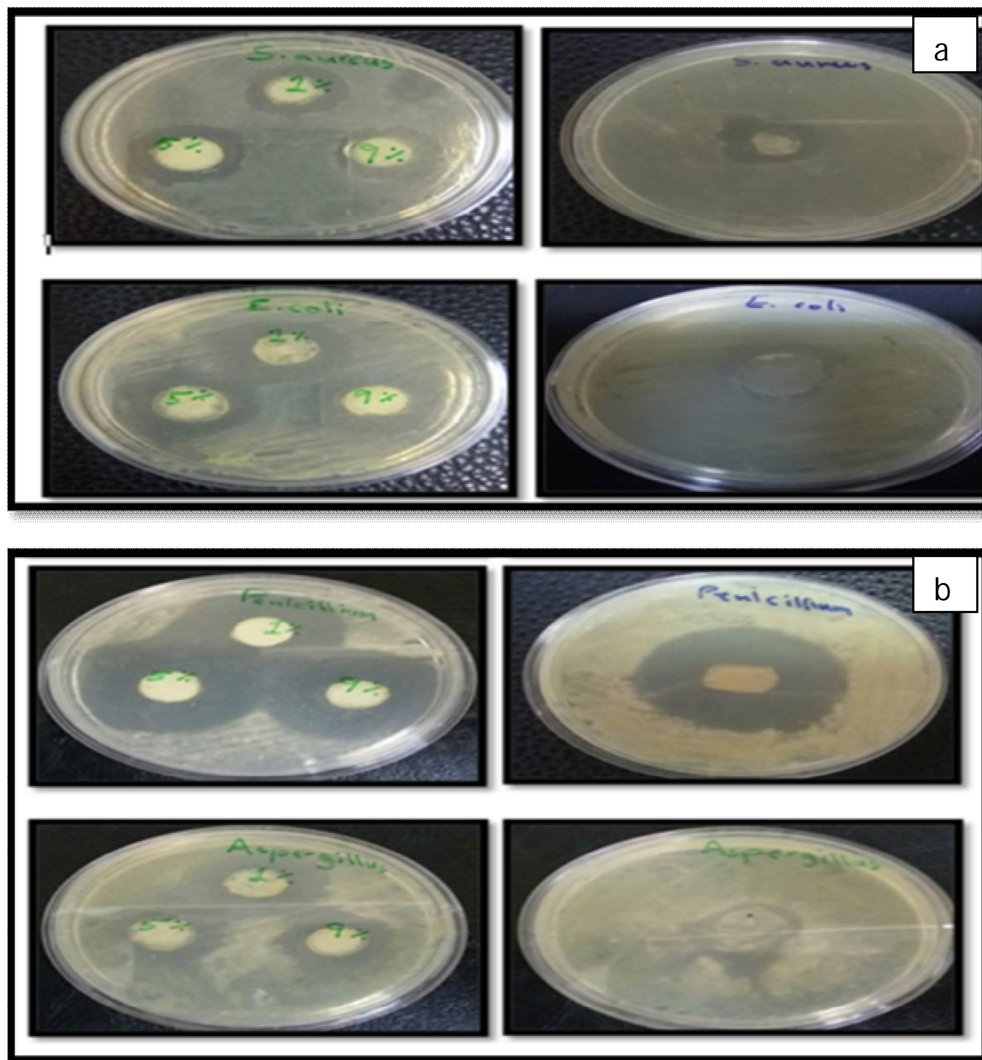

Figure 8. a-Anti bacterial and b-Anti fungal activity of epoxy with ZrO_2 nano composites

RESEARCH ARTICLE

Effect of Different Molar Ratio and Temperature on the Hydrophobic and Structural Properties of Nanoporous Silica Cryogels

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ABSTRACT

Cryogels with a high porosity and high specific surface area were fabricated using freeze-drying method using tertiary butanol as a reaction solvent. Alco-gels were synthesized by acid-base catalysis using tetraethoxysilane as a silica precursor at molar ratio X = 11 and pH = 8 at different freezing temperature. The cryogels samples were examined by SEM, FTIR, nitrogen absorption/desorption isotherms and hydrophobic property. We found that the best sample prepared at -30^{0}C. Increasing the preparation temperature cause to reduce the samples quality specially at -5^{0}C.

Keywords: Vacuum freeze-drying; tertiary butanol; silica cryogels.

INTRODUCTION

Aerogels, the lightest transparent solids known, are a class of low-density solid-state materials obtained from a gel by replacing the pore liquids with air while maintaining the network structure as it is in the gel state. They are also known as frozen smoke because of its hazy blue appearance or air-glass and are comprised of particles with typical dimensions below 10 nm and pore sizes < 50 nm in diameters. Aerogels are micro- and mesoporous networks composed of randomly interlocking nano-scale filaments. The highly porous, open cell structure gives aerogels its unique physical properties. Although 99% of aerogels volume are open pore space, they are solid materials. Among the more potentially useful properties of silica Aerogels possess a wide variety of exceptional properties such as high specific surface area (1,000 m^2 /g) low dielectric constant (~1.0–2.0), low thermal conductivity (~0.01 W/m.K), low refractive index (~1.05), high optical transmission (90%) in the visible region, high porosity (~99%), and low sound velocity (100 m/s)[1–4].

Owing to these properties, aerogels find applications in a number of fields such as acoustic insulation, catalysis, controlled drug delivery, optical coatings, Cerenkov radiation detectors, dielectrics, thermal insulation, collectors of hyper-velocity particles in space capacitor electrodes, electronics, ultrafiltration membranes, and oil spill cleanup kits [5-17]. In freeze drying method the solvent was removed from the gel by lowering the gel temperature below solvent freezing temperature and reducing the pressure (sublimation). The product of this process is usually called a cryogel. Tertiary butanol is the most attracted solvent that have been examined in many researches over the past years in freeze-drying technique. As a matter of fact, tertiary butanol can mixed with water, has a low toxicity profile and exhibits suitable physical properties for use in freeze drying process such as high solid vapor pressure and a low sublimation enthalpy. The water + tertiary butanol mixture compositions, at any ratio, sublime at a higher rate than neat water at same process parameters. This study focus on the effect of molar ratio TEOS/TBA and freezing temperature of the reaction solvent tertiary butanol (TBA) on the properties of cryogels prepared by a novel vacuum freeze drying are investigated.

MATERIALS

The chemicals used for the preparation of alcogels were tertiary butanol(TBA) (99%), tetraethoxysilane(TEOS, SiO_2> 28.5%), ammonia (NH_4OH, 28%) and hydrochloric acid (HCl, 36.5%), all were supplied from Sigma Aldrich. The double-distilled water (H_2O) was prepared in the authors' laboratory.

METHODS

Silica gels were prepared by acid-base catalysis sol-gel procedure. The TEOS and TBA were used as precursor and solvent, respectively.Firstly, of catalyst of hydrochloric acid (HCl) and ammonia (NH_4OH) at 0.12 mol/L and 1.44 mol/L, respectively. (HCl) was added to induce the hydrolysis of the precursor, and then the irreversible polycondensation reaction took place in the presence of a base catalyst (NH_4OH). Firstly, the molar ratios TBA/TEOS were chosen as X = 7, 9, 11, 13 and 15. Then, a diluted HCl (0.12 mol/L) was added into the mixture under continuous stirring for 30 min at 50 °C. Secondly, diluted NH_4OH (1.44 mol/L) was added drop wise with rapid stirring for 5 min, until the pH became about 8. The alcohols were then transferred to mould at room temperature, and gelled in 10-60 min. The molar ratio of TEOS: H_2O: HCl: NH_4OH was 1: 3.7: 1.08×10^{-4}: 1.24×10^{-3}. Thirdly, the transparent alcogels were aged to strengthen the gel networks. for 24 h in a sealed cabin at room temperature. The alcogels were washed with TBA at 50 °C for each time per 8 h.

The surface modification was carried out by immersing the gels in a mixture of TBA containing 5 % volume solution of TMCS at 50 °C for ~24 h, the modification times were changed from 0 to 3 times. Finally, the alcogels were dried by a vacuum freeze dryer. using a special procedure, Alcogels were dried in a vacuum-freeze dryer (Homemade chamber) in two steps. Firstly, the alcogels were freeze to -30 ºC in the ice condenser chamber and kept there for 4 h. Secondly, the drying procedure started by vacuum the chamber. The temperature was increased from (-30 to 10) ºC at 10 ºC steps and a fixed pressure of 0.12 mbar and was kept for 1.5 h at every step. Then, it was increased from (10 to 25) ºC at 5 ºC steps and was kept for 15 min at a pressure of 0.63 mbar at every step. The temperature was raised with rate of 0.65 ºC min^{-1}. During this process, the frozen liquid was sublimed from solid to gas and removed using the vacuum pump. The obtained cryogels were very fragile, opaque powder.Then after the selection of optimum TEOS/TBA molar ratio, the effect of freezing temperature, in the values begin freeze (-30, -20, -10, -5), on the properties of prepared cryogels were examined. The samples were surface modified by TMCS.

Characterization Methods

The porosity of the cryogels were determined by nitrogen absorption/desorption measurements at 77.35 K using an ASAP2010 Micromeritics apparatus (USA). The pore size distribution and specific surface area were analyzed by the

density functional theory (DFT) methods and Brunauer–Emmett–Teller (BET), respectively. The morphology was examined by Scanning Electron Microscopy (SEM, JSM-6330F, Japan).The variation in molecular bond were studied by Fourier Transform Infrared spectroscopy (FT-IR, Bruker TENSOR 27, Germany), while the hydrophobicity of water were studied by measuring the contact angle of the water dropped on the performing cryogels.

RESULTS AND DISCUSSIONS

By varying the TBA/TEOS ratio as (7, 9, 11, 13 and 15) and fix other parameter (pH8 and -30 ºC temperature) we observed that the density of the cryogel samples without TMCS (hydrophilic) is least than the cryogel samples with TMCS (hydrophobic). The best samples, which have least density, at X11 molar ratio as shown in Figure 1. This means that this sample has the largest specific surface area. Generally, high surface area cryogels has low density[18], which depend on the pore size and distribution N_2 absorption/desorptionisotherm for cryogels with different molar ratio were shown in Figure 2 (A) and (B). The largest absorption molar ratio was at X11. The porosity and the pore size distribution of silica cryogels was investigated by the N_2 absorption/desorption, the physic-sorption isotherm is of type IV, which is typical of mesoporous materials[22] as shown in Figure 3 (A). The SiO_2cryogels with X = 11 exhibit an extremely high specific surface area. Figure 3 (B) show the pore size distribution and show a high density of micro- and mesopores. Large surface area and small pores that distribution in the sample can be attributed not only to the excellent dispersion function, but also to the smaller molecular size of the solvent used in this technique, which absorbs fluid easily and then make pores.

Selecting the best molar ratio (X11) and variation temperature (-30,-5) ºC and by comparing between low value (-30) ºC and high value (-5) ºC as shown in Figure 4 (A) that the SiO_2 cryogels prepared at (-30) ºC exhibit an extremely high specific surface area of 800 m^2/g and mean pore diameter 5.0818 (nm). Figure 4 (B) shows that the pore size distribution in the range of (0.3–19) nm with a high density of micro- and mesopores (0.37–8 nm). Decreasing the preparing temperature to (-5) ºC leads to decrease the surface area to 711 m^2/g and mean pore diameter to 5.3446 (nm) while the pore size distribution in the range of (0.3–21) nm with a high density of micro- and mesopores (0.3- 9 nm).Using the best TBA/TEOS molar ratio (X=11) with pH8 at different temperatures (-30, -20, -10 and -5) ºC, we observed that the best sample (with least density and high surface areas) at temperature (-30) ºC as shown in Figure 5, which is agree with the BET completely. Generally, high surface area cryogels has low density [18]. This is in good agreement with the results of SEM images as shown below.

The surface morphology of the cryogels samples prepared at different freezing temperature (-30, -20, -10 and -5) ºC were examined by SEM at magnification power (1kx) as shown in figure 6 (A, B, C and D) respectively, using the reaction solvent tertiary butanol (TBA) at X=11 molar ratio and pH=8. It was observed that the network of silica cryogels well constructed. The effect of preparation temperature values on the cryogel morphologies was very clear. The (-30 and -20) ºC sample, images (A and B),has homogenously network with high porosity and narrow pore size. The main pores size was 20 nm, the separation between particle and another is very clear. This behavior gave the sample its lowest density compare with other temperatures. The (-10 ºC) image (C) seems more aggregated, increase particles and pore size. The (-5 ºC) (D) observation very high aggregation more increase particles size microstructure that appears to be with low porosity and increase in pore size. The reason due to the sample at this temperature is in critical condition and may not reach the complete freezing state of the liquid inside, so during the polymerization reaction if the mechanical strength of the polymer structure is not high enough to counteract the capillary force, the structure could completely collapse, giving rise to non- porous materials is fuse. The variation of molecular bonds for cryogel, at different preparation temperatures, was examined by FT-IR spectroscopy as shown in Figure 7. Comparison of the four curves shows no variation in molecular structure, all curves have bond located at (3470 and 1600) cm^{-1} corresponding to O-H groups, and another ones the for $–CH_3$ located at (2960, 1380 and 860) cm^{-1} corresponding to $–Si-(CH_3)_3$ groups attached to the silica networks which indicate the hydrophobic feature.

The cryogels hydrophobicity was examined by measuring the contact angle of the water droplet on the cryogels surface as illustrated in Figure 8. The contact angle decreased from 145º to 138 º when increasing the preparation temperature from (-30 to -5) ºC as shown in table 1. The density of the samples prepared at different temperatures was measured as shown in figure 5, for the samples prepared with the addition of TMCS (hydrophobic) and without (hydrophilic) addition. The Figures (1 and 5) shows that the density is higher in all samples hydrophobic than others. On the other hand, the density of the samples increased by increasing the preparation temperature from -30°C to -5 °C as shown in Figure5.

CONCLUSIONS

Hydrophobic silica cryogels were prepared by freeze-drying method using TBA as solvent by a locally manufactured chamber at different freezing temperature. Choosing a freezing temperature has a significant effect on the quality of the product. The freezing temperature of -30°C was sufficient to obtain a porous material with low density and surface area with high hydrophobicity, but at higher temperatures the product specifications were of lower quality, especially when reaching a temperature of -5°C.

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Table 1. Contact angle of the cryogels prepared with TBA at different temperature.

Temperature	-30	-20	-10	-5
Contact angle	145	142	140	138

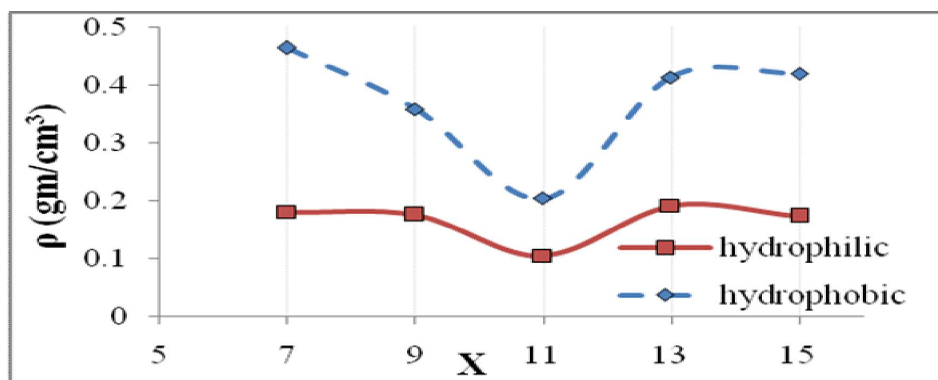

Figure 1. Variation of cryogel density prepared with TMCS (hydrophobic) and without TMCS (hydrophilic) with different molar ratio TBA/TEOS.

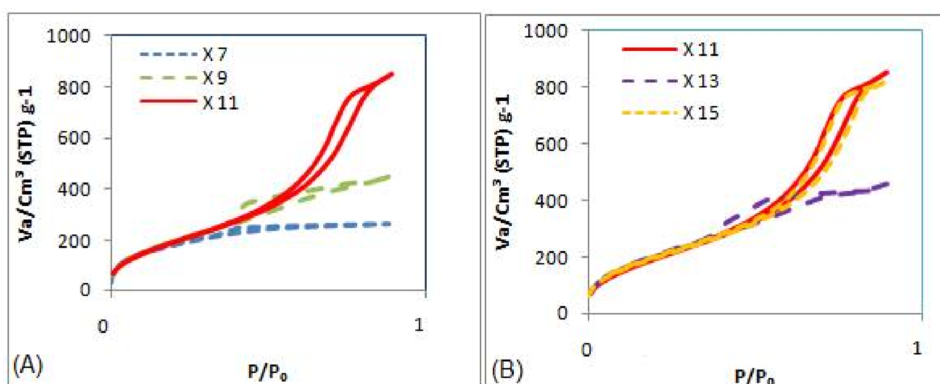

Figure 2. SiO_2cryogels with different molar ratio by N_2 absorption/ desorption isotherm

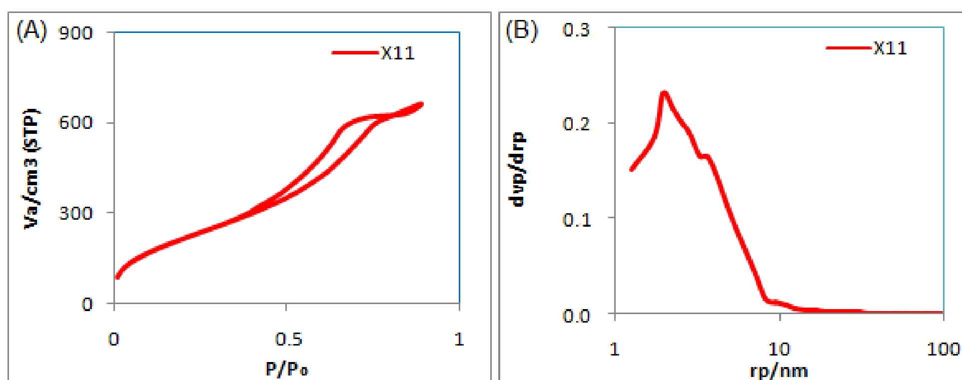

Figure 3. SiO_2cryogels with X = 11 : (A) N_2 absorption/ desorption isotherm and (B) pore size distributions obtained by the BJH method

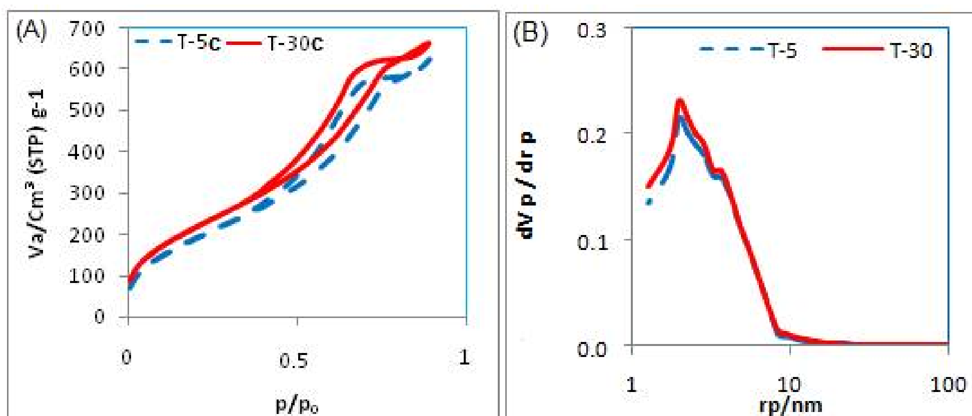

Figure 4. SiO_2 cryogels with X = 11 with pH8 prepared at different freezing temperature : (A) N_2 absorption/ desorption isotherm and (B) pore size distributions obtained by the BJH method.

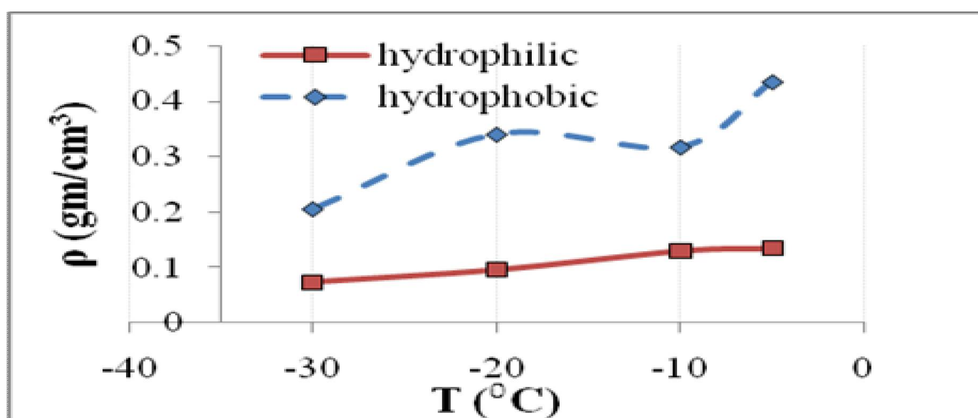

Figure 5. Variation of cryogel density prepared with TMCS (hydrophobic) and without TMCS (hydrophilic) at different temperature.

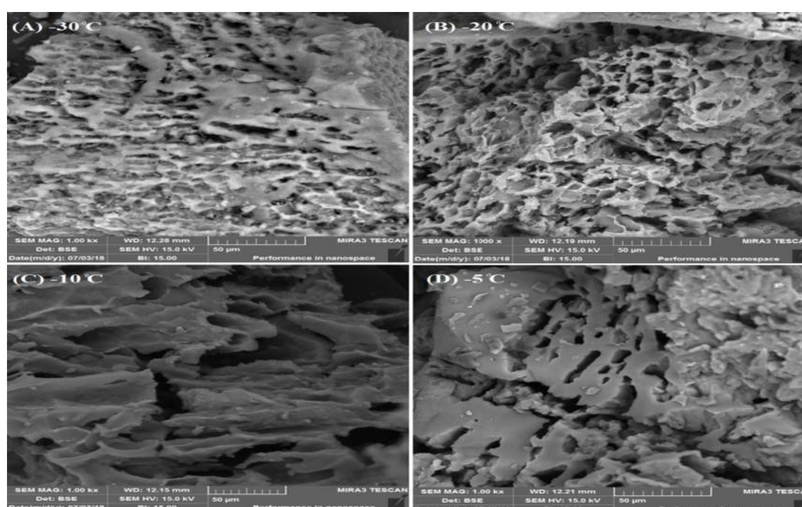

Figure 6. SEM of cryogel at different temperature (-30, -20, -10, -5) (A, B, C, D) respectively.

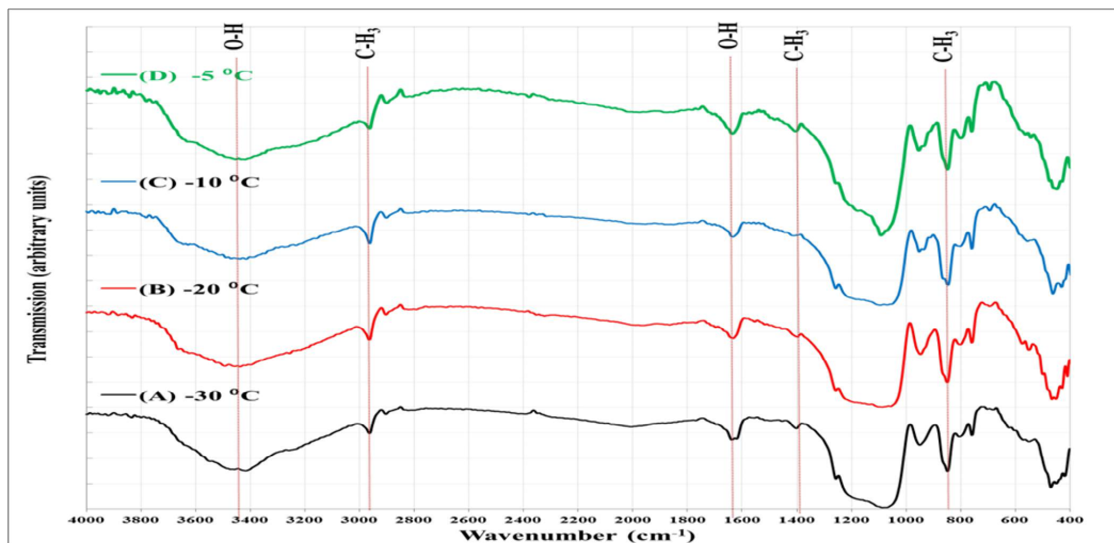

Figure 7. Shown FT-IR spectroscopy for samples cryogel different temperature (-30, -20, -10, -5) with surface modification by (TMCS).

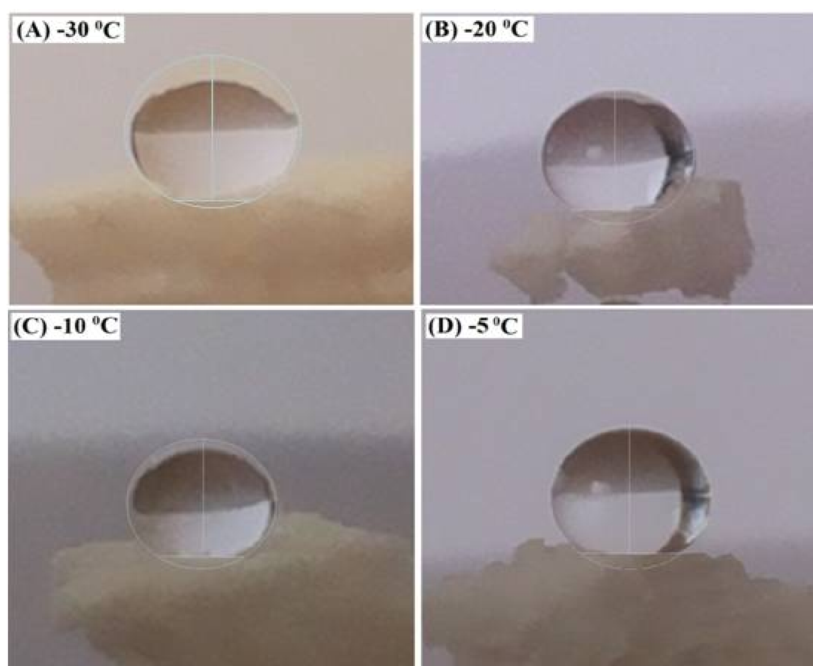

Figure 8. Calculation of contact angle of the cryogels prepared with TBA at different temperature.

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RESEARCH ARTICLE

Approximate Solution for the System of Nonlinear Volterra Integral Equations of the Second Kind by Taylor Series Expansion

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ABSTRACT

In this work, we applied Taylor series expansion to find an approximate (sometimes exact) solution for nonlinear system of Volterra integral equations of the second kind (VIEK2). This method gives the solution for integral equation in a simple and closed form. Also some illustrative examples are presented, to elucidate the accuracy of this method.

Keywords: Taylor series expansion, system of nonlinear Volterra integral equation, Leibnitz generalized formula.

INTRODUCTION

Taylor-Series methods have the useful feature that, whenever they are applicable, they produce the exact Taylor coefficient of the solution; this is an advantage in case an approximation has been obtained and later higher accuracy is wanted.Taylor-series expansion are used to find an approximated solution of different type of equations and it was the base for deriving some important numeric techniques for solving the different equations such as Euler method, modified Euler method, Runge-Kutta method and etc. (Staek, 1970), (Hall, 1976), (Jain, 1979), (Conte, 1972). This approach gives an approximate simple and closed form solution for some class of integral equations, such as Volterra integral and integro-differential equations of the second kinds, nonlinear Volterra-Fredholm integral equations, linear integro-differential equation, linear Fredholm integro-differential equations, Volterra – Fredholm integro – differential equations (linear and nonlinear), Lokta integral equations, linear Fredholm integro-differential-differenc equations, see (Kanwal, 1989), (Ren,et.al,1999), (Maleknejad, 2005), (Goldfine, 1977), (Mahmoudi, 2005), (Yalçinbaş, 2002), (Karamete, 2002), (Nas, et.al, 2000), (Yalçinbaş, 2000), (Maleknejad,2003), (Pollaed, 1970), (Gülsu , 2005).

Also Taylor series expansion used by (Saeed, 2006), for solving linear system of VIEK2 and linear system of Volterra integro-differential equations of the second kind. In this paper, we also use Taylor series method but for solving a system of nonlinear VIEK2. The rest of this paper is organized as follows:

In section 2, proposition have been proved to finding the nth derivatives for a system of non-linear VIEK2's.
In section 3, Taylor-series expansion is formulated which is needed in the result.
In section 4, Taylor-series expansion has been used (presented) to solve a system of non-linear VIEK2.
In section 5, The accuracy of solution has been presented.
In section 6, numerical results are given for illustrations, depending on the least square errors.

Leibnitz Generalized Formula

For solving system of nonlinear Volterra integral equations

$$U(x) = F(x) + \int^{x} K(x, t, U(t))dt \,, \qquad (1)$$

where

$$U(x) = (u_1(x), ..., u_m(t))^T \,,$$

$$F(x) = (f_1(x), ..., f_m(x))^T \,,$$

$$K(x, t, U(t)) = (k_1(x, t, U(t), ..., k_m(x, t, U(t)))^T$$

we need the following Leibnitz generalized formula

$$\frac{d}{dx}\int_{(x)}^{(x)} F(x, t)dt = \int_{(x)}^{(x)} \frac{\partial F(x, t)}{\partial x} dt + F(x, \ (x)) \frac{d \ (x)}{dx} - F(x, t)\frac{d \ (x)}{dx} \qquad (2)$$

This is a generalization of the fundamental theorem of integral calculus

$$\frac{d}{dx}\int_{a}^{x} F(t)dt = F(x)$$

To prove (2), see (Jerri, 1985).
Here, we drive formulas concerning the nth derivative of a system of non-linear VIEK2's which is needed in the sequel.

Proposition 1:

The nth derivative of a system of non-linear VIEK2's (1) is given by the following formula

$$u_i^{(n)}(x) = f_i^{(n)}(x) + \int_a^x \frac{\partial^{(n)} K_i(x, t, U(t))}{\partial x^n} dt + \sum_{s=0}^{n-1} \frac{d^{n-1-s}}{dx^{n-1-s}}\left(\frac{\partial^s K_i(x, t, U(t))}{\partial x^s}\right) \qquad (3)$$

Proof

The principle of mathematical induction is used to prove (3). The following steps are needed

Step one

We must show that (3) is true for n=1. Differentiate equation (1) one time with respect to x and use equation (2) we obtain:

$$u_i'(x) = f_i'(x) + \int_a^x \frac{\partial K_i(x,t,U(t))}{\partial x} dt + K_i(x,x,U(x)), \quad i=1, 2, \ldots, m.$$

This is equation (3) for n=1. Hence, the proposition is true for n=1.

Step two

Suppose that equation (3) is true for n= l, i.e.

$$u_i^{(l)}(x) = f_i^{(l)}(x) + \int_a^x \frac{\partial^l K_i(x,t,U(t))}{\partial x^l} dt + \sum_{s=0}^{l-1} \frac{d^{l-1-s}}{dx^{l-1-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right) \Bigg|_{t=x} \qquad (4)$$

Step three

We must prove that equation (3) is true foe n=l+1, that is we want to prove that

$$u_i^{(l+1)}(x) = f_i^{(l+1)}(x) + \int_a^x \frac{\partial^{l+1} K_i(x,t,U(t))}{\partial x^{l+1}} dt + \sum_{s=0}^{l-1} \frac{d^{l-s}}{dx^{l-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right) \Bigg|_{t=x}.$$

Differentiate equation (4) with respect to x and using equation (2), we get:

$$\frac{d}{dx} u_i^{(l)}(x) = \frac{d}{dx} f_i^{(l)}(x) + \frac{d}{dx} \int_a^x \frac{\partial^l K_i(x,t,U(t))}{\partial x^l} dt + \sum_{s=0}^{l-1} \frac{d}{dx} \frac{d^{l-1-s}}{dx^{l-1-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right) \Bigg|_{t=x}.$$

This means that

$$u_i^{(l+1)}(x) = f_i^{(l+1)}(x) + \int_a^x \frac{\partial^{l+1} K_i(x,t,U(t))}{\partial x^{l+1}} dt + \frac{\partial^l K_i(x,t,U(t))}{\partial x^l} \Bigg|_{t=x} + \sum_{s=0}^{l-1} \frac{d^{l-s}}{dx^{l-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right) \Bigg|_{t=x}$$

Rewrite above equation as follows:

$$u_i^{(l+1)}(x) = f_i^{(l+1)}(x) + \int_a^x \frac{\partial^{l+1} K_i(x,t,U(t))}{\partial x^{l+1}} dt + \sum_{s=0}^{l} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right) \Bigg|_{t=x}$$

Hence equation (3) is true for n=l +1, so it is true for all n. This completes the proof of the proposition 1.

Taylor - Series

A function $f(x)$, must satisfies the condition of continuity and possesses all the derivatives we need within some region of interest. Taylor-series expansion of the function f in powers of x-a , where a, x in some interval I is expressed as

$$f(x) = {}_0 + {}_1(x-a) + {}_2(x-a)^2 + \quad + {}_n(x-a)^n + {}_{n+1}(x-a)^{n+1} + \qquad (5)$$

If we take the derivative on both sides of (5), n times we get

$$f^{(n)}(x) = n! \quad {}_n + (n+1)! \quad {}_{n+1}(x-a) +$$

Setting $x =$
a , we have

$$f^{(n)}(a) = n! \ {}_n \text{, where } {}_n = \frac{f^{(n)}(a)}{n!}, n = 0, 1, 2, \ldots .$$

Thus, the Taylor series is

$$f(a) + \frac{f'(a)}{1!}(x-a) + \frac{f''(a)}{2!}(x-a)^2 + \quad + \frac{f^{(n)}(a)}{n!}(x-a)^n + . \qquad (6)$$

For more about Taylor-series expansion, see (Stark, 1970), (Jain, 1979), and (Conte, 1972).

The Method of Solution for System of non-linear VIEK2

The technique is based on, first, differentiating both sides of the integral equation (1) n times and then substituting the Taylor series for the unknown functions in the resulting equation. We assume that the solution of (1) is expressed in the form

$$u_j(x) = \sum_{k=0}^{\infty} \frac{1}{k!} u_j^{(k)}(a)(x-a)^k \qquad (7)$$

which is Taylor-series about 0 $x = a$, where $u(k)(a)$, k=0, 1, 2, ... are coefficients to be determined. In equation (3) for n=1, we get

$$u'_i(x) = f'_i(x) + \int_a^x \frac{\partial K_i(x,t,U(t))}{\partial x} dt + K_i(x,x,U(t)) \quad , \quad i=1, 2, \ldots, m \qquad (8)$$

Also, for $n=2$, we get:

$$u''_i(x) = f''_i(x) + \int_a^x \frac{\partial^2 K_i(x,t,U(t))}{\partial x^2} dt + \left. \frac{\partial K_i(x,t,U(t))}{\partial x} \right|_{t=x} + \frac{\partial K_i(x,x,U(x))}{\partial x}$$

$$i=1, 2, \ldots, m. \qquad (9)$$

In general, from (3) we have

$$u_i^{(n)}(x) = f_i^{(n)}(x) + \int_a^x \frac{\partial^n K_i(x,t,U(t))}{\partial x^n} dt + \sum_{s=0}^{n-1} \frac{d^{n-1-s}}{dx^{n-1-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial x^s} \right)\Bigg|_{t=x}$$

$$i=1, 2, \ldots, m \qquad (10)$$

Now, put $x=a$, and substitute it in equation (1) and equations (8) - (10) gives

$$u_i(a) = f_i(a) \ , i=1, 2, \ldots, m.$$

$$\left.\begin{aligned} & u'_i(a) = f'_i(a) + K_i(a,a,U(a)), i = 1,2,\ldots,m \\ & u''_i(a) = f''_i(a) + \frac{\partial K_i(x,t,U(t))}{\partial x}\bigg|_{t=x=a} + \frac{\partial K_i(x,x,U(x))}{\partial x}\bigg|_{x=a}, i = 1,2,\ldots,m. \\ & \vdots \\ & u_i^{(n)}(a) = f_i^{(n)}(a) + \sum_{s=0}^{n-1} \frac{d^{n-1-s}}{dx^{n-1-s}} \left(\frac{\partial^s K_i(x,t,U(t))}{\partial^s x} \right)\bigg|_{t=x=a}. \end{aligned}\right\} \qquad (11)$$

Solve the above system for the quantities *u j* ' (*a*), *u j* " (*a*), …, *u* (*n*) *j* (*a*), *j*=1, 2, …, *m* using forward substitution. Then, these values are substituted in equation (3.6) to obtain the solution

$$u_j(x) = u_j(a) + u'_j(a)(x-a) + \frac{1}{2!}u''_j(a)(x-a)^2 + \ldots + \frac{1}{n!}u_j^{(n)}(a)(x-a)^n,$$

$$j=1, 2, \ldots, m; \ n=1, 2, \ldots. \qquad (12)$$

The Algorithm (VIETM)

Step (1): In equation (1), put *x*=*a* to get *u*(*a*) = *f* (*a*) , *i*=1, 2 ,…, *m*.
Step (2): Find *u* (*x*) *i*= , *u* (*x*) *i*= , ..., *u*(*n*) (*x*) *i* , *i*=1, 2 ,…, *m* from equations (8)-(10).
Step (3): In **step (2)**, put *x*=*a*, to obtain a system (11).
Step (4): Solve resulting system, using forward substitution to find *u* (*a*) *i*= , *u* (*a*) *i*= , ..., *u*(*n*) (*a*) *i* , *i*=1, 2, …, *m*.
Step (5): After calculating the values of *u* (*a*) *i*= , *u* (*a*) *i*= , ..., *u*(*n*) (*a*) *i* ; *i*=1, 2, ..., *m*, substituted in equation (7) for finding *u*(*x*) , *i*=1, 2, …, *m*.

Accuracy of Solution:
We can easily check the accuracy of the solution obtained in the form (12) as follows: The truncated Taylor-series (12) is an approximate solution of equation (1), when the solution *u*(*x*) substituted in equation (1), the resulting equation must satisfied approximately; the equation (1) for *x* = *x* = [*a*,*b*], *r* = 0,1,2,= *r* that is

$$R_{iN}(x_r) = u_i(x_r) - f_i(x_r) - \int_a^{x_r} K_i(x_r,t,U(t))dt \cong 0 \quad \text{or} \quad |R_{iN}(x_r)| \le 10^{-k_r} \ (k_r$$

are any positive integers) Thus, we can get better the solution (7) or (12) by choosing *k* appropriately. So that 10 = *k* is very close to zero.

Illustrative Examples

The method of this chapter is useful in finding the solutions of the system of non-linear VIEK2 in terms of Taylor-series. We illustrate it by the following examples

Example 1: (Babolian, 2000)

Solve a system of non-linear VIEK2's:

$$u_1(x) = x - x^2 + \int_0^x (u_1(t) + u_2(t))dt$$

$$u_2(x) = x - \frac{1}{2}x^2 - \frac{1}{3}x^3 + \int_0^x (u_1^2(t) + u_2(t))dt$$

The exact solution of this system is:
u (*x*) = *x* 1 and *u* (*x*) = *x* 2 .
When applying Taylor's method with $n \geq 1$ in equation (12), we obtain the exact solution.

Example 2: (Jumaa, 2005)
Solve a system of non-linear VIEK2's:

$$u_1(x) = 1 - e^{\sin(x)} + \int_0^x \cos(t)e^{u_1(t)}dt + \int_0^x u_2(t)dt$$

$$u_2(x) = \sin(x) + x\ln(\cos(x)) + 1 + \int_0^x x\sin(t)\frac{1}{u_2(t)}dt - \int_0^x u_3(t)dt$$

$$u_3(x) = e^x\cos(x) + \cos(x) - e^x + \int_0^x e^x u_1(t)dt + \int_0^x u_2(t)dt$$

The exact solution of this system is:

$$u_1(x) = \sin(x) \ , \quad u_2(x) = \cos(x) \quad \text{and} \quad u_3(x) = \sin(x) + \cos(x)$$

When applying Taylor's method with $n=3$ in equation (12), we obtain the approximate solution

$$u_1(x) \cong x - \frac{x^3}{3!} + \frac{x^5}{5!} \ , \quad u_2(x) \cong 1 - \frac{x^2}{2!} + \frac{x^4}{4!} \quad \text{and} \quad u_3(x) \cong 1 + x - \frac{x^2}{2!} - \frac{x^3}{3!} + \frac{x^4}{4!} + \frac{x^5}{5!}.$$

Example 3: (Waswas, 2005)
Solve a system of non-linear VIEK2's

$$u_1(x) = \sec(x) - x + \int_0^x \left((u_1(t))^2 - (u_2(t))^2\right)dt$$

$$u_2(x) = 3\tan(x) - x - \int_0^x \left((u_1(t))^2 + (u_2(t))^2\right)dt$$

The exact solution of this system is

$$u_1(x) = \sec(x) \quad \text{and} \quad u_2(x) = \tan(x)$$

When applying Taylor's method with n=5 in equation (12), we obtain the approximate solution

$$u_1(x) \approx 1 + \frac{x^2}{2!} + \frac{5x^4}{4!} \quad \text{and} \quad u_2(x) \approx x + \frac{x^3}{3}$$

Also for n=10, we obtain the approximate solution

$$u_1(x) \approx 1 + \frac{x^2}{2!} + \frac{5x^4}{4!} + \frac{61x^6}{6!} + \frac{1385x^8}{8!}$$

$$u_2(x) \approx x + \frac{2x^3}{3!} + \frac{16x^5}{5!} + \frac{272x^7}{7!} + \frac{7936x^9}{9!}.$$

Example 4: (Jumaa, 2005)
Solve a system of non-linear VIEK2's:

$$u_1(x) = \frac{1}{4} - \frac{1}{4}e^{2x} + \int_0^x (x-t)u_2^2(t)dt$$

$$u_2(x) = -xe^x + 2e^x - 1 + \int_0^x te^{-2u_1(t)}dt$$

The exact solution of this system is

$$u_1(x) = -\frac{1}{2}x \quad \text{and} \quad u_2(x) = e^x$$

When applying Taylor's method with n=3 in equation (12), we obtain the approximate solution

$$u_1(x) = -\frac{1}{2}x \quad \text{and} \quad u_2(x) \cong 1 + \frac{x}{1!} + \frac{x^2}{2!} + \frac{x^3}{3!}.$$

CONCLUSIONS

The Taylor series method is effective for cases where the known functions have sufficient derivatives in the given interval. After we applied this method we conclude that: To obtain the best approximation solution of the given system, the degree n of the approximate solution must be chosen sufficiently large. We get the exact solution if $f(x)$ i, i=1, 2, …, n are polynomial. The Taylor-series expansion is not given only at some point but also by a function. The method has the best advantage when the known functions in an equation can be expanded to Taylor series which converge rapidly.

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Table 1. a comparison between the exact solution and the approximate solution of Example 2.

x	$u_1(x)$		$u_2(x)$	
	Exact	Taylor	Exact	Taylor
0	0	0	1	1
0.1	0.099833417	0.099833417	0.995004165	0.995004167
0.2	0.198669331	0.198669333	0.980066578	0.980066667
0.3	0.295520207	0.295520250	0.955336489	0.955337500
0.4	0.389418342	0.389418667	0.921060994	0.921066667
0.5	0.479425539	0.479427083	0.877582562	0.877604167
0.6	0.564642473	0.564648000	0.825335615	0.825400000
0.7	0.644217687	0.644233917	0.764842187	0.765004167
0.8	0.717356090	0.717397333	0.696706709	0.697066667
0.9	0.783326909	0.783420750	0.621609968	0.622337500
1	0.841470984	0.841666667	0.540302306	0.541666667
L.S.E.		4.90948×10^{-8}		2.55123×10^{-6}

Table 2. a comparison between the exact solution and the approximate solution of Example 2.

x	$u_3(x)$	
	Exact	Taylor
0	1	1
0.1	1.094837582	1.094837583
0.2	1.178735909	1.178736000
0.3	1.250856696	1.250857750
0.4	1.310479336	1.310485333
0.5	1.357008100	1.357031250
0.6	1.389978088	1.390048000
0.7	1.409059874	1.409238083
0.8	1.414062800	1.414464000
0.9	1.404936878	1.405758250
1	1.381773290	1.383333333
L.S.E.		3.30656×10^{-6}

Table 3. a comparison between the exact solution and the approximate solution of Example 3.

x	Exact solution of $u_1(x)$	Taylor	
		n=5	n=10
0	1	1	1
0.1	1.005020918	1.005020833	1.005020918
0.2	1.020338845	1.020333333	1.020338843
0.3	1.046751602	1.046687500	1.046751516
0.4	1.085704428	1.085333333	1.085702867
0.5	1.139493927	1.138020833	1.139478799
0.6	1.211628315	1.207000000	1.211529751
0.7	1.307459259	1.295020833	1.306968539
0.8	1.435324199	1.405333333	1.433305763
0.9	1.608725810	1.541687500	1.601498997
1	1.850815718	1.708333333	1.827405754
L.S.E.		2.5873×10^{-2}	6.04578×10^{-4}

Table 4. a comparison between the exact solution and the approximate solution of Example 3.

x	Exact solution of $u_2(x)$	Taylor	
		N=5	n=10
0	0	0	0
0.1	0.100334672	0.100333333	0.100334672
0.2	0.202710036	0.202666667	0.202710035
0.3	0.309336249	0.309000000	0.309336233
0.4	0.422793219	0.421333333	0.422792821
0.5	0.546302489	0.541666667	0.546297674
0.6	0.684136808	0.672000000	0.684099160
0.7	0.842288380	0.814333333	0.842069697
0.8	1.029638557	0.970666667	1.028610570
0.9	1.260158218	1.143000000	1.256017537
1	1.557407725	1.333333333	1.542504409
L.S.E.		6.8365×10^{-2}	2.4036×10^{-4}

Table 5.a comparison between the exact solution and the approximate solution of Example 4

x	$u_1(x)$		$u_2(x)$	
	Exact	Taylor	Exact	Taylor
0	0	0	1	1
0.1	-0.050000	-0.050000	1.105170918	1.105170917
0.2	-0.100000	-0.100000	1.221402758	1.221402667
0.3	-0.150000	-0.150000	1.349858808	1.349857750
0.4	-0.200000	-0.200000	1.491824698	1.491818667
0.5	-0.250000	-0.250000	1.648721271	1.648697917
0.6	-0.300000	-0.300000	1.822118800	1.822048000
0.7	-0.350000	-0.350000	2.013752707	2.013571417
0.8	-0.400000	-0.400000	2.225540928	2.225130667
0.9	-0.450000	-0.450000	2.459603111	2.458758250
1	-0.500000	-0.500000	2.718281828	2.716666667
L.S.E.		0		3.52931×10^{-6}

RESEARCH ARTICLE

Some Pathogenic Bacteria Isolated from Cockroaches in Baghdad

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ABSTRACT

The prevalence of pathogenic bacteria carried by cockroaches has been documented in the present study, 100 cockroaches were collected from houses in three areas in Baghdad city /Iraq, (Al-Hurryiah, Hay Al-Salam and Al-Khadhraa) from September 2017-March 2018. Blood agar, selective and differential media were used to culture cockroaches gut content after disinfecting their outer surfaces using 10 ml of 70% aseptic alcohol. Total infection rate of cockroaches with pathogenic bacteria was 100% . The higher percentage was in Al-Hurryiah city 45% followed by Hay Al-Salam 30% and the lower was in Al-Khadhraa city 25%. The pathogenic bacteria that were isolated are *E.coli* 49(49%), *Staphylococcus spp* 29(29%) , *Salmonella* 13(13%) , *Klebsiella* 12(12%) and *Shigella* 12(12%).

Keywords: Cockroaches, Baghdad, bacteria, biochemical tests, selective media, pathogens .

INTRODUCTION

Cockroaches are insects belong to Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Subclass: Pterygota, Order: Dictyoptera, Sub Order: Blattaria, Family: Blattelidae, Genus: Periplaneta [1,2]. They live in warm, humid and dark places nearly to humans in houses, hospitals, restaurants, sewage drains, garbage, kitchens, bakeries , toilets, wall slits, baseboards [3,4,5], they fed on rubbish, human and animal excreta so they play an important role in transport of various pathogens mechanically and through their gut [6,7,8]. [9]Thyssen et al. (2004) found that cockroaches carry helminthes eggs like Hook worm, Giant human worm, Pin worm, Tape worm, worm Round, Whip worm. Other studies have recorded that cockroaches carried 17 species of fungus: *Aspergillus* , *Alternaria spp.*, *Geotrichum* , *Fusarium spp.*, *Penicillium spp.*, *Trichoderma spp.*, *Chrysosporium spp.*, *Mucor spp.*, *Cladosporium spp.*, *Rhodotrula spp.*, *Candida spp.*, *Saccharomyces cerivisiae* and *Rhizopus spp.*[10], in addition to large number of bacteria that cause food poisoning like *Salmonella spp*, *Shigella*, *Escherichia coli* O157, *Serratia marcescens*, *Proteus spp*, *Enterococcus* spp, *Pseudomonas aeruginosa* , *Klebsiella pneumoniae Staphylococcus aureus*, and *Bacillus cereus* [11,12].

Studies have revealed that exposure to antigens of cockroaches like salivary secretions, fecal pellets may have an important effect on allergies especially skin sensitivity (Itching, swelling of the eyelids) and tightness of breath (asthma) which consider another important threat to public health [13]. The current study was suggested to study the prevalence of pathogenic bacteria that cockroaches carry in their gut in 3 locations of Baghdad city.

MATERIALS AND METHODS

A total of 100 household cockroaches were collected from 3 locations in Baghdad (Al-Hurryiah , Hay Al-Salam and Al-Khadhraa) from September 2017-March 2018 and were transported to the lab of Zoonotic diseases unit in Veterinary Medicine College / University of Baghdad .They were classified as *Periplaneta Americana* [14] , 10 ml of 70% aseptic alcohol was added to each cockroach in 150ml cups and wait for few minutes to decontaminate its external surface then let air dry outside the cup , cotton swabs were used to transport content of gut on blood agar and incubated to 24 hours in 37°C then the selective and differential media were used, including Eosin methylene blue agar, Mannitol salt agar, *Salmonella -Shigella* agar and MacConkey agar. Biochemical tests were used also like Triple Sugar Iron (TSI), Urease, citrate utilization test and Voges Proskauer test (MR/VP) to identify bacteria in addition to gram staining and colony morohology [15,16].

RESULTS AND DISCUSSION

This study was conducted to know the prevalence of pathogenic bacteria carried by cockroaches in Baghdad province as they consider as a great source of environment pollution with pathogens [16]. The finding of our study show that the prevalence of pathogenic bacteria carried by *P. Americana* in 3 regions in Baghdad city was 100% this result is higher than 35.6% recorded by [17] from household cockroach species in Quetta city, and higher than 93.33% recorded by [18] from *P. Americana* collected from households of 10 districts of Tangier, Morocco, but so closely to 99.9% recorded by [19] from 40 households in Kaohsiung City and Kaohsiung County, Taiwan. The higher percentage was in Al-Hurryiah city 45% followed by Hay Al-Salam 30% and the lower was in Al-Khadhraa city 25% (table 1) this may be due to high population density, poor sanitary procedures like poor sanitary drainage, accumulation of garbage all these factors make a good environment for cockroaches to live and proliferate. The pathogens isolated in our study were gram positive & negative bacteria and their percentage was as the following *E.coli* 49(49%), *Staphylococcus spp* 29(29%), *Salmonella* 13(13%), *Klebsiella* 12(12%) and *Shigella* 12(12%). This finding was lower than that recorded by [20] in their study about pathogens of domestic cockroaches in human dwelling localities in Iran *E.coli* (61.5%), *Klebsiella* (36.9%), Non pathogenic *Staphylococcus* (30.8%) and lower than results recorded by[18] which were *Salmonella* 48%, *Staplylococcus* 68%, *Shigella spp* 52%, *Klebsiella spp*.26%, *Escherichia coli* 62% and(21)*in All the collected cockroaches were selected for the presence of pathogenic bacteria. From the collected Periplaneta americana, pathogenic species of bacteria like E.coli, salmonella, staphylococcus and streptococcus were isolated*. Cockroaches carry many pathogens, they pick up from contaminated places such as: drains, sewers, garbage, landfills, toilets,and bathrooms,. Many health risks come along with a cockroach infestation. These cockroaches are typical to causes many intestinal diseases and illnesses. The results of our study show a high infection ratio of *Periplaneta Americana* with pathogenic bacteria which will lead to a great pollution to the environment due to their living near to human localities, this high percentage may be due to poor sanitary conditions in studied areas

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Table 1. Percentage of pathogenic bacteria isolated from 3 regions in Baghdad city

Areas	no. of samples	Infected samples	*E.coli*	*Klebsiella*	*Shigella*	*Salmonella*	*Staph.*
Al-Khadhraa	25	25(25%)	13(26.5%)	2(16.6%)	5(41.6%)	3(23%)	8(27.5%)
Al-Hurryiah	45	45(45%)	21(42.8%)	3(25%)	4(33.3%)	6(46.1%)	15(51.7%)
Hay Al-Salam	30	30(30%)	15(30.6%)	7(58.3%)	3(25%)	4(30.7%)	6(20.6%)
Total	100	100(100%)	49(49%)	12(12%)	12(12%)	13(13%)	29(29%)

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RESEARCH ARTICLE

Evaluation of Maize Germplasm against Stem Borer *Chilo partellus* (Swinhoe) Under Natural Condition

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ABSTRACT

Field trial was conducted to screen 104 entries (Trial 75 Late Maturity - 40 Nos., Trial 76 Medium maturity - 38 Nos., Trial 77 Early maturity- 15 Nos., Trial 78 Extra Early maturity – 11 Nos.) against maize stem borer, *Chilopartellus* under natural condition. The entries were sown in two replications. Each line was sown in single row with 2.5m row length. The number of seeds was sown per row was 15 and 12 seedlings were retained. The regular package of practices was followed for this trial.All the entries were screened for *C. partellus* under natural conditions and the observations on leaf injury scale was recorded on 35 days after sowing and analyzed. Among 104 DMR entries screened against maize stem borer under natural condition, 21 were found to be resistant, 47 were moderately resistant and remaining entries were susceptible.

Key Words: Maize, germ plasm, screening, *Chilo partellus*.

INTRODUCTION

Maize is a promising option for diversifying agriculture in upland areas of India. The area is slowly expanding to meet out food and feed demands in livestock and poultry industry besides blooming as commercial crop for farmer's livelihood. On the other hand, continuous cropping of maize will lead to the occurrence of pests. About 139 insect-pests cause varying degree of damage to maize crop. Among all the insect pests of stem borer *Chilo partellus*is Swinhoe the most notorious pest found throughout India. The survey conducted all over Tamil Nadu on the incidence of pests on maize revealed that maize stem borer is emerging as serious pest which causes greater yield

loss. The economic injury level is 2.09 to 2.42 larvae per plant (Manjunath and Mallapur. 2017). Among several factors, insect pests have significant contribution for low yields (75%) and even total crop failure in case of severe infestation. Several insect species attack maize crop in the field but maize stem borer is the most notorious pests and cause heavy damage crop in Asia and Africa, which ultimately affect our food security and agricultural economy. The phenomenon of plant resistance to insects is a quality that enables a plant genotype to avoid, tolerate or recover from the effects of oviposition or feeding that would cause greater damage to other genotypes of the same species under similar environmental conditions. Mihm (1989) reported that breeders and entomologists had to rely on natural infestations for screening and breeding work before developing efficient techniques for large scale mass production of insect pests. The technology regarding germplasm resistant to insect pests is efficient, economical and environmentally concerned approach to manage many pests. It will also lead to substantial reduction in the use of insecticides. Keeping in view the risks and difficulties in chemical and biological control of insects, identification of resistant germ plasm against *C. partellus* becomes essential.

MATERIALS AND METHODS

Field trial was initiated during kharif 2012 at Maize research Station, Vagarai with 104 maize entries (Trial 75 Late Maturity - 40 Nos., Trial 76 Medium maturity - 38 Nos., Trial 77 Early maturity- 15 Nos., Trial 78 Extra Early maturity – 11 Nos.). The experiment was laid out in Randomized Block Design (RBD) in two replications. Each line was sown in single row with 2.5m row length. The number of seeds was sown per row was 15 and 12 seedlings were retained. The regular package of practices was followed for this trial. All the entries were screened for *C. partellus* under natural conditions. The data on LIR (1-9 scale in Table 1) was recorded on 30 and 45 days after sowing.

RESULTS AND DISCUSSION

The observations on leaf scale injury (LIR) screened for *C. partellus* under natural conditions revealed that among forty late maturity entries ten were found to be resistant (LIR: 1.0-2.7), eighteen with moderately resistant (LIR :3.2-5.6) and twelve were susceptible (LIR: 6.4 to 8.4) to maize stem borer, *C. partellus* (Table 2). In medium maturity entries (38 Nos.), eight were found resistant with LIR score ranging from 1.5 to 2.5, fifteen each as moderately and susceptible entries (3.2-5.5 and 6.4-9.0, respectively) (Table 3). The entry PE 701 of early maturity with LIR 1.7 was resistant to maize stem borer and eight entries (LIR 3.6-5.3) and six entries (LIR 6.5-7.4) were moderately resistant and susceptible (Table 4).. But in extra early maturity entries PE 804 (LIR 2.4) and PE 809 (LIR 1.8) were resistant to stem borer whereas PE 808, PE 803, PE 802 (LIR-6.8, 7.2 and 7.5, respectively) were susceptible (Table 5). Among 104 entries screened against maize stem borer under natural condition, 21 were found to be resistant, 47 were moderately resistant and remaining entries were susceptible (Fig.1). Pathak and Othieno (1992), used 10 parent diallel analysis to evaluate the potential for genetic improvement of resistance to Chilo partellus on the basis of leaf injury, dead hearts, stem tunneling and number of entry and exit holes.

The research was conducted by Liaqat Ali *et al.*, (2002) to investigate the comparative resistance of nine maize cultivars/lines i.e. W-2, FRY-4, EV-9808, FRY-1, EV-9806, W-19, Ghori, Pahari and Babar, to maize stem borer and found that lowest significant infestation was recorded on EV-9806 (5.29%), EV-9808 (6.21%) and FRY-1 (6.32%), while the lowest number of dead hearts were observed on FRY-1 (4.10%), EV-9808 (5.40%), Pahari (5.47%) and W-2 (5.51%), and FRY-1 was comparatively the most resistant followed by EV-9808, Pahari and W-2. The maize lines, ICZ5, IC92M2 and IC92M5 were found to be a compatible component for IPM for maize stem borer (Ajala *et al.*, 2010). Among the cultivars evaluated, Pmh-117, Buland and Parkash cultivars recorded moderately resistant. Bio-9637, Seedtech-2324, Hqpm-7, Bio-9681 and Hybrid maize gs-802 cultivars recorded moderately susceptible (Vishvendra *et al.*, 2017).

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Table 1. Rating scale for *C. partellus*

Rating	Description
1	Apparently healthy plant
2	Plant with parallel, oval or oblong holes, slightly bigger than pin sized (2 – 3 mm) on 1 – 2 leaves
3	Plant with more elongated holes (4 – 5 mm or matchstick – head sized) or shot holes on 1-2 leaves
4	Plant with injury (oval holes, shot holes and slits of 1-4 cm) in about one third of total number of leaves and midrib damage on 1 – 2 leaves
5	Plant with about 50 % leaf damage, oblong holes, shot holes, slits and streaks of 5 – 10 cm and mid rib damage on leaves
6	Plants with a variety of leaf injuries to about two thirds of the total number of leaves (ragged appearance) or one or two holes or slits at the base of the stem (>10 cm streaks are observed)
7	Plants with every type of leaf injury and all the leaves damaged (ragged or crimpled appearance), with tassel stalk boring or circular dark ring at the base of the stem
8	Plants with stunted growth in which all the leaves are damaged
9	Plants with dead heart

The entries were categorized based on mean LIR as given below

Mean LIR		Category
1- 3	:	Resistant
3.1 - 6	:	Moderately resistant
6.1 - 9	:	Susceptible

Table 2. Leaf Injury Rating (LIR) for Late maturity entries screened under natural condition at MRS, Vagarai

Trial 75 Late Maturity		R- 10	MR- 18	S -12	
Resistant	**LIR**	**Mod. Resistant**	**LIR**	**Susceptible**	**LIR**
DMR 502	2.4	DMR 501	3.4	DMR 505	7.2
DMR 503	1.5	DMR 507	4.0	DMR 511	8.0
DMR 504	2.0	DMR 508	4.2	DMR 515	7.5
DMR 506	2.7	DMR 509	5.1	DMR 516	6.8
DMR 510	1.2	DMR 512	4.7	DMR 517	6.4
DMR 521	1.8	DMR 513	4.0	DMR 522	7.5
DMR 525	1.5	DMR 514	4.2	DMR 523	8.4
DMR 526	1.2	DMR 518	5.5	DMR 524	7.8
DMR 535	1.0	DMR 519	5.0	DMR 527	7.9
DMR 536	1.6	DMR 520	5.6	DMR 533	6.5
		DMR 528	3.5	DMR 534	6.4
		DMR 529	4.2	DMR 537	7.5
		DMR 530	4.6		
		DMR 531	3.7		
		DMR 532	3.2		
		DMR 538	5.4		
		DMR 539	5.4		
		DMR 540	5.0		

LIR- Leaf Injury rating R- Resistant MR- Moderately Resistant S -Susceptible

Table 3. Leaf Injury Rating for Medium maturity entries screened under natural condition at MRS, Vagarai

Trial 76 Medium maturity		R- 08	MR- 15	S -15	
Resistant	**LIR**	**Mod. Resistant**	**LIR**	**Susceptible**	**LIR**
PE 604	2.2	PE 601	3.4	PE 605	8.3
PE 608	2.0	PE 602	5.0	PE 606	8.0
PE 609	1.5	PE 603	4.2	PE 607	7.5
PE 622	2.0	PE 614	4.6	PE 610	7.2
PE 623	2.4	PE 618	5.0	PE 611	6.5
PE 631	1.6	PE 620	4.7	PE 612	7.5
PE 632	2.1	PE 621	5.3	PE 613	7.8
PE 633	2.5	PE 624	5.5	PE 615	6.4
		PE 625	3.7	PE 616	6.5
		PE 626	3.2	PE 617	6.8
		PE 627	5.2	PE 619	7.5
		PE 628	5.0	PE 634	7.8
		PE 629	4.6	PE 636	9.0
		PE 630	4.8	PE 637	6.4
		PE 635	4.4	PE 638	7.5

LIR- Leaf Injury rating R- Resistant MR- Moderately Resistant S -Susceptible

Table 4. Leaf Injury Rating for Early maturity entries screened under natural condition at MRS, Vagarai

Trial 77 Early maturity		R- 01	MR- 08	S -06	
Resistant	**LIR**	**Mod. Resistant**	**LIR**	**Susceptible**	**LIR**
PE 701	1.7	PE 702	4.0	PE 704	6.5
		PE 703	4.3	PE 710	7.0
		PE 705	5.1	PE 711	7.2
		PE 706	5.3	PE 712	7.4
		PE 707	3.4	PE 713	6.8
		PE 708	4.7	PE 714	7.0
		PE 709	5.2		
		PE 715	3.6		

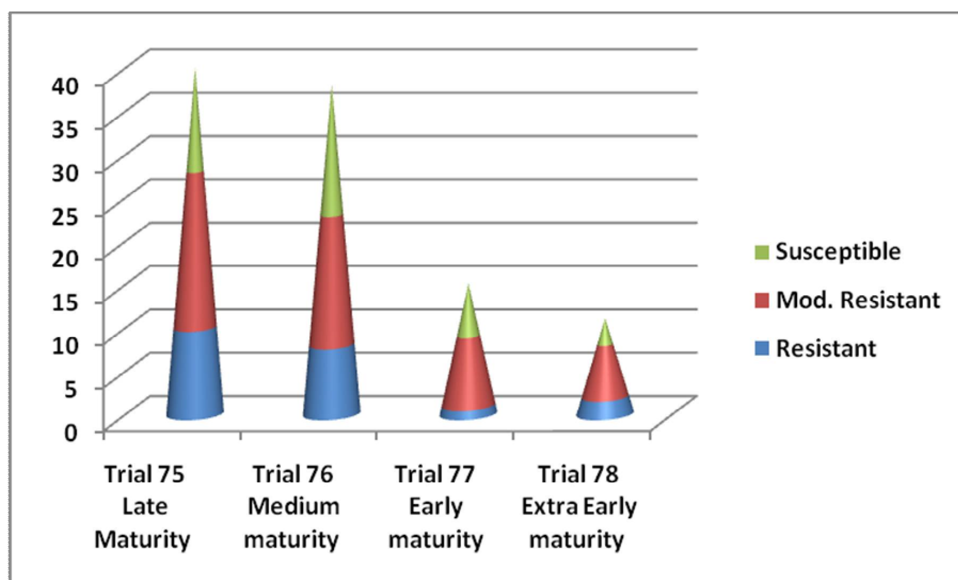
LIR- Leaf Injury rating R- Resistant MR- Moderately Resistant S -Susceptible

Table 5. Leaf Injury Rating for Extra early maturity entries screened under natural condition at MRS, Vagarai

Trial 78 Extra Early maturity		R- 02	MR- 06	S -03	
Resistant	**LIR**	**Mod. Resistant**	**LIR**	**Susceptible**	**LIR**
PE 804	2.4	PE 801	3.4	PE 802	7.5
PE 809	1.8	PE 805	3.3	PE 803	7.2
		PE 806	3.5	PE 808	6.8
		PE 807	4.0		
		PE 810	3.8		
		PE 811	4.5		

LIR- Leaf Injury rating R- Resistant MR- Moderately Resistant S –Susceptible

Figure 1. Pooled result on entries screened under natural condition at MRS, Vagarai



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RESEARCH ARTICLE

Social & Cultural Impact of FM Radio in Pakistan

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ABSTRACT

This research paper is an attempt to assess and evaluate the Social & Cultural impact of FM radio in Pakistan. At the time of independence, Radio Pakistan was the main & only electronic medium available in the country. Radio medium was and still is, exceptionally compelling, very effective communication mode in the country. Radio has been the most powerful and useful informative & educative tool for the reason that it has excellent outreach as compared to other electronic media but radio medium has been turning into a neglected medium in past due to the policy issues which has made radio medium for proliferation of particular agendas instead of disseminating education, highlighting different social & cultural issues and awareness campaigns for the masses. Undoubtedly, FM radios have played vital role to resuscitate radio broadcast in Pakistan. Currently, more than two hundred (200) FM radios (Commercial & Non-Commercial) are operational and airing educational, entertainment and informative programs for the listeners. Commercial & Non-Commercial radios are working successfully and contributing their role by spreading awareness, education and information to the masses especially for the youth.FM radios are influential upon the audience socially & culturally. Radio has the capacity to outreach in distant areas as well as radio can transmit message and voice without distinguishing its audience whether young or old , literate or ill-literate, men or women etc. Concrete and stunning efforts are still required to get maximize yield and benefit from the radio medium in Pakistan.

Keywords: FM radio, Social, Cultural, audience.

INTRODUCTION

In the era of Information technology, radio is still a very useful and effective mode of communication today. In view of handiness and easy accessibility its uniqueness can never be mixed-up with other tools of communication. Radio is so easy medium that it can be tuned even while driving, studying or retiring in bed. In this age of revolution, FM medium has gained tremendous popularity and impression as it operates as community radio, airs programs in both local and regional languages which attract the large population of local community (Iqbal, 2015). Radio is a leading media and lively media in rural areas particularly where cable and TV transmission is not easily accessible. Radio is popular among people of all age groups, sex and gender significantly youth. Radio has raised social and cultural awareness of the masses, significantly the youth as the majority of radio listeners belongs to youth (Chaudhary, 2011).

Radio has played a vital role in independence movement. Quaid-e-Azam Muhammad Ali Jinnah, had used radio (All India Radio) to address the people of Sub-continent. His addresses and speeches were enthusiastically listened by the Muslims especially the youth which have motivated the Muslims of Sub-continent to struggle for their independent homeland. The announcement of creation of Pakistan was also made on radio. On 14th August 1947, very first broadcast aired simultaneously from two different cities Lahore and Peshawar (Siddiqui, 1991). The monopoly of Government to control radio was changed with the establishment of Pakistan Electronic Media Regulatory Authority (PEMRA) in year 2002, which had made possible private radio ownership in Pakistan (PEMRA, 2016).

FM band was introduced in Pakistan in year 1995. Initially, its service was limited to three big cities Karachi, Lahore and Islamabad. However, in year 1998 Pakistan Broadcasting Corporation (PBC) has started their own FM radio broadcast, under the umbrella of FM-101. In beginning, service of FM-101 was available in Karachi, Lahore and Islamabad. Later on, it was extended to other cities. Now ten FM-101 stations are working with twenty four regular FM stations throughout Pakistan. At the moment, PBC has the ownership of sixty four radio stations i.e. 23 MW, 7 SM & 34 FM radios. FM radio has revived the declined radio industry in Pakistan; radio audience was reduced in the country after arrival of TV and other electronic media and a clear transformation in broadcast industry was observed in the country (PBC, 2016). Presently, 187 FM radios (142 commercial and 45 non-commercials) are operational in different sectors in Pakistan. Commercial FM radios are airing infotainment, entertainment and musical programs. These programs are designed to cater commercial based needs and the demands of public, particularly youth. Whereas, non-commercials FM radios are established in different public universities and departments with an aim to promote knowledge, awareness and bridge the communication gap between urban and rural population. These FM radios are also providing a platform to the students so as to get confidence, practical experience and demonstrate skills and potential. Non commercial radios established in different public sector organizations and departments such as education Sector (Campus radios), Judiciary (FM Radio Meezan, Peshawar High court), City Traffic Police Lahore (FM RASTA 88.6), National Highway & Motorway Police (FM 101) and PBC FM radio-101 etc. All these FM radios are performing role of social mobilizer, airing public awareness campaigns and also disseminating key information to update and keep informed their listeners (PEMRA, 2016).

FM radios are not only entertaining their listeners but also educating them and it is a medium which contributes to raise various social and cultural issues. FM radio had also been illegally used by the extremists and militants as propaganda tool to mobilize people to support their agendas. A large number of illegal radio stations had been blocked /suspended by the military during the operation in insurgent areas.FM Radio is handy medium in promotion of cultural values and issues. It creates impact on the listeners whether living in urban areas or rural. FM Radio aired programs on cultural heritages of local area which are equally listened by the people of other areas / regions. People of remote areas might be remained unaware of the cultural trials of other areas if FM radio waves could not reach them. (Tyagi, 2015).

Keeping in view and importance of FM radio, the purpose of this research is to explain and investigate Social & Cultural impact of FM radio in Pakistan.

Objectives of the Research

The major objectives of this research work are:
1. To know the background of FM radio in Pakistan
2. To know social impact of FM radio on the listeners
3. To know and evaluate cultural impact of FM radio on the listeners
4. To know and explore as how FM radio can achieve its objectives

Research Questions

1. Whether the radio industry has revived after emergence of FM medium in Pakistan?
2. Whether radio listenership has increased due to establishment of FM radio in Pakistan?
3. Whether FM radio programs fulfill the modern requirements of radio listeners with their healthy contents?

Significance

The rapid growth of FM radios due to which revival of radio listening in the Pakistan become possible, has grabbed the attention of the researcher. Social & cultural impact of FM radio in Pakistan will be examined as this dimension needs to be addressed. In preceding researches, this aspect was not discussed. So, this research is intended to investigate and explore the revival of radio listening in Pakistan through FM channels and significantly analyze social & cultural impact of FM radio in Pakistan.

Delimitations of the study

Keeping in view the requirements of the Ph.D dissertation and available facilities, present study is delimitized to the Social and Cultural impact of FM radio in Pakistan.

Statement of the problem

This research paper will investigates the history and background of FM radio in Pakistan. Furthermore, it will also elaborate how FM radio programs are socially and culturally impacting in Pakistan.

REVIEW OF LITERATURE

According to Abila (1998) the listenership of FM-100 has been expanded in Pakistan because of its adaptability and versatility. Over 80% individuals had conceded that their propensity for radio listening has been expanded due FM-100 as the programs aired on FM-100 are significantly more enlightening and additionally conveys amusement and resultantly it has picked up prominence. Ahmad (1999) depicts that after emergence of FM, radio business in Islamabad and Rawalpindi has been expanded around 70 %. The analysts also describes that around 70% youth love to listen FM radio transmission aired in Rawalpindi and Islamabad. This expanding pattern of FM listening portrays the prominence of FM radios among audience in Pakistan. Raza (2001) portrays that radio is a less expensive mode of communication which provides helpful information to its audience particularly agriculturists. Furthermore, farmers are getting advantage from the transmission exceptionally intended for them by the radio. Radio programs are anticipating in our cultural and social values which are affecting the listeners.

According to Wahgra (2002) prominence of FM tuning in Lahore can be gauged from this reality that after establishment in 1998, transmission of FM-100 has been expended and radio listening propensity in Lahore has been increased from 40.45% to 82.02% within three years. Programs of FM-100 have gained enormous popularity and acknowledgment in very short period in urban communities of Pakistan. Discourse and discussion amongst guest and radio presenter (DJs) are live communicated which pulls in the general population and individuals appreciate FM radio tuning in. Rawan and Siraj (2003) portray the viability of radio and depicts that radio in Pakistan has taken and carried the obligation of mass communication. Different educational and informative programs are communicated on radio for mass awareness. Radio audience consists of house spouses, agriculturists, general public and students etc.

Abbas (2004) describes that FM medium have pulled the attention promoting experts and professionals because FM radios are exceptionally cost effective. FM channels are liked for delivering informative and entertaining programs with quality of voice for the listeners of various age groups.Osunkunle (2008) express that radio has brought sociological changes in the society because of its varsity and simplicity in programming, undoubtedly radio is a device for social change. In South Africa, community radios have been utilized to illuminate, inform and educate. These radio stations also engage their audience by providing them a platform to participate and contribute meaningfully in national and community development issues. Sally D. Berman (2008) portrays that radio is a viable and traditional tool which is utilized for distance learning programs. The researcher also contends that the capability of radio medium is not appropriately used and this handy medium is being ignored. Radio is extremely powerful tool for rustic mass awareness.

As indicated by Ali (2009) the popularity of FM radio has been enhanced due to the fact that it is effective in highlighting various social and cultural issues for example, fanaticism , militancy, feudalism, murdering and disorder etc. and it also provide information to such people who have no access to newspapers, television etc.Jumani and Fazal-ur-Rahman (2009) state that radio is extremely powerful mass medium which can be utilized to engage and involve individuals in national development process. Radio medium is also useful is opinion building of mass audience as it has capacity to address substantial fragment of populace. Radio has tendency to provide guidance and awareness to masses on various social issues.Dutta (2010) portrays that most territories in India, radio is used as informative and entertaining tool. People get news from radio as other source of medium are inaccessible for them. Yaser et al (2011) depict that radio is known to be significant wellspring of information in far flung areas. A larger population utilized radio medium to listen news, get information and listen entertaining programs. Noticeably, after the development of FM, radios are also gaining popularity in urban areas as well.According to Naqvi et al (2011) mass audience offer inclination to FM broadcasting because of its intriguing programs contents which are popular among youth and enthusiastically listened by the listeners of all age groups. Kwakwa (2012) features significance and distinctive viewpoints about radio tuning in rustic regions. The scientist contends that lion's share of open listened radio in light of the fact that it provides information, training, diversion, current issues, sports etc. and also airs programs on religious topics and social issues including health and gender problems.

Aldrich (2012) depicts that radio programs are influential on listeners extensively. Impact of radio programs is seen on male and female audience members is very extraordinary. It is has been observed that audience members who listen peace programs on radio frequently, partakes in urban exercises and also helpful in changing viewpoints of audience members whereas hate radio programs expands animosity amongst audience. It clearly shows that radio transmission can change norms and standards. Thus, radio transmission can be used to counter fear based oppression through communicating delicate programs.As indicated by Venkatalakshmi and Chandraleka (2013) private FM radios are run commercially, however some kind of social mindfulness information is shared by private FM radios to their audience members such as environment, health, gender issues, education and information etc. FM radio moderators regularly talk on different social issues and announce public service messages for attention of masses.

Rahman-ullah (2013) describes that FM radios airs education, information and entertaining stuff to satisfy the necessities and prerequisites of the audience members of Peshawar city. Audience members use to listen FM radio to

get latest information. According to Rehman (2014) FM radios are observed as more viable and handy community media as it can discuss national and community based issues, promote regional and local voices. The stance of non sensationalism make FM radios more reliable and more dependable media among audience members as compared with other electronic media in the country i.e. cable, Television etc.

RESEARCH METHODOLOGY

To analyze Social & Cultural impact of FM radio in Pakistan, the researcher will examine the means and ways by which FM radio has revived radio listening in Pakistan. Survey method has been used to study this phenomenon. A research questionnaire has been prepared and designed which is consisted of close ended questions. Sample size for said survey comprises of 2100 respondents.

Population

The population of this survey is comprised of FM listeners particularly youth having age between 15 to 35.

Sampling

Two thousand one hundred (2100) respondents have been selected as a sample population for this research. The participants, who were common radio/FM radio listeners consists between age 15 to 35 are selected which comprises journalists, doctors, agriculturists, engineers, teachers, students and common public etc. Sampling frame designed for the said research consisted of education sector, public sector and private sector. Questionnaires in hard and soft form were distributed manually and online. Seventeen hundred (1700) questionnaires were manually distributed. Out of which, 1633 respondents have returned back the questionnaires which were scrutinized by the researcher and among these 1633 respondents, 1600 respondents have accurately filled out the questionnaires, left over respondents either have filled incomplete questionnaire or made cutting such questionnaire were rejected and 1600 responses were selected. Google Survey Form was utilized to get responses online. Accordingly, a link of Google Survey Form was shared online on social media as well as e-mailed to different peoples. Resultantly, 457 responses were received wherein, 400 responses were selected and left over responses were rejected due to incomplete entries. Total, 2000 responses were selected from the respondents.

Analysis of Data

Present study has been undertaken with the help of a detailed target based line survey to analyze and find out Social and Cultural impact of FM radio in Pakistan. A questionnaire was designed to conduct survey which contains close ended questions. The results of survey are accumulated and statistical analysis of results was performed by using SPSS (Statistical package for the social sciences).

Data Analysis & Interpretation

Demographical analysis of participants

Table 1 shows the gender variables of the study wherein total participants of the study were 2000, out of which 1,258 were male participants and 742 were female participants.

Radio listening habit of respondents

According to the figures in the above table, 73.1% respondents listen to radio and 26.9% respondents occasionally listen radio programs. The results reveal that 100% respondents listen radio because of the fact that during the survey only such participants were selected who use to listen radio.

Frequency of Radio listening

The Table shows that 15% participants regular radio listeners. 24.1 % participants are those who listen radio in a week time whereas 21.5% participants listen radio few times in a month and 38.9% participants are rarely radio listeners.

FM Radio listening habit of respondents

The Table depicts that 100% respondents use to listen FM radio as survey was conducted from such participants who are FM radio listeners.

Purpose of FM Radio listening

According to the results, about 50% participants listen FM radio programs for amusement whereas 35.8% respondents use to listen FM radio programs to get information, 9.2% participants listen FM radio for education, 3.4% respondents are such listeners who use to listen FM radio for development purpose and only 2.0% participants listen FM radio for persuasion.

Daily time spent on listening FM radio transmission

Results depict that 59% respondents listen FM radio programs for 1 to 2 hours daily, 33.1% respondents are such listeners who used to listen FM radio more than 2 hours daily and 8.0 % participants listen FM radio more than 3 hours.

Helpfulness of *FM* radio in highlighting different social issues

Participants of the survey were asked to share their views about the effectiveness of FM radio programs in terms of raising various social issues. Results of the survey depicts that, 56% participants have the opinion that FM radios programs are handy in highlighting different social issues. 31.6 % participants are of the opinion that to some extent FM radio programs are effective in raising social issues.7.8% respondents consider that FM radio programs are not much handy in highlighting social issues whereas 4.9% participants consider that programs on FM radio do not air and raise different social issues.

Types of social issues mostly highlighted in FM radio programs

According to the results, 23.8% participants bear the opinion that FM radio programs flourished issues associated with fitness and health. 38.0% participants would of the assumption that FM radio programs raise educational issues while 13.2% viewed as that issues identified with female privileges are highlighted on FM radio programs. 10.7% participants opined that issues related with gender orientation are project on FM radio whereas 7.3% responses have been received from such respondents who consider that arable and Agricola issues are promoted on FM radio. 6.4% participants opined that poverty related issues highlighted and 0.8% respondents have the idea that basically unemployed issues are raised on FM radio programs.

The outcome of the results reveals that education related issues are mostly project on FM radio programs which seems to be a handy sign keeping in perspective those angle that mostly audience of FM radio programs belongs to young generation.

Influence of FM radio in highlighting health related issues

The results depict the idea viewing the impact of FM radio on the audience as far as brining social transformation of raising health issues. Lion's share of respondent's i.e. 66.6% were of the idea that FM radio programs projects health issues and influential on the audience. 25.0% participants consider that FM radio averagely impacted the audience on health related issues while 5.5% participants acknowledged that FM radio programs on health issues rarely impacted audience whereas 3.0% respondents opined that health issues are not much broadcasted on FM radio programs, therefore didn't impacted the audience.

Overall results reveals that audience vivaciously tune in health programs, especially female are main audience as special health programs are aired to discuss health issues of female listeners e.g. programs on beauty tips, mother care, family health etc. Health campaigns are also launched on FM radios for attention and awareness of society i.e. campaign on Hepatitis, Dengue Virus, HIV aid etc. Pakistani society demands and requires proper media attention for promotion of health issues and FM radios are working very delicately in this sector.

Influence of FM radio in promoting Education and mass awareness

Results delineate the effect of FM tuning in for instructive and educational issues. 66.3% participants have the opinion that FM radio programs projects educational issues and impacted the audience while 24.5% respondents recognized that FM radio averagely impacted the audience with respect to educational issues, 6.6% respondents viewed as that FM radio projects rarely impacted the audience in terms of educational issues whereas, 2.8% participants consider that instructive and educational issues are not much discussed on FM radio programs and didn't impact the audience as well. Overall results uncovers that instructive and educational issues discussed on FM radio programs are influential on the audience. There are many education related issues of the audience particularly students such as career counseling, admissions issues, academic coaching, student teacher relationship, scholarships etc. A significant majority of respondents are of the view that FM radios are working very delicately and positively in this sector and trying to raise and highlight educational issues for guidance of audience particularly youth.

Influence of FM radio in promoting law abidance

According to the results, 30.8% participants responded that FM radio programs projects law abidance issues and influential on the audience. 46.5% participants share their views that FM radio averagely impacted the audience on account of law abidance issues while 16.7% respondents recognized that FM radio programs on law abidance issues have rarely impacted the audience. Furthermore, 6.1% participants consider that law abidance issues discussed on FM radio are not influential upon audience.Overall results uncover that majority of participants opined that law abidance issues discussed/broadcasted on FM radio very less influential on the audience. Numerous law abidance issues which are going through our community such as smoke within public, pedestrian crossing on highways & Motorways, wrong side driving on highways & Motorways, usage of seat belts while driving, over speeding etc. Law abidance issues are very rarely project on FM radio programs. Mostly participants consider that FM radio are averagely working in this sector and not appropriately tending and projecting law abidance issues for awareness of audience.

Influence of FM radio in improving spoken language

According to the results, 35.3% participants recognized that FM radio projects and impacted the audience in terms of change in spoken language while 34.3% participants consider that FM radio averagely projects and impacted the audience in terms of change in spoken language, 23.2% respondents opined that FM radio programs are rarely influential on the audience by virtue for dialect whereas 7.3% participants recognized that FM radio programs are not influential on audience by virtue for dialect utilization. Overall results depict that significant majority of participants consider that FM radio programs impacted the audience by virtue for dialect utilization. Presenters of radio programs are motivational ad inspirational heroes of young audience. Youngsters attempts to duplicate the accent and dialect they utilized. Radio presenters or RJs didn't take after those ethics or values from claiming social order. They utilized Hindi expressions throughout their discussions alternately utilized unscrupulous language, resultantly it has been observed that youth speaks Hindi expressions in their discussions and if this thing did not control at this stage it would hamper our social and cultural values. FM radio authorities should broadcast such programs which highlight this aspect for awareness of audience particularly youth.

Types of cultural issues highlighted in FM radio programs

The results depicts that more space is given to project traditional festivals in FM radio programs. 31. 8% participants favoured the argument whereas 30.2% respondents consider that FM radio programs projects and supportive in highlighting traditional customs. 19.1 % participants are of the opinion that that FM radio programs projects regional languages while 19. 0 % respondents viewed as that FM radio programs projects conventional living style.

Influence of FM radio in cultural change & living style

Results reveal that significant majority of participants i.e. 51.8 % have opined that FM radio programs are influential on audience in terms of brining cultural change in living style. 5.7% participants disagreed while 11.6 % participants have the opinion that FM programs are rarely influential on cultural dwelling style of audience rest of participants i.e. 31.0 % considered that FM radio programs have partially brought cultural change in terms of living style of the audience. Overall results show that FM radio programs have influenced its listener in terms of bringing change in their cultural living style.

Influence of FM radio in celebration of cultural festivals

Participants were asked to share their opinion regarding manipulation of FM radio on the audience in terms of bringing change in celebration of cultural festivals. A significant majority i.e. 59.5% of participants have favoured the statement while 4.5% participants disagreed with the statement, whereas 10.1 % participants have the opinion that FM radio programs are rarely influential upon celebration of cultural events. 36.1% participants opined that FM radio programs have partially brought cultural change on the audience in terms of celebration of cultural events.

Level of ethical values in FM radio Programs

Participants were requested to consign their views about the FM radio programs in accordance with the values. According to figures of table, dominant group of participants have the view to the amount that FM radio programs are average rated. 54.2% participants considered that programs aired on FM radio are average rated. 33.3% participants have the view that FM radio programs are morally good whereas rests of participanti.e.12.6% are about the choice as the programs aired of FM radio are unethical.

Overall impact of FM radio programs

According to the results, 45.5% participants have the opinion that FM radio programs have positive impact on the audience. 13.8 % participants opined that FM radio programs have negative impact on the audience while 32.1% participants have impartial views on the issue. 6.0% participants viewed as that FM radio programs rarely influential on the audience and 2.6% participants consider that FM radios programs are not influential on the audience. Overall results of survey are encouraging and ambitious considering the aspect that youth is the most intact audience with FM radio and the results portrays very excellent sign for community whereas less number of participants consider that FM radio make negative impact on the audience.

FINDINGS AND DISCUSSION

FM radio stations are working in almost all the major cities & towns in Pakistan and gratifying the needs of their audience by airing informative, educational and entertaining stuff since their inception. Medium of FM radio is rapidly grown up in Pakistan because of the fact that, the listenership of traditional radio (PBC) was significantly diminished in recent past. In Pakistan, Radio was facing many issues including accessibility, voice quality etc. from long time but after its conversion from AM medium (analogue) to FM medium (digital), now it is progressing with every passing day. Undoubtedly, FM radio has reached to a large number of disconnected and isolated communities and groups. FM radio is playing significant role in development of society by keeping the audience well aware of their surroundings. FM radio has got the attraction and attention as well of the audience, yet many discrepancies and deficiencies exist over numerous areas and zones for example programming contents, program balancing , professionalism etc. such deficiencies could be addressed with skilled and professional staff and by adopting latest techniques and technological advancement. Following are some findings that were observed during this research work: FM radio helps individuals to exchange of information. Individuals get information looking into tuning in news through radio. It gives most recent news to listeners without any obstructions. Radio gratifies the audience without gender discrimination. The most persuasive and influential feature of FM radio which attracts the young audience is music as it creates a lot of space for wonder.

FM radio presenters also anticipates a part on setting of an impact on the audience as young audience get inspiration from them and perform the same course as they listen from their favorite personality and it is also observed young audience spent their money in getting similar things e.g. clothing, jewelry etc. of their role models. FM radio provides a platform to introduce new talent. Teenagers learn many good things through FM radio programs as it enhances vocabulary and increases knowledge. Quiz programs, debate competitions and speeches of famous personalities are a big source of inspiration and enhancement of knowledge & information for audience particularly youth. Opinion polls are also conducted on FM radio which permits the audience to partake and share their thoughts with respect to different cultural, political & social issues. Audience participates in such assessment polls via e-mails, live calls etc. New FM radio stations are being established in private sector exorbitantly and the disappointment and concerning factor is the appointment of less educated and untrained staff by commercial radios. Excessive tuning in of FM might cause health problems. Youngsters spent hours in listening FM radio programs. This may distract & harm their innocent minds, persuasive on mental development and divert their attention from studies. PEMRA charged overwhelming sum from private FM radios on account of license issuing fee, resultantly, commercial FM radios relies upon airing musical and entertaining stuff instead of airing informative and constructive stuff.

CONCLUSIONS

FM radio is persuasive and effective communication medium. FM medium has setup new standards and revived radio listening in the Pakistan as the traditional radio had lost its credibility among the audience in recent past. FM radio got attraction and gained popularity among audience by providing uninterrupted service, better program

quality, versatile programming, excellent voice quality, easy access and approachability in very short span of time. FM radio is being utilized by all age groups, male or female, literate or illiterate, professionals or unprofessional etc. No doubt FM radio is social mobilizer, creating mass awareness and raising cultural and social issues, either pertains to local or national level. Many social awareness health related campaigns i.e. Hepatitis, Polio, Dengue, HIV/AIDS virus etc. and other social campaigns to address public issues such as unemployment, poverty, law abidance, literacy etc. are also aired on FM for awareness of audience.Many commercial and non commercial (Campus) FM radios are working in Pakistan. Commercial FM radios are information & entertainment channels whereas Campus radios are working in public sector organizations and educational institutes. Similar to other part of the world, FM medium is being successfully used as result oriented educational tool in Pakistan as well. FM radios are very efficiently transmitting educational and informational stuff to the audience. Particularly, Allama Iqbal Open University (AIOU), Islamabad is effectively using Campus FM to run distance education programs all over the country.

One thing should be kept in mind that in very highly competitive atmosphere and growing market, serious and concrete efforts are always required to develop and enhance the quality and standards which FM radio programs essentially requires. The necessity of era is to effectively use the potential of this powerful media for betterment of society, by setting out not only rules for broadcasting industry but also ensuring implementation of these rules so that predictability and certainty be ensured. This will also provide safeguard to expected standards and requirements of audience. Many efforts are still required to get maximum output and benefit from the very effective and useful radio medium in Pakistan. It has the capacity to outreach in far flung areas of the country as well as it can spread the message and its voice without discriminating its audience whether literate or ill-literate, young or old, men or women etc. It is the obligation and responsibility of Govt., PEMRA as well as management of FM radio to evaluate their policy, plan, prepare & devise their programs wherein the key concentration is on raising social awareness, cultural integrity, national *solidarity* etc. as the magic box is undoubtedly a tool of social and cultural change.

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Table. 1. Gender

	Male	Female
Frequency	1258	742
Percentage	62.9%	37.1%

Table 2. Social Background

	Rural	Urban
Frequency	702	1298
Percentage	35.1%	64.9%

Table. 3. Age group

	15-20	20-25	25-30	30-35
Frequency	536	636	486	342
Percentage	26.8%	31.8%	24.3%	17.1%

Table. 4. Education level

	Literate	Matric	FA/F.Sc	BA/B.Sc	Above
Frequency	95	140	301	651	813
Percentage	4.8%	7.0%	15.0%	32.6%	4.7%

Table.5. Profession

	Students	Journalist / Media persons	Engr.	Agriculturists	Businessmen	Govt. Servants	Others
Frequency	1199	35	96	54	113	204	114
Percentage	60%	1.8%	4.8%	2.7%	5.7%	10.2%	5.7%

Table. 6 Radio listening habit of respondents

	Yes	No	To some extent
Frequency	1462	0	538
Percentage	73.1%	0.0%	26.9%

Table.7.Frequency of Radio listening

	Regularly	A few times in a week	A few times in a month	Rarely
Frequency	312	481	430	777
Percentage	15.6%	24.1%	21.5%	38.9%

Table.8.FM Radio listening habit of respondents

	Yes	No	To some extent
Frequency	2000	0	0
Percentage	100.0%	0.0%	0.0%

Table.9.Purpose of FM Radio listening

	Entertainment	Information	Education	Development	Persuasion
Frequency	994	716	183	68	39
Percentage	49.7%	35.8%	9.2%	3.4%	2.0%

Table.10.Daily time spent on listening FM radio transmission

	1-2 Hours	> 2 hours	> 3 Hours
Frequency	1179	662	159
Percentage	59.0%	33.1%	8.0%

Table.11. Helpfulness of FM radio in highlighting different social issues

	Greater extent	Great	To some extent	Rarely	Not at all
Frequency	337	780	631	155	97
Percentage	16.9%	39.0%	31.6%	7.8%	4.9%

Table.12.Types of social issues mostly highlighted in FM radio programs

	Health issues	Education	Women rights	Agriculture	Gender issues	Poverty	Unemployment
Frequency	475	760	263	146	213	127	16
Percentage	23.8%	38.0%	13.2%	7.3%	10.7%	6.4%	0.8%

Table.13.Influence of FM radio in highlighting health related issues

	Greater extent	Great	To some extent	Rarely	Not at all
Frequency	631	700	499	110	60
Percentage	31.6%	35.0%	25.0%	5.5%	3.0%

Table.14.Influence of FM radio in promoting Education and mass awareness

	Greater Extent	Great	To some extent	Rarely	Not at all
Frequency	501	823	489	131	56
Percentage	25.1%	41.2%	24.5%	6.6%	2.8%

Table.15.Influence of FM radio in promoting law abidance

	Greater extent	Great	To some extent	Rarely	Not at all
Frequency	167	447	930	334	122
Percentage	8.4%	22.4%	46.5%	16.7%	6.1%

Table.16.Influence of FM radio in improving spoken language

	Greater extent	Great	To some extent	Rarely	Not at all
Frequency	292	414	685	464	145
Percentage	14.6%	20.7%	34.3%	23.2%	7.2%

Table.17. Types of cultural issues highlighted in FM radio programs

	Promotion of regional languages	Promotion of traditional festivals	Traditional customs	Traditional living style
Frequency	382	635	603	380
Percentage	19.1%	31.8%	30.2%	19.0%

Table.18. Influence of FM radio in cultural change & living style

	Greater Extent	Great	To some extent	Rarely	Not at all
Frequency	455	580	620	232	113
Percentage	22.8%	29.0%	31.0%	11.6%	5.7%

Tanle.19.Influence of FM radio in celebration of cultural festivals

	Greater Extent	Great	To some extent	Rarely	Not at all
Frequency	517	671	522	201	89
Percentage	25.9%	33.6%	26.1%	10.1%	4.5%

Table.20.Level of ethical values in FM radio Programs

	Ethical	Unethical	Average
Frequency	665	252	1083
Percentage	33.3%	12.6%	54.2%

Table.21. Overall impact of FM radio programs

	Positive	Negative	Neutral
Frequency	995	302	703
Percentage	49.8%	15.1%	35.2%

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RESEARCH ARTICLE

Laser Power Effect of Pulsed Laser Deposition (PLD) on Optical Energy Gap of Diamond Like Carbon Thin Films

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ABSTRACT

In this work, Thin films of diamond like carbon(DLC) were prepared by pulsed laser deposition(PLD).Theoptical energy gap of DLC decreased from (2.04,2.02,1.96)ev with increasing in laser power from (600 ,700, 800) mj,respectively,optical absorbance, extinction coefficient of the (DLC) films were evaluated and the result are discussed. Fourier transform infrared (FTIR) spectra for DLC used to the know presence of bonding where ratio SP2 increased and decreasing of SP3 with increasing of laser power from (600, 700, 800) mj, respectively, also decreasing of the transmittance for all the films.

Keywords: DLC, thin film, optical, laser.

INTRODUCTION

The carbon is a sixth element in the periodical table that exists in various compounds and forms; graphite, Diamond and nanotubes.The carbon occurs in another form as diamond like carbon (DLC), which has amixture of sp2 and sp3 bonding. DLC film is amorphous structure, very smooth, and can be deposited on different substrates at temperatures less than 325^{0}C[1,2]. The main parameters of interest in these materials is the content of sp3 fraction, sp2 clusters in the films, hydrogen content, and the orientation of sp2 phase.The wide properties range of DLC is coming from atomic orbitals are hybridized during making chemical bonds and the complex structure involving different bonding configurations, and the incorporation of hydrogen in the films [5-3], At all events, that the diamond like carbon (DLC) films consist of sp2 clusters embedded in the sp3 bonded matrix Carbon. The properties of diamond like carbon (DLC) films depend on the process conditions and deposition technique. DLC have the great variety of unique properties, include high thermal conductivity, optical transparency, wide band gap semiconductor from (1-4)eV, chemical inertness, high hardness, and low mobility semiconductor. Therefore, DLC film was used in various applications such as magnetic storage disks additionally DLC films used as semiconductors coatings for

electronic application [7-8].The wide range of characteristics of carbon allotropes which result from thehybridization of atomic orbitals through the making of the chemical bonds.The presence of sp3 bonds to confer the DLC film mechanical propertieswhile the sp2 bonds are found to control the electronic properties [4-6].

EXPERIMENTAL

Started the work from of clean the slides (substrates) by using detergent with water. The processing has been replaced by replacing the distilled water with pure alcohol solution.Were prepared thin films by pulsed laser deposition (PLD) using Nd:YAG laser (wave length 1064 nm , f = 6Hz , E= 600 , 700 , 800 mj) at room temperature under the vacuumwith angle 45^0 to the normal of the surface . The substrate was located 2 cm from the target. The target diameter of 2cm and thickness 0.5cm. The optical properties of(DLC) thin films were studied in the wavelength range (330 –1100 nm) by using UV-VIS shimadzu 1800 spectrophotometer. The output data of wavelength, transmittance and absorbance are used to deduced the optical absorption edge and all optical constants. electronic structure determined by Fourier Transformed Infrared Spectrum of DLC was recorded by shimadzu double beam FTIR spectrometer for DLC thin film were studied in the wave number range (400 – 4000 cm^{-1}).

RESULTS AND DESCUSSION

FTIR Spectroscopy

The chemical composition of DLC thin films can be investigated byFourier Transform Infrared (FTIR) spectroscopy. Figure (1) shows the Fourier Transform Infrared (FTIR) spectra for (DLC) deposited at (600,700,800) mj/ 6H on the glass substrate, many bands are evident in the spectra of DLC films in the range (400-4000 cm^{-1}).The analysis of the deposited DLC films shows peaks in the range (2800-3100 cm^{-1}). There is a common agreement with the predicted frequency, where that region is an indication of the existence of (C-H) bonds [9,16]. From figure (1a) shows the peaks at (2926) cm^{-1} correspond to the sp3 (CH2) asymmetric stretching vibrations [5,7,15]. The peak located at (1643.17) cm^{-1} is correspond to the Olefinic sp2 [5] . The peak located at (1110.92) cm^{-1} is due to the (C=O). The peak observed at 3429.2 cm^{-1}, can be attributed to (O-H) stretching vibrations [5,20]. From figure (1b) shows the peaks at (2920) cm^{-1} correspond to the sp3 (CH2) asymmetric stretching vibrations [5,7,14]. The peaks found at 1518 cm^{-1} attributed to the stretch of mixed sp2-sp3 (C-C) bond [5,10]. The peak located at (1643) cm^{-1} is correspond to the Olefinic sp2 [5] . The peak located at (1064) cm^{-1} is due to the (C=O) [5,11]. The peak observed at 3429.2 cm^{-1}, can be attributed to (O-H) stretching vibrations [5,20].

From fig. (1c) shows the peaks at (2924) cm^{-1} correspond to the sp3 (CH2) asymmetric stretching vibrations [5,7,12,13]. Modes indicating the presence of small amount of sp3 bonded carbon in sp2 matrixwhich is the characteristic of (DLC) film. The peaks found at 1517 and 1237 cm^{-1} attributed to the stretch of mixed sp2-sp3 (C-C) bond [5,10]. The peaks at (1638) cm^{-1} correspond to the Olefinic sp2 [5]. The peak observed sp2 (C-H) stretch at 3172 cm^{-1} and we have observed sp (C-H) (stretch) to 3251cm^{-1} [5]. The peak observed at 3452.34 cm^{-1}, can be attributed to (O-H) stretching vibrations [5,20]. The peak at 590.18 cm^{-1} is refer to (C-H) bend and is attributable to the graphite vibrational [5,11]. We have observed the peak located at (2375.35)cm^{-1} is may be due to ($C\equiv C$) stretch.Figure (1) show that the FTIR Spectrum for DLC films at the different of laser fluency at (a,b,c). Show that from the figure (1) that the transmittance of thin films decreasing with increasing of the laser intensity and decreased sp3 with decreasing the optical energy gap of DLC thin films and increased sp2.

The absorbance spectra

The optical absorbance of DLC thin films grown on the glass substrate asa function of wave length at room temperatureis shown in Fig. (2) absorbance spectra of all DLC films under study were recorded in the wave length

range between 330 and 1100 nm.Show that from the figure that the film has very high absorbance in the UV region and decreases exponentially with the increasing of wavelength, which indicates that DLC have high response in the UV region and this behavior is typical for many semiconductors due to internal electric field within the crystal and inelastic scattering of charge carriers by photon. Figure (2) show that the absorbanceSpectrum for DLC films at the different laser flounces. Show that from the figure (2) that the absorbance of thin films decreases with increasing of the laserpower. From the figure (2) that the film has high absorbance near wave length (357 ,356, 354) nm for the powers (600 ,700,800) mj, respectively, and whichindicates on happen surface Plasmon resonance (SPR) within that the values.

Optical energy band gap

The optical energy band gap values (E_g) for (DLC) thin films have been determined by using (tauc formulas),the relation between the absorption coefficients (a) and incident photon energy (hv) can be written as [19,25] :

$$\alpha h\nu = B\left(h\nu - E_g\right)^{1/2}$$

Where B is the a constant which depends on the type of the material , hv is the photon energy (eV) , E_g is the optical energy gap (eV) , r is constant and many take values 1/2 , 3/2 , 2 and 3 depending on the material and the type of the optical transition . In fig. (3) the value of the optical energy gap of (DLC) is calculated to be (2.04,2.02,1.96) eV to laser power at (600,700,800) mj, respectively. These results are similar to those from laser deposited DLC films in other reports [21,22,23]. Absorbance spectra of (DLC) films under study were recorded in the wavelength range between 330 and 1100 nm, and from the absorbance spectra, the optical bandgap was calculated in an amorphous material like DLC. He optical band gap (Eg) of DLC films can be obtained from the Tauc relationship, $(\alpha h\upsilon)^2$ versus photon energy plots of DLC films prepared at 600mj/6H laser power under 10^{-3} mbar. From fig. (3) shows change the optical band gap (Eg) with change laser power andshow that the optical band gap (Eg) decreased with increasing in the laser power.

Figure(3) shows the $(\alpha h\upsilon)^2$ versus photon energy plots of DLC films prepared at (600,700,800) mj/6H forlaser power. This figure confirms the transition type is direct, the band gap Eg can be determined from extrapolating the linear part of this figure to hυ= 0 points. The values of the optical band gap of films were (2.04,2.02,1.96) eV respectively. It can be find that increasing the optical energy gap with decreasing sp2 bond was attributed to decrease the width of π-π* bands with constant of π-π* separation.This result agrees withreported results [26,27]. The localized state may by exist in optical energy gap due to various clusters of sp2 sites (including graphitic phase), and free sp3 sites (not saturated with hydrogen atoms). This would mean that a distribution of clustersize and shape would determine the value of optical energygap and theTauc plot may give an average value of Eg [17,18]. Figure (4) shows the variation of (Eg) as a function to laser power.

Optical Constants of DLC thin film

The extinction coefficient, which is related to the exponential decay of the wave as it passes through the medium, is defined as:

$$k = \frac{\alpha\lambda}{4\pi}$$

Where λ is the wavelength of the incident radiation.
the extinction coefficient (k) values could be calculated from the absorption coefficient. (k) has typical value switch agree with other studies [24]. Figure (5) shows the variation of (k) as a function of wavelength, figurebehavior of the extinction coefficient (K) is nearly similar to the corresponding absorbance and absorption coefficient for DLC thin film at room temperature. It can see that the value of extinction coefficient (K) increasing with increasing of the

wavelength to 574nm and decreasing with increasing of the wavelength. The maximum value of extinction coefficient (K=0.118,0.16,0.241) at wavelength 573nm, at (600,700,800) mj respectively.

CONCLUSIONS

1. The DLC thin films deposited at laser fluence were uniform, smooth and compact.
2. From the images of X-Ray Diffraction(XRD) measurement indicated that the DLC grown are amorphous in nature.
3. The optical properties appear the deposited DLC films have a direct band gap and decreasing with increasing of the laser power and shows that the absorbance of thin films increases with increasing of the laser power.
4. FTIR measurement indicated that the DLC films have increase of sp2 and decrease from sp3 with increase laser power.

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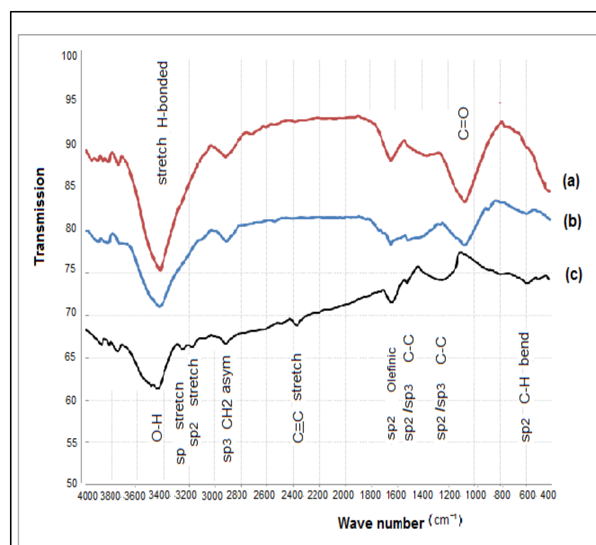

Fig. 1.The images of FTIR spectra of DLC films: (a) at 600mj/6H, (b) at 700mj/6H, (c) at 800mj/6H

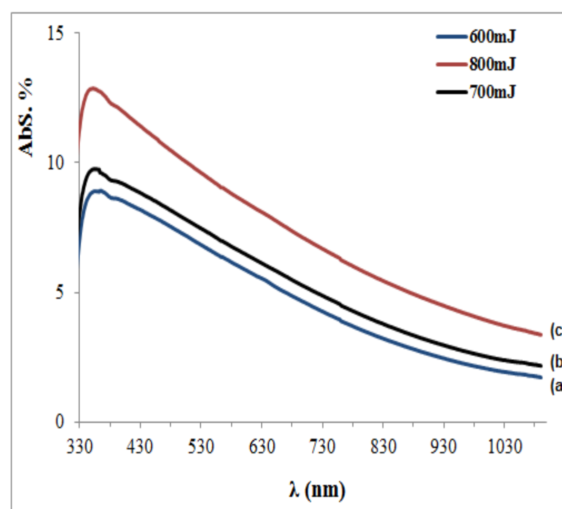

Fig .2. The absorbance spectra for DLC films: (a) 600mj/6H, (b) 700mj/6H, (c) 800mj/6H.

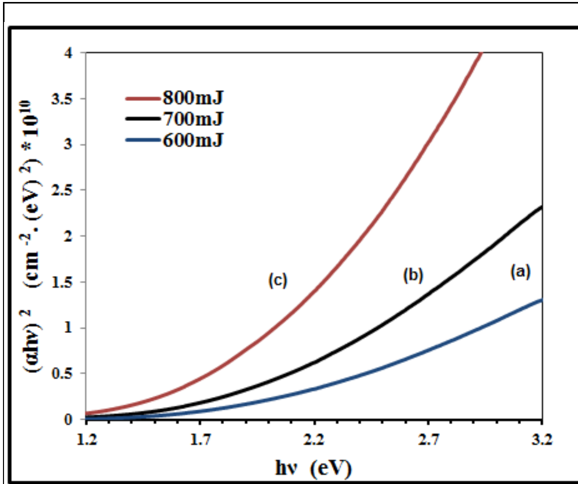

Fig .3. ($\alpha h\nu$)2 versus hν plots for DLC films: (a) 600mj/6H, (b) 700mj/6H,(c) 800mj/6H

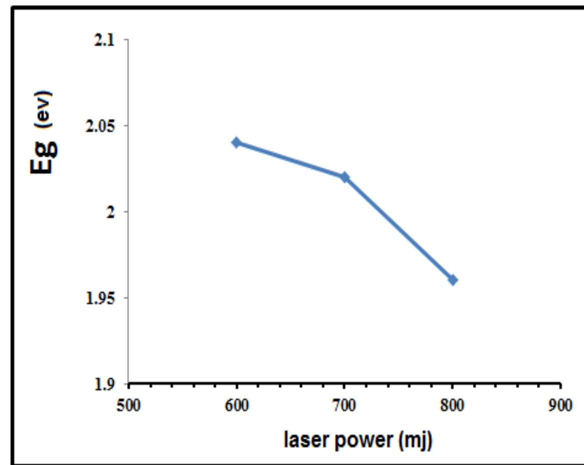

Fig.4. the variation of (Eg) with the laser power

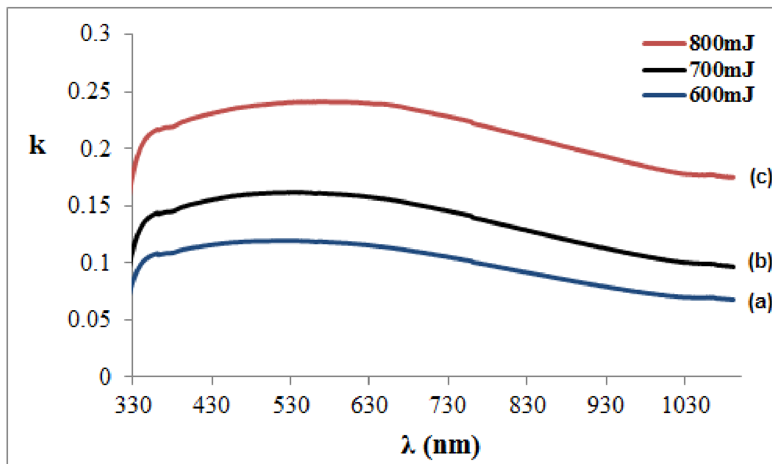

Fig. 5. Extinction Coefficient for DLC thin film: (a) 600mj/6H, (b) 700mj/6H, (c) 800mj/6H.

RESEARCH ARTICLE

Physical Stress Manifestation in Gestation: Assessing Physical Stress Symptoms and Levels

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ABSTRACT

Changes in health status are experienced during and after the gestational period which include alterations in physiological and psychological health conditions. Exposure to prenatal stress affects hypothalamic-pituitary-adrenal axis regulation which is shown to impart long-term consequences on maternal and fetal health outcomes. This study aimed to assess physical stress symptoms and physical stress levels in gestation. Participants filled in a study questionnaire which analysed sample characteristics. Sadaf Stress Scale was utilized for assessment of physical stress that it divides into nineteen symptoms, and physical stress levels that it categorizes into four states termed normal, mild, moderate and severe. Participants in first trimester, second and third trimester reported for presence of varied spectrum of physical stress symptoms, where the mean physical stress scores were highest for participants in first trimester compared to that of participants in second and third trimester. Overall, 44.36%, 23.31%, 16.54% and 15.79% of the study population were assessed to experience normal, mild, moderate and severe physical stress intensities. Pearson's correlation test identified a significant association between physical stress scores and age in second trimester of gestation. Taken together, the findings of this study exhibited the manifestation of physical stress in gestation, suggesting the need of its timely evaluation and management for alienating adverse associated outcomes.

Keywords : Maternal Stress, Maternal Physical Stress, Sadaf Stress Scale, Physical Stress.

INTRODUCTION

Substantial alterations in health state are experienced during and after gestational period [1], including changes in psychological and physiological health conditions [1-3]. This may be linked to situation of altered heath functioning [4]. Exposure to prenatal stress is found to affect hypothalamic-pituitary-adrenal axis regulation [5, 6], and is shown to have long-lasting impact on maternal and fetal health consequences [7]. Stress is considered as one of the biopsychosocial factors [8], that contributes to adverse gestational outcomes, including preterm delivery [8-19], low birth weight [20-25], preeclampsia and intrapartum complications [26-28]. Gestational outcomes such as, preterm are found to be mediated by stress triggering neuroendocrine responses [29-33]. Moreover, several studies have suggested role of maternal stress in transducing its impact on offspring health conditions [34], including fetal neurobehavior [35], and fetal development [29, 36, 37]. Prenatal maternal stress is considered to stem out from an imbalance between multiple environmental needs includingstressors of acute and chronic nature in alignment with resources available [20, 29, 36]. This may lead to a pathway of elevated stress perception and increased possibility of emotional responses such as anxiety and depression, collectively contributing to poor health results [38]. Prenatal stress is found to impact maternal physiology and immune function in such a way that it potentiates the chances of gestational complications development [39]. Previous study has presented an effect of maternal psychosocial milieu on state of maternal physiology [29].

Behavioral risk factors, such as diet intake, prenatal care, and maternal smoking are included in models of prenatal maternal stress [32, 40-43]. These behaviors have been studied as possible links between conceptsof prenatal maternal stress and perinatal complications [29, 32, 40-44].Chronic stress in gestation is found to reflect upon underpinning characteristics that drive adverse birth outcomes[42]. Gestation is a period of myriad physiological changes involving adaptation at systemic and local organ level, which enable maternal body to cope with demands of gestation. In light of existing literature, timely evaluation and management of prenatal maternal stress standspivotal, as this period denotes the window when stress is found to impact maternal and fetal health settings. In view of the physiological and psychological alterationsin gestation, understanding and analysing the upheaving stress levels, which may include physical stress,is important to delineate its impacts on maternal and fetal health. This study aimed to determinethe presence of diverse array ofphysical stress symptoms and physical stress levels in gestation, and tofurther decipher the relationship of physical stress and age, weight, and body mass index(BMI) of study participants in first, second and third trimester of gestation.

MATERIALS AND METHODS

This study comprised a total of 133 participants in first (N=15), second (N=35) and third (N=83) trimester. Participants without any severe psychological and mental illness, complicated gestational period and chronic health disease, were included.This study was approved by an independent ethics committee. Informed consent was obtained from each volunteer, prior participation in this study. Participants filled in a questionnaire which aimed to assess sample characteristics (Table 1). Sadaf Stress Scale (SSS)was used in this study for assessment of physical stress in gestation[45]. Following 19 physical stress symptoms were assessed using this scale:

1. Breathlessness
2. Churning Stomach
3. Diarrhea/Constipation
4. Dizziness
5. Dry Mouth
6. Excess Sweating
7. Fatigue
8. Headaches

9. Increased Colds/Flu
10. Increased Heart Rate
11. Indigestion
12. Nausea Feeling
13. Feeling of Palpitations
14. Feeling Heartbeat
15. Rapid and Shallow Breathing
16. Sleeping Problems
17. Tingling in Hands/Legs
18. Tremor in Hands/Legs
19. Weight Loss/Gain

Participants reported the presence or absence of physical stress symptoms as:

- Never (Score = 0)
- Rarely (Score = 1)
- Sometimes (Score = 2)
- Very Often (Score = 3)
- Always (Score = 4)

SSS categorized the physical stress into four levels:

1. Normal (Score = ≤ 29)
2. Mild (Score =30 – 37)
3. Moderate (Score =38 – 45)
4. Severe (Score =≥ 46)

Data were analysed by using IBM SPSS Statistics 24, and presented as mean ± standard deviation. Linear relationships between assessed variables (physical stress scores, and age, weight and BMI) were identified using Pearson's correlation.

RESULTS

Participants in this study (N=133; sample characteristics provided in Table 1) had a mean age of 26.62 ± 4.84 years. SSS assessed various physical stress symptoms experienced by participants, reported as never, rarely, sometimes, very often and always (Figure1). SSS assessed the physical stress level as normal (44.36%), mild (23.31%), moderate (16.54%) and severe (15.79%), which exhibited the presence of varying degrees of physical stress among study participants (Figure 2). Participants in the first, second and third trimester reported for presence of multiple physical stress symptoms as defined by SSS, which were utilized to assess physical stress intensities (Table 2). Among all participants in first trimester, 20% (N=3), 26.67% (N=4), 40% (N=6), and 13.33% (N=2) were analysedto experience normal, mild, moderate and severe physical stress levels, respectively. Participants in second trimester, 51.43% (N=18), 22.86% (N=8), 14.28% (N=5), and 11.43% (N=4) were assessed to have presence of normal, mild, moderate and severe stress levels, respectively. Participants in third trimester, 45.78% (N= 38), 22.89% (N=19), 13.25% (N=11), and 18.07% (N=15) were assessed to experience normal, mild, moderate and severe stress levels, respectively.

Mean physical stress scores of participants in first, second and third trimester were 34.2 ± 12.37, 29.68 ± 12.30 and 31.35 ± 11.37, respectively, which displayed highest mean physical stress score in first trimester compared to that of second and third trimester(Figure 3). Pearson's correlation test identifiedsignificant positive relationship between

physical stress scores and age in second trimester(*r=0.361*, *p*=0.033). Associations between physical stress scores and weight in first (*r*=0.340, *p*=0.215), second (*r*=0.185, *p*=0.287) and third (*r*=0.146, *p*=0.187) trimester of gestation, and between physical stress scores and BMI in first (*r*=0.216, *p*=0.439), second (*r*=0.153, *p*=0.380) and third (*r*=0.110, *p*=0.324) trimester of gestation remained statistically insignificant. Moreover, relationships between physical stress scores and age in first (*r*=0.399, *p*=0.141) and third trimester (*r*=0.051, *p*=0.648) of gestation were determined to be statistically insignificant (Table 3).

DISCUSSION

Gestational period brings about many alterations in the body [46]. It is the state of progressive changes attributable to physical variations[47]. The results of this study presented the occurrence of physical stress symptoms in gestation using SSS. This study further explored parameters such as, age, weight and BMI to identify its association with maternal physical stress. Gestation is marked by changes and shifts in the maternal body triggering adaptive pathways[48]. These changes in health conditions[1], entailalterations in psychological and physiological health status[1-3]. SSS assessed 19 physical stress symptoms of the study participants (Figure 1) and also, differing levels of physical stress (Table2).Multiple adaptations at physiological and psychological health situation may underpin mediation ofphysical stress in gestation. The maternal respiratory function is altered during gestation in order to fulfil additional demands of oxygen of fetus [49]. Elevated levels of progesterone are indicated to governphysiological changes in the respiratory system[50], as it is determined to increase the respiratory center sensitivity to carbon dioxide [51]. Slow bowel movement during gestation is found to cause severe, or chronic abdominal pain [52].

During gestation, the major cardiovascular changes encompass elevated plasma blood volume, augmented cardiac output, and reduced systemic vascular resistance [53]. Heart rate is found to escalate above non-gestational period by 15% in gestation at the end of first trimester, and this increases further to 25% by the end of the second trimester, withpotentially no majorchange suggested to happenin third trimester [52]. In gestation, indigestion and hypochlorhydria in the gastric juice are reported to develop[54].Also, in gestation, nausea and vomiting are most common symptoms which are found to impact 50-90% of the women[55]. Moreover, the early stages of gestation comprising morning sickness, coupled with nausea, vomiting and dizziness aretermed attributable to hormonal imbalances[54]. Increased sympathetic activity during gestation may account for multiple physiological changes, which are markedly observed in gestation[52].There is general excitement of nervous system during gestation, which may lead to the psychological imbalance, such as precipitating change in the mood, excitement or depression in the initialphase[54].The weight gain is presented to be evident in third trimester as compared to first and second trimester of gestation[54].

SSS evaluated physical stress levels as normal, mild, moderateand severe for study participants (Figure 2). A high proportion of participants were found to have physical stress levels as normal,followed by mild, moderateand severe stress ranks.Previous study depicted that theraised intensities of maternal stress, anxiety and in general depressive symptoms are related to enhancedmotor growth in children and mental development, however stress levels of mild and moderate nature in healthy population may play role in raising fetalmaturation[56]. Participants in first, second and third trimester presented differing levels of physical stress assessed as normal, mild, moderate and severe (Table 2). Participants in third trimester depicted higher frequency for severe stress as compared to participants in first and second trimester. The mean physical stress scores of participants were assessed more for first trimester as compared to that of second trimester and third trimester (Figure 3). Previous study has displayed that with advancing stage of gestation, the susceptibility for effects of stress is decreased, and this drop in sensitivity for stress may exhibitelevated protection from adverse pregnancy outcomes [9]. Moreover, previous research study haspresented a biobehavioral model relating prenatal stress to outcomes of fetal development, which considered that theinfluence of maternal stress are mediated by type, length, and period of stressduring gestation[57].Pearson's correlation identified

significant positive linear association between physical stress scores and age in second trimester of gestation, whereas insignificant relation was analysed between physical stress scores and weight in first, secondand thirdtrimester of gestation, and between physical stress scores and BMI in first, secondand third trimester of gestation. Moreover, insignificant relation was obtained between physical stress scores and age in firstand third trimester of gestation (Table 3). Previous study presented findings denoting occurrence of prenatal depressive symptoms in women, older in age [58].Furthermore, an earlier reportexhibited inverse correlation between BMI and depression in gestation[59].In this regard, it is suggested that studies should uncover underlying factors in gestation paving way to maternal stress. It is recommended that future studies should focus on the physiological changes leading to prenatal stress development. It is suggested that studies should explore categorically varying stress forms and intensities, firstly for better understanding of the existence of maternal stress types, and secondly for contributing to the field forprecise evaluation and management of prenatal maternal stress. It is encouraged that longitudinal research study designs should be conducted to elucidate extent of severity and altering stages of stress throughout the period of gestation, to avoid related adverse outcomes.

CONCLUSIONS

Gestation is a period of several physiological and psychological changes, which enable maternal body to cope with requirements of gestation. This study presented findings which came together to collectively indicate manifestation of physical stress symptoms in gestation, coupled with varying levels of physical stress. The findings displayedthat the highest frequency of study participantsexperienced normal physical stress level,which was followed by mild, moderate and severe stress intensities.Elevated mean physical stress score was determined for participants in first trimester compared to mean physical stress score of participants in second and third trimester. Moreover, significant association was identified between physical stress scores and age of participants in second trimester. It is suggested that future studies should consider evaluation of different stress types to add to the existing knowledge of prenatal maternal stress management, and thereby alienate itspotential adverse maternal and fetal health outcomes.

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Conflict of Interest

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Table 1. Characteristics of study participants.

Variable	Parameters	N	%
Education	No schooling completed	3	2.26%
	Primary	2	1.50%
	Middle	7	5.26%
	Matriculation	31	23.31%
	Intermediate	34	25.56%
	Associate ordinary bachelor	29	21.80%
	Bachelor	24	18.04%
	Masters	1	0.75%
	Other(s)	2	1.50%
Family	Nuclear	30	22.56%
	Extended	103	77.44%
BMI	Underweight	4	3.01%
	Normal weight	65	48.87%
	Overweight	43	32.33%
	Obesity	21	15.79%
Gravidity	Primigravida	55	41.35%
	Multigravida	78	58.65%
Trimester	First	15	11.28%
	Second	35	26.32%
	Third	83	62.41%

Table 2. Participants with assessed normal, mild, moderate and severe physical stress levels in first, second and third trimester.

Physical Stress Level	Trimester					
	First		Second		Third	
Normal	N=3	20%	N=18	51.43%	N=38	45.78%
Mild	N=4	26.67%	N=8	22.86%	N=19	22.89%
Moderate	N=6	40%	N=5	14.28%	N=11	13.25%
Severe	N=2	13.33%	N=4	11.43%	N=15	18.07%

Table 3. Pearson's correlation between assessed physical stress score, and age, weight and BMI.

Variables Assessed			*r*	*p*
First trimester	Physical stress score	Age	0.399	0.141
		Weight	0.340	0.215
		BMI	0.216	0.439
Second trimester	Physical stress score	Age	0.361*	0.033
		Weight	0.185	0.287
		BMI	0.153	0.380
Third trimester	Physical stress score	Age	0.051	0.648
		Weight	0.146	0.187
		BMI	0.110	0.324

*. Correlation is significant at the 0.05 level (2-tailed).

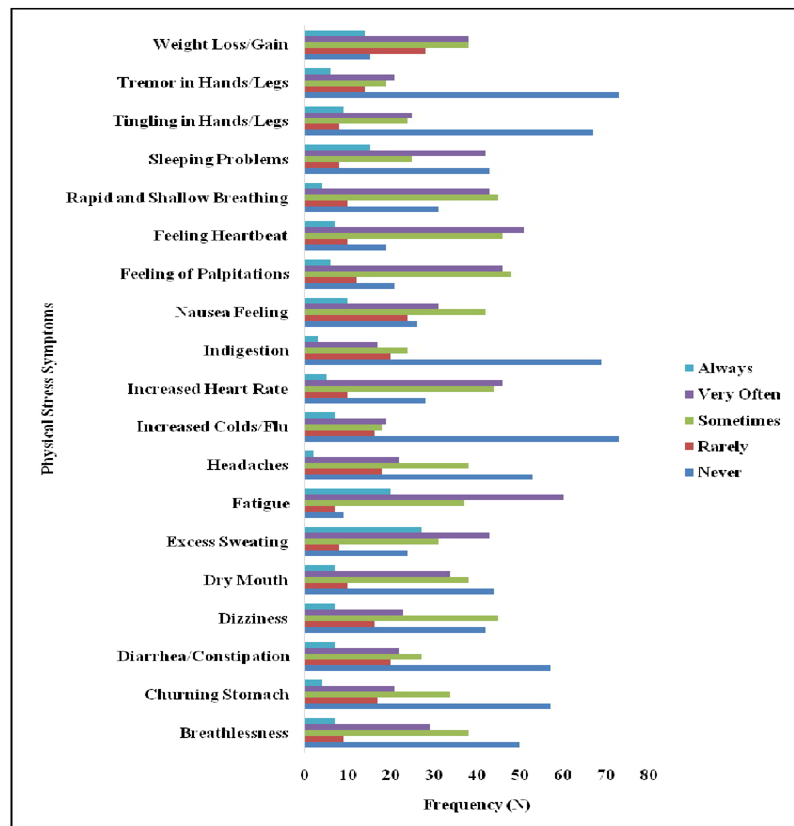

Figure 1. Physical stress symptoms. The frequencies of physical stress symptoms experienced as never, rarely, sometimes, very often and always reported by study participants.

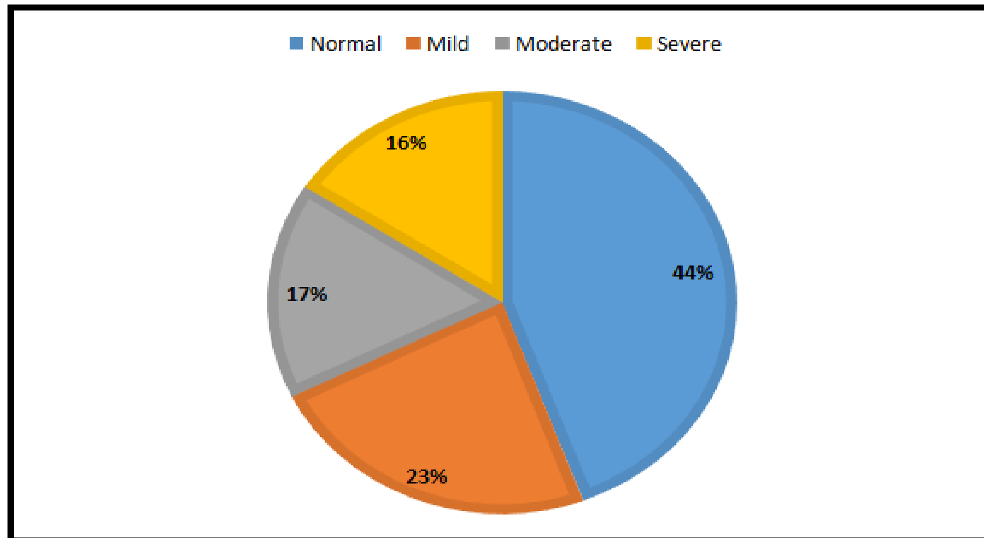

Figure 2.Pie chart representing percentage of study participants assessed to have normal (N=59, 44.36%), mild (N=31, 23.31%), moderate (N=22, 16.54%) and severe (N=21, 15.79%) physical stress level.

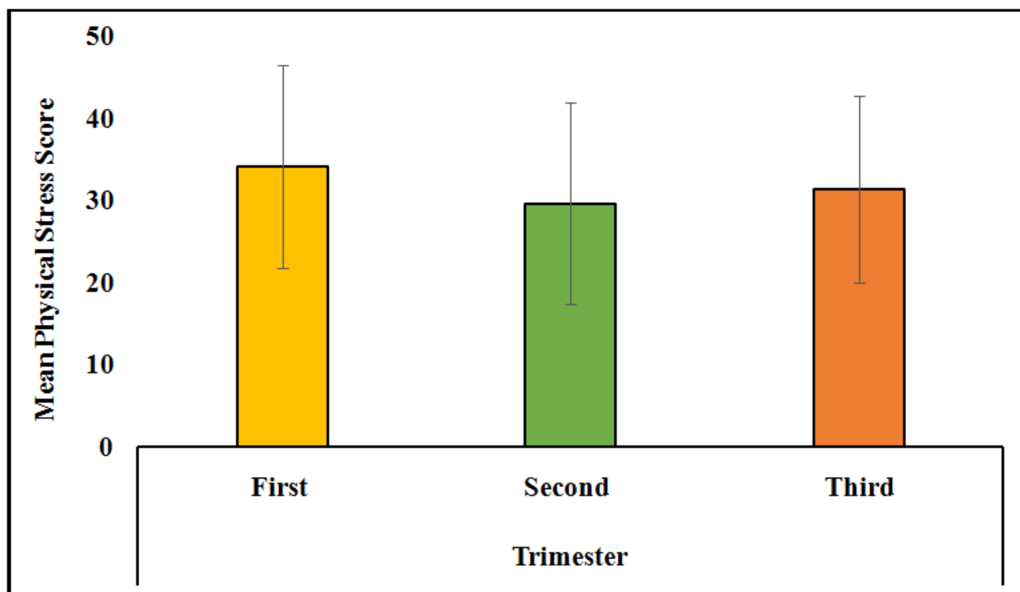

Figure 3. Vertical bar graph representing mean physical stress score values of participants in first (score: 34.2 ± 12.37), second (score: 29.68 ± 12.30) and third (score: 31.35 ± 11.37) trimester.

RESEARCH ARTICLE

Effect of Supplementation of Rumen Protected Fat on Postpartum Uterine Involution in Crossbred Cows

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ABSTRACT

Postpartum negative energy balance leads to delayed uterine involution and hampers the resumption of cyclic ovarian activities. The present study evaluated the effect of dietary supplementation of rumen protected fat in postpartum uterine involution and vaginal cytology. The experiment was carried out in four experimental groups of six cows each (GI – control, GII- 200 g bypass fat, GIII – 200g bypass fat daily + Ovsynch protocol, GIV – Ovsynch protocol alone). The mean number of days required for complete uterine involution were 31.16±2.71, 29±1.09, 28.33±1.50 and 32.33±1.96 in group I, II, III and IV respectively. By day 30 postpartum 100 per cent of animals completed uterine involution in group II and III. In group I all the animals completed uterine involution by day 36 whereas; in group IV all the animals completed involution by day 34 which was confirmed by ultrasonography. The results of present study showed that dietary supplementation of rumen protected fat during postpartum period helps in early involution of uterus, thus promoting the resumption of cyclic ovarian activities in crossbred cows.

Keywords: Negative energy balance, Bypass fat, Uterine involution, Vaginal cytology.

INTRODUCTION

Animal agriculture is a major source of livelihood for the famished and rural population across the sphere. The agricultural economy of India considerably depends upon animal husbandry activities especially dairying in

supporting the livelihood requirement of the majority of rural population. India is the leading milk producing country in the globe and the milk production has recorded impressive growth after independence which has jumped from 17 million tonnes in 1950-51 to 155.5 MT in 2015-16. The annual milk production of Kerala was recorded as 2.65 MMT (2015-16). The annual growth rate of milk production in Kerala was far below than the national average with less than 2.5 percent for the last few years and contributed only 1.7 percent of total milk production of the country (Economic Review, 2016). Even though the average productivity of the crossbred cows in Kerala is high comparing to national average, the production in the state is lagging behind the actual domestic requirement. Hence the Govt. of Kerala is targeting to achieve self-sufficiency in milk production in the state in coming two years and implementing integrated schemes in the dairy sector to ensure fair returns to dairy farmers (The Hindu, 2017). To achieve this goal, improving the reproductive efficiency of available female cattle population is of utmost importance.

Productivity largely depends upon reproduction, and one of the major reasons for reduced life-time milk production by an individual dairy animal is transient loss of fertility or infertility. The reproductive efficiency is influenced by several physiological, pathological, nutritional and environmental factors. Anoestrus and repeat breeding are two of the major reproductive problems affecting 30 to 40 per cent of total cattle and buffalo population of India (Chakurbar *et al*., 2008). In Kerala, the incidence was reported to be even higher (61%) and this is mostly attributed to the negative energy balance in high producing cows during the postpartum period (Kutty and Ramachandran, 2003). The postpartum negative energy balance in dairy cows adversely affects the uterine involution thus leading to delay in resumption of cyclic ovarian activities.Supplementation of rumen protected or rumen bypass fat in the ration of dairy animals has been reported to have great positive impact on their production and reproduction performances (Naik, 2013). It is reported that reproductive efficiency of dairy cows had improved by the supplementation of bypass fat, by increasing the conception rate and reducing the days open (Sklan *et al*., 1994, Naik *et al*., 2009). But these studies were preliminary in nature and did not provide any concrete confirmation about the mechanism of action and the exact benefit of its supplementation. Hence the present study was carried out to evaluate the rate of postpartum involution of uterus following bypass fat feeding and the vaginal cytology characteristics.

MATERIALS AND METHODS

The investigation was carried out on a total of 24 apparently healthy normally calved, crossbred cattle of similar age and parity with a body score of 3 to 3.5 out of 5 maintained at University Livestock Farm and Fodder Research Station, Mannuthy.The animals were randomly allotted to four groups of six cows each. All the animals in these four groups were fed as per standard feeding practices based on ICAR recommendations. Animals in Group I was not given any supplementation and kept as control. The animals in Group II were fed with 200 g bypass fat per day (Calcium salt of palm fatty acid, containing crude fat - 84 %, calcium - 9%, acid insoluble ash - 4% and moisture - 3%) from 5 days after calving till 90th day along with compounded cattle feed, every morning. The animals in Group III were fed 200 g of bypass fat per day from 5th day of calving till 90th day along with compounded cattle feed every morning. In addition, they were subjected to Ovsynch protocol as described earlier (Hagen et al., 2015). Animals in Group IV were not supplemented with bypass fat. But they were subjected to Ovsynch protocol on Day 45 postpartum. All the animals in Groups I and II were inseminated during natural oestrus exhibited after Day 45 postpartum. Animals in Groups III and IV were subjected for timed AI and observed for induced oestrus. The time at which foetal membranes were fully expelled was noted. Those animals in which the foetal membranes were expelled after 12 hours were not included in the study. Uterine involution in all the experimental animals was assessed by rectal palpation and trans-rectal ultrasonography on day 10 post-partum and subsequently from day 26 to 36 at two days interval.

Trans-rectal scanning of uterus and ovary were done with a real time Doppler ultrasound scanner (ESAOTE™ My Lab Gamma, Italy) equipped with linear array, 5-10 MHz frequency trans rectal transducer. The gain, brightness and contrast were set optimally for each examination. The changes in the average thickness of body of the uterine wall

were measured by ultrasonographic examination on Day 10 postpartum and subsequently at ten days interval till day 90 postpartum in all the four groups. The uterine involutions and ovarian structures were recorded and measured in millimeter with the inbuilt scale provided with the ultrasound scanner. Uterine involution was considered to be completed when no more significant changes recorded in the uterine wall thickness on the succeeding examination. The data recorded were analyzed statistically using statistical software SPSS (SPSS, Version 14, USA).

RESULTS AND DISCUSSION

The mean uterine wall thickness (mm) on day 10, post-partum in groups I, II, III and IV were 10.61±0.26, 10.94±0.30, 10.45±0.23 and 10.76 ±0.32, respectively. The mean uterine wall thickness (mm) on day 20 post-partum in groups I, II, III and IV were 7.72±0.29, 7.06±0.29, 6.97±0.22and 7.28±0.31, respectively. The Corresponding values on day 30 post-partum in groups I, II, III and IV were 6.29±0.33, 6.08±0.27, 5.82±0.24and 6.18±0.18, respectively. The mean uterine wall thickness (mm) on day 40 post-partum in GI, GII, GIII and GIV were 6.25±0.41, 6.04±0.26, 5.88±0.34 and 5.96±0.12, respectively. The mean thickness of the uterine wall did not reveal any significant difference between groups (Figure 1-3).The mean number of days required for complete uterine involution among different groups an assumed by trans-rectal ultrasonograpic examination is given in Table 1. The mean numbers of days required for complete uterine involution among different groups as assessed by trans-rectal examination were 31.16±2.71, 29±1.09, 28.33±1.50 and 32.33±1.96, respectively in group I, II, III and IV. By day 30 postpartum, 100 percent of animals in group II and III completed uterine involution. In group I and IV all the animals completed uterine involution by days 36 and 34 respectively. There was significant difference in rate of involution in group II and III ($p<0.05$) compared to group I and IV. Percent of cows completed uterine involution on various days in different groups, as assessed by rectal palpation is presented in Table 2. All the cows in group II and III uterine involution were completed by day 30 post-partum. But in groups I and IV (no bypass fat supplementation) uterine involution was delayed up to day 36 and 34 respectively.

As per the available reports, the average time required for complete cervical involution is 30 days and that for complete uterine involution is 25 to 50 days (Noakes *et al*. 2009). In present study, the mean numbers of days taken for complete uterine involution among different groups were 31.16±2.71, 29±1.09, 28.33±1.50 and 32.33±1.96 respectively in group I, II, III and IV. By day 30th post-partum all the animals in group II and III completed uterine involution. In group I, the time taken for completing the uterine involution in all animals was 36 days, whereas that in group IV was 34 days.The days taken for complete uterine involution was 29±1.09 and 28.33±1.50 respectively in those groups supplemented with bypass fat compared to 31.16±2.71 and 32.33±1.96, respectively in non-treated groups This is in concurrence with the earlier findings of Tyagi *et al.* (2010), who supplemented the animals with 2.5 per cent calcium salts of fatty acids and observed that uterine involution in cows treated with bypass fat was completed in 35.40 days compared to 49.44 days in control group. This is also in concurrence with Gowda (2014) who observed that the mean duration taken for uterine involution in bypass fat supplemented animals were 23.14±0.86 and 22.29±0.75 days respectively.It is well established that factors such as limited energy intake and low body reserves are responsible for delayed uterine involution hampering the early resumption of postpartum ovarian cyclic activity (Theodore *et al*., 2016). Thus, the bypass fat feeding in present study has helped improve the body reserves and the energy balance, there by promoting early involution of uterus after calving.

CONCLUSIONS

The results of present study showed that the supplementation of rumen protected fat during postpartum period helps in early involution of uterus, thus promoting the resumption of cyclic ovarian activities in crossbred cows.

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Table 1. Days required for complete uterine involution in cross bred cows as assessed by ultrasonography

S. No.	Group (n=6)	Days taken for complete uterine involution (Mean±SE)*
1	GI	31.16± 2.71[a]
2	GII*	29± 1.09[b]
3	GIII*	28.33± 1.50[b]
4	GIV	32.33± 1.96[a]

GI-control cows, GII- Cows supplemented with bypass fat (BF), GIII- Cows supplemented with BF and subjected to ovsynch on day 45 PP; GIV- Cows subjected to ovsynch day 45 PP. *Values bearing different superscripts differed significantly (p<0.05)

Table 2. Per cent of cows completed uterine involution on various days in different groups, as assessed by rectal palpation

S. No.	Group (n=6)	Day 26	Day 28	Day 30	Day 32	Day 34	Day 36
1	GI	0	16.67	33.33	0	16.67	16.67
2	GII	0	50	50	0	0	0
3	GIII	16.67	50	33.33	0	0	0
4	GIV	0	0	33.33	16.67	50	0

GI-control cows, GII- Cows supplemented with bypass fat (BF), GIII- Cows supplemented with BF and subjected to ovsynch on day 45 PP; GIV- Cows subjected to ovsynch day 45 PP. All the cows in group II and III uterine involution were completed by day 30 PP. But in groups I and IV (no BF Supplimentaion) uterine involution was delayed up to day 36 and 34 respectively.

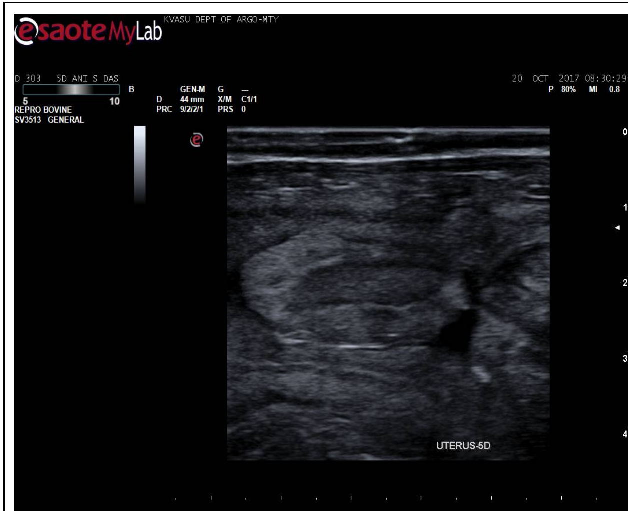

Figure 1. Ultrasonographic image of uterus on Day 5 postpartum

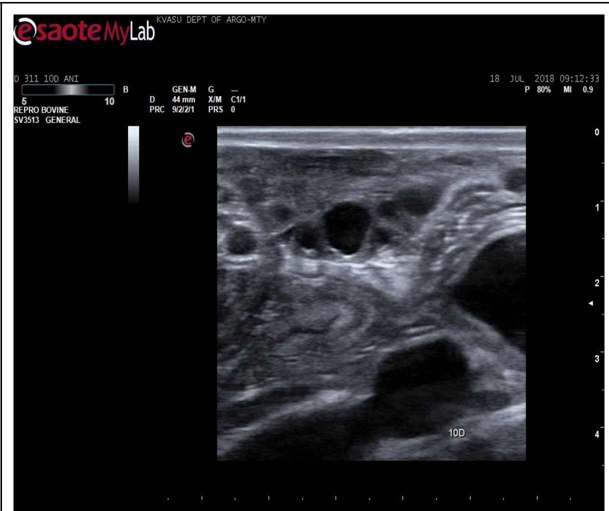

Figure 2. Ultrasonographic image of Uterus on Day 10 postpartum

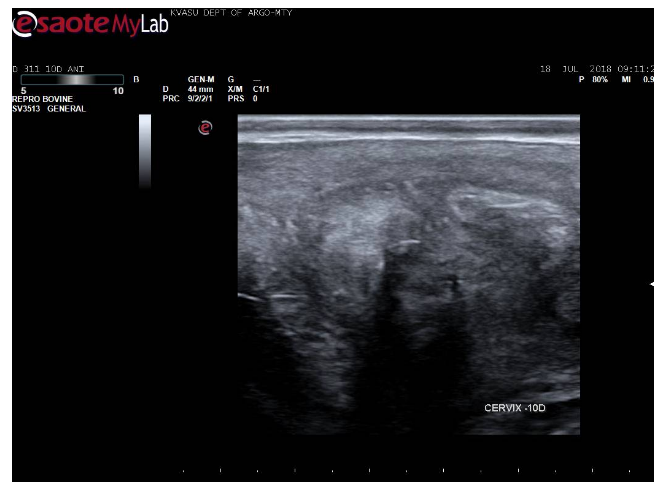

Figure 3. Ultrasonographic image of Cervix on Day 10 postpartum.

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RESEARCH ARTICLE

Association of Body Mass Index with Cardiovascular Parameters and Serum Cortisol Level in Gestation

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ABSTRACT

Maternal obesity is found to play an important role in promoting multiple health complications and adverse perinatal outcomes. Studies have associated maternal obesity with cardiovascular risks. These cardiovascular risks coupled with obesity during gestation include gestational hypertensive disorders, antepartum venous thromboembolism and other complications. Obesity related hypertension in gestation may arise due to endothelial dysfunction, hyperinsulinemia, unbalanced lipids profile and upregulation of inflammatory status. Cortisol is found to play an important role in maternal biochemical alterations and its elevated levels are considered to indicate the maternal health physiological challenges. Corticosteroids are reported to regulate blood pressure mediated by glucocorticoid action at renal and extra-renal sites including the central nervous system and the vasculature. The role of cortisol in inducing hypertension and regulating cardiovascular parameters in maternal obesity remains largely elusive and needs to be determined. This study aimed to evaluate and compare relationships between BMI, blood pressure and serum cortisol level in gestation. Participants with uncomplicated gestation were categorized by BMI into obese (BMI ≥ 30) and non-obese (BMI < 30) group. Analysis of resting blood pressure was performedusing mercury sphygmomanometer, and blood was drawn for serum cortisol estimation. Welch's t-test was applied for comparing means of two groups (obese and non-obese) and Pearson's correlation was utilized to identify linear relationship between variables. This study identified elevated systolic blood pressure, diastolic blood pressure and serum cortisol level in obese group compared with non-obese group. Furthermore, enhanced systolic blood pressure was determined for obese group in third trimester versus non-obese group. Serum cortisol concentration was assessed to be elevated for obese group in first, second and third trimester compared to that of non-obese group.

Pearson's correlation identified significant association between maternal BMI scores, and systolic blood pressure, diastolic blood pressure, and serum cortisol level. These findings may encourage weight management, to avoid obesity associated complications, including obesity related hypertension during gestation and the precise role of cortisol mediating cardiovascular parameters in maternal obesity or overweight status is suggested to be assessed by future studies.

Keywords : Gestation, Body Mass Index, Maternal Obesity, Cortisol, Systolic Blood Pressure, Diastolic Blood Pressure, Cardiovascular Parameters

INTRODUCTION

Obesity in gestation islinked to diverse maternal and fetal health complications [1-4]. These include higher risk of gestational diabetes mellitus [5], preeclampsia [6], caesarean delivery [7], newborn macrosomia [8] and other adverse perinatal outcomes [9]. Increase in weight gain during early gestation is found related to poor cardio-metabolic status of offspring, and childhood adiposity is considered to possibly mediate this effect [10]. Obesity coupled with increased gain in weightis assessed to elevate gestation related hypertension risk [11]. Studies have demonstrated relationship between maternal pre-pregnancy body mass index (BMI) and preeclampsia risk [6]. Preeclampsia is found associated with endothelial dysfunction [12]. Another study reported increased levels of circulating E-select in and endothelial cells as predictors of preeclampsia [13]. Moreover, the events of preeclampsia are found to impact glucocorticoids release[14]. Study conducted on nulliparous women found pre-pregnancy obesity and also over weight BMI status to be related with complications in gestation [15].It is reported that maternal obesity may present various cardiovascular risks possibly as manifestation of hyperinsulinemia, unbalanced lipids profile, alteration in functioning of endothelial cells and upregulation of inflammatory status of the body [16]. Further more, maternal obesity and blood pressure during each trimester are reported to be inter linked [17]. The cardiovascular risks coupled with obesity during gestation is associated with gestational hypertensive disorders [17], antepartum venous thromboembolism and other complications [18].

Cortisol plays an important role in maternal biochemical alterations and its elevated levels are considered to indicate the maternal health physiological challenges [19]. Research study has presented findings indicating role of maternal cortisol in influencing intrauterine growth of fetus [20].More over, regulatory role of the hypothalamic-pituitary-adrenal (HPA) axis in gestation with effects concerning growth and development of fetus is shown to vary by the status of parity [14]. Cortico steroids are reported for their role in the regulation of blood pressure, which is mediated by glucocorticoid action at renal and extra-renal sites including central nervous system and the vasculature [21].Glucocorticoids are found to regulate alpha 1 adrenergic receptors expression, contributing to enhanced sensitivity of vascular smooth muscle cells to the action of catecholamines [22]. Also, 11β-hydroxysteroid dehydrogenase is found to govern glucocorticoid action in central nervous system [23]. In view of existing literature, it stands important to understand relation between maternal BMI and blood pressure, and serum cortisol concentration. Thus, the aim of this study was to analyze relationship between maternal BMI and cardiovascular parameters as well as serum cortisol level to elucidate the role of obesity in mediating blood pressure regulation and cortisol secretion during gestation.

MATERIALS AND METHODS

Women with uncomplicated gestation were randomly invited to participate. This study received ethical approval from an independent ethics committee. Participants were informed about study objectives and provided written informed consent. Study participants were categorized intoobese group (BMI ≥ 30) and non-obese group (BMI <30) after assessment of their BMI, which was determinedby Quetelet's Index, weight (kg) /height (m^2). Resting blood

pressure of each individual was measured using mercury sphygmomanometer. Blood was drawn for afternoon mean serum cortisol level quantification, which was analyzed by chemiluminescent microparticle immunoassay, termed Chemiflex (Architect Cortisol, Abbott Laboratories, USA). Descriptive data were presented as mean ± SEM. Welch's t-test was utilized for comparing means of two groups (obese and non-obese). Pearson's correlation wasapplied for identification of linear relationship between variables. p value ≤ 0.05 was considered statistically significant. Data were analyzed by SPSS version 24.

RESULTS

This study comprised a total of 98 participants.Out of total study participants, 9% (N=9) were in first trimester, 28% (N=27) were in second trimester and 63% (N=62) were in third trimester, who were categorized into obese group (N=35) and non-obese group (N=63).Mean age of participants in the obese group and non-obese group was found to be 24.26 ± 0.95 years and 24.68 ± 0.61 years, respectively. The mean systolic blood pressure of obese and non-obese group was 130.28 ± 3.95mmHgand 118.87 ± 1.99 mmHg, respectively (Figure 1). The mean value fordiastolic blood pressure of obese group was determinedto be 83.17 ± 1.91 mmHg,whereas it was 77.32 ± 1.44 mmHgfor the non-obese group (Figure 2). Serum cortisol estimation presented mean value of 22.22 ± 0.59µg/dl for obese group and mean value of 18.03± 0.34µg/dl for non-obese group (Figure 3).Welch's t-test identified a significant difference between mean values of systolic blood pressure (p=0.013) (Figure 1), diastolic blood pressure (p=0.017)(Figure 2) and the serum cortisol level (p<0.001) (Figure 3) of the obese and non-obese group.

Obese and non-obese participants in first, second and third trimester were also assessed for changes in blood pressure and serum cortisol concentration (Table 1). Systolic blood pressure remained statistically indifferent between obese and non-obese participants in first and second trimester, however, it remained elevated in third trimesterin obese group versus non-obese group reaching statistical significance (p=0.041) (Figure 4). Diastolic blood pressure remained statistically unaltered in first, second and third trimester between obese and non-obese group (p>0.05) (Figure 5). Furthermore, serum cortisol level was identified to be statistically enhanced for obese group in first (p=0.006), second (p=0.017) and third (p<0.001) trimester versus non-obese group (Figure 6). Pearson's correlation was utilized to determine linear association between variables assessed in this study (Table 2). A significant positive association was identified between BMI score and systolic blood pressure (r=0.287, p<0.01), BMI score and diastolic blood pressure (r=0.268, p<0.01), and BMI score and serum cortisol level (r=0.534, p<0.001) (Figure 7).

DISCUSSION

Maternal obesity is found associated with multiple adverse health outcomes [3, 24, 25]. This may include an enhancedgestational diabetes, pregnancy related hypertension and pre-eclampsia risks [26].Previous study has reportedthat excessive weight gain coupled with existing obesity potentiates the risk of gestation associated hypertension [11]. This study presented higher mean values of blood pressurein obese group compared to that of non-obese group. There was found a significant difference between mean values of systolic blood pressure (Figure 1) and diastolic blood pressure (Figure 2) of obese and non-obese group. Systolic blood pressure remained substantially elevated in third trimester of gestation in obese group, as compared to non-obese group (Figure 4). Diastolic blood pressure remained statistically indifferent between two groups in first, second and third trimester (Figure 5). Obesity in pregnancy is found associated with impaired functioning of microvascular endothelium, hyperinsulinemia, disturbed lipids profile, and up-regulation of inflammatory status [16].The risk of pre-eclampsia development is found to be doubled with 5-7 kg/m^2 increment in the BMI during pre-pregnancy period [6]. In this study, mean values of blood pressure of obese group and non-obese group were below the range considered for preeclampsia, i.e.blood pressure ≥ 140/90 mmHg, also there are other conditions for specifically diagnosing preeclampsia which were not considered in this study [15]. Elevatedserum cortisol level was quantified for obese group compared to that of non-obese group (Figure 3). Moreover, serum cortisol was determined to be markedly enhanced in obese group, in

first, second and third trimester versus non-obese group (Figure 6). A previous research study presented findings suggesting role of BMI, and not the perceived stress and waist circumference of obese non-pregnant women in predicting chief portion of variation in diurnal adrenal cortisol secretion [27].It is suggested to explore the molecular mechanisms driven by cortisol in inducing obesity related hypertension during gestation in future studies. Another study reported that maternal HPA axis and corticotropin binding globulin elevation during late gestation is mediated by enhanced levels of adrenocorticotrophic hormone and unbound secretion of cortisol during this period [28]. In gestation, the secretion of arginine vasopressin from the parvocellular paraventricular nuclei may regulate the variation in diurnal plasma cortisol levels [29].

This study presenteda positive relation between BMI and systolic blood pressure, BMI and diastolic blood pressure, and BMI and serum cortisolconcentration (Table 2; Figure 7). The role of corticosteroids is reported for regulation of blood pressure [21].Findings of previous study indicated reduced activation of the HPAaxis in obese participants as compared to lean participants during gestation[30]. This decreased HPA axis state in maternal obesity may be an underlying factor for macrosomia and the event of prolonged gestation [31]. The low level of maternal cortisol may impact the fetal HPA axis and other programming of diseases [32]. Further more, the placental corticotropin-releasing hormone may function as an important modulator of the HPA axis in pregnancy [33]. There exists raised level of corticotrophin releasing hormone and comparatively decreased level of the corticotrophin-releasing hormone binding protein in pre-eclampsia, enabling increased level of free hormone to mediate this pathological state [34]. Obesity during gestation has been found associated with increased pro-inflammatory markers [35]. Endothelial dysfunction is reported in pre-eclampsia [12]. Findings of previous study has presented elevated levels of circulating endothelial cells as well as E-selectin in preeclampsia [13]. Glucocorticoids play significant role in the regulation of circadian rhythm; and it is influenced by the events associated with preeclampsia [14]. The limitations of the study were recognized, in that, it was a cross-sectional study and it is suggested to further explore the association and mechanism by which obesity, cardiovascular parameters and cortisol are interlinked in longitudinal study design to confirm the presented preliminary findings. More over, the study did not consider evaluation of endocrine factors other than cortisol which may be involved in mediating response of elevated blood pressure in maternalobesity.

CONCLUSION

Weight management during gestation may be important to avoid obesity associated complications which may include obesity related hypertension during gestation and the role of cortisol mediating cardiovascular parameters in maternal obesity or overweight status needs further in-depth assessment by future studies.

ACKNOWLEDGMENTS

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Conflict of Interest

None.

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Table 1. Frequency of participants in first, second and third trimester categorized by BMI as obese and non-obese.

Trimester	Obese (N)	Non-Obese (N)
First	3	6
Second	8	19
Third	24	38

Table 2. Pearson's correlation between BMI and, systolic blood pressure, diastolic blood pressure and serum cortisol level.

Variables Assessed		*r*	*P*
BMI Score	Systolic Blood Pressure (mmHg)	*0.287*	*0.004*
	Diastolic Blood Pressure (mmHg)	*0.268*	*0.008*
	Serum Cortisol (µg/dl)	*0.534*	*<0.001*

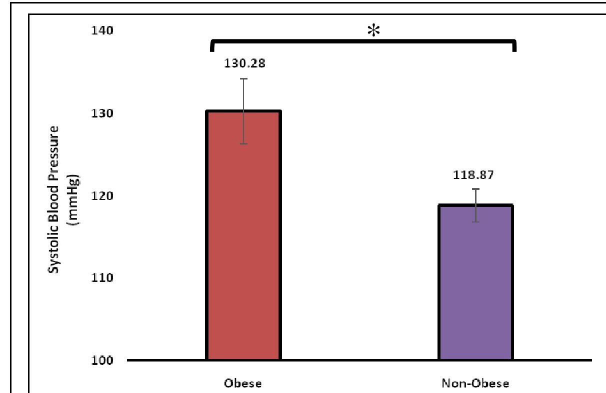

Figure 1. Mean values of systolic blood pressure for obese group and non-obese group.Systolic blood pressure of obese group was found to be markedly elevated (N=35, 130.28 ± 3.95mmHg, *p*=0.013)as compared with non-obese group (N=63, 118.87 ± 1.99 mmHg). * represents *p*<0.05

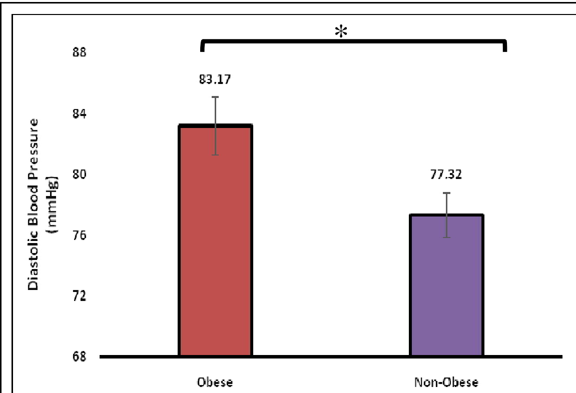

Figure 2. Mean values of diastolic blood pressure for obese group and non-obese group. Diastolic blood pressure of obese group (N=35, 83.17 ± 1.91 mmHg, *p*=0.017) remained statistically enhanced compared with non-obese group (N=63, 77.32 ± 1.44 mmHg). * represents *p*<0.05.

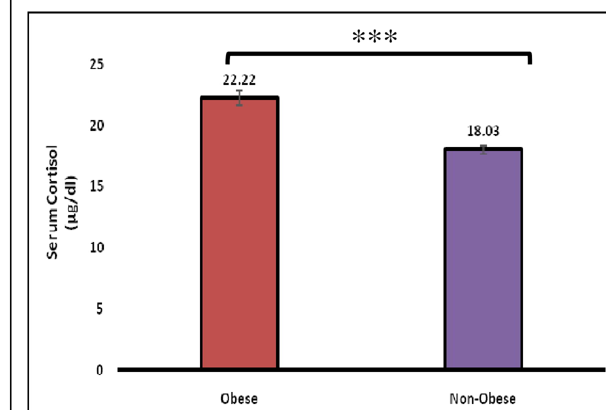

Figure 3.Mean values of serum cortical for obese and non-obese group. Serum cortical concentration was found to be substantially escalated for obese group (N=35, 22.22 ± 0.59 µg/dl, *p*<0.001) compared with non-obese group (N=63, 18.03 ± 0.34 µg/dl). * represent *p*<0.001.**

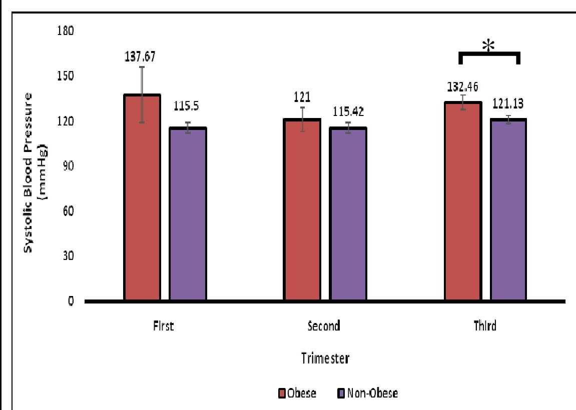

Figure 4.Systolic blood pressure of participants in obese and non-obese group in first, second and third trimester.Participants in obese group presented mean values of 137.67 ± 18.76 mmHg (N=3), 121 ± 8.17 mmHg (N=8) and 132.46 ± 4.61 mmHg (N=24), whereas participants in non-obese group exhibited mean values of 115.5 ± 3.61 mmHg (N=6), 115.42 ± 3.36 mmHg (N=19) and 121.13 ± 2.76 mmHg (N=38) for systolic blood pressure, in first (*p*=0.359), second (*p*=0.543) and third (*p*=0.041) trimester, respectively. * represents *p*<0.05.

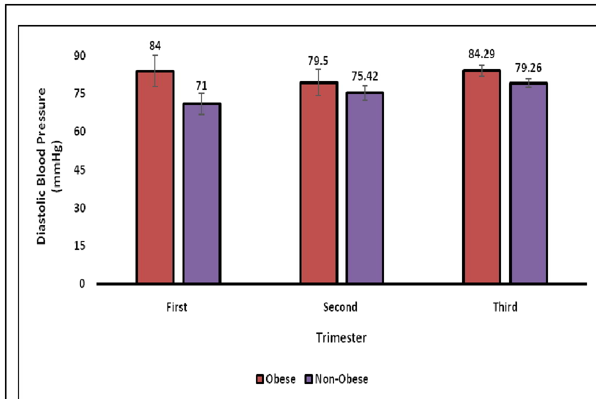

Figure 5. Diastolic blood pressure of participants in obese and non-obese group in first, second and third trimester. Participants in obese group exhibited mean values of 84 ± 6.08 mmHg (N=3), 79.5 ± 5.18 mmHg (N=8) and 84.29 ± 2.11 mmHg (N=24),whereas participants in non-obese group displayed mean values of 71 ± 4.17 mmHg (N=6), 75.42 ± 2.82 mmHg (N=19) and 79.26 ± 1.78 mmHg (N=38) for diastolic blood pressure in first (*p*=0.153), second (*p*=0.503)and third (*p*=0.075) trimester, respectively.

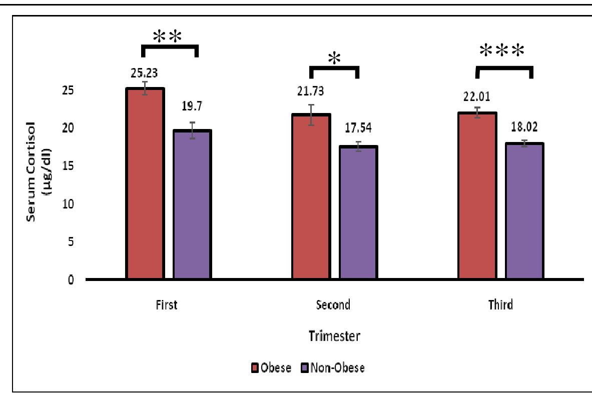

Figure 6.Serum cortisol concentration of participants in obese and non-obese group in first, second and third trimester. Participants in obese group depicted mean values of 25.23 ± 0.88 µg/dl (N=3), 21.73 ± 1.34 µg/dl (N=8) and 22.01 ± 0.72 µg/dl (N=24), and participants in non-obese group showed 19.7 ± 1.06 µg/dl (N=6), 17.54 ± 0.61 µg/dl (N=19) and 18.02 ± 0.44 µg/dl (N=38) for serum cortisol level in first (*p*=0.006), second (*p*=0.017) and third (*p*<0.001) trimester, respectively. *,,*** represent *p*<0.05, *p*<0.01, *p*<0.001, respectively.**

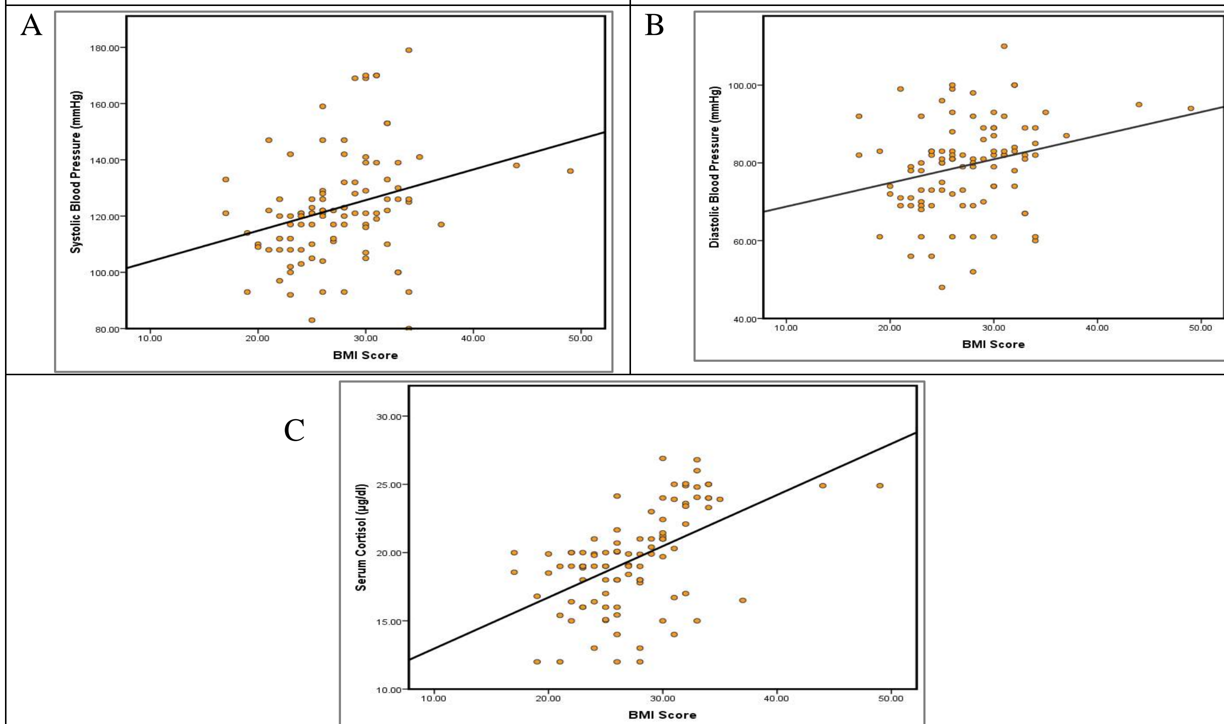

Figure 7.Correlation analysis for assessed variables. Pearson's correlation between BMI score and, systolic blood pressure (Panel A), diastolic blood pressure (Panel B) and serum cortisol level (Panel C).

RESEARCH ARTICLE

Study of Histopathological Changes in Sample of Iraqi Women with Uterus Cancer

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ABSTRACT

Uterine cancer is the most common cancer occurring in a woman's reproductive system. Uterine cancer begins when healthy cells in the uterus change and grow out of control. The aim of this study was to indicate some histological changes in the infected uterus of sample of Iraqi women with uterine cancer. Some infected samples of endometrial cancer were collected from menopausal women with uterus cancer for histological examination during their attendance at the center of cancer in Medicine City Hospital of Baghdad in Iraq. Histological examinations of infected endometrial tissues with cancer showed hypertrophy and hyperplasia of epithelial lining cells of uterus, mitotic activity of cells, vacoulation and hyperchromatic pleomorphic cancer cells. Collagenous fiber was also appeared in the effected region. Severe hemorrhage and inflaminatory cells were found in basal layer.

Keywords: cancer, reproductive system, women, endometrial, tissues, histological

INTRODUCTION

Uterus is a major female sexual reproductive organ that responsive to hormones in humans and most other mammals, especially steroids which have a big effect on endometrial cells [1].Structurally, uterus has three parts; the first part is superior to the opening of the oviducts called the fundus which appears like a dome, the middle part of it is called the body of uterus (corpus), which is the place of fetus grows and the narrow inferior portion of the uterus is the cervix, which is project into the vagina [2]. Cervix produces secretions of mucus that will be stringy and thin under the influence of high concentrations of systemic plasma estrogen, these secretions can facilitate the movement of

sperm through the tract of reproductive system [3] Histologically, the wall of uterus is composed of three layers. From out to the inner: Perimetrium or serous membrane, of visceral peritonium, it covers the outer surface of the uterus [4]. Myometrium, the next layer of uterus composes of smooth muscle [5]. Which can undergo both hypertrophy and hyperplasia and usually responds to increase demands by doing both, myometrium is increase enormously in both bulk and power during the 9 months of pregnancy in preparation for the immense contractile effort required at childbirth [6].Endometrium, the third layer, which is the innermost one in the uterus, it consist of lining of connective tissue, lamina propria, and lamina epithelial, The lamina propria or stroma of the endometrium contains primarily nonbundled type III collagen fibers with abundant fibroblasts and ground substance, it's type is simple columnar epithelial lining has both ciliated and secretary cells [7]. The latter line the numerous tubular uterine glands that penetrate the full thickness of the endometrium [8]. Endometrium has two concentric zones: The basal layer adjacent to the myometrium has a more highly cellular lamina propria and contains the deep basal ends of the uterine glands, the superficial functional layer has a spongier lamina propria, richer in ground substance, and includes most of the length of the glands, as well as the surface epithelium, The functional layer undergoes profound changes during the menstrual cycles, while the basal layer remains relatively unchanged [7].

Uterine cancer is any type of cancer that emerges from the tissue of the uterus, more than 43,000 women have uterine cancer each year in the USA, most are over 55 years old [9]. The grade of a uterine cancer is based on how much the cancer cells form glands that look like the glands found in normal healthy uterus (such as the glands that produce the lining that is shed monthly as a "period") [10]. In lower-grade cancers (grades 1 and 2) more of the cancer cells forming glands looking like normal shape, while, in higher-grade cancers (grade 3) more of the cancer cells are kind of jumbled up and do not form normal glands, higher grade cancers tend to grow faster and are more likely to spread to the other parts than lower-grade cancers [11] .There are some types of uterine cancer**:** Cervical cancer**,** Uterine sarcomas**,** Gestational trophoblastic disease and Endometrial cancer [12].Endometrial cancer (cancer of the inner lining of the uterus) is the second most common type in world, and fourth most common cancer in women from developed countries [13]. If caught early, most types of uterine cancer can be cured using surgical or medical methods, when the cancer has extended beyond the uterine tissue, more advanced treatments including combinations of chemotherapy, radiation therapy, or surgery may be required [14]. Endometrial cancer occurs most commonly after menopause [15]. Hormones are important agents in sex-related cancers, such as endometrial cancer [12]. Development of an endometrial hyperplasia is a significant risk factor because hyperplasia can and often do develop into adenocarcinoma, also, cancer can develop without the presence of a hyperplasia [16]. Endometrial cancer frequently metastasizes to the ovaries and fallopian tubes when the cancer is located in the upper part of the uterus, and the cervix when the cancer is in the lower part of the uterus [17]. More distant metastases are spread by the blood and often occur in the lungs, as well as the brain, liver, and bone [18]. Endometrial cancer metastasizes to the lungs 20–25% of the time, more than any other gynecologic cancer [19].

Signs and symptoms of endometrium arevaginal bleeding or spotting in women after menopause occurs in 90% of uterine cancer [20]. Bleeding is especially common with adenocarcinoma, occurring in two-thirds of all cases [15]. Abnormal menstrual cycles or extremely long, heavy, or frequent episodes of bleeding in women before menopause may also be a sign of endometrial cancer **[18].** Symptoms other than bleeding are not common; other symptoms include thin white or clear vaginal discharge in postmenopausal women [20]. More advanced disease shows more obvious symptoms or signs that can be detected on a physical examination, the uterus may become enlarged or the cancer may spread, causing lower abdominal pain or pelvic cramping [18]. Painful sexual intercourse or painful or difficult urination are less common signs of uterine cancer, the uterus may also fill with pus [21].

MATERIALS AND METHODS

Samples of endometrial tissue from menopausal women with uterine cancer were taken for assessment of histopathological changes. The preparation of histological section depended on standard methods [22]. The histology

and Histopathological study was performed with help of two books the first of them was Junquira and Carneiro [7] and the second was Kumar *et al.* [23].

RESULTS AND DISCUSSION

Sections in the uterus of menopausal woman with endometrial cancer showed several changes in uterus tissue. Figure (1) showed hypertrophy and hyperplasia of epithelial lining cells of uterine gland and goblet cells that lead to close its lumen with over secretion of mucin. Figure (2) showed mass of cancer cells expressed pleomorphic with mitotic figures nuclei and vaculation of dysorganized epithilal cells. This result agree with Kurman *et al.* who examined the pretreatment endometrial samples were evaluated the histological features of gland-to-stroma ratio, glandular cellularity, mitotic activity, cytologicatypia, and cytoplasmic changes including mucinous, secretory, squamous, and eosinophilic metaplasia, these histological changes could decrease by progestin-treated endometrial [24]. This result also agree with another studies [25] [26].Figure (3) showed abnormal proliferation of epithilal lining cells of endomaterium that form sheath of pleomorphic cancer cells extended to the lumen of the uterus, the cells expressed high mitotic division. Sheath of malignant cancer cells surrounded by squamous cells with vacuolation of pleomorphic cancer cells with high mitotic activity (adenocarcinoma) were showed in figure (4). Mitotic activity, up normal shape and up noraml size of cells may be duo to estrogen mitogenic activity on uterus cell especially with decreae level of PRG. This result was agree with Patel *et al.* which he referred to that if progesterone effects are disrupted, the epithelium can become hyper-plastic in response to unopposed estrogen which increase mitotic activity of cells [27]. Mitosis can be readily and accurately demonstrated on routine H and E stained sections of uterine cancer [28].

Histological examination in figure (5) showed fewy vacoulation in addition to hyperchromatic pleomorphic cancer cells arranegment as cord separated by thick strands of collagenous fibers tissue, this collagen was deposition by smoth muscles sells and caused increase in size and weight of fibroids. The tumor cell in figure (6) consist from chondrosarcomatous tissue separed by thickness fibrous contective tissue infiltrated by mononuclear cells (mixed tumor uterus). Khan *et al.* noted they are monoclonal tumors of the uterine smooth muscle cells and consist of large amounts of extracellular matrix that contain collagen, fibronectin, and proteoglycan [29]. Also, this result agreed with Wallach and Vlahos [30] and Ryan *et al.* [31]. In other section ,the uterus expressed proteinous materials in cystic dilation of uterine glands and inflammatory cells infiltration in proliferation of fibrous connective tissue between mucosal glands, multiple masses of compact cancer cells were showed in figure (7) these cells surrounded by severe inflmmatory cells infiltration in basal layer in addition to converted of cancer cells to secreted mucin type (adenocarcinoma). Figure (8) showed that hyperplasia of epithelial cells of uterine glands which become secreted types that secreted protinous materials in their lumens with proliferation of fibrous connective tissue between mucosal glands. This result agreed with Jaafar *et al.* and Matsuo *et al.* who found that different histological subtypes are presents with endometrial cancer that include: hyperplasia, inflammatory cells, serous, mucous and other histological changes [32] [33] .Severe hemorrhage in basal layershowed in figure (9), dysorganization of abnormal proliferation of epithelial layer of endometerium that extended to basal layer in addition to inflammatory cells infiltration in basalis layer and cancer cells in the lumen of the uterus as well as necrosis. Figure (10) showed hyperchromatic pleomorphic cancer cells arranement as cord, glandular or masses surrounded by thick fibrous connective tissue infiltrated by inflammatory cells, the tumor mass expressed hemorrhage and necrosis in basalis layer (adenocarcinoma). Hemorrhage and bleeding are most common symptoms in uterine cancer [34] [35].

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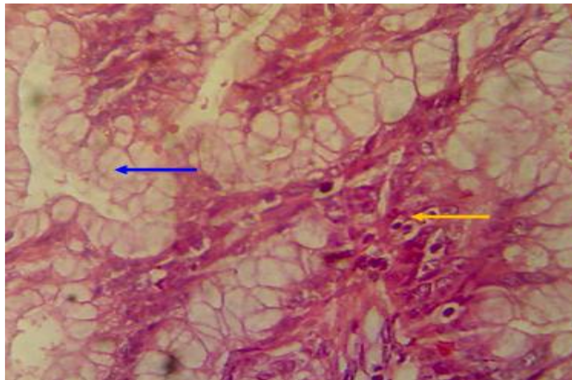

Figure 1. Section in uterus of menopusal women with endometrial cancer showing (blue arrow) hypertrophy and hyperplasia cells (yellow arrow) (H & E stain) (40X).

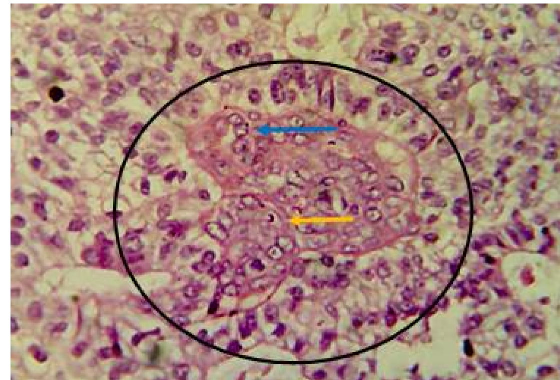

Figure 2. Section in uterus with endomatreum cancer. showing mass of cancer cells (circle) expressed pleomorphic (blue arrow) and mitotic figures nuceli (yellow arrow) (H & E stain) (40X).

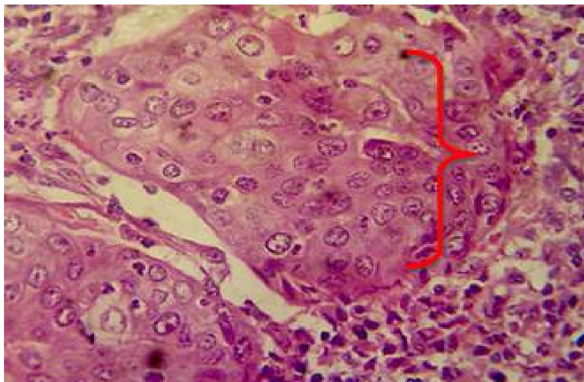

Figure 3. Section in uterus with adenocarcinoma show pleomorphic cancer cells with high mitotic division (red brace) (H & E stain) (40X).

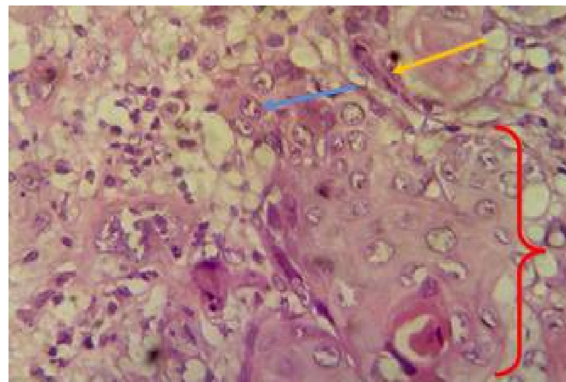

Figure 4. Section in uterus with adenocarcinoma showing sheath of cancer cells surrounded by squamous cells (yellow arrow) with vacuolation of pleomorphic cancer cells (blue arrow) and high mitotic activity (H & E stain) (red brace) (40X).

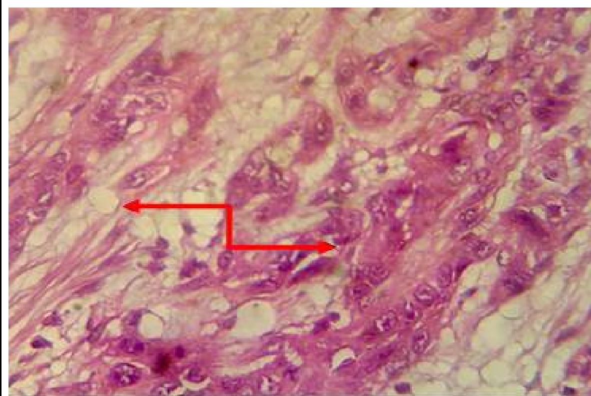

Figure 5a. Section in the uterus of woman shows hyperchromatic pleomorphic cancer cells arranement as cord surrounded by thick fibrous connective tissue in basalis layer (fibroadenocarcinoma) (H & E stain 400X)

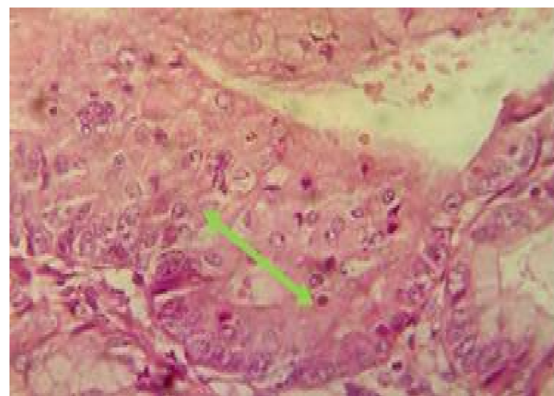

Figure 5b. a: Section in uterus with fibro_ adenocarcinoma (H & E stain) (40X).

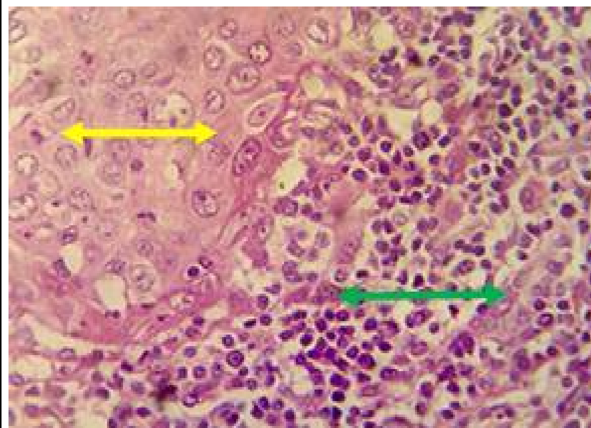

Figure 6. Section in uterus with mixed cancers: adenocarcinoma And fibroadenocarcinoma (H & E stain) (40X).

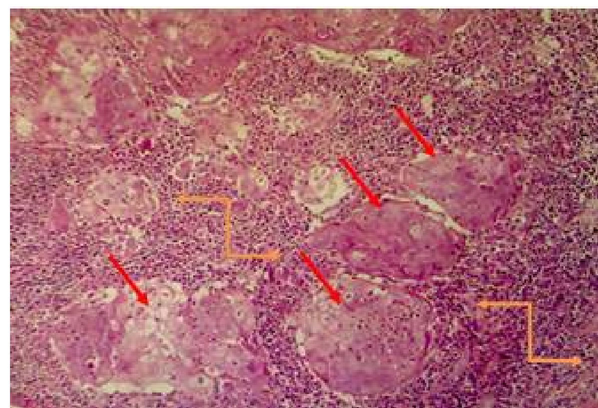

Figure 7. Section in uterus with adenocarcinoma showir multiple masses of cancer cells surrounded by severe inflammation (H & E stain) (10X).

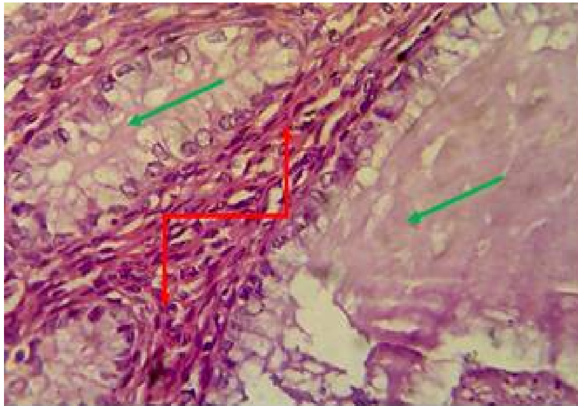

Figure 8a. Section in uterus with edenocarcinoma showing protinous materials in their lumens of glands with proliferation of fibrous connective tissue between there glands (H & Estain) (40X)

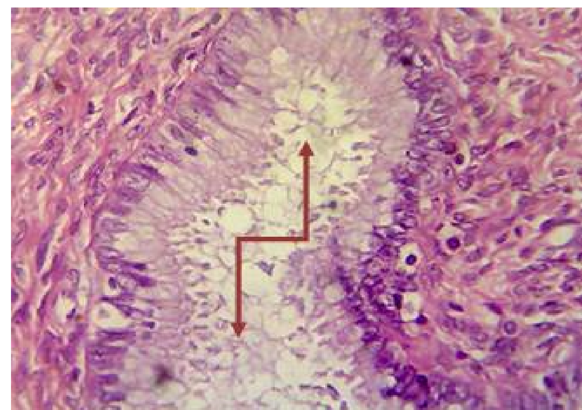

Figure 8b. Section in uterus with edenocarcinoma sho protinous materials in their lumens of glands (H & Estain) (40X)

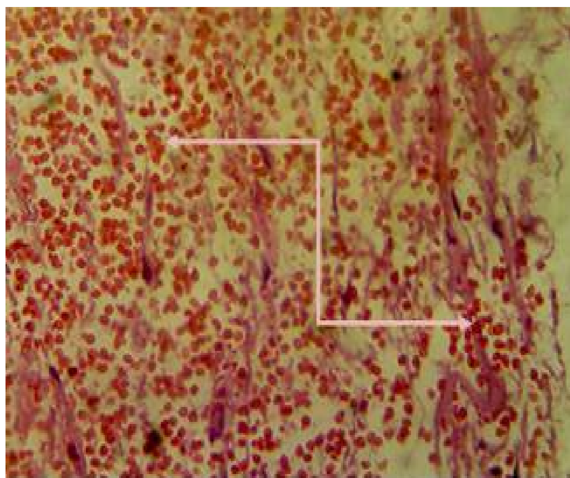

Figure 9. Section in uterus with adenocarcenoma showing severe hemorrhage (H & E stain) (40X).

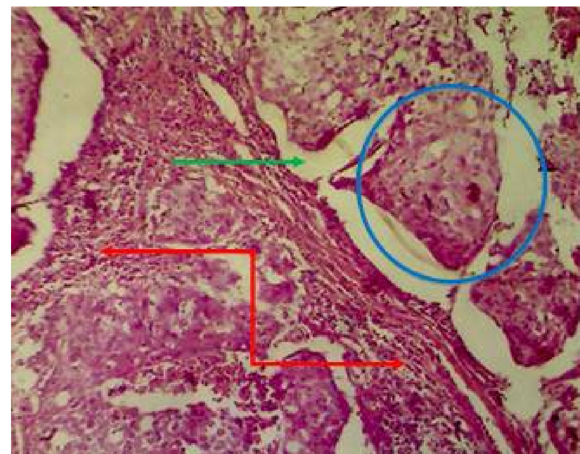

Figure 10. Section in uterus of menopausl women with adenocarcinoma showing cancer masses surrounded by thick fibrous connective tissue infiltrated by inflammatory cells the tumor mass expressed hemorrhage and necrosis (H & E stain) (10X).

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RESEARCH ARTICLE

Quality and Shelf Life Attributes of Carabeef Treated with Curd, Lemon Juice and Blade Tenderization

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ABSTRACT

Fresh carabeef (buffalo meat) samples were treated with blade tenderization—T_1, curd (20%)—T_2, lemon juice (20%)—T_3 and combination of blade tenderization with curd (20%)—T_4 and blade tenderization with lemon juice (20%)—T_5 and compared with control—C. The samples were aerobically packed and stored in the chiller (4±1°C) and analyzed on days 0, 1, 3, 6 and 9 or until spoilage, whichever is earlier. T_1 had significantly ($p<0.01$) lower cooking loss, shear force value, drip loss, collagen content, 'L' value, but higher 'a' value and myofibrillar fragmentation index. T_1, T_3 and T_4 showed significantly lower ($p<0.01$) TBA numbers on all storage days. There was significant ($p<0.01$) difference in the aerobic plate counts between the samples on all storage days. The highest count was observed for T_4 closely followed by T_3. T_1 had superior sensory scores on zeroth day and maintained the scores throughout the storage period. T_2 and T_4 attained similar sensory scores as that of T_1, only on day nine. T_3 and T_5 had the lowest sensory scores on all storage days as of C. On storage in chiller, sensory attributes improved in all the samples stressing the importance of ageing. Blade tenderization is recommended as the most preferred tenderization technique. As a cost effective tenderization treatment, application of curd alone can make the meat tender in nine days, with similar sensory scores as of blade tenderization.

Keywords: Carabeef, curd, lemon, blade tenderization.

INTRODUCTION

Carabeef (buffalo meat) is obtained mainly from spent animals. Meat from such animal is tough and coarse in nature with high amount of connective tissue, but is superior in flavour. Palatability and eating quality characteristics are the major criteria of consumer preference and toughness of meat is attributed as the most undesirable factor by the consumers (Kemp *et al.*, 2010). Blade tenderization is a commercially effective method of tenderization commonly used in buffalo meat industry. Obuz *et al.* (2014) stated that techniques like blade tenderization and post mortem ageing improved the eating quality of beef. Curd is one of the most popular milk products in India and is widely consumed throughout the country. Lactic acid is the major component in curd that contributes to tenderness of meat. Lemon (*Citrus limon*), a common and popular fruit in India is a rich source of citric acid and lemon juice is also used in tenderization of meat. Curd and lemon juice, two naturally occurring sources of weak acids are commonly used in Indian kitchens for meat marination as well as ingredients in day-to-day cooking. However, scientific studies on their effects on meat quality and shelf life are very limited. Also the single and combined effects of mechanical tenderization and application of curd and lemon juice on quality attributes of meat need to be studied

MATERIALS AND METHODS

Treatment of buffalo meat

Longissimus dorsi muscles from carcasses of buffaloes aged eight to nine years old were procured from Malabar Meat Factory, Wayanad, Kerala and transported under refrigerated conditions. The muscles were divided into six equal groups and subjected to the treatments viz., C- Control, T_1- Blade tenderization, T_2- Application of curd (20%), T_3- Blade tenderization + Application of curd (20%), T_4- Application of lemon juice (20%), T_5- Blade tenderization + Application of lemon juice (20%). Salt at a level of 1.5% was added to control as well as treatments. For treatments 1, 3 and 5, blade tenderization was carried out in blade tenderizer (Biro, USA) using tenderizer blades at a speed of 15 rpm. Curd and lemons were purchased locally and lemons were squeezed manually using a squeezer. Application of curd/lemon juice in treatments 3, 4, 5 and 6 was carried out for a period of two hours under chiller conditions (4±1°C). The samples were then aerobically packed and stored in the chiller (4±1°C). Analyses were conducted on days 0,1, 3, 6 and 9 or until spoilage or until spoilage, whichever is earlier for both control and treatments. The spoilage of samples was assessed by physical examination like odour, colour and slime formation on the samples.

Physico-Chemical Characteristics

The drip loss was calculated as per the procedure of Honikel (1987). The cooking loss was calculated as per Boccard et al. (1981). pH of the samples was measured using a digital pH meter as described by AOAC (2012). The water holding capacity was calculated as per Wardlaw *et al.* (1973). Thiobarbituric acid numbers were determined as per Witte *et al.* (1970) with modifications. Tyrosine values of the samples were estimated as per the method described by Pearson (1968). Colour values of the samples were determined objectively as per Page *et al.* (2001) using Hunter Lab Mini Scan XE Plus Spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination. The Warner-Bratzler shear force (WBSF) of each sample was determined by the method outlined by Wheeler *et al.* (1997) using Shimadzu Texture Analyzer Model EZ-SX 500N (Shimadzu (Asia Pacific) Pvt. Ltd, Singapore) cross head at a speed of 200 mm/min. Total collagen content of selected buffalo muscles was determined as per Stegman and Stadler (1967). The myofibrillar fragmentation index of each sample was determined by procedure outlined by Davis *et al.* (1980).

Microbiological Characteristics

All the microbiological parameters were determined by following standard methods of APHA (2001). The aerobic plate count was evaluated as per the procedure of Morton (2001). The psychrotrophic count was expressed as per the procedure of Beuchat and Cousin (2001).

Organoleptic Characteristics

Sensory evaluation of buffalo meat was conducted by a panel consisting of semi-trained members. Panelists evaluated samples for colour, flavour, juiciness, sourness, tenderness and overall acceptability.

Proximate Composition

Samples were analyzed for proximate principles like moisture on zeroth, first, third, sixth and ninth day or until spoilage and fat, protein and ash on zeroth day. The proximate composition was estimated as per AOAC (2012).

Statistical Analysis

Data recorded were analyzed statistically as per Snedecor and Cochran (1994) by using SPSS Software Version 21.0. Repeated ANOVA measures, Pearson's correlation and Sperman's correlation, Kruskal- Wallis test, Wilcoxon signed rank test, Friedman test, Mann Whitney test were used

RESULTS AND DISCUSSION

Physico-Chemical Characteristics

There was significant ($p<0.01$) difference in drip loss between the samples on all storage days. Samples treated with lemon juice had significantly ($p<0.01$) higher drip loss followed by treatments having curd. In T_2, T_3, T_4 and T_5 the drip loss significantly ($p<0.01$) increased across the storage period. Similar findings were observed by Diamante and Tran (2016) and Payne *et al.* (1997) in chilled beef. Higher drip losses in curd and lemon juice treated samples might be due to comparatively lower pH of these treatments which might have resulted in lowered water holding capacity as reported by Kahraman *et al.* (2012). Lemon juice treated samples showed the lowest pH and hence the highest drip loss. Cooking loss was significantly ($p<0.01$) higher for T_3, T_4 and T_5 samples and lowest for T_1 sample. Lowered pH of T_4 and T_5 might have resulted in the comparatively higher cooking losses of these treatments due to reduced water holding capacity. This was also in agreement with the findings of Aktas *et al.* (2003) and Klinhom *et al.* (2015) where a higher cooking loss was observed in beef treated with citric acid.

The pH values were significantly ($p<0.01$) different between samples on all days of storage with T_4 and T_5 showing significantly ($p<0.01$) lower pH on zeroth, third and sixth day and T_3, T_4 and T_5 showing lower pH on ninth day. Lemon juice due to high acidity might have resulted in the lowered pH of T_4 and T_5. Curd due to its lactic acid content, though not as acidic as lemon juice, might have resulted in the lower pH of T_2 and T_3 compared to control and T_1. The pH value reduced significantly ($p<0.01$) on day one in all the samples and thereafter there was a significant increase till day nine. The significant reduction in pH on day one can be attributed to post mortem glycolysis of meat and the accumulation of lactic acid following anaerobic glycolysis. This was in agreement with the observations of Pearson and Young (1989). According to Kandeepan *et al.* (2013), the major quality determining factor of meat was pH and it was related to water holding and emulsifying capacities. Sample T_5 showed the lowest water holding capacity, followed by T_4 and then T_3 on all storage days. This might be due to the comparatively lower pH of the samples and the fall in pH might have resulted in the reduced availability or unavailability of reactive group of

proteins for water holding. This was also reflected in the drip and cooking losses of T_3, T_4 and T_5 samples. The findings were in agreement with that of Nuraini *et al.* (2013) and Kahraman *et al.* (2012). The result with respect to Thiobarbituric acid (TBA) numbers during refrigeration storage is shown in Fig I. There was significant ($p<0.01$) difference between samples with regard to TBA number on all storage days with control and T_1 showing significantly ($p<0.01$) higher TBA numbers when compared to other samples on all storage days. The T_3 and T_5 samples showed significantly ($p<0.01$) lower TBA numbers on all storage days. This might be due to the metal chelating properties of curd and lemon juice and increased penetration of curd and lemon juice into the meat samples due to blade tenderization. Ke *et al.* (2009) observed that application of citric acid on meat improved the texture of meat as well as inhibited lipid oxidation. There was significant ($p<0.01$) increase in TBA numbers of all samples on storage from zeroth day to ninth day. Increasing trend in TBA value as observed by Elgadir *et al.* (2009).

There existed significant ($p<0.01$) difference in tyrosine values (mg tyrosine equivalent per 100 g of sample) between samples on all storage days with T_5 showing significantly ($p<0.01$) higher value than all other samples and T_4 showing the next highest value. This might be attributed to the increased proteolysis by the low pH of lemon juice as suggested by Aktas and Kaya (2001). A significant ($p<0.01$) rise in tyrosine value was observed in all the samples as the storage progressed which might be due to the increased proteolysis that hadoccurred during storage of the samples. Similar results were observed in in buffalo meat stored in refrigerator by Kandeepan and Biswas (2007) and Anandh (2014). There existed significant ($p<0.01$) difference in Hunter 'L' values between samples on all storage days. 'L' values were significantly ($p<0.01$) lower in C and T_1 when compared to others. Higher 'L' values in T_2, T_3, T_4 and T_5 might be due to the bleaching effect of acids in lemon juice and curd. Acidic pH may cause denaturation of proteins, lowered water holding capacity and leaching off of myoglobin. Klinhom *et al.* (2015) and Kahraman *et al.* (2012) evidenced a similar higher 'L' value for lemon juice treated *longissimus dorsi* muscle of beef. There existed significant ($p<0.01$) difference in the redness or 'a' values between all samples, with C and T_1 showing significantly higher values and T_4 and T_5 showing the lowest values. There was no significant difference in 'a' values on storage in T_3, T_4 and T_5. Lower 'a' values were evidenced by Klinhom *et al.* (2015) in citric acid treated beef sample and Kahraman *et al.* (2012) in lemon juice treated *longissimus dorsi* muscle of beef and might be due to the bleaching effect of lemon juice on meat. All the samples differed significantly ($p<0.01$) in yellowness or 'b' values on all days of storage except on third day. The T_2 and T_3 had significantly ($p<0.01$) higher 'b' values on almost all days. A significant ($p<0.05$) increase in 'b' value was observed by Elgadir *et al.* (2011) in beef treated with citric, lactic, acetic and tartaric acid and stored under refrigerator conditions at 5ºC.

There existed a significant ($p<0.01$) difference in WBSF between samples. The results of WBSF are presented in Table I. T_1 showed a significantly ($p<0.01$) lower and C showed a significantly higher shear force value throughout the storage period. It could be attributed to the cutting of muscle fibers and physical disruption of connective tissues due to blade tenderization (Pietrasik and Shand, 2004). A significant ($p<0.01$) reduction in shear force values was encountered in all the samples except in T_3 on storage as also observed by Koohmaraie (1996) and Kandeepan and Biswas (2005) in beef stored under chiller condition. Similar reduction in shear force readings was recorded by Burke and Monahan (2003) and Elgadir *et al.* (2011) in organic acid treated beef slices upon storage at 5ºC. The reduction in shear force value was attributed to the low pH attained as a result of the application of acid on meat. Onenc *et al.* (2004) observed that this might be due to the increased proteolysis of muscle proteins especially cathepsins on application of weak acids. Collagen is the most abundant connective tissue protein in meat and is the major contributing factor that determines the tenderness and texture of meat. There existed significant ($p<0.01$) difference in collagen content between the samples on all storage days. Among the samples the lowest ($p<0.01$) collagen content was observed for T_1, followed by T_2 and the highest for A significant ($p<0.01$) reduction in collagen content was observed in all the samples as the storage progressed. Serdaroglu *et al.* (2007) opined that treatment with acidic marinating agent increased the rate of proteolysis and also accelerated the conversion of collagen to gelatin on cooking.

A significant ($p<0.01$) difference existed between samples on all storage days in MFI with control showing the highest values and T_1 showing the lowest values. The values reduced significantly ($p<0.01$) on storage for all the samples. In T_1 the lowest MFI might be due to the cutting of muscle fibres into shorter segments and the physical disruption of connective tissues (Pietrasik and Shand, 2004). The reduction in MFI on storage might be due to the increased proteolysis by calpain enzyme. Similar reduction in MFI was observed by Shin *et al.* (2009) and Rajagopal and Oommen (2014)

Microbiological Characteristics

There existed a significant difference ($p<0.01$) in the aerobic plate counts between the samples on all storage days. The highest count was observed for curd treated samples which might be due to the high levels of lactic acid bacteria in curd. Lemon juice treated samples had lower total viable counts when compared to curd treated samples, this was in agreement with the findings of Kim *et al.* (2015). Psychrotrophic count was not detected up to ninth day of storage in control and in treatment similar to the findings of Luckose *et al.* (2015); Kurian (2016) and Pavan (2016)

Organoleptic Characteristics

There was significant difference ($p<0.01$) in colour and appearance scores between samples on zeroth, third and sixth day with C and T_1 showing higher scores. No significant difference was found between the samples during the storage period except for T_4, where the score significantly ($p<0.05$) increased. On zeroth, first and sixth day, C scored highest in flavour. However, decline in the flavour scores of meat on refrigerated storage was noted in C, T_4 and T_5. On day ninth, significantly ($p<0.01$) higher flavour scores were observed for T_2 and T_3 and T_1 maintained the flavour scores throughout the storage period. During post mortem storage of muscle, nucleotides break down into hypoxanthine and inosinic acid, contributing to the flavour of meat (Miller *et al.*, 1985). Ziauddin *et al.* (1995) reported increased score for flavour in buffalo beef samples marinated with organic acids like lactic acid. Flavour scores were higher for buffalo meat obtained from culled animals compared to young animals (Kandeepan *et al.*,2013) and this was attributed to the higher fat content in the meat from spent animals (Kandeepan *et al.*, 2009). Scores for sourness were undesirable only in samples T_4 and T_5 which remained same on storage also. This finding was in agreement with Klinhom *et al.* (2015). There existed significant ($p<0.01$) difference in the juiciness scores between samples on all storage days. T_1, T_2 and T_3 scored significantly ($p<0.01$) higher on all days of storage whereas, C had similar higher scores only on sixth and ninth day and this might be due to the post mortem ageing of meat in chiller. T_4 and T_5 had significantly ($p<0.01$) lower scores throughout the storage period and this might be due to significant reduction in the pH and thus reduced water holding capacity of lemon juice treated beef sample. In agreement to this, lower juiciness score for citric acid treated beef samples was also reported by Klinhom *et al.* (2015). Corbin *et al.* (2015) evidenced that meat with more juiciness score was perceived by consumers as more tender.

For tenderness score, significant difference was noted between control and treatments and as well as between storage periods. Tenderness scores were highest for T_1 on all storage days followed by T_2 due to relative reduction in detectable connective tissue. This was in agreement with the findings of Savell *et al.* (1982) and Obuz *et al.* (2014). C, T_4 and T_5 had the lowest tenderness scores. On storage the scores did not differ significantly in all the samples except for C and T_3 where the scores increased significantly ($p<0.01$). Vitale *et al.* (2014) observed an increase in tenderness value on storage of beef for eight days in chiller. There existed significant ($p<0.01$) difference in overall acceptability scores between samples on all storage days. T_1 was found to have the highest overall acceptability score among all the samples followed by T_2. Similar results were observed by Obuz *et al.* (2014) in blade tenderized beef. In C and T_3, the overall acceptability scores increased significantly ($p<0.01$) on storage and might be due to the increased tenderness values scored during storage of the meat samples. Platter *et al.* (2003) opined that consumers perceived overall acceptability of beef primarily based on the flavour of beef. T_1 and T_2 maintained high flavour scores and T_4 and T_5 maintained lower flavour scores during the storage period.

Proximate Analysis

Proximate analyses for fat, protein and ash were done for all samples on zeroth day and analysis for moisture was done on all storage days. There existed no significant difference in protein, fat, ash, carbohydrate and energy values between samples.

CONCLUSION

Blade tenderization is recommended as the most preferred tenderization technique. As a cost effective tenderization treatment, application of curd alone can make the meat tender in nine days, with similar sensory scores as of blade tenderization.

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Table 1.Warner-Bratzler shear force (N/cm2) values of control and treatments on different storage days

	Day 0	Day 1	Day 3	Day 6	Day 9	F-value (p-value)
Control	59.99±1.14aA	48.67±1.85aB	38.63±0.85aC	33.76±0.73aD	32.09±0.68aE	151.830** (<0.001)
T 1	34.61±0.83bA	22.99±0.16dB	20.46±0.53eC	19.59±0.43dC	15.92±0.44dD	170.584** (<0.001)
T 2	38.08±0.46bA	34.56±0.36cbB	32.03±0.46cC	28.51±0.58bD	23.43±0.67cE	132.404** (<0.001)
T 3	31.63±5.60b	32.35±0.49c	29.94±0.49d	25.63±0.39c	24.79±0.36c	1.763ns (0.241)
T 4	37.74±0.29bA	37.02±0.25bAB	36.31±0.28bB	32.84±0.70aC	28.16±0.65bD	75.681** (<0.001)
T 5	37.34±0.37bA	35.01±0.47bB	31.46±0.70cC	32.10±0.86aC	24.84±0.76cD	82.009** (<0.001)
F-value (p- value)	18.24** (<0.001)	100.11** (<0.001)	118.56** (<0.001)	92.88** (<0.001)	78.09** (<0.001)	

**-significant at 1% level, *- significant at 5% level, ns-non significant; Means ± SE having different alphabets (lower case) as superscript are significantly different within a row and means with different alphabets (upper case) as superscript are significantly different within a column

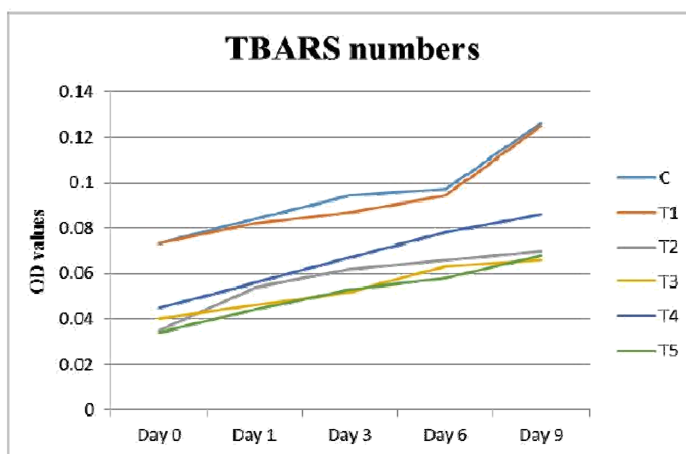

Fig. 1. Thiobarbituric acid (TBA) numbers of control and treatments on different storage days

RESEARCH ARTICLE

Is Osteoprotegerin Gene Polymorphism g.27522G>A Protective SNP in Type 2 Diabetic Women

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ABSTRACT

Globally, diabetes can be considered among the most frequent metabolic disorders, also it is spreading quickly, thus it is referred to as "a silent epidemic.It is now estimated that 415 million people aged between 20 and 79 years will have diabetes in 2015, and is expected to reach 552 million by 2030and 642 million by 2040.Osteoprotegerin (OPG) is the inflammatory cytokine receptor involved in bone remodeling". "It is a soluble glycoprotein for the tumor necrosis factor superfamily, which is responsible for inhibition of osteoclastogenesis". There might be an interaction between osteoprotegerin and TNF-related apoptosis-inducing ligand (TRAIL) and avoiding the development of antiapoptotic bodies, that could be mineralized. And was associated in many human diseases such as myocardial infraction, diabetes and its complications. Aim: The most importantpurpose of this study is highlighting the relation of g.27522G|A SNP in exon5 of osteoprotegerin gene with T2DM in the population of Iraq. Methods: In the diabetic center of Karbala-Iraq, a case-control study has been conducted consisting of 82 healthy women and 82 women experiencing T2DM. Healthy and patient persons have been genotyped for g.27522G|A polymorphism through utilizing the approach of polymerase chain reaction-restriction fragment length polymorphism, which could be abbreviated to (PCR-RFLP).Results: The genotype frequency related to the g.27522G/A SNP has beennotably different in the two groups (control and patients). The odds ratio regarding those related to GA genotype has been 0.522 (95% CI =(0.178-1.35), P = 0.236) and 0.489 (95% CI =(0.185_1.509), P = 0.213) for those related to AA genotype. The minor allele frequency A has beenconsiderably high in healthy women(64.02%) in relation to the group of patients (59.14%).These results indicated a protective effect from T2DM for carriers of GA and AAgenotypes respectively. Conclusion: The g.27522G|A SNP regarding theosteoprotegerin gene is involved in the protection from T2DM in Iraqi premenopausal women for carriers of the GA and AA genotypes .

Keywords: Osteoprotegerin gene polymorphism,diabetic, women,type 2.

INTRODUCTION

Ninety percent of diabetes are considered to be T2DM and it is indicatedvia chronic hyper glycaemia happen because of flaws in insulin action and/or insulin secretion and metabolic disorders of protein and lipids (Toan, Hoan, Cuong, Dung, & Dung, 2018). (T2DM), previously called adult diabetes or non-insulindependent diabetes might be considered as an multivariate disease whichimplicates complex interactions between immune system abnormalities, genes, environmental factors and behavior that affect health, and represents the seriousness of the public health problem in many developed countries(Y. Shen *et al.*, 2017) The genetic susceptibility of type IIdiabetes seems to be decidedviavarious variables familiar to many low-impact genetic sites. Although at least 36 genes associated with diabetes have been recognized, approximately ten percent of the genetics regarding the type IIdiabetes might bedescribed.(Herder and Roden, 2011). Osteoproteger in might be considered as the inflammatory cytokine receptor that is involved in bone remodeling". "It is a soluble glycoprotein for the tumor necrosis factor superfamily, which is responsible for inhibition of osteoclastogenesis" (Daniele *et al.*, 2018). it is produced by osteoblasts and by other cells like peripheral blood lymphocytes" (Pandey et al., 2018)."It includes various biological functions, like strong inhibitor regarding osteoarthritis and possible therapeutic agent to treat osteoporosis (Moon *et al.*, 2016).

The human TNFRSF11B gene can be considered as a monoclonal gene"on the 8q23-24 chromosome with 5 exons that span 29 kb Because of its important function in the biology of bones, the human genome TNFRSF11B, the encoding of osteoprotegerin, was identified as candidate genes for osteoporosis"(Boronova *et al.*, 2014). A number of individual nucleotide polymorphisms (SNPs) were identified in the OPG gene and were associated with bone mineral density, osteoporosis fractures, and coronary artery disease" (Mohamed et al., 2009). g.27522G>A, this genetic polymorphism causes G→Amutation. It is a synonymous mutation in exon 5 at 27522 position of OPG gene "(p. cysteine (Cys) 319Cys, reference sequences Gen- Bank IDs: NG 012202.1, NM 002546.3, and NP 002537.3) (J. J. Zhao, Feng, Cheng, Lu, & Wang, 2014).

MATERIALS AND METHODS

A case control study was conducted on164 individuals, consisted of 82 patients T2DM and 82 healthy individuals. Patients with type 2 diabetes mellitus were randomly selected from the center of diabetes in kerbala governorate-Iraq.. The control group randomly selected from the general population and they were free of any chronic disease. The mean age of the patients group was47.59±8.11 year, while that of the control group was45.29±11.23year. Demographic parameters of the subjects obtained, included the age, , height, weight and BMI (Table 1). The study was approved by the Clinical Research Ethics Committee of Kerbala Medical College and carried out in the department of biochemistry, College of Medicine, University of kerbala at the period, November 2017–May 2018. DNA was extracted from complete blood samples using a DNA purification kit (Promega). The g.27522G|A SNP in OPG was genotyped by using polymerase chain reaction-restriction fragment-length polymorphism analysis.The DNA fragment was amplified using 2 primers (Wang et al., 2014), the forward; 5′– GAG-CAG-CTT-CGT-AGC-TTG-ATG –3′ and the reverse primer; 5′– TTG-TGA-AGC-TGT-GAA-GGA-ACC –3′. The amplification reaction was performed in a 25-μl volume containing 5 μl of AccuPower®PcrPreMix (Bioner),1.5 μl of each primer, 8 μl genomic DNA and 9 μl sterile nuclease free water. Thermocycling conditions for g.27522A|G consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 60°C and 30 s at 72 °C with a final extension at 72 °C for 8 min. The PCR product (253 bp) was digested by 2 U of SphI restriction enzyme (Biolab) at 37 °C for 1 h according to the manufacturer's protocol and separated on 2.5% agarose gel electrophoresis. To examine the reliability of the results and to determine the genotyping error rate, 10% of the studied samples were selected randomly and reassessed. The consistency was obtained to be 100%. Frequencies were estimated using the gene counting method. Polymorphisms were tested for Hardy-Weinberg's equilibrium using the chi-squared ($\chi2$) test.

Frequencies of alleles were also calculated and compared by the χ2 test and multinomial logistic regression analysis. A significant change was considered when the p value was < 0.05. The polymorphic fragments related to g.27522 G/A SNP have been amplified through the use of certain primers leading to 253 bp product. On digestion, the former offered two fragments, 80 and 173 bps for the carriers of homo-variant genotype (AA), three fragments, 80, 173 and 253 bps for those of hetero-variant genotype (GA) and singlefragment, 253 bp, for those of wild genotype (GG) (Fig. 1). Examining the genotype and allele frequencies regarding g.27522G|A SNP in osteoprotegerin gene of control individuals and T2DM has been conducted under dominant, codominant and recessive models. According to the codominant model, healthy control femaleswith AA have been slightly (OR =0.489, CI 95% =0.158-1.509, P = 0.213) elevatedin comparison to the group of patients, healthy control females with GA has beenslightly (OR = 0.522, CI 95% = 0.178-1.53, P = 0.236) raised than those of the group of patients. These result suggest a protective from T2DM in the case whenpersonshaveone or two A alleles regarding g.27522G|A SNP respectively. According to the dominant model, healthy control females with genotypes GA+AAhave beenappeared to be slightly (OR = 0.222, CI 95% = (0.043-1.136), P = 0.070) elevatedin comparison to the group of patients (Table 2). Also, the examination under recessive and additive models showedslightraise in the group of healthy controls throughout a comparable estimation. The frequency of minor allele A in healthy women (64.02%) patients was insignificantly (OR =0.8135, CI 95% =0.52-1.27, P =0.364) elevated when compared to the group of controls (59.14%) (Table 3). The adjustment of data for age, and body max index increased the significant levels in all statistical analyses

DISCUSSION

T2DM is a complex metabolic disease Which accompanied insulin resistance and weak beta cell Leads to high blood sugar, ahallmark of the disease (Brunetti & Indolfi, 2018).New suggestion indicates that the risk factors could prompt chronic inflammation, that is also related to the T2DM like diet, sedentary lifestyle and obesity (Denova-guti et al., 2018). Osteoprotegerin is abundantly formed via the osteoblasts at the surface of the bone and inhibits the activity of osteoclast, acting as key regulator of bone homeostasis (Orita .y.et al.,2007). The human TNFRSF11B gene is a single-copy gene located on the chromosome 8q23-24 with 5exons that spans 29 kb of human genom (Boroňová.I.,et al.2014). Osteoprotegerinwas well-thought-out to be an anticalcifying protein due to the fact that it prevents the receptor activator of NF-κB (RANK) binds to its ligand (RANKL)"."By this mechanism, osteoprotegerin inhibits the transcription of pro-calcifying genes as bone morphogenetic protein- 4 (BMP-4) and alkaline phosphatase (ALPL)". "In addition, osteoprotegerin may interact with TNF-related apoptosis inducing ligand (TRAIL) and avoid the formation of anti-apoptotic bodies, which can be mineralized" (Luna-Luna et al., 2017)."and wasinvolved in many human diseases such ascancer, chronic kidney disease, aortic aneurysm, diabetes and its complications, stroke, aortic stenosis, atrial fibrillation and myocardial infarction (Tschiderer.,et al 2017) ".

The results related to the assessment of genotype distribution regarding g.27522G|ASNP indicated an insignificant elevationin the A allele in healthy control womenover different inheritance model. The data indicated a potentialfunctionregarding A allele in the protection from T2DM. Furthermore, it has been identified that the minor allele frequency has been considerablygreater in healthy control women (64.02%) compared tothe group of patients (59.14%). The precise methodthrough which the variations in osteoprotegerin geneplay a role in the protection from T2DM is not fullysrecognized.The results also show the effect of different parameters on type 2 diabetes, The level of PO4 were considerably lesser in the groups of patients than in the group of controls, this may indicate a potential adverse effect of high blood sugar on serum phosphorus". "It is also known that low phosphate levels affect those who have type 2 diabetes, because insulin has been reported to affect the secretion of phosphorus by renal tubules".This results confirmed by Hamad et al., who found that serum phosphorus levels were lower in diabetic patients than in healthy individuals". "and the study also give good agreement with other study by Najeeb et al., who showing low phosphate levels in newly diagnosed diabetic patients"(Hamad et al., 2013)(Najeebet al., 2014).
The results indicated that E2 levels decreased considerably in the T2DM patient compared with control, E2 may regulate the action of insulin directly through actions on insulin-sensitive tissues or indirectly by regulating factors

such as oxidative stress, which contribute to insulin resistance" (Gupte, Pownall, & Hamilton, 2015).and this consisted with other study by Jassim et al. "Who found that a significant reduction in estradiol level in women with type 2 diabetes compared to control "(Jassim et al., 2017) and also confirmed by Preetiyadav et al. "Who found that estradiol levels (E2) were significantly lower in female cases than controls" (Preeti Yadav, Shashi Seth, Kiranchugh, 2016). there was also a significant increase in serum alkaline phosphatase from the patients group compared with the control group, increased activity of ALP in diabetic patients could be because of certain liver toxicity resulting fromparticular drug therapy (Angel et al., 2018). this result agree with other study published by Thanpari et al. "who found that total alkaline phosphatase was found to be highest in all diabetic patients versus control group with positive correlation with glycemic index"(Thanpari, 2013)."

And the result also confirm by Angel et al who found that statistically,the ALP level has been elevated among diabetics" ."On other hand, this result disagree with study by Haddad et al who found thatThere has been no considerable difference in the level of alkaline phosphatase among diabetes patients and control"(Haddad, 2018).There was a significant increase in bone mineral density for diabetics compared to control group, The use of thiazad which is expected to be higher in diabetic patients was also associated with high bone mineral density in different locations of the skeletal". "Similarly,Statin (also more common in diabetic patients) is also associated with high bone mineral density "(Lili Ma et al., 2012).And this result consist with the study by Maghbooli et al. "who found that In diabetic patients, bone mineral density values in the spine were higher than those without diabetes but the difference was only significant in the subgroup after menopause" (Maghbooli et al., 2007) and the results also consist with L Ma et al. "who found that individuals with diabetes had higher bone mineral density (BMD) levels than non-diabetics independently of skeletal, sex, age, BMI or drug use" (L Ma et al., 2012).

But the result different in other study by Dutta et al. "who was found that diabetics showed low bone mineral density in the hip and spine, glitazones, metformin, and insulin associated with a decrease in bone mineral density at the spine and hip, while sulfonyl urea was associated with an increase in bone mineral density"(Dutta et al., 2012).the different may be to the difference in sample size and the ethnicity there has been no considerable difference in total calcium level among patients group and the control group may be due to the measurement of total calcium instead of the form of ionic calcium, this result Emphasizes by Hamad et al. who found that levels of calcium in the blood between patients and control remained unchanged without significant effect of hyperglycemia associated with diabetes" (Hamad et al., 2013)."but this results oppose with sultan et al. who found that In patients with type 2 diabetes, the average level of serum calcium has beenconsiderably lesser than the group of controls " (Sultan et al., 2008).the difference may be to the difference in sample size and method.It is highly important to state the strength points regarding this study. The 1st point is that there has been no database handling the genetic background regarding the majority ofcritical diseases in the population of Iraq, specifically T2DM, thus, this is considered as the major effort to handle this issue of missing data. The other point of strength is that this study might be considered as a pilot study or an initiative for following studies inT2DM genetic background. Similarly, we must indicate the points of weakness regarding this study. The 1st point is the quite slight sample size which showed a genetic power of (9.03%). Therefore, and because of financial issues it is difficult to increase the sample size. The other point is the lack of osteoprotegerin measurement, in fact, and also because of financial issues, it could not be accomplished.

CONCLUSION

Osteoprotegerin gene polymorphism regarding the g.27522G|A is involved in the protection from T2DM women in the population of Iraq. Carriers of 1 or 2 A alleles are protect from T2DM of relative to those of the wild type.

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Table 1 Featuresrelated to the groups of study.

	Control(Mean±SD)	Patient(Mean±SD)
BMI	30.09± 5.05	32.10±5.40
Age	45.29±11.23	47.59±8.11

Table 2 Genotype distribution of osteoprotegerin gene polymorphism g.27522G>A in T2DM patient and control groups analyzed under the codominant and dominant models

Genotypes	Control (n=82)	Patients(n=82)	Un adjusted OR	P value	Adjusted OR	P value
Co-dominant GG(reference)	6	11				
GA	47	45	0.522 (0.178_1.35)	0.236	0.540 (0.179_1.629)	0.274
AA	29	26	0.489 (0.158_1.509)	0.213	0.453 (0.142-1.442)	0.180
Dominant AA+GA	76	71	0.509 (0.179-1.450)	0.206	0.504 (0.172-1.475)	0.211

Table 3. The genotype distribution related to theosteoprotegerin gene polymorphism (g.27522G>A) in control groups and T2DM patients examined under additive and recessive models and the minor allele frequencies

Genotypes Recessive GG+GA (reference)	Control(n=82)	T2DM(n=82)	Un adjusted OR	P value	Adjusted OR	P Value
AA	29	26	0.848 (0.443_1.623)	0.619	0.763 (0.391_1.491)	0.429

Additive 2(AA)+GA	105	97	0.503 (0.179_1.414)	0.193		
MAF%	64.02	59.14	0.8135 (0.52-1.27)	0.364		

Table 4. Biochemical parameters among diabetic and healthy control women characteristics

parameters	T2DM subject(n=82)	Control subject(n=82)	p-value
Ca(mg/dl)	5±0.89	5.94±1.43	0.529
Po4 (mg/dl)	1.87±0.87	3.67±1.85	0.00
ALK (king units/100ml)	10.80±2.81	8.03±1.44	0.00
BMD	0.87±0.12	0.80±0.15	0.014
E2(pg/ml)	119.85±41.65	179.62±40.8840.88	0.00

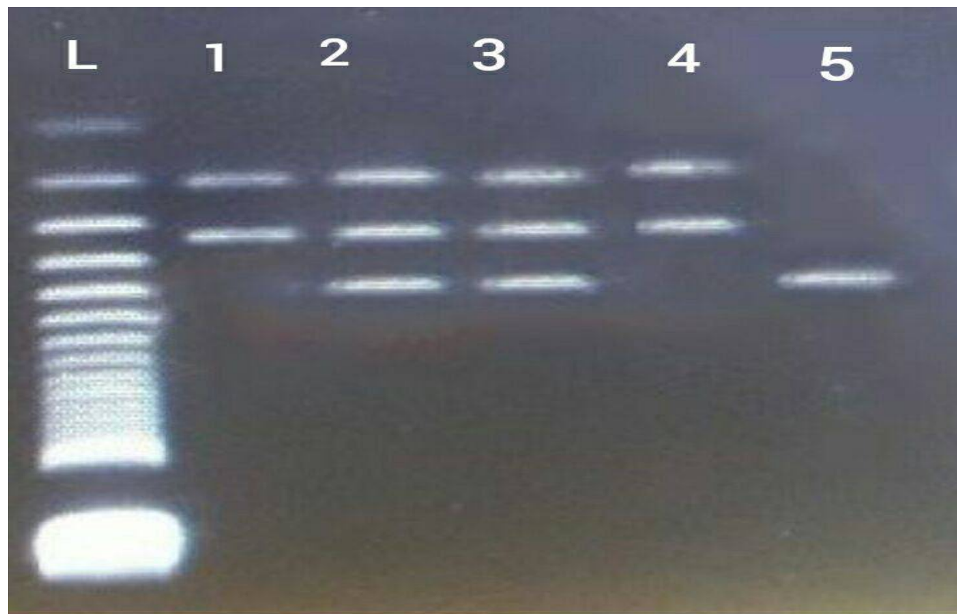

Fig. 1. PCR product of osteoprotegerin gene polymorphism (g.27522G>A)digested by restriction enzyme and electrophoresed 2.5%agarose gel. Lane L: DNA Marker (Ladder 50bp), Lanes 1, 4: AA genotype (80 & 173) bp, Lanes 2,3: GA genotype (253,173 & 80) bp, Lanes 5 GG genotype (253) bp.

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RESEARCH ARTICLE

Changes in Immunohistochemical Expression of Leptin Receptors in the Neurons of Dentate Gyrus of Hippocampus in Pregnant Rat

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ABSTRACT

The Dentate Gyrus (DG) is the most important region of hippocampus that deal with memory consolidation of information from short-term memory to long-term memory, and in spatial memory that enables navigation. The Dentate Gyrus have three histological layers molecular layer, granule layer, and the hilus or polymorph layer. This study aims to compare immunohistochemically between pregnant and non-pregnant adult rat using Anti-leptin Ab. markers expression to evaluate the functional activity of this tissue with neurogenesis process in the Dentate Gyrus region. Sample of 40 adult female rats with 300 ± 50 g body weight was used in this study. The animals were euthanized with chloroform and the brains were fixed with formalin after making in section in the head region and processed to paraffin blocks for hematoxylin and eosin (H&E) staining and immunohistochemical labeling with Anti-leptin Ab. markers. Reactivity of the dentate gyurs to these markers was assessed with Aperio ImageScope v.12.2 software for total positivity. Statistical analysis was done using SPSS 25.0 V software, labeling intensity recorded for dentate gyrus of hippocampus. The pattern of the Immunohistochemical labeling of Anti-leptin Ab. Give Statistical analysis reactivity in the dentate gyrus of hippocampus in with non-pregnant female (control) group gave mean values 0.620± 0.029 pixel/micron^2 and pregnant group at 7th day of gestation gave 0.606± 0.025 pixel/micron^2,When the pregnant group at 14th day of gestation gave mean values 0.703± 0.030 pixel/micron^2 and pregnant group at 19th day of gestation gave 0.552 ± 0.034 pixel/micron^2, respectively, with a wider range of reaction intensity in the pregnant group at 14th day of gestation than other groups. that lead to the neuroactivity unstable during pregnancy and the leptin hormone have role in the neural activity .

Keywords: Hippocampus , Dentate Gyrus , Leptin , Immunohistochemistry , Gestation ,neurogenesis

INTRODUCTION

Hippocampus is a essential region for memory consolidation, that important for learning and memory, development of the hippocampus is a multistep process under the guidance of a complex program, many factors effect on hippocampus development in terms of volume, morphology, and cell genesis to form Four main regions have been identified for the hippocampus, CA1, CA2, CA3, and dentate gyrus (DG) the last one deal with neurogenesis process (Khoshdel-Sarkarizi, H., et al 2019). The Dentate Gyrus (DG) as part of the hippocampus that as cortical region that is an integral portion of the larger functional brain system called the hippocampal formation that structure location started at a dorsomedial position, in close proximity to the most caudodorsal part of the septal complex to a ventrolateral position in close proximity to the most caudomedial parts of the amygdaloid complex. , considered to play a crucial role in associative memory(Scharfman, H. E., 2011). The Dentate Gyrus have three layers molecular layer, granule layer, and the hilus of the dentate gyrus as the third, inner, or polymorph layer.

The molecular layer will be further subdivided into inner, middle, and outer one-thirds. The molecular layer and granule neuron layer together form the fascia dentate. This laminar pattern appears to be conserved among mammals (Hevner, R. F. 2016). The adult dentate gyrus (DG) that a main portion neurogenesis. (Deroche-Gamonet, V., et al .,2018) Adult hippocampal neurogenesis is a type of structural and functional plasticity in the DG. (Hevner, R. F. 2016). Adult hippocampal neurogenesis is the process for adding new glutamatergic neurons to the dentate gyrus granule cell layer (Bulin, S. E., et al ., 2018).The neurogenesis can be divided into four phases: a precursor cell phase, an early survival phase, a postmitotic maturation phase, and a late survival phase. Based on cell morphology and a set of marker proteins, six distinct milestones can be identified, which to date still somewhat overemphasize the precursor cell stages of adult neurogenesis (Kempermann, G., et al .,2015). The neurogenesis of Adult has been seen to occur at low levels compared with development adult, and in only two regions of the brain the adult subventricular zone (SVZ) of the striatum, and the dentate gyrus of the hippocampus.

Neurogenesis in Adult is regulated by physiological and pathological events and modulated by pharmacological manipulations at any of three primary stages: cell proliferation, differentiation, and survival (Lim, D. A., and Alvarez-Buylla, A. 2016). Leptin hormone produce by white adipose tissue (WAT), the tissue have ability to releasing a growing number of products into the blood stream.Leptin, a peptide hormone that was first reported from obese (ob/ob) mice and plays a pivotal role in appetite regulation, metabolism, and energy homeostasis, and induces the brain to regulate food intake and peripheral tissues to increase energy expenditure (Lei, M. M., 2018).Leptin hormone that governs energy expenditure, acts in the central nervous system as a negative feedback signal to regulate appetite and metabolism by activating its receptor (ObR) on target cells ,The ObR has six isoforms (ObRa to ObRf) with an identical extracellular domain (Mazor, R., et al 2018). The stimulates of Leptin adult neurogenesis by increasing progenitor proliferation without any maturation (Whitfield et al ., 2015). Leptin receptors are have expressed in the central nervous system and target leptin hormone to other many brain areas ,one of them hippocampus organ , leptin is a potent modulator of hippocampal excitatory synaptic function (McGregor, G., ; Harvey, J., 2017) .

The hippocampus has been identified as extra-hypothalamic target for leptin, with increasing evidence revealing important cognitive enhancing and neuroprotective roles for leptin in this brain region (McGregor, G., Harvey, J., 2017). Leptin and its receptors share structural and functional similarities with interleukin-6 family of cytokines, (Ghasemi, M., et al ., 2016) High levels of Leptin receptors positive immuno-labelling evident in many extra brain regions including the brain stem ,thalamus, hippocampus and cerebellum , cellular distribution of leptin receptors found high levels of expression at synaptic contact in hippocampal neurons (McGregor, G., Harvey, J., 2017). Multiple factors control leptin levels, including sex steroids, insulin, and cytokines. In the hypothalamus, leptin was discovered to regulate the synthesis of neuropeptides involved in the control of food intake and energy balance (Lv, D., et al 2019) During pregnancy their expression increases with advancing gestation , These receptors are important to both maternal and fetal systems.(Briffa et al ., 2014)(McGregor, G.,& Harvey, J., 2017).

METHODS

The study need to take random (40) experiment female rats, were chosen from the animal house of Biotechnology Research Center / Al-Nahrain University \iraq . (30) pregnant rats and (10) non- pregnant rats . the wight range between (250 -400) g. All rats of experiment study take care with ethics law for animals. The tissues were immediately fixed in formalin solution for 24 h at room temperature (RT), dehydrated in a graded series of ethanol and embedded in paraffin wax. Then, any samples were cut in transverse 5–6 lm thick serial section. Paraffin sections 5 lm thick were mounted on slides coated . These sections were de-waxed with xylen and rehydrated with graded ethanol. Endogenous peroxides activity was blocked with 3% hydrogen peroxides for 15 min. Slides were washed with distilled water for 5 min, placed in a EDTA buffer 0.01M (pH 8.0) and heated in a water bath 95c for 15 min. Background blocking was performed with normal goat serum prior to incubation with specific antiserum. The tissue sections were incubated with a rabbit polyclonal antibody no. (orb10980) diluted to 1:100 in Tris–HCl 0.05M buffer, 0.5M saline, pH 7.6 (TBS) overnight at room temperature and washed in TBS. The detection system used was the HRP method (Biorbyt , UK) consisting in a secondary antibody(HRP Polymer), goat antirabbit Ig, coupled to a peroxidase labeled dextran polymer. The sections were incubated with this reagent for 30 min at room temperature, washed with TBS for 5 min and with 0.1M sodium acetate–acetic acid buffer, pH 6.0 for 5 min. The peroxidase activity was revealed in 0.03% 3,30-diaminobenzidine (DAB) in 0.1M sodium acetate–acetic buffer acid, pH 6.0, containing 2.5% nickel ammonium sulphate, 0.2% b-Dglucose, 0.04% ammonium chloride, and 0.01% glucose oxidase. The color reaction was stopped by a wash in TBS. Finally, the sections were mounted with DPX.

RESULTS

In the coronal sections of rat brain showed the dentate gyrus (DG) with sections appear the three layers of the DG after stain with H&E, the layers of hippocampus also have been seen, Histological view of DG layers showed the three disrupted layers (molecular , granular and polymorphic) layer ,the most abounded cell layer with 3-6 rows that is the granular layer ,that closely packed around the hilus ,this structure give the DG crone-shape tree of spiny dentate(figure 1.). when labeling with anti –leptin marker the Serial sections seen under light microscope of adult rat brain non- pregnant female that labeled with anti-leptin Ab showed high positivity seen as dark brown color in comparison to other dentate gyrus region in hippocampus of brain regions in this group(figure 2). The sub granular cell layer of DG showed high positivity to anti-leptin Ab marker that when mitotic activity occur. Similar positivity was detected in the granular zone that have more number cell row in DG ,the positivity for this cell deferent in same cell to other cell ,Molecular cell layer of DG showed weaker positivity to anti-leptin Ab marker, still less than that of Polymorphic layer ,that founded in this group(figure .3).

The pregnant female group at day 7th of gestation(G 7th)labeled with anti-leptin Ab showed high positivity seen as dark brown color in comparison to other dentate gyrus region in hippocampus of brain regions in all this group ,The granular cell layer of DG showed high positivity to anti-leptin Ab marker that have more number cell row in DG the positivity for this cell deferent in same cell to other,Similar positivity was detected in the sub granular zone ,also the positivity for this cell deferent in same cell to other,Molecular cell layer of DG showed weaker positivity to anti-leptin Ab marker, still less than that of Polymorphic layer ,that founded in all group. (figure 4. In the adult female rat brain pregnant at a day 14th of gestation(G 14th) labeled with anti-leptin Ab showed high positivity seen as dark brown color in comparison to other dentate gyrus region in hippocampus of brain regions in all taken group ,The sub granulr cell layer of DG showed high positivity to anti-leptin Ab marker that when mitotic activity occur, Similar positivity was detected in the granular zone that have more number cell row in DG ,the positivity for this cell deferent in same cell to other ,Molecular cell layer of DG showed weaker positivity to anti-leptin Ab marker, still less than that of Polymorphic layer ,that founded in all this group(figure 5).

lastly adult rat brain female that pregnant at day 19th of gestation (G 19th) labeled with anti-leptin Ab showed moderate positivity seen as dark brown color in comparison to other dentate gyrus region in hippocampus of brain regions in all taken group .The sub granular cell layer of DG showed low positivity to anti-leptin Ab marker that when mitotic activity occur,Similar positivity was detected in the granular zone that have more number cell row in DG ,the positivity for this cell deferent in same cell to other . Molecular cell layer of DG showed weaker positivity to anti-leptin Ab marker, still less than that of Polymorphic layer, that founded in all group (figure 6). Statistical analysis reactivity of anti-leptin Ab in the dentate gyrus of hippocampus in non-pregnant female (control) group gave(mean values ± St.Error) : 0.620± 0.029 pixel/micron² and pregnant group at 7th day of gestation gave 0.606± 0.025 pixel/micron²,When the pregnant group at 14th day of gestation gave mean values 0.703± 0.030 pixel/micron² and pregnant group at 19th day of gestation gave 0.552 ± 0.034 pixel/micron², respectively, with a wider range of reaction intensity in the pregnant group at 14th day of gestation than other group. Four sample assuming equal variances t-Test revealed a significant difference between these values P-value ≤ 0.05 (Tables 1.1).

DISCUSSION

The DG most important area of hippocampus in brain that related with learning and memory ,most former studies reveal on memory cell activity and learning behaviors for DG and leptin with food behavior , in this study linking the neurogenesis in memory cell and learning activity with most important leptin hormones ,the effect of food behaviors with healthy cell memory in healthy human will be studded in hand and we linking the pregnant female with this hormone in this area of memory storage and mitotic division cell activity in adult in other hand. showed the neurogenesis activity in group of pregnant adult rat female have deferent events in histological field and Immunohistochemical study , many differences within that tissue in various aspects, including its embryological stages. Morphogenesis of the dented gyrus is in fact, an early event in CNS development and occurs in vertebrates.
In this study, Immunohistochemical labeling of the DG of hippocampus with Anti-leptin Antibody was analyzed with software Aperio ImageScope with reaction intensity of cells which were stained with Anti-leptin Antibody marker. The result intensity was categorized into four groups: strongly positive, positive, weakly positive and negative, while areas that did not show any staining were reported as negative. Sub granular cells of DG and granular cells in control group female non- pregnant were stained with Anti-leptin Antibody. This is in agreement with other Immunohistochemical methodologies performed in rat brain where Anti-leptin Antibody protein is detected in brain with Leptin receptors are have expressed in the central nervous system and indicate that in targets many brain areas ,one of them hippocampus organ specific in DG , leptin is a potent modulator of hippocampal excitatory synaptic function(McGregor, G., ; Harvey, J., 2017) .

The study in (McGregor, G., Harvey, J., 2017) show the hippocampus has been identified as a key with hypothalamic region target for leptin in brain, with increasing evidence revealing important cognitive enhancing and neuron protective roles for leptin in this brain region. In this study ,show result leptin receptor found in the dentate gyrus with medium level of leptin target in DG that impotent in adult neurogenesis activity of brain neurons that linking with the in vivo and in vitro experiment of (Pérez-González et al., 2011) that leptin increases adult hippocampal neurogenesis stimulated neurogenesis mainly by leptin to from increased cell proliferation, that no significant effect on cell differentiation and survival ,that found in the rat hippocampus was found at up to 11 months of age with neurogenesis (Kaplan and Bell, 1984).In pregnant group of rat female with (G 7th) day ,the result with software Aperio ImageScope with reaction intensity of cells which were stained with Anti-leptin Antibody marker show the same level of positivity in the DG of hippocampus in brain region when compare with first control group ,that deduction mention to the neurogenesis cell activity remaining with same plane line after gestation, This is in agreement with other study of Leptin as a novel placenta-derived hormone in humans, and suggests the physiologic and pathophysiological significance of leptin in fetal growth in normal and complicated pregnancy and that increases with advancing gestation ,suggested that these receptors are important to both maternal and fetal systems During pregnancy (McGregor, G.,& Harvey, J., 2017).

The Anti-leptin Antibody marker is highly expressed level in pregnant group of rat female at day (G 14th) of gestation ,that increasing of leptin level suggested related with sexual hormonal (steroid hormones) of pregnant female is altered significantly throughout life in response to steroid hormone fluctuations occurring during the estrous cycle effect on leptin activity, reproduction, stress, and aging also. that found in other study the Steroid hormones are important mediators of neurogenesis and cell living during development .The roles of these new neurons in the female brain have yet to be determined and the ability of steroid hormones to act as 'growth factors' during development may have extended into adulthood. Furthermore, many species have significant seasonal pressures which are linked to neural plasticity, increased levels of leptin receptors due that the increase in neurogenesis in the SVZ was due to increased levels of prolactin during pregnancy at a mid period of gestation that was reporter in (Pawluski, J. L., et al ., 2009) (figure 5). The report of (Tanapat, P.,et al ., 1999) study sight the new olfactory neurons may play an important role in maternal behaviors and recognition of young female, Many of estrogen's physiological and behavioral effects are modulated by progesterone, an ovarian hormone that also fluctuates across the estrous cycle with peak levels found during proestrus at mid gestation and the specific role of progesterone in regulating hippocampal neurogenesis is not fully elucidated ,that lead to suggesting the leptin hormones effected with the with these factors ; however it does mediate estradiol's effects on hippocampal neurogenesis. Using a hormone regimen that is known to induce female sex behaviors , circulating estrogen levels are positively correlated with cell proliferation and are negatively correlated with cell death .lastly Adult female rats have 50% more newly proliferating cells and fewer pyknotic cells in the dentate gyrus during proestrus (the high estrogen stage) compared to male rats or adult female rats in either the estrous or diestrous stage ,when estradiol levels are much lower.

CONCLUSIONS

The Anti-leptin Antibody marker is highly expressed level in pregnant group of rat female at day (G 14th) of gestation ,that increasing of leptin level suggested related with sexual hormonal (steroid hormones) of pregnant female is altered significantly throughout life in response to steroid hormone fluctuations occurring during the estrous cycle effect on leptin activity, reproduction, stress, and aging also. That found in other study the Steroid hormones are important mediators of neurogenesis and cell living during development. The roles of these new neurons in the female brain have yet to be determined and the ability of steroid hormones to act as 'growth factors' during development may have extended intoadulthood. And lower expressed level in pregnant group of rat female this decreasing in leptin receptor level in the last group of pregnant that deal with the neurogenesis process found in pregnant female at last period of gestation but same resistant in the DG found due to same effects.

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Table 1. Descriptive statistics of anti-leptin Ab marker labeling in the dentate gyrus of hippocampus.

Descriptive Statistics	Control	(G 7th)	(G 14th)	(G 19th)
Mean	0.620	0.606	0.703	0.552
Stander division	0.182	0.154	0.185	0.207
Range	0.55	0.54	0.58	0.74
variance	0.033	0.026	0.033	0.048
observation	37	37	37	37
Stander Error	0.029	0.025	0.030	0.034

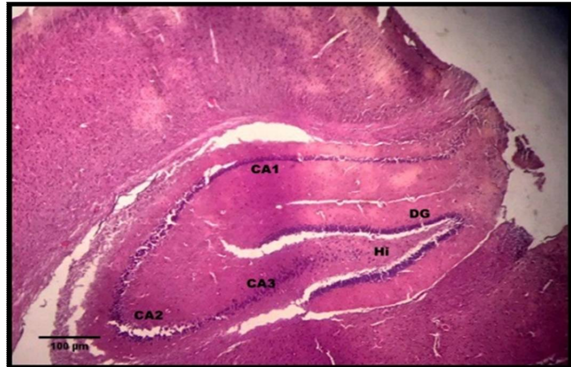

Figure 1. Hippocampal regions, dentate gyrus (DG), CA1,CA2 and CA3(Cornu Ammonis) 40X

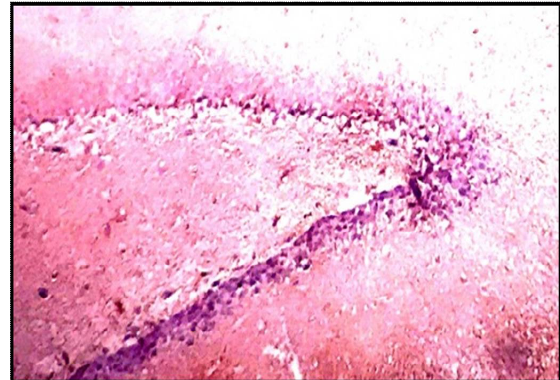

Figure 2. Coronal section of non-pregnant female rat brain showing the dentate gyrus (DG) Cornu Ammonis of hipoocompus (CA 3) labeled with anti Leptin Ab marker.(100 X)

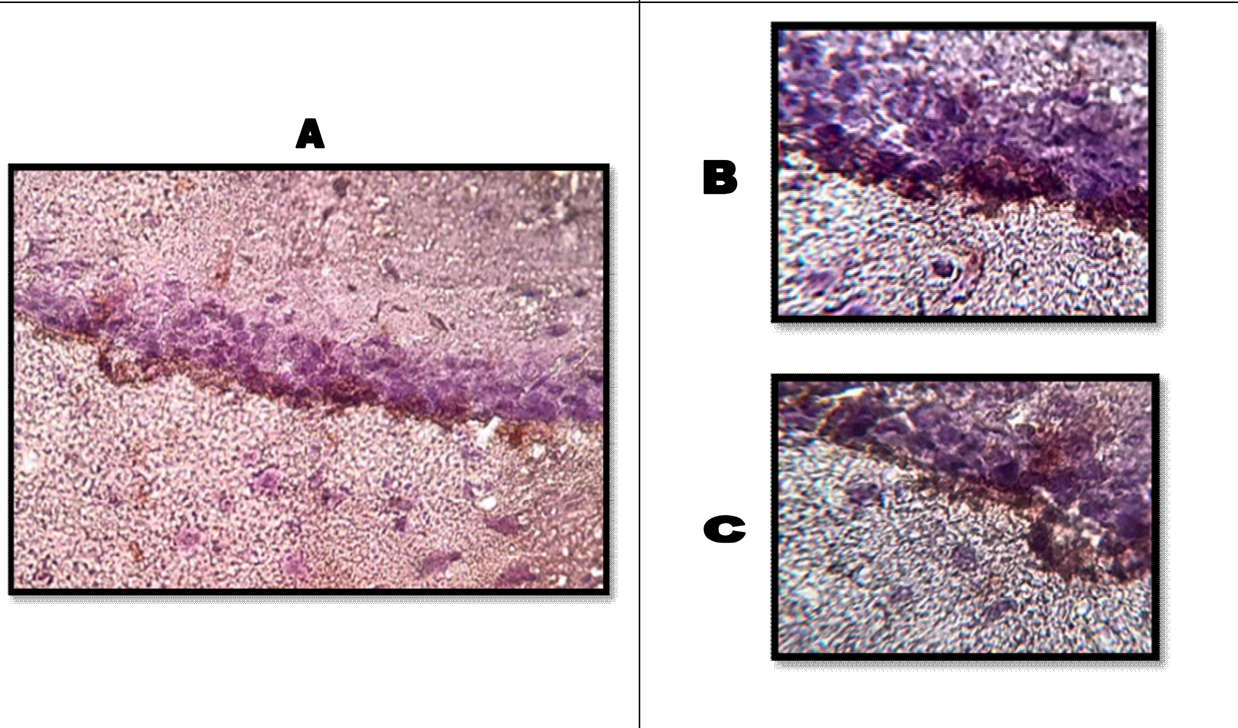

Figure 3. A: Higher magnification of Dentate gyrus (DG)) in non-pregnant female rat brain showing dark brown color labeling with anti leptin Ab marker in sub granular layer (Sub) B:Inset showing large granular cell with mitotic activity stained with hematoxylin counter stain C: Inset showing large granular cell with dark brown color labeling with anti leptin Ab marker .(A) 400X (B)(C) 1000X.

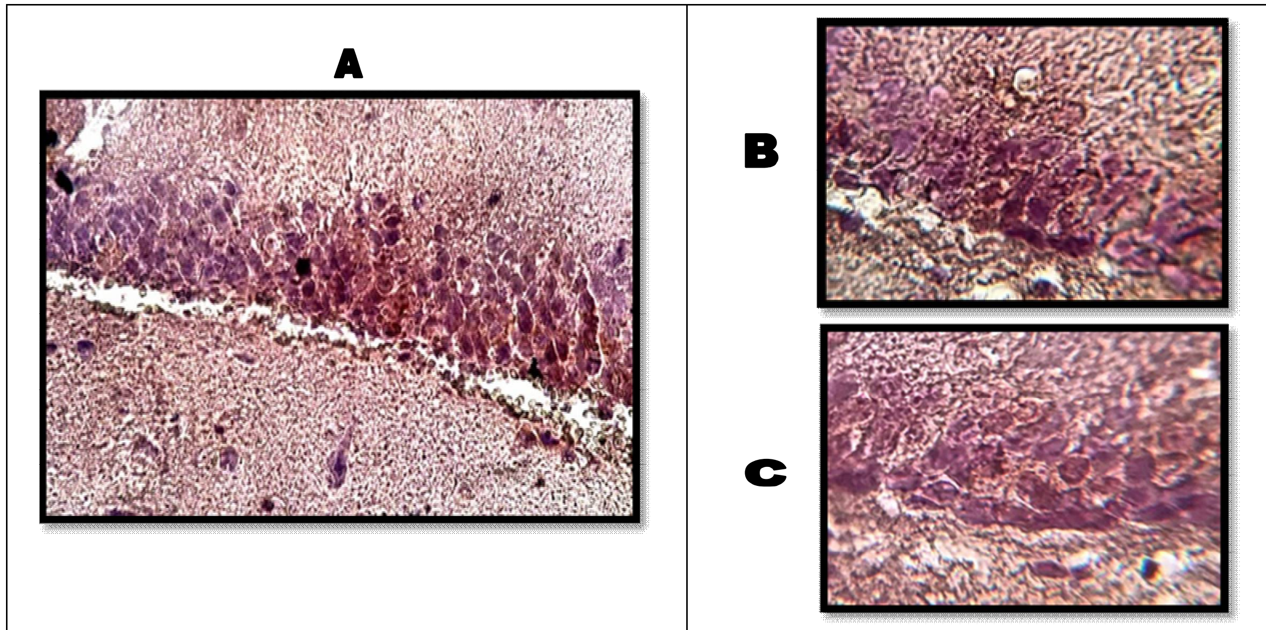

Figure 4. A: Higher magnification of Dentate gyrus (DG)) in female pregnant at 7th gestation rat brain showing dark brown color labeling with anti leptin Ab marker in sub granular layer (Sub) B:Inset showing large granular cell stained with hematoxylin counter stain C: Inset showing large granular cell with dark brown color labeling with anti leptin Ab marker .(A) 400X (B)(C) 1000X

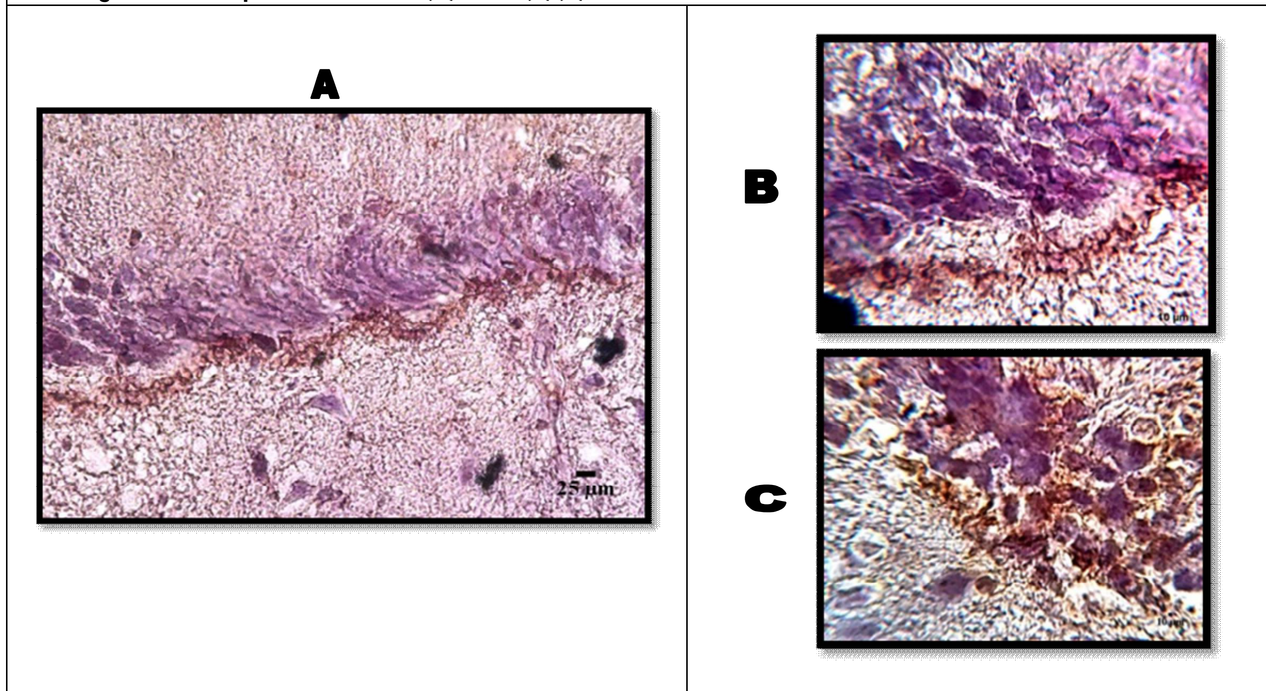

Figure 5. A: Higher magnification of Dentate gyrus (DG) in day 14th of gestation pregnant female rat brain showing dark brown color labeling with anti leptin Ab marker in sub granular layer (Sub) B:Inset showing large granular cell with mitotic activity stained with hematoxylin counter stain C: Inset showing large granular cell with dark brown color labeling with anti leptin Ab marker .(A) 400X (B)(C) 1000X.

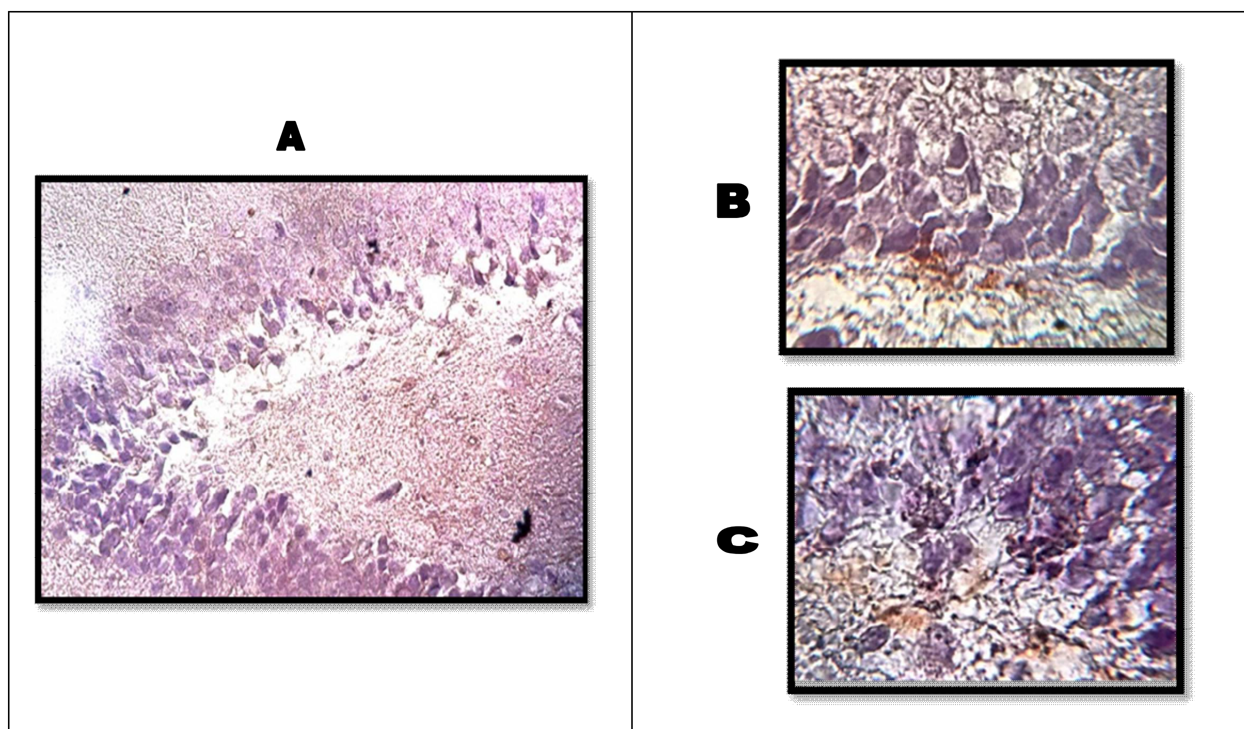

Figure 6. A: Higher magnification of Dentate gyrus (DG)) in day 19th of gestation pregnant female rat brain at 19th day of gestation showing dark brown color labeling with anti leptin Ab marker in sub granular layer (Sub) B:Inset showing large granular cell with mitotic activity stained with hematoxylin counter stain C: Inset showing large granular cell with dark brown color labeling with anti leptin Ab marker .(A) 400X (B)(C) 1000X.

RESEARCH ARTICLE

The Expression of TLR3 in Ovarian Benign and Malignancy Tissues

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ABSTRACT

Ovarian carcinoma (OC) is one of commonest cancer ranks 8th in both incidence and mortality among women world wide, it is the most lethal gynecologic cancer. It affects women in all ages, but is most commonly diagnosed in 55 to 64 years of age. Ovarian tumors arises from surface epithelium ,sex cord stroma and germ cells, surface epithelial tumor is the most common type of ovarian cancer, comprising more than 95% of cases. This study aimed to evaluation the role of mRNA TLR3 gene expression in solid ovariancancer,benign tissues and control group using Real Time PCR.Our result revealed that ovarian cancer tissues showed overexpression for TLR3 comparison with benign tissues and control group, the folding were (1. 95 *vs.* 1.51 vs. 1) respectively. The current results concluded that the overexpression of TLR3 in ovarian cancer may be due to the chronic inflammation in ovarian cancer patients which lead to ovarian cancer.

Keywords: Ovarian, Cancer,Benign, TLR3,PCR, Gene expression,Inflammation

INTRODUCTION

Ovarian cancer (OC) is the eighth most common cancer in women worldwide, accounting for 4,4% of cancer in woman ,approximately 295.414 women were diagnosed with this disease in 2018 with about 1.9% mortality rate [1].The high mortality is primarily due to difficulties in diagnosing early stage disease. The majority of women (75%) are diagnosed at an advanced stage (stage III or IV) [2]. Ovarian cancer is the most lethal gynecologic cancer [3]. It affects women of all ages, but is most commonly diagnosed in those 55 to 64 years of age [4]. Ovarian tumors arise from surface epithelium, sex cord stroma and germ cells. Overall incidence of ovarian neoplasm is surface epithelial (65%), germ cell (15%), sexcord stromal (10%), metastases (5%) as per WHO[5].Symptoms most predictive of ovarian cancer include persistent abdominal distension, postmenopausal bleeding, appetite loss, and early satiety .These symptoms usually only manifest themselves late in the disease process; hence, 70% of women are diagnosed with

advanced stage ovarian cancer [6].Standard treatment involves debulking surgery followed by a combination of taxane and platinum-based therapy. Initially most women respond to platinum-based therapy, but the majority suffer disease recurrence due to drug resistance. It is therefore essential to introduce new therapeutic approaches to improve treatment at diagnosis and/or provide an effective second line treatment [7]. Toll like receptors (TLRs) are part of the basic mechanisms that are involved in the activation of the innate immune system, with extensive activation of various transcription factors that subsequently lead to an adaptive immune response, making them key players that modulate the inflammatory response and tumor dynamics. In the same time, activation of TLRs on OC cells can lead to a different type of response that favors an aggressive phenotype and tumor progression [8]

MATERIALS AND METHODS

Molecular Study of TLR 3 Gene

Current study was involved 35 ovarian tissues, 10 samples of them were diagnosed as malignancy tissues, while 20 samples of them recorded as ovarian benign tumortissues and 5 as control group. Patients were confirmed by pathologists from AL Karamah and AL Zahrah Teaching Hospitals in Wasit Province-Iraq with no other disease such as autoimmune and other chronic diseases.For isolation total RNA from. formalin-fixed paraffin-embedded (FFPE) samples, the samples were cutting into small pieces with 4-8 cm thick, 25-40 µgm of samples were treated by xylene 3 times in 1.5 eppindrofe tubes and then washing by absolute ethanol,70% and 50% for 2 times and centrifuged at 2,000 rpm for 5 minutes. Samples were incubated with 10µl proteinase K at 56°C and 100 µl D.W for overnight.

Total RNA extraction with AccuZol (TRIzol)

We used ovarian benign and cancer tissues in Formalin-Fixed Paraffin-Embedded to extraction RNA.Total RNA of all samples was extracted using the *AccuZo*l reagent following the protocol as follows: For each 250µlof sample was added 750µlof AccuZol (trizol). Lyse cells in the sample suspension by passing the suspension several times through a pipette or vortexing. 200µl of chloroform were added per 1ml of samples -*AccuZol* tubes and shake vigorously for 15 Sec. The mixture incubated on ice for 5 minutes. Mixture was centrifuged at 12,000 rpm for 15 minutes at 4℃. Following centrifugation, the mixture was separated into a lower organic phase (green color), an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The aqueous phase was transferred to a new 1.5ml tubes and add equal volume of isopropyl alcohol. The tubesmixed by inverting five times and incubated at -20℃for 10 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes at 4℃., then carefully removed the supernatant. 1ml 0f 80% ethanol was added to the tube and mixed well by inverting or vortexing. The tube was centrifuged at 12,000 rpm for 5 minutes at 4℃., then carefully removed the supernatant. The pellet was dried by electric fan. RNA dissolved in RNase-free water, by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60C. Total RNA directly converted to cDNA. Or also can be in 100 % formamide (deionized) and store at - 70C.RNA concentration was measured by Nano Drop ND-1000 spectrophotometer (Nano Drop). RNA concentration more than 1.6 n.ml was used in reverse transcription reactions

cDNA Synthesis

Total RNA was reversely transcribed to complementary DNA (cDNA) usingcDNA kit (СИНТОЛ Company / Russia). The procedure was carried out in a reaction volume of 25 µl according to the manufacturer's instructions. Three main steps were needed to conversion:

PCR Programming

The reaction volume were centrifuged at 2000rpm for 20 second .Tubeswere placed in conventional thermo cycler and programmed as in the following table (1).

EVA GREEN Real Time qPCR

Lyophilized primers were diluted in a D.W.as mentioned in manufacturer's instructions. Primers solutions were stored at –40°C until using .Primer sequences of TLR3 and GAPDH were mentioned in appendix [1].Program and cycling condition for amplifying TLR3 gene were seen in tables (2)

Ethical consent

The study was submitted and approved by the College of Science, University of Wasit in cooperation with ALKarama and AL-Zahraa Teaching Hospitals, Wasit, Iraq.

Statistical analysis

The datawere calculated by using 2_DDCt method.

RESULTS AND DISCUSSION

TLR3 Gene expression 3

In our study we were used formalin fixed paraffin embedded samples to investigate the gene expression of TLR3 by Real Time QPCR, using 2-^DDCT method. The results revealed that ovarian cancer tissues showed overexpression for TLR3 comparison with benign tissues and control group, the folding were (1. 95 *vs.* 1.51 vs. 1) respectively, our results indicated that TLR3 expression in malignancy patients was higher than benign tissues, in which was higher than control group. The results were shown in table (3) and figures (1, 2).Kelly *et al.*, (2006) cleared that TLR2, TLR3, TLR4, TLR5 were the most common expression in human ovarian cancer cells[9]. Sato et al., (2009) showed functional TLRs are expressed not only on immune cells but also on cancer cells. Moreover, TLRs play an active role in tumor progression and carcinogenesis during chronic inflammation that involves the tumor microenvironment. Damage-associated molecular patterns (DAMPs) derived from injured normal epithelial cells and necrotic cancer cells appear to be present at significant levels in the tumor microenvironment, and their energizing of specific TLRs can foster chronic inflammation. Carcinogenesis, cancer progression, and site-specific metastasis are related to interactions between cancer cells, immune cells, and DAMPs through TLRs activation in the tumormicroenvironment[10].Zhou et al., (2009) have shown that TLR3, was highly expressed on the normal ovarian epithelium, as well as on neoplastic ovarian epithelial cells, TLRs as a part of the basic mechanisms that are involved in the activation of the innate immune system, with extensive activation of various transcription factors, that subsequently lead to an adaptive immune response, making them key players that modulate the inflammatory response and tumor dynamics. In the same time, activation of TLRs on ovarian cancer cells can lead to a different type of response that favors an aggressive phenotype and tumor progression.

Tumor cell expression of TLRs can promote inflammation and cell survival in the tumor microenvironment [11]. Muccioli and Benencia, (2014) signaled toll-like receptor (TLR) can play an important role in ovarian cancer progression. TLR activation in immune cells can help activate an anti-tumor response, while TLR signaling in tumor cells themselves is often associated with cancer-promoting inflammation. TLR expression in ovarian cancer cells is associated with more aggressive disease (likely due to recruitment of pro-tumoral leukocytes to the tumor site) and

has also been implicated in resistance to mainstream chemotherapy. The delicate balance of TLR activation in the tumor microenvironment in different cell types altogether help shape the inflammatory profile and outcome of tumors growth or regression [12]. On the other hand, Allhorn*et al.*, (2008) showed in their study on TLR3 expression in healthy and diseased human endometrium thatTLR3 proteins was mostly localized to the glandular and luminal epithelium. TLR3 levels did not show significant changes during the menstrual cycle. In patients with peritoneal endometriosis, mRNA TLR3 expression decreased significantly in proliferative diseased endometrium compared to controls. Ectopic endometriotic lesions showed a significant increase of TLR3 gene expression compared to corresponding ectopic tissues, indicating a local gain of TLR expression. Endometrial adenocarcinoma and hyperplasia revealed significantly reduced receptor levels when compared with postmenopausal controls. The lowest TLR expression level was determined in poor differentiated carcinoma (grade 3)[13]. Shcheblyakov*et al.*, (2010) showed that TLRs have had the opposite effects on tumor progression. On the one hand, TLR agonists can promote the survival of malignant cells and increase their resistance to chemotherapy, and TLR3 when ligands can suppress tumor growth [14]

CONCLUSIONS

Our resultsconcluded that the overexpression of TLR3 in ovarian cancer may be due to the chronic inflammation in ovarian cancer patients which lead to ovarian cancer.

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Table 1.Program of conventional thermo cycler

Step	Temperature	Time
1	40 °C	30 min
2	92 °C	5 min
3	4 °C	5 min

Table 2. Program and cycling condition for amplifying TLR3 gene by RT-qPCR

Cycling Step	Temperature	Time	Cycles
Enzyme activation	95 °C	5 min	1
Denaturation	95 °C	30 sec	X 35
Annealing	59 °C	30 sec	
Signal stabilization	72 °C	40 sec	
Melting	25 °C	6 sec	

Table 3. Fold of TLR3 Gene Expression Depending on $2^{-\wedge DDCt}$ method, normalized with GAPDH

Cases	Ct target gene mean	Folding of TLR4 Mean	Folding range
Ovarian cancer patients	24.28	1.95	1.13 - 6.53
Ovarian benign patients	14.54	1.51	0.16 - 6.36

Appendix 1. Primers sequences used in RT- qPCR (15)

Primer	Sequences	Tm
TLR3	FW 5-CCT GGT TTG TTA ATT GGA TTA ACG A-3 RE 5-TGA GGT GGA GTG TTG CAA AGG-3	59 C°
GAPDH	FW 5-ATG GCT ATG ATG GAG GTC CAG-3 RE 5-TTG TCC TGC ATC TGC TTC AGC-3	59 C°

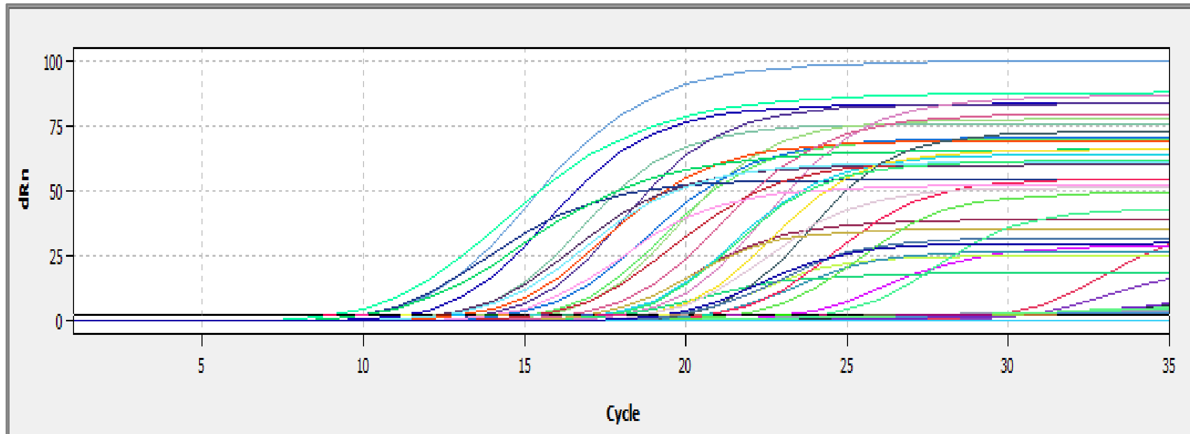

Figure 1.TLR3 and GAPDH amplification plots by qPCR. Thershold (0.3)

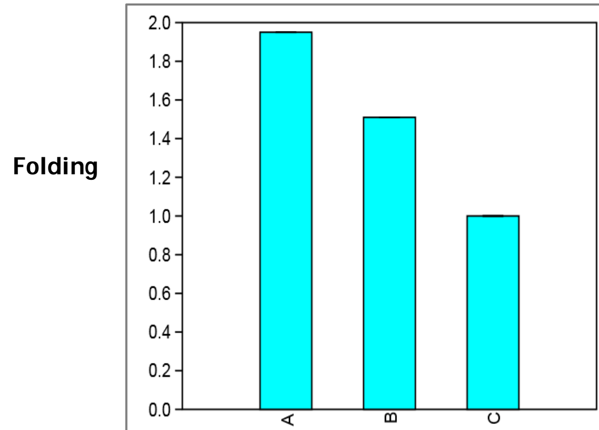

Figure 2. TLR3 expression in ovarian cancer tissues, ovarian benign tissues and control group, normalized with GAPDG A: Ovarian cancer tissues, B: Ovarian benign tissues, C: Control group

RESEARCH ARTICLE

Effect of the Training of Sakio in Some Physiological and Biochemical Variables According to the Lines of Play for the Players of Wasit University Football Team

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ABSTRACT

The basic physical abilities that characterize performance of soccer players demonstrated their level of quantitative and qualitative performance of virtual players, no matter how different levels of performance depends essentially on the sports body's energy systems because the direction and quality of training depends on the method and system Energy supply base, and as the game of football game with various requirements according to each player's duties or status of the problem was the inability of trainers use a variety of exercises related to the nature and performance of duty required by lines of play than lose the players ability to perform their duties as Appropriate to the nature center play and this caused a clear divergence in the level of the overall performance of the team, so the researchers considered using this new technology in training. A sample of Wasit University football player's football applied them playing lines sakiodrills and hammer drills used int to positive "impact on the level of research sample members. The researcher recommended that need attention to aerobic workouts and anaerobic training programming commensurate with performance requirements for playing lines and boundaries.

Keywords: physical, performance, playing, football, game, players, exercises,

INTRODUCTION

Research and its importance as a goal for coaches is to get players to sports that qualifies them to enter competitions and the possibility of achieving positive results, so they should develop training curricula forms and amounts of muscle activity and efficiency requirements as well as Energy production systems operating in accordance with the

muscular action artist. The basic physical abilities that characterize performance of soccer players demonstrated their level of body condition and functional adequacy in performing characteristically, stop construction and its evolution in terms of quantity and quality at the level of the player's virtual performance, no matter how different levels of performance relies, in essence Energy systems in the body because the direction and quality of training and a determined in accordance with the regulations of the power supply.As coaches, players and specialists are constantly looking for new training methods to improve the level of athletic performance and gain competitive advantages, the researchers found that the training of the sack is one of the latest methods and techniques used in the field of sports as it improves the efficiency of performance by developing the ability to perform rapid movements and A clear effectiveness in improving the physical and motor abilities of the players in many sports events, including football, practicing the training of the Sakio as an additional program and supplemented with resistance training to take advantage of the output of muscle strength acquired from the training And died and transferred to field performance through these exercises.

This type of exercise and motor performance certainly has its effects on some physiological and biochemical variables such as heart rate, lung capacity, hormone cortisol, lactic acid, maximum oxygen consumption, viscosity ratio in blood and hemoclobin for football players, and their effect varies according to the physical and motor variables performed by Players whose performance is different depending on the requirements of the play positions and the centers of the players, which vary according to the need of each center. From here the research gained its importance towards the latest techniques and methods of training and modernity and to achieve the positive results that contribute to maintaining the abilities and efforts of the players to continue performance for as long as possible and distinctly using the training Sakio to see its impact in some physiological and biochemical variables according to the play centers for the players of Wasit University football.

Research problem

Football is one of the most varied and varied games that vary in nature, duration, speed and performance on various systems of energy production. It is related to roles and tasks that lead to different forms and skills of movement according to the center, place and duty of the player and the nature of his or her individual or collective performance. On the body of functional sports,Noted that there is a lack of interest of the trainers in the use of various exercises related to the nature of performance and duty required and according to the play centers, which deprived the players the ability to perform their duties properly to their lines and places of play, which caused a clear disparity in the overall performance of the team and most of the exercises are traditional, A modern technique in training is the training of the Sakio to raise the level of physical, motor, skill and functional abilities commensurate with the nature of performance according to the centers of play and knowledge of the impact of these exercises on some physiological andbiochemical variables for players elected University of Wasit football.

Research Objectives

Preparation of exercises to use the method and technology of the Sakio in the training of physical and motor skills and skills. Knowledge of the effect of the training of the Sakio on some physiological and biochemical variables (heart rate and bio-capacity of the lungs and hormone cortisol and lactic acid and blood viscosity and hemoglobin) according to the play centers for the players of Wasit University football. Comparison of some physiological and biochemical variables according to the centers of play in the remote tests of the research sample.

Research hypotheses

There are significant statistical differences between the results of pre and post tests in some physiological and biochemical variables for the players of Wasit University football team.

Research Domains

Human domain: Wasit University football players soccer school year 2017-2018. Temporal domain: 5/12/2017 and 15/3/2018. Space domain: football Athletic Club Stadium Kut – Hall of the Faculty of physical education and Sports Science/University of Wasit lab of dr.Mountadher al- Saidi/Wasit.

MATERIALS AND METHODS

Research Methodology

The experimental method of the most efficient in accessing reliable knowledge.

Sample and Research Society Research

Society determines elected Basra University players in soccer and their number (12) players excluding the goalkeeper and two guards of the players for a lack of commitment and one player to injury. It was divided into three equal groups according to the lines of play (4) players per group and take advantage of other players in the exploratory experience, in order to adjust the variables and differences between players and homogenization process was performed as described in table (1).

Means devices and tools used in research

- medical device to measure the heart rate.
- Spirometer for measuring vital capacity Type Truzone American-made.
- Length and weight measuring device - Medical injection (5cc) Number of 75
- Blood transfusion Chinese number 30.
- Cooling box for transfusion blood samples.
- Various kites.
- Football team + Indoor hall
- Laptop Calculator Type (hp).
- Electronic Stopwatch Type Japanese Casio Number 3 .
- Balls Number 30.- Iron a floor - Various weights + Various assorted heights - 60 - Different medical balls weights + different height barriers.

Methods of collected information

- Arab and foreign sources
- International Information Network
- Observation
- Interview
- Physical and physiological and biochemical tests and its measurements.

Field research procedures

Identify physiological and biochemical variables

After the researcher learned a lot of Arab and foreign sources and taking the opinion of specialists and experience in the field of training and physiology of training were identified the following variables (maximum consumption of oxygen - biological capacity - lactic acid - cortisol hormone - pulse rate - hemoglobin blood - blood viscosity).

Exploratory experiment

In order to identify the method of testing and physiological and chemical measurements, the researcher conducted a pilot experiment on a sample of (3) players at 10 am on Sunday, 3/2/2017, as the pulse rate was measured at rest time - the vital capacity - the maximum consumption of oxygen - Blood withdrawal to measure the level of lactic acid and cortisol and level of viscosity of blood and hemoglobin blood) and through the results of the experiment has been identified the timing necessary to conduct these measurements and ensure the safety of players and after (3) days was re-experiment on Wednesday, 6/12/2017 same steps And procedures .

pre-Tests

The tests were conducted at 10 am on Sunday 10/12/2017 in the hall of the Faculty of Physical Education and Sports Sciences, where the physiological tests (pulse at rest time - the vital capacity of the lungs - the maximum consumption of oxygen) and was followed by the withdrawal of blood (On the same day) by the assistant medical staff of the laboratory of Dr. Muntadar al-Saidi, where the timings for the preservation of blood and according to groups and numbered in the refrigeration fund to measure the level of lactic acid and cortisol hormone and the proportion of blood viscosity and hemoglobin in the blood before the implementation of the main experiment and will be returned after To the end of the application of the exercises as an important functional indicator used to evaluate the training load (difficulty) in sports activities and index of energy systems.

Main Experiences

After identifying the results of the pre- tests and levels of players, special training was conducted according to the requirements of the Sakio suit to the needs of players in each line of toys of physical and motor abilities, which included general endurance exercises and special speed and speed and strength and explosive training exercises and flexibility and agility, The experiment on the three groups according to a special mechanism under the supervision of the assistant staff of the researcher on Wednesday, 13/12/2017 at the club Al Kut Sports Club, and the application of the experiment was completed on Monday, 12/2/2018.

1- The duration of the training program is 2 months.
2- The number of training units (24) training modules.
3 - Number of weekly training units (3) training modules.
4- Weekly training days (Saturday - Monday - Wednesday).
5- Training time (90) minutes.

Post Tests

After finished the application of the training program prepared by the researcher was conducted tests of the dimension of the sample of the research were conducted physiological tests (pulse resting time - the vital capacity of the lungs - the maximum consumption of oxygen) at ten o'clock on Saturday, 17/2/2018 in the College Hall Physical Education and Sports Sciences / Wasit University. The blood flow was then measured to measure cortisol, lactic acid, blood viscosity, and hemoglobin in the blood, ensuring that the same conditions and data were performed in the pre-tests.

Statistical means

The researcher used the SPSS to handle the results (mean, standard deviation, mean, torsion, single variance analysis, independent sample test, least significant difference, L.S.D).

RESULTS AND DISCUSSION

We find that the defense players have outperformed this variable and then the midfielders and this is due to the privacy of the tasks performed by the defenders and the midfield, which require short and continuous movements in the short and medium defense in order to cut and break the ball from the attackers and the opposing team in the middle of the movements of support for the attackers And assistance to defenders, which requires continuous movements, short and medium and this moral came because of these players to the regular training contributed to the development of functional efficiency of vital organs (blood circulation and respiratory), which helped to distinguish these players few heartbeat improved the level of "An increase in the size of the stroke or the amount of blood pumped by the heart in each stroke made the heart more efficient in its work and its ability to meet the needs of the body with fewer strikes" (3:29).

Presentation, analysis and discussion of pre and post tests and the value of L.S.D for the study of the variables of the biological capacity of the three lines of play. We find that the midfielders have exceeded the other lines of play and this is a strong relationship to the nature of the work of the players of this center and their continuous movement and varied, which contributed to the development of the lungs and high efficiency to the extent that enabled them to perform their duties as required and this is due to the nature of various exercises and comprehensive development of their aerobic capabilities, which contributed In the occurrence of functional adjustments in the respiratory system, which leads to an increase in the adequacy of the lungs to absorb the largest amount of oxygen at the inhalation as a result of large size of the lungs or to increase the flexibility of the muscles of the rib cage, which increases the possibility of expansion and widening because " Diverse sports training regularly leads to the occurrence of positive and functional changes in the respiratory system, including increasing the vital capacity "(2: 142).

Presentation, analysis and discussion of the results of post and pre-tests and the value of L.S.D for the sample of the search in the pulse variable at rest time. In terms of the results obtained in Table (8) for the post-test in the pulse variable at the time of rest, we find that there is a significant difference achieved. The calculated value (P) is 8.217, which is greater than the value of (P) of the scale (3.48) at the probability level (0.009) (LSD) from the results of the three lines of play, we find that the defense line had a preference in the pulse level at the time of rest and the specificity of the duties performed by all systems of aerobic and anaerobic energyWhich is adopted in the case of the progress of attribution and return for the purpose of cutting and the risk of the goal, which gave an opportunity to increase the efficiency of the heart and thus the number of strikes at rest as a result of the performance and application of the comprehensive training vocabulary for training endurance and speed of all kinds and on a regular basis, "Regular training leads to access to vital organs to adapt and the improvement of the efficiency of the circulatory and respiratory organs, and thus a decrease in pulse rate "(8: 344).

Presentation, analysis and discussion of the results of tribal and remote tests and the value of L.S.D for the sample research in the variant Lactic acid. There are significant differences between the results of the third game lines and for the line of defense and then the attack because of the characteristics of the players here of the movements of fast depends on the performance on the anaerobic energy system and was preferable to the line of defense because of his rapid movements of the pieces of the ball and try to prevent competitors from access to areas of danger to areas goal and followed by a line The attack also required the players to adopt fast and medium distances to overcome competitors. Therefore, the exercises carried out by the players had a great impact on the development of the ability of the body to work in conditions of high intensity and for relatively long periods of timeAnd good tolerability due to

increased metabolism of lactic acid in the working muscle and increase the ability to get rid of it. This is what Carlo *et al*. (2004) points out that "the maximum performance during repeated effort is influenced by the nature of that effort and the following period of hospitalization because high voltage requires anaerobic glucose (6: 224). Abdulrahman Zaher (2011) noted that lactic acid is an important indicator of progress in training, as the training is very intense under the extreme as in the exercises prepared by the researchers of the maximum consumption of oxygen "improves the ability of the athlete to perform high-level is an indicator of assessment of anaerobic pregnancy and knowledge Whether the training load is sufficient to adapt the body systems to increase the proportion of lactic acid in blood or not "(4 - 465).

Presentation, analysis and discussion of the results of pre and post tests and the value of L.S.D for the sample of the search in the blood viscosity variable. There is a significant difference between the results according to the lines of play and for the benefit of midfielders, where the performance here is characterized by continuity and rapid movements and slow and different distances, which increases the consumption of fluids and loss of which "causes increased blood concentration and increase of its viscosity " (1 - 255).

Presentation, analysis and discussion of the results of pre and post tests and the value of L.S.D for the study sample in the cortisol hormone variable. There is a significant difference between the results according to the three lines of the game and for the players of the line of attack and then the players of the line of defense and then midfielders, as the researcher attributed that the attackers and defense are characterized by the element of explosive capability, the defender through cutting and dispersion of the ball and reaction and speed of expectation and respond to the requirements Defensive positions and the striker through his quick and short start as well as scoring on the goal with maximum force to surprise the opponent, and since most of these movements are explosive nature, we find that the hormone cortisol responds and increases the level of concentration in the serum because the exercisesexplosive power of working in this direction as it increases after a violent physical effort, RissanKhreibt, from Lamb 1984, states that "explosive force exercises in football training increase the levels of cortisol in serum and that there is a claim that there is a balance between the primary activity of metabolism represented in the so-called free cortisol rate" (2: 94). Prepared by the researcher informed the impact in developing the capabilities of the players to implement movements of explosive nature effectively and effectively contributed to the completion of their dutiesoffensive and defensive.

View, analyze and discuss the results of tribal and remote tests and the value of L.S.D for the sample research in the hemoglobin variable. The results of the three lines of play showed that the midline had the advantage in the level of hemoglobin blood to distinguish the duties of the movement of continuous and medium speed and for various distances, which contributed to increase the number of red blood cells, which reflected on the increase of Hemoglobin blood and thus gave the opportunity for players of this line to continue to perform effectively, The exercises that were applied by them and characterized by high intensity contributed to the increase in blood volume and thus increase in the amount of hemoglobin blood. In this regard, JabbarRahima Al-Kaabi (2010) "increases the amount of hemoglobin they have (16 g) for every (100 cm 3) Whenit was in endurance players reached to more than (18 g) for each (100 cm 3) of blood "(11: WWW).

CONCLUSIONS

1) The training of the Sakio positive effect in some physiological and chemical variables of the research sample
2) There was a significant difference between the physiological and chemical variables according to the lines of play.
3) A condition of adaptation in the indicators of the chemistry of the members of the research sample according to the privacy of the lines of play.
4) Superiority of defensive players in the maximum variables of oxygen consumption and pulse rest time and lactic acid while superior

5) The midfielders in the dynamic capacitance variables are hemoglobin and the line of attack in cortisol.

Recommendations

1. Necessity of the training of the saqio by coaches in training football players because of their important role in developing the levels of players.
2. Attentionon aerobic and anaerobic training in the development of training programs in line with the requirements of the performance of lines and centers of play.
3. It is necessary to conduct continuous biochemical and physiological tests at each stage of the training to determine the level of development.
4. Make a researchesand studies on other age groups and other chemical, biological and physiological indicators.

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Table 1. The homogeneity of the research sample

Variable	Measuring unit	x⁻	y⁻	Mediator	Torsion coefficient
Age	year	18.85	0.679	18	- 0.574
Length	cm	177.5	4.655	178	- 0.322
Weight	kg	72.45	2.35	72	0.574
Training age	year	2.3	0.315	2	2.857

Table 2. The analysis of the variance, the calculated and tabular value and the level of significance of the differences between the three play lines in the post-test of the variable of the maximum consumption of oxygen Vo2Max.

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P)value	Tabular (p) value	Probability	Significance of differences
Between groups	0.841	2	0.420	5.316	3.48	0.030	Significant
Within groups	0.712	9	0.079				

Table 3. Shows the results of the L.S.D test for the distance comparisons of the three play lines in the maximum oxygen consumption variableVo2Max.

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	0.632	0.011	0.01	Sig.
Defense - Attack	1 – 3	0.192	0.358	0.05	Sig.
The middle - the attack	2 – 3	0.440	0.440	0.05	Sig.

Table 4. The analysis of the variance and the calculated and tabular value (q) shows the level and significance of the differences between the different play lines in the post-test.

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P)value	Tabular (p) value	Probability	Significance of differences
Between groups	2744.278	2	1372.139	7.206	3.48	0.344	Significant
Within groups	10239.86	9	1137.763				

Table 5. Shows the results of the test (L.S.D) after comparisons of different play lines at critical amplitude variable.

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	34.67	0.181	0.05	Sig.
Defense - Attack	1 – 3	5.865	0.811	0.05	Sig.
The middle - the attack	2 – 3	38.742	0.259	0.05	Sig.

Table 6. The analysis of the variance and the calculated and tabular P value and the level of significance of the differences between the three play lines in the post-test

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P) value	Tabular (p) value	Probability	Significance of differences
Between groups	10.500	2	5.250	8.217	3.48	0.009	Significant
Within groups	5.750	9	0.639				

Table 7. Shows the results of the L.S.D for comparisons the dimensionality of the three play lines in the variable pulse time rest

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	1.50	0.026	0.05	Sig.
Defense - Attack	1 – 3	0.750	0.217	0.05	Sig.
The middle - the attack	2 – 3	2.250	0.003	0.05	Sig.

Table 8. The analysis of the variance and the calculated and tabular P value and the level of significance of the differences between the three play lines in the post-test of the lactic acid variable

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P) value	Tabular (p) value	Probability	Significance of differences
Between groups	13.134	2	6.567	47.074	3.48	0.000	Significant
Within groups	1.256	9	0.14				

Table 9. The results of the L.S.D test are shown for the distance comparisons of the three play lines in the lactic acid variable

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	2.56	0.000	0.05	Sig.
Defense - Attack	1 – 3	1.157	0.004	0.05	Sig.
The middle - the attack	2 – 3	1.405	0.001	0.05	Sig.

Table 10. The analysis of variance, the calculated and tabular value (q), and the level of significance of differences between the three play lines in the post-test of the viscosity variable show the blood.

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P) value	Tabular (p) value	Probability	Significance of differences
Between groups	69.055	2	34.528	14.99	3.48	0.001	Significant
Within groups	20.718	9	2.302				

Table 11. Shows the results of the L.S.D test for the three dimensional comparisons of the three play lines in the blood viscosity variable.

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	5.01	0.001	0.05	Sig.
Defense - Attack	1 – 3	0.225	0.838	0.05	Sig.
The middle - the attack	2 – 3	5.335	0.001	0.05	Sig.

Table 12.The analysis of variance, the calculated and tabular value (q), and the level of significance of differences between the three play lines in the post-test of the cortical.

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P) value	Tabular (p) value	Probability	Significance of differences
Between groups	639.500	2	319.75	13.48	3.48	0.002	Significant
Within groups	213.410	9	23.72				

Table 13. The results of the L.S.D test are shown for the dimensional comparisons of the three play lines in the cortisol variable.

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	10.065	0.027	0.05	Sig.
Defense - Attack	1 – 3	7.750	0.012	0.05	Sig.
The middle - the attack	2 – 3	17.730	0.077	0.05	Sig.

Table 14. The analysis of variance and the calculated and tabular P value and the level of significance of differences between the three play lines in the post-test of the hemoglobin variable are shown.

Source of variation	Total squares	The degree of freedom	Average squares	Calculated (P) value	Tabular (p) value	Probability	Significance of differences
Between groups	2.232	2	1.116	26.780	3.48	0.000	Significant
Within groups	0.375	9	0.042				

Table 15. The results of the L.S.D test are shown for the post-comparisons of the three play lines in the hemoglobin variable.

Line playing	The value of differences between the computational circles of the playing lines	Value (P)	L.S.D value	Level of significance	Significance of differences
Defense-center	1 – 2	0.770	0.054	0.05	Sig.
Defense - Attack	1 – 3	0.007	0.038	0.05	Sig.
The middle - the attack	2 – 3	0.762	0.056	0.05	Sig.

RESEARCH ARTICLE

Knowledge and Prescribing Practices of Clinicians Regarding Typhoid Fever in Pakistan

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ABSTRACT

Background:Typhoid fever is highly contagious and can transfer easily from one individual to another. According to the previous statistical reports Typhoid fever is highly prevalentin Pakistan. Although the advanced treatment modalities have been introduced but factors responsible for typhoid relapse are still the major concern for this region.Objective: The main aim of the study was to gather information regarding health-care provider's knowledge and practices during clinical examination and treatment of typhoid fever. Methodology: A cross-sectional multi-center study was conducted from May - July 2018. Data was collected from 1135 health-care providers, of all four provinces of Pakistan. Data was collected by means of a structured questionnaire. Clinical features, preference among the prescribing practices like clinical examination and diagnostic tests were inquired. Moreover, factors leading to typhoid relapse, factors for resistance and drug of choice among health care providers were also part of the survey. Statistical analysis was done on statistical package for social science (SPSS version 22.0). Results:18.1% of Health Care Providers refer patients for blood culture and 30.7% preferred Typhidot Test always. 45.7% health care providers preferred empirical antibiotics. Ciprofloxacin 82.5% was most commonly prescribed by the health care providers in suspected typhoid fever patients followed by Cefixime 10.20%. Incomplete course of treatment 40.57% and inappropriate dosage 27.47% remains the leading cause of typhoid relapse.Conclusion: There were notice able differences in the clinical examination, prescribing practices and diagnostic techniques used by these professionals in all four provinces of Pakistan. Moreover, Typhoid management was not done according to guidelines provided by the Ministry of National health services, Regulations & coordination's.

Keywords: Enteric Fever, Typhoid Fever, Prescribing Practices, Typhidot, Blood Culture.

INTRODUCTION

Typhoid fever has grown into an endemic systemic infection induced by Gram negative bacteria; *Salmonella entericaserotype typhi* [1].The disease is found more prevalent in low to middle-income countries due to lack of resources and poor treatment modalities. This fatal condition is transmitted via oral and fecal route as well [1&2]. According to the recent statistics, up to 21 million cases of typhoid cases are reported worldwide of which 128, 000 to 161, 000 typhoid-related deaths are recorded annually [3].Being more prevalent in Asia and Africa i.e. 93% of the global episodes of typhoid are reported from Asia [4]. A total of 110 cases/ 100,000 population are reported from Southeast Asia i.e. the third highest incidence rate for any region[4].High incidence rate of typhoid fever, especially among youngsters were reported[5&6],although there are very less number of studies targeting typhoid incidence rate in Pakistan. The major reason for the increasing prevalence among these under-developed countries is mainly unhygienic lifestyle, unavailability of safe drinking water and constant exposure to such unhealthy environmental risk factors on daily basis. Despite being treatable, the severity of the infection is dependent upon the body's immune response, micro-organism's ability and minimal infective dose [7].

The emerging antibiotic resistance to *S. Typhi*s train is a rising concern and is significantly associated with increased morbidity and mortality [8&9].While delayed diagnosis also plays a key role in increasing the risk of adverse outcome and mortality [8&9].According to the report published by World Health Organization (WHO), there is an outbreak of extremely drug resistant (XDR) typhoid fever in Pakistan. Out of 8188 cases of typhoid, around 5274 cases of XDR typhoid cases were reported by the Provincial Disease Surveillance and Response Unit (PDSRU) in Sindh of them 69% cases were reported from Karachi, 27% from Hyderabad and 4% in other districts of the province [10]. The clinical diagnosis of typhoid fever is difficult as the representation is non-specific and similar to various other common illnesses. Research shows that there is still not a single test that is 100% sensitive for the diagnosis of enteric fever [11].Among all treatment modalities, blood cultures are considered as the gold standard for diagnosis of typhoid fever despite of the fact that it gives delayed results (almost 2-3 days)[12].The sensitivity of blood cultures for the diagnosis of typhoid is far much greater than the others. Blood cultures are found to be 60% sensitive and depends on the volume of blood utilized for culture. A profound reason behind the declining sensitivity might be the initiation of antibiotic use even before the diagnostic confirmation of the disease. Due to delayed diagnostic results through blood culture, the health care providers now prefer serological test namely Typhidot test for timely diagnosis and efficient management according to the disease status [11]. Appropriate diagnosis and management of typhoid are necessary measures as the disease is backed up by a high index of relapse cases i.e. typhoid relapse occurs in almost 5-10% of the cases even after proper antibiotic treatment[13].The main aim of this study was to evaluate the knowledge and prescribing practices of healthcare providers across Pakistan. And to figure out the causes behind typhoid relapse in the opinion of these health care providers.

MATERIALS AND METHODS

A cross-sectional multi-center study was conducted from May - July 2018. Study was designed under declaration of Helsinki and independent ethical committee approval was also obtained from Pakistan Medical Association Committee on ethics (Reference Number: OB/212/PMC/16). Data was collected from 1135 health-care providers, of all four provinces of Pakistan.Written and signed informed consent from physicians was obtained prior to inclusion in the study. A paper based form was designed for systematic data collection. Data collectors designated by the investigator, fully informed the health-care providers about terms and conditions, objectives, constraints and duration. Data was collected by means of a structured questionnaire including eight closed-ended questions. Clinical features, preference among the prescribing practices like clinical examination and diagnostic tests were inquired. Moreover, factors leading to typhoid relapse, factors for resistance and drug of choice among health care providers were also part of the survey.

The collected data was then coded and entered into a database. Double entry of data was done by 3rd party data punch operator on Epidata v.3.1. Statistical analysis was done on statistical package for social science (SPSS version 22.0).

RESULTS AND DISCUSSIONS

According to the World Bank, low or low-middle income countries are at risk of the outbreak of typhoid fever yearly [14]. Poor diagnostic and laboratory resources are the leading cause of this endemic condition, while under-estimation is the next promoting factor of the disease burden in countries like Pakistan [14&15].As stated earlier the aim of our study was to evaluate knowledge and prescribing practices of clinician in different provinces of Pakistan. Participation from the Punjab province was highest i.e. 56.9% while only 2.7 % health care providers participated from Baluchistan province (Table 1). In the present study, 45.7% health care providers preferred empirical antibiotics for treatment of typhoid fever, while 9.1% never prescribed empirical antibiotics (Figure 1). Moreover, Ciprofloxacin 82.5% was most commonly prescribed by the health care providers in suspected typhoid fever patients followed by Cefixime 10.20%, Chloramphenicol 1.90%, Amoxycillin 1.60%, Azithromycin 2.20% and others 1.60% (Figure 2). These findings are also supported by another study conducted in Rawalpindi where Ciprofloxacin was recommended as the first line treatment for sensitive and multidrug resistance S.Typhi and Paratyphi strains. Moreover, it was also suggested that Ceftriaxone from third generation can also be utilized but only in complicated multidrug resistance cases [16].

In addition to the preference for treatment modalities, the recommended diagnostic test for enteric fever was also inquired and according to our results 18.1% of health care providers refer patients for blood culture and 30.7% preferred Typhidot Test always. While 43.3% of Health Care Providers diagnose Typhoid Fever through clinical examination, most of the time (Figure 3). Although the blood culture is standard diagnostic method for Enteric fever [17,18-21]but its sensitivity (30-40%) is less in comparison to Typhidot test (68-95%) [17&22]. In contrast, another study reported that the results of blood culture overruled typhidot test showing greater sensitivity therefore it was suspected as a better choice for typhoid fever diagnosis [23].There are a number of clinical factors that lead to typhoid fever and are also associated with the subsequent relapse of the disease [24].Takashi et al., in their study concluded that the delayed initiation of the treatment and increased duration of recovery influences typhoid relapse [24].While according to another study ineffective treatment and antibiotic resistance are mainly associated with typhoid relapse [25]. Moreover the typhoid carriers are more likely to develop typhoid as compared to other counterparts [25]. Also indicated through our results, incomplete course of treatment 40.57% and inappropriate dosage 27.47% remains the leading cause of typhoid relapse as suggested by the health care providers (Figure 4). Recently health care providers issued health alerts in Sindh due to the drastic outbreak of XDR [26]. So, in order to manage such circumstances, it is very important to disengage the transmission pathways. Which includes assurance of healthy lifestyles, safe and clean drinking water, better resources, improved clinical setups, better management waste. Among typhoid endemic countries like Pakistan, vaccination and eradication campaigns are required on a large scale.

CONCLUSIONS

Our aim through this study was to evaluate the knowledge of our health care providers regarding the major clinical aspects related to typhoid fever. As this endemic condition is widespread in Pakistan, vast variation in the knowledge of typhoid fever was observed among the health care providers. There were notice able differences in the clinical examination, prescribing practices and diagnostic techniques used by these professionals in all four provinces of Pakistan. Moreover, Typhoid management was not done according to guidelines provided by the Ministry of National health services, Regulations & coordination's.

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Table 1. Provincial Distribution of Health-care provider'sparticipation in the study

Provinces	n(%)
Punjab	646 (56.9)
Sindh	390 (34.4)
Khyber Pakhtunkhwa	68 (6.0)
Baluchistan	31 (2.7)

*(n=1135)

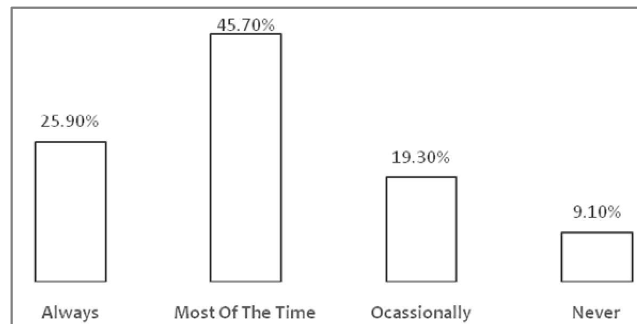

Figure 1. Empirical antibiotics prescribed by health care providers for Typhoid Fever

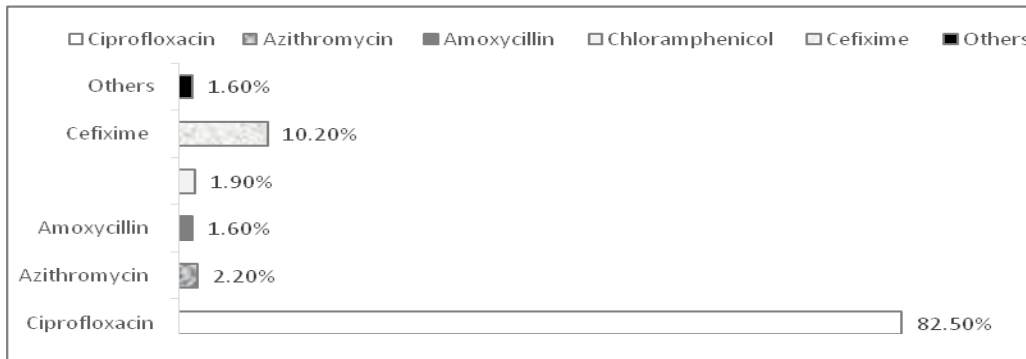

Figure 2. Choice of drugs prescribed by health care providers for typhoid patient

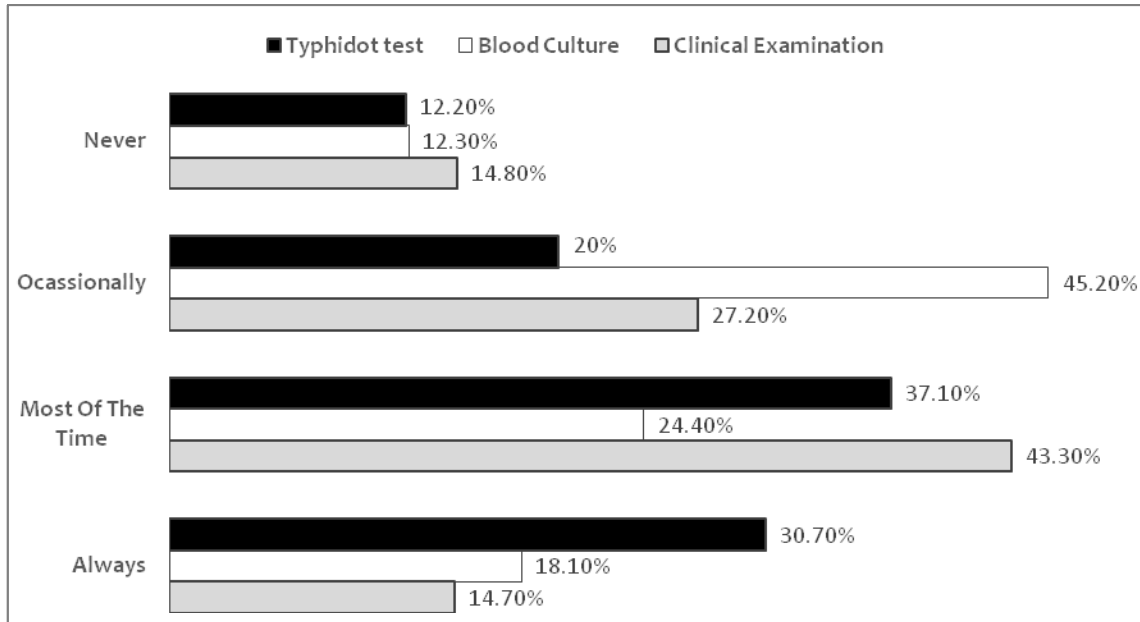

Figure 3. Different methods referred for diagnosis of Typhoid Fever by health care providers

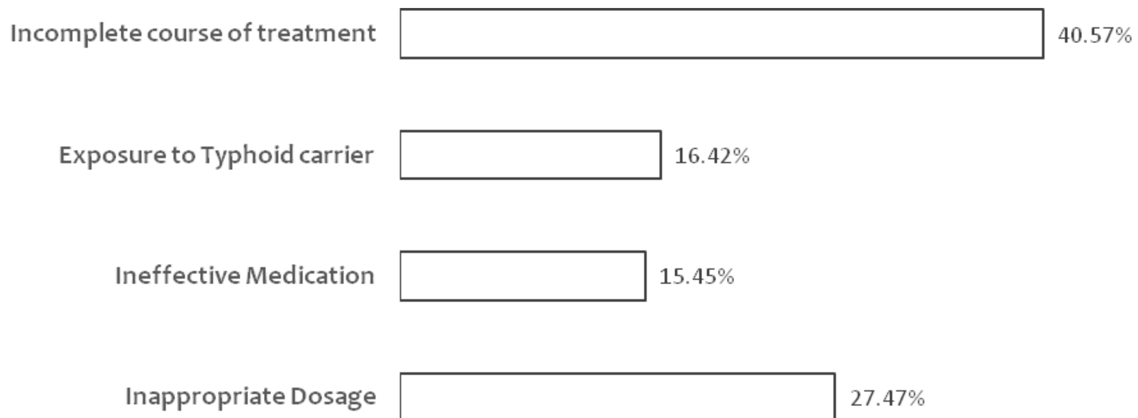

Figure 4. Major factors leading typhoid relapse according to health care providers

RESEARCH ARTICLE

Leishmania Spp Isolated in Patients by Microscopy, Culture and PCR

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ABSTRACT

Leishmania spp were isolated from skin of 786 patients clinically diagnosed with cutaneous leishmaniosis from 8 hospitals in different localities in Iraq from October 2012 to December 2013. *Leishmania* types were identified by microscopy, culture and PCR. By Giemsa-stained smear and culture of all samples we detected 225(28.6%) and (172)21.9% to be positive to Leishmania spp respectively, We then randomly selected 160 samples for PCR and found 95.6 % to be positive. Of those identified by PCR ~58% were *L. major,* and ~38% *L tropica*. We found that young patients (<11yrs) suffered highest incidence (24%) compared with those aged 11-20 years (~12%). We found leishmania in 55% males and 46% in females ~46% with lesions of the upper extremity more common in children ~54% and ulcers mostly humid (78%) rather than dry (22%) types. Higher prevalence occurred in patients from Najaf (25%) and Wasit (22.5%), Iraq. Results suggest that PCR is a more sensitive for detecting Leishmania from skin scrappings of patients clinically diagnosed with cutaneous leishmaniosis.

Keywords: Cutaneous leishmaniasis, PCR, Culture, Human

INTRODUCTION

CL is endemic in many parts of the world especially in the Middle East countries including Iraq. In Iraq, zoonotic CL (ZCL) is caused by *L. major* and transmitted by *P. papataci* sand fly from rodents are the reservoir (WHO, 2003) and anthroponotic CL caused by *L. tropica* and transmitted by *P. sergenti* (Alvar *et al*., 2012). The cutaneous form is self-healing but without treatment lesions can cause significant scarring (Gillis *et al*., 1995). Untreated healing times vary 2 to 15 months depending on leishmania type (Gillis *et al*., 1995). Clinical leishmaniosis exists as self-healing cutaneous leishmaniasis (CL), muco-cutaneous, life threatening visceral or post kala-azar forms (Postigo, 2010). Conflict in Iraq has been related with incidence of cutananeous leismaniosis. For example in 1991 incidence was 8,779/100,000 but in 2001, only 625 cases/100,000 (AlSamarai and AlObaidi, 2009) Traditionally, Giemsa stains or culture have been used to confirm diagnosis (Ramirez *et al*., 2000). However only recently has PCR a more sensitive method to detect DNA antigen has become available in Iraq. We report incidence of Leishmania in patients in Iraq determined by microscopy, culture and PCR methods.

MATERIALS AND METHODS

Ethics statement and patients

This study was approved by the Ethics Committee of Wasit University, Iraq. Suspect patients were interviewed and then physically examined. Those who signed an informed consent willing to participate were recruited. A total of 786 patients with clinical lesions pathognomonic of cutaneous leishmaniosis of different ages, gender, locations (8) and occupations were studied. The population under the study was selected from the CL suspected patients who were referred to the Laboratory of parasitology by dermatologists.

Location

This study was carried out in Wasit, Baghdad, Basrah, Diyala, Kirkuk, Najaf, Anbar and Salah-Adin, Iraq, from October 2012 to December 2013.

Specimens

Routine diagnosis of cutaneous leishmanial is done by giemsa stained smears and cultures of dermal scrapings (Robinson *et al*., 2002). In this study, patient lesions were cleaned with 70% alcohol and the scraped with scapel from different sites around of the lesion as described by Robinson *et al*.,(2002). The samples were smeared on glass slides and then stained with Giemsa for amastigote(s) inside or outside macrophages (Robinson *et al*., 2002). Other skin scrappings were inoculated into the liquid phase of Novy-McNeal-Nicolle (NNN) medium as described by Herwaldt, (1999). The culture media was incubated at 25 $\pm$ 1°C and examined for parasite growth using inverted microscope every 4 days until promastigotes were seen or up to one month before being discarded and discarded as negative. The cultures were done in duplicates for each patient.

Genomic DNA Extraction

A third skin scrapping sample was used for Genomic DNA extraction as described in AccuPrep®Genomic DNA extraction kit (Bioneer. Korea). Nested-PCR Master Mix for detection *Leishmania spp.* in the first-round was prepared according to kit instructions (AL-Hucheimi, 2005). All these components of Nested-PCR master mix reaction was added into *AccuPower* nested-PCR PreMix tube that contain PreMix pellet of all other components. Then, the PreMix pellet was resuspended and mixed using vortex. Then, Nested-PCR was used for identification of *Leishmania* spp. Nested-PCR.

Thermocycler conditions were done by using Optimase PCR protocol writer online in Simple PCR Thermocycler (TECH-Belgium) as following:

Forward primer sequence: CGAGTAGCAGAAACTCCCGTTCA(Tm= 59ºC)

Reverse primer sequence: ATTTTTCGCGATTTTCGAGAACG(Tm= 53.7ºC)

PCR product length: 750 bp

Protocol type: Touchdown PCR protocol

Forward primer sequence: ACTGGGGGTTGGTGTAAAATAG (Tm = 55.1ºC)

Reverse primer sequence: TCGCAGAACGCCCCT(Tm = 51.1ºC)

PCR product length: 750 bp

Protocol type: Touchdown PCR protocol

The final Nested- PCR products were subjected to gel electrophoresis.

RESULTS

Figure 1 shows incidence of CL in various localities in Iraq.

DISCUSSION

Diagnosis of cutaneous leishmaniosis (CL) by Giemsa stain or culture requires a high number of viable or morphologically intact parasites particularly in the chronic phase when parasite levels in skin lesions are very low (Abdulsadah *et al.*, 2013). In contrast, because PCR detects *Leishmania* in both acute or chronic lesions we found a very high incidence of 95% (n= 160) compared with only ~19-24% (n=758) by stain or culture (Abdulsadah *et al.*, 2013). This could suggest prevalence of the chronic form of cutaneous leismaniosis in our subjects. Diagnosis and treatment at early stages can prevent development of the chronic form with disfiguring scars (Herwaldt, 1999). In endemic areas such as Iraq Ie, CL is diagnosed mostly by epidemiological and clinical assessment and secondary infection or mistreatment can misdiagnose and delay correct treatment (Hepburn,2001 ; Jarallah, 2009). By Giemsa stain we found only 18.6% patient positive to Leismania spp, much lower than reported previously by Rodrigues *et al.*, 2002 , AL-Hucheimi, 2005) or Shaaban *et al.*, (2004), who found 83.3%. Al-Samarai and Al-Obaidi, (2009) found similar high incidence of 73% (Iraq). We found highest prevalence in young patients < 10 years most likely due to child contact with sandfly vector contact in villages (Momeni and Aminjavaheri,1994; Yaghoobi-Ershadi *et al.*, 2003; Fellah *et al.*, 2007). Our finding resemble those of Abdula (2000) who found that <7 year old were mostly affected in Baghdad boil. AL Mashhadani, (2002) and AL-Janabi, (2001)) reported high incidence in 10-15 year olds in Nayaf whereas Altvili, (2003), found most ulcers in >15 year patients in Hawija. Leishmania can be spread from villages up to 15 miles associated with some types of leishmaniasis parasite such as *L. tropica* from sandflies or from animals such as *L. major* (Vergel *et al.*, 2006). Our findings confirm that *L. major* and *L. tropica* predominate as agents of cutaneous leishmaniosis in Iraq with a slight preference for *L major* (57%). We find that CL is endemic in Iraq and recommend that PCR be used over direct methods as it is a more sensitive test.

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Table 1. *Leishmania* detected in skin scrappings by direct Giemsa stain in 768 patients by age.

Age (Year)	Gender	Direct smear examination		Subtotal	Total	%
		+VE	-VE			
≤ 11	M	50	50	100	186	23.7
	F	56	30	86		
11-20	M	52	00	52	100	12.7
	F	45	03	48		
21-30	M	44	46	90	157	20.0
	F	43	24	67		
31-40	M	40	48	88	170	21.6
	F	42	40	82		
≥ 40	M	45	48	93	173	22.0
	F	39	41	80		
Total		231	192	423	786	100
		225	138	363		

Table 2. Type of *leishmania* ulcer lesions in 786 patients by age.

Age (Year)	Type of ulcer lesion		Total	%
	Wet	Dry		
≤ 10	116	70	186	23.7
11-20	67	23	90	11.4
21-30	95	62	157	20
31-40	92	78	170	21.6
≥ 41	107	76	183	23.3
Total	477	309	786	100

Table 3. *Leishmania spp.* detected by nested-PCR in 160 patients by gender and age.

Age (Year)	Gender	Nested- PCR		Subtotal	Total	%
		+VE	-VE			
≤ 10	M	24	3	27	51	31.9%
	F	23	1	24		
11-20	M	15	1	16	28	17.5%
	F	12	0	12		
21-30	M	12	0	12	26	16.2%
	F	14	0	14		
31-40	M	13	0	13	27	16.9%
	F	14	0	14		
≥ 41	M	12	1	13	28	17.5%
	F	14	1	15		
Subtotal	M	76	5	81	160	100%
	F	77	2	79		
Total %		153(95.6%)	7(4.4%)			

Table 4. Types of *Leishmania* spp detected by PCR in 160 patients by gender and age.

Age (Year)	Gender	*L. major*	*L. tropica*
≤ 10	M	16	8
	F	17	6
11-20	M	9	6
	F	7	5
21-30	M	6	6
	F	7	6
31-40	M	8	6
	F	7	7
≥ 41	M	7	5
	F	8	6
Total %	153 (95.6%)	92(57.5%)	61 (38.1%)

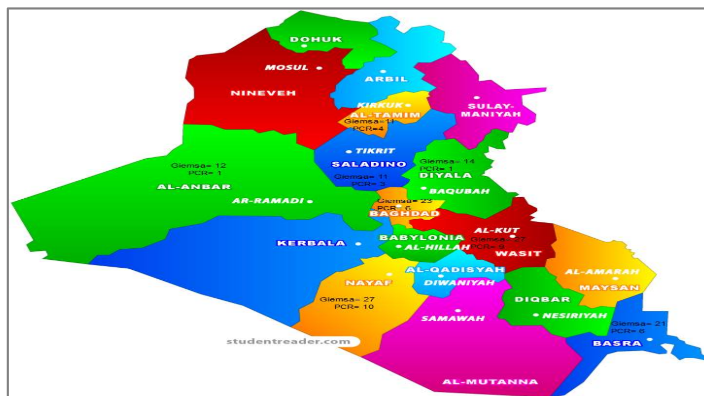

Figure 1. Cutaneous leishmaniosis detected by Giemsa stain in 768 subjects and by PCR in 160 of those same patients

RESEARCH ARTICLE

Profile of Malignant Disease in Wasit Province, Iraq

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ABSTRACT

Back ground: Blood cancers affect the production and function of blood cells. Most of these cancers start in bone marrow where blood is produced. Stem cells in bone marrow mature and develop into three types of blood cells: red blood cells, white blood cells, or platelets, In most blood cancers, the normal blood cell development process is interrupted by uncontrolled growth of an abnormal type of blood cell, These abnormal blood cells, or cancerous cells, prevent your blood from performing many of its functions, like fighting off infections or preventing serious bleeding.Objective: The main objective of this study was to determine the prevalence of hematological malignancy in pediatric age group in Iraq Material and method: In this cross section study we take age , sex, place, diagnosis and prognosis of the hematological malignancy of (110) patients . The data analyzed by using (SPSS 20).Results: In our study about the prevalence of hematological malignancy,one hundred ten patients with hematological malignancy were studied and we found that (66) patients (60%) complaint from (leukemia) Conclusion: leukemia is common hematological malignancy in Wasit. Iraq, and is common in male than female.

Keywords : Malignant disease, Leukemia, Wasit.

INTRODUCTION

Blood cancer is a term that's used to describe many different types of cancers which affect the blood, bone marrow or lymphatic system.[(1)] There are three main types of blood cancer: leukaemia, lymphoma, myeloma. Within these main groups, there are many different types of conditions - this is particularly the case for leukaemia and lymphoma, Most blood cancers start in the bone marrow, where blood is made. Many different types of blood cells are made in the bone marrow: broadly speaking,the type of blood cancer you have depends on the type of blood cell that's affected [(2)].

In most blood cancers, the affected blood cells stop developing in the normal way and become cancerous. This leads to you having either too many or not enough of the affected type of blood cell. These abnormal blood cells, or cancerous cells, stop your blood doing what it normally does, such as fighting off infections[3]. Tumors of the hematopoietic and lymphoid tissues or hematopoietic and lymphoid malignancies are tumors that affect the blood, bone marrow, lymph, and lymphatic system. As those elements are all intimately connected through both the circulatory system and the immune system, a disease affecting one will often affect the others as well, making myeloproliferation and lymphoproliferation (and thus the leukemias and the lymphomas) closely related and often overlapping problems [3]. An estimated 6000 new cases (3400 male and 2600 female) of acute lymphoblastic leukaemia (ALL) are diagnosed annually in the US[4]. Patients are predominantly children; approximately 60% of cases occur at age[5]. The survival rate of childhood ALL is approaching 90%[6-9]

While uncommon in solid tumors, chromosomal translocations are a common cause of these diseases. This commonly leads to a different approach in diagnosis and treatment of haematological malignancies [10]. Hematological malignancies may derive from either of the two major blood cell lineages: myeloid and lymphoid cell lines. The myeloid cell line normally produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells; the lymphoid cell line produces B, T, NK and plasma cells. Lymphomas, lymphocytic leukemias, and myeloma are from the lymphoid line, while acute and chronic myelogenous leukemia, myelodysplastic syndromes and myeloproliferative diseases are myeloid in origin[10].A subgroup of them are more severe and are known as haematological malignancies (American spelling hematological malignancies) or blood cancer. They may also be referred to as liquid tumors [10].

MATERIALS AND METHODS

This study was conducted in 110 patients complaint from hematological malignancy , 66 complaint from leukemia,9 wilm s tumor ,8 osteosarcoma ,6 NonHodgkin lymphoma ,4 Ewing sarcoma, 4 Rhabdosarcom ,4 neuroblastoma ,4 other ,attending from hematological center in wasit , de qar , mesan, karbala and dewanya .a questionnaire was designed by the researcher. Consent was taken from college of medicine / Wasit University. Information was collected from patients' records that maintained at the center and including age , sex, address ,type of hematological malignancy ,diagnosis and prognosis. Data were entered into Statistical Package for Social science (SPSS) program version 20 for Windows 7. Quantitative variables were summarized by finding mean and qualitative variables were summarized by finding frequency and percentage.

RESULTS

Table 1: show prevalence data for patients including sex male (62) (56.4%) female (48) (43.6%) ,age (64) (58.2%) child (46) (41.8%) young, prognosis (100)(90.9%) treatment (3)(2.7%) relapse (7)(6.4%) die, diagnosis (66)(60.0%) leukemia (4)(3.6%) neuroblastoma,(8)(7.3%) osteosarcoma (4)(3.6%) ewing sarcoma (6)(5.5%) non hodgkin lymphoma (9)(8.2%) wilms tumor (5) (4.5%) hodgkin lymphoma (4) (3.6%) rhabdomyosarcoma (4)(3.6%)others and place (52) (47.3%) wasit-center (46)(41.8%) wasit-rurle(7)(6.4%) de qar(2)(1.8%)mesan (2) (1.8%) dewanya(1)(0.9%) karbala.

Table 2 leukemia: sex (36)(56.3%) male (28)(43.8%) female , age (37) (57.8%)child (27)(42.2%)young , place (26)(40.6%) wasit-center (27)(42.2%) wasit-rurle (6)(9.4%) de qar (2)(3.1%) mesan (1)(1.6%) dewanya and karbala and prognosis (58)(90,6%) treatment (2)(3.1%) relapse (4)(6.3%) die. The studies in the world show that Acute lymphocytic leukemia the most common type of leukemia in young children age specific incident rate are highest in infant aged 0-4 years old and drop sharply through childhood and most common in male[11],and this similar to the result of our study about leukemia in Iraq.

Table 3 solid tumor: (24)(54.5%) male (20)(45.5%) female , age (27)(61.4%)child (17)(38.6%)young , place (24)(54.5%) wasit-center (19)(43.2%)wasit-rurle (1)(2.3%) de qar and prognosis (39)(88.6%) treatment (2)(4.5%) relapse (3)(6.8%) die. the studies in world show brain and spinal cord tumors are the second most common cancers in children, making up about 26% of childhood cancers. about 6% of childhood cancers are neuroblastomas. This type of cancer develops in infants and young children. It is rarely found in children older than 10 , Wilms tumor It is most often found in children about 3 to 4 years old, and is uncommon in children older than age 6, Hodgkin lymphoma accounts for about 3% of childhood cancers. It is more common, though, in early adulthood (age 15 to 40, usually people in their 20s) and late adulthood (after age 55). Hodgkin lymphoma is rare in children younger than 5 years of age , Non-Hodgkin lymphoma makes up about 5% of childhood cancers. It is more likely to occur in younger children than Hodgkin lymphoma, but it is still rare in children younger than 3 , Rhabdomyosarcoma This is the most common type of soft tissue sarcoma in children. It makes up about 3% of childhood cancers , Osteosarcoma is most common in teens , Ewing sarcoma is a less common type of bone cancer(12).

DISCUSSION

The most common hematological malignancy that occur in pediatric age group is leukemia that occur in male and child in Iraq and the same prevalence in us show about 245,000 people in the United States are affected with some form of leukemia, including those that have achieved remission or cure. Rates from 1975 to 2011 have increased by 0.7% per year among children (13). Approximately 44,270 new cases of leukemia were diagnosed in the year 2008 in the US(14). This represents 2.9% of all cancers (excluding simple basal cell and squamous cell skin cancers) in the United States, and 30.4% of all blood cancers(15) Among children with some form of cancer, about a third have a type of leukemia, most commonly acute lymphoblastic leukemia(14). A type of leukemia is the second most common form of cancer in infants (under the age of 12 months) and the most common form of cancer in older children(16). Boys are somewhat more likely to develop leukemia than girls, and white American children are almost twice as likely to develop leukemia than black American children(16). Only about 3% cancer diagnoses among adults are for leukemias, but because cancer is much more common among adults, more than 90% of all leukemias are diagnosed in adults(13).Another study in UK show Overall, leukaemia is the eleventh most common cancer in the UK (around 8,600 people were diagnosed with the disease in 2011), and it is the ninth most common cause of cancer death (around 4,800 people died in 2012)(17).

CONCLUSIONS

leukemia is common hematological malignancy in Wasit. Iraq, and is common in male than female and this corresponding to world study about the leukemia

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Table 1. Prevalence Data

variable		frequency	percentage
sex	male	62	56.4
	female	48	43.6
age	child	64	58.2
	young	46	41.8
prognosis	treatment	100	90.9
	relapsed	3	2.7
	die	7	6.4
diagnosis	leukemia	66	60.0
	neurofibromatosis	4	3.6
	osteosarcoma	8	7.3
	Ewing sarcoma	4	3.6
	Non hodgkin lymphoma	6	5.5
	Wilms tumer	9	8.2
	Hodgkin lymphoma	5	4.5
	rhabdomyosarcoma	4	3.6
	others	4	3.6
place	Wasit-center	52	47.3
	Wasit-rurle	46	41.3
	De qar	7	6.4
	mesan	2	1.8
	dewanya	2	1.8
	karbala	1	0.9

Table 2 leukemia

variable		frequency	percentage
sex	male	36	56.3
	female	28	43.8
age	child	37	57.8
	young	27	42.2
place	Wasit-center	26	40.6
	Wasit-rurel	27	42.2
	De qar	6	9.4
	mesan	2	3.1
	dewanya	1	1.6
	karbala	1	1.6
prognosis	treatment	58	90.6
	relapse	2	3.1
	die	4	6.3

Table 3. solid tumor

variable		frequency	percentage
sex	male	24	54.5
	female	20	45.5
age	child	27	61.4
	young	17	38.6
place	Wasit-center	24	54.5
	Wasit-rurle	19	43.2
	De qar	1	2.3
prognosis	treatment	39	88.6
	relapse	2	4.5
	die	3	6.8