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EDITORIAL BOARD - 2013

FORWARD

IJST is coming for you all today in its recent issue of volume eight for year 2013, as our deep belief in continuing the steps we began since eight years ago.

As what we intended to add valuable steps on our journey through IJST, one of the most successful achievement had been done during October 2013, ***when IJST owned an international indexed value issued by Indexed Copernicus International and scored ICV: 4.32,*** *which increased the prestigious level of the journal to the international scientific society, as Indexed Copernicus is considered one of the high standard level indexing organizations for scientific journals all around the world. We congratulate ourselves firstly for all the editorial board members who performed faithful efforts to IJST to raise its value among the scientific journals, and for the advisory groups who gave times and efforts to evaluate the researches published in IJST, and we congratulate ourselves for being here today with you all to celebrate this promising achievement, with a promise to continue what we began. Special thanks and appreciations must be presented to significant members whom joined and shared in our journey from the beginning until now, Dr. Abdullah Al- Shebani from Al- Kufa University, Dr. Atheer Al- Douri from Baghdad University, Prof. Teghreed Al- Noor from Ibn- Al- Haitham College for Education of Baghdad University, Prof. Jamal Abbas from Al- Kufa University, Dr. Hayder Abdul-Hameed from Baghdad University, and finally to Prof. Waleed Al- Murrani from Plymouth University – UK. Thanks for your efforts, deep beliefs in our scientific message to the Arab World, and your endless supports and encourageable words to IJST.*

It is my pleasure to welcome you and present you a new issue of our Journal, Volume 8, No. 4 (2013), the fourth issue of this year, with diversity of researches and elite experts of the Editorial Board and Advisory Group. The current issue comes to you while the World is celebrating a new year 2014 , which gives me an opportunity to send you all my deep wishes and faithful prays to Allah for peaceful times.

The members of Editorial Board, the ICAST and TSTC teamwork and I hope you will find this collection of research articles useful and informative.

IJST had the honor to welcome new editorial board members from Pakistan and India who will join the editorial board in 2014, which is another step toward the internationality of IJST.

Finally, on behalf of the International centre, I would like to express my special thanking to the Editorial Board Secretary for her faithful efforts in managing the scientific, design, technical and administrative aspects of the Journal and for preparing this issue for final printing and publishing.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

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ENGLISH SECTION

Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on some productive and physiological characteristics of heat stressed broiler

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ABSTRACT

Two stage-study was carried out at poultry station of Animal Resources Department, College of Agriculture at Kufa University from the 6th of September to the 20th of November 2010 as an attempt to reduce growth rate of birds in the early ages and release it later to get advantage of compensatory growth in comparison with supporting restricted diet of those birds with a mixture of vitamin C, salicylic acid and betaine and investigate the effect of these treatments on some productive and physiological characteristics of heat stressed broiler.

Two hundred twenty five unsexed one-day-old chicks of Cobb breed with average weight of 40g were used in the study. Birds were reared on ground and were randomly allocated into 3 treatments with 25 chicks per each. The 1st stage of the study was extended for 9-19 days of age for birds of control treatment and were offered free feeding until the end of the this stage and the whole study (T_1), while birds of treatment 2 and 3 were fastened for 10 hours a day from 8 a.m to 6 p.m until the end of this stage. The 2nd stage was extended for 20-35 days of age during which free feeding for birds in T_2 and T_3 was resumed and a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed was added to the diet of birds in T_3only.Heat and humidity inside house as an average were 26-36 C° and 60-70% respectively.

Results of the study were as follows:

1- Birds of T_3 during 2-5 weeks of age achieved significantly ($P<0.05$) higher average body weight (BW) and average weight gain (WG) as compared with those of other treatments.
2- Feed intake by birds of T_1during 2-5 weeks of age was significantly ($P<0.05$) higher as compared with those of T_2 and no differences were observed between birds of T_1vs. T_3 and T_2 vs. T_3.
3- Feed conversion ratio (FCR) of birds in T_2 and T_3during 2-5 weeks of age was significantly ($P<0.05$) higher as compared with those in T_1.
4- Dressing percentage (DP) of bird's carcasses in $T_3$was significantly ($P<0.05$) higher as compared with that of birds in T_1 and T_2 with no differences among treatments in carcasses main cuts except wings.
5- Abdominal fat in carcasses of birds in T_2 and T_3 was significantly ($P<0.05$) lower as compared with that of T_1birds and no difference was observed among treatments in interior eaten parts.
6- Lower ($P<0.05$) mortality rate was recorded in T_3 as compared with other treatments.
7- Lymphocytes (L) ratio was significantly ($P<0.05$) increased, whereas, Heterophil (H) and H/L ratios were significantly ($P<0.05$) decreased in blood samples withdrawn from T_2birds as compared with those of T_1.

Keywords: **broiler, salicylic acid, betaine, nutritional restriction regimen**

الملخص باللغة العربية

أجريت هذه الدراسة في حقل الطيور الداجنة / قسم الثروة الحيوانية / كلية الزراعة – جامعة الكوفة . نفذت التجربة الحقلية خلال المدة من 6 – ايلول و لغايـــة 20 تـــشرين الاول 2010 هدفت الدراسة الحالية الى محاولة تقليل سرعة النمو للافراخ في الاعمار المبكرة و اطلاق نموها بعد ذلك للحصول على النمو التعويضي و من ثم مقارنة هـــذه المعاملة مع المعاملة التي تم تدعيمها بخليط فيتامين C و السالسليك اسيد و البيتين بالعلف في بعض الصفات الانتاجية و الفسلجية لفروج اللحم المعرض للاجهـــاد الحـــراري. استخدم 225 فرخ فروج لحم غير مجنس بعمر يوم واحد سلالة Cobb و بمعدل وزن 42 غرام لطير, ربيت الافراخ على فرشة ارضية و قسمت عشوائيا بعمر يوم الى ثلاث معاملات بواقع ثلاث مكررات للمعاملة الواحدة شملت كل مكرر 25 فرخا, كانت المعاملات على مرحلتين , المرحلة الاولى من عمر 9 الى 19 يوم و تضمنت المعاملات T1 (السيطرة بدون أي اضافة و كانت التغذية حرة اما المعاملتين الثانية وا لثالثة T2 و T3 تضمنت قطع العلف لمدة 10 ساعات من الساعة (800) الى الساعة (1800). امـــا المرحلة الثانية من عمر 20 يوم لغاية 35 يوم حيث تم اضافة (خليط فيتامين C 100ملغم + بيتين 800 ملغم + ساسليك اسيد 50 ملغم)/ كغم علف للمعاملة الثالثة T3 فقط و كانت المعاملة T1 و T2 بدون اضافة. كان معدل درجات الحرارة في القاعة 26–36–26 مْ و الرطوبة النسبية (60–70%) و قد اظهرت نتائج التجربة ما يلي:

1– تفوقت معنويا ($أ<0.05$) معدلات وزن الجسم و كذلك معدلات الزيادة الوزنية التراكمية خلال المدة من عمر 2– 5 اسابيع لطيـــور المعاملـــة T3 مقارنـــة مـــع بـــاقي المعاملات.

2– تفوقت معنويا ($أ<0.05$) طيور المعاملة T1 على طيور المعاملة T2 في كمية العلف المستهلك التراكمية للفترة (2–5) اسبوع و لم يكن هناك فارق معنوي بين المعاملتين T1 و T3 و بين المعاملتين T2 و T3.

3– كانت كفاءة التحويل الغذائي التراكمية خلال فترة (2–5) اسبوع من العمر لطيور المعاملتين T2 و T3 افضل معنويا ($أ<0.05$) من طيور معاملة السيطرة T1.

4– حصل ارتفاع معنوي ($أ<0.05$) في نسبة التصافي لطيور المعاملة T3 مقارنة مع المعاملتين T1 و T2 و لم يكن هنالك فارق معنوي بين المعاملات في النسبة المئويـــة لقطيعات الذبيحة عدى الجناحين.

5– حصل انخفاض معنوي ($أ<0.05$) في دهن البطن لطيور المعاملتين T2 و T3 مقارنة مع طيور معاملة السيطرة و لم يكن هنالك فارق معنوي بين المعاملات للجـــزاء الداخلية المأكولة.

6– لوحظ انخفاض معنوي ($أ<0.05$) في نسبة الهلاكات لطيور المعاملة T3 مقارنة مع باقي المعاملات.

7– ارتفعت معنويا نسبة الخلايا اللمفية (L) و انخفضت معنويا نسبة الخلايا هيتروفيل (H) و نسبة H/L لطيور المعاملة T2 مقارنة مع طيور معاملة السيطرة T1.

INTRODUCTION

Recent traditional broiler breeds are characterized with fast growth and high FCR due to genetic selection efforts (1). However, this improvement in growth was negatively reflected on resistance of birds to diseases (2) leading to increase rate of mortality in these breeds due to high sensitivity to diseases, nutritional disorders and sudden death syndrome. Summers (3) and Gonzales *et. al*. (4) reported that pathologic cases increase in high growth flocks during first 3-4 weeks of age. Improvement in growth rate of broiler breeds caused an increase in fat deposition especially abdominal fat which is considered undesired and harmful for human health, therefore the objective of the current study is reducing growth rate of broiler chicks and trying to increase that rate later to get advantage of compensatory growth directly occurred after feed restricting period. It was proved that birds can compensate growth retard previously taken place when suitable conditions are existed (5-7). Since birds in the current study were reared in heat stress conditions, feed was supported with vitamin C, salicylic acid and betaine in order to bring about convenient circumstances and reduce the effect of stress that birds may exposed to through increased feed intake and subsequent increase in heat released from metabolism processes (8-10).

MATERIALS AND METHODS

This study was conducted with two stages at poultry station belonging to Animal Resources Department, College of Agriculture at Kufa University from the 6th of September to the 20th of November 2010**.** two hundred twenty five unsexed one-day-old broiler chicks of Cobb breed with average weight of 40g were used in the study. Birds were reared on 5 cm depth of sawdust litter. Feed and water were offered to birds *ad-libitum* using upside down feed and water plastic containers. 24-hours continuous light system was used throughout the study. Birds were weighed at age of 7 days and randomly allocated into 3 treatments of 3 replicates of 25 chicks of semi equal weight per each. 9 individual pens of 2×1.5 m^2.The 1st stage of the study was extended for 9-19 days of age during which birds of control treatment were offered free feeding until the end of the this stage and the whole study (T_1), while birds of treatment 2 and 3 were fastened for 10 hours a day from 8 at morning to 6 at evening until the end of this stage. The 2nd stage was extended for 20-35 days of age during which free feeding for birds in T_2 and T_3 was resumed and a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed was added to the diet of birds in T_3 only. Birds were fed starting ration from 1 to 19 days of age and finishing ration from 20 to 35 days of age. Table (1).

Table (1): composition of experimental diets

Ingredient	Starter diet (1-14day) %	Finisher diet (15-35day) %
Yellow corn	37.0	37.0
Wheat	22.0	30.0
Soybean meal	30.0	22.0
Protein concentration (1)	8.0	8.0
Sunflower oil	2.0	2.0
Limestone	0.7	0.7
Salt	0.3	0.3
Calculated chemical structure (2)		
Crude protein (%)	22.23	19.63
ME, K cal / kg feed	2958.90	3030.10
Lysine	1.2114	1.021
Methionine	0.445	0.407
Calcium	0.37	0.35
Available phosphorus	0.29	0.26

(1) Protein concentration used was Dutch. This concentration provided per Kg. 40% crud protein, 2100 ME, K cal/kg. 5% crud fat, 2% crud fiber, 5.6% calcium, 2.3% sodium, 4% available phosphorus, 3.85% Lysine, 3.7% methionine and 4% methionine + cystine. (2) Chemical structure was calculated according to the analysis of diamaterial in NRC (11).

House temperature was recorded triple times a day, 6 a.m, 12 p.m and 6 p.m using three thermometers located in the front, centre and back of house, where, periodically temperature during a day was 26-36-26. Relative humidity was recorded using hygrometer, where, it was 55-65%.

Average live body weight (BW), weight gain (WG), feed intake, feed conversion ratio (FCR) and rate of mortality were recorded at the end of 2, 3, 4 and 5 weeks of age and to the whole this period (2-5 weeks).

Dressing percentage (DP) was estimated according to live BW without interior eaten parts as described by (12) as follows:

DP = [weight of carcass without eaten interior eaten parts (g)/live BW (g)] × 100

Relative weight of carcass cuts (RWCC) were estimated as described by the same workers as follows:

RWCC = [weight of carcass cut (g)/weight of clean carcass (g)] × 100

Fat of interiors and round abdomen parts was descended, weighed and Relative weight of abdominal fat was estimated as equation mentioned above.

Regarding blood tests, blood samples were withdrawn from the wing vein of 3 males and 3 female's birds of each treatment 12 o'clock at noon the end of 5th week of age, and then withdrawn blood was placed in K-EDTA tubes. Whole blood was directly used to determine haemoglobin according to method described by (13), packed cell volume according to method described by (14), and differential white cell count according to method described by (15).

Data obtained was statistically analyzed according to the complete randomized design (CRD) procedure (16). Duncan's multiple range tests was used to determine the significance of differences between treatments means (17).

RESULTS AND DISCUSSION

Live body weight (LBW) and weight gain (WG)

Effect of early nutritional restriction regimen and supporting with a mixture of vitamin C, salicylic acid and betaine on LBW and WG for 2, 3, 4, 5 weeks of age and total period of 2-5 weeks is shown in table (2). LBW of birds in T_1 was significantly ($p<0.05$) superior as compared with that of T_2birds, no significant difference between LBW of birds in T_2 and T_3 at 3 weeks of age. This superiority may due to stop offering feed to birds in T_2 and T_3 for 10 hours a day during the 1st stage of the study. Teeter and Smith (18) demonstrated that there is a positive significant correlation (r = 0.89) between feed intake and growth rate of broiler. However, there were no significant differences in BW and total WG for total period of 2-5 weeks between birds in T_1 and T_2 though feed was not offered for 10 hours in T_2 for a period of 9-19 days. This result is agreed with that observed by other workers (19-21) who did not notice significant differences in BW between feed restriction and control treatments at marketing. This may attribute to completeness of compensatory growth occurred for birds of T_2. Proudfoot and Hulan (22) and proudfoot *et. al*. (23) reported that free feeding after feed restriction led to compensatory growth which improved growth rate and enlarged digestive tract. Results also showed that BW and WG achieved by birds of T_3 were significantly higher than those achieved by birds of T_1 and T_2. This may be referred to completeness of compensatory growth of T_3 birds that may be occurred due to role of body and alleviating heat stress of birds leading to increase feed intake and FCR. Two of additives added to feed of birds T_3 in the current study were tested in other studies. Skomura *et. al.* (24) and Zarei *et. al*. (25) observed that addition of betaine to diet of heat stressed birds increased BW and WG significantly. Significant improvement was occurred in BW due to addition of Vitamin C to diet and drinking water of heat stressed birds (26,27).

Feed intake and feed conversion ratio

Effect of different treatment on feed intake and feed conversion ratio (FCR) of broiler for 2, 3, 4, 5 weeks of age and total period are shown in table (3). There were no significant differences among treatments for 2 and 3 weeks in both feed intake and FCR, in week 5, birds of T_1 consumed significantly higher ($P<0.05$) feed as compared with those of T_2 and T_3, whereas, feed intake for total period (2-5) weeks by birds in T_2 was significantly decreased as compared with that of T_1 birds, this may attribute to the effect of 10 hours a day starving in the 1st stage of the study. Whereas feed intake was not significantly differed between birds in T_2 and T_3 and between birds in T_1 and T_3, this may attribute to the effect of a mixture added to the diet of birds in T_3 which may help in reducing body heat in heat stress condition leading to increase feed intake to compensate reduction occurred due to fasting procedure. Three compounds were found to be effective in reducing body heat and increased feed intake, vitamin C(28); aspirin (29) and betaine (10). Birds of T_3 were achieved significantly higher ($P<0.05$) FCR for 4th and 5th weeks than that in T_2 and control treatment (T_1). However, for total period from 2-5 weeks birds of both T_2 and T_3achieved significant ($P<0.05$) improvement in FCR as compared with control treatment. This improvement is in agreement with finding of (30, 20, 21).

Percentage of carcass cuts and dressing percentage

Effect of different treatment on dressing percentage and carcass's cuts percentages in 5th week of age are shown in table (4). Results revealed that the addition of mixture of vitamin C, salicylic acid and betaine significantly ($P<0.05$) increased dressing percentage of birds in T_3 as compared with that of birds in T_1 and T_2. Lohakara *et. al.* (26) reported that addition of vitamin C to diets of Ross heat stressed broiler improved dressing percentage. Similar result was observed by (31) with addition of salicylic acid. Addition of betaine improved carcass yield of broiler at 42nd of age (32,33). Results of carcass quality also showed that wings weight of birds in control treatment was significantly higher than that of birds in T_2 and T_3. Moreover, percentage of carcass cuts was not differed significantly among treatments.

Percentage weight of abdominal fat and interior parts

Effect of different treatment on percentage weight of abdominal fat and interior eaten and uneaten parts are shown in table (5). Results revealed that percentage weight of abdominal fat of birds in T_2 and T_3 was significantly ($P<0.05$) decreased as compared with that of birds in control treatment. Similarly, (30, 20) and (21) noticed that early feed restriction treatment significantly decreased percentage weight of abdominal weight at marketing age.

Results of the current study also showed that percentage weight of glandular stomach was significantly ($P<0.05$)higher in carcasses of birds in T_2 and T_3 as compared with that of birds in T1. However, no significant differences were shown in the ratio of weight of interior to body weight among treatments.

Table (2): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on live body weight and weight gain of heat stressed broiler

Treatments	Items	1	2	3	4	5	Total (2-5)Weeks
Average live BW (g/bird)	T_1	160±3.1 a	388±1.2 a	763±9.3a	1230±8.4 b	1873±7.2 b	1873±7.9 a
	T_2	165±4.2 a	375±5.1a	716±6.2b	1215±7.3 b	1903±8.4 b	1903±7.3 b
	T_3	163±1.2 a	365±3.2a	746±7.3 ab	1282±3.2 a	1978±3.2 a	1978±5.3 a
Level of significance		**	**	**	*	NS	NS
Average WG (g/bird)	T_1	-	228±8.2a	375±6.3a	467±8.3 b	643±7.3 b	1713±2.9 b
	T_2	-	210±5.3a	341±7.2a	499±7.2 a	688±4.3 a	1738±3.2 b
	T_3	-	202±6.2a	381±6.9a	536±7.9 a	696±8.8 a	1815±6.5 a
Level of significance		-	NS	NS	*	*	*

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively
T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively.

Table (3): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on feed intake and feed conversion ratio of heat stressed broiler

Treatments	Items	2	3	4	5	Total (2-5)Weeks
Average feed intake (g/bird)(g/week)	T_1	385±14.7 a	525±8.9 a	900±3.1a	1300±3.1 a	3110±3.1 a
	T_2	340±13.9 a	470±7.2 a	965±3.1 a	1226±3.1 b	3001±3.1 b
	T_3	340±9.2a	519±5.6 a	960±3.1 a	1235±3.1b	3054±3.1 ab
Level of significance		NS	NS	NS	*	*
Feed conversion ratio (g/bird) (g/g weight gain)	T_1	1.69±0.10 a	1.40±3.1 a	1.92±3.1 a	2.02±3.1a	1.76±3.1 a
	T_2	1.62±0.08 a	1.38±3.1a	1.93±3.1 a	1.78±3.1 a	1.68±3.1 b
	T_3	1.68±0.03 a	1.36±3.1a	1.79±3.1 b	1.77±3.1 b	1.65±3.1 b
Level of significance		NS	NS	*	*	*

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively
T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively.

Table (4): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on carcass cuts percentage and dressing percentage of heat stressed broiler at 5th weeks of age

Treatments	Percentage of carcass cuts					Dressing percentage without interior eaten parts
	Neck	Back	Wings	Thigh	Chest	
T_1	34.45±3.20	4.30±29.6	10.2±0.20a	20.4±1.20	0.30±5.75	68.9±0.33 b
T_2	34.84±3.20	28.78±2.30	9.1±0.35 b	21.2±1.45	6.10±0.19	67.8±0.19 b
T_3	34.6±2.20	30.0±1.90	0.36±9.1 b	20.0±1.60	5.5±0.20	0.25±70.5 a
Level of significance	NS	NS	*	NS	NS	*

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively
T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively.

Table (5): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on percentage weight of abdominal fat and interior eaten and uneaten parts in carcasses of heat stressed broiler at 5th weeks of age

Treatments	Percentage weight of interior parts						
	abdominal fat	Heart	Liver	Hazard	Glandular stomach	Fabrishia	Spleen
T_1	3.20±0.10 a	0.64±0.03	2.80±0.15	b0.12±1.85	0.33± 0.01b	0.11±0.01	0.16±0.01
T_2	2.65±0.08 b	0.68±0.03	2.65±0.2	1.91±0.1	0.04±0.53 a	0.13±0.01	0.03±0.19
T_3	2.66±0.07 b	0.61±0.06	2.66±0.09	1.80±0.13	0.45±0.05 a	0.11±0.01	0.22±0.02
Level of significance	*	NS	NS	NS	*	NS	NS

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively
T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively

Mortality ratio

Effect of different treatment on mortality ratio (MR) is shown in table (6). Results showed that lower significant reduction in total MR was recorded in birds of T_3 as compared with that in birds of T_1 and T_2. Lower significant reduction was also recorded in T_2 as compared with T_1. Similar results were noticed by (34, 6 and 21) who indicated the significant effect of feed restricting on MR. The significant reduction in MR of T_2 birds may be occurred due to early nutritional restriction for 9-19 days of age and its effect in reducing exposure of birds to heat stress by increasing feed intake and growth rate during this period and preventing mortality due to skeleton diseases and sudden death syndrome. The positive effect of nutritional restriction and its role in alleviating heat stress together with addition of experimental mixture was the reason for the significant reduction in MR of T_3 as compared with that of T_1 and T_2. This truth can be supported by the significant reduction in Lymphocytes (L) to Heterophil (H) ratio of birds in T_2 and T_3 as compared with that of birds in control treatment (T_1). H/L was 0.41, 0.35 and 0.31 for T_1, T_2 and T_3 respectively (table 7). Zulkifil *et. al.* (35) and Turkyilmaz (36) reported that H/L ratio increased under condition of heat stress, and then this ratio can be used as evidence for environmental stress.

Blood characteristics

Effect of different treatment on size of blood packed cell volume (PCV), hemoglobin concentration (Hb),percentage of Lymphocytes (L), Heterophil(H) and H/L ratio of blood samples withdrawn from heat stressed broiler at 5th weeks of age are shown in table (7). Results showed that PCV, Hb concentration were not significantly differed among treatments, whereas, percentage of H cell and H/L ratio in blood samples withdrawn from birds of T_3were significantly ($P<0.05$)lower than that in blood samples withdrawn from birds of control treatment accompanied with significant ($P<0.05$) increase in percentage of L cell.

The significant decrease in the percentage of L cell in blood samples withdrawn from birds of T_1as compared to that in blood samples withdrawn from birds of T_3 can be explained by the heat stress that birds of this treatment had exposed to leading to increase secretion of corticoid gland hormones that caused death and lysis L cells leading to reduce the percentage of these cells (37). Significant increase in H/L of blood samples withdrawn from birds of T3 can be considered as evidence for exposure birds of this treatment to chronic heat stress. Davis *et. al*. (38); Turkyilmaz (36) and Zulkifli *et. al.* (353) pointed out that exposure to heat stress increase H/L ratio and supported using it as evidence for presence of environmental stress. Significant reduction in this ratio on blood samples withdrawn from birds of T_3 can be explained by reducing body temperature of birds due to addition of vitamin C, salicylic acid and betaine leading to alleviate heat stress that birds exposed to. Zulkifli *et. al.* (35) and Attia *et. al.* (9) demonstrated that addition of betaine to broiler diets had a conclusive role in reducing body temperature. Moreover, it was observed that body temperature of heat stressed broiler was significantly decreased by the addition of vitamin C with drinking water and diet (9,39). Kafi (31) found that addition of vitamin C and salicylic acid to drinking water offered to heat stressed broiler significantly decreased body temperature.

According to the previously mentioned findings in consistent with the results of the current study, role of addition of mixture in reducing body temperature of birds in T_3 becomes very clear leading to reduce H/L ratio as compared to that of control treatment (0.31 vs. 041).

Table (6): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on mortality ratio of heat stressed broiler at 1-5 weeks of age

Treatments	Mortality % a week					Total mortality
	1	2	3	4	5	
T_1	-	-	-	1.33±0.03 a	0.09± 2.66 a	3.99±0.08 a
T_2	-	-	-	-	1.33±0.02 a	1.33±0.04 b
T_3	-	-	-	-	-	0.0±0.0 b
Level of significance	NS	NS	NS	*	*	*

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively

T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively

Table (7): Effect of early nutritional restriction regimen and supporting with mixture of vitamin C, salicylic acid and betaine on size of blood packed cell volume (PCV), hemoglobin concentration (Hb),percentage of Lymphocytes (L), Heterophil (H) and H/L ratio of blood samples withdrawn from heat stressed broiler at 5th weeks of age

Treatments	PCV (%)	Hb g/100 ml	H cells (%)	L cells (%)	H/L ratio
T_1	30.20± 0.45	10.20±0.55	26.60±1.15 a	65.10±1.45 b	0.41±0.01
T_2	30.90±0.30	10.80±0.40	23.45±0.88 ab	67.15±1.52 ab	0.35 b
T_3	31.20±0.25	11.10±0.30	21.50±0.70 b	96.25±1.20 a	0.31±0.02
Level of significance	NS	NS	*	*	**

* and ** different letters vertically refer to significant differences at 0.05 and 0.01 respectively

T_1, T_2 and T3 represented treatments of control, 10 hours a day fasting for 9-19 days of age followed by free feeding without and with the addition of a mixture containing 100 mg of vitamin C and 50 mg of salicylic acid and 800 mg of betaine per 1 kg of feed respectively.

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Biological treatment of organ chlorinated pesticide using local bacterial isolates

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ABSTRACT

The aim of this research was to isolate microbial isolates with ability of growth in medium with presence of added pesticide to be used for bioremiation of pesticides contaminated sites (Soil and water).
Bacterial groups, which capable of degradation of chlorinated organic pesticides were isolated from many agricultural soil and contaminated water.
After purification the strains were assessed in order to discover their ability to degrade (COP) (α propachlor) with concentration of 25mg/L as carbon source in mineral medium and in rich medium. However, the best three pure strains were able to grow in M.M includes (COP) without enrichment.
This group which was composed of three isolates characterized based on their morphological and biochemical characteristic. The isolates were presumptively identified as rhodococcus spp. and *Streptomyces albus.* Using growth curve as a parameter of (COP) compounds, optimum conditions (Pesticide concentration, temperature, and time) of selected degrading bacterial strains were studied. Results indicated that the optimal temperature was 37°C for all isolates, with best growth at 100mg/L of propachlor by *str. albus,* in addition of its efficiency to degrade the compound during 48 hr at incubation.

Key words: biotreatment, bacteria, pesticides, soil pollutants.

الملخص باللغة العربية

يهدف البحث الحالي الى عزل أحياء مجهرية محلية لها القابلية على النمو في وسط غذائي يحوي على المبيدات كمصدر كربوني, وبهذا تكون هذه العزلات قادرة على تفكيك وأزالة المبيدات من المناطق الملوثة بها سواء كانت تربة أو مياه. تم العثور على مجموعة من العزلات البكترية التي تم عزلها من نماذج تربة ومياه مختلفة و ملوثة بالمبيدات, حيث لم يتم الحصول على اي نوع من الفطريات أو الخمائر أثناء العزل. نقيت هذه العزلات البكترية , ودرست قابليتها على تفكيك المبيد المستخدم في هذه التجربة وهو (propachlor)،في وسط الأملاح المعدنية بألاضافة الى أعادة التجربة بأستخدام وسط غني بمصدر كربوني اخر. تمكنت ثلاث عزلات فقط من النمو بصورة جيدة في وسط الأملاح الحاوي على المبيد كمصدر كربوني وحيد و لم تتمكن العزلات الأخرى من النمو الابصورة ضعيفة او بوجود مصدر كربوني آخر يشجع النمو. شخصت العزلات بدراسة خواصها المظهرية وبعض الفحوصات البيوكيميائية , ووجد ان اثنان منها تعود للجنس *Rhodococcus* , اما العزلة الأخرى فهي تعود للنوع : *Streptomyces aslbus* . درست الظروف المثلى (درجة الحرارة , الوقت , تركيز المبيد)للعزلات الثلاثة , حيث لوحظ ان درجة الحرارة 37م° هي المفضلة للعزلات الثلاثة مع نمو افضل للعزلة للعزلتين Rho1 و *Streptomyces albus* خلال ال48 ساعة الأولى للحضانة بالاضافة الى قابلية الاخيرة على النمو بكفاءة اعلى عند التركيز للمبيد البالغ 100mg/l .

INTRODUCTION

The class of natural and synthetic chemical compound called pesticides holds a very important place in agriculture and economics sati factory crop yields are impossible without the use of pesticides despite the public an awareness that now exists about harmful effects of pesticides use (1). Many chlorinated pesticides have been banded for use because of their short and long toxicity, carcinogenicity, and environmental persistence. Despite the fact that most of these chlorinated pesticides are now illegal to use, organ chlorines are still potential source of pesticides poisoning (2,3).

The primary goal of our study was to develop an approach for enhancing pesticide degradation using microorganisms. Isolation of indigenous bacteria capable of metabolizing chlorinated organ pesticide has received considerable attention because these bacteria provide an environmentally friendly method by detoxification. Biodegradation is an economic friendly, cost effective, high efficient approach and can be considered a superior alternative to physical and chemical methods, which are not only technically laborious and costly; also are not sufficient to completely degrade organic toxins.

Complete biodegradation of pesticides involve the oxidation of parent compound to form carbon dioxide and water. This process provides both carbon and energy of the growth and reproduction of microbes if appropriate microorganism is absent in soil or if biodegrading microbial population has been reduced due to toxicity of pesticide in that case a specific microorganism can added or introduced in soil to enhance the activity of the existing population (4).The use of the bacteria for the degradation and detoxification of numerous toxic chemical such as pesticides is an effective tool to decontaminate the Polluted sites (5). Degradation by microbes depends not only on the presence of degradative enzymes, but also a wide range of environmental parameters; temperature, pH, water potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factor for pesticide degrading microorganisms, which requires further exploration and their biochemical activities (4). A divers group of bacteria, including members of the genera Alcaligenes, flavobacterium, Pseudomonas, and Rhodococcus, metabolize pesticides. (1,6). Actinomycetes have considerable potential for the biotransformation and biodegradation of pesticides (1). Figure (1).

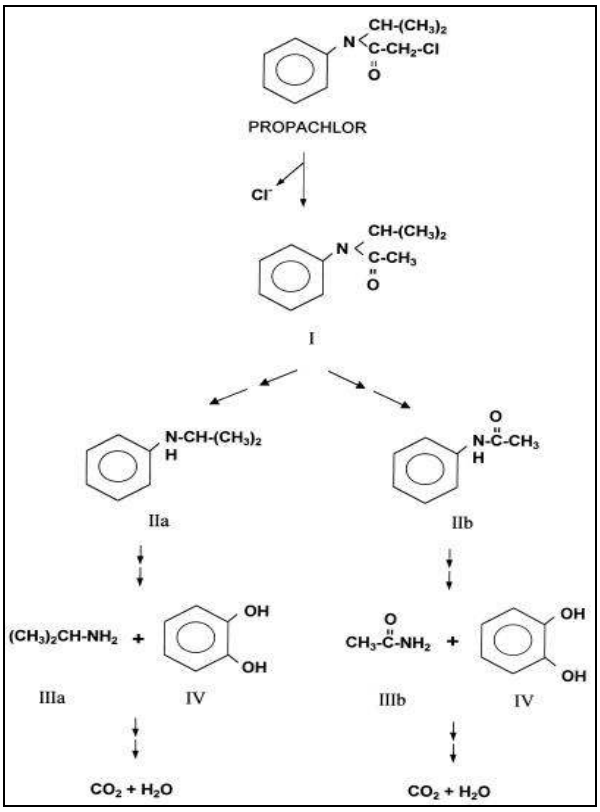

Figure (1): Schematic pathways proposed for the degradation of propachlor (7)

Co- metabolism is the ability of microorganisms to catalyze transformation or partial degradation of compound that do not support their growth. It is probably the most wide spread mechanism for pesticide degradation (8). Complete mineralization of chemical is more likely to occur in mixed populations than with single microorganisms (9).

MATERIALS AND METHODS

Soil sampling

Four soil samples were collected from potato and cornfield in some Baghdad's farms, which had been treated with pesticides twice a year for the previous 30 years and contaminated water from Tigris and Diyala Rivers.

Preparation of inoculums

One gram of soil was suspended in 5ml of sterile (Mineral Medium) and this suspension was considered the inoculums .The MM had the following composition (per liter): 0.2g KH_2PO_4, 0.5g K2HPO4 (Sterilized separately at 125°C per 5 min. and added to the rest of the salts; 1g $(NH_4)SO_4$;0.2g $MgSO_4.7H_2O$;0.2g NaCl; 0.05g $CaCl_2.2H_2O$; 0.025g $FeSO_4.H_2O$; 0.005g Na_2MoO_4; 0.0005g $MnSO_4$(pH7±0.3).(9), were supplemented with 25mg/ L of (propachlor) as

the carbon source with or without Yeast extract (0.1%) in duplicate for each. (10).

Water samples preparation:

One milliliter of water sample was added to 99 ml of D.W., mixed by vortexing and regard as inoculums (11).

Pesticide sampling:

One gram (gm) of Pesticide (propachlor) was weighted and filtrated after added to 25ml of methanol using (Millipore membrane, pore size 0.25mm) the methanol was evaporated to dryness (12).

Isolation of microorganisms:

Hundred milliliters of sabaroud broth for fungi isolation and similar flask contain L. Broth with 50 µg/L of antifungal (cycloheximide) for isolation of bacteria, supplemented with 25 mg/L of propachlor (in duplicate) and inculcate with 0.2 ml of each contaminated sample and incubated in shaker incubator at 37 ºc with 15 rpm for 7 days (13).

Evaluation of Bacterial growth

We carried out the growth and degradation experiment with the pure bacterial colonies using flasks with 250 ml containing 100 ml of (MM) Mineral Medium supplemented with series of pesticide dose and incubation at different temperature.
Cell growth was measured used spectrophotometer at O.D 600 and CFU/ml. After preparing of serial fold dilution of liquid culture in D.W, cell growth of Streptomyces was measured by CFU/ml only**.**

Characterization of isolates

The morphology of bacterial isolates was determined characterized by some biochemical tests(14).

RESULTS AND DISCUSSION

Three soil samples, representing different agricultural soil and tow contaminated water samples, were using as inoculums to isolate microorganisms capable of utilization type of chlorinated organ pesticides(propachlor).
After culturing samples in special media. there were no observed fungal isolates. There was five bacterial isolates in addition of tow strains of Actionmyces, which were resistant at 25mg/L of pesticide).

Table (1) reveals the bacterial efficiency growing on mm media with or without carbon source; and shows that three of the isolates Rh1spp, Rh2 spp and Strept1, shows good growth without addition of carbon source for enrichment. It can be observed that when the bacteria were cultured in presence of a rich medium, Including a carbon source (0.1%of yeast extract), capacity of degradation will be increase.

Table (1): Bacteria efficiency of growing on MM (with or without carbon source)

Bacteria Isolates	MM. without carbon source	MM with carbon source
Flav. 1	6×10^4	7×10^6
Flav . 2	2×10^4	2×10^6
Rho1	2×10^6	8×10^7
Rho2	2×10^6	5×10^7
Rho3	3×10^5	3×10^6
Spp1	5×10^6	8×10^7
Spp2	4×10^5	6×10^6

The physiological base for co- metabolism is not well known, but the most accepted hypothesis is related to the specificity of enzymes(15).
Table (2) shows the various phenotypical characteristic of the selected isolates. They were presumptively identified as *Rhodococcus* spp. and *Streptomyces albus.*

Table (2): Characterization of Bacteria Isolates

Isolates	Type of test	Result
Streptomyces albus	Chain of spores	+
	Gram stain	G+ve
	Growth at 40º	+
	White aerial mycelium	+
	Starch utilizing	+
	Formation of organic acids	+
Rhodococcus 1& Rhodococcus 2	Gram stain	G+ve
	Colony texture	Smooth , pigmented, orange color
	Casein degradation	+
	Ramose fermentation	-
	Growth at crystal violate 0.0001gm/l	+

These strains are interesting since it has rarely been isolated from clinical samples and therefore its pathogencity is low. Optimum conditions of selected isolates were studied, including (time, temperature and concentration of pesticide). Figure (2), shows the efficiency of *Streptomyces albus* and *Rho1*, through their high growth during first 48hr, in the presence of pesticide.

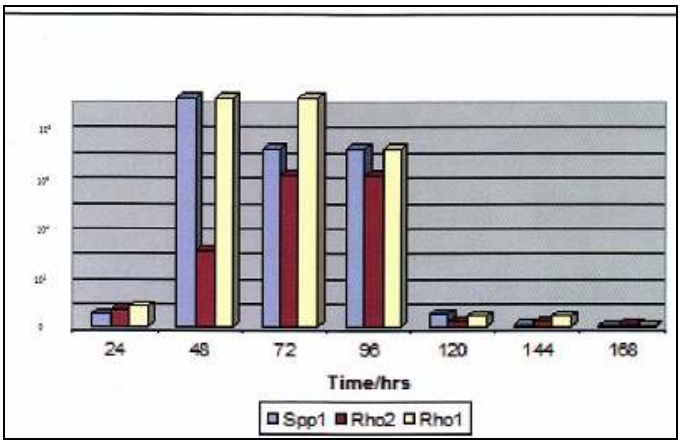

Figure (2):growth rate of bacterial strain in oresence of 25mg/l of pesticides during 168 hrs.

Figure (3) shows results obtained from measuring growth rate of the bacterial culture, it can be seen that is the most growth in comparison to other strains on MM containing 100mg/L of pesticide, belong to Strept1 isolate. Strains showed the best growth at 37ºC without significant differences between them. Figure.(4). It would be useful to test these strains with other organic pesticides in order to find catalytic activity that might make it recommendable treatment of wastes or polluted environment, with a low potential effect public health. It has been observed that shortly after repeated applications of degradable pesticide, the soil become richer in bacterial populations, which are capable of degradation.

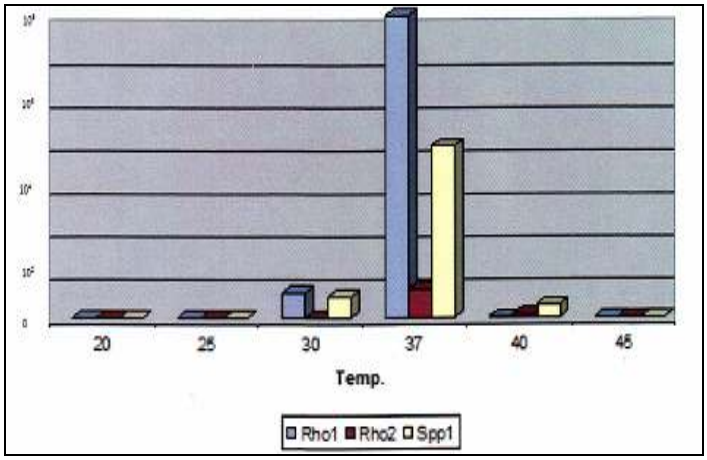

Figure (3): growth rate of bacterial strain in MM with pesticide at different tempertatures after 72 hrs.

So it would dramatically reduce the effectiveness of subsequent pesticide applications (15).

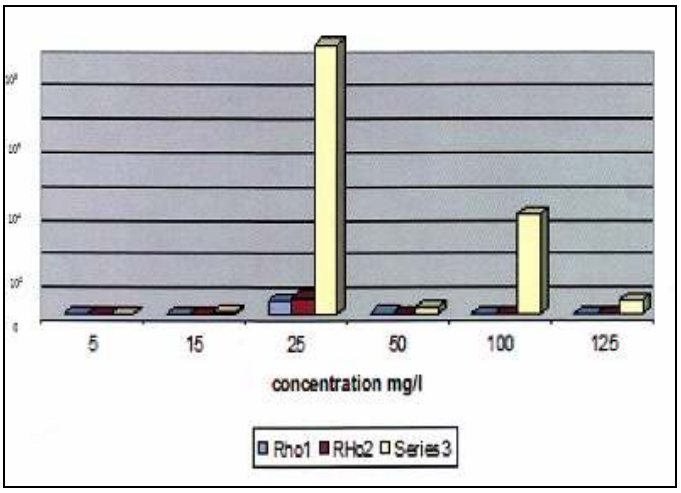

Figure (4): growth rate of bacterial strain in MM with pesticide after 72 hrs. on different concentrations of chlornade

This study concludes that the isolated microbes can be used for biodegradation and bioremediation of pesticides contaminated soil or water. The results also suggested that is the co metabolism increase the ability of bacterial utilizing of pesticide, so we can culture the strains as a consortium, several of bacteria interfering processes, so there is no need to add nutrients.
Environmental conditions, soil pH, agricultural management of pesticides added are important factor for bacterial use of xenobiotic compounds (such as pesticides) as a growth substrate.

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Expression in *Escherichia coli* of a single-chain variable-fragment (scFv) against the amino acid motif DELLA of plant transcription factor is toxic to *E. coli*

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ABSTRACT

The aim of the current study was to generate scFv that targeted a DELLA motif of DELLA proteins by immunization with a synthetic peptide- KLH conjugate. Expression level of scFv by *E. coli* was low due to toxicity. However, the mechanism of toxicity caused by scFv over expression may not indicate a single target. We used *P. pastoris* system as alternative, but we found that, the purified scFvs, unable to interact with their target (*At*) RGA recombinant protein. The experimental results suggested that, the cellular toxicity in *E.coli* was due to binding of scFv with host's protein. None of the irrelevant 8g8 scFv and an irrelevant MAB anti-CIN 1 protein interacted with hosts proteins, or influenced the viability of the cells. It is desirable to do a protein blast search for presence of the peptide in the expression host in case scFv needed to be obtained from MAB generated from immunization with that synthetic peptide from highly conserved domain of a protein in addition to investigation of the reactivity of MAb to hosts cells.

Key words: DELLA proteins , (*At*) RGA, synthetic peptide- KLH conjugate, scFv *, P. pastoris*, *Escherichia coli, Venturia inaequalis, Arabidopsis thaliana*

الملخص باللغة العربية

تم توليد اس سي اف في (ScFv) التي لها القدره على الاتحاد مع الجزء من الاحماض الامينيه دي ل ل ي (DELLA) لانواع من البروتينات الحاويه عاى الجزء من الاحماض الامينيه دي ل ل ي (DELLA) وذلك عن طريق التحصين بستخدام الببتايد المركبه كي ال امKLM) ٠ .(لقد كان مستوى الانتاج لل اس سي اف في (ScFv)في المضيف منخض جداً نتيجت الذيفيه لل اس سي اف في (ScFv) للمظيف اشريكيا كولي. (E.coli) ان اليه الذيفيه التي سببتها اس سي اف في (ScFv)قد لا تدل على آليه واحده . وعليه فلقد تم استخدام مظيف اخر هي بيشيبا باستورس (Patia pastoris) كبديل٠ لقــد وجــدنا ال اس ســي اف فــي (ScFv)المنتجه غير فعاله وليست لها القدره على التفاعل مع البروتينات الحاويه عاى الاحماض الامينيه دي ل ل ي . (DELLA) ان هذه النتائج تدل مــن ان الذيفيه لخلايا اشريكيبا كولاي (E.coli) بسبب اتحاد ال اس سي اف في (ScFv)مع البروتينات الحاويه عاى دي ل ل ي (DELLA) للمظيف. لم يحصل هــذا الاتحاد باسستخدام انواع اخرى من ال اس سي اف في (ScFv) المحضره ضد بروتينات لا تحوي الاحماض الامينيه دي ل ل ي (DELLA) ومثال هو الاجسام الظاده من وحيده الخليه (MAB) والمحظره ضد . CIN 1 ان هذا الاتحاد لم يتم مع بروتينات المضيف. بناء عاى ذلك فمن الافظل اجراء بحث لمعرفــه وجــود الببتايد في المضيف المنتج لل اس سي اف (ScFv) والتي تكون الحاجه الى الحصول لاضداد نوعيه من التحصين باستخدام ببتايد مركبه مختبريا و هذه الببتايد هي جزء محفوظ من البروتين اضافه الى بحث النشاط للاضداد التي تم االحصول عليها(MAB) .

INTRODUCTION

The DELLA family of plant proteins are named after the DELLA amino acid motif in the N-terminal domain and are part of a larger family of plant transcription factors named the GRAS family (1) that contain a variable N-domain but a highly conserved C-domain. Five DELLA proteins have been identified in *Arabidopsis thaliana* (2-4). The DELLA proteins act to depress plant growth by repression of genes required for cell elongation and differentiation (5). RGA is involved mostly in the repression of juvenile growth and phase change (6,7) and together with minor contribution from RGL-1 and RGL-2 to control the transition of shoot apical meristem to infloresence meristem (8,9). These authors also confirmed that RGL-2, together with some contribution from RGA, GAI and RGL-1, is the prominent germination repressor and that RGA and RGL-2 are predominant but RGL-1 has a role in controlling floral organ growth. To provide insight into the localization and function of DELLA proteins, we generated a single-chain antibody for use in immunolocalization, biochemical, and bioassay studies. Single-chain variable antibody fragments carry the complete antigen-binding site and have a monomeric structure that remains stable even at low concentration and physiological temperature (10).

Heterologous expression in various bacterial systems can allow the production of suitable amounts of eukaryotic proteins. *E. coli* remain the host of choice for recombinant protein expression. Its culture is simple, fast, inexpensive, and highly efficient. Unfortunately, toxic genes severely interfere with the physiology of *E. coli*. As a result, expression yields are dramatically diminished, and sometimes abolished (11).

In this work, we found that, over-expression of 6c8 scFv is toxic to *E. coli.* The experimental results suggested that, the cellular toxicity in *E.coli* was due to binding of scFv with host's protein. None of the irrelevant 8g8 scFv (a derivative of a murine Mab which recognizes an azinphos-methyl insecticide) and an irrelevant Mab anti-CIN 1 protein (Mab that recognize a *Venturia inaequlis* EST's Cin 1 protein), interacted with *E. coli* cells.

The yeast *P. pastoris* provides several advantages as a heterologous protein expression system (12). The strong, tightly regulated, and inducible promoter of the alcohol oxidase I gene, AOX1, (13) is generally employed, since the product of genes directed by it can constitute up to 30% of total cell proteins (14). Finally, it releases comparatively few endogenous proteins into the culture medium while secreting large amounts of recombinant protein; this being a major advantage for subsequent protein purification (15,16). We used *P. pastoris* clone with a Mut^S phenotype for expression of the 6c8 scFv and 5e1scFv. We found that, the purified scFvs, were unable to interact with their target (*At*) RGA recombinant protein.

MATERIALS AND METHODS

Bacterial strains and plasmids

E. coli strains DH5α were used as a host for cloning and *E. coli* BL21 (DE3) were used as a host for protein expression. Recombinant DNA techniques were performed according to established protocols as compiled by (17). Plasmids were digested using *Sfi*I (New England Biolabs) and gel purified. The scFv fragment was ligated into previously digested pAK 300 plasmids using T4 DNA ligase (Promega) according to the manufacturer's recommendations, at a vector to insert molar ratio of 2:1. Plasmids were transformed into expression strain *E. coli* BL21 (DE3) using chloramphenicol to select for recombinant bacteria.

Expression of scFv fragments in *E. Coli*

For expression of scFv antibody fragments, 500 ml of LB medium, supplemented with 25 µg/ml Chloramphenicol, was inoculated with an overnight culture of *E. coli* BL21 (DE3) harbouring the expression plasmid pAK 300 containing the scFv insert. This was incubated at 37 °C with shaking (200 RPM). Expression was induced when $O.D._{600\ nm}$ reached 1.0–1.2 with 0.3 mM isopropylthiogalactopyranoside (IPTG). After 90 min of incubation at 37 °C, Cells from 5 ml culture was collected by centrifugation to analyze for solubility of the fusion protein

resuspended in a lysis buffer (20 mM Tris, pH 7.4, 100 mM NaCl, 0.1 mM phenyl methyl sulfonyl fluoride (PMSF), 1 mM EDTA, and 0.5 mM dithiothreitol) and frozen at -20 °C. The bacteria were thawed and lysed by sonication on ice. The bacterial lysates were subjected to centrifugation to analyze for solubility of the fusion protein.

For purification of the scFv, Tris–sucrose–dithiothreitol (TSD) hypertonic buffer method was used as follows. Cells were washed three times in 20 mM Tris-HCl, (pH 7.5) before being resuspended in 2 ml of the hypertonic buffer containing 100 mM Tris–HCl, pH 8, 30% sucrose and 1 mM dithiothreitol (TSD). The cell suspension was incubated on ice for 10 min. After centrifugation at 12,000 x *g* for 10 min at 4 °C, the cell-pellet was resuspended gently into

2 ml of deionized sterile water and incubated on ice for an additional 10 min. Cells were then pelleted at 12,000 x *g* for 10 min at 4 °C and the supernatant was removed and labeled as periplasmic fraction. Periplasmic and cell-pellet fractions from each treatment were analyzed by 12% w/v sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot. Purification of scFv was carried out by using MAb D9 (Anti-R-tag antibody) affinity chromatography as described previously (18).

Yeast strain and plasmids

P. pastoris strain KM71H (Mut^S) and the pPICZαC expression vector were purchased from Invitrogen. Yeast extract–peptone–dextrose medium (YPD) contained 2 % peptone, 1 % yeast extract, and 2 % dextrose, whereas YPDS was supplemented with 1.0 M sorbitol. Zeocin was added to a final concentration of 100 µg / mL. The buffered minimal glycerol-complex medium (BMGY) was prepared with 2 % peptone, 1 % yeast extract, 1 % glycerol, 1.34 % yeast nitrogen base with ammonium sulphate but without amino acids, and 4×10^{-5} % biotin in 100 mM potassium phosphate buffer. The phosphate buffer was adjusted to pH 6.0. The buffered minimal methanol-complex medium (BMMY) was the same as BMGY, except 0.5 % methanol replaced glycerol.

Plasmid modification

The vector used in the production of scFv molecules in a *P. pastoris* expression / secretion system was derived from plasmid pPICZαC (Invitrogen). The expression cassette is under the control of the strong *AOX* I promoter, and downstream of the α- mating type signal secretary sequence. The multiple cloning site located downstream of the signal sequence was initially cut to introduce two *Sfi* 1 sites and R-tag sequence (19) followed by two stop codons. To introduce these sites with the Rtag sequence, two complementary oligonucleotides were synthesized (Invitrogen) (flushed with the Kex2 cleavage site of the signal sequence processing site),TCGAGAAGAGAGAGGCCCAGCCGGCCA TGGTGGAGGCCTCGGGGGCCCCGGATCAGT ATGAATACAAATATCCGTGATAGCTCGAG and CTCGAGCTATCACGGATATTTGTATTCATAC TGATCCGGGGCCCCCGAGGCCTCCACCATG GCCGGCTGGGCCTCTCTCTTCTCGA were heat denatured and annealed by slow cooling down to room temperature. The annealed oligonucleotide possessed *Xho* 1 sites at each end. To obtain the modified pPICZαSR vector, the dimer was ligated by using DNA Ligase (Roche) to the pPICZαC vector cut with *Xho*1. The resulting plasmid pPICZαSR (Fig.1A) retains the Kex2 cleavage site of the signal sequence processing site, with two *Sfi*1 sites, and R-tag sequence (19) was cloned into Top 10 *E.coli* (Invitrogen) by electroporation.

RNA was purified from hybridoma cell line 6c8 using mRNA direct purification kit (Dynal). RT-PCR was performed with Superscript II One Step RT-PCR Kit (Invitrogen) to amplify light and heavy chains with primer sets (20). Heavy and light chain was assembled in a second amplification reaction (Platinum *Pfx* DNA polymerase-invitrogen). The assembled single-chain PCR product was cloned *SfI* 1 into Phagmid pHB110 (20). Phage was screened by ELISA on antrgen plates of the immunizing KLH peptide and a single clone chosen. The 6c8 scFv DNA was gel-purified and quantified using a spectrophotometer (Eppendorf BioPhotmeter, Germany), digested with *Sfi* I restriction enzyme (New England BioLabs) and purified using gel extraction kit. The 6c8 scFv gene was cloned into the *Sfi* 1site of pPICZαSR (Fig. 1a and 1b) that was derived from plasmid pPICZαC (Invitrogen). This was cloned into Top 10 *E. coli* (Invitrogen) by electroporation.

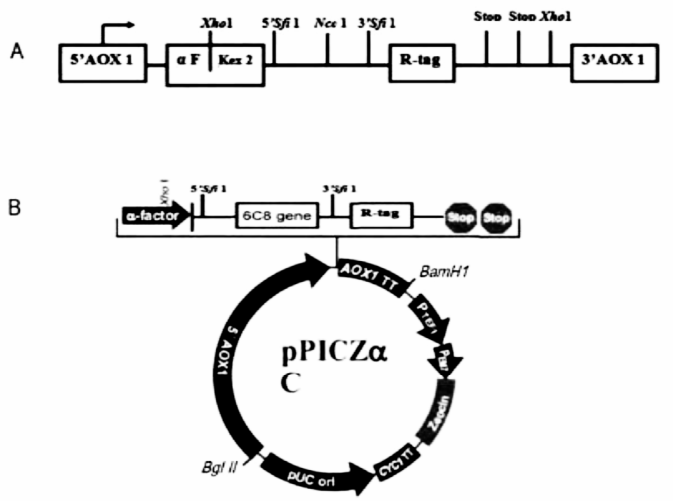

Figure (1): Schematic drawing of the expression cassette used in the expression of 6c8 scFv protein.
A- pPICZαSR vector construction. The original vector used for cloning was pPICZαC (Invitrogen). This plasmid was modified so that its multiple cloning sites are replaced with *Sfi1* sites and R-tag sequence followed by two stop codon. The cloning strategy for obtaining these vectors is described in the text.
B- 6c8 scFv **gene was cloned into the pre-digested modified pPICZαSR vector. The modified expression vector was used to electroporate the yeast *P. pastoris*, strain KM71H (Mut^S) (Invitrogen) as described in the text. The symbols used in the scheme are: 5' AOX 1: 3' AOX 1: Alcohol oxidase 1 gene promoter fragment; α-F: α-factor secretion signal. Digestion with *Sfi*1 restriction enzyme allowed the introduction of the scFv of 6c8 into the vector**

Expression of scFv in *P. pastoris* expression/ secretion system

The construct was linearized with *Sac*1 and 10 µg was used to transform *P. pastoris* KM71H (Mut^S) cells by electroporation. Colonies that grew on YPDS agar medium containing 100 µg/ mL Zeocin were examined for 6c8 scFv expression, by inoculating 10 ml of BMGY medium with the clones, and incubating at 29 °C for 18 h with shaking at 200 rpm. The cells were harvested by centrifugation at 1000 RPM (SS34 rotor; Sorval RC 5b) for 5 min, suspended in 1 ml of BMMY medium, and induced overnight at 29 °C. The culture supernatant was obtained by centrifugation at 1000 RPM for 2 minute, analysed using SDS–PAGE and Western blotting for the presence of R-tag peptide as described below. *P. pastoris* KM71H (Mut^S) cells transformed with pPICZαSR (empty vector) and 8g8 scFv were prepared as described above.

Purification of 6c8 scFv from culture of *P. pastoris*

A single colony expressing recombinant 6c8 scFv was used to inoculate 10 mL BMGY medium and incubated overnight at 29 °C with shaking at 200 rpm. The volume was increased to 500 mL in a 2-L baffled flask and incubated at 29 °C overnight with shaking at 200 rpm until the OD600 nm reached 15-20. The cells were harvested and suspended in BMMY at an absorbance OD600 of 40. Fifty ml of cell suspension was inoculated into four of 2-L baffled flasks. The flasks were incubated at 14°C with shaking at 200 rpm. After 15 h of induction, the cultures were harvested by centrifugation at 2000 RPM for 10 min. The supernatants were isolated by centrifugation at 18000 RPM, for 20 min and concentrated ten times by using stirred cell filteration device (Pall Filteron Technology Corporation, MA, USA). The protein was precipitated by addition of ammonium sulphate to 80% and collected by centrifugation. The protein pellet was dissolved in 10 ml of 20 mM sodium acetate buffer pH 4.5. After several times of sheering (using 10 ml Nichipet) the protein was precipitated as described above and the precipitate finally dissolved in 5 mL of 20 mM sodium acetate buffer pH 4.5 and desalted by using PD-10 column equilibrated with the appropriate buffer. The sodium acetate buffer eluent was loaded into a 5 mL HiTrap SP column (GE Healthcare Life Sciences) equilibrated with 20 ml of sample, the column washed with 20 ml sample buffer, and the protein was eluted at 1 mL/min, on an AKTA Prime (Amersham Biosciences) with a gradient of 0–1000 mM NaCl in sample buffer.

Preparation of *P. Pastoris* and *E.coli* Total membrane

P. pastoris cells were broke by employing 0.25–0.30 mm glass beads. *E. coli* cells were broke by sonication. Intact yeast cells and bacterial cells debris were removed by low-speed centrifugation. Total membrane pellets were collected by centrifugation at 15000 RPM at 4 °C for 30 min (21).

Interaction of Mab 6c8 with *E.coli* and *P. pastoris* cells

The effect of Mab 6c8 on the viability of *E.coli and P. pastoris* cells were studied as follows:
a- Yeast cell transformed with pPICZαSR (empty vector) cultured in 20 ml of BMGY medium and incubated at 30 °C for 18 h with shaking at 200 rpm. The cells were harvested by centrifugation, suspended in 1 ml of BMMY medium.The interaction of Mab 6c8 with *P. pastoris* cells was carried out by mixing of 10 µg of Mab 6c8 with 1 ml of OD_{600} 20.0 yeast cells. After the addition of Mab 6c8 to yeast cells, the mixtures incubated for 10 min at room temperature. The number of viable cells was counted by the addition of Trypan blue which penetrates only dead cells and results in blue staining of the cellular contents. The percentage of dead cells under each treatment was determined by counting several hundred cells under the microscope. Controls carried out were the use of irrelevant antibody or no antibody.
b- *E.coli* BL21 (DE3) cells cultured in 20 ml of LB medium and incubated at 37 °C for 18 h with shaking at 200 rpm. The cells were harvested by centrifugation, suspended in 1 ml of LB medium.The interaction of Mab 6c8 with *E.coli* cells was carried out by mixing of 2 µg of Mab 6c8 with 1 ml of OD_{600} 1.0 cells. After the addition of Mab 6c8 to cells, the mixtures incubated for 30 min at room temperature. The number of viable cells was counted as described above.

Surface plasmon resonance

Binding activity of the purified 6c8 scFv was measured by surface Plasmon resonance using a BIACORE 2000 instrument (BIACORE, Piscataway, NJ) and an (*At)*RGA antigen-coated Biosensor chip. Samples were buffer exchanged and diluted in running buffer [Hepes-buffered saline (HBS). Samples were injected at a flow rate of 30µl /min, allowed a contact time of 5min, and dissociation time of 15min. After the dissociation period, stripping buffer (50mM citrate buffer, pH 3.0) was flowed over the surface of the chip for 30 s.

SDS–PAGE and immunoblotting

Centrifuged culture supernatants samples, chromatographic fractions, whole yeast, whole bacteria, total membranes, and outer membranes were analyzed by electrophoresis on 10% SDS–PAGE according to standard protocols (17). 15 µl of samples were used for all SDS–PAGE analyses.
The proteins from gels of whole yeast cells, whole bacterium, cell envelop, and cytoplasmic proteins, were transferred by electrophoresis to a nitrocellulose membrane (Trans-Blot Transfer, Bio-Rad Laboratories, CA, USA). The membranes were blocked in 0.5% I-block (Tropix, Bedford, MA, USA) in phosphate buffered saline containing 0.1 % Tween 20 (PBST) for 2 h at room temperature, then incubated for 1 h in blocking buffer containing 500 ng of MAb 6c8 or MAB anti-CN1 (control). The membranes were washed three times in PBST (22) and incubated for 1 h in blocking buffer containing peroxidase–labelled goat anti-mouse IgG (Fc specific, 1:20000; Sigma Chemicals). The membranes were washed three times in PBST and developed using the ECL system (NEN Western Lightning Plus).
The proteins from gels of cultural supernatants was treated as above except that the membranes incubated for 1 h in blocking buffer containing 500 ng of MAB D 9 instead of MAB 6c8.

Protein determination

Purity of the scFv was assessed by electrophoretic analysis using Coomassie blue R stained SDS gels image densitometry software, Image J. Briefly, samples were run on an SDS–PAGE, Coomassie stained, and dried using a gel drying kit. Dry gels were

then scanned on an HP ScanJet 4300C and loaded onto the ImageJ software. Soluble protein concentration was also determined using the BCA assay (Pierce chemicals) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Expression level of scFvs in *E. Coli*

The advantages of *E. coli* protein expression system are cost effective, faster and more easily regulated expression than eukaryotic systems and the ability to produce recombinant proteins in a controllable manner (16). The scFvs fragments were ligated into pAK 300 Plasmids and transformed into expression strain *E. coli* BL21 (DE3).

However at 37 °C, although expression of 6c8 scFv and 5e1 scFv using an optimal concentration of IPTG of 0.3mM were tested with several clones, western blot analysis of samples (boiled and non-boiled) separated by SDS-PAGE showed several bands of varying intensity. The molecular weights of these bands from non-boiled SDS sample ranged between 27 -180 KDa (Fig. 2a, lanes: 5, 6, 7,) and from boiled SDS sample ranged approximately between 56-185 KDa. None of the bands could be detected from non-boiled SDS sample of an irrelevant 8g8 scFv recombinant protein (Fig. 2a, lanes: 8 and 9).

Estimation of protein bands by using ImageJ software showed that approximately 95% of the scFvs interacted with *E. coli* proteins and sediment by high speed centrifugation (calculation not shown). To explain the presence of these bands, we have carried out protein blast search for the presence of DELLA motif in proteins of *E. coli* BL21 (DE3). The search revealed 59 proteins with DELLA motif and molecular weights ranged from 10-160 KDa. Some of these proteins are; translational initiation factor, cofactors and enzymes. We investigated the reactivity of MAb 6c8 to *E. coli* by carrying immunoblotting experiments with whole-cell, total membrane, and cytoplasmic associated proteins preparations. The MAb reacted with several proteins that, associated with whole cells and cytoplasmic proteins (Fig. 3a, Lanes: 4 and 5) and weakly reacted with three of the cell envelope associated proteins (Fig.3a, lane 3). None of the proteins interacted with an irrelevant MAB anti Cin1 (Fig.3b, lanes: 1, 2, and 3). The approximate molecular weight of the proteins interacted with MAB 6c8; were; 22, 24, 27-28, 35-36, 43-45, 51-53 54-56, 75-77 and 95-100 KDa. The proteins with DELLA motif with approximate molecular weights closer to these proteins are; Ferrochelatase (35 KDa),Gamma-Glu-putrescine synthase (53.2 KDa), DNA adenine methylase (32 KDa), isoleucyl-tRNA synthetase (104 KDa), pyruvate dehydrogenase (99.6 KDa), Ferrochelatase (36.2 KDa), Molybdopterin biosynthesis protein A (37.3 KDa). GTP diphosphokinase (75 KDa), Aminopeptidase N (95 KDa), chemotactic sensory histidine kinase (72 KDa), tRNA(Ile)-lysidine synthetase (47 KAa), dehydrogenase (46 KDa). Binding of scFvs into proteins that involve in the import or synthesis of nutrients could resulting in bacterial cell lysis or death.

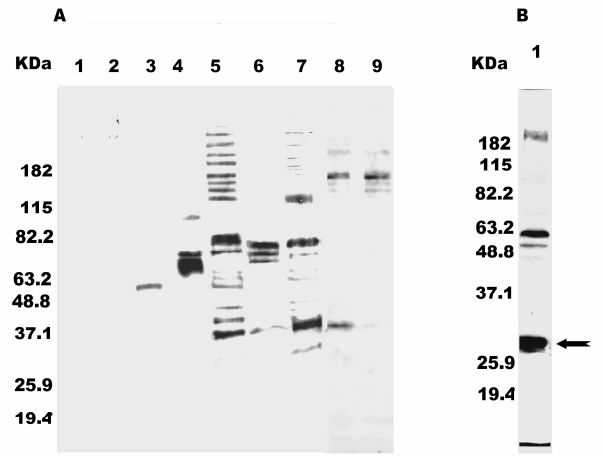

Figure (2): Western blot analysis of expression of scFvs recombinant proteins by *E. coli* BL21.
A- 6c8 scFv and 5e1 scFv expressed in *E. coli* BL21 (DE3) by using denaturing gel electrophoresis (un-boiled sample). Lane 1; total cell lysate of uninduced culture, lane 2;
***(At)*Scarecrow recombinant protein (control), lane 3; Scarecrow R-tagged recombinant protein (control), lane 4; supernatant of the cell lysate after induction (6c8 scFv), lane 5; sediment of cell lysate after induction (6c8 scFv), lane 6; supernatant of the cell lysate after induction (5e1 scFv), lane 7; sediment of cell lysate after induction (5e1 scFv), lane 8; supernatant of the cell lysate after induction (8g8 scFv), lane 9; sediment of cell lysate after induction (8g8 scFv),B- Total cell lysate after induction (6c8 scFv) (boiled sample).Western blot was developed with peroxidase–labelled goat anti-mouse IgG (Fc specific Sigma chemicals) and developed with chemiluminesence. Arrow indicates scFV proteins**

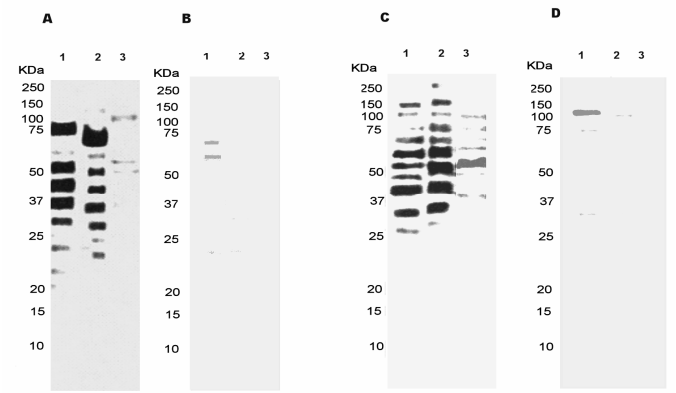

Figure (3): Immunoblotting experiments of, whole-cell, total membrane, and cytoplasmic associated proteins preparations of *E.coli* BL21 (DE3) *and P. pastoris* with MAb 6c8. A- Immunoblotting of protein profiles of *E.coli* with MAb 6c8. Lane 1*;* whole cells, lane 2; cytoplasmic associated proteins, lane 3; total membranes. B- Immunoblotting of protein profiles of *E.coli* with MAb Anti Cin1. Lane 1*;* whole cells, lane 2; cytoplasmic associated proteins, lane 3; total membranes. C- Immunoblotting of protein profiles of *P. pastoris* with MAb 6c8. Lane 1; Whole cells, lane 2; cytoplasmic associated proteins, lane 3; total membranes, D- Immunoblotting of protein profiles of *P. pastoris* with MAb Anti Cin1. Lane 1; Whole cells, lane 2; cytoplasmic associated proteins, lane 3; total membranes Molecular mass markers (sizes in kilodaltons are indicated to the left) Western blot was developed with peroxidase–labelled goat anti-mouse IgG (Fc specific Sigma chemicals) and developed with chemiluminesence. Arrow indicates scFV proteins.

6c8 scFv overexpression inhibits *E. coli* growth

Expression from pAK 300 plasmid containing the 6c8 scFv inserted under the control of the strong IPTG-induced promoter strongly inhibited *E. coli* growth of strain DE3 (Fig.4 1a). Strain carrying the empty vector, or uninduced had no effect (Fig. 4a). The toxicity of 6c8 scFv depends on cell culture phase at the time of 6c8 scFv production. *E.coli* was grown overnight at 37 °C, diluted 1:100, and treated with different IPTG concentrations (50 µM, 100 µM, 250 µM, 500 µM, and 1 mM) to determine the effect of 6c8 scFv expression and concentration. Figure 4a shows that cell growth, as determined by $O.D._{600}$, negatively correlated with increasing inducer concentration. Figure 4b shows that scFv 6c8 sensitivity was growth phase dependent. We found that 6c8 scFv blocked cells from exiting stationary phase, and was less toxic to cells in middle or late log phase. Exit of bacterial cell from stationary phase would indicate that the synthesis of host proteins reached its maximum, and subsequent inhibition of translation would not have an immediate effect upon the expression of the scFv and the survival of the bacteria. After 5 h of induction, a drop of approximate 40- 50 % in the O.D. was observed in culture induced by 500 µM and 1 mM IPTG with the appearance of flocculation materials an indication of the lysis of the host bacterial cells. The density of the cells decreased until 6 h had passed, at which time normal growth resumed (recovery). The addition of IPTG to these recovered cells did not influence cell density or result in 6c8 scFv protein expression. This could be attributed to Plasmid instability, i.e., loss of plasmid or sequence rearrangements is frequently observed in heterologous gene expression (17).

Mab 6c8 induced clumping of *E. coli* cells

Mixing of 4 µg of Mab 6c8 with 1 ml of OD_{600} 1.0 *E. coli* cells resulted in clumping, followed by lysis of about 26% of the bacterial cells (Figure not shown), while the addition of 50 µg of MAB anti-Cin 1 had no effect. The cell envelope of the Gram-negative bacterium *E. coli* is a complex structure, and many of the proteins found in the bacterial envelope are involved in import mechanisms (22). The binding of scFv into one or more of the following proteins; putative membrane protein, lipoprotein, inner membrane protein yhbX and periplasmic binding protein with DELLA motifs can cause cell death. However, the toxicity caused by 6c8 scFv expression may not indicate a single target.

Interaction of Mab 6c8 with *P. pastoris* cells

Before our attempt to express scFvs, we have carried out a protein blast search for the presence of DELLA motif in the proteins of *P. pastoris*. The search revealed eight proteins with DELLA's motif, four of these are autophagy-related proteins and one of them is DNA polymerase gammaprotein (116 KDa). The reactivity of MAb 6c8 with the proteins of *P. pastoris* was also carried out using immunoblotting experiments with whole-cell, total membrane, and cytoplamic associated proteins preparations. The MAb 6c8 reacted with several proteins found in whole cells and cytoplsamic associated proteins (Fig. 3c, lanes: 1 and 2). In regards to envelope protein, the MAb reacted with few proteins (Fig. 3c, lane 3). None of the proteins interacted with an irrelevant MAB anti-cin1 (Fig.3d).

We also examined the addition of Mab 6c8 onto yeast cells transformed with pPICZαSR (empty Vector). We found that mixing of 60 µg of Mab 6c8 with 1 ml of OD_{600} 20.0 yeast cells ($4x10^9$ cells/ml) resulted in clumping of the cells, followed by gradual lysis of more than 50% after 10 min of incubation at room temperature (Fig.5a). The reaction specifies was examined by mixing of 40 µg of DELLA protein (*At*) RGA with 60 µg Mab 6c8. After incubation of the mixture for ½ hr on a rotor, the mixture was added to *P. pastoris* cells. We found that (*At)* RGA inhibited clumping and lysis of the cells. Negative controls included an irrelevant Mab anti-CIN 1 protein. Mixing of 200 µg Mab Anti-CIN-1 with 1 ml suspension of OD_{600} 20.0 of yeast cells causes no agglutination or lysis of the cells (Fig.5b). Despite of the above results, 6c8 scFv and 5e1 scFv, fused to carboxyl-terminal R-tag to allow for immunodetection and affinity purification, were expressed in *P. pastoris* (see materials and methods).

Western blot analysis of samples of 6c8 scFv (non-boiled and boiled) separated by SDS-PAGE, showed a band of monomer in addition to a second band of approximate molecular weight of 60 KDa (Fig. 6a, lane 1) while a boiled sample of supernatant, showed only a single band of the 6c8 scFv (Fig. 6b, lane 1). Western blot analysis of samples of 5e1scFv (non-boiled and boiled) separated by SDS-PAGE, indicated a very low expression of recombinant protein (a very faint band of monomer) (Fig. 6c, lane 1). The supernatant of an irrelevant 8g8 scFv gave a single band in both boiled and non boiled samples (Fig. 6a and 6b, lane 2). The recombinant protein was purified by affinity chromatography and the level of expression for 6c8 scFv was generally in the order of 35 mg/l culture. The purified 6c8 scFv was unable to interact with their target (*At*) RGA recombinant protein. The lack of the activity of scFv was in accord with the absence of the toxicity to the host.

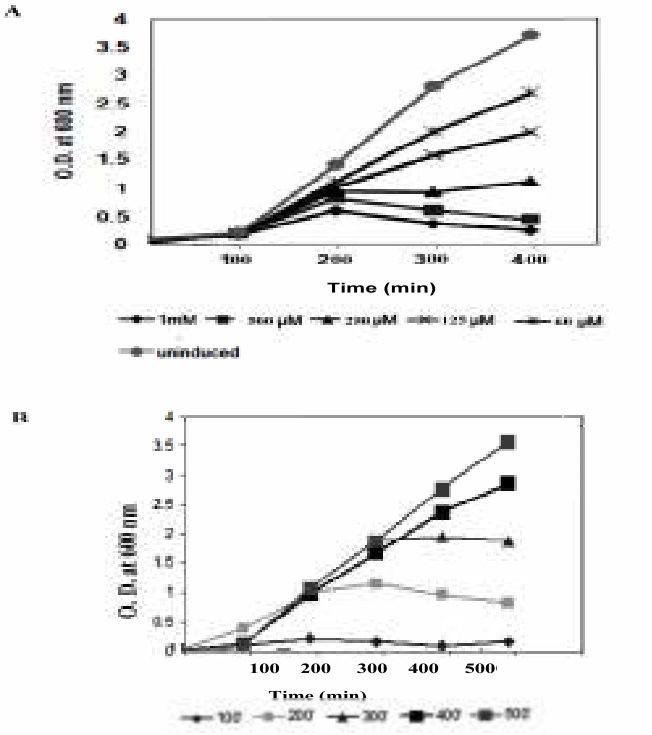

Figure (4): *E. coli* outgrowth from stationary phase is inhibited by 6c8 scFv overexpression. Strain *E. coli* BL21 (DE3) was grown overnight at 37 °C in LB+chloramphenicol (50 µg/ml) and diluted 1:100. (a) IPTG was added at time 0 at the indicated concentrations to induce 6c8 scFv; (b) Expression of 6c8 scFv was induced with 1 mM IPTG at the indicated times following dilution. All experiments were performed at 37 °C

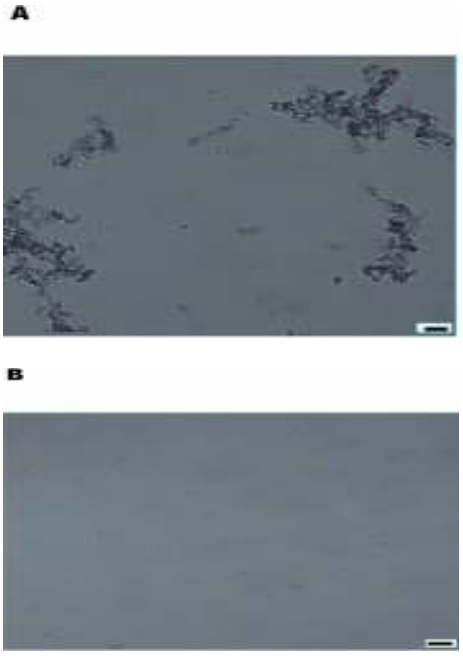

Figure (5): Interaction of of Mab 6c8 with *P. pastoris* cells.
A- Yeast cell transformed with pPICZ αSR(empty vector) (Control)
B- Mab 6c8 (60 µg) mixed with 1 ml of OD_{600} 20.0 yeast cells transformed with pPICZ αSR ($4x10^9$ cells/ml). After addition of Mab 6c8 to yeast cells, the mixture incubated for 30 min at 29°C with shaking at 200 rpm. The number of viable cells was counted by the addition of Trypan blue. Dead cells (coloured blue) were observed under a light microscope (see material and method for details)

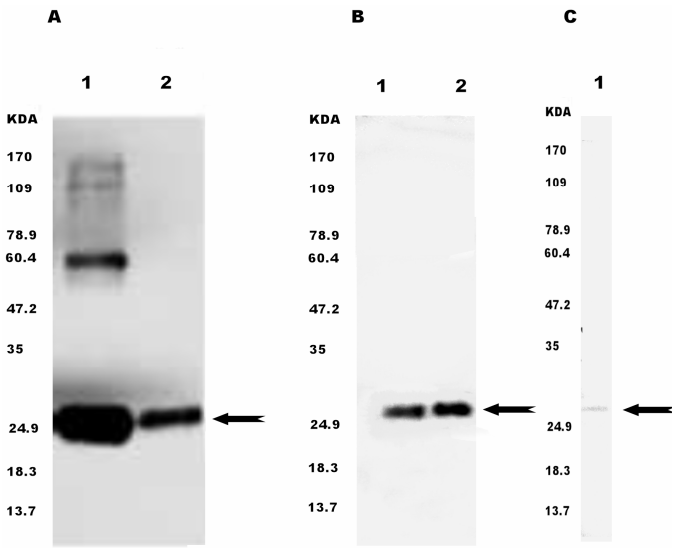

Figure (6): Western blot analysis of expression of scFvs recombinant proteins by *P. pastoris*
A- 6c8 scFv and 8g8 scFv expressed in *P. pastoris* by using denaturing gel electrophoresis (un-boiled samples). Lane 1; 6c8 scFv, lane 2; 8g8 scFv. Arrow indicates scFv proteins
B- A- 6c8 scFv and 8g8 scFv expressed in *P. pastoris* by using denaturing gel electrophoresis (boiled sample). Lane 1; 6c8 scFv, lane 2; 8g8 scFv. Arrow indicates scFv proteins
Molecular weight markers (sizes in kilodaltons are indicated to the left). Western blot was developed with MAb D9 and peroxidase–labelled goat anti-mouse IgG (Fc specific Sigma chemicals) and developed with chemiluminesence. Arrow indicates scFv proteins.

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Transformation of *Agrobacterium tumefaciens* GV3101 with pSoup plasmid via electroporation

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ABSTRACT

The present study aims to investigate transformation of *Agrobacterium tumefaciens* GV3101 with the pSoup plasmid isolated from *Escherichia coli* DH5α by electroporation. The maximum transformation frequency was $0.21x10^{-6}$ transformants/ µg DNA achieved at field strength of 19.0KV/cm with pulse of 2.0msec (200Ω). The number of transformants was found to increase (13, 16, 18 and 21) with increasing cell density and DNA concentration, but further increase decrease transformation frequency. The transformed *Agrobacterium* will reacted with cabbage (Brassica olaraceae var. capitata) plants in next work.

Keywords: Agrobacterium tumefaciens GV3101, pSoup plasmid, Electroporation, Transformation

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الملخص باللغة العربية

سعت الدراسة الحالية إلى الكشف عن التحول الوراثي لبكتريا *Agrobacterium tumefaciens* GV3101 ببلازميد pSoup المعزول من بكتريا *E.coli* DH5α بوساطة التثقيب الكهربائي. وسجل تكرار التحول الوراثي 0.2 x 10^{-6} خلية محولة / مايكروغرام DNA في ظروف 19 kv/cm ونبضة امدها 2.0 ملـــي ثانيـــة (200Ω) . واظهرت النتائج زيادة اعداد المستعمرات البكتيرية المحوّلة وراثيا(13, 16, 18, 21) مع زيادة تركيز الخلايا والحامض P-DNA , ولوحظ عند تجاوز هذه الزيادة انخفاض نسبة التحول الوراثي المتحققة.

INTRODUCTION

One of the basic techniques used in plant biotechnology is the transfer of the genetic information from one organism into another. This is to enhance the production of recombinant organisms or to generate an organism with new recombinant properties. The soil-borne bacterium *Agrobacterium* has been involved in the genetic engineering of plants (1).

Agrobacterium tumefaciens is gram-negative bacterium that genetically transforms host plants and cause crown tumors at wound sites in tobacco (2). The genetic material that's introduced is called T-DNA(transferred DNA) which is located in Ti plasmid, a circular piece of DNA found in bacteria(3).The proliferated tissue in the tumor provides the bacterium with opines unusual amino acid, which are an important carbon and nitrogen source (4).

Agrobacterium tumefaciens strain GV3101 is useful for Ti-vector based plant transformation (3). The *Agrobacterium*-mediated transformation method was improved by developing modern binary Ti vector after the removal of all the genes for tumor induction and opine synthesis, Ti plasmid without the tumor inducing function is called disarmed plasmid (non-oncogenic Ti plasmid).This plasmids have been engineered to separate T-DNA and *Vir* regions into two distinct plasmids resulting in a binary vector and a *Vir* helper plasmid respectively (5).Since disarmed binary plasmids containing the T-DNA region do not have the ability to move a T-DNA in to the plants, they need the help of another separate plasmid containing the *Vir genes* such as pSoup plasmid (Fig.1) ,which provides replication function in transfer pGreen plasmid (2).

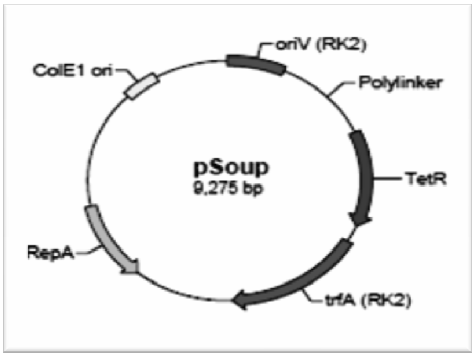

Figure (1): pSoup plasmid (6)

The introduction of P-DNA fragment into an organism cell without the involvement of biological agents leading to stable transformation such as electroporation, particle bombardment and microinjection called direct gene transfer (7).

Electroporation leads to the formation of tiny temporary holes in the cell through which the DNA can pass (8). This study aimed to transport pSoup plasmid into *A. tumefaciens* GV3101 by electroporation.

MATERIALS AND METHODS

Bacterial strain used

Data in table (1) indicate the bacterial strains and their genetic markers.

The strains were kindly supplied from Prof. P. Meyer, Faculty of Biological Sciences, University of Leeds, UK.

Table (1): Description of bacterial strains used

Bacterial strain	plasmids	Genetic marker	Conc. µg/ml	Media	Ref.
A. tumefaciens Gv3101		Gent.$^{Rest+}$	50	YEB	(9)
E.coli DH5α	pSoup	Tetr.$^{Rest+}$	12.5	N.agar N. Broth	

Plasmid DNA isolation

Modified method of Birnboim and Doly (10) was used for the isolation of pSoup plasmid from *E.coli.* Single colony of *E.coli* containing pSoup plasmid was picked and inoculated into 50 ml N. Broth (11) supplemented with 12.5µg /ml of tetracycline for selection in a sterile culture flask.

Cultures were incubated overnight at 37˚C with shaking at 50rpm,when the optimum density O.D.= 1.0 at 600 nm they decanted into 1.5ml microcentrifuge tubes and centrifuged at 8000 rpm for 15 min and the cells harvested. The supernatant was discarded and the pellet was re-suspended in 2.0 ml of the solution containing 20 % Glucose, 0.25 M EDTA and 1 M Tris-Hcl pH 8 with the addition of 0.1 ml of (50 mg/ml) lysozyme solution. The supernatant left for 10 min at room temp, then 4.0 ml of solution (10 M NaOH and 10 %SDS) added to it with mixing using the vortex. The mixture left in ice bath for 10 min, and 3.0 ml of 5M cold sodium acetate (pH 4.8) was added. The supernatant left on ice bath for 10 min and finally centrifugated at 1000 rpm for 10 min. For protein removal equal amount of (chloroform: isoamyl alcohol 24:1) added to the clear fraction and centrifugated at 1000 rpm for 10 min (three times). The supernatant was transferred to a new microcentrifuge tube with addition of 1/10 of its volume of solution 3M CH_3COONa-$3H_2O$ and two volumes cold absolute ethanol, the prep. was left for 30 min at -20˚C for DNA precipitation and then, centrifugated at 1000 rpm 10 min. The precipitant washed by 5.0ml of 70% ethanol and resuspended in 0.5 ml of TE buffer then kept in freeze at -20 ˚C.

Preparation of competent *A. tumefaciens* cell.

Grew eight ml of over night culture of *A. tumefaciens* GV3101 in liquid YEB medium provided with 50 µg/ml Gentamycine. Inoculate the 8.0 ml overnight culture into 192ml of YEB in the absence of antibiotic with shaking at 28˚C until optical density achieved about 0.5.The culture was centrifugated at 4000 rpm /15 min at 4.0˚C, resuspend pellets in 10 ml ice-cold 10 mM Tris-Hcl pH 7.5 and centrifugated for 15 min at 4000 rpm at 4.0˚C.Finally resuspend pellets in 20 ml cold YEB medium and kept in deep freeze at -80˚C (12).

Transformation of competent *A. tumefaciens* cells with pSoup plasmid

Competent cells of *A. tumefaciens* strain GV3101 are defrosted in ice, different volumes of miniprep. DNA (pSoup) was mixed with different volumes of electro competent *A. tumefaciens* cells. Each mixture was placed in an ice cold electroporation cuvette and electroporated (13) under conditions of Capacitance 25 µF, Setting 200 Ω, charging volt 1.9 KV and Pulse length 2 msec followed in Plant Genetic Manipulation Lab. /Dept. of Biology/ College of Education/ Mosul University.

Immediately after electroporation 1.0 ml liquid YEB medium added using gilson pipette, the mixture transferred to sterile eppendrof tube and incubated with vigorous shaking at 28˚C for 2.0 hrs.

Decimal dilutions were prepared from each transformation mixture and 0.1 ml aliquots from previous dilutions overspread on agar-solidified YEB medium supplemented with the appropriate antibiotics. In addition to 0.1 ml from competent cells overspread as control samples and 0.1 ml from competent cells overspread on YEB medium to confirm that the cells are competent for DNA uptake. Petri dishes were incubated at 28˚C for 48hrs.(14).

Transformed colonies were calculated and purified on the same medium, a transformation frequency obtained depending on the standard formula (15).

$$\text{Transformation frequency} = \frac{\text{No. of transformed colonies/ µg PDNA}}{\text{No. of viable colonies}}$$

RESULTS AND DISCUSSION

The results in table (2) express the variations of transformation frequency affected by the concentrations of plasmid DNA and competent *A. tumefaciens* GV3101 cells. The transformation frequency increased to maximum value (0.21×10^{-6}), other concentrations resulted in less transformation frequency.

Table (2): Transformation frequency of *A. tumefaciens* GV3101 affected by concentration of pSoup plasmids

Competent GV3101 (volume / µl)	pSoup plasmid/(µl)	Pulse/ Volt/cm.	Time/ (msec).	No. Colonies after (48hrs)	Transformation frequency ($\times 10^{-6}$)
40	2	1900	2	0.136	13
50	12.5	1900	2	0.168	16
200	25	1900	2	0.189	18
200	50	1900	2	0.210	21
(control) 200	50	0.0	2	0.0	0.0

Each value represents three replicates

The reduction of transformation frequency may be due to the presence of deleterious chemicals in the DNA preparation which might enter the cell during electroporation as noted in *Rhizobium*, the relative genus to *Agrobacterium* (16).

The growth of the transformed colonies on agar solidified YEB medium supplemented with gentamycine and tetracycline (Fig.2) proved that pSoup plasmid has been transferred to cells of *A. tumefaciens*. This due to the presence of tetracycline resistance gene in the constructed pSoup plasmid (Fig 2. A). comparing with the number of colonies of the competent cells on YEB medium (Fig 2. B).

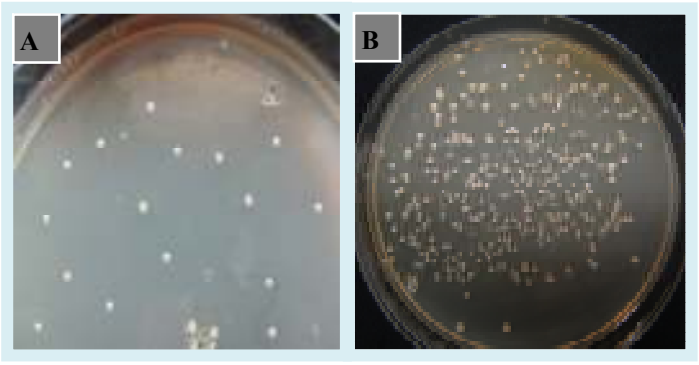

Figure (2): A- Transformed A. *tumefaciens* colonies grown on agar-solidified YEB medium supplemented with gentamycine and tetracycline. B- *A. tumefaciens* competent cells grown on YEB medium.

It seems likely that electroporation improved the mediated DNA uptake in *Agrobacterium tumefaciens* GV3101, through stabilizing the field strength and pulse length coupled with variable concentrations of both competent cells and plasmid DNA. This is important for efficient inclusion of plasmid DNA into cells of *Agrobacterium tumefaciens* GV3101.

Many researchers mentioned that electroporation yielded reversible and irreversible permiabilization of the cell membrane as a function of electrical pulse voltage parameters, which means amplitude, length, shape of the pulse, and cell type and developmental stage. Since the first report of gene transfer by electroporation (17), it became a standard method for transformation of cells (18). Electroporation has several advantages, it is technically simple, can be used to treat whole population of cells, has broad application for transfer of any macromolecule, provides greater efficiency of transfection for many cell lines, and can be applied equally successfully to prokaryotic and eukaryotic cells (19). In addition, plasmid size doesn't affect transformation frequency using electroporation technique. The plasmid pSoup used in current study had low or medium copy number and that's explaining the low number of transformed colonies developed in the transformation plate (20). Intrestingly this study was designed to improve electroporation mediated DNA uptake in *Agrobacterium* as other workers reported (8).

Acknowledgement

Many thanks to Prof. Peter Mayer and to Mr. Ayad H.H. from university of Leeds for their assistant in providing the bacterial strains Agrobacterium tumefaciens GV3101 and E.coli DH5α contained pSoup plasmids

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Obesity in England: an analysis of the health survey for England (HSE) and Hospital Episode Statistics (HES) (2001-2011)

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ABSTRACT

Obesity as a disease is one of the important preventable risk factor associated with increasing diseases burden and death worldwide. Despite its recognition as a separate disease in the International Classification of Diseases (ICD) since 1948, not many studies assessed it separately among hospital and general population. This study aims to present a descriptive analysis of obesity prevalence among children and adults across different age groups and genders in addition to admissions, Finished Consultant Episodes (FCE), and the numbers of days in which hospital beds were occupied by obesity inpatients in England between 2001 and 2011.

The results of analysis over a study period indicate the preponderance of women in respect of adult's obesity prevalence and the percentage of FCE recorded. Mainly, the age group which accounts the highest obesity prevalence was (11-15) years among children and (55-64) years among adults whereas the highest percentage of FCE recorded among (15-59) years age group. During the study period in which a total of 94,268 adults and 43,050 children participated in HSE obesity prevalence among adults outstripped those recorded among children. Patients admissions, and FCE recorded in 2011 were around eleven times more than what was recorded in 2001 in addition the total number of days patients occupied hospital beds due to obesity increased sharply to four times what it was in 2001. On the other hand, the mean number of days obese patients spend in hospital appears to have continually decreased to one-third of that recorded in the beginning of the study period.

Keywords: Obesity, Health Survey for England, Hospital Episode Statistics

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الملخص باللغة العربية

تعتبر السمنة مرضا وهو احدى العوامل الخطرة المهمة بارتباطة للوقاية من العبء المرضى والموت فى انحاء العالم. وعلى الرغم من تصنيفها كمرض من قبل التصنيف الدولى للامراض منذو 1948 لكن العديد من الدراسات لا تصنفه كمرض مستقل فى المستشفيات او بين عموم الناس. تهدف هذه الدراسة الى تقديم تحليل وصفى لانتشار السمنة عند الاعمار المختلفة من كلا الجنسين وعدد الايام التى شغلت فيها الاسرة بالمستشفيات نتجية مرض السمنة فى انجلترا للفترة من 2001 الى 2011. اشارت نتائج الدراسة الى رجحان السمنة عند النساء فيما يتعلق بالبالغين بين الفئة العمرية 55–64 وعند الاطفال بين سن 11–15 حسب احصائية مستشار الحلقات النهائية. بلغ عدد المشاركين بالدراسة 94268 من البالغين و43050 من الاطفال وكانت نسبة السمنة عند البالغين اكثر من الاطفال وكذلك سجلت حالات السمنة فى عام 2011 اكثر 11 مرة من مثيلاتها فى عام 2001 بالاضافة الى عدد ايام التى شغلت فيها الاسرة بالمستشفيات نتجية مرض السمنة باربعة اضعاف مما كانت عليه فى عام 2001 ومن ناحية اخرى انخفضت هذه النسبة الى الثلث فى نهاية الدراسة مقارنة ببدايتها.

INTRODUCTION

Obesity and overweight can be considered as a one of the major health problems in England and all over the world. Obesity was introduced in the International Classification of Diseases (ICD) for the first time in 1948, since then the major debate has been about whether obesity can be described as a disease or what is the exact definition and body weight categorizing criteria of obesity (1). National Institute for Heath and Care Excellence (NICE) clinical guidelines defined it as a disorder and risk factor for other diseases in which weight gain reaches a level that can lead to considerable risk to health (2).

The popular and widely utilized method for evaluating and categorizing peoples' weight is the body mass index (BMI) which is the person's weight in kilograms divided by their height in meters squared (3).

Adults are categorized as overweight if their BMI falls between (25−29.9 kg/m^2) and if it exceeds (30 kg/m^2) they can be categorized as obese (4).

Obesity is caused by the disparity between input and disbursement of energy linked with many risk factors such as lifestyle, diet, physical activity in addition to genetic factor (which is linked to childhood obesity) and assortative mating (5).

World Health Organization (WHO) stated that since 1980 obesity has been multiplied internationally, in 2008 (35%) of adults 20 years old and over were classified as overweight, 11% were categorized as obese globally (6).

In 2011, there were internationally 40 million or more under five years children who were overweight (6). Three in ten girls and boys (2-15 years of age) in England were categorized as obese or overweight in 2010 (7).

In England over one fourth (26%) of adults of both genders aged 16 years or over, measures a BMI of 30 kg/m^2 or more, were assessed as obese in 2010. 42% of men compared with 32% of women classified as overweight BMI (25-30 kg/m^2) in the same year (7). Being obese or having weight increase can lead to increasing risk of developing significant health problems so decreasing quality of life and life expectancy. Hypertension, type II diabetes, coronary heart disease, stroke, certain cancers, osteoarthritis are examples of obesity related diseases (2). Furthermore, obesity is associated with increasing risk of gallstones and benign prostatic hyperplasia (8).

In addition obesity in women can lead to more serious problems with their health such as sleep apnoea, psychological disease such as depression, pregnancy complication such as gestational diabetes and hypertension (9).

Obesity is a preventable condition and strongly related to peoples' lifestyle and physical activity. In 2010, in England based on Health Survey For England (HSE) data just 41% of respondent stated that they walk 20 minutes three times a week, 23% made it just once or twice a week, 20% walks 20 minutes (once yearly or never) (7). This can be linked with the percentage of obese people and according to that it is possible to conclude that not many people follow the Department of Health (DH) physical activity recommendation which is 30 minutes for at least five days a week (10).

In addition to life style modification which include recommendation about diet, physical activity and exercise some drugs can be used as an alternative approach (8). Two drugs have been most commonly prescribed or used for the management of this condition in England as an alternative if the life style modification is not beneficial; Orlistat and Sibutramine (suspended after 2010) which accounted for 1.1 million prescriptions for obesity treatment in 2010 as compared to 2000 in which there was just 157,000 prescriptions. An increase of around seven times so the economic consequences of just the cost of drugs used for treatment in 2010 was £36.9 million in contrast to 2000 when the cost of the treatment was £6.6 million (7).

As another treatment approach NICE clinical guidelines recommends that people with more than or equal 40 BMI or people with a BMI of 35 or more as well as other diseases such as diabetes, hypertension, sleep apnoea should be given the offer of weight loss surgery "Bariatric Surgery" this would mean that £1.3 billion will be spent if one fourth of obese people, who met the NICE criteria, undergo surgery between 2010 and 2013 (11).

These direct obesity treatment costs in addition to cost of other diseases developed because of obesity raise a serious economic and health crisis in England and require a rapid intervention to solve this continually increasing problem.

Definition of terms:

Health Survey for England (HSE) is an annual series of survey assessing health and its related behaviours including both adults and children who are living in private households in England. Blood pressure, height, weight, BMI, waist circumference, smoking, alcohol, physical activity, fruits and vegetables consumption, diabetes, cardiovascular diseases, hypertension, general health are the elements mentioned in the last published survey in 2011. The survey was carried out by Research Department of Epidemiology and Public Health at UCL (University College London), Joint Health Surveys Unit of National Centre for Social Research and the National Centre for Social Research, managed and released by Health and Social Care Information Centre (12).

Hospital Episode Statistics (HES) is a data repository containing all admissions, accident and emergency attendances and outpatient appointments for all NHS trusts comprising primary care trusts, acute hospitals and mental health trusts in England (13).

Finished Consultant Episodes (FCE) is a calculation of the number of HES records transferred to Secondary Uses Service (SUS) from hospital providers in regards to the care episodes of admitted patients ending during the financial year (1 April till 31 March) (13).

Admission Episodes is the enumeration of the proportion of the first episodes for the treatment of admitted patients to secondary health care units (13).

Bed Days is the total number of days in which patients engaged hospital beds through the financial year of HES (1 April to 31 of March) (13).

Mean Length of Stay (MOS) is the mean number of days patients stay in NHS hospitals due to specific reasons.

METHODS

The design of the study is categorized as descriptive analytical research, as the Hospital Episode Statistics and Health Survey for England data have been collected from the Health and Social Care Information Centre website {www.hscic.gov.uk} through the years 2001 to 2011 in a quantitative manner and then analysed descriptively to provide a description for these data to make it easy for understanding or more meaningful.

The core English population is represented in this study due to the inclusion of all NHS trust's admissions, accident& emergency attendances and outpatient appointments in Hospital Episode Statistics data (more than 150 million of accident and emergency, outpatients and admitted patient's records have been processed each year) (13) together with the inclusion of a representative sample of people who live in private household in England in Health Survey for England data.

As this study includes a secondary analysis of publically available online data, the ethical approval is not required for this type of data used in this research. The full methodology of the HSE and HES are detailed included in each year report and published in the above mentioned website.

Obesity in adults is defined in the data used in this study as those having a Body Mass Index (BMI) equal to or more than 30, while childhood obesity defined through the use of the British 1990 growth reference (UK90) which is equal or more than 98th centile for clinical assessment and 95th centile for population monitoring(12,13).

In this study, Hospital Episode Statistics data was selected based on primary diagnosis of obesity which is coded as {E66} as a summary of all subclasses, but the subclasses or subdivisions of obesity which are: obesity due to excess calories {E66.0}, drug induced obesity {E66.1}, extreme obesity with alveolar hypoventilation {E66.2}, other obesity {E66.8}, and obesity: unspecified {E66.9} were not included or clarified in this study to avoid disorganization or confusion. The HES included codes are gleaned from the International Classification of Diseases (ICD) 10th revision.

The statistical analysis of this study was performed via using of "SPSS 20.0". After collecting data based on its year, the data has been summarized and tabulated in clear tables as discussed in the results section and descriptive statistics has been used such as percentages, minimum and maximum in addition to linear graphs, bar and pie charts have been plotted to indicate clearly the relationship between different age groups and genders in regards to obesity admissions, FCE, bed days and prevalence and to illustrate the true size of obesity as one of the major health and economic problems in England.

RESULTS

Health Survey for England

The Health Survey for England data regarding obesity prevalence among children and adults over a period of eleven years from 2001 to 2011 has been collected, summarized and tabulated clearly as in the tables below in addition to linear graphs, bar and pie charts plotted to show the differences between age groups, genders and years in respect of obesity prevalence.

The results in Table (1) summarize and illustrate obesity prevalence among children in England across different age groups from 2001 to 2011, a total data of 43,050 children with an age range of (2-15) years have been utilized in this study obtained from HSE during the eleven years. It can be noticed that the minimum prevalence recorded was in 2011 among boys aged (2-10) years which was 12.4%, while the maximum was 26.7% among girls aged (11-15) years in 2004.

Table (1): Childhood obesity prevalence in England (2001-2011)

Year	Age (2-10) Boys %	Age (11-15) Boys %	Age (2-10) girls %	Age (11-15) girls %	All Children (2-10) %	All Children (11-15) %	All Children (2-15) %
2001	13.6	19.0	13.0	18.0	13.3	18.5	15.2
2002	15.5	20.3	16.1	19.8	15.8	20.0	17.4
2003	15.4	20.4	12.7	22.2	14.1	21.3	16.9
2004	16.2	24.3	12.8	26.7	14.6	25.5	18.9
2005	17.1	20.5	17.4	21.1	17.3	20.8	18.6
2006	17.4	17.9	13.5	17.3	15.5	17.6	16.3
2007	16.5	18.2	14.6	19.4	15.5	18.8	16.8
2008	14.4	20.6	13.3	18.3	13.9	19.5	16.0
2009	13.7	19.7	15.2	15.4	14.4	17.7	15.7
2010	15.3	19.9	13.9	16.6	14.6	18.3	16.0
2011	12.4	23.8	15.5	16.5	13.9	20.2	16.3

The linear graph plotted (Figure 1), for clarity and ease of comparison shows the data in Table 1. in respect of childhood obesity prevalence for the age groups and time periods. Childhood obesity prevalence fluctuation among years and age groups are clearly seen in Figure (1). Children aged (11-15) years have higher obesity prevalence as compared to those aged (2-10) years in England. Obesity prevalence among English adults is presented across different age groups and genders during the eleven years period (2001-2011) in Table (2). From this table, it is easy to recognize the increasing manner of obesity prevalence from year to year in addition to the proportion of women who suffer from this condition appear to be more than men. It is possible to conclude from Table (2) that more health-wise data (less obesity prevalence) can be distinguished in 2011 in comparison to 2010.

A total data of 94,268 adults have been included in this study obtained from HSE during eleven years (2001-2011).

Figure (2) clarifies the differences among age groups regarding adult's obesity prevalence in England from 2001 to 2011. Among adult's age groups in the specified time periods, the predominant age group in respect of the highest obesity prevalence was (55-64) years which accounts for six out of eleven years almost 55%. On the other hand (65-74) year's age group peaked in 2003, 2005, 2006 and 2008. 2007 was the only year (from the specified time period) in which age group (45-54) years was recorded to be the age group which accounts the highest obesity prevalence among adults in England.

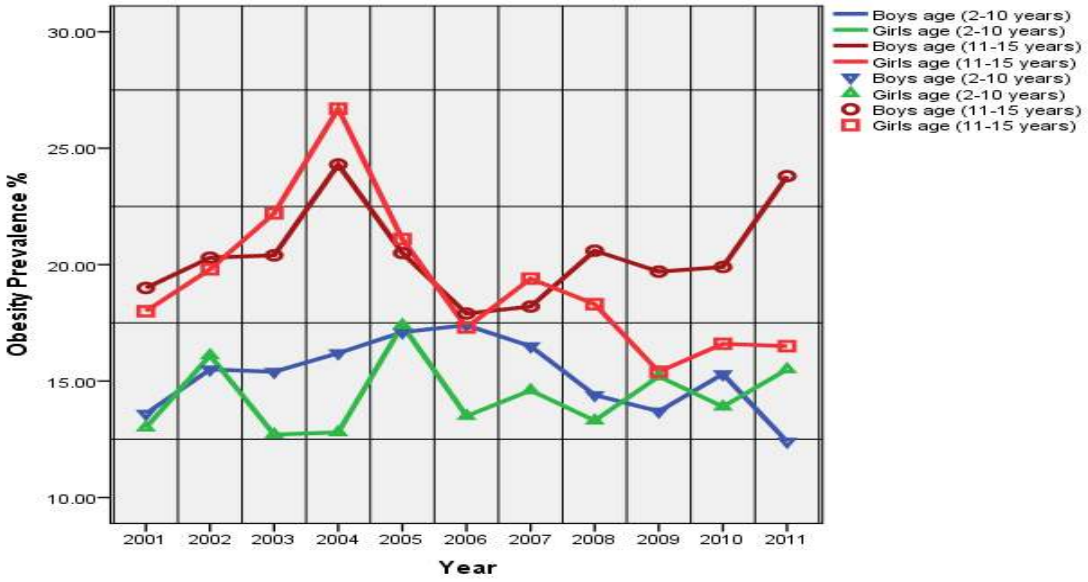

Figure (1): Childhood obesity prevalence in England (2001-2011)

Table (2): Adult's obesity prevalence in England (2001-2011)

Year	Obesity prevalence across age group %							All Adults %	All men %	All women %
	16-24	25-34	35-44	45-54	55-64	65-74	75+			
2001	10.8	17.8	22.4	26.9	28.7	27.5	19.4	22.4	21.0	23.5
2002	10.3	19.4	23.3	26.5	28.6	27.5	21.1	22.5	22.1	22.8
2003	10.8	17.8	23.8	27.3	27.4	29.4	24.1	22.6	22.2	23.0
2004	9.9	17.4	24.6	28.5	31.1	28.3	19.9	22.9	22.7	23.2
2005	9.9	17.7	26.3	28.2	28.3	30.5	22.6	23.2	22.1	24.3
2006	10.5	19.5	24.4	27.7	31.6	32.9	23.4	23.9	23.7	24.2
2007	9.7	17.2	24.2	32.3	31.0	30.2	24.6	24.0	23.6	24.4
2008	10.7	18.2	25.7	29.8	32.4	33.1	24.5	24.5	24.1	24.9
2009	10.9	14.9	22.7	30.3	30.5	30.4	24.4	23.0	22.1	23.9
2010	12.0	20.3	26.9	32.3	34.1	32.4	26.6	26.1	26.2	26.1
2011	10.7	18.6	22.6	31.8	31.9	31.2	30.3	24.8	23.6	25.9

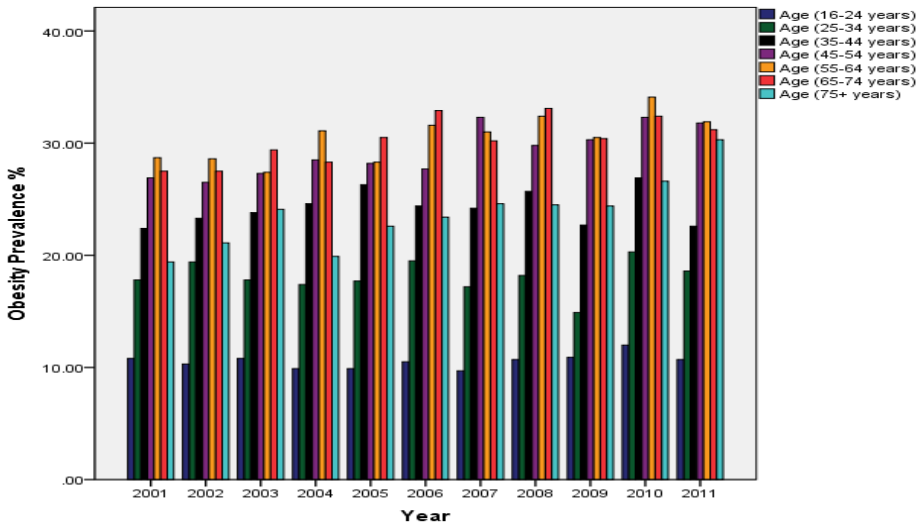

Figure (2): Adult's obesity prevalence in England (2001-2011)

Gender differences among adults in England in respect of obesity prevalence can be seen in Figure (3), and from this figure it is possible to conclude that women suffer from obesity more than men during the whole period of study.

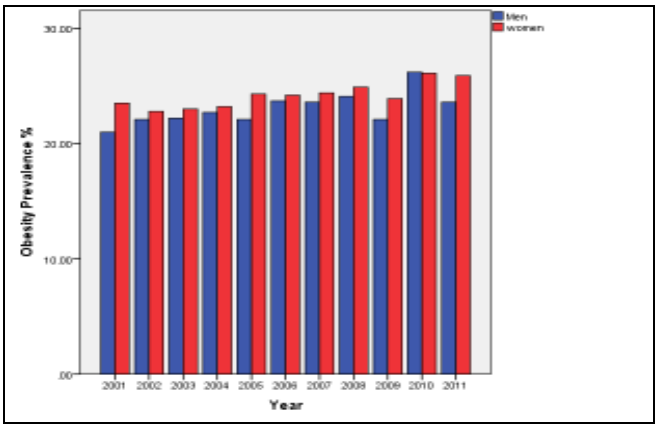

Figure (3): Gender differences in obesity prevalence among English adults (2001-2011)

Adult's age group differences or the percentages of age group regarding obesity prevalence in 2011 can be easily seen in Figure (4) below.

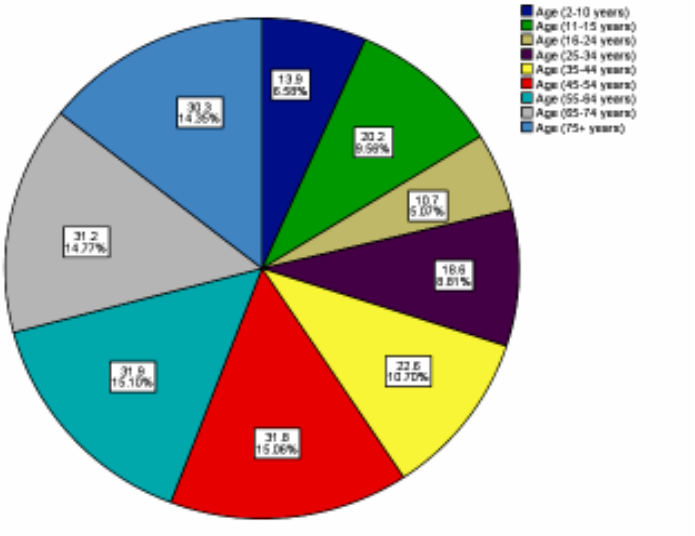

Figure (4): Obesity prevalence based on age groups among English adults in 2011

The variation between children and adults based on obesity prevalence can be seen in Figure (5). From this figure, it can be seen that obesity prevalence is lower in children compared to adults.

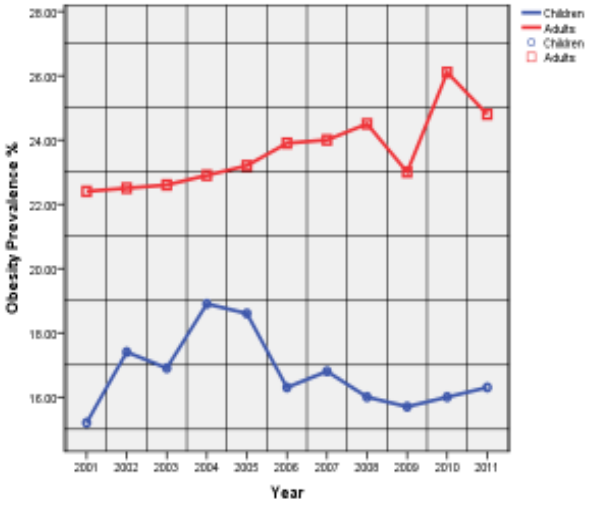

Figure (5): Differences between adults and children in obesity prevalence (2001-2011)

Hospital Episode Statistics

The results in table (3) proved that a total of; 63,349 Finished Consultant Episodes which includes 16,878 males. The number of admissions was 60,096 and 172,769 bed days have been recorded from all NHS trusts in England during the eleven years (2001-2011). In addition the age group which accounts the highest percentage of Finished Consultant Episodes was (15-59 years).

The increasing trend in all variables shown in table (3) can be recognized easily with the exception of the mean days patients spend in the hospitals which appear to be dropping off.

As a gender differences in respect of Finished Consultant Episodes during the specified time period (2001-2011), males reported having a lower percentage (26.6%) of FCE compared to females as clarified in the table (3).

Between 2001 and 2011 Finished Consultant Episodes based on obesity as a primary diagnosis increased from 1,148 to 12,567.

Figure (6) shows the extraordinary increase in hospital admissions in respect of obesity as a primary diagnosis rising from 1,038 admissions in 2001 to 11,905 admissions in 2011.

Table (3): Hospital Episode Statistics (2001-2011)

Year	Finished Consultant Episodes	Admissions	Male	Mean length of stay (days)	Mean Age	Age 0-14	Age 15-59	Age 60-74	Age 75+	Bed Days
2001	1,148	1,038	327 (28.5%)	9.9	36	224 (19.5%)	781 (68.0%)	110 (9.6%)	27 (2.4%)	6,803
2002	1,442	1,297	480 (33.3%)	9.6	33	358 (24.8%)	933 (64.7%)	117 (8.1%)	34 (2.4%)	7,244
2003	1,894	1,744	563 (29.7%)	7	34	517 (27.3%)	1,200 (63.4%)	137 (7.2%)	40 (2.1%)	8,234
2004	2,215	2,063	639 (28.8%)	6.9	35	495 (22.3%)	1,559 (70.4%)	118 (5.3%)	42 (1.9%)	9,334
2005	2,765	2,576	815 (29.5%)	6.3	37	529 (19.1%)	2,007 (72.6%)	202 (7.3%)	24 (0.9%)	10,879
2006	4,082	3,876	1,123 (27.5%)	4.8	39	581 (14.2%)	3,134 (76.8%)	310 (7.6%)	57 (1.4%)	13,174
2007	5,333	5,056	1,517 (28.4%)	4.2	40	642 (12.0%)	4,214 (79.0%)	393 (7.4%)	80 (1.5%)	15,009
2008	8,451	8,085	2,234 (26.4%)	3.9	42	694 (8.2%)	6,946 (82.2%)	711 (8.4%)	97 (1.1%)	20,284
2009	11,173	10,716	2,695 (24.1%)	3.7	43	553 (4.9%)	9,397 (84.1%)	1,102 (9.9%)	118 (1.1%)	25,322
2010	12,279	11,740	3,180 (25.9%)	3.4	45	457 (3.7%)	10,291 (83.8%)	1,359 (11.1%)	162 (1.3%)	27,431
2011	12,567	11,905	3,305 (26.3%)	3.3	45	442 (3.5%)	10,425 (83.0%)	1,499 (11.9%)	179 (1.4%)	29,055
Total	63,349	60,096	16,878 (26.6%)							172,769

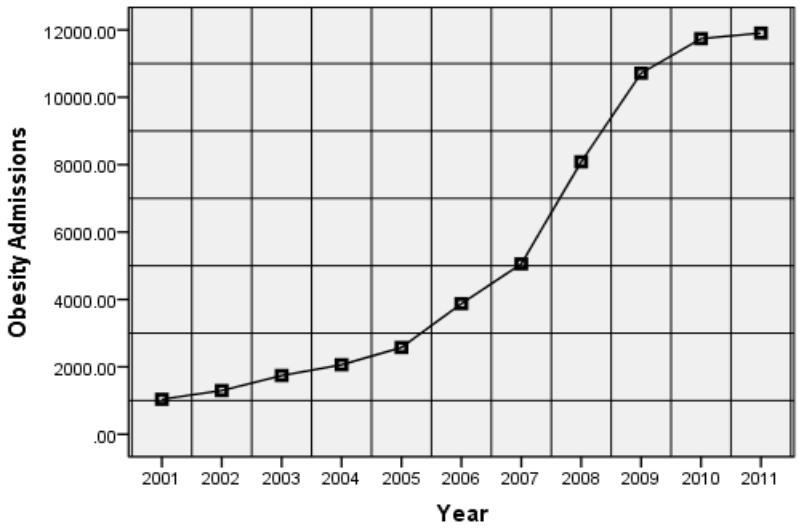

Figure (6): Obesity hospital admissions (2001-2011)

Apparent differences in the percentage of FCE between males and females in England have been reported and indicate that more females have been recorded than males in respect of FCE. This is clarified in Figure (7).

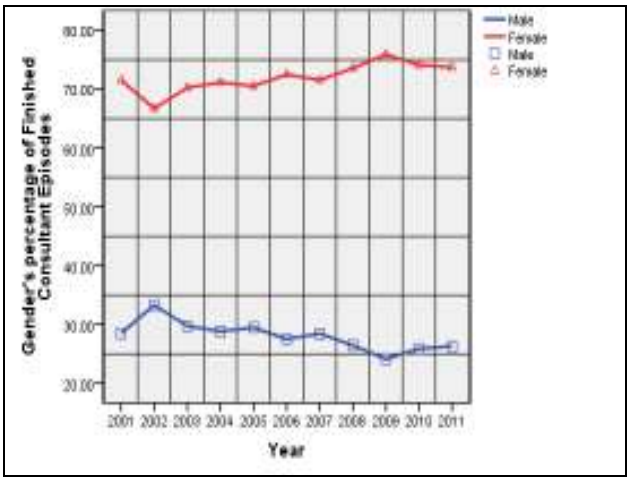

Figure (7): FCE percentage's differences between genders (2001-2011)

In England, the number of days in which hospital beds were occupied by obesity patients has increased over the years, growing from 6,803 days in 2001 to 29,055 days in 2011.

Figure (8) provides information about the differences between age groups according to the percentage of FCE, the highest percentage of FCE recorded during the eleven years (2001-2011) is linked with people aged (15-59) years in England.

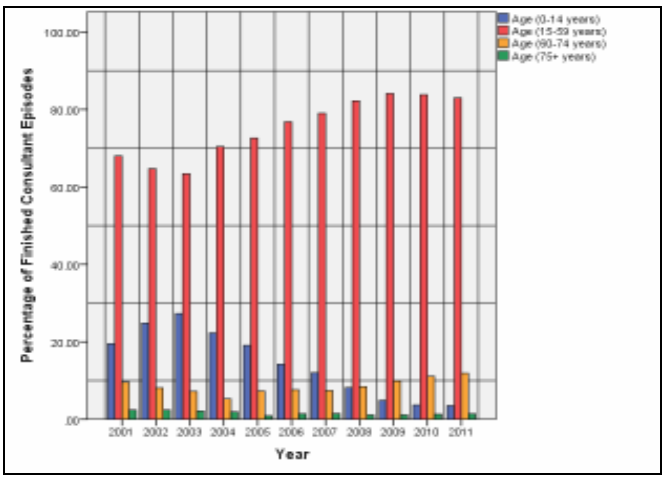

Figure (8): Differences between age groups according to FCE percentage (2001-2011)

DISCUSSION

From the results of analysis of the study which is, to my knowledge, is the first study that utilized all HES and HSE data ,which are large and nationally representative sources, which reflected general and hospital population in England to examine obesity and its prevalence among genders and different age groups over an eleven year period (2001-2011), many observations can be derived.

When obesity prevalence examined among a total of 43,050 children who participated in the annual HSE between 2001 and 2011, fluctuations which account for almost small amount of difference from year to year with the predominance of children aged (11-15) years as compared to (2-10) years can be distinguished. The analysis of results indicates that during the study period no specific gender preponderance regarding the highest obesity prevalence among children across the two specific age groups in England was found as shown in Figure (1).

In the representative sample of England 94,268 adults contributed to the HSE study over eleven years. During the study period, generally, across both genders and age groups obesity among English adults started increasing at the beginning of the period until 2009 in which a small decrease can be noticed, after that it reached the highest prevalence in 2010 then slightly declined by the end of the study period.

Not surprisingly adult's obesity prevalence were proportionally greater in women than in men over the study period. The predominance of women regarding obesity prevalence noticed in the results of study analysis as shown in Figure (3) have been noted by other authors in other studies who assessed this issue in different time period and age groups among English adults (14-19).

Considering adult obesity prevalence in relation to their ages, obesity is most common among people aged (55-64) years and (65-74) years with a decline among younger adults. The possible reasons for this are manifold, but can be illustrated by difficulty in losing weight once have gained and lower physical activity level among these age groups.

The study results indicate that obesity prevalence among adults outstripped that among children between 2001 and 2011 in England.

Despite the importance of the effect of obesity (as a disease and a risk factor for other diseases) recognition among hospital inpatients on the improvement and implementation of weight management clinical guidelines, very limited or no studies have assessed this issue. Obesity continues to be rising at an accelerating rate among English hospital population, the study results suggest that there was a sharp increase in the obesity, eleven times as much, (as a primary diagnosis) patient admissions to NHS hospital between 2001 and 2011, which recorded a total of 60,096 admissions during these years.

In the same manner the number of FCE which is total care time provided by one consultant to the patient, delivered from hospital providers to SUS continually and sharply increased and reached in 2011 around eleven times more than what was recorded in 2001 through a total of 63,349 FCE registered over the study period. In addition the FCE trend dramatically agreed with that in adulthood prevalence in respect of gender differences as women were more likely than men to have obesity FCE record. It should be noted that people aged (15-59) years account for the highest FCE recorded while no significant differences were found

between other age groups which account for lower percentage of recorded FCE.

On the other hand, the mean number of days, patients stay in NHS hospitals (MOS) appears to be continually decreasing from year to year and by 2011 reached just one-third of what it was in 2001. While the total number of days in which NHS hospital beds were occupied by patients due to obesity continually increased to more than four times between 2001 and 2011. One possible explanation of this may be the improvement in bariatric surgery as a secondary procedure for those who were diagnosed with obesity so that less surgery complications occurred and therefore required less days for patients to remain in the hospitals, as between 2012 and 2013 the average hospital stay length for all bariatric surgery was 2.5 days in addition to this during the four financial years (2009-2012) the survival rate for all bariatric surgery was 99.89% (20). This means while the rising trends in the admissions and so bed days, more patients have been treated efficiently with better outcomes.

The study results indicate that, although a slight decrease in obesity prevalence among English adults have been recognized by the end of the study period, obesity rising rates and its proportion among hospital and general population over the study period continues to be a public health challenge and reached an alarming proportion in England which requires a rapid intervention to prevent and treat it and so its co-morbidities which placed a burden on both community and health care system in England.

Study Limitations

This study has limitations, which may bias or under represent the results and its interpretation. These limitations are summarized as follows:

1. The HSE data includes only people who are living in private households and does not take into account institutionalized individuals, although those subgroups do not account for a high percentage of people in England, but this may under represent the actual number of obese people in the study.
2. The HES data includes only patients treated in NHS hospitals, people treated privately in independent sectors (limited number) are not included, and so hospital inpatient may be more than what is reported in this study.
3. Although HSE is considered as a good representation of people in England, response bias for HSE should be considered as its response was around 60% during the study period (66% in 2011) (12).
4. The HES data used in this study includes only inpatient with a primary diagnosis of obesity. As obesity is associated with many diseases, many obesity patients were admitted to the hospitals with conditions other than obesity, but they are obese. Those people are not included in the data used in this study which leads to decrease in the total number of obese inpatients recorded in this study.
5. The HES age groups data presentation in the online form were not distributed equally as it is presented as; children (0-14) years, adults (15-59) years, (60-70) years and more than 75 years. Due to a wide band range of the (15-59) years age group, this age group accounts for the highest number of obesity cases which leads to bias and affects the study results and so its interpretations.
6. Despite its efficacy as a measurement tool for obesity, BMI has some limitations such as its inability to differentiate between lean and fat body mass or body fat and muscle mass, which result in misleading information among some people such as athletes and elderly (21).

CONCLUSION

The study results indicate that with the increasing rates of obesity prevalence among adults and children through genders and age groups, in addition to the sharp increase of obesity as a primary diagnoses among hospital inpatients, obesity in England can be considered as a growing health crisis that requires multi-approach interventions for treatment as well as prevention.

The alarming increasing trends of obesity among hospital inpatients discussed in this study results provide a partial image of the problem size as obesity is associated with many diseases and risks such as type II diabetes, hypertension, high cholesterol, coronary heart disease, orthopaedic problems, asthma, sleep apnoea, psychological problems, and fatty liver diseases (22). Those obese patients treated in the hospitals with a primary diagnosis of these diseases other than obesity are not involved in the study results. In 2011, 53% and 44% of obese men and women correspondingly were found to have hypertension. These obesity consequences increase the burden of obesity which according to DH costs £5 billion a year (23), and is estimated to rise to £49.9 billion per year by 2050 (24).

According to the evidence that obesity is linked with eating habits together with the level of physical activity or people lifestyle, the key clues for obesity management will be promoting healthy eating and changing dietary behaviors in addition to encouraging people to meet the recommended level of physical activity (25).

Some specialists believe that obesity increasing trends is linked to increasing sedentary behavior of people, while there is a belief by others that people have not changed or become less active in recent years, but the food people eat and the access to cheap or bad quality food and the environment are the only things that have changed (26). This can be linked to fast food and its association with poorer quality of diet and higher net total energy (27). However, due to the television shows that focus on supporting and educating people about healthily eat, a slight eating behaviour changes was noted which resulted in the closing down of 25 and 21 stores of Mc Donald and Burger King respectively between 2006 and 2007 due to the sales drop which shows the importance of media in managing this problem (28,29).

The UK government supports a policy for the improvement of school meals to make it healthier and so reduce obesity prevalence among children. In addition to this the new UK government policy released in June 2013 was set up to make people select healthier food via providing a color based consistent system of nutritional labels which contains red, amber and green color labels to show the amount of calories, sugar and salt, fat and saturated fat in food products (30). These policies are important strategies in tackling obesity in England. However, across sectional study includes a UK representative sample which indicates that 61% of people link the cause of obesity to food environment and so 71% of UK population supports

the campaigns of healthy lifestyles, and 66% food labeling, on the other hand only 32% supports unhealthy food taxes as obesity tackling strategies or policies.

Above all, providing a free weight loss or management service for obesity assessment and treatment in pharmacies similar to smoking cessation service and improving pharmacists and other health care professionals knowledge about obesity prevalence and promoting awareness about other diseases and death rates linked with obesity via media and streets advertisements in addition to providing calories content of food or the minutes and types of physical activity required to burn food attached to the restaurants menus could be a possible strategy in improving people lifestyles and so solving this health problem (31).

Future studies should be more focused on true recognition of obesity as a separate disease in both general and hospital population as the exact evaluation of this issue and then increasing people's knowledge and awareness about the true size of the problem and its link to other serious health problems will be the key solution for encouraging and improving people's lifestyles. This is linked to tackling obesity as a growing health problem in England.

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Relationship between ischemic heart disease and oral hygiene

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ABSTRACT

The aim of this study was to explain the effect of oral hygiene on ischemic heart disease by studying isolates of *Staphylococcus aureus* depending on the sensitivity test , minimal inhibitory concentration (MIC) values for selected antimicrobial agents against the study isolates and plasmid curing .
Thirty three patients admitted to Ramadi Teaching Hospital in the lobby of intensive care were included in this study . Sensitivity test using minimal inhibitory concentration (MIC) method was done for selected antimicrobial agents against the study isolates to explain the level of resistance . Furthermore plasmid curing for resistant isolates for cephalexin antibiotic .
Out of 25 isolates of *Staphylococcus aureus* , MIC was done for three isolates which were resistant for cephalexin antibiotic . Furthermore these three isolates were still resistant to cephalexin antibiotic .
It is concluded that good oral hygiene could be more effective against heart diseases in particular in people who had a history of heart diseases among their families.

Keywords: Ischemic heart disease , oral hygiene

الملخص باللغة العربية

الهدف من هذه الدراسة هو لتوضيح تأثير صحة الفم على مرض القلب الوعائي بواسطة دراسة عزلات من بكتريا المكورات العنقودية الذهبية بالاعتماد على اختبار الحساسية ، قيم التركيز المثبط الادنى للمضادات المايكروبية المختارة ضد العزلات المدروسة وتحييد البلازميد . ثلاثة وثلاثون مريض دخلو مستشفى الرمادي التعليمي في وحدة العناية المركزة . اختبار الحساسية وطريقة التركيز المثبط الادنى قد عمل للمضادات المايكروبية لتوضيح مستوى المقاومة . بالاضافة الى ذلك استخدام طريقة تحييد البلازميد للعزلات المقاومة للمضاد الحيوي السيفاليكسين . (25) عزلة من بكتريا المكورات العنقودية الذهبية قد عمل لثلاثة منها طريقة التركيز المثبط الادنى وكانت مقاومة للمضاد الحيوي السفاليكسين . بالاضافة الى ذلك هذه العزلات الثلاثة بقيت مقاومة للمضاد الحيوي السيفاليكسين . نستنتج بأن صحة الفم الجيدة او العناية بصحة الفم اكثر فاعلية تجاه امراض القلب خاصة هؤلاء الذين لديهم وراثة بأمراض القلب ما بين عائلاتهم او تعرضوا لأزمة قلبية مسبقة . اذن صحة الفم هي ضرورية قبل وبعد الأصابة بأمراض القلب .

INTRODUCTION

Ischemic heart disease is also known as coronary heart disease (CHD) which represents the leading cause of premature death in the developed world and it is estimated to become, by 2020 the major cause of death worldwide. Diseases of the coronary arteries are always due to atherosclerosis and its complications, particularly thrombosis. Atherosclerosis is a progressive inflammatory disorder of the arterial wall , characterized by focal lipid - rich deposits of atheroma that remain clinically silent until they become large enough to impair arterial perfusion or until disruption of the lesion results in thrombotic occlusion or embolisation of the affected vessel . The pathogenesis of atherosclerosis is complex but several risk factors have been identified as the following: Age and sex, family history, hypertension, hypercholesterolaemia, Diabetes mellitus and lifestyle factors (cigarette smoking) (1,2). Association between poor dental health which included (bleeding gums, gingivitis and periodontitis) and coronary heart disease (CHD) have been reported recently (3-6). The connection between gum disease and heart attacks is higher than the connection between high cholesterol and heart attacks (7).The current theory for pathogenesis is that bacteria present in infected gums can come loose and move throughout the body. The same bacteria that cause gum disease and irritate our gums might travel to your arteries (8). Periodontitis seems to influence the occurrence and the severity of coronary proposes two hypotheses for this occurrence. One hypothesis is that periodontal pathogens could enter the bloodstream, invade the blood vessel wall and ultimately cause atherosclerosis. (Atherosclerosis is a multistage process set in motion when cells lining the arteries are damaged as a result of high blood pressure , smoking , toxic substances and other agents) . Another hypothesis is based on several studies that have shown that periodontal infections can be correlated with increased plasma levels of inflammatory factors like fibrinogen (this creates blood clots) , C - reactive protein , or several cytokines (hormone proteins) (9). Researches reveal that diseased gums pump high levels of harmful bacterial components into the bloodstream . Oral Mucosa is very rich with blood vessels and if outside bacteria and the toxins which they produce it get into the bloodstream, they are off and running throughout the body (7,10). Some of the proposed mechanisms of the gum disease (Periodontitis) and heart disease association are :

A- Studies showed that the oral bacteria like *Streptococcus sanguis* does cause the clumping of blood platelets . This clumping can be the first stage in the development of a blood clot , the cause of a heart attack . The periodontal infection would reduce the health of the lining of the gum tissues, which, in turn, can allow bacteria from the mouth to enter into the underlying tissues (11). B- Patients with periodontitis have significantly higher levels of inflammatory products such as fibrinogen and white blood cells, which are well known risk factors for acute heart attacks (12). C- Dental bacterial components affects the body's response to infection and can play a role in the development of atherosclerosis (13). D- New development in medical research is further raising concerns that bacteria can cause heart attacks . One bacteria such as *Chlamydia pneumonia*e has been found in the wall of the blood vessel of patients who have had heart attacks (14).

PATIENTS AND METHODS

Thirty-three swabs were taken from both sexes of patients admitted to Ramadi Teaching Hospital, in Ramadi during the period from January to March 2013. Out of 33 patients, 20 were male and 13 were female as shown in table (1). The age of the patients was varying between 20 years and 80 years old with mean of 50 years.

Table (1) : Sex distribution of patients and control

Age group (years)	Patients		Control	
	Males	Females	Males	Females
(20-50)	8	4	7	3
(51-80)	12	9	0	1

Methods

Swabs were cultivated on blood agar, chocolate agar, MacConkey agar and incubated aerobically for 24 - 48 hrs at 37 C°. Bacterial isolates were identified using Direct Gram stained smears and biochemical test as described by (15).Bacterial isolates were kept frozen in glycerol brain heart infusion (10%) to be used for Antimicrobial Sensitivity test.

Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried to study the multi - drug resistant patterns of *Staphylococcus aureus* isolates. Different antibiotics namely Rifampin, Cefodizime , Gentamicin , Cephalonia , Nalidixic , Trimethoprim , Amoxicillin and Clavulanic acid were tested . Muller Hinton agar plates were surface inoculated with 20 Ml of 24 hrs. bacterial inoculum and then discs of different antibiotics were placed uniformly on the surface of the plate . Plates were incubated at 37 C° for 24 hrs . The sensitivity and resistance of the isolates were determined according to (16).

Preparation of antimicrobial solutions

They were prepared as described by (17). Cephalexin 500 mg / ml were prepared as stock solution of l000 µg/ml of antibiotic powders in distilled water, sterilized by filtration through Millipore filter 0.22 µm and stored at - 20 C °.

Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) were using to know the level of resistance for antibiotics which were as the following :
The double fold dilutions of antimicrobial in five ml volumes of broth were prepared. A starting range of about eight fold higher than the normal MIC for the species has been tested and extended to at least one dilution below that of the control organism . A drug free control tube was included , thereafter one set of tubes was inoculated with a drop of well grown broth culture of the test organism diluted one in hundred (about 10^5 organism) and the other with the control organism similarly diluted and then incubated overnight . After incubation , the last tube which diluted and then incubated overnight . After incubation , this tube which shows no growth will represent the minimal inhibitory concentration (MIC) (18).

Plasmid curing

To demonstrate whether the resistance for antibiotics for the selected isolates were plasmid or chromosomal origin, plasmid curing was carried out according to (19). One ml of brain heart infusion broth medium containing 10 % SDS was inoculated with 10 µl of an overnight culture then incubated at 37 °C for 24 hrs. Two fold dilutions were made, after that, 10 µl was streaked over nutrient agar medium plates and incubated at 37 °C for 24 hrs. The separate colonies (mutants) were re-subcultured on nutrient agar plates to ensure their purity. These isolates were tested for their antibiotic sensitivity and presence of plasmids.

RESULTS

Results of this study showed that three isolates of *Staphylococcus aureus* , out of twenty five bacterial isolates as shown in table (2) , were resistant for cephalexin, which determined by disk diffusion test. In order to determine the proper concentrations of antibiotics to be used in the selective media for the minimal inhibitory concentration (MIC) and plasmid curing . Bacterial strains were cultured on media containing different concentrations of antibiotics to determine the level of resistance of these strains to each antibiotic and at what concentration can be used for the selection of plasmid curing. For this reason, the exact MICs for several antibiotics were not determined, because some bacterial strains were able to grow on the highest concentration used in the study.
Results were showed that both strains of *Staphylococcus aureus* No.1 and *Staphylococcus aureus* No.3 were resistant for cephalexin antibiotic and the MICs were 10 µg/ml . While the strain of *Staphylococcus aureus* No.2 was also resistant to cephalexin and the level of resistance was 15 µg/ml .The minimal inhibitory concentrations (MIC) of curing agent used in this study was determined in brain heart infusion broth using *Staphylococcus aureus* No.1, No.2 and No.3. The curing concentration used for each bacterial strain was the highest concentration of curing agent that still allows bacterial growth. Bacterial growth in different concentrations of curing agents was monitored visually, and the lowest concentration that inhibited the growth considered as the MIC. The minimal inhibitory concentrations of sodium dodecyl sulphate SDS (10%) for *Staphylococcus aureus* No.1 was not found (There is no inhibitory concentration for this strain) while the MIC for *Staphylococcus aureus* No.2 was 5 µg/ml and subminimal inhibitory concentration (the highest concentration allows bacterial growth) was 10 µg/ml also the MIC for *Staphylococcus aureus* No.3 was 10 µg/ml while the subminimal inhibitory concentration was 15 µg/ml .

Table (2): Distribution of bacterial types among patients and control

Bacterial spp.	Patients		Control	
	Male	Female	Male	Female
Staphylococcus aureus	15	10	7	3
Micrococcus	3	1	1	1
Streptococcus pyogenes	11	6	5	4
Candida	6	5	0	0
Yeast	0	4	0	0
Diplococci	7	6	4	3

DISCUSSION

Plasmids are extra chromosomal circular DNA molecules found in most bacterial species and in some species of eukaryotes . The molecular weights of plasmid DNA range from 10^6 Dalton for the smallest plasmid to slightly more than 10^8 dalton for the largest one (20).In nature , plasmid can be lost spontaneously from a very few bacterial cells but the probability of this loss is extremely low , ranging from 10^{-5} to 10^{-7} (20,21). However the majority of plasmids are extremely stable , and require the use of curing agents or other procedures that might increase the plasmid loss , and these form the basis of artificial plasmid elimination (20-22). Sodium dodecyl sulphate as a detergent is known to act on the bacterial membrane (23). According to (24), SDS might gain access to the membrane via the pili that the plasmids are attached to membrane close to the pili and may thus be damaged . Exposure to SDS lead to selection of clones

completely resistant to pilus - specific phages and they concluded that non - susceptibility to SDS is strongly correlated with failure to produce pili (25). Our results on curing experiments revealed that *Staphylococcus aureus* No.1 , No.2 and No.3 were very resistant and the MIC was higher than the highest concentration used in this study 15 µg/ml . Samples from subminimal inhibitory concentration sub MIC (the highest concentration allows bacterial growth) were taken to test their antibiotic sensitivity and presence of plasmids (20).

Many plasmids can not be cured (refractory) according to (26). It should be mentioned that failure of plasmid curing does not imply that the trait is not plasmid - encoded . After the treatment of bacterial strains with the curing agent sodium dodecyl sulphate SDS (10%) no cured cells were obtained from *Staphylococcus aureus* No. , No.2 and No.3 which means that these cells have not lost resistance marker for cephalexin and this resistance marker were encoded chromosomally. The efficiency of curing generally varies from less than 0.1% to more than 99% depending upon the agent involved , the bacterial strain and the conditions used and he assumed that curing activity is generally related to the ability of these compounds to intercalate into the DNA molecule (27) . After treatment of bacterial strains with curing agent sodium dodecyl sulphate SDS (10%) no cured cells were obtained from *Staphylococcus aureus* No.1 , No.2 and No.3 which means that these cells have not lost resistance marker for cephalexin and this resistance marker for cephalexin were encoded chromosomally .

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Mathematical expression model for the suspended solid parameters in Al-Yarmook water treatment plant

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ABSTRACT

Removal of the suspended solids from water is one of the major parts of the water treatment processes. The high cost of the usage of chemical for water treatment has initiated various researches into finding alternative methods of improving potable water quality. In This research, a mathematical expression model was developed and conducted of the parameters that affecting the removal efficiency of the suspended solids from water. The change in the concentration and turbidity value as a water quality parameter was studied. Mathematical models for suspended solid (S.S), were developed based on the relationships between the suspended solids (S.S) concentration in water with, temperature ,turbidity, settling time, alkalinity ,power of hydrogen and mass of the suspended solids. Results showed that for both the suspended solid and turbidity of water is the cumulative effect of the individual parameters/factors affecting the system. Polymath3 software was used for the assessment the reliability of the model's equation. A model equation for the evaluation and prediction of a clarifier's performance was developed according to the degree of removal for the suspended solids, and the improvement in the turbidity values for Al-Yarmook treatment plant.
It found from the model that the removal efficiency related strongly to the settling time and suspended solid mass, and less for the other parameters. It found that the model followed the expression as:
$S= S_o(-2.14801+0.120911x10^{pH}+0.0020108T+ 0.01619C_{alk}+0.392\ t_{sett.}+0.2201M_{s.s})$ The developed model will aid the predictive assessment of water treatment plant performance.

Keywords: Suspended solid, turbidity, pH, Alkalinity, Mathematic model, Polymath 3

الملخص باللغة العربية

تشكل عملية إزالة الشوائب الصلبة العالقة في المياه جزءا هاما من عملية تنقية ومعالجة المياه ، وقد أدت التكاليف الباهظة الناتجة من استخدام الطرق الكيميائية لمعالجة المياه إلى تشجيع البجانب البحثي لإيجاد حلول أخرى أكثر فاعلية وأقل تكلفة ، وعليه، فقد سعى البحث الحالي إلى تطوير وتنفيذ نموذج مقترح للتعبير الرياضي لبعض المقاييس التي تؤثر في فعالية إزالة الشوائب الصلبة من المياه، وقد أظهرت نتائج البحث فعالية هذا النموذج في إزالتها وتنقية المياه من التعكر بنسبة فعالة.

INTRODUCTION

Water plays an essential role in community development since a reliable supply is a prerequisite for establishing a permanent settlement (1)
There is a vast amount of water present in the earth and surrounding atmosphere. About 7% of the earth's mass is made up of water. 97% of this is found as saline water in oceans, about 2.3% is in the polar caps and only 0.7% exists in fresh water lakes, rivers, aquifers and in the atmosphere (2).
Water that is pure is not found in nature; even water vapour condensing in air contains solids and dissolved gases (3). As it condenses and falls, it sweeps up other materials from the air, it becomes still more contaminated on reaching the ground, as it runs running over soil surface and percolate the soil strata.
Wastewater resulted from human activities, either industrial or domestic, considered one of the major pollutants that affecting the quality of the water resources (4). These pollutants introduce even more contamination load than any natural sources into water bodies.as a result, a treatment plant is clearly necessary to improve the water quality. Water supply to a community goes through the following stages; the community water demand is carefully estimated with allowances for population growth; the most suitable raw water source is identified and analysed; then a water treatment plant to effect the required changes is designed, constructed and operated along with its own distribution network (5).
One of the major important issues that reflecting the degree of the performance of any water treatment plant is a periodic review of plant performance to ascertain if, or otherwise, the plant works according to prediction (6,7). Okun (8) agreed that record–keeping and periodic reviews of plant performance are necessary decision tools when the plant requires expansion or when operational problems arise.
The aim of this research is to model the suspended solids and turbidity in water as function of several other parameters that will aid in assessing the performance of a Al-Yarmook water treatment plant over a period of time and to suggest ways of improving plant performance

MATERIALS AND METHODS

Al-Yarmook drinking water compact unit treatment plant located (25km) south west of Messan governorate on the western-bank of Tigris River. The water supplies to the plant by four in river intakes, the plant consists of two clarifiers with a total capacity of ($100m^3$/hr.) that their functions are to reduce the solid content in the water coming from the river to pass through the sub-sequent treatment stages till pumps through the network. A sample of water was taken every 10 min. and according to the standard method of water and waste water analysis a gravimetric method was used in order to determine the suspended solid concentration.

Determination of Temperature

The temperature of the sample of water was determined with the aid of thermometer, beakers. The water samples were collected in beakers and labelled appropriately to avoid mixing up. The thermometer is dipped into the beaker in turn, the readings are recorded.

Determination of Turbidity

This involves the use of turbid meter (HASH2105). The sample cells were washing with sample water, and was discarded and refilled with the same sample. The reading shows-up on a digital display and the units are in NTU.

Determination of pH

The PH of the sample was determined with aid of a portable PH meter (Hanna 305). The probe first calibrated with a provided buffer solution, and then dipped into the sample and the reading was displayed on the LCD display of the meter.

Determination of Total Alkalinity

The total alkalinity was determined using titration. Using of analytical method (titration) to determine the total alkalinity of the sample, a (100ml.)of the sample was put into a conical flask(250ml.), and the base reagent used was NaOH(2M) , and the sample was titrated using Methyl-Orange indicator(1-2 drops /sample) to determine the end point of the titration , as the indicator was introduced to the sample, it colour will turn to orange , and the titration is stopped as the colour of the sample turns to pink colour. The alkalinity then can be calculated mathematically from the general expression for the alkalinity determination.

Modelling For Suspended Solid Removal

In order to construct a model for the (S.S) removal some assumptions may introduced in order to reduce the probability of the mismatched items in the final expression , and as follows:
1. Constant flow rate.
2. The suspended solids (and hence turbidity) removal is a fixed fraction of inlet suspended solid.
3. The particle size distribution is constant over a time period.
4. No change of the (S.S) concentration with respect to the time.
5. The flow is uniform and steady state condition was implemented.
6. Brownian motion effect (for particles) is valid along the flow rate pattern.

Then suspended solid removal is:

$(S_o - S) / S_o = k_{os}$ (1)

Where:

S_o: initial suspended solid concentration (mg./lit), S: treated water suspended solid concentration (mg. /lit), k_{os} = constant

$1 - (S/S_o) = k_{os}$, $S/S_o = 1 - k_{os}$, let $(1 - k_{os}) = k'$,

$\therefore S = (S_o)(k')$ (2)

In reality, as from the practical and operational perspectives for the water treatment plants all the operating conditions do vary (9). Therefore k_{os} , k' are only one of the functions by which water quality may be measured (10). Other essential parameters such as temperature, pH and alkalinity are also affecting this performance (11). It is well established that these parameters exhibit independent and cumulative effect on both suspended solids and turbidity.

Effect of Water Temperature

Water molecules and impurity particles (suspended solid as organic and non-organic matters) that causes turbidity are in thermal Brownian motion whose intensity is directly proportional to temperature (11). It is clear that the probability of collision of individual particles with one another and their consequent aggregation depend on their relative velocities i.e. on thermal Brownian motion (and therefore on water temperature) (12).

Again, it shall be assumed that the temperature profile within the clarifier is constant and is equal to that of the clarified water samples (13). Then; quantities in equation (1) will proportional to the temperature of the water.

$(S_o - S) / S_o \alpha T$ (3)

Where T = temperature in °C.

Effect of settling time

As the process for removing the suspended solid (coarse, medium sizes) is depending strongly on the gravitational forces and allowable settling time, and as settling time can be considered (almost) constant(unless there are a variation in the suspended solid concentration and size distribution)(14) then equation (1) can be re-written as:

$(S_o - S) / S_o \alpha t_{sett}$ (4)

Effect of Alkalinity

Suspended solids and turbidity removal is preceded by and dependent on the formation of chemical flock. The equation of the overall process is:

$Al_2(SO4)_3 + 3Ca(HCO_3)_2 \rightarrow 2Al(OH)_3 + 3CaSO_4 + 6CO_2$

Obviously, good coagulation is dependent on the presence of sufficient alkalinity (HCO_3^-) and therefore:

$(S_o - S)/S_o \alpha C_{alk.}$ (5)

Where $C_{alk.}$ = alkanity of water in (mg/lit.).

Effect of pH

Suspended matters in water are surface–charged particles and it is a function of the coagulant to neutralize the charges. Different particles types have been seen to have a particular pH at which the net charge on them is zero and coagulation optimum (11). This pH is the electro-balance point (pH_{e-b}). A large difference between pH of the water medium and pH_{e-b} confers greater anticoagulation properties. This effect is confirmed by (3) where turbidity removal is reduced as the water pH deviates from an optimum value of about 7.0.

Therefore, it can be concluded that:

$(S_o - S)/S_o \alpha 1/(pH - pH_{e-b})$ (6)

Assuming pH_{e-b} as 7 then pH – 7 = ΔpH has a positive value.

$pH = -\log[H^+]$

An expression was used by (11) to express the lime dosage required to being about a desired change in the pH of water to pH_{e-b} of its contaminants.

Effect of suspended solid mass

As in a conventional treatment plants, the criterion for the removal of the suspended solids depends strongly on the mass of the suspended particles that may exist in the water. Due to the effect of the gravitational forces that lead to settle and later sediments these particles, the mass of these suspended solids plays a major role on the efficacy and the time of their removal from the water stream.

$(S_o - S) / S_o \alpha M_{s.s}$ (7)

Where $M_{s.s}$= mass of the suspended solids (kg)

Introducing constants into equations 3,4,5,6 and 7:

$(S_o - S) / S_o = k_1 T$ (8)

$(S_o - S) / S_o = k_2 t_{sett.}$ (9)

$(S_o - S) / S_o = k_3 C_{alk.}$ (10)

$(S_o - S) / S_o = k_4 / 10^{-\Delta pH}$ (11)

$(S_o - S) / S_o = k_5 M_{s.s}$ (12)

Other factors, such as organic content do exert influence on suspended solids removal; their contribution can be accounted by introducing a rate of the degradation of such material under the influence of bacterial activities (15), which is beyond the scope e of this research.

Assuming that hydrodynamic conditions are approximately constant, the influences of temperature, settling time pH, and alkalinity on the

suspended solid and turbidity of water respectively, in a clarifier basin may be additive or multiplicative (16). However a change in one will cause dis-equilibrium in the overall turbidity (17). Such changes are accounted for by the various constants. Using the additive method:

$$(S_o\text{-}S)/S_o=k_o+k_1T+k_2t_{sett.}+k_3C_{alk.}+k_4/10^{-\Delta pH}+k_5M_{s.s} \quad (13)$$

From which:

$$S = S_o\,(1 - k_o + k_1\,T + k_2t_{sett.} + k_3\;C_{alk.} + k_4/10^{-\Delta pH} + k_5M_{s.s}) \quad (14)$$

Because $1 - k_o$ is a constant, let put k_o in place of:

$$S=S_o(k_o + k_1\,T + k_2\,t_{sett} + k_3\,C_{alk.} + k_4/10^{-\Delta pH} + k_5M_{s.s}) \quad (15)$$

The values of these constants may be determined using the least – square method for multiple regression as outlined below adapted from (14).

Let "S_i" be an observed value of outlet suspended solid concentration, the value predicted by the model equations are then:

$$S_i = S_{oi}\,(k_o + k_i\,T + k_i\,t_{sett.} + k_i\,C_{alk.} + k_i/10^{-\Delta pH} + k_iM_{s.s}) \quad (16)$$

Then the error of prediction E_i will be given by:

$$E_{i,susp.}=S_i\text{-}S_{oi}(k_o + k_i\,T + k_i\,t_{sett.} + k_i\,C_{alk.} + k_i/10^{-\Delta pH} + k_iM_{s.s}) \quad (17)$$

The square of the error is:

$$E_{i,susp}^2=[S_i\text{-}S_{oi}(k_o + k_i\,T + k_i\,t_{sett.} + k_i\,C_{alk.} + k_i/10^{-\Delta pH} + k_iM_{s.s})]^2 \quad (18)$$

For all data sets, by summing:

$$\Sigma\,E^2_{i,susp} = \Sigma\,[S_i - S_{oi}\,(k_o + k_i\,T + k_i\,t_{sett.} + k_i\,C_{alk.} + k_i/10^{-\Delta pH} + k_iM_{s.s})]^2 \quad (19)$$

Expanding the brackets gives:

$$\Sigma E_{i,susp}{}^2\Sigma S_i{}^2+k_o{}^2\Sigma S_{oi}{}^2+k_1{}^2\Sigma S_{oi}{}^2S_i{}^2T^2+k_1{}^2\Sigma S_{oi}{}^2+\;k_2{}^2\;\Sigma S_{oi}{}^2S_i{}^2\;t_{sett}{}^2+\;k_3\Sigma\;S_{oi}{}^2\;\;C_{alk.}+\;\;k_4/(10^{\Delta pHi})^2\;\;+k_i\Sigma S_{oi}{}^2M_{s.s}+k_2{}^2\Sigma S_{oi}{}^2S_i{}^2+k_3{}^2\Sigma S_{oi}{}^2t_i{}^2-\;2k_ok_1\Sigma S_{oi}{}^2/(10^{\Delta pHi})-2k_ok_2\Sigma S_{oi}{}^2S_i\text{--}\;2k_ok_3\Sigma S_{oi}{}^2C_{alk.}+2k_1k_2\Sigma S_{oi}{}^2/(10^{\Delta pHi}+2k_1k_3\Sigma S_{oi}{}^2t_i/(10^{\Delta pHi})+2k_2k_3\Sigma S_{oi}{}^2T_i\;t_i-2k_o\Sigma S_iS_{oi}\;+2k_1\Sigma S_iS_{oi}/(10^{\Delta pHi})+2k_2\Sigma S_iS_{oi}S_i\;+\;2k_3\Sigma S_iS_{oi}\;t_i+2k_o\;k_i\Sigma\;S_i{}^2S_{oi}{}^2M_{s.s}\text{……..}$$

Partial differentiation of this equation with respect to the constants k_o, k_1 gives:

$$\partial(\Sigma E_{i,susp}{}^2)/\partial k_o = 2\Sigma\;S_i(\partial\;S_i\;/\partial k_o) + 2k_o\Sigma\;S_{oi}{}^2 - 2k_1\Sigma\;S_{oi}{}^2 - 2k_2\Sigma S_{oi}{}^2S_i - 2k_3\Sigma S_{oi}{}^2\;S_i - 2\Sigma S_iS_{oi} -2k_4\Sigma\;S_{oi}{}^2\;/\;(10^{\Delta pHi})^2\text{-}2k_5\;\Sigma\;S_{oi}{}^2 \quad (20)$$

$$\partial(\;\Sigma E_{i,susp}{}^2)/\partial k_1 = 2\Sigma\;S_i(\partial\;S_i\;/\partial k_1) + 2k_1\Sigma\;S_{oi}{}^2/(10^{\Delta PHI})^2 - 2k\Sigma S_{oi}{}^2/(10^{\Delta PHI}) + 2k_2\Sigma S_{oi}{}^2S_i/(10^{\Delta PHI}) + 2k_3\Sigma S_{oi}{}^2\;C_{alk}\;/(10^{\Delta PHI}) + 2\Sigma S_iS_{oi}/(10^{\Delta PHI}) + 2k_4\Sigma S_{oi}{}^2\;S_i{}^2+\;2k_5\Sigma S_{oi}{}^2S_i{}^2/(10^{\Delta PHI})\;M_{s.s} \quad (21)$$

Applying the same procedures in equations(20)and(21) for k_3,k_4,k_5,and Equating all the derivatives to zero yields the following system of equations:

$$k_o - k_1\Sigma\;S_{oi}{}^2\text{-}\;k_2\Sigma S_{oi}{}^2t_{sett.} - k_3\Sigma S_{oi}{}^2C_{alk.}+\;k_4\Sigma S_{oi}{}^2/10^{-\Delta pH} + k_5\Sigma S_{oi}{}^2M_{s.s} = \Sigma S_iS_{oi}\;T_i \quad (22)$$

$$k_o - k_1\Sigma\;S_{oi}{}^2\text{-}\;k_1\Sigma\;S_{oi}{}^2T_i - k_2\Sigma S_{oi}{}^2C_{alk.}+\;k_4\Sigma S_{oi}{}^2/10^{-\Delta pH} + k_5\Sigma S_{oi}{}^2M_{s.s} = \Sigma S_iS_{oi}\;t_{sett.i} \quad (23)$$

The same procedure again can be applied for the remained equations. The constants k_o, k_1, k_2, k_3, k_4, k_5 could be obtained from the solution of the generated (6x6) matrix numerically and analytically. The alternatively the developed models can be solved software packages such as **Polymath3** (18).**Polymath3** , is a proven computational system which has been specifically created for educational and professional use, The various **Polymath's** programs allow the user to apply effective numerical analysis techniques during interactive problem solving on personal computers. Results are presented analytically for easy understanding and for incorporation into papers and reports.The software package was used to find the coefficients in the model (http://www.polymath-software.com/hagen/). The developed model is presented below:

$$S= S_o(\text{-}2.14801 + 0.120911x10^{pH} + 0.0020108T + 0.01619C_{alk}+0.392\;t_{sett.}+0.2201M_{s.s})\;S= S_o(\text{-}2.14801 + 0.120911x10^{pH} + 0.0020108T + 0.01619C_{alk}+0.392\;t_{sett.}+0.2201M_{s.s}) \quad (24)$$

RESULTS

The results of the various experimental methods are as presented in table (1).

Table (1): weekly water quality parameters values

Mins.	Quality parameters(indicators)					$M_{s.s}$
	S_o	T	$t_{sett.}$	ΔpH	$C_{alk.}$	
1	192	24	17.15	0.11	22.82	12.33
2	177	26	16.24	0.23	23.80	12.10
3	181	24.7	19.11	0.14	24.00	14.98
4	175	24.6	18.22	0.11	22.70	13.76
5	178	24.4	19.90	0.10	22.94	14.42
6	186	24.9	20.11	0.10	22.59	11.43
7	192	24.4	18.25	0.80	22.85	13.76
8	187	25.2	19.18	0.220	24.50	12.87
9	193	25.5	18.30	0.40	31.07	12.98
10	196	24.3	19.44	0.100	23.20	15.77

DISCUSSION

Simulation results of the models showed that the model to a large extent will give a better suspended solid prediction. It showed also that factors affecting suspended solid values are mainly independent in operation. The suspended solid concentration of water is the cumulative effect of the individual parameters/factors affecting the system.

From the model, the change in suspended solid mass, settling time caused by one of the dependent variables. The cumulative effects of individual contributions of T_o, $t_{sett.}$, and $C_{alk.}$the relationship of the suspended solid parameters is as the synergy or interdependence of the variables. The nature of the

model equation gives suspended solid change a dimensionless significance.
The discrepancies between the experimental and simulated values could be attributed to the assumptions made during the formulating of the model. The numbers of variables considered were quite small. The effect of coagulation and settling processes and much other need to be considered to enhance the reliability of the model.

CONCLUSION

The model conducted and presented in equation (24) gives an approximate estimation for the suspended solid concentration, hence efficiency of removal from the clarifiers. Limitations of the models are as a result of insufficient variables considered during the conceptualization process.

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Spectral Laguerre technique for integro- differential equation

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ABSTRACT

A computational method based on Laguerre spectral technique is presented in this paper to solve the linear integro- differential equations (IDE). The method approximates the unknown function by a finite Laguerre series olunklown parameters.
The IDE resulted in a system of algebraic linear equations for the coefficients, which can be solved more easily than the original problem. Moreover, the explicit results that simplify the implementation of the method is presented also. The numerical results are coincident to the exact solution.

Keywords: Spectral laguerre , integro- differential equation, parameters

الملخص باللغة العربية

عرضت في هذا البحث طريقة حسابية بالاعتماد على أسلوب لاكير الطيفي لحل المعادلات التكاملية الخطية، وتعمل هذه الطريقة على تقريب الدالة المجهولة بعدد منتهي من سلسلة لاكير ولمتغيرات مجهولة.
إن النتيجة للمعادلات التفاضلية التكاملية هي منظومة معادلات جبرية خطية للمعاملات التي يمكن حلها بسهولة أكثر من المعادلات الأصلية، علاوة على ذلك، هذا البحث يعطي نتائج واضحة تبسط تنفيذ الطريقة، حيث إن النتائج العددية التي ظهرت اتفقت بشكل مقبول مع الحل المناسب لهذه المعادلات.

INTRODUCTION

Integro-Differential equation (IDE) is an equation that the unknown function appears under the sign of integration, also contains the derivatives of the unknown function.
Many different types can arise and there is no straight forward classification (1).
In the science and other branches of mathematics such as mathematic modeling or ordinary differential equations, the results of the work needs solutions of integro- differential equation (2).
In the engineering field, numerical approaches are practiced to obtain an approximation Solution for IDE there are many methods that car be used to solve IDE such as generalized minimal residual method (3), collocation method with trigonometric Wavelet (4). Homotopy analysis method solves linear integro-differential equations (1), and variation iteration method solves nonlinear IDE (5) and others (6).
In this study, spectral Laguere parameterization will be used for solving IDE.

METHODS AND APPLICATIONS

The Laguerre Integration Matrix B_N^m

In this paper, an operational matrix B_N^m is proposed to approximate the integral, as follows:

If a function f(t) can be approximated using Laguerre series of length N

$$f(t) = \sum_{i}^{N} a_i L_i(t)$$

or in a vector from f(t): a L (t) where a =[a_0 a_1 a_2 … a_N], and L (t) = [L_0 L_1 L_2 … L_N]^T

Then the integral of f (t) can be represented as:

$$\int_0^x f(t)dt = a \int_0^x L_i(t)dt$$

The integral of L(t) that is given by [1], is as follows:

$$\int_0^x L_0(t)dt = L_0(x) - L_1(x)$$

$$\int_0^x L_1(t)dt = L_1(x) - \frac{L_2(x)}{2}$$

$$\int_0^x L_2(t)dt = L_2(x) - \frac{L_3(x)}{3}$$

$$\int_0^x L_N(t)dt = L(x) - \frac{L_{N+1}(x)}{N+1} \qquad ...(1)$$

which can also be written in a matrix form, as*:*

$$I_M^1 = B_L^1 L(x)$$

Where $I_M^1 =$

$$\int_0^x L_N(t)dt \qquad , M = 0, 1, \ldots N$$

L (x) = [L_0 L_1 L_2 … L_N L_{N+1}]^T , and

$$B_L^1 = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & \ldots & 0 \\ 0 & 1 & \frac{-1}{2} & 0 & 0 & \ldots & 0 \\ 0 & 0 & 1 & \frac{-1}{3} & 0 & \ldots & 0 \\ & & & & & & \\ \square & 0 & 0 & & 0 & 1 & \frac{-1}{N+1} \end{pmatrix}$$

The matrix B_L^1 is an Nx(N+1) matrix furthermore, from Eq. (1), we can conclude that

$$\int_0^x \int_0^t L_N(t)dt\,dx = L_N(x) - 2\frac{L_{N+1}(x)}{N+1} + \frac{L_{N+2}(x)}{(N+1)(N+2)}$$

Therefore, we can get

$$I_M^2 = B_L^2 L(x) \qquad ...(2)$$

Where $I_M^2 = \int_0^x \int_0^t L_N(t)dt\,dx,$

$I_M^2 = 0, 1, \ldots, N,$

L (x) =[L_0 L_1 L_2 … L_N L_{N+1} L_{N+2}]^T , and the matrix B_L^2 is an Nx(N+2) matrix given by*:*

$$B_L^2 = \begin{pmatrix} 1 & -2 & \frac{1}{2} & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 1 & -1 & \frac{1}{6} & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 1 & \frac{-2}{3} & \frac{1}{12} & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \cdots & 1 & \frac{-2}{N+1} & \frac{1}{(N+1)(N+2)} \end{pmatrix} \quad ...(3)$$

Finally, we can conclude from Eq. (2), that the operational Laguerre matrix of the successive integrate is

$$I_M^N = B_L^N L(x) \quad ...(4)$$

Where

$$I_M^N = \int_0^x \int_0^x \int_0^x \cdots \int_0^x L(t_0) dt_0\, dt_1 \ldots dt\, dx \quad ...(5)$$

The matrix B_L^N is an Nx(N +N), and is called laguerre integration operational matrix.

Problem Statement

The problem we are considering to find the solution y (x) of the class of IDE is defined as:

$$\dot{y}(x) = q(x) + \int_0^x y(t) dt \quad ...(6)$$

with the initial condition

y(0) = a and x > 0

Integrate Eq. (6) to get,

$$y(x) = h(x) + \int_0^x \int_0^t y(t) dt\, dx, \quad ...(7)$$

where h (x) =

$$a + \int_0^x q(t) dt \quad ...(8)$$

This preliminary integration often has a very good effect on accuracy.

Problem Approximation

The numerical algorithm of this is based on applying a spectral method using Laguerre polynomials (2) to approximate the IDE problem into a system of algebraic equations. The unknown function y(x) in Eq.(6) is approximated by a finite length Laguerre series of unknown parameters as follows:

$$y(x) = \sum_{i=0}^{N} a_i L_i(x) \quad (9)$$

where a_i, i = 0, 1, …, N gives (N+1) unknown parameters. Eq. (9) can be rearranged in a vector form as:

$$Y(x) = a\, L(x) \ldots (10)$$

where a= [a_0 a_1 … a_N], and L (x) =[L_0 L_1 … L_N]^T
The initial condition can also be expressed using Laguerre polynomials to obtain,

Since $L_i(0) = n!$, therefore

$$y(0) = n! \sum_{i=0}^{N} a_i = a \quad ...(12)$$

Substituting Eq. (9) into eq. (7), we have,

$$\sum_{i=0}^{N} a_i L_i(x) = h(x) + \int_0^x \int_0^t \sum_{i=0}^{N} a_i L_i(t) dt\, dx \quad (13)$$

where,

$$h(x) = n! \sum_{i=0}^{N} a_i + \int_0^x q(t) dt \quad (14)$$

Eqs. (13) and (14) can be rearranged as follows,

$$aL(x) = h(x) + aB_L^2 L(x) \quad ... (15)$$

Also, the function h(x) can be written as in terms of Laguerre series, since the relation between power series and Laguerre polynomials is given by,

$$x^n = \sum_{k=0}^{n} \frac{(-1)^k (n!)^2 L_k(x)}{(k!)^2 (n-k)!}, n = 0, 1, \ldots \quad (16)$$

That is,
$1 = L_0$
$X = L_1 + L_0$
$X^2 = L_2 - 4\, L_1 + 2L_0$
$X^3 = -\, L_3 + 9\, L_2 - 18\, L_1 + 6\, L_0$
$X^4 = L_4 - 16\, L_3 + 72L_2 - 96\, L_1 + 24\, L_0$

Finally, to determine the unknown coefficients , i = 0,1, .., N, the coefficients at Laguerre polynomials will be equated to get a linear system of algebraic equations.

Numerical Example

A numerical example to illustrate the proposed method is presented in this section.

Example:
We consider the IDE,

$$y(x) = x - \frac{x^3}{3} + \int_0^x y(t)dt \quad y(0) = 1 \text{ and } 0 \le x \le 1 \tag{17}$$

The exact solution is $y(x) = 1 + x^2$
The integrated form of Eq. (17) is

$$y(t) = h(x) + \int_0^x (t)dt\,dx \tag{18}$$

where $$h(x) = 1 + \int_0^x \left(t - \frac{t^3}{3}\right)dt$$

First approximate y(x) in Eq.(18) by third order Laguerre series

$$y(x) = a_0 L_0(x) + a_1 L_1(x) + a_2 L_2(x) \tag{19}$$

Second, write the function h (x) in terms of Laguerre series using Eq. (16), we have

$$h(x) = 6L_1 - \frac{11}{2}L_2 + \frac{4}{3}L_3 - \frac{1}{12}L_4 \tag{20}$$

Inserting Eq. (19) and (20) and using Eq.(2) to obtain,

$$(a_0 a_1 a_2)\begin{pmatrix} L_0 \\ L_1 \\ L_2 \end{pmatrix} - (a_0 a_1 a_2)\begin{pmatrix} 1 & -2 & \frac{1}{2} & 0 & 0 \\ 0 & 1 & -1 & \frac{1}{6} & 0 \\ 0 & 0 & 1 & \frac{-2}{3} & \frac{1}{12} \end{pmatrix}\begin{pmatrix} L_0 \\ L_1 \\ L_2 \\ L_3 \\ L_4 \end{pmatrix}$$

$$= 6L_1 - \frac{11}{2}L_2 + \frac{4}{3}L_3 - \frac{1}{12}L_4$$

or

$$a_0 L_0 + a_1 L_1 + a_2 L_2 - a_0 (L_0 - 2L_1 + \frac{1}{2}L_2) - a_1 (L_1 - \frac{1}{6}L_3) - a_2 (L_2 \frac{2}{3}L_3 + \frac{1}{12}L_4) = 6L_1 - \frac{11}{2}L_2 + \frac{4}{3}L_3 - \frac{1}{12}L_4$$

Equate the coefficients of Laguerre polynomials to get the algebraic equations

$2 a_0 = 6$

$$-\frac{1}{2}a_0 + a_1 = -\frac{11}{2}$$

$$-\frac{1}{6}a_1 + \frac{2}{3}a_2 = \frac{4}{3}$$

Therefore the unknown parameters are
$a_0 = 3$, $a_1 = -4$, and $a_2 = 1$
and the approximate solution is
$y(x) = 3 L_0(x) + (-4) L_1(x) + L_2(x)$

CONCLUSION

The IDE is reduced to a linear system of equation using spectral method with Laguerre polynomials basis. The example shows that the numerical solution convergence to the exact solution, which presents the efficiency of Laguerre polynomials in the solution of IDE.
We also conclude that, this approximate method can be easily generalized to the solution of other equation such as,

$$y^{(m)}(x) = q(x) + \int_0^x y^{(m)}(t)dt, \qquad n > m$$

$$\alpha_i = y^{(m-i)}(0); i = 1, 2, \ldots, n.$$

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Catfish (*Clarias lazera*) as bio- indicator to estimate the levels of heavy metals in lentic aquatic ecosystem in Sebha man-made lake –South Libya

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ABSTRACT

Catfish (Clarias lazera) have been used as bio-indicator to estimate the level of heavy metals (Ni, Co, Fe, Zn, Cu, Cd, and Pb) in Sebha man – made lake, South Libya. The result revealed that the of all metals studied were present in the tissues and organs (muscle, either all of them plural or singular, kidney, liver and gonad) of the fish used as a bio-indicator.
The results of present study revealed that the levels of accumulation of different metals (Co, Ni, Zn, Cu, Cd, Pb) were 0.023, 0.536, 0.481, 0.206, n.d., 0.137 ppm in male fishes, and 0.007, 0.550, 0.412, 0.237, n.d., 0.166 for female fishes respectively. On the other hand Fe levels were recorded much higher levels of accumulation as it reached to 24.44 ppm and 9.34 ppm in male and female fishes respectively. The accumulation levels can give suggestion to use this species of fish as bio-indicator especially for Fe.

Keywords: catfish, bio- indicator, lentic aquatic, man – made lake.

الملخص باللغة العربية

استخدمت أسماك القط المرقط نوع (***Clarias lazera***) كدليل حيوي لتقدير مستوى المعادن الثقيلة (النيكل، الكوبلت، الحديد، الزنك، النحاس، الكادميوم والرصاص) في مياه بحيرة سبها الاصطناعية في جنوب ليبيا. بينت النتائج ظهور جميع المعادن المدروسة في الأنسجة والأعضاء بشكل منفرد أو كلي في (العضلات، الكلية، الكبد والغدد التناسلية).
كما أوضحت نتائج الدراسة الحالية أن مستوى العناصر (الكوبلت و النيكل و الزنك والنحاس و والكادميوم والرصاص) كان بالتراكيز (0.023, 0.536, 0.481, 0.206, n.d., 0.137) **في عينات الذكور و بالتراكيز** (0.007, 0.550, 0.412, 0.237, n.d., 0.166)جزء بالمليون جزء (ppm) في عينات الإناث وعلى التوالي. ومن جهة أخرى سجل عنصر الحديد مستوى جدا من التراكم وصل إلى 24.44 **و** 9.34 جزء بالمليون جزء في كل من الذكور والاناث على التوالي.
هذا المستوى من التراكم يشجع على استخدام هذا النوع من الأسماك كدليل حيوي وبشكل خاص لعنصر الحديد.

INTRODUCTION

There is an increasing concern regarding the roles and fates of trace metals in the environment of many countries of Asia and Africa. Much of this concern arises from the low level of available information on the concentration of these metals within the environment (1-3).The contamination of aquatic food resources by trace metals is a potential problem to man. Aquatic organisms accumulate metals to concentrations many times higher than present in water. The potentially toxic metals are lead, zinc, nickel, chromium, arsenic, selenium, vanadium, beryllium and barium (4, 5).Natural and anthropogenic activities result in gaseous emissions and waste water discharges into air, water and land. When the substances in the emissions and effluent discharges in the environment are in very minute amounts or in low concentrations, are not toxic to plants and animals and have short residence time in the environment, they are described as 'contaminants'(6- 8). Living organism's require trace amounts of some heavy metals, including iron, copper, manganese, molybdenum, vanadium, strontium, and zinc, but excessive levels can be detrimental to the organism (9- 11). Other heavy metals such as mercury, lead and cadmium (with exception of cadmium) are toxic metals, they have no known vital or beneficial on organisms, and their accumulation over time in the bodies of different organisms can cause serious effects (12, 13). The pathway for toxic effects on humans is normally (14- 16).

Heavy metals are dangerous because the tend to bioaccumlate, for example, marine organisms can consume a particularly dangerous form of mercury called methylmercury (17, 18).When fish eat these organisms, the methmercury is not excreted, but retained in bodily tissues. The older fish and the more contaminated organisms have consumed the greater amount of methyl mercury in its tissues. When another fish eats the first fish, the accumulated methylmercury is passes up the food chain, eventually becoming hundreds or thousand of time its original concentration. Any organism at the top of the food chain (humans, polar bears etc.) faces a serious risk of mercury poisoning by eating such fish. Further, in order to indicate the level of heavy metals in the aquatic bodies (19). Many species of organisms were used as Bioindicators such as Crustacea, Mollusca, Fishes, Algae, and Aquatic plants. So many studies reported about to examining the ability of Catfish (*Ictalurus punctatus*, *Synodontis claria*, *Silurus triostegus* , *Clarias gariepinus* and others as a monitor for water pollution (4, 16, 20 , 21**).**

(*Clarias lazera)* were studied. These fish are commonly used as an environmental cleaner at the bottom of lakes and as predators of insects including mosquitoes, and harmful crustaceans (22, 23) Catfish can reproduce in large numbers (12000 to 13000 eggs per fish) in a season, and the species is able to endure shortages of oxygen, high organic contamination, high temperature and other adverse factors this hardiness renders useful in different regions of the world, especially in Asia and Africa; see for example (24 -28).

Site of the Study

This study was carried out in the Sebha man-made lake / Libya, which was designed to receive purified waste water from the city of Sebha, as showing in figure (1).

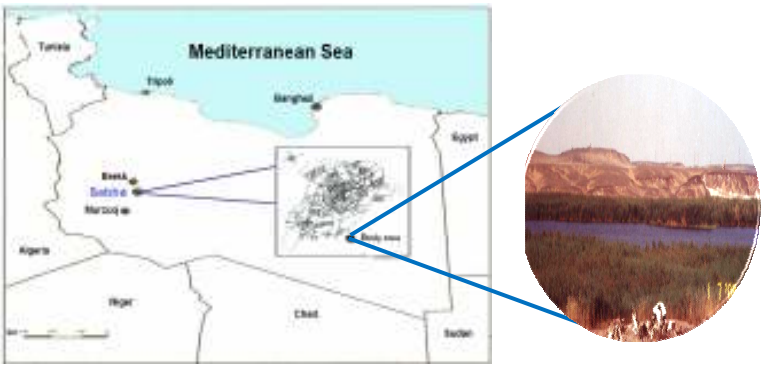

Figure (1): The site of Sabha at the map of Libya and general view of man-made lake

Aim of the study

This research aims to determine the concentrations of some heavy metals in the cat fish (*Clarias lazera*) (Ni, Co, Fe, Zn, Cu, Cd, and Pb) to establish whether it ingests major pollution of the habitat and whether the fish could be a hazard to local people.

MATERIALS AND METHODS

Twenty five of *(C. lazera*) fishes were caught and divided into two groups (male and female). The weight of fish to the nearest 0.1gm and total length of the nearest 1mm were recorded (Table1).

Table (1): Analysis of Weight /g and Length/cm, properties of catfish (male and female)

		Length/cm	Weight/g
Male	$\bar{x} \pm S_{\bar{x}}$	41.30000 $\pm$ 2.38258	710.00000 $\pm$ 87.62293
	C.V.	18.2430	39.0265
Female	$\bar{x} \pm S_{\bar{x}}$	39.80000 $\pm$ 5.31574	636.74667 $\pm$ 93.15459
	C.V.	51.7281	56.6609
t-test		0.194848 Ns	0.4052126 Ns

No. of Males = 10 No. Of Females = 15, $\bar{x}$ ***: Mean ,*** $S_{\bar{x}}$ ***: Standard deviation ,C.V.: Coefficient of variation, Ns: Non- Significant***

Fishes were placed in 10% formalin and taken directly into the laboratory. The muscle, kidney, liver and gonad were removed and then extracted to measure the level of some heavy metals. Twenty five (25) gm was taken of each of the muscle, kidney, liver and gonad. The samples were dried by ordinary air and cut into small pieces. Then were milled separately by using the glass ponder (crusher) which changed it into powder. Subsequently, 5 grams of each sample was taken at random and digested chemically.

Chemical digestion

The soft (wet) digestion method of (29) was used:

1) 5 grams of dried fish tissue was putted into a clean 100 ml conical decanter and then 5 ml. of concentrated nitric acid (NHO_3) and 5 ml of concentrated H_2SO were added and then left for a period of 5 - 10 minutes.
2) The solution volume was heated at 60 degrees for 30 minutes until reduced to 2 ml. on a hot-plate to test for reactions.
3) After cooling down the solution 10 ml of HNO_3 was added to it and heated until 120 degrees and thereafter again to 150 degrees until reduced to blackened powder.
4) The residue was left to cool before carefully adding one mm of peroxide hydrogen, reheating again. After that the solution was to be returned once more for heating up to the boiling point.
5) The solution was left to cool and then one ml of peroxide hydrogen was added again until the blackness disappeared. Filter it by using a filtration paper (Ashless Whitman No. 4). and left to cool down and then a suitable quantity (about 50 ml) of distilled water was added, then filtered (by using Ash less Whitman No.4) in order to be analyzed for heavy metal content.

The results were analyzed statistically according to (30) to determine the effect of the area of samples location in lake by using *The Linear model*.

RESULTS

Table (2) provides a broad representation of the heavy metals in the male and female catfish. Co, Ni, Fe, Zn, Cu, Cd, and Pb in the male muscles were 0.023, 0.521, 22.62, 0.444, 0.188, and, 0.075 respectively. Table 2 indicates the level of accumulation the female muscles were 0.015, 0.556, 6.014, 0.385, 0.171, and 0.171 respectively. The male livers were 0.536, 25.14, 0.524, 0.221, 0.128 respectively, whereas in the female livers were 0.001, 0.541, 16.54, 0.528, 0.349, 0.175 respectively. As for the kidney in the males were Na, 0.543, 25.57, 0.476, 0.211, 0.210 whereas in the female (gonad) the results were 0.005, 0.554, 5.480, 0.325, 0.193, and 0.152 respectively. Comparing the averages of concentration of heavy metals (Co, Ni, Fe, Zn, Cu, Cd, and Pb) in the males and the females, Co in the males was 0.0076, whereas in the females was 0.007 ,Ni in the males was 0.533 and in the females 0.550 ,Fe was 24.44 in males and 9.344 in the female ,as for the Zn it was 0.481 in males and 0.412 in females ,the Cu was 0.206 in males and 0.237 in the females ,Cd wasn't found in either the males and females, whereas Pb was 0.137 in males and 0.166 in females. It is important to note that the concentration of Cd is examined in different organs selected in this study but the results showed no evidence to record it.

It can be seen from Table 2 that the levels of accumulation, except Fe which the level reached ppm (24.44) in male fishes and ppm 9.34 in female fishes, were less concentrated, respectively ppm 0.023, 0.536, 0.481, 0.206, nd., 0.137, Co, Ni, Zn, Cu, Cd, Pb in the samples of the male and ppm 0.007, 0.550, 0.412, 0.237, n.d., 0.166 in the samples of the female. Based on these rates, the concentration is relatively lower than expected and this emphasizes that the plants take up big quantities of such elements in the water environment and the nutrition of such fishes basically on the decayed materials and insects and organic wastes and the contents of the bottom, particularly the shore areas of the lake in which the biology increase of the total plants that provide a place for growth and protection from the hot sunshine and where the canes grow profusely up to an area 50 meter inside the lake. This is described properly in (*figure 1*) which represents the general view of the lake and the area of study.

This kind of nutritional phenomenon impacts on the level of accumulation of the minerals in the fishes and concurs with what is mentioned by the researchers (10, 31- 34). In that, the nutritious conduct and the proximity to and separation from the source of pollution and the nature of the pollutant material are all factors which impact on the level of the heavy minerals in the fish. **(**4, 33, 35**)** found from the applied study on the *Roach, Perca sp* , in two different locations in the same river and *Aristichthys nobilis* grass carp *Ctenopharyngodon idellus*, and mandarin fish *Siniperca chuatsi* were collected from different fish ponds in the Pearl River Delta that, the concentration of the minerals taken from the rivers and ponds were double the concentration in the fish available in the clean river spring. It was found same in Pyeong Chang River in Korea (3). Also (36) found that the concentration Cd and Pb in sardines and baize, was different, being 0.48, 0.42 ppm in sardines and 6.48 and 6.35 ppm respectively and this is imported to the areas from which such fish are imported as well as the kind of pollutants the fish is exposed to and this conclusion concurs with (2, 35, 37).

Table (2): Analysis of properties of heavy metals accumulation in different tissues and organs of catfish (male (M) and female (F)

		Cd ppm	Cu ppm	Zn ppm	Fe ppm	Ni ppm	CO ppm	Pb ppm
M. Muscle	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.18800 ± 0.00322	0.44400 ± 0.00828	22.62000 ± 0.31692	0.52100 ± 0.00736	0.00158 ± 0.02300	0.07500 ± 0.00184
	C.V.	*N/A*	3.83569	4.16819	3.13289	3.15679	15.37189	5.49747
F. muscle	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.17080 ± 0.00287	0.38500 ± 0.00316	6.01360 ± 0.15812	0.55600 ± 0.00212	0.00170 ± 0.01500	0.17100 ± 0.00369
	C.V.	*N/A*	3.75803	1.83664	5.87944	0.85313	25.38591	4.82235
t-test			2.82178**	5.15791**	34.95774**	3.69331**	2.43600*	17.35444**
M. liver	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.22100 ± 0.00665	0.52400 ± 0.00769	25.14000 ± 0.16420	0.53600 ± 0.00369	*N/A*	0.12800 ± 0.00184
	C.V.	*N/A*	6.72673	3.28333	1.46043	1.53847	*N/A*	3.22118
F. liver	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.34900 ± 0.00311	0.52800 ± 0.00681	16.54000 ± 0.06450	0.54100 ± 0.01415	0.00032 ± 0.00100	0.17500 ± 0.00354
	C.V.	*N/A*	1.99547	2.88476	0.87196	5.84963	70.71068	4.51754
t-test		----- --	13.11101**	0.27575 Ns	37.60499**	0.28026 Ns	3.16228**	8.73696**
M. kidney	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.21100 ± 0.00354	0.47600 ± 0.00369	25.57000 ± 1.14018	0.54300 ± 0.00330	*N/A*	0.21000 ± 0.00330
	C.V.	*N/A*	3.74677	1.73240	9.97071	1.35956	*N/A*	3.51543
F. Gonad	$\bar{x} \pm S_{\bar{x}}$	*N/A*	0.19300 ± 0.00330	0.32500 ± 0.00806	5.48000 ± 0.15815	0.55400 ± 0.00957	0.00071 ± 0.00500	0.15160 ± 0.00242
	C.V.	*N/A*	3.82508	5.54700	6.45299	3.86298	31.62278	3.57055
t-test		-----	----------	----------	----------	----------	----------	----------

No. of Samples = 5: $\bar{x}$ *: Mean :* $S_{\bar{x}}$ *: Standard deviation :C.V.: Coefficient of variation *: Significant at the 0.05 level. **: Significant at the 0.01 level. :Ns :Non-significant. M: Male. F: Female.*

When following up on the bioaccumulation of every component from the studied parts of Catfish (*Clarias lazera)* as represented in the muscles, kidney, liver and gonads, it was found that such elements recorded differences in the nature of the targeted organ and its biological discrimination and the levels of the accumulation as shown in Table (2) it can be seen that the elements of Co, Fe, and Zn accumulated in the samples of the male are higher than that in the female ppm, 0.023, 25.57, 0.528 respectively, while the elements of Ni, Cu and Pb accumulated in the female are higher than in the male with a recorded concentration of 0.556, 0.349, 0.175 ppm respectively. But when taking the general averages for the tested elements and accumulated in the different organs of the fish body, it was found that the accumulation is higher in the male than in the female.

It is believed that this difference in the rates in both categories is due to the active movement and nutrition and the making and gathering of the fish schools in the areas they used to feed in it (4, 12, 38, 39). Moreover, it was found that most of the fish of the lake gather at the point that connects the canal and the storage lake of as shown in figure (2) and the beach regions which are nearer to the eastern part of the lake where there is a lot of animal organic waste due to pasturing activity on the plants of the lake and those who deposit domestic waste.

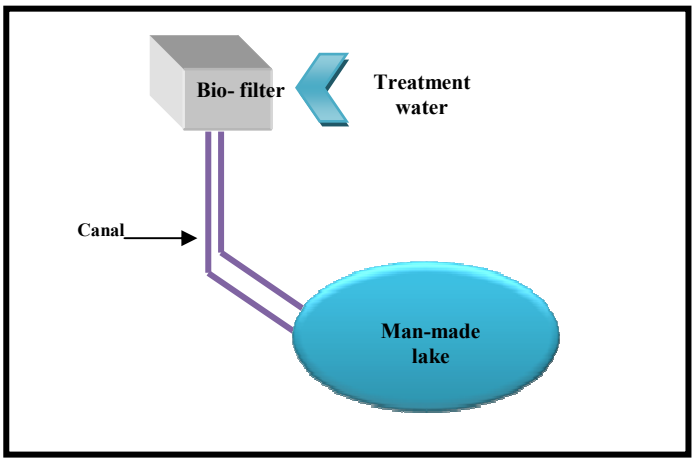

Figure (2): Diagram outlining the general features of the filtering station's link via canal to the artificial lake

These environmental circumstances have created a high medium in organic analysis and the growth of insects and different hydro-organisms that draw these fish, particularly the male fish. These results accord with research done by (9, 31, 40, 41) in that the activity of the male is more than that of females, which explains its higher rate of contamination. When following up the relation between the ratio of accumulation for the heavy element and targeted organs it was found that Co has targeted the muscles only, whereas Ni targeted kidney, liver and muscles. Iron targeted kidney, liver and muscles. Zinc targeted liver, kidney and muscles. Cu targeted liver, kidney and muscles. Cd did not show in any of the studied organs; whereas the lead element was found concentrated in the kidney, liver and the muscles of male fish.

The studied organs in females (i.e., muscles, gonad, and liver) it was found that Co and Ni had similarly targeted both muscles, gonad and liver, whereas Zn and Fe had similarly targeted liver, muscles and gonad. Cu had firstly targeted liver, gonad and muscles. Cd did not show in any organs at female. Lead was found to have targeted the liver first, then the muscles and gonad. These results accord with (13, 36, 42, 43**).** (44), in those elements differ in their distribution and accumulation in various organs of the body. (37,45) found that lead had accumulated in muscles and internal tissues whereas Cd did not show in internal tissues of fish.(45) found when studying the fish in Krugersdrift dam that heavy minerals accumulated in liver and kidney respectively. (42) found during his study on four types of fish (*Catla catla, Labeo wohita, Tiapia, Channa punctata)* that minerals accumulated in liver, brain, fine organs and muscles, while (11,43) found that Cu and Cd when in water targets the blood constituents, enzymes in fish and mammalians. (37) found during his study on 10 species of freshwater fishes that lead accumulated in muscle with average 2.67-19.1 *m*g/kg¯1.

This study showed that the behavior of an element like Cd and Pb in accumulating in tissues and organs of fish was inverse, Cd though it exists in the water of the lake, did not show in any of the examined fish, whereas in contrast, in the case of Pb, it did not show in the water samples but had accumulated in all the organs which were examined. This indicates the existence of other elements in the lake that have not yet been examined, and may play some role in the accumulation of any of the elements as the other elements are affected by their existence.

As well, minerals are present and results accord with (12, 45, 46, 47) who indicated that the existence of certain elements like Cu, Zn, Si, and Se together in the water can affect the behavior of accumulation of Pb, Hg and Ag in fish and aquatic plants. (1, 48) indicated that accumulation of Pb in fish has been affected by the minerals of Pb or nitrate. Based on the follow up of the results of the statistics analysis and the comparison between the male and the female as shown in the characteristics' analysis *(Table 2)* in order to know the abstract impact for the targeted organ in the accumulation of the heavy elements, it can be seen that there is an abstract difference with the possibility of 0.05 between the muscles and the element of Co whereas the abstraction was greater and under the possibility 0.01for the elements Ni, Fe, Zn, Cu, Pb. When following up the abstract difference in the liver, the possibility of 0.01 for the elements Co, Fe, Cu, and Pb was found but the elements of Ni, Zn. were not present. The liver of the male contained the highest average of Fe whereas highest average for the elements of Cu, Pb was found in the female liver. As for the t- test performed in order to know the abstract differences for the characteristics of length and weight of these fishes, as shown in *Table 1*, no abstract differences between the male and female were noticed. The reason for not appearance of Cd in the organs perhaps due to the fact that the element appeared in very low concentration in the study area (49).

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Detection of *gyrA* gene in Methicillin resistant *Staphylococcus spp.* in Mosul – Iraq

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ABSTRACT

Total numbers of 37 clinical Staphylococcus spp. were isolated from patients in Al-Salam General Teaching hospital in Mosul city. Antimicrobial susceptibility was assayed by disk diffusion and broth dilution method (MIC). Isolates were examined for resistance to ciprofloxacin (CIP^{10}) and Methicillin (ME^{10}). In order to reefers the Ciprofloxacin resistant to genes, molecular study was conducted using PCR technique to detect the *gyrA* gene. About 64.2% of isolates were resistant to ciprofloxacin, with MIC value (128µg/ml) and all isolates contained *gyrA* gene (280bp.)

Keywords: *Ciprofloxacin, gyrA*, *Staphylococcus spp.*

الملخص باللغة العربية

تم عزل 37 عزلة سريرية تابعة لجنس *Staphylococcus spp.* من المرضى في مستشفى السلام العام التعليمي في مدينة الموصل، شخصت العزلات وحددت مقاومتها للمضادين Ciprofloxacin (CIP^{10}) و Methicillin (ME^{10}) معا بطرق الأقراص، التركيز المثبط الأدنى وجزيئيا لتحديد جين المقاومة *gyrA* باستخدام تقنية تفاعل البلمرة المتسلسل، كانت 64.2% من العزلات مقاومة للمضاد الحيوي Ciprofloxacin (CIP^{10}) وقاومته كل العزلات في اختبار التركيز المثبط الأدنى اذ بلغت قيمة MIC 128 مايركوغرام/مل وتبين عند تحديد جين المقاومة *gyrA* أن كل العزلات كانت على هذا الجين عند 280bp.

INTRODUCTION

Staphylococci can be found in air, dust, food and water and are primarily isolated from skin and mucous membranes of humans and other mammals, they are found in the hospital environment and also the most frequent blood culture contaminants, and it is often difficult to distinguish between clinical infection and contamination, although several approaches have been investigated (1-3).

Staphylococci are classified as coagulase-positive or coagulase negative depending on their ability to produce the enzyme coagulase, and therefore clot blood plasma. *Staphylococcus aureus (S.aureus)* is the most clinically significant among the coagulase-positive staphylococci. *S. epidermidis* comprises 65% to 90% of all coagulase negative staphylococci isolated from humans and is the most clinically important species of coagulase negative staphylococci in humans (4, 5).

Researches over the past several decades identified *Staphylococcus spp.* depending upon cell wall-anchored proteins, secreted toxins, capsular and exo-poly- saccharides, iron-transport systems, and modulators of host immune functions in addition to antibiotic-resistance genes as important virulence factors (6). Methicillin resistance is available in all β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and their derivatives, occurs in staphylococci when the bacteria acquire the *mecA* gene that encodes an alternative penicillin-binding protein with low affinity for most β-lactam antibiotics (7).

Ciprofloxacin belongs to fluoroquinolones (Quinolone) group of synthetic antimicrobial agent. Their mechanism of action is interfering with the bacterial deoxyribonucleic acid (DNA) replication. They target and inhibit the enzymes DNA gyras and topoisomerase IV, which are involved in the folding and supercoiling of the DNA after replication, leading to rapid bacterial cell death (8). The resistance is due to mutational alterations in the target genes, *gyrA* and *gyrB* for DNA gyras and *parC* and *parE* for topoisomerase IV, or over expression of efflux pumps (9).

The aim of this study was undertaken to detection the *gyrA* gene in ciprofloxacin and methicillin resistance staphylococci.

MATERIALS AND METHODS

Bacterial isolates

Thirty-seven staphylococcal isolates were collected clinically from patients (superficial wounds, pus, urine and blood) of both sexes' sufferings from bacterial resistant to most antibiotics at Al-Salam General Teaching Hospital, in Mosul city from March to July, 2012. Samples were collected by transport media and sterile swabs damped in normal saline.

Conventional Method for Identification *Staphylococcus spp.*

Staphylococcal isolates were identified by Grams staining; catalase production, haemolysis on blood agar, coagulase test and mannitol fermentation. Identification for the fourteen isolates was confirmed at the species level using API ID 32 STAPH system (Bio Merieux, Marcy l'Etoile, FRANCE) according to manufacturer's instructions (10- 13).

Disk Diffusion Test

The test was carried out on Mueller-Hinton agar, following the recommendations given by CLSI zone diameters were measured after 24h of incubation at 35 ˚C and were interpreted according to the CLSI recommendations (14). All antibiotic disks were used from (Bioanalyzer Company, Turkey). Table (1).

Table (1): Antibiotics disks under study

Antibiotics	Abbreviation and Concentration(µg/ml)
Penicillin	P^{10}
Gentamicin	CN^{30}
Ciprofloxacin	CIP^{10}
Methicillin	ME^{10}
Norfloxacin	NOR^{10}
Tobramycin	TOB^{10}
Cefotaxime	CTX^{10}
Doxycycline	DO^{10}
Oxacillin	OX^{10}
Imipenem	IPM^{10}
Cefitraxone	CRO^{10}
Ampicillin	AM^{25}

Measurement of MIC value for Ciprofloxacin

Ciprofloxacin, (SDI Samarra-IRAQ) stock solution was prepared at concentrations 256 µg/ml in sterile distilled water. Antibiotic was serially diluted in Brain-Heart infusion broth to give working concentrations of 128, 64, 32, 16, 8, 4 and 2 µg/ml. Bacterial suspension of 0.5 McFarland turbidity standards 1.5×10^8cfu/ml was added to all the tubes and were incubated for 24h at 35 ˚C (14,15). The value of MIC for ciprofloxacin *against Staphylococcus spp*. if less than 1 µg/ml was sensitive and more than 4 µg/ml was resistant.

Bacterial DNA Extraction

Whole genomic DNA extractions of 14 bacterial isolates were done using the Wizard® Genomic DNA extraction kit (Promega Corporation-USA) and the modification of this test was performed without using lysostaphin (16).

Colonies were grown overnight at 37 ˚C in Trypticase soy broth and centrifuged at 13000 X g for 2 min, cell pellets were re-suspended in 500µl of

sterile distilled water (with 700 µl of lysozyme) and the mixture were incubated at 37 ˚C for 1hr. Other prepared solutions from the extraction kit were added according to the manufactures recommendations. Extracted DNA was stored at -20 ˚C until PCR was performed (17).

***gyrA* Primer (18)**

F (5-AGTACATCGTCGTATACTATATGG-3),
R (5-ATCACGTAACAGTTCAAGTGTG-3)

Polymerase Chain Reaction (PCR) mixture

The mixture prepared like Master Mix recommendation "GoTaq" Green Master Mix, 2X, Cat. # M7122, Promega Corporation- USA, the mixture was composed from 12.5 µl GoTaq Green Master Mix, 5.5 µl Nuclease Free Water**,** 1 µl Forward Primer**,**1 µl Reverse Primer and 5 µl Extracted DNA. All PCR amplifications were carried out using PCR thermal cycler, eppendrof, master-cycler personal model Eppendrof AG, 22331, Hamburg, GERMANY (18).

Detection of *gyrA* (280 bp.) gene

The amplification was confirmed with modification after Goswitz, et al. and Benhamed and Kihal (18,19). The PCR products were electrophoresed by agarose gel (2%) for 50 volt at 75min and visualization under a UV trans-illuminator (model MUV21-312, TAIWAN) and the gel was photographed using Sony digital camera (model DSC-HX1, JAPAN) (20,21). Table (2).

Table (2): Program for detection of *gyrA* gene

Initial Denaturation		30 Cycles						Final Extension		Cooling	
		Denaturation		Annealing		Extension					
Tem	Time.	Tem	Time.	Temp	Time.	Temp	Time.	Temp	Time.	Temp	Time.
94	4 min	94	1 min	55	1 min	72	30 Sec	72	5 min	4	3 min

RESULTS

All isolates were Gram positive, catalase positive, out of 37 isolates; 26 isolates were hemolysis blood, 10 positive to coagulase test and 5 fermented mannitol salt. API ID 32 STAPH System test was used to identify the isolates. Table (3).
Among 14 *Staphylococcus spp.*, 14 (100%) were resistant to ampicillin and penicillin, 6 (42.8%) ,1 (7.1%) and 7 (50%) were resistant, intermediate and sensitive respectively to gentamicin, on other hand, 9 (64.2%) were resistant and 5 (35.7%) were sensitive to ciprofloxacin and methicillin, 8 (57.1 %) were resistant, 2 (14.2 %) were intermediate and 5 (28.5 %) were sensitive to norfloxacin and doxycycline, 13 (92.8 %) were resistant and 1 (7.1%) were intermediate to tobramycin, 11 (78.5 %) were resistant, 2 (14.2 %) were intermediate and 1 (7.1%) were sensitive to cefotaxime, 12 (85.7%) were resistant and 2(14.2 %) were sensitive to oxacillin, 3 (21.4%) were resistant, 2(14.2 %) were intermediate and 9 (64.2%) were sensitive to imipenem and 13 (92.8 %) were resistant and 1 (7.1%) were intermediate to cefitraxone. Table (4). Resistant isolates had MIC 128µg/ml but sensitive isolates MIC 2 µg/ml and 4 µg/ml to Ciprofloxacin (CIP^{10}).
All of methicillin resistant *Staphylococcus spp*. isolates were had *gyrA* (280bp.) gene and gave resistant to ciprofloxacin (CIP^{10}) resistant by broth dilution method, but in disc diffusion method just 9 isolates were resistant to ciprofloxacin (Fig. 1and Fig. 2).

Table (3): Number of species of *Staphylococcus* genus which identification by API System

Staphylococcus spp.	**Number of isolates**
Staph. xylosus	5
Staph. aureus	2
Staph. hominis	2
Staph. epidermidis	2
Staph. lugdunensis	2
Staph. haemolyticus	1
Total	14

Table (4): Number of *Staphylococcus spp*. which gave resistant to methicillin, oxacillin and ciprofloxacin

Species	**Ciprofloxacin CIP^{10}**	**Methicillin ME^{10}**	**Oxacillin OX^{10}**
Staph. xylosus	3	3	5
Staph. aureus	1	2	2
Staph. hominis	0	1	1
Staph.epidermidis	2	2	2
Staph. lugdunensis	2	2	2
Staph. haemolyticus	1	0	1

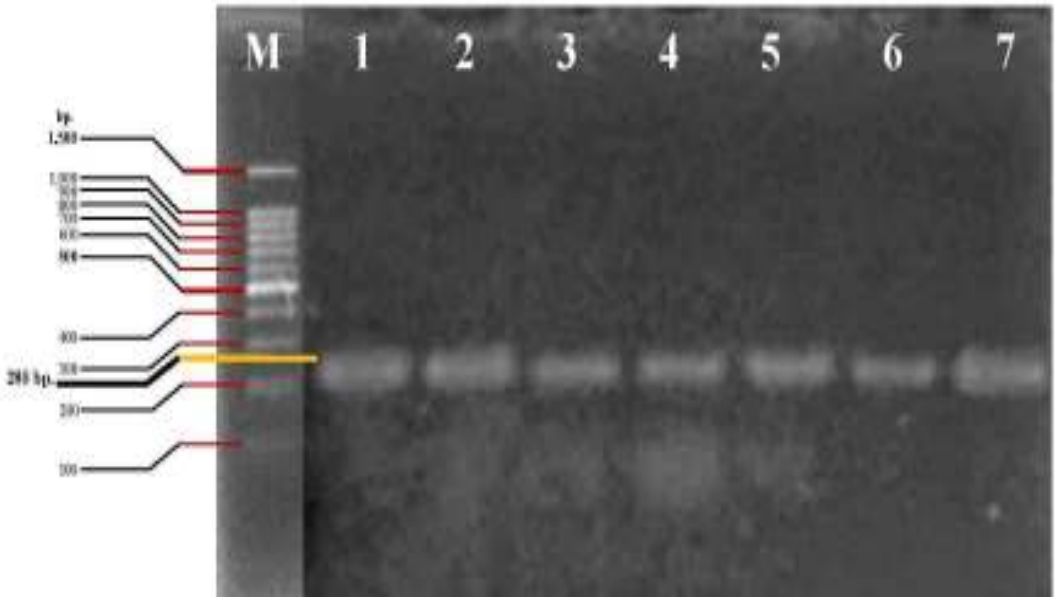

Figure (1): Lane M 100bp DNA Ladder, Lane 1 *Staph. hominis*, Lanes 2 and 5 *Staph. epidermidis*, Lane 3 *Staph. aureus*, Lane 4 *Staph. haemolyticus*, Lane 6 and 7 *Staph. xylosus*

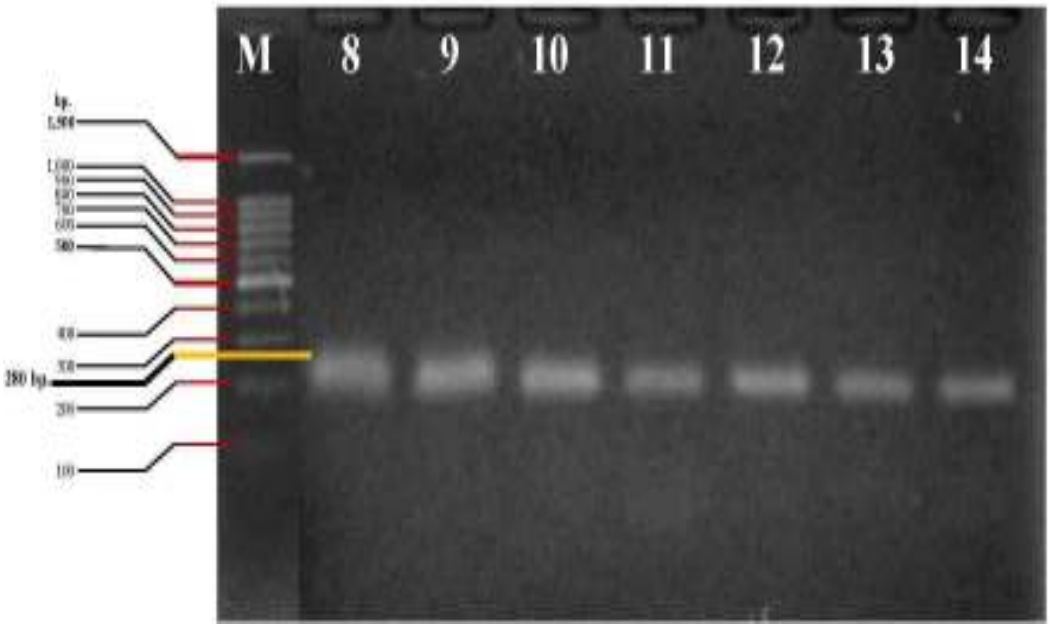

Figure (2): Lane M 100bp DNA Ladder, Lanes 8, 9, 10 and 12 *Staph. xylosus*, Lanes 11 and 13 *Staph. lugdunensis* and Lane 14 *Staph. hominis*

DISCUSSION

The results showed that all isolates were resistant to ampicillin (AM^{25}) and penicillin (P^{10}); a similar finding was reported by other researcher (22).

The results very similar to Chaieb *et al.* (23) they found that *Staphylococcus spp.* were resistant to ampicillin by (93.8%) and similar to. Rahimi *et.al.* (24). Study the antibiotic susceptibility pattern among *Staphylococcus spp.* with emphasis on detection of *mecA* gene they found that (99%) of these isolates were resistant to ampicillin AM^{25}.

We found that (42.8%) from isolates were resistant to gentamicin (CN^{30}) but Chaieb *et.al.* (23) Found that (15.9%) of isolates were resistant to gentamicin (CN^{30}), the differentiation may be belonged to bacteria have resistant to gentamicin (CN^{30}) from other bacteria by bacterial conjugation or it may contain resistant plasmid to gentamicin came from prolonged exposure to antibiotic.

Ciprofloxacin (CIP^{10}) and methicillin ME^{10} (64.2%) of *Staphylococcus spp*. were resistant, in previous study about antibiotic susceptibility of *Staphylococcus spp*. the researchers were found that (87%) of these bacteria were resistant to ciprofloxacin (CIP^{10}) and other reported demonstrate that *mecA* gene when found in the *Staphylococcus spp*. make it a highly resistant to other antibiotics like ciprofloxacin (CIP^{10}) therefore we found that all *Staphylococcus spp*. resistant to methicillin (ME^{10}) are resistant to ciprofloxacin (CIP^{10}) except in disk diffusion test (18).

In previous studies founded that (77.1%) of MRS isolates were resistant to norfloxacin (NOR^{10}), doxycycline (DO^{10}) and resistant also to tobramycin TOB^{10} (81.4%), but we found that (57.1%) resistant to the first group of antibiotics and (92.8%) were resistant to tobramycin (TOB^{10}) and ceftriaxone CRO^{10}, (78.5%) were resistant to cefotaxime (CTX^{10}) Rahimi and others, 2009 who they found that (62 %) of *Staphylococcus spp*. were resistant to cefotaxime (CTX^{10}) (22,24).

The researchers studies on *Staphylococcus aureus* from burnt patients in Sulaimaniyah city found that (88.9%) of isolates were resistant to oxacillin we also found that (85.7%) of isolates were resistant to oxacillin (25).

The result indicated that (21.4%) of bacteria under study were resistant to imipenem.

MIC of ciprofloxacin (CIP^{10}) were 128µg/ml and all isolates were resistant to antibiotic and resistant to methicillin because all of them have *gyrA* gene in 280bp. Benhamed and Kihal, were work on cows mastitis caused by *Staphylococcus aureus* found that *gyrA* gene in 280bp (19).

The resistance mechanisms have mostly been related to specific mutations that lead to amino acid alterations in the quinolone resistance-determining regions (QRDRs) in *gyrA*, Staphylococcal *gyrA* mutations associated with ciprofloxacin resistance in all methicillin resistant *Staphylococcus spp.* this mutation of ciprofloxacin resistant causes the shift of MIC from 16 to 128µg/ml (18).

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The effect of Hypothyroidism on cytokines

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ABSTRACT

Recent evidence indicates the presence of bidirectional interactions between the hypothalamic-pituitary –thyroid (HPT) axis and the immune system. L-thyroxin (T4) and triiodothyronine (T3) are modulators of the immune response. This work aimed to study the effect of hypothyroidism on immune system by assessing the changes in serum levels of IL-10 and IL-2R.

Twenty-one patients with hypothyroidism and 13 healthy controls were enrolled in this study. The sera were processed for the determination of T3, T4, TSH, IL-10 and IL-2R concentrations.

The results are expressed as mean± SD. The data were analyzed using student's "t" test (unpaired, one tailed), and confidence interval (C.I.95%) taking $p \leq 0.05$ as the lowest limit of significance.

The level of serum IL-2R (6.49 ± 4.59 U/ml) in patients with hypothyroidism was significantly lower ($p<0.05$) than that of healthy control (10.8± 6.6 U/ml) while the level of serum IL-10 (390.7± 305.3 pg/ml) was significantly higher ($p<0.05$) than that of control (232.5 ±174.3 pg/ml). The $C.I._{95\%}$ of IL-2R of the control subjects was 7.254-14.45 therefore, thirteen patients had significant low serum IL-2R, on the other hand the $C.I._{95\%}$ of IL-10 of the control subjects was 133.85-330.15 therefore, and eight patients had significant high serum IL-10.

There was no significant correlation between serum IL-2R and thyroid hormones. Serum IL-10 was significantly correlated with serum thyroxin level. Although serum IL-10 negatively correlated (r=-0.370) with serum thyroid stimulating hormone, it did not reach to significant level.

From the results of the present study we conclude that Thyroid hormones directly modulate circulating markers of cell mediated immune response in humans.

Keywords: Hypothyroidism, Cytokines, T3, T4, IL-10 and IL-2R

الملخص باللغة العربية

تشير الدلائل الحديثة الى وجود تفاعل ثنائي الاتجاه بين المحور الوطائي–النخامي–الدرقي والجهاز المناعي.يعتبر هورموني الدرقين والثايرونين ثلاثي اليود مغيران للاستجابة المناعية. يهدف هذا العمل الى دراسة تاثير قصور الدرقية على الجهاز المناعي عن طريق تقييم التغييرات في مستويات الانترلوكين– 10 ومستقبلات الانترلوكين– 2 في مصل الدم.

شملت هذه الدراسة(21) فردا من المصابين بقصور الغدة الدرقية و (13) فردا من الاصحاء كمجموعة سيطرة.تمت معاملة مصل الدم لقياس تراكيز الثايرونين ثلاثي اليود, الثايرونين رباعي اليود,الهورمون المنبه للدرقية, الانترلوكين– 10ومستقبلات الانترلوكين– 2.

تم عرض النتائج على شكل المعدل± الانحراف المعياري كما تم تحليل المعطيات احصائيا باستخدام اختبار T ومسافة الثقة.

لقد كان مستوى مستقبلات الانترلوكين– 2 في مصل الدم لمرضى قصور الدرقية اقل وبشكل يعتد به من مستواه عند الاصحاء, بينما كان مستوى الانترلوكين–10 أعلى وبشكل يعتد به من مستواه عند الاصحاء. ان مسافة الثقة لمستقبلات الانترلوكين– 2 عند الاصحاء كانت 7.254_ 14.45 لذلك فان (13) مريض كانت مستويات مستقبلات الانترلوكين– 2في مصل الدم عندهم منخفضة وبشكل يعتد به, بينما كانت مسافة الثقة للانترلوكين–10 عند الاصحاء 133.85–330.05 لذلك فان (8) مرضى كانت مستويات الانترلوكين–10 في مصل الدم لديهم مرتفعة بشكل يعتد به.

لم تكن هناك علاقة يعتد بها بين هورمونات الغدة الدرقية و مستقبلات الانترلوكين–2 بينما كان هناك علاقة طردية يعتد بها بين مستوى هورمون الدرقين ومستوى الانترلوكين–10 في مصل الدم , في حين ان العلاقة العكسية بين الهورمون المنبه للدرقية والانترلوكين –10 لم يصل الى مستوى يعتد به.

من نتائج الدراسة الحالية نستنتج ان هرمونات الغدة الدرقية تؤثر بشكل مباشر على معلمات الاستجابة المناعية المتواسطة بالخلايا عند الانسان.

INTRODUCTION

Cytokines have been classified on the bases of their biological responses into pro- or anti – inflammatory cytokines. Cytokines act in networks or cascades. Major cytokines include the interleukins (IL). Many of the cytokines act locally like autocrine hormones and their targets are cells of 2the same or similar type as the cytokines producing cells (1). The function of some cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6 and IL-10 is closely associated with the interaction between B-cells and T- cells (2).

IL-10 is a protein of about 160 amino acids that contains four conserved cysteines involved in disulphide bonds(1). it is produced mainly by T-helper lymphocytes of type 2 (3). First recognized for its ability to inhibit activation and effect or function of T-cells, monocytes, and macrophages, is a multifunctional cytokine with diverse effects on most hemopoietic cell types. The principle routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses (4) by suppressing the production of pro-inflammatory cytokines (5).

In addition to these activities, IL-10 regulate growth and/or differentiation of B-cells, natural killing cells (NK), cytotoxic and helper T –cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells (4).

IL-10 plays a key role in differentiation and function of a newly appreciated type of T-cells, the T-regulatory cell, which may figure prominently in control of immune response and tolerance in vivo. Uniquely among hemopoietic cytokines, IL-10 has closely related homologs in several viruses (genomes) (4).

Soluble cytokine receptors naturally arise from genes encoding membrane- bound receptors or are direct derivatives of the receptors themselves. There is mounting evidence that soluble receptors play important roles in human disease states (6).

The high affinity IL-2 receptor is a cell membrane heterodimer composed of α-, β- and γ- subunits. T lymphocyte stimulation is followed by shedding of the α-subunit (also known as CD25), which may be measured in the serum. This fraction is termed soluble interleukine-2 receptor (sIL-2R) and is considered a specific serum marker of T cell proliferation and activation (7).

Irrespective of whether SIL-2R expression is increased in response to a non-specific stimulus such as inflammation or result directly from release from proliferating tumor cells, it is important to consider that soluble IL-2R is likely to have intrinsic biological activity and may act as an immunosuppressant (6). IL-2 R signals may also promote cell survival, effect function, and apoptosis (8).

There is evidence indicate the presence of bidirectional interactions between the hypothalamic –pituitary- thyroid (HPT) axis and the immune system (9). L-thyroxin (T4) and tri-iodothyronine (T3) are modulators of the immune response. In monocytes, macrophages, leukocytes, natural killer cells, and lymphocytes, a wide range of immune functions such as chemotaxis, phagocytosis, generation of reactive oxygen species (ROS) and cytokines synthesis and release are altered under hypo- and hyper-thyroid conditions. Thyroid hormones also affect natural killer cell activity and cell mediated immune response (10).

Thyroid hormones seen to be able to modulate the immune system by a combination of rapid nongenomic responses interacting with the classical nuclear response (11), also thyroid hormone participate in primary and secondary lymphopoiesis (12).

The presence of functional receptors for HPT hormones on lymphocytes as well as the frequent immune alteration observed during physiological or pathological fluctuations of thyroid hormones strengthen the interaction between the HPT axis hormones and the immune system (9).

This work aimed to study the effect of hypothyroidism on immune system by assessing the changes in serum levels of IL-10 and IL-2R in patients with hypothyroidism and analyze the relation to disease severity.

MATERIALS AND METHODS

This study was conducted at department of medical laboratories, Al-Yarmouk teaching hospital, in Baghdad, Iraq from October 2012 to May 2013.

Patients with hypothyroidism from the outpatient clinic were enrolled in this study. All patients were diagnosed by clinical examination, and laboratory investigation (thyroid function tests). The criteria of inclusion included patients with different stages of hypothyroidism with and without treatment. The criteria of exclusion were concomitant diseases including hypertension, diabetes mellitus, renal failure and pregnant women.

Twenty-one patients (19 females and 2 males) with median age of 48 years were included in the study. The distribution of patients in respect of severity of disease was as follow: 11 patients with overt hypothyroidism (High TSH and low T3 and T4) and 10 patients with sub-clinical hypothyroidism (High TSH and normal T3 and T4).

In addition, 13 subjects matched to the patients group with regard to age and gender were allocated randomly as a control group. All subjects were examined and investigated by routine laboratory tests and they appeared healthy.

Venous blood samples were collected from patients and healthy subjects, the sera were separated by centrifugation (3000 rpm for 5 min) and processed for the determination of TSH, T3, T4, IL-10, and IL-2R.

Thyroid stimulating test (TSH), Triiodothyronin (T3) and Thyroxin (T4) were measured by combining an Enzyme Immunoassay Competition

method with a final fluorescent detection (ELFA) using mini VIDAS, bioMérieux , France.
Interleukine-10 and IL-2R were measured by Enzyme – Linked Immuno -Sorbent Assay (ELISA) kit (Invitrogen, ELISA kit, USA) using ELISA reader from Sanofi Diagnostic Pasteur, PR 2100, USA.

Statistical analysis

All statistical analysis were performed using Microsoft office excel, 2007. The results were expressed as mean± SD and percentage on need. The data were analyzed using student's "t" test (unpaired, one tailed), simple correlation test, and confidence interval (C.I.95%) taking $p \leq 0.05$ as the lowest limit of significance.

RESULTS

Table (1) showed significant low level of serum IL-2R and high level IL-10. The $C.I._{95\%}$ of IL-2R of the control subjects was 7.254-14.45 therefore, 13 patients had significant low serum IL-2R, on the other hand the $C.I._{95\%}$ of IL-10 of the control subjects was 133.85-330.15, therefore eight patients had significant high serum IL-10.

Table (1): The characteristics, thyroid function tests and related biomarkers

	Control (n=13)	Hypothyroidism (n=21)
Gender (Female: Male)	10:3	19:2
Age (year)	44.5±6.4	46.7±11.3
Serum triodothyronine(nmol/L)	1.8±0.27	1.2±0.5*
Serum thyroxine(nmol/L)	83±10.2	55.98±25.36*
Serum thyroid stimulating hormone (µIU/ml)	1.8±0.72	23.98±19.6*
Serum IL-2R (U/ml)	10.852±6.620	6.497±4.598*
Serum IL-10 (pg/ml)	232.5±174.36	390.7±305.34*

** p < 0.05 compared with control values*

Table (2) shows significant low levels of serum IL-2R in both overt and sub-clinical hypothyroid patients in comparison with that of control, While there is significant high level of IL-10 in sub-clinical hypothyroid patients in comparison with control.
However, there was no significant difference between overt and sub-clinical hypothyroid patients regarding the levels of IL-10 and IL-2R.

Table (2): Serum IL-10 and IL-2R levels in overt and sub-clinical hypothyroid patients and control group

Cytokines	Overt HOT (n=11) (52.4%)	Subclinical HOT (n=10) (47.6%)	Control (n=13)
IL-2R (U/ml)	6.5 ± 4.3 *	6.4 ± 5.1 *	10.9 ± 6.6
IL- 10 (pg/ml)	313 ± 235	469 ± 358 *	232 ± 174.4

** p < 0.05 compared with control values*

Figure (1) shows significant reverse correlation between age and TSH level (r= -0.46, p<0.05).

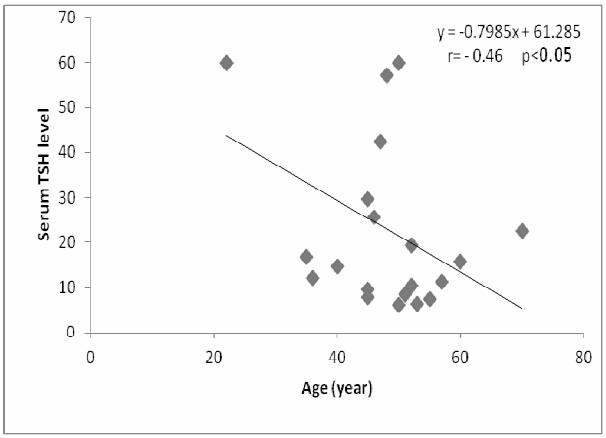

Figure (1): significant correlation between age and serum TSH level.

Serum IL-10 was significantly correlated (r=.0461, p<0.05) with serum thyroxin level. Figure (2). There was no significant correlation between serum IL-2R and thyroid hormones. Although serum IL-10 negatively correlated (r=-0.370) with serum thyroid stimulating hormone, it did not reach to significant level.

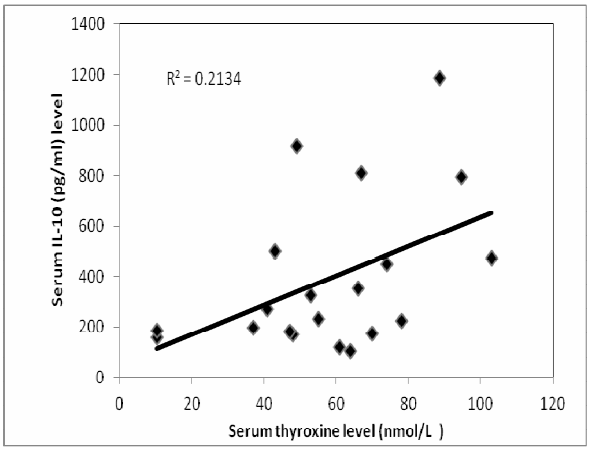

Figure (2): Significant correlation between Serum IL-10 and Serum thyroxin level

DISCUSSION

The results have shown low level of serum IL-2R in hypothyroid patients in comparison with normal control group; similar results were already observed by Koukkou *et al* and Mariotti *et al.* (13,14).Also there was significant low level of serum IL-2R in both subclinical and overt hypothyroid patients in comparison with normal control group, these results confirm the results of previous studies by Zwirisk *et al*. and Jose *et al*. (15,16).

Regarding the serum level of IL-10 we found that there is significant increase in hypothyroid patients in comparison with healthy control which agree with results of previous study by (17). Also, its level was higher in both overt and sub-clinical hypothyroidism but only in sub-clinical hypothyroid it reach the level of significance.

Thyroid hormone participates in primary and secondary lymphopoiesis. On other hand, the presence of functional receptors for hypothalamic-pituitary-thyroid hormones on lymphocytes as well as the frequent immune alterations observed during physiological and pathological fluctuations of thyroid hormones strengthens the interactions between HPT-axis hormones and the immune system (18-20).

Interleukin- 10 has various immunomodulatary actions depending on the target cell type, some of these effects have been shown to be owing to its ability to down regulate surface expression of markers, for example HLA-DR on microphages and IL-2 receptor on B- cells (21) and this may explain the low level IL-2 receptor in hypothyroid patients enrolled in this study.

Serum TSH level was negatively correlated with age which agree with previous studies (22-24). Normal aging is accompanied by a slight decrease in TSH release (25). This may be due to an increased sensitivity of the thyrotrophes to the negative feedback by T4 but other mechanisms such as a reduced hypothalamic- Thyrotropin releasing hormone (TRH) secretion cannot be excluded (26).

Serum IL-10 was significantly directly correlated with serum thyroxin level. This relation may be explained by the study of Balahan *et.al.* who demonstrated a significant IL-10 release after thyroid hormone induction were the level of IL-10 significantly enhanced after L-thyroxine administration (27).

This correlation also explain the significant increase in serum level of IL-10 in sub-clinical hypothyroid patients while the increase in overt hypothyroid patients not reach the level of significance in comparison with control group.

CONCLUSION

From the results of the present study we conclude that Thyroid hormones directly modulate circulating markers of cell mediated immune response in humans and IL-10 but not IL-2R is affected by severity of hypothyroidism.

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The role of Hepatitis B surface antigen level in correlation with viral load in untreated patients with chronic Hepatitis B in Ramadi City, West of Iraq

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ABSTRACT

Hepatitis B virus (HBV) infection is an important global health problem and detection of serological markers is the mainstay of diagnosis of HBV infection. HBsAg level is the hallmark of HBV infection. This study has been undertaken to reveal if there is any relationship between viral load (as HBV DNA) and HBsAg levels in untreated patients with chronic hepatitis B. A total of 117 patients with chronic hepatitis B who were admitted to GIT center in Ramadi Teaching Hospital and Private Clinics during the period from January, 2012 to June, 2013 were included in this study. Liver function tests, viral load, hepatitis B profile including HBsAg, HBeAg, anti HBeAg antibodies and anti HBc antibodies were analyzed. An HBsAg level was measured accurately using TOSOH-Auto analyzer. Further, HBV DNA copies were detected by quantitative real-time PCR. Regarding results, all patients (100%) were HBsAg and HBcAb positive. Sixteen (13.7%) of them were positive for HBeAg while 101 (86.3%) were negative. The median (IQR) of HBsAg level was 3.47 (30.02 - 3.89) log10 IU/ml, and those for HBV DNA was 4.60 (3.9 - 5.5) log10 IU/ml. The Median for SGOT, SGPT and TSB were 25 (18.15 - 30), 20 (15 - 26) and 0.8 (0.7 - 0.9) respectively. The study revealed that 16 patients with positive HBeAg had higher median HBsAg levels log10 IU/ml and HBV DNA log10 IU/ml levels higher than 101 patients with negative HBeAg, (3.97 vs. 3.44 , P= 0.006) and (8.0 vs. 4.3, P=0.0013), respectively. The correlation was direct and significant between both groups of patients (16 HBeAg Positive and 101 HBeAg negative), and it was stronger HBeAg positive group than HBeAg negative group (R=0.560, P=0.012 vs. R= 0.101, P=0.003) respectively. The study suggested that both of viral loads by RT-PCR and HBsAg level play an important role in the diagnosis and follow up of patients with chronic hepatitis B. Furthermore, overall HBsAg level and HBV-DNA by viral load appeared highly significant direct correlation.

Keywords: HBsAg level, HBV-DNA, Real time PCR

الملخص باللغة العربية

إن الإصابة بفايروس الكبد نوع B هو مشكلة صحية واسعة الانتشار ويعد تحديد الدلائل المصلية هو المحور لتشخيصه. إن مستوى المستضد السطحي للفايروس هو الحجر الأساس لتشخيص الإصابة . وضعت هذه الدراسة لتحديد فيما إذا كانت هناك علاقة بين عدد الفايروسات (الحمل الفايروسي) ومستوى المستضد السطحي في المرضى المصابين بالالتهاب الكبد الفايروسي نوع B المزمن والذين لم يتعرضوا للعلاج. شملت هذه الدراسة 117 مريض مصابين بالفايروس أعلاه دخلوا إلى مركز الجهاز الهضمي في مستشفى الرمادي التعليمي والعيادات الخارجية خلال الفترة الممتدة مابين كانون الثاني 2012 إلى حزيران 2013. تم إجراء التحليلات واختبارات وظائف الكبد والحمل الفايروسي والأجسام المضادة والمستضدة للفايروس .تم قياس مستوى المستضد السطحي باستخدام جهاز TOSOH كذلك تم تحديد نسخ الدنا باستخدام جهاز (Real time- PCR). أظهرت الدراسة بان جميع المرضى (100%) أظهروا نتائج موجبة لكل (HBsAg and HBcAb). 16(13.7%) منهم اظهروا نتيجة موجبة لفايروس (HBe Ag) بينما كان 101 (86.3%) نتائجهم سالبة كان الوسيط الحسابي لمستوى المستضدات السطحية هو 3.47 وللدنا الفايروسي 4.60 فيما كانت هذه القيمة لكل من S.GPTوال TSB 25و20و0.8 على التوالي . أظهرت الدراسة أن 16 مريض ممن يحملو المستضد السطحي يملكون وسيط حسابي عالي لمستوى المستضد السطحي وان الدنا الفايروسي كان أعلى من 101 مريض لايملكون المستضد السطحي أعلاه. أن العلاقة كانت مباشرة وملحوضه لكلا المجموعتين من المرضى وكانت أوضح في المرضى الذين يملكون الانتجينات السطحية مقارنة بالمجوعة الأخرى معامل الارتباط (R=0.560 للمجوعة الأولى و R=0.101 للمجوعة الثانية). تستنتج الدراسة ان كلا للحمل الفايروسي باستخدام RT-PCR ومستوى المستضد السطحي يلعب دور مهم في تشخيص ومتابعة المرضى المصابين لالتهاب الكبد الفايروسي نوع B المزمن كما ان العلاقة بين كلا من مستوى المستضد السطحي وعدد نسخ الدنا الفايروسي كانت علاقة مباشرة وذات فرق معنوي إحصائي عالي.

INTRODUCTION

Chronic infection is usually defined as an individual remaining positive for HBsAg for 6 months or longer. Chronicity rate has been estimated to be 90% for newborns, 25–30% for children less than 5 years of age and less than 5% for adolescents and adults (1-3).

Serum hepatitis B surface antigen is a reliable marker of overt hepatitis B virus infection (4). Quantification of serum HBsAg has been recently standardized by automated quantification assays leading to an increased interest in clinical utilization of this marker (5). HBsAg serum levels result from a balance between virus biology and a host immune system as the indirect expression of transcriptionally active covalently closed circular DNA rather than the product of the viral replication (6). The highest correlations between HBV DNA and HBsAg levels are found in early phases of infection, during acute infection, immune clearance and HBeAg positive hepatitis B persistent infection (7).

Chronic hepatitis B is a major global problem, affecting more than 350 million chronic Hepatitis B worldwide (8) and leading to 1 million deaths each year (9). Chronic hepatitis B patients with high viral loads are at increased risk of cirrhosis and hepatocellular carcinoma (HCC). In patients with low viral loads, higher hepatitis B surface antigen (HBsAg) levels have been shown to predict HCC development (10).. This study has been undertaken to detect the role of viral load by real time PCR and HBsAg level in the diagnosis of chronic hepatitis B. Also, to reveal if there is any relationship between viral load (as HBV DNA) and HBsAg levels in untreated patients with chronic hepatitis B.

PATIENTS AND METHODS

Total number of 117 patients with chronic hepatitis B who were admitted to GIT center in Ramadi Teaching Hospital and Private Clinic during the period from January, 2012 to June, 2013 were included in this study. All clinical information were obtained from those patients and reported in a requestionary sheet including age, sex, clinical presentation and treatment. Liver function test, viral load by quantitative real time-PCR, hepatitis B profile including (HBsAg, HBeAg, Anti HBe, and Anti HBc) will be recoded and then analyzed. Written consent was taken from the patients. Inclusion criteria include untreated patients with CHB. Exclusion criteria include patients with CHB on treatment.

Serological part of study

Blood samples were collected and transferred into plain tubes and serum will be separated and stored immediately at -20°C.

Measurement of HBsAg levels and liver enzymes

HBsAg levels will be measured accurately using TOSOH-Auto analyzer. Sera from blood samples were tested for ALT and AST aminotransferases by use of a Roche Cobas Mira Autoanalyser.

Molecular part of study

The assay was carried out using commercial Smart Cycler instrument, U.S.A and primers specific to the S gene designed to amplify a 98 base pair product. The detection limit of the qPCR assay was 200 copies per ml and quantified accurately samples with greater than 2.6×10^2DNA copies per ml. Test samples falling above the top of the standard curve will be re-assayed at a dilution of 1:100. The assay was 100% specific when tested against HBV seronegative sera from ten subjects and coefficient of variation obtained from intra-and inter assay was 1.08 and 1.72 respectively. Serum HBV DNA levels greater than 10^5 copies/ml was considered as high viral load and less than 10^5 copies as low viral load. An arbitrary value of 100 copies/ml was assigned to samples with undetectable HBV DNA for statistical comparisons (11).

DNA extraction:- DNA was extracted from 150 µl of plasma with DNA extraction kit (Ribo-Sorb kit, Sacace, Italy) using the silica based technology according to the manufacturer's instructions, using fluorescent reporter dye probes specific for HBV and HBV Internal Control. Briefly, patient's plasma was subjected to lysis at 70^o C with 600µL lysis buffer and 20µL of protease reagent. The DNA was extracted from the lysate using 600µL absolute ethanol and subsequently purified using spin columns. Finally, purified DNA was eluted from the spin columns using 50 µl RNase-free H_2O. The extracted DNA was subjected to amplification (11,12)**.**

Gene amplification:-Amplification was carried out using Real Time kit for the quantitative detection of hepatitis B virus in human plasma (Sacace biotechnologies, Italy). Amplification mixtures comprised of 300µL RT-PCR-mix-1-TM; 200µL of RT-PCR-mix-2-TM and 20µL of Hot Start Taq Polymerase. Sixteen PCR tubes were prepared: three for HBV Standard (QS1 HBV, QS2 HBV, and QS3 HBV), three internal controls Standard (QS1 IC, QS2 IC, QS3 IC), seven for test samples, two for positive controls and one for negative control. "12.5"µl of Reaction mixture added into each tube. 12.5µl of extracted DNA sample added to the appropriate tube with Reaction Mixture and mixed by pipetting. 12.5 µl of controls and standards added to the appropriate tube with Reaction Mix. The tubes closed and transferred into the thermal cycler. DNA amplification was carried out in Smart Cycler II instrument (Cepheid) (12).

Amplification program:- The amplification was performed as follows: initial hot start denaturation at 95°C for 15 min, followed by 42 cycles of denaturation at 95°C for 20 sec., annealing and extension at 60°C for 40 sec. Real-time monitoring was achieved by measuring the fluorescence at the end of the extension phase for each cycle. The quantitative analyses were conducted by using Smart Cycler II analysis software version 2.0 following the manufacturer's instructions (Cepheid). The concentration of HBV DNA for each control and patient specimen, calculated using the following formula:

HBV DNA IC DNA × coefficient* = copies HBV/ml
* Coefficient factor for kit= 2.7×10^5

Statistical analysis

Depending on SPSS software for windows, data of patients were entered, checked for any errors or inconsistency and analyzed with appropriate statistical tests. Descriptive statistics for the continuous variables were presented as median and inter-quartile range (IQR). Categorical variables were presented as frequencies and proportions (%). Pearson's correlation test was used to assess the correlation between HBsAg and HBV DNA levels, and to find the correlation coefficient value (R), and the significance of the correlation,. Partial correlation test was used to assess the correlation with adjustment for age and gender. Curve estimation regression test was used to assess the direction and the nature of correlations. Comparison of medians in between two groups was conducted by using non parametric tests.
Further analysis with multiple logistic regressions was performed to assess the correlation in between HBsAg and HBV DNA levels in addition to other variables. Level of significance in all comparison was two tailed and set at P≤ 0.05 considered as significant, < 0.001 considered as highly significant (HS). Finally all findings presented in tables and figures with appropriate explanatory paragraphs.

RESULTS

A total number of 117 patients with CHB were recruited in this study, their descriptive characteristics were shown in the following table. The median (IQR) age of patients was 32 (26 - 43) years. They were 68 male (58.1%) and 49 females (41.9%). All patients (100%) were HBsAg positive and HBcAb positive, 16 (13.7%) of them were HBeAg positive vs. 101 (86.3%) were HBeAg negative. Table (1).

Table (1): Descriptive characteristics of 117 patients with chronic hepatitis B

Variable		value
Age	Median (IQR)	32 (26-43)
Gender N (%)	Male	68 (58.1)
	Female	49 (41.9)
HBsAg N (%)	Positive	117 (100.0)
	Negative	0 (0.0)
HBeAg N (%)	Positive	16 (13.7)
	Negative	101 (86.3)
HbeAb (%)	Positive	101 (86.3)
	Negative	16 (13.7)
HBcAb N (%)	Positive	117 (100)
	Negative	0 (0.0)
HBs Ag Level log10 IU/ml	Median (IQR)	3.47 (30.02-3.89)
Viral load (HBV DNA) log10 IU/ml	Median (IQR)	4.60 (3.9-5.5)
HBsAg\ HBV DNA ratio	Median (IQR)	0.73 (0.54-0.85)
SGPT	Median (IQR)	25 (18.15-30)
SGOT	Median (IQR)	20 (15-26)
TSB	Median (IQR)	0.8 (0.7-0.9)

The median (IQR) HBs Ag level was 3.47 (30.02 - 3.89) log10 IU/ml, and the median for HBV DNA was 4.60 (3.9 - 5.5) log10 IU/ml. The Median for SGOT, SGPT and TSB were 25 (18.15 - 30), 20 (15 - 26) and 0.8 (0.7 - 0.9) respectively.

Relationship between HBV DNA and HBsAg level

On Bivariate correlation analysis, the correlation between the overall HBsAg level and the viral load was highly significant direct correlation (R=0.460, P<0.001), and this correlation still highly significant after adjustment for age and gender (R= 0.356, P<0.001). (Table 2 and figure 1).

Table (2):The relationship between HBV DNA and HBsAg levels of 117 patients with CHB

HBV DNA and HBsAg correlation	R	P. value (2-tailed)
Before adjustment for age and gender	0.416	< 0.001(HS)*
After adjustment for age and gender	0.356	< 0.001(HS)

** HS; highly significant*

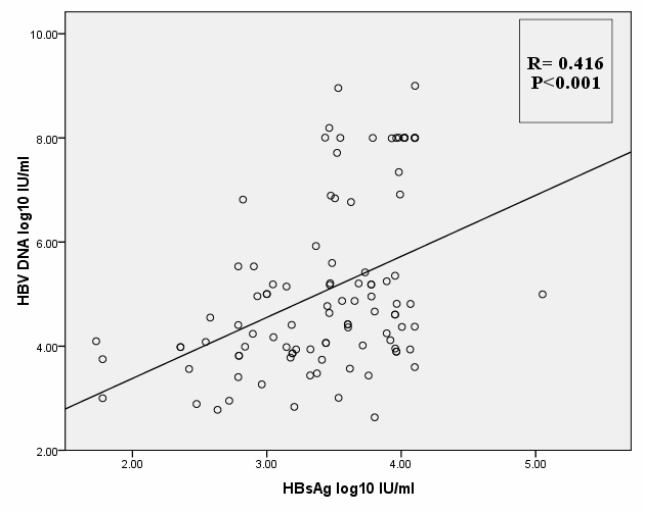

Figure (1): Curve estimation of the correlation between the levels of HBsAg and HBV DNA among study group

Relationship between HBsAg level and HBV DNA levels according to HBeAg status

It had been significantly found that the 16 patients with positive HBeAg had higher median HBsAg levels $\log_{10}$ IU/ml and HBV DNA $\log_{10}$ IU/ml levels than the 101 patients with negative HBeAg, (3.97 vs. 3.44 , P= 0.006) and (8.0 vs. 4.3, P=0.0013), respectively, these findings are shown in table 3.

Table (3): Comparison of HBsAg level and HBV DNA levels in between HBeAg positive and HBeAg negative groups

Variable	HBeAg		P value
	Positive (n=16)	Negative (n=101)	
HBsAg level $\log_{10}$ IU/ml median (IQR)	3.97 (3.53 -4.08)	3.44 (2.94 - 3.80)	0.006
HBV DNA $\log_{10}$ IU/ml median (IQR)	8.0 (7.16 - 8.1)	4.3 (3.86 - 5.16)	0.0013

From other point of view, curve estimation of this correlation revealed that the correlation was direct and significant between both groups of patients (16 HBeAg Positive and 101 HBeAg negative), and it was stronger HBeAg positive group than HBeAg negative group (R=0.560, P=0.012 vs. R= 0.101, P=0.003) respectively according to figure (2-a & B).

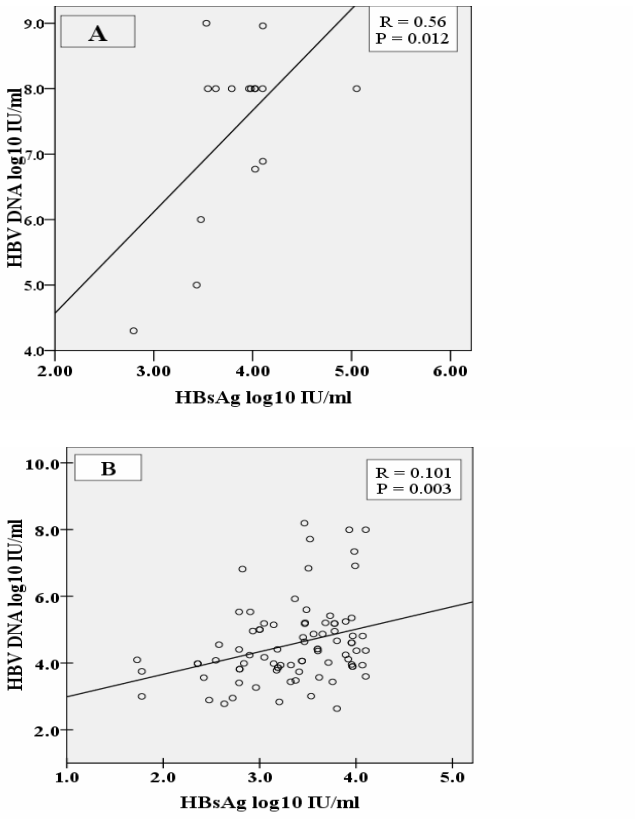

Figure (2): Curve estimation of the correlation between HBsAg and HBV DNA levels:
A- in 16 HBeAg positive patients. B- in 101 HBeAg negative patients

Correlation with other variables

The bi- variate analysis to assess the correlation between each of HBsAg and HBV DNA levels with other variables is shown in table (4). A direct significant correlation had been found between SGPT and HBsAg (P=0.003), and HBV DNA (P=0.032), i.e. levels of SGPT were significantly increased with the increase in the HBsAg and HBV DNA levels. SGOT showed significant direct correlation only with HBsAg level (P=0.001), patients with higher HBsAg levels had the higher SGOT values. While the correlation was not significant with the HBV DNA level. On the other hand, total serum bilirubin showed no significant correlation with either HBsAg or HBV DNA levels, in both correlations P>0.05. However, by further analysis with multiple logistic regression, the correlation between HBsAg and HBV DNA levels remained highly significant (P<0.001), while the correlation with other variables became not significant, this indicated a strong correlation between the HBsAg level and HBV DNA levels.

Table (4): Results of multiple logistic regression analysis for the correlation in between HBsAg and HBV DNA levels and with other variables

	HBsAg level		HBV DNA level	
	Standardized Coefficients (β)	*P*	Standardized Coefficients (β)	*P*
HBV DNA	0.310	<0.001	-	-
Age	-0.136-	0.152	-0.047-	0.023
SGPT	0.348	0.068	0.090	0.566
SGOT	-0.073-	0.696	-0.105-	0.495
TSB	0.085	0.347	0.002	0.980

DISCUSSION

A number of sensitive and specific diagnostic tests have lead to a deeper understanding of the natural history of HBV infection (13). Serological markers are indispensable in the diagnosis of HBV infection. Hepatitis B surface antigen (HBsAg) is the hallmark of HBV infection and is the first serological marker to appear in acute hepatitis B, and persistence of HBsAg for more than 6 months suggests chronic HBV infection, but HBsAg does not provide information about active virus replication (14). Moreover the presence of serum HBV DNA in chronic hepatitis patients indicates active virus replication. HBV DNA levels are detectable by 30 days following infection (13).

In our study, males showed the high rates of HBV infections than females. The median (IQR) age of patients was 32 (26 - 43) years, they were 68 male (58.1%) and 49 females (41.9%).This result was in consistent with those observed by Chu and Liaw (15); Khan *et al*. (16). This was probably due to occupational and other risk factors associated with the exposure of males.

Our study revealed that most patients are HBeAg negative (86.3%). This result was in agreement with those observed by Vaezjalali *et al.* (17). Our study revealed highly significant direct correlation was observed between HBsAg level and the viral load (P<0.001). This result was inconsistent with those observed by Ozdil and co-workers (18) and in agreement with the results of Beasley (19); McMahon (20); and Coursaget (8).

Serum HBsAg concentration was related to HBV DNA replication level; nevertheless, it is not feasible to use HBsAg concentration to monitor HBV replication levels (21). Our study revealed that patients with positive HBeAg had higher median HBsAg levels and HBV DNA levels than the patients with negative HBeAg, (P= 0.006) and (P=0.0013), respectively this result was in agreement with those observed by Coursaget (21); ;Rotman (7) ; Lutgehetmann (22). This observation is in line with a previous report indicating that pre-S deletion involved in HBeAg negative HBV infection decreases the synthesis of small surface antigens (23).

Patients who have high HBsAg level might harbor more hepatocytes with HBV integration than those who have low HBsAg level. Therefore, the higher risk of HCC in former patients can be attributed to the increased genomic instability as a result of integrated viral sequences, which plays an important role in hepato-carcinogenesis (24).The study suggested that both of viral load by RT-PCR and HBsAg level play an important role in the diagnosis and follow up of patients with chronic hepatitis B. Furthermore, overall HBsAg level and HBV-DNA by viral load appeared highly significant direct correlation.

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In-vitro study of cytotoxic effect of *cydonia oblonga* seeds extract on some cancer cell lines

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ABSTRACT

The inhibitory activity of crude extract of cydoniaoblongaseeds on cancer cells was evaluated and its cytotoxicity on different cancer cell lines was measured.
Glycosides, tannins, and phenolic compounds were extracted from seeds, which considers as the main active principals, by methanol.
Cytotoxic effects of different concentrations (62.5, 125, 250, 500, and 1000 µg/ml) of cydoniaoblonga seeds extract on two cell lines (RD and L20B) was studied after 24, 48, 27 and 144 hrs of exposure to the extract.
It was found that there was a significant effect of high concentrations (1000 and 500 µg/ml) on both cell lines within the first 24hrs exposure. Other concentrations of extract (250,125 and 62.5 µg/ml) have no effect on L20B cell line throughout the days follow of the experiment. The concentration 250 µg/ml starts to act on RD cell line at the second day (48hrs) but not so far. At the third day (72hrs), the effect of 250 and 125 µg/ml on the RD cells were very clear. The effect of 62.5 µg/ml was very obvious after 144 hrs of incubation.
From previous results, it can be concluded that cydoniaoblonga seeds' methanolic extract have a cytotoxic effect on different cancer cell lines and differ in its potency from cell line to other at different exposure times.

Keywords: cydoniaoblonga, cell lines, RD, L20B, methanolic extract

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الملخص باللغة العربية

هدفت الدراسة إلى تقييم التأثير السمي لمجموع المواد المستخلصة من بذور السفرجل على خطين من الخلايا السرطانية ولفترات تعرض مختلفة.
تم استخلاص المكونات الفاعلة الرئيسية الموجودة في البذور باستخدام الميثانول. في دراسة مسبقة اثبتت وجود بعض المكونات الفاعلة كالمواد الفينولية والتانينات والكلايكوسيدات.
استخدمت التراكيز 62.5 و 125 و250 و 500 و 1000 µg/ml في دراسة التأثير السمي لمجموع المواد الفاعلة في مستخلص بذور السفرجل على خطي الخلايا السرطانية (RD, L20B)ولفترات تعرض مختلفة (24 و 48 و 72 و 144 ساعة).
اظهرت نتائج هذه الدراسة ان هنالك تأثير سمي سريع جدا لكلا الخطين في التراكيز العالية (500 و 1000µg/ml) خلال فترة التعرض الاولى (24 ساعة) بينما لم يظهر للتراكيز الاخرى المستعملة خلال نفس الفترة اي تأثير سمي.
لم تظهر التراكيز المتبقية (62.5 و 125 و 250 µg/ml) اي تأثير سمي على الخط السرطاني (L20B) خلال فترات التعرض الداخلة في هذه الدراسة (48 و72 و144 ساعة).
في فترة التعرض 48 ساعة، كان للتركيز 250 µg/ml تأثيرا سميا بسيطا واصبح واضحا في فترة التعرض 72 ساعة كما هو الحال للتركيز 125 µg/ml حيث بدا واضحا ان له تأثيرا سميا خلال هذه الفترة (72 ساعة)، اما التركيز 62.5 µg/ml فلم يظهر اي تأثير الا خلال فترة التعرض 144 ساعة.
مما سبق تم الاستنتاج ان للمستخلص الميثانولي لبذور السفرجل تأثيرا سميا على الخطوط السرطانية المستخدمة في هذه الدراسة يختلف بإختلاف الخطوط السرطانية وفترات التعرض ولنفس التراكيز.

INTRODUCTION

Cancer is one of the major public health problems facing our world. (1) Cancer results from disruption in the control normally exerted over production and differentiation. One key aspect of this abnormal differentiation is the greatly prolonged life spans of cancer cells compared with those of their normal counterparts. Cancer cells are essentially immortal. Another aspect is the failure of the cancer cells to develop the specialized functions of their normal counterpart. (2)

The herbal medicines achieve their antineoplastic effect through various ways. Moreover, some medicine can bring on several actions, for example, they may directly inhibit the growth of tumor as well as indirectly exert an antineoplastic effect by enhancing the bodily immunologic function. Generally, they elicit no significant adverse effect on the human body and this is a strong point of herbal medicine for antineoplastic treatment.(3)

With respect to the former field ,and over the last two decades, an expanding body of evidence from epidemiological and laboratory studies has demonstrated that some edible plants as a whole, or their identified ingredients, have substantial protective effects on humanmutagenesis and/or carcinogenesis. (4) In this regard, a progress was made to understand the biochemical mechanisms of dietary and medicinal anti-mutagens and anti-carcinogens, and the investigators have broaden the horizons to cover various aspects of chemoprevention by edible photochemical or their mixtures. (5)

One of the current strategies for drug discovery involved the study of plant materials based on the ethnobotanical usage. The search for anticancer drugs, use of a plant or plant materials for the treatment of certain cancer-related disease can provide a guide for further studies, this includes, cancer treatment, immune disorders, infectious diseases, parasitic diseases and viral diseases. (6)

Few researches investigated the activity of cydoniaoblongamethanolic extract on selected cell lines using isolated phytochemical compounds of the whole fruit. (7,8).

MATERIALS AND METHODS

Plant collection

The seeds were collected from herbal drugs shop in Baghdad-Iraq.

Preparation of plant extracts

For extraction of cydoniaoblonga, ethanol 99.9% was used as solvent, thirty grams of the seed powders were extracted with 300ml of ethanol by using soxhlet apparatus for 10hr.(9) Then the extract was filtered by using whatman No.1 filter paper and the solvent was evaporated using rotary distillation apparatus. In order to obtain a completely dry extract, the resultant extract was transferred to beaker and was left in 50°C oven for 24hrs. The extract left at 4°C until assessments of anti-proliferative activities.

Tannins, phenolic compounds, and glycosides were found to be the active principals of this extract. They have a lot of proved biological activities. (10)

Cell lines

Cell lines were kindly provided by Central Public Health Laboratory, Ministry of Health, Baghdad-Iraq:

1. Rhabdomayosarcoma RD Human cell line was derived from biopsy specimen obtained from a pelvic rhabdomyosarcoma of 7 years old Caucasian girl. (11)
2. L20B Cell Line: This cell line is a murine cell line derived from mouse L cells (fibroblasts) expressing the human poliovirus receptor. (12)

Five of RD cells and five of L20B cells were cultured. The seeds extract was dissolved in phosphate buffer saline (PBS), so five concentrations (62.5, 125, 250, 500 and 1000) µg/ml were prepared. 10µl of each concentration directlyinjected to cultured RD and L20B cells thenincubated at (37ºC). After 24, 48, 72,and 144 hours, the cells were checked under the microscope and see the effect of the extract on the cells, if there any proliferation seen within the cells it meant that concentration has a cytotoxic effect.

RESULTS

Tables (1 and 2) summarize the results of this study (the concentrations of the extract, the period of exposure and the cytotoxic effect of the extract).

The results revealed a significant effect of high concentrations (1000 and 500 µg/ml) on both cell lines within the first 24hrs exposure. (tables 1 and 2)

The concentration 250 µg/ml starts to act on RD cell line at the second day (48hrs) but not so far. At the third day (72hrs), the effect of 250 and 125 µg/ml on the RD cells were very clear. The effect of 62.5 µg/ml was very obvious after 144 hrs of incubation. (table 1 and figure 1)

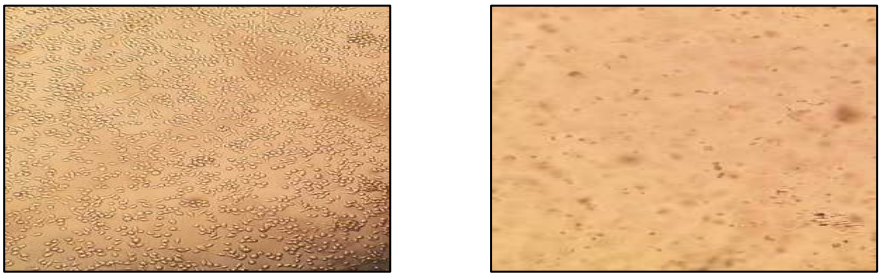
Other concentrations of extract (250,125 and 62.5 µg/ml) have no effect on L20B cell line throughout the days follow of the experiment. (table 2 and figure 2).

Table (1): The cytotoxic activity of cydoniaoblonga seeds extract on RD cell lines

concentrations	Exposure period			
	24 hrs	48 hrs	72 hrs	144 hrs
1000 µg/ml	+ve cytotoxic	-----	-----	-----
500 µg/ml	+ve cytotoxic	-----	-----	-----
250 µg/ml	-ve	Start to act	+ve cytotoxic	-----
125 µg/ml	-ve	-ve	+ve cytotoxic	-----
62.5 µg/ml	-ve	-ve	-ve	+ve cytotoxic

Table (2): The cytotoxic activity of cydoniaoblonga seeds extract on L20B cell lines

concentrations	Exposure period			
	24 hrs	48 hrs	72 hrs	144 hrs
1000 µg/ml	+ve cytotoxic	-----	-----	-----
500 µg/ml	+ve cytotoxic	-----	-----	-----
250 µg/ml	-ve	-ve	-ve	-ve
125 µg/ml	-ve	-ve	-ve	-ve
62.5 µg/ml	-ve	-ve	-ve	-ve



A before exposure B after exposure

Figure (1): RD cell lines before and after exposure to cydoniaoblonga seeds extract

A before exposure B after exposure

Figure (2): L20B cell lines before and after exposure to cydoniaoblonga seeds extract

DISCUSSION

Herbal remedies and alternative medicines are used throughout the world, and in the past, herb often represented the original sources of most drugs. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powder (13).

Fruits, vegetables, and whole grains contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics and carotenoids and thus, help to protect cellular systems from oxidative damage and could lower the risk of chronic diseases (14).

Phenolic compounds are found in cydoniaoblonga (10)have antimutagenic activity by blocking the metabolicactivation of the mutagens and scavenging the freeradicals produced from mutagen metabolism. Phenoliccompounds can also reduce the DNA-adduct formationby binding to the target sites in the DNA to preventthe binding of the mutagen (15). Tannins is anothercompound, which can be extracted from cydoniaoblonga seeds and they are considered to have cancer preventive properties (16).

Carvalho *et al.* (2010) investigated the bioactivity of the methalonic extracts of leaf, pulp, peel and seed of quince by determining phenolic profiles and suppresion effects of the extracts on the prolifration of selected human cancer cells using 3-(4,5 dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) bioassay. The antiproliferative activities of the extracts were tested on human renal (A-498 and 796-P) and colon (Caco-2) cancer cell lines. Quince leaf extract possesses concentration dependent growth inhibitory effect on Caco-2 cells and no effect was observed on renal cancer cell lines. Seed extract inhibits the proliferation of renal cancer cell lines at the highest tested dose (500 mg/kg), whereas no significant inhibition is observed at lower concentrations (7). This is a valuable finding since renal cellcarcinoma is highly resistant against current chemotherapeutic agents (17).

Alesiani *et al.* (2010) investigated the antiproliferative activities of the isolated phytochemicals from quincepeels against murine melanoma B16-F1 cells in which the most active phytochemical to inhibit the growth of melanoma cells was ursolic acid with the IC50 of 10.2 μM. (8).

CONCLUSION

By comparing the sensitivity of the two cell lines tocydoniaoblonga seeds' extract, it is clear that RD cell line is more sensitive to cydoniaoblonga seeds' extract than the L20B cell line. Further studies will be needed to determine the effects of compounds isolated from cydoniaoblonga and other more advanced anticancer assay must be applied.

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The effect of green tea (*Camilla sinensis* L.) on blood clotting after tooth extraction

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ABSTRACT

The methods (alcoholic and watery extracts) were applied to obtain the active compounds from green tea(*Camilla sinensis*) ,the results of the watery extract showed that compounds contained phenols, tannins ,flavonoids alkaloids ,while alcoholic extract of green tea included (all above compounds) in addition Resins and Coumarins. Catechin compound was separated from green tea plant and was diagnosed by FT-IR. The effect of green tea bag and catechin compound as were also studied *in vivo* and *in vitro*, green tea bag has been successful administered for checking hemorrhage after tooth extraction from 3-8 min. tannin has ability to agglutination RBCs of blood type A at 0.78g/bag ,while Catechin compound have the ability to reduce clotting time within 4-10 min. and the faster clotting time by using 1024µg/ml catechin within 4mins. In this study we suggest that green tea, tannins more effective than catechin compound in clot blood and stop bleeding after tooth extraction.

Keywords: green tea, polyphenols compounds, tooth extraction, catechin compounds

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الملخص باللغة العربية

تم استخلاص المركبات الفعالة الموجودة في نبات الشاي الاخضر (*Camilla sinensis*) بطريقتين هما المائي والكحولي , وقد بينت النتائج بان المستخلص المائي احتوى على المركبات الفينولات ,التانينات , الفلافونات والقلويدات , اما المستخلص الكحولي فقد احتوى بلاضافة على المركبات اعلاه على مواد راتينجية وكومارينات , تم فصل الكاتشين (Catechin) من نبات الشاي الاخضر , وتم تشخيصها بتقنية FT-IR. تم دراسة تاثير اكياس الشاي الاخضر ومركب الكاتشين داخل وخارج الخلية , وقد اظهرت اكياس الشاي الاخضر قدرة على ايقاف النزف بعد قلع السن من 3-6 دقائق واظهرت التانينات قدرة على تلزن كريات الدم للانسان من نوع ا بتركيز 0.78غم/ كيس , بينما اظهر الكاتشين قدرة على تقليل زمن التخثر بين 4-10 دقائق وكان اسرع وقت للتخثر بتركيز 1042مايكروغرام/ مليليتر خلال 4 دقائق و من خلال هذه الدراسة تبين بان التانينات فعالة اكثر من مركب الكاتشين في تخثر الدم وايقاف النزف بعد عملية قلع السن.

INTRODUCTION

Green tea is produced from leaves of *Camilla sinensis* , which is native to Eastern Asia ,traditional Chinese medicine has recommended drinking green tea for the prevention of disease , and in Asia this is still regarded as a healthy practice (1-3).

Tea plant belongs to the family Theacea , all tea black, green and oolong comes from the leaves of the *Camellia sinensi s*plant but they differ in the production process(4,5).Tea (*Camellia sinensis*) is a source of dietary polyphenol, which is an astringent , bitter polyphenolic compound , also found in many other plants, those in green tea are mainly flavan-3ols (catechins), catechins constitute about 25% of the dry weight of fresh tea leaf (6,7).Although tea contains a variety of compounds like minerals, vitamins , caffeine and tannins which are in actuality called polyphenols which include flavonoids, give tea a boost of health benefits that help to prevent cancer, heart disease and stroke , a subgroup of polyphenol in tea is called catechin and one of the most powerful catechins in tea , especially green tea , is called EGCG (epigallocatechingallate), which is said to be a particularly strong antioxidant(8,5,9).

Tannins are a diverse group of polyphenols that are formed as secondary metabolites in plants and have anti- oxidant qualities , these antioxidants help prevent damage to the cells in our bodies and strengthen our immune system(9-11) and include a wide range of oligomeric and polymeric polyphenols(6,12).The tannins in green tea have anti-inflammatory ,antibacterial , antiviral and antiparasitic effects ,on way in which the tannin in green tea can be helpful to human is by killing the bacteria that lead to gingivitis or gum disease(12,13).

Also tannins contained in tea are useful for healing burns and stopping bleeding, additionally tannins stop infection while continuing to heal the wound internally , in the event an infection has already begun , tannins have the ability to form green tea was bought a protective layer over the exposed tissue to stop the infection from spreading(12,14).The purpose of the present study is to investigate *in vivo* and *in vitro* the efficacy of tea plant ,*Camilla sinensis*, to stop bleeding which may occur for several hours after tooth extraction ,and the efficacy to clots blood groups.

MATERIALS AND METHODS

Sample collection

Green tea was bought from local markets in powder of dried leaves in bag coated with , this kind of green tea Chinese origin and was filled in Syria – Damascus, then transferred sample to the laboratory for classification.

The weight of the tea bag 1.56 gram /bag, then attended three weight included 0.78 g/bag ,0.39 g/bag ,and 0.19g/bag, then has been detected on the effectiveness of tea bag in clotting time after tooth extraction by hold a smaller size tea bag in place with bite and lips , or bear down on the tea bag with fingers and keep the mouth as closed as possible and agglutination of Red Blood Cells *in vitro* .The study done on 20 patients males and females from different age ,also same cases taken is infected tooth and we see the clot and blood stopping from 3-6 min .

Hot watery extract

Dissolved 50g from tea bag powder in 500ml distal water for 10 min, then filtered the supernatant, this supernatant is concentrated by rotary vacuum evaporation then incubate at 37^0C for 48hrs. and kept at 4^0C.(15).

Gold alcoholic extract

Moister 50g from green tea in 500ml methanol 99% for 24hr.in shaking incubator after this, supernatant is filtered by WatmanNo.1, and then concentrated by rotary vacuum evaporation and incubate at 37^0C for 48hrs. and kept at 4^0C.

Detection of Phenols

Phenols were detecting using ferric chloride (1%),then added 3ml green tea extract to 2ml ferric chloride , the positive result by the existence of a bluish green color (16).

Detection of Alkaloids

Added 3ml of tea extract to test tube containing either Mayer's reagent{a- dissolved 1.35g $Hgcl_2$ in 60 ml D.W. b-dissolved 5g KI in 10 ml D.W and mixed 60ml from (a)+ 10ml from (b) and completed to 1000ml by D.W which gives white precipitation or added Marquis's reagent {prepared according to (16) by ,mixed 1 ml formaldehyde 40% with 10ml H_2SO_4} , the green color evidence the presence of alkaloids.

Detection of Tannins

Tannins were detected according to Harbone (1973),boiling 10 gm from green tea powder in 50 ml distil water then taken the supernatant which was divided into two parts .Add to the first part 1%Lead acetate and the positive result upon tannin is white precipitation gelatinous texture .While add to the second part 1% ferric chloride , the green color indicates the existence of tannin.

Prepare of green tea powder for extraction of Catechins

Weighing 200 g of green tea and put in the electric furnace and adjusted to 100^0Cuntil constant weight for the purpose of creating the sample extraction.

The extraction and separation of active compounds (Catechins) from green tea were done by the method described by (17).

Diagnosis of Catechin compound by FT-IR technique

The KBr disk of the purified compound was done and measured the spectrum, Catechin were compared with the standard compound in Ministry of Industry and mineral /Ibn Sina state company.

The effect of Catechin on blood clot after tooth extraction

Catechin concentration which are used in this experiment are (1024 , 512 and 256µg/ml) from Catechin stock according to (18), moister a piece of cotton with each concentration and placed over each extraction site with bite and lips, or bear down on the tea bag with fingers , and keep the mouth as closed as possible.

Preparation of Erythrocyte Suspension

The blood used was human blood ,was mixed with phosphate buffer solution (PBS)(PH9.8).This suspension is centrifuged at 800xg for 10 min, the compact red blood cells were suspended in 3% formalin maintained at 37^0C for 12-24hrs.After this, sample is centrifuged several times until the supernatant is clear , the cell suspension was kept at 4^0C(19).

Agglutination activity of Catechins

Use the same previous concentration of Catechin (1042, 512 and 256µg/ml), it was mix 1ml of blood suspension with each concentration in the test tube and determine the clotting time for human blood .

RESULTS AND DISCUSSION

Two methods (watery and alcoholic extracts) were applied to obtain the active compound from green tea. the results of the watery extracts showed that compounds contained phenols, tannins ,flavonoids, and alkaloids , the alcoholic extract of green tea included (all the above)in addition Resins and Coumarins, and this agree with(20,21)the Catechins compound were extracted and diagnosed by FI-TR, the spectrum included the following peak:

- 3387(cm-1) board peak for absorption of OH group.
- 2890, 2929 (cm-1) two clear peaks for absorption of aliphatic group C-H.
- 3060(cm-1) integral with OH peak for absorption of aromatic group C-H.
- 1516, 1612 (cm-1) tow peaks for absorption of aromatic group C=C.
- 1049(cm-1) clear peak for absorption of ether group C-O-C.(Figure 1).

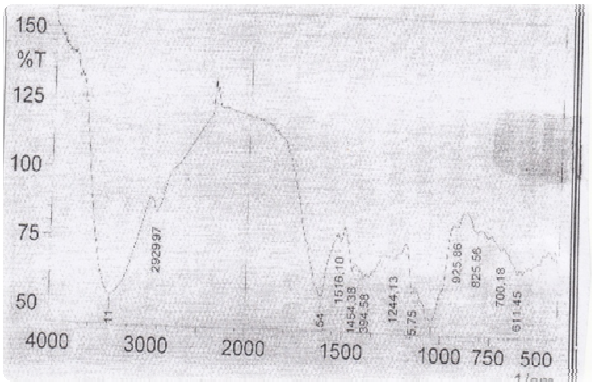

Figure (1): FT -IR spectrum for pure Catechin from green tea

From these spectrum (FI-TR), there is comparable between Catechins and the standard compound, this result indicate the high purity of Catechin (Figure 2).

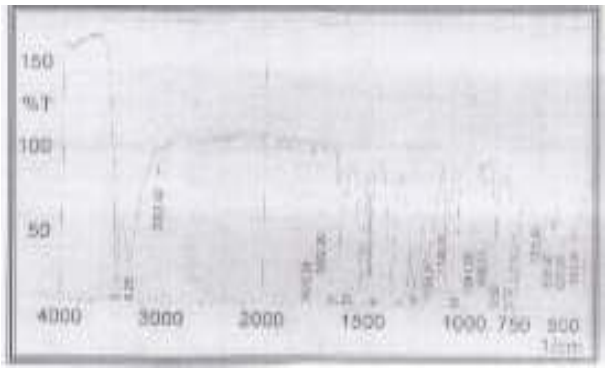

Figure (2): FT-IR spectrum of Catechin standard

The effect of green tea bag to clot and stop bleeding *In vivo* and In *vitro*

In the initial survey for the presents of tannins from the powder of green tea only blood type A agglutination faster then other blood groups in concentration 0.78g (dry green tea)/bag through 2min and 3min, 4min at the concentration 0.39g/bag and 0.19g/bag respectively, and the longer clotting time 6 min at 0.95 g/ bag occurred in B blood group (Figure 3).

In normal clot and stop bleeding after tooth extraction need 30 min. at least to stop oozing the blood from the socket (22, 23). A systematic review by (24, 5) found that tannins constrict blood vessels, which well stop the bleeding and they reduce the swollen tissues that cause soreness.(25) and(7) also demonstrated that What is even more exciting is that tea bags can be used for more than tooth extraction , it also use to control bleeding that occurs as the result injuries to the soft tissues, which include the tongue ,cheeks ,gum and lips.

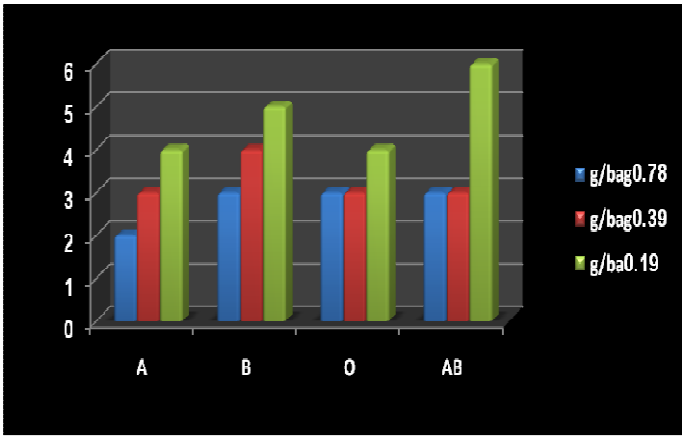

Figure (3): Effect of green tea on blood clotting after tooth extraction

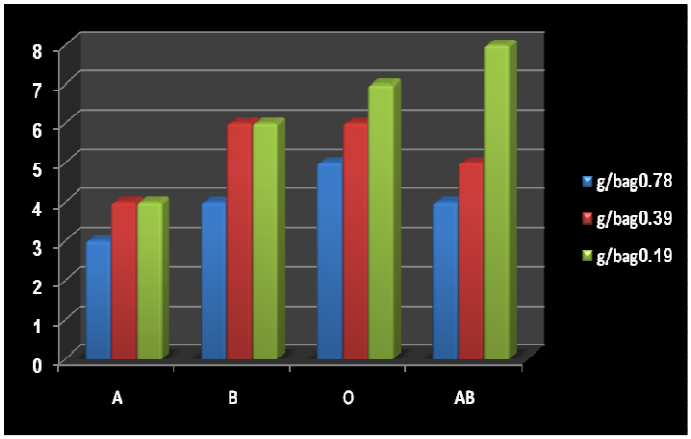

Figure (4): Effect of green tea on agglutination of human blood (*in vivo*)

Our result, disagree with (26) who said that green tea has been successful administered for checking hemorrhage after tooth extraction in from thirty min. to one and one half hours time.

Several published studies are in accordance with these data , a study by (27,26)demonstrated that tea bag constringes the tissue, temporarily decreasing their vascularity ,and causing contraction of their blood vessels ,it arrests secretion and condenses relaxed and feeble tissue when mixed with blood ,it forms a clot rapidly on account of coagulation of the albumen.

The tea extract was tested In vitro for clotting time, from figure (4),we notice that the agglutination using human blood was done at different time and tea bag concentration , the agglutination was observed in all blood groups , but the faster agglutination show with RBCs of A group at 3 min. by using 0.78g(dry green tea)/bag and the longer clotting time occurs in AB group at 0.19g/bag .Most studies agree with the present one (28,29) revealed that the human blood types have different sugar moieties, on the surface of the cell ,type A has n-acetyle-D-galactosemins ,D-galactose for type B, L-fucose in type O blood ,type AB contains the sugar determinates for both A and B .Agglutination occurs when the tannin in terraces with these sugar moieties.

Raza and John (2008) and Lee *et.al.*,(2004) also demonstrated that the active constituent in green tea are powerful antioxidants called polyphenols(catechin) and flavonoids, tannins in tea are large polyphenol molecules and form the bulk of the active compounds in green tea , while catechins make up nearly 90% of the tannins ,several Catechins are present in significant quantities:epicatechine(EC), Epigallocatechin(EGC), Epicatechingallate(ECG) , epigallocatechingallate(EGCG)(5,1).

Figures (5) and (6) showed the role of Catechins compound in clot blood and stop bleeding after tooth extraction. The results show that the longer clotting time after tooth extraction by using o.78g/bag dry green tea through 5 min. and 12 min by using 0.19g/bag dry green tea in AB blood group.

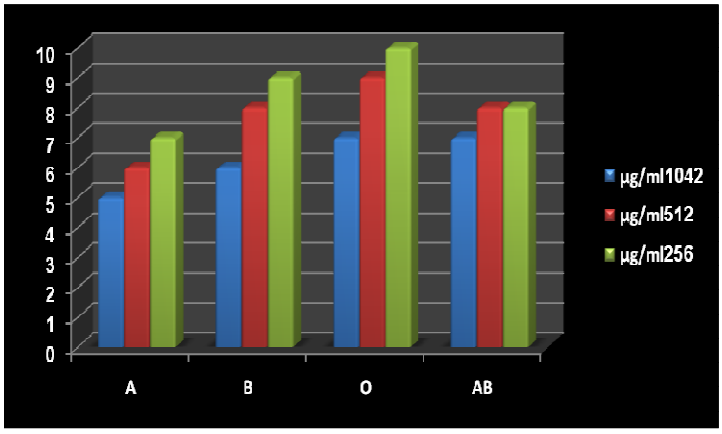

Figure (5): Effect of Catechin compound from green tea on blood clotting after tooth extraction

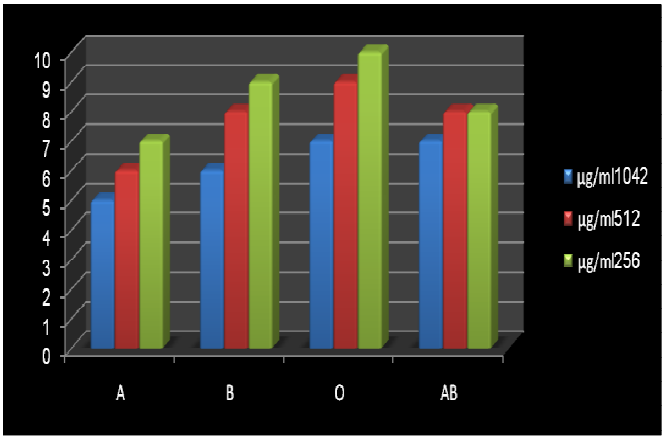

Figure (6): Effect of Catechin compound from green tea on human blood groups *in vitro*

This observation suggest that green tea could be used as potential tool for reduce the clotting time after tooth extraction and to help even the infected tooth after extraction to be clot in a very suitable time.

Based on this observation we suggest that green tea, tannins more effective than Catechin compound in clot blood and stop bleeding after tooth extraction.

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Evaluation of biosynthesis of nanoparticles using medicinal plant extract of its anti oxidant and anti microbial activities

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ABSTRACT

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. In the synthesis of metal nanoparticle by the reduction of the corresponding metal ion salt solutions. Nanoparticles are often referred to as clusters, nanospheres, nanorods and nanocups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Nanoparticles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects. Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. In this study, we report the synthesis of silver nanoparticles using the. *Zingiber officinale, Curcuma longa and Syzygium aromaticum.* Synthesized particles are characterized by UV –The silver nanoparticles formation was confirmed by the colour change of plant extracts (SNPs) and further confirmed with the help of UV-Vis spectroscopy and detection nano particle shape by SEM. These silver nanoparticles were tested for antibacterial and antifungal activities using disc diffusion method. The test cultures are *Proteus, Pseudomonas, Klebsiell,* and *E.coli* species of bacteria and *Aspergillus* of fungal were used. The microbial property of silver nanoparticles was analyzed by measuring the inhibition zone. For the last two decades extensive work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice.

Keywords: Nanoparticlesm, *Zingiber officinale, Curcuma longa and Syzygium aromaticum,* SEM

الملخص باللغة العربية

تهتم الكيمياء الخضراء بتصميم وتطوير وتنفيذ المنتجات والعمليات الكيميائية لتقليل أو الحد من استخدام وتوليد المواد التي تشكل خطورة على صحة الإنسان والبيئة، والنانوبارتكلز أو الجسيمات المتناهية الصغر تسهم في تخفيض نسب المعادن الثقيلة المذابة في شكل أملاح، يتراوح حجم هذه الجسيمات ما بين 1−100 نانومتر، وتتمتع هذه الجسيمات بعدد من الخواص بالنسبة إلى المواد السائبة، وغالبا ما يكون لها خواص مرئية فريدة نظرا لصغر حجمها بشكل يكفي لحصر الاليكترونات وإنتاج آثار كمية.

ويعبر تطوير عمليات صديقة للبيئة لبناء الجسيمات المعدنية مهما في مجال تطبيقات النانوتكنولوجي، في هذه الدراسة تم تطبيق النانوتكنولوجي على بعض النباتات الطبية مثل الزنجبيل والكركم والسيزية العطري، وتم اختبار الجسيمات النانوية الفضية للأنشطة المضادة للجراثيم والفطريات باستخدام طريقة الانتشار القرصي.

INTRODUCTION

A lot of strategies are employed for the synthesis of silver nanoparticles (AgNPs), but the green methods have been gained considerable interest because of use of environmentally benign materials (1,2). So, the synthesis and design of nanomaterials through biological routes (called *biosynthesis*) have attracted great interest. Among the biological systems, the living plants (3,4) are considerably preferred for biosynthesis of silver nanoparticles due to the diversity richness of plant kingdom that provides phytochemicals with strong antioxidant properties. It is well known that plants have been used by humans for a very long time to treat many diseases.
Nanotechnology is expected to open new avenues to fight and prevent disease using atomic scale tailoring of materials. The most promising nanomaterial with antibacterial properties are metallic nanoparticles, which exhibit increased chemical activity due to theirlarge surface to volume ratios and crystallographic surface structure (5). Metallic nanoparticles are mostly prepared from nobel metals such as Gold, Silver, Platinum and Lead. Among the nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine (6).

MATERIALS AND METHODS

Plant material and synthesis of silver nanoparticle

The *Zingiber officinale, Curcuma longa* and *Syzygium aromaticum.* were grounded to a fine powder. Silver nitrate3 mM was added to the plant extract to make up a final solution of 200 ml and centrifuged at 10,000 rpm for 25 min.
The supernatants were heated at 80ºC. A change in the color of the solution was observed during heating of process with in 10-15 minutes. The color changes indicate the formation of silver nanoparticles (SNPs). The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

Microorganisms

Culture of, *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoneae* species of bacteria and *Aspergillus niger.*

Antibacterial activity

The antibacterial activities of SNPs were carried out by disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10 mg/ml) and placed in the nutrient agar plate and kept for incubation at 370C for 24 hours. Zones of inhibition for control, SNPs and silver nitrate were measured. The experiments wererepeated thrice and mean values of zone diameter were presented(6).

Antifungal activity

Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates with 40 cell / ml. The sterile discs were dipped in silver nanoparticles solution (10mg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition was measured(7).

Synthesis of silver Nanoparticle:

About 3 ml of each extract were added to 20 ml of AgNO3. The mixture was boiled at 80°C for 20 minutes, while heating the colour of solution was changed from pale to dark brown. The reduction of Ag^{+} ions to Ag^{0} was monitored by measuring the UV-vis spectrum of various concentration of reaction mixture.

UV-Vis Spectra analysis

The reduction of pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer at the range of 300-700 nm and observed the absorption peaks at 420-450 nm regions, which are identical to the characteristics UV-visible spectrum of metallic silver and it was recorded.

SEM analysis of silver nanoparticles

The pellet was subjected for SEM analysis. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis.

RESULTS AND DISCUSSION

UV–visible spectroscopy is an important technique to determine the formation and stability of metal Nanoparticle in aqueous solution. The reaction mixture changes the colour by adding Ag^{+} metal ions. These color changes arise because of the excitation of surface plasmon vibrations in the silverNanoparticle (8). It shows yellowish to dark brown in colour. The dark brown colour of silver colloidis accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced byan interacting electromagnetic field (9). The strong surface plasmon resonance

band appears at the range of 400-500 nm and the broadening of peak indicated that the particles are monodispersed. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. *Zingiber officinale* synthesized silver nanopartcles within 5min where as *Curcuma longa* 12 min *Syzygium aromaticum* 15 min took to synthesize nanoparticles.

The UV-Vis spectrum of colloidal solutions of SNPs synthesized from *Zingiber officinale* have an intense peak was observed in the UV-spectrophotometer at 450nm while *Curcuma longa and Syzygium aromaticum* appear at 500nm the broadening of peak indicated that the particles are poly-dispersed (Fig1).

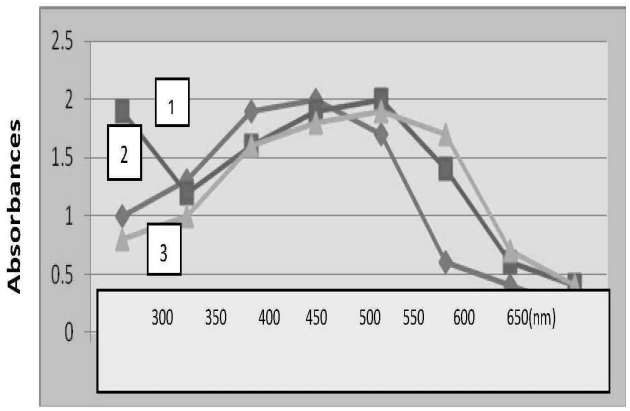

Figure (1): UV-Vis absorption spectroscopy of silver nanoparticles *1-Curcuma longa 2- Syzygium aromaticum. 3- Syzygium aromaticum*

After the reduction of silver ions by the *Zingiber officinale, Curcuma longa and Syzygium aromaticum* extracts, The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles. The second biogenic route is the energy (or) electron released during Glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of Ag(NO3)2 to form nanoparticles and the another mechanism is releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions(9,10). The synthesised Nanoparticles morphology were characterised by scanning electron microscope. The silver Nanoparticle formed were predominantly with uniform shape (Fig. 2). It is known that the shape of metal Nanoparticle considerably change their optical and electronic properties. The SEM image exposed that the formed nanoparticle was spherical cubic hexagounal in shape formed with the size range of 22-30nm for zingiber, 40-55nm for *Curcuma longa, 35-60nm for Syzygium aromaticum.*

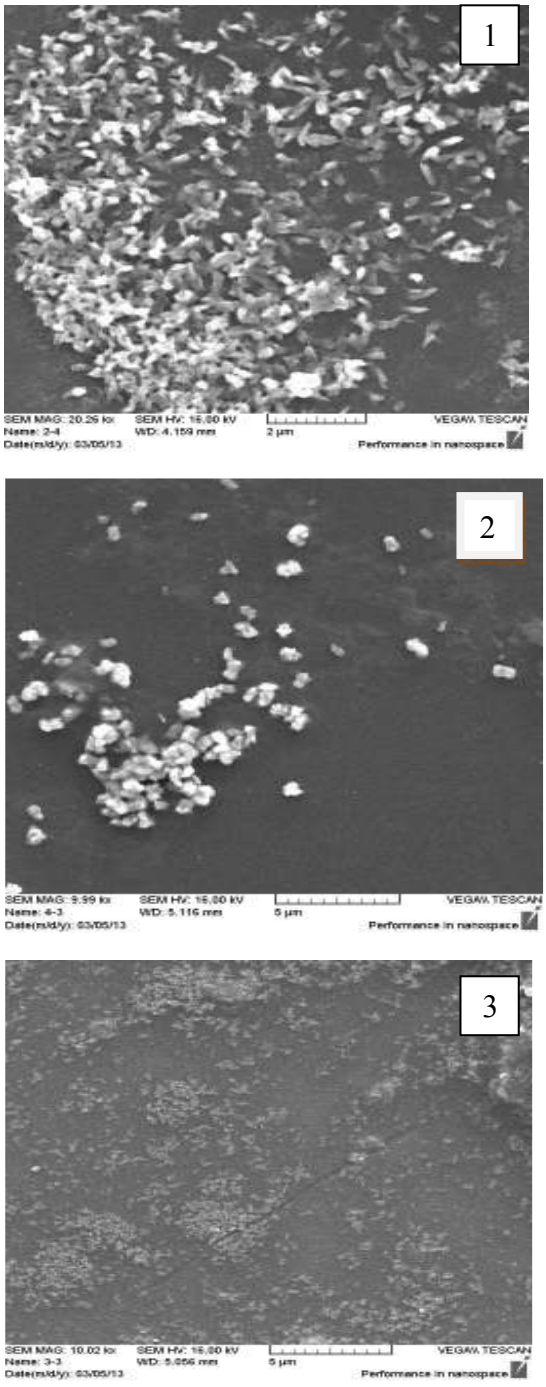

Figure (2): The SEM images of silver Nanoparticle synthesized from :1- *Zingiber officinale,2- Curcuma longa and 3- Syzygium aromaticum* extracts at various magnification.

Antioxidant activity

Antioxidant activity revealed as scavenging DPPH staible free radical by *Zingiber officinale, Curcuma longa and Syzygium aromaticum* extracts and nano particle biosynthesis we found that the nano particle of all extracts had given *highest scavenging activity than the extracts for all plants Zingiber officinale nanoparticle shwoed* highest scavenging activity (97%)IC_{50} 25 µgml-1followed by *Curcuma longa and Syzygium aromaticum* (95% IC_{50} 37.7 µgml-1, 93% IC_{50} 44.6 µgml-1), respectively Fig(3). antioxidant behavior of these silver phytonanosystems makes them useful in therapy of many diseases caused by oxidative stress(11).

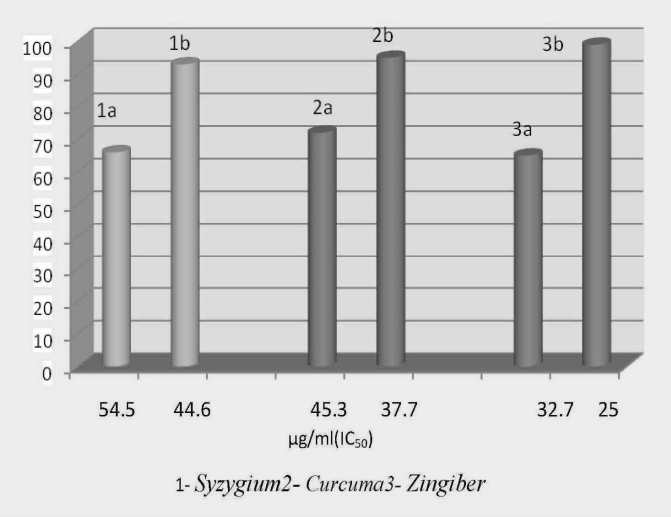

Figure (3): Antioxidant activity of *Zingiber officinale, Curcuma longa and Syzygium aromaticum* extracts and synthesis Nanoparticle

Table (1) shows the antimicrobial activity of synthesized Ag nanoparticles against four different bacteria and fungi such as *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoneae,* and phytopathogenic *Aspergillus niger.* As it showed a clear inhibition zone, the synthesized Ag nanoparticles were highly effective in their activity against *pathogenic bacteria* and phytopathogenic *Aspergillus niger* than the all other plant extracts . The silver nanoparticles synthesized via green route are highly toxic towards fungal species also when compared to bacterial species. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity(12). Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions(13). our findings of suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane.

Table (1): Antimicrobial activity of medicinal plants

S. No.	Baterial species	Inhibition zone (mm)					
		Zingiber		*Curcuma*		*Syzygium*	
		Extract	Nano	Extract	Nano	Extract	Nano
1	*E.coli*	8	19	8	18	6	14
2	*Klebsiella*	10	20	6	17	8	19
3	*Proteus*	7	18	7	14	9	14
4	*Pseudomonas*	9	16	5	11	6	12
5	*Aspergillus*	6	15	6	10	5	13

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Prevalence of resistant bacteria among patient in Islamic hospital in Jordan

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum β lactamases (ESBLs) and MRAB (Multidrug-resistant - *Acinetobacter baumannii*) continue to be a major challenge in clinical setups world over. This study, was made to study the prevalence of resistant bacteria from clinical isolates in Islamic Hospital-Amman, Jordan.

A total of thirty five collected isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* and *E.coli* (ESBLs) and *Acinetobacter baumannii* (MRAB) were studied for their susceptibility patterns to various antibiotics and detection of resistant producers by disc diffusion test. Out of 35 clinical isolates, 9 were MRSA, 24 were ESBL (among them 21 *E.coli* and 3 *K.pneumoniae*), and 2 were *Acinetobacter baumannii*. The resistant detected according standard procedures. ESBL producers were more in hospital isolates (69%) compared to MRSA (26%). Maximum percentage of ESBL was *E.coli* that isolated from urine and representative high percentage of isolates. Among the 35 bacterial isolates tested for their antibiogram; the Acinetobacter were resistant to all tested antibiotics except colistin, while *K.pneumoniae* were resistant to first, scenod and third generation of cephalosporin, but susceptible to Entrapenem, impenem and meropenem, the other ESBL isolates was *E.coli* has similar patterns, but they are resistant to 3rd-generation cepholosporins in 95% instead of 100% and was susceptible to levofloxacin and ciprofloxacin in percentage between 40 and 20% respectively. This study concluded that the bacterial resistant in Jordan still regards as problem facing clinician and the monitoring for drug resistant is necessary in clinical setting for proper disease treatment.

Keywords: MRSA, ESBLs, MRAB

الملخص باللغة العربية

تشكل البكتيريا المقاومة للمضادات الحيوية مثل المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) والبكتيريا ذات الطيف الواسع لإنزيم البيتالاكتميز (ESBLs) ومتعدد المقاومة للمضادات الحيوية مثل *Actinobacter Baumanni* التحدي الأكبر للمستشفيات في العالم.

هدفت الدراسة الحالية لتسجيل هذه الأنواع من البكتيريا في المستشفى الإسلامي في مدينة عمان في المملكة الأردنية الهاشمية.

تم مع 35 عزلة تمثل المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) و *K.pneumoniae* و *E.coli* و (ESBLs) و *Actinobacter Baumanni* (MRBA). ودرست حساسية ومقاومة هذه العزلات للمضادات الحيوية باستعمال طريقة قرص الانتشار بالكار.

من مجموع 35 عزلة، كانت 9 عزلات تعود إلى (MRSA) و 4 عزلات تعود إلى (ESBLs) ضمنها 21 عزلة (E.coli) و 3 عزلات *Actinobacter Baumanni* وعزلتين (MRBA).

تم الكشف عن المقاومة باستعمال الطرق القياسية المعتمدة، وجد أن الأكثر شيوعا هي ESBLs حيث مثلت 69% مقارنة بنسبة 26% ل (MRSA)، كما وجد بأن الأكثر نسبة هي E.coli بين ESBLs والتي عزلت من البول.

وعند دراسة المخطط المضاد للمضادات الحيوية وجد بأن *Actinobacter Baumanni* مقاومة لكل المضادات الحيوية ما عدا كلوستين، بينما وجد أن *K.pneumoniae* مقاومة للجيل الأول والثاني والثالث للسيفالوسبورين لكنها حساسة لكل من Entrapenem، impenem ، meropenem وكانت *E.coli* لها نفس الصورة لكنها مقاومة للجيل الثالث بنسبة 95% بدلا من 100% وأنها حساسة لكل من levofloxacin وciprofloxacin بنسبة بين 20–40% بالتتابع.

استنتجت الدراسة بأن هذه الجراثيم المقاومة تتواجد بنسب عالية في الأردن وأنها تمثل تحديا ومشكلة تواجه المعنيين بمعالجة الأمراض التي تسببها هذه الجراثيم.

INTRODUCTION

Antibiotic resistance among pathogenic bacteria is a well documented phenomenon that has severe consequences for the treatment of infections in the hospital setting and increasingly in the community. A sharp decline in the number of newly approved antibiotics has further complicated the treatment process (1). Multidrug resistant pathogens (MDR) are very common today. The list of such resistant pathogens has multiplied from the popular MRSA (methicillin-resistant *Staphylococcus aureus*) to VISA (vancomycin-intermediate *S.aureus*) ,VRSA (vancomycin-resistant *S.aureus*), ESBL (Extended spectrum beta lactamase) ,VRE (Vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*) and MRAB (Multidrug-resistant - *Acinetobacter baumannii*). There is no doubt that antibiotic resistant bacteria existed before the widespread of use of antibiotics, MRSA have been isolated from patient in medical city in Baghdad-Iraq, they never exposed to Methicillin (2). But their extensive use has put an unnoticed evolutionary pressure on the pathogens leading to the development of drug resistant populations and the spread of resistance between bacterial species (2,3).

Antibiogram of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Hospital Samples has been extensively documented (4,5). ESBL are plasmid mediated, TEM-1, TEM-2 and SHV-1 derived enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem (4). The first ESBL-producing organism was isolated in Germany in 1983. The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option (4).

Acinetobacter are a key source of infection in debilitated patients in the hospital. Multidrug-resistant *Acinetobacter baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. Antimicrobial resistance among *Acinetobacter* species has increased substantially in the past decade (6). Strains that demonstrate resistance to all antimicrobial agents, including polymyxins, have also been reported in the literature, making treatment of these infections extremely difficult and in some cases impossible (6). *Acinetobacter* species possess a wide array of beta-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins, and carbapenems. Tigecycline, a relatively new glycylcycline agent, has bacteriostatic activity against multidrug-resistant *Acinetobacter* species.

It is vital to test for antimicrobial resistance in pathogenic isolates obtained from infected patients where the organism is known to harbor resistance or susceptibility pattern is unpredictable. This study was investigated the prevalence of resistant pathogenic bacteria in Islamic hospital in Amman-Jordan.

MATERIALS AND METHODS

Clinical sample collection

Thirty five human isolates were collected from Islamic hospital (Amman-Jordan) patients that show resistance to known antibiotics. These clinical specimens included: Urine , blood , diabetic foot , nasal , pus , wound , ear ,and high vaginal swabs.

Characterization of bacterial isolates

Characterization of the thirty five bacterial isolates that were shows resistant to antibiotics by standard methods were documented (NCCLS approved standard M100-S17) (7).

Methicillin-Resistant *Staphylococcus aureus* was identified by showing resistant to oxacillin, second generation cephalosporin (cefoxitin) and third generation cephalosporin (cefotaxime ,ceftizoxime ,ceftriaxone) . *Escherichia coli* and *Klebsiella pneumonia* ESBL's were identified by the synergism effect of two antibiotics (cefotaxime) and (amoxicillin + calvulanic acid), and resistant to first generation cephalosporin (cephalexine), second generation cephalosporin (cefuroxime and cefoxitin) and third generation cephalosporin (cefotaxime, ceftizoxime ,ceftriaxone).

Identification of *Acinetobacter spp.* was done by Gram staining which shows gram- negative cocci , Remel RapID ^{TM} ONE system which used for oxidase-negative, Gram – negative bacilli conformation. All bacterial isolates that shows resistant to antibiotics were confirmed by Sensititre ARIS® 2X SWIN® Software which is standard for accurate detection of antimicrobial resistance (8).

Tests procedure

Two methods were used for inoculums preparation direct colony suspension at log phase growth, and broth methods, the turbidity of the test suspension were standardized to match that of a 0.5 McFarland standard (7). Three to five colonies were selected rather than just one, to increase the chances of detecting higher resistance. Colonies were suspended in saline (Sterile distilled water) Then inoculums turbidity was adjusted equivalent to a 0.5 McFarland standard (corresponds to approximately 1.5 X 10^8 CFU/ml). The adjusted suspensions were used as inocula within 15 minutes (7). Nutrient agar plates were warmed up at room temperature for 10–15 minutes. Vortex the organism suspension to make sure it is well-mixed. Then, a fresh, sterile cotton swab was dipped into the suspension. Excess

liquid was removed from the swab by pressing it against the side of the tube. Inoculums' was spread evenly over the entire surface of the plate by swabbing in three directions then plates were allowed to dry before applying discs. Discs were applied to the surface of the agar within 15 min of inoculation.

Disk diffusion test

The thirty five clinical isolate strains were subsequently screened against 23 antibiotics purchaser from Bioanalyse Company (Amman/ Jordan) , including natural products (such as oxacillin, vancomycin), and completely synthetic molecules (such as ciprofloxacin) according to NCCLS Document M100-S17(7). Disks were placed on the plate one at a time using an ethanol dipped and flamed forceps. Finally, disk was pressed down firmly to ensure complete, level contact with the agar surface ,space between the antibiotic disks were made sufficiently to prevent overlapping zones of inhibition . The plates were then inverted and incubated at 37°C for 24 to 48 hours (7). Inhibition zone area were measured to nearest millimeter, the sizes of the zones of inhibition are interpreted by referring to standards of the NCCLS M100-S17. The organisms were reported as either susceptible or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given. The susceptible (S) category implies that isolates are inhibited by the usually achievable concentration of antimicrobial agent while the resistance (R) category implies that isolate are not inhibited by the usually achievable concentration of the agent (7, 9).

RESULTS

Characterization of resistant bacteria from clinical samples: Thirty-five bacterial species were studied which shows resistance to known antibiotics. These species were grouped after reconfirmation of antibiotic profile, and were found that nine bacteria belong to methicillin resistant *Staphylococcus aureus* (MRSA), twenty one *Escherichia coli* - ESBL, three *Klebsiella pneumonia*- ESBL and two belong to *Acinetobacter spp.* (Table1). The resistance to known antibiotics of these clinical isolates depend upon their resistant to at least five of mentioned antibiotics: oxacillin (OX), amoxicillin plus calvulanic acid (AMc), cephalexine (CL), cefuroxime (CXM) , ceftizoxime (ZOX) ceftriaxone (CRO), cefotaxime (CTX), ciprofloxacin (CIP), levofloaxcin (LEV) and sulfamethoxazole + trimethoprim (SXT) . However they were sensitive to at least one of these antibiotics gentamicin (GN), tigecycline (TGC), teicoplanin (TEC), vancomycin (VA), amikacin (AK), etrapenem (ETP), imipenem (IPM) and meropenem (MEM).

Table(1): Number and type of resistant bacteria isolated from clinical samples

Type Of Isolated Resistant Bacteria	No. of Clinical Isolates
Methicillin Resistant *Staphylococcus aureus* (MRSA)	9
Escherichia coli – ESBL	21
Klebsiella pneumonia - ESBL	3
Acinetobacter spp.	2

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates

Antibiotics disc potencies and acceptable zones of inhibition were based on NCCLS standards. A total of nine MRSA isolates described as Z100, X100, M100, A100, U100, E100, S100, D100 and Y100 were tested. Table (2) and figures (1-6) show the results of zone of inhibition (mm) as compared with standard antibiogram. All (MRSA) isolates were resistant to amoxicillin+ calvulanic acid, oxacillin, first ,second ,third and fourth cephalosporin generations except for five strains U100, E100 ,S100 ,D100 and Y100 showed susceptibility to cefuroxime (second generation) and cefepime (fourth generation). Out of 9 isolates 5 isolates were found to be sensitive to second generation fluroquinolones (ciprofloxacin) and third generation fluroquinolones (levofloxacin) with good zone of inhibition range (26 – 32 mm). On other hand most of the strains showed sensitivity to Aminoglycosides group including (amikacin and gentamicin), glycylcyclines group including (tigecycline), sulfonamides with antifolate drugs (trimethoprim+sulfamethoxazole) and glycopeptide antibiotic (vancomycin and teicoplanin).

***Escherichia coli* ESBL Isolates**

A total of twenty one *Escherichia coli* isolates described as YZ1 ,YZ2 ,YZ3 ,YZ4 ,YZ5 ,YZ6 ,YZ7 ,YZ8 ,YZ9 ,YZ10 ,YZ11 , YZ12 ,YZ13 ,YZ14 ,YZ15 ,YZ16 ,YZ17 ,YZ18 ,YZ19, YZ20 , YZ21 were tested. The value of zone of inhibition in (mm) of these strains were shown in (table3).

Table (2): Antibiogram of Methicillin-Resistant *Staphylococcus aureus (MRSA)* that were collected from Islamic Hospital in Amman

Antimicrobial agent	Zone of clinical Isolates (mm)								
	Z100	X100	M100	A100	U100	E100	S100	D100	Y100
Amoxicillin/ Calvulanic acid	12 (R)	10 (R)	Zero (R)	Zero (R)	14 (R)	18 (R)	24 (S)	12 (R)	27 (S)
Oxacillin	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	9 (R)	7 (R)	Zero (R)	12 (R)
Sulfamethoxazole/ Trimethoprim	24 (S)	Zero (R)	Zero (R)	20 (S)	25 (S)	26 (S)	Zero (R)	24 (S)	24 (S)
Teicoplanin	15 (S)	16 (S)	Zero (R)	16 (S)	16 (S)	16 (S)	15 (S)	15 (S)	15 (S)
Vancomycin	18 (S)	19 (S)	Zero (R)	19 (S)	18 (S)	17 (S)	22 (S)	18 (S)	18 (S)
Tigecycline	17 (R)	18 (R)	13 (R)	16 (R)	20 (S)	20 (S)	20 (S)	18 (S)	18 (S)
Amikacin	13 (R)	17 (S)	28 (S)	12 (R)	21 (S)	23 (S)	22 (S)	22 (S)	22 (S)
Gentamicin	Zero (R)	18 (S)	22 (S)	Zero (R)	20 (S)	20 (S)	20 (S)	20 (S)	19 (S)
Cephalexine	Zero (R)	Zero (R)	Zero (R)	Zero (R)	15 (R)	17 (R)	17 (R)	Zero (R)	17 (R)
Cefuroxime	Zero (R)	Zero (R)	Zero (R)	Zero (R)	21 (S)	21 (S)	22 (S)	20 (S)	18 (S)
Ceftizoxime	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)
Ceftriaxone	Zero (R)	Zero (R)	Zero (R)	Zero (R)	12 (R)	14 (R)	12 (R)	Zero (R)	16 (R)
Cefepime	Zero (R)	Zero (R)	Zero (R)	Zero (R)	23 (S)	21 (S)	20 (S)	21 (S)	19 (S)
Levofloxacin	12 (R)	Zero (R)	15 (R)	13 (R)	31 (S)	29 (S)	29 (S)	32 (S)	29 (S)
Ciprofloxacin	Zero (R)	Zero (R)	Zero (R)	Zero (R)	30 (S)	26 (S)	26 (S)	29 (S)	27 (S)

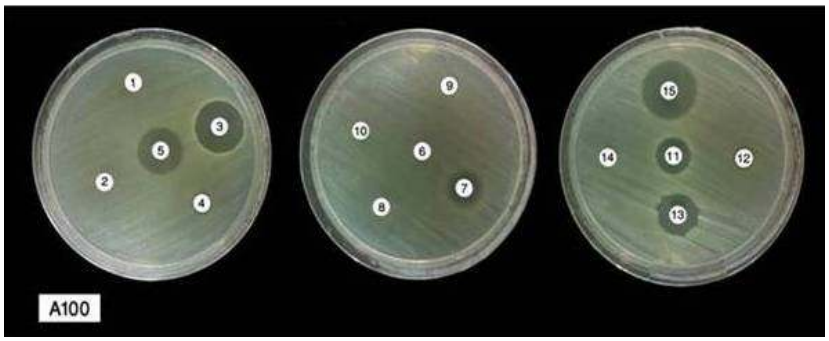

Figure (1): MRSA spp. A100: showing resistant to 1(ZOX), 2(FEP), 4(OX), 5(TGC), 6(CL), 7(LEV), 8(CRO), 9(GN), 10(CIP), 11(AK), 12(AMc), 14(CXM) and sensitivity to 3(VA), 13(TEC) and 15(SXT).

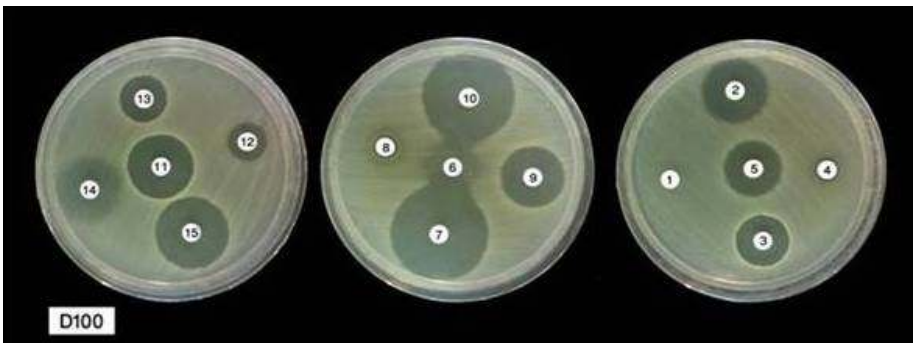

Figure (2): MRSA strain D100 showing resistant to 1(ZOX), 4(OX), 5(TGC), 6(CL), 8(CRO), 12(AMc), 13(TEC) and sensitivity to 2(FEP), 3(VA), 7(LEV),9(GN), 10(CIP), 1(AK), 14(CXM), 15(SXT)

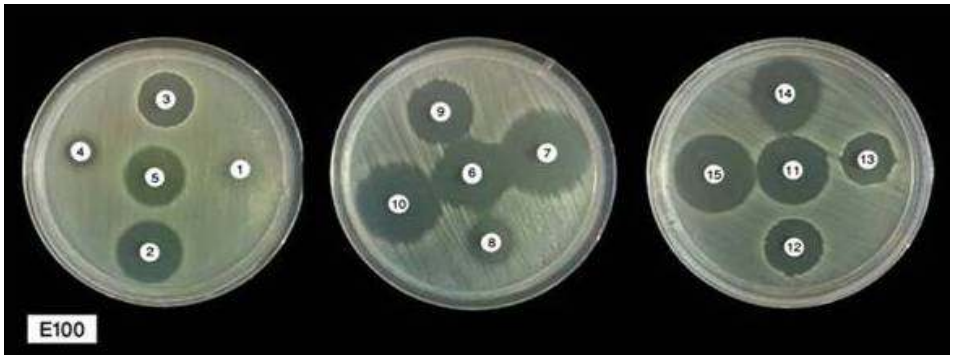

Figure (3): MRSA strain E100 showing resistant to 1(ZOX), 4(OX), 6(CL), 8(CRO), 12(AMc) and sensitivity to 2(FEP), 3(VA), 5(TGC), 7(LEV), 9(GN), 10(CIP), 11(AK), 13(TEC), 14(CXM).

Figure (4): MRSA strain U100 showing resistant to 1(ZOX), 4(OX), 6(CL), 8(CRO), 12(AMc) and sensitivity to 2(FEP), 3(VA), 5(TGC), 7(LEV), 9(GN), 10(CIP), 11(AK), 13(TEC), 14(CXM)

Figure (5): MRSA strain X100 showing resistant to 1(ZOX), 2(FEP), 4(OX), 5(TGC), 6(CL), 7(LEV), 8(CRO), 10(CIP), 12(AMc), 14(CXM) , 15(SXT) and sensitivity to 3(VA), 13(TEC), 9(GN), 11(AK).

Figure (6): MRSA strain S100 showing resistant to 1(ZOX), 4(OX), 6(CL), 8(CRO), 15(SXT) and sensitivity to 2(FEP), 3(VA), 5(TGC), 7(LEV), 9(GN), 10(CIP), 11(AK), 12(AMc), 13(TEC), 14(CXM).

Table(3): susceptibility and resistance for *Escherichia coli* - ESBL pathogens collected from Islamic Hospital-Jordan to standard antibiotics

Antimicrobial agent	Zone of clinical Isolates (mm)																				
	YZ 1	YZ 2	YZ 3	YZ 4	YZ 5	YZ 6	YZ 7	YZ 8	YZ 9	YZ 10	YZ 11	YZ 12	YZ 13	YZ 14	YZ 15	YZ 16	YZ 17	YZ 18	YZ 19	YZ 20	YZ 21
Amoxicillin/ Calvulanic acid	9 (R)	9 (R)	9 (R)	Zero (R)	13 (R)	10 (R)	Zero (R)	13 (R)	Zero (R)	Zero (R)	16 (R)	Zero (R)	11 (R)	Zero (R)	Zero (R)	10 (R)	Zero (R)	9 (R)	8 (R)	17 (R)	Zero (R)
Sulfamethoxazole/ Trimethoprim	25 (S)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	27 (S)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	23 (S)	Zero (R)	27 (S)	Zero (R)
Amikacin	21 (S)	21 (S)	21 (S)	20 (S)	21 (S)	22 (S)	19 (S)	20 (S)	24 (S)	24 (S)	22 (S)	22 (S)	21 (S)	21 (S)	21 (S)	22 (S)	20 (S)	19 (S)	24 (S)	24 (S)	Zero (R)
Gentamicin	19 (S)	9 (R)	20 (S)	Zero (R)	20 (S)	9 (R)	20 (S)	18 (S)	Zero (R)	20 (S)	23 (S)	18 (S)	20 (S)	21 (S)	Zero (R)	20 (S)	Zero (R)	9 (R)	24 (S)	21 (S)	Zero (R)
Ertapenem	30 (S)	30 (S)	30 (S)	19 (S)	30 (S)	30 (S)	28 (S)	34 (S)	29 (S)	30 (S)	28 (S)	29 (S)	31 (S)	23 (S)	30 (S)	28 (S)	31 (S)	31 (S)	30 (S)	32 (S)	27 (S)
Imipenem	31 (S)	32 (S)	30 (S)	24 (S)	29 (S)	32 (S)	29 (S)	31 (S)	30 (S)	30 (S)	31 (S)	31 (S)	30 (S)	24 (S)	28 (S)	30 (S)	27 (S)	32 (S)	30 (S)	31 (S)	28 (S)
Meropenem	29 (S)	33 (S)	30 (S)	25 (S)	29 (S)	31 (S)	29 (S)	37 (S)	35 (S)	30 (S)	32 (S)	32 (S)	31 (S)	30 (S)	34 (S)	29 (S)	30 (S)	34 (S)	31 (S)	30 (S)	30 (S)
Cephalexine	Zero (R)	Zero (R)	Zero (R)	Zero (R)	11 (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	10 (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	7 (R)	Zero (R)	Zero (R)	Zero (R)
Ceftizoxime	13 (R)	14 (R)	18 (R)	11 (R)	23 (S)	13 (R)	9 (R)	18 (R)	Zero (R)	13 (R)	18 (R)	14 (R)	23 (S)	18 (R)	16 (R)	12 (R)	12 (R)	16 (R)	Zero (R)	19 (R)	Zero (R)
Ceftriaxone	Zero (R)	Zero (R)	Zero (R)	Zero (R)	13 (R)	Zero (R)	Zero (R)	12 (R)	Zero (R)	Zero (R)	Zero (R)	17 (R)	13 (R)	24 (S)	Zero (R)	Zero (R)	Zero (R)	11 (R)	Zero (R)	Zero (R)	Zero (R)
Cefepime	18 (S)	20 (S)	17 (R)	14 (R)	24 (S)	14 (R)	11 (R)	20 (S)	19 (S)	14 (R)	22 (S)	27 (S)	22 (S)	31 (S)	18 (S)	15 (R)	14 (R)	18 (S)	12 (R)	23 (S)	Zero (R)
Levofloxacin	Zero (R)	16 (R)	12 (R)	Zero (R)	35 (S)	14 (R)	Zero (R)	13 (R)	Zero (R)	13 (R)	35 (S)	23 (S)	33 (S)	Zero (R)	9 (R)	28 (S)	8 (R)	11 (R)	26 (S)	32 (S)	Zero (R)
Ciprofloxacin	Zero (R)	Zero (R)	11 (R)	Zero (R)	35 (S)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	35 (S)	22 (S)	32 (S)	Zero (R)	Zero (R)	25 (S)	Zero (R)	Zero (R)	26 (S)	32 (S)	Zero (R)
Tigecycline	18 (R)	18 (R)	17 (R)	16 (R)	16 (R)	17 (R)	15 (R)	19 (R)	15 (R)	19 (R)	20 (S)	15 (R)	16 (R)	14 (R)	16 (R)	17 (R)	15 (R)	16 (R)	20 (S)	20 (S)	16 (R)
Cefuroxime	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	16 (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)
Cefotaxime	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	20 (R)	Zero (R)	22 (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)	Zero (R)

***Acinetobacter spp.* Isolates**

Calibrations for the strains, antibiotics, disc potencies and acceptable zones of inhibition were based on NCCLS standards. Two *Acinetobacter spp.* isolates described as A5 and A14 were tested. Table (4) revealed that the tested *Acinetobacter spp.* were resistant to all mentioned antimicrobial agents.

Table (4): Antibiogram of *Acinetobacter spp.* pathogens that were collected from Islamic Hospital – Jordan

Antimicrobial agent	Zone of Clinical Isolate (mm)	
	A 5	A14
Amoxicillin/Calvulanic acid	Zero (R)	Zero(R)
Sulfamethoxazole/Trimethoprim	Zero (R)	Zero (R)
Amikacin	12(R)	Zero (R)
Gentamicin	Zero (R)	Zero (R)
Ertapenem	Zero (R)	Zero (R)
Imipenem	Zero (R)	8 (R)
Meropenem	Zero (R)	Zero (R)
Cephalexine	Zero (R)	Zero (R)
Ceftizoxime	Zero (R)	Zero (R)
Ceftriaxone	Zero (R)	Zero (R)
Cefepime	12 (R)	Zero (R)
Levofloxacin	11 (R)	12 (R)
Ciprofloxacin	Zero (R)	Zero (R)
Tigecycline	10 (R)	12(R)
Colistin	12 (R)	11 (R)
Cefuroxime	Zero (R)	Zero (R)
Cefotaxime	Zero (R)	Zero (R)

Klebsiella pneumonia Extended-spectrum beta-lactamase Isolates

Three Klebsiella *pneumonia* ESBL isolates described as K 1, K2 and K3 were tested. Table (5) revealed that tested *Klebsiella pneumonia* were resistant to amoxicillin + calvulanic acid, tigecycline, sulfamethoxazole +trimethoprim ,ciprofloxacin ,levofloxacin, gentamicin ,first ,second ,third and fourth cephalosporin generations except for (K2) which showed sensitivity to cefepime .

While all of the isolates were susceptible to amikacin and carbapenems group including ertapenem , imipenem and meropenem.

Table(5): The antibiogram profile of *Klebsiella pneumonia* ESBL

Antimicrobial agent	Zone of clinical Isolates (mm)		
	K1	K2	K3
Amoxicillin/Calvulanic acid	9 (R)	17 (R)	9 (R)
Sulfamethoxazole/Trimethoprim	Zero (R)	Zero (R)	Zero(R)
Amikacin	19 (S)	22 (S)	21 (S)
Gentamicin	Zero (R)	18 (S)	Zero (R)
Ertapenem	28 (S)	30 (S)	28 (S)
Imipenem	27 (S)	25 (S)	28(S)
Meropenem	25 (S)	28 (S)	30 (S)
Cephalexine	Zero (R)	Zero (R)	Zero (R)
Ceftizoxime	12 (R)	19 (R)	16 (R)
Ceftriaxone	Zero (R)	Zero (R)	Zero (R)
Cefepime	12 (R)	22 (S)	16 (R)
Levofloxacin	9 (R)	7 (R)	10 (R)
Ciprofloxacin	Zero (R)	Zero (R)	Zero(R)
Tigecycline	12 (R)	14 (R)	14 (R)
Colistin	Zero (R)	Zero (R)	Zero(R)
Cefuroxime	Zero (R)	Zero (R)	Zero(R)
Cefotaxime	Zero (R)	Zero (R)	Zero(R)

Table (6) shows the percentage of antibiotics resistant for the isolates investigated in this study. Comparative assessments of antibiotic resistance to one or more antibiotics in the standard panels for all bacterial isolates were indicated.

Table (6): Resistance percentage of MRSA, *Escherichia coli* ESBL , *Klebsiella* ESBL and *Acintobacter* species against tested antibiotics

Antibiotic Disks	Resistance Percentage %			
	Acinetobacter spp.	*Klebsiella pneumonia ESBL*	*Escherichia coli* ESBL	*Methicillin Resistant S.aureus*
AMC	100	100	100	78
FEP	100	67	42	44
ZOX	100	100	90	100
OX	ND	ND	ND	100
CXM	100	100	100	44
CRO	100	100	95	100
CL	100	100	100	100
LEV	100	100	67	44
VA	ND	ND	ND	11
CTX	100	100	100	ND
TEC	ND	ND	ND	22
AK	100	Zero	5	22
GN	100	67	38	22
TGC	100	100	85	67
CIP	100	100	62	44
SXT	100	100	81	33
ETP	100	Zero	Zero	ND
IPM	100	Zero	Zero	ND
MEM	100	Zero	Zero	ND
CT	Zero	ND	ND	ND

ND: Not Determined

DISCUSSION

This study confirms previous study concern the picture of antimicrobial resistant by bacteria in Jordan (10). This phenomenon raised up year by year, but the problematic of this issue not only because it is highly prevalent but also because it has become resistant to almost all available antibiotics. The precipitous spread of bacterial resistant to antimicrobial drug has posed new challenges for governments, healthcare systems, and drug research. Our study showed that a high degree of resistance of MRSA to oxacillin , ceftriaxone ,ceftriaxone and cephalexine. However, fearing MRSA, clinicians may exploit vancomycin, especially when a sensitivity study is not performed (11). The present study reports that antibiotics other than vancomycin, for instance, amikacin, ciprofloxacin, levofloaxcin and sulfamethoxazole +trimethoprim with good zone of inhibition obtained (17-28 mm),(26-30 mm) ,(29-32 mm) and (20-26 mm) respectively which can be promising if a susceptibility testing is done, reserving vancomycin for life-threatening infections. Similar findings have been reported from other studies as well (12,13) Ciprofloxacin was proposed to be an alternate therapy for MRSA infection (14). Although rapidly developing resistance to ciprofloxacin in Jordan has been reported (14) a (56%) susceptibility against MRSA was reported in this study. This is perhaps due to the differential clonal expansion and drug pressure in the community (15). The organisms exhibited 44% resistance against ciprofloxacin and levofloaxcin, this extent resistance is similar to various studies

conducted in different parts of the world (16-20). Ciprofloxacin resistance among *S. aureus* isolates is comparable to that reported in 2010 from Pakistan (16) and South India (15) 21.95% and 31.8% respectively. There has been an increase in resistance to fluoroquinolones among isolates of *S. aureus* in recent years. In this study, the highest resistance was shown by 100% for *Acinetobacter spp.* and 67% for *K. pneumonia*. This increased resistance showed that *K. pneumoniae* had adapted to survive in presence of ciprofloxacin and on the basis of these results, it is not considered as an effective therapy choice to eradicate the infections caused by *K. pneumonia*, a simialr results obtained by syeda et al. with 72.22% resistance to *K. pneumoniae* (21).

Escherichia coli a Gram negative bacterium and considered as a major source for urinary tract infections, The clinical isolates of *S. aureus* in the current study showed less resistance than *Escherichia coli*. Amikacin showed good activity against *E. coli*, about 5 % resistance was shown by this pathogen. Production of ESBLs did not influence levofloxacin and ciprofloxacin antibacterial activity against *Escherichia coli* which markedly susceptible to these quinolones with zone of inhibition range (23-35 mm) and (22-35 mm) respectively on seven of the isolated strains. Overall etrapenem, imipenem and meropenem had the broadest spectrum of activity against all *Escherichia coli* and *K. pneumoniae* with 100% susceptibility.

Recently, researchers have been exploring the bacterial riches of the ancient Lechuguilla cave in New Mexico, which contains vast amounts of naturally antibiotic-resistant bacteria. In fact, the bacteria in the cave, which have not been exposed to any antibiotics, appear resistant to virtually every antibiotic known. The scientists are hoping that by studying these germs, they may find clues to dealing with (MRSA) (22).

The most widely used (MRSA) active antibiotics is Vancomycin, which is known as a glycopeptide antibiotic.

In Jordan and others part of the world the bacteria harboring ESBL increase in community dwellers (21-29). *E.coli* that isolated from UTI's from patients visiting Islamic hospital shows highly prevalence of ESBL, Pitout *et al* described the clonal spread of two closely related strains harboring CTX-M-14, isolated most often from urine samples(30,31). These *E. coli* are also resistant to quinolones, aminoglycosides, and sulfonamides(32,33).

Also this study confirm the occurrence of bacteria resistant to all most the common and widely used antibiotics in Jordan, and the picture were cleared by isolation of (MRSA), ESBL and MRAB (Acinetobacter) bacteria from patients in Islamic Hospital in Amman.

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Use of panel of immunohistochemistry markers in the diagnosis of soft tissue sarcomas

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ABSTRACT

Immunohistochemistry has been introduced in the 80's. Because of its relatively low cost. simple technique and the availability of a large number of increasingly sensitive and / or specific antibodies, it has become the main diagnostic tool in soft tissue tumors .. A retrospective study of 39 cases of soft tissue sarcomas , classified according to Sharon W.Weiss and John R.Goldblum classification , presented to the Central Public Health Laboratory /BAGHDAD/IRAQ between 1st of January 2009-13th of March 2012 for verification of the diagnosis provided by the routine Hematoxylin &Eosin stain by immunhistochemicalstudy The mean age of the 39 cases was 34.04 years with a range between 7-80 years. Most of the cases were found between 10-29 years (50%) . Twenty cases were males(51.2 %) and nineteen cases were females(48.7 %) . majority of cases (42.5%)presented with lower limb mass, and a limited number of cases (5%)presented as head and neck mass.the most common type of tumors were malignant peripheral nerve sheath tumor MPNST 12.5% and least common type were liposarcoma & dermatofibrosarcoma protuberancs 2.5% . two panels of immunohistochemical markers were used a general panel including (vimentin, actin, desmin, myosin, myoglobulin)and specific panel were used according to the presumptive diagnosis including (NSE- neuron specific enolase,S100 protein,CK8- cytokeratin 8,EMA epithelial membrane antigen ,HMB45 –melanosome ,CD34,collagen IV, ferritin, VWF-vonwilbrand factor). The goal of the this study is to show that panels of immunohistochemistry markers should be used to reach a definite diagnosis in soft tissue sarcomas after an early presumptive diagnosis and that Immunohistochemistry should used as complement of the morphological analysis.

Keywords: immunohostochemistry, marker, sarcomas

الملخص باللغة العربية

تعد دلالات الاورام المناعيه احدى الوسائل المهمه في تشخيص الاورام السرطانيه منذ ظهورها في حقبه الثمانينات حتى الوقت الراهن كونها متاحه و متنوعه حيث اصبحت احدى اهم الوسائل التشخيصيه لاورام الانسجه الرخوه . البحث هو دراسه استرجاعيه ل 39 حاله من حالات اورام الانسجه الرخوه تم جمع الحالات في شعبه النسيج المرضي / مختبر الصحه العامه المركزي في بغداد ابتداء من شهر يناير 2009 حتى الثالث عشر من اذار 2012 وتم صبغ الحالات بصبغات الهيماتوكسياين والايوسين H.&E لغرض التشخيص النسيجي الاولي ثم صبغها بصبغات دلالات الاورام المناعيه التاليه لغرض التاكد من التشخيص الاولي عدد الذكور 20 (51.2%) وعدد الاناث 19 (% 48.7) .بلغ متوسط اعمار المرضى 34.4 وتراوحت الاعمار بين 7–80 سنه وتركزت اغلب الحالات(50%) للفئه العمريه 10–29 سنه , 42.4% من الاورام ظهرت في الاطراف السفلى و5% من الاورام ظهرت في مناطق الراس والعنق , اكثر الاورام شيوعا في هذه الدراسه ورم الاعصاب المحيطيه الخبيث بنسبه 12.5% واقل الاورام شيوعا في هذه الدراسه هي ورم النسيج الشحمي الخبيث و ورم الخلايا الليفيه الجلدي بنسبه 2.5% .

تم استخدام مجموعتين من صبغات دلالات الاورام المناعيه المجموعه الاولى صبغات عامه وتشمل vimentin, actin, desmin, myosin, myoglobulin والمجموعه الثانيه صبغات خاصه وتشمل NSE- neuron specific enolase,S100 protein,CK8- cytokeratin 8,EMA epithelial membrane antigen ,HMB45 –melanosome ,CD34,collagen IV, ferritin, VWF-vonwilbrand factor) لغرض تاكيد التشخيص الاولي للاورام . الغرض من الدراسه هو اثبات ان اورام الانسجه الرخوه تحتاج الى استخدام مجموعه من صبغات الدلالات الورميه المناعيه لغرض التوصل الى التشخيص النهائي بعد التشخيص الاولي المعتمد على الشكل النسيجي للورم بعد صبغه بصبغه الهيماتوكسيلين والايوسين.

INTRODUCTION

Soft tissue tumors are uncommonly found in general pathology and represent less than 5% from all malignant tumors. They are rare tumors, but at the same time are responsible for 2% cancer-related deaths. In many cases, the pathologic diagnosis is difficult because these tumors are extremely heterogeneous. As many authors previously showed, sarcomas are poorly understood, especially due to their histogenesis and behavior. As a consequence, their treatment is still poorly adjusted to the pathologic diagnosis and seems to be inadequate in the large majority of cases.
The role of the pathologist in the diagnosis of a soft tissue tumor is crucial, because she or he must decide if the lesion is reactive or a tumor, and if it is a tumor, to establish its benign or malignant character. The accuracy of the diagnosis is essential for the clinical behavior and therapy. On the other hand, the classic methods of pathology are seldom enough for the diagnosis. As mentioned by Enzinger and Weiss, less than 60% of cases are clarified if only haematoxylin-eosin stained slides are examined (1).
That is why, currently, immunohistochemistry is widely accepted as a useful method, not for the diagnosis of malignancy itself, but for the immunophenotyping of a soft tissue tumor. It is estimated that immunohistochemistry confirms the diagnosis in about 30 to 40% of cases, is useful to direct the diagnosis in 50 to 60% of cases, and it is non-contributive in 1-2% of cases (2). The incidence of immunohistochemical procedures is significantly higher in soft tissue tumors than in carcinomas. This is why our aim was to investigate the value of primary immunophenotyping for the diagnosis in soft tissue tumors and to establish a specific diagnosis protocol (1).
Immunohistochemistry has been introduced in the 80's. Because of its relatively low cost, simple technique and the availability of a large number of increasingly sensitive and / or specific antibodies, it has become the main diagnostic tool (2).
Immunohistochemistry (IHC) plays an important role in STT(soft tissue tumors) diagnosis. The first approach consists in ruling out a nonmesenchymal tumor, followed by trying to define mesenchymal cell lineage. This approach, achieved with a panel of commonly used antibodies, helps narrow down the differential to a more manageable level. In addition ,there are specific tumors requiring a more refined set of immunehistochemical antibodies .(3).
Immunohistochemistry is required for the diagnosis of a variety of sarcomas, including synovial sarcoma (EMA , CK) (4), epithelioid sarcoma (EMA, CK (4), clear cell sarcoma (S100, HMB45 (5), GIST (gastrointestinal stromal tumors (CD117, CD34)(6) rhabdomyosarcoma (myogenin, myo D1)& desmoplastic small round cell (CK, desmin, NSE) (7) , epithelioid angiosarcoma (CD31, CD34)(8) .
It is also useful in the diagnosis of leiomyosarcoma (smooth muscle actin 95% sensitive , muscle specific actin 91 % sensitive , calponin 88% sensitive) (9).

MPNST- malignant peripheral nerve sheath tumor) (recent studies suggested nestin as a more sensitive marker than S100P , CD56)(10), dermatofibrosarcoma protuberans (CD34 , *CD10*) (11) Dedifferentiated liposarcoma (MDM2,CDK4)(12) , CD99 and FLI-1 are useful in the diagnosis of Ewing sarcoma / PNET(primitive neuroectodermal tumor) (13), but in view of their specificity (CD99 may be positive in a large variety of tumors including poorly differentiated synovial sarcoma, desmoplastic small round cell tumor, lymphoblastic leukemia) or sensitivity (FLI-1 is only positive in 94% of cases of Ewing sarcoma), molecular proof of the translocation is increasingly required(14).

Most useful antibodies

Epithelial markers

Cytokeratins should be included in the immunohistochemical panel of most spindle and pleomorphic cell malignant tumors. One of the most sensitive antibodies is CytoKeratin AE1-AE3, when a carcinoma is suspected clinically (15)
Cytokeratins are also expressed by myoepithelial neoplasms, (90%), epithelioid sarcoma (90-100%), synovial sarcoma (70-90%),desmoplastic round cell tumor (80%), chordoma . "Unexpected" positivities include epithelioid vascular neoplasms (20-30%), leiomyosarcoma(40%), Ewing sarcoma / PNET (20%) (16).
In addition to epithelial and myoepithelial neoplasms, epithelial membrane antigen (EMA) is expressed in epithelioid sarcoma (95%), synovial sarcoma (90%) and can be expressed by a large number of other soft tissue neoplasms: perineurioma, low-grade fibromyxoid sarcoma, epithelioid fibrosarcoma, superficial acral fibromyxoma (17).

Melanocytic / "neural" markers

S-100 protein is positive in more than 95% of melanomas and is useful in the distinction between benign peripheral nerve sheath tumors (strong and diffuse expression) and MPNSTs (focal positivity if any tumor cells are positive) (18) Its also expressed unexpectedly by synovial sarcoma , myxoid chondrosarcoma, myxoid &round cell liposarcoma , PNET (15) HMB-45 is expressed by melanoma, clear cell sarcoma (19) . and the vast majority of PEComas which are usually negative for S-100 protein (20)

Lymphoid markers

The histopathological distinction between lymphoma and soft tissue sarcoma is routinely determined by IHC. Leucocyte common antigen (LCA) (lymphoid marker) and vimentin (mesenchymal marker) are commonly used for this purpose. Further IHC markers showed the neoplastic cells in hodhkins disease were reactive towards CD30 and CD15 The cells were however, non-reactive towards anaplastic lymphoma kinase (ALK) protein.. The neoplastic cells were also noted to be non-reactive towards cytokeratin, epithelial membrane antigen (EMA), smooth muscle actin, desmin, HMB45 (melanoma marker), CD31 (vascular marker), CD34 (fibrohistiocytic marker), CD1a (dendritic cell marker), CD_3 (T cell marker), and CD_{20} (B cell marker). (21)

Muscle markers

Smooth muscle actin was the most sensitive antibody (95%), followed by muscle-specific actin (91%), calponin (88%), desmin (73%), caldesmon (66%), and myosin (64%). Caldesmon and myosin were usually coexpressed, and were highest in retroperitoneal tumors (94%). There was no discernable correlation noted between histologic differentiation and smooth muscle marker expression.those are the most important used as a panel for identification of leimyosarcomas (9). Myogenin and MyoD1 were equally sensitive (positive for 97% of RMS cases), with both also showing similar specificity (90% vs. 91% of cases) for the diagnosis of RMS (7). The vast majority of GISTs (95%) are strongly and diffusely positive for C-KIT (CD117), which makes the C- KIT to be a very specific and sensitive marker in the differentiating GIST from other mesenchyma tumors in the GI tract , Several new antibodies for the diagnosis of GIST have been discovered based on the molecular studies. DOG1 (discovered on GIST1), has been found specifically in GISTs and has emerged as a promising biomarker for GISTs & has even higher sensitivity and specificity than C-KIT (CD117) and CD34 with 75% to 100% overall sensitivity (22).

Vascular markers

The most sensitive and specific marker is CD31, which is expressed by 95-100% of benign and malignant vascular tumors. However, the expression of CD31 by macrophages is a potential pitfall (7). CD34 is expressed by virtually all vascular neoplasms but also by a wide variety of mesenchymal tumors: dermatofibrosarcoma protuberans (100%°), solitary fibrous tumor (90%), spindle cell / pleomorphic lipoma (100%), GISTs (70%), peripheral nerve sheath neoplasms, epithelioid sarcoma (50%).
Occasional positivities have also been reported in leiomyosarcoma (+/- 10%),
myxofibrosarcoma, myxoma. The presence of non-tumoral CD34 positive dendritic cells is a potential pitfall in cutaneous tumors (3,23).

PATIENTS AND METHODS

Case Selection and Slide Review

A retrospective study of 39 cases(twenty cases were males(51.2 %) and 19 were females(48.7 %)) , The morphologic analysis of usual H&E(hematoxyline &eosin) stained sections allowed a presumptive diagnosis and classification of cases according to Sharon W.Weiss and John R.Goldblum classification (24). Cases were presented to the Central Public Health Laboratory/Baghdad/Iraq between 1st of January 2009- 13th of March 2012. The specimens were fixed in10% formalin , and paraffin embedded. Five sections (4 micron in thickness) were cut from each paraffin block. A general panel of immunohistochemical markers were used including (vimentin, actin, desmin, myosin, myoglobulin)and specific immunomarkers were used according to the presumptive diagnosis including (NSE- neuron specific enolase,S100 protein,CK8- cytokeratin 8,EMA epithelial membrane antigen ,HMB45 – melanosome ,CD34,collagen IV, ferritin, VWF-vonwilbrand factor) using positively charged slides.
Immunohistochemical stainig protocol:
Pretreatment of the positively charged tissue sections was done according to DAKO educational guide,as follows(25):
1. deparaffinize and rehydrate tissue sections.
2. Fill container with enough retrieval solution to cover slides and equilibrate to 95-99 °C in water bath.
3. Immerse slides in preheated retrieval solution, cover container with lid, and incubate for specified time within the 20-40 minutes range after the set temperature has been reached.
4. Remove the container from the water bath and cool the contents with the lid in place for 20 minutes at room temperature.
5. Rinse with tris buffered saline at room temperature. When removing the slides from the container it is very important that the slides do not dry out
6. Rinse with tris buffered saline.
7. Proceed with IHC staining according to dako education guide 2009(25).
The staining intensity in neoplastic cells was scored subjectively as: 0 = negative; 1 = weak; 2 = intermediate; 3 = strong slides were scanned at a low resolution (4x PF) and high resolution (40 PF×) using Olympus microscope(26).

RESULTS

The mean age of the 39 cases was (34.04) years with a range between(7-80) years. Most of the cases were found between 10-29 years (50%) .(Table1)

Table (1): Age distribution of 39 cases with soft tissue sarcomas

Age in years	Number of cases	%
<10 years	1	2.5%
10-19 years	10	25%
20-29 years	10	25%
30-39 years	4	10.2%
40-49 years	7	17.5%
50 years and above	7	17.5%

The clinical presentation of the 39 cases with soft tissue sarcomas was as follow:
Seventeen cases (42.5%)presented with lower limb mass, 8cases(20%) presented with upper limb mass, 8 cases(20%) presented with abdominal wall mass,4 cases(12.5%) presented with a retroperitoneal mass, and 2 cases(5%)presented with head and neck mass.
According to table (2), twenty cases(50%) were showing positive immunoreactivity for vimentin(3cases with score+, and 17cases with score+++), 14 cases(35%) were positive for actin(9cases with score+, 1case with score++, 4cases with score +++), 3 cases(7.5%) were positive for desmin(1case with score+, 2 cases score+++), one case(2.5%) with score + was positive for myosin, and 2 cases(5%) were positive for myoglobulin(1 case with score +, 1case with score +++).

Table (2): The immunoreactivity of the 39 cases with soft tissue sarcomas to the different immunomarkers

Immunomarker	Score +	Score ++	Score +++	Total
Vimentin	3		17	20
Actin	9	1	4	14
Desmin	1		2	3
Myosin	1			1
Myoglobulin	1		1	2
Total				40

(+)= weak positive staining of tumor cells
(++)=intermediate positive staining of tumor cells.
(+++)= strong positive staining of tumor cells.(25)

An additional immunohistochemical marker was applied to some of the cases based on the histopathological differential diagnosis provided by the routine H&E stain. Table (3)

Table (3): The different morphological types of the 39 cases with soft tissue sarcomas

Morphological types of soft tissue sarcomas	Number of cases	%
Liposarcoma	1	2.5%
Fibromatosis	3	7.5%
Dermatofibrosarcoma protuberans	1	2.5%
Fibrosarcoma	4	10%
MFH	3	7.6 %
Rhabdomyosarcoma	4	5 %
Leiomyosarcoma	1	2.5%
MPNST	5	12.5%
Ancient schwannoma	2	5%
Clear cell sarcoma	1	2.5%
Granular cell tumor of unusual site	1	2.5%
Synovial sarcomas	3	7.5%
Extra GIST	2	5%
Extra skeletal myxoid chondrosarcoma	2	5%
PNET/Extraskeletal Ewing tumor	3	7.5%
Soft tissue sarcoma without a definite differentiation	3	7.5%

Given the bewildering number of STTs and likewise continuously growing list of IHC antibodies used in STT diagnosis, this article concentrates on pathologic entities as broad categories and discusses the applicability of IHC (or lack thereof) instead of providing a detailed discussion of individual antibodies. Emphasis is placed on specific cases for which IHC application has proven to be particularly useful in diagnosis. The impact of immunohistochemistry in pathology may be explained by three major advances: the availability of numerous good- quality antibodies applicable on routine formalin-fixed tissues; improvements in antigen retrieval techniques and particularly heat-induced epitope retrieval (HIER) which provides consistent and reliable results; and the availability of sensitive detection systems. Moreover, immunohistochemistry procedures are now more and more standardized, reliable and consistent thanks to automation and external quality-assurance programs (27).

DISCUSSION OF THE MOST COMMON CASES

Representative cases of fIbroblastic /myofibroblastic differentiation

One case was diagnosed as dermatofibrosarcoma and he was 30 years male with palm mass. Final diagnosis was dermatofibrosarcma protuberance , immunomarkers showed score+++ for vimentin, CD34 score+ ,diffused mildly expressed this was

supported by (3). These markers used to differentiate dermatofibrosarcoma from fibrosarcoma where CD34 is lost and to differentiate DFSP(dermatofibrosarcoma protuberance) from scare tissue were its generally negative to CD34. NSE and collagen IV were negative , collaen 4 was not expressed in DFSP since that the only type of collagen fibers expressed in DFSP in a variable degree is collagen I(28) .NSE is negatively expressed this was supported by (29) .The histopathological findings and clinical findings goes with diagnosis of DFSP . its relatively common lesion which is more frequent in males presented as intradermal lesion composed of spindle cell monotonous with tight storiform pattern , exact cell type is not clearly characterized (histiocytic, perineural , pericytic,endothelial origin all variably suggest to to develop feature of fibroblasts ,IHC shows diffused positive expression of CD34& actin(30).

Four cases were diagnosed as fibrosarcoma:

One of the cases was 13 years boy with leg mass, by H&E we put a differential diagnoses including MFH(malignant fibrous histiocytoma) , fibrosarcoma but by immunomarkers proved to be fibrosarcoma where vimentin showed score+++, while NSE, actin,myosin,desmin and myoglobulin were all negative. Fibrosarcoma is a Malignant tumor of fibroblasts with herringbone architecture and variable collagen its Rare (up to 3% of adult sarcomas) (31) , Some limit diagnosis to those age 10+ years, most patients are ages 40-55 years Many cases formerly called fibrosarcoma are actually dedifferentiated liposarcoma, fibromatosis, fibrosarcomatous DFSP, low-grade fibromyxoid sarcoma, MPNST, synovial sarcoma or MFH-pleomorphic variant , Usually deep soft tissue of lower extremities or trunk are involved. Immunohistochemical stains are positive vimentin and negative muscular markers and neuronal markers which goes with immunoprofiling of this case for which diagnosis of low grade fibrosarcoma done excluding MPNST(malignant peripheral nerve sheath tumor) and SS(synovial sarcoma).Three cases were diagnosed as MFH (malignant fibrous histiocytoma): One case was 53years male with recurrent arm mass diagnosed as pleomorphic MFH where actin was score+, α1 antitrypsin score+++, while desmin, myoglobulin, CD34, CK8, S100P, and Ferritin were negative. The strong positive expression of elfa one antitrypsin was supported by (32) , (33). muscular marker expression was noticed as by (34), who also estimated that 30% soft tissue pleomorphic MFH can express smooth muscle markers and have a morphology identical to that of MFH without myofibroblastic differentiation. They also estimated that MFH are always negative to S100P, sporadic expression of CD34 might be seen in pleomorphic MFH which is supported by (34) . Negative expression of CK was supported by (35). MFH is a designation used for poorly differentiated sarcomas that do not show any specific differentiation except perhaps fibroblastic / myofibroblastic differentiation .The diagnosis is therefore made by exclusion of other specific diagnoses (3).

MFH was found to share a less degree of expression of smooth muscle cell markers such as actin ,desmine and other muscular markers revealing a myofibroblastic differentiation in approximately 50% of these tumors the others have only myofibroblastic or undifferentiated nature tumor cells (34).

CD34 reactivity was expressed in approximately 38% of cases indicating dendritic fibroblastic cells mostly seen in a subset of myxoid MFH that has a histogenesis distinct from pleomorphic MFH. Reactivity for epithelial markers CK&EMA was negative excluding the possibility of epithelial origin of such tumors (34). S100P is not a specific marker and it does not indicate the origin of tumor cells since it has seen in many tissue types (36).

The variation in immunereactivity of vimentin makes it of no diagnostic value due to lack of specificity where its expressed in many cell line linage fibroblasts , endothelial cells & smooth cells (36). According to the latest WHO classification of soft tissue tumors the so called MFH can no longer be regarded as a definable entity & now viewed as as synonym for un differentiated pleomorphic sarcoma (34).

Representative Cases of skeletal muscle tumors

Four cases out of 39 with soft tissue sarcomas were diagnosed as rhabdomyosarcoma:

An interesting case was 28 years male with recurrent arm mass, diagnosed as poorly differentiated alveolar rhabdomyosarcoma where immunomarkers showed focal weak-moderate actin positivity, the other markers were all negative (desmine, ,S100P& HMB45). Amita *et.al.* (37) estimated that Desmin and muscle-specific actin, although highly sensitive markers for RMSs, are not specific for these tumors because they also stain smooth muscle neoplasms and desmoids small round cell tumors. El - hasani *et.al.* (36) estimated that 95% of rhabdomyosarcomas and smooth muscle cell tumors express desmine and can be seen even in myofibroblasts,

The proportion of desmin-positive cells may vary from case to case depending on the number of differentiated cells, and desmin expression is usually mirrored by muscle-specific actin immunoreactivity because desmin immunostain exhibits occasional non specific reactivity in smooth muscle cells and rhabdomyoblasts as well as other tumors such as desmoplastic small round cell tumor, desmin should never be used as a sole marker for diagnosis but as a part of a panel of immunostains while all rhabdomyosarcomas will stain with these markers this variant appears to demonstrate a higher degree of differentiation owing to the consistency of the expression of late myogensis markers (38).

S100P is negatively expressed, which is compatible with the findings of AL Flope et.al. (39). As for HMB45 this marker is used to differentiate malignant melanoma from poorly differentiated rhabdomyosarcomas, which shows no expression. (40) as intermediate-high grade alveolar rhabdomyosarcoma ,vimentin was score+++,actin score+ .while other markers as desmine,myosin ,NSE,HMB45, ,EMA,CK8&S100P all were negatively expressed . epithelial and neuroendocrine markers are negatively expressed in rhabdomyosarcoma yet Bahram *et.al.* (41) reported a positive expression of these markers (30-40 % for epithelial and neuroendocrine), while Vimentin is expressed in 30-50% according to (39), which emphasis the need for implication of a panel of markers including myogenic markers to verify the diagnosis.

Representative Cases of Peripheral nerve tumors

Five cases were found to be Malignant Peripheral Nerve Sheath Tumor (MPNST) :

The first case was 55 years female with multiple nodules in the thigh diagnosed by immunohistochemistry markers as MPNST where vimentin score +++,actin score +++, desmin score+++, myoglobulin score+++, collagenVI score++, chromogranin A score+,S100P score+, while CK8 and EMA were negative. the light microscopy revealed spindle cell fascicles with brisk mitoses and necrosis the differential diagnosis was MPNST, Fibrosarcoma. The applied IHC stains strong expression of NSE&CD57 with mild expression of chromogranin A and S100P all support the histogenesis of nerve sheath tumor with limited neuroendocrine differentiation .NSE and CD57 both are focally expressed in MPNST according to (42). S100 P seen to be positive in 30-67% of MPNST which correlates with ultrastructural evidence of Schwann cell differentiation (43), while hasegawa *et.al.* (34) found that 100% of MPNST expressing S100P .chromogranin A was mildly expressed indicating a limited neuroendocrine differentiation (44). myolobin positivity indicating a skeletal muscle differentiation suggesting possibility of being a subtype of MPNST called malignant triton tumor. CK and EMA are both negative excluding an epithelial differentiation (glandular pattern of MPNST) (45). According to the WHO definition, MPNST are malignat tumors arising from peripheral nerves or extraneural soft tissues and shows nerve sheath differentiation diagnosis of MPNST has a lack of specific morphological criteria and /or ancillary IHC , MPNST shows significant histological overlap with synovial sarcoma , fibrosarcoma , rhabdomyosarcoma& angiosarcoma , a rare subset of MPNST shows a perineural differentiation demonstrated by EMA a distinctive subtype characterized by epitheloid cells predominance arising in from pre-exisiting shwann cells , epitheloid MPNST should be differentiated from M.M (malignant melanoma) ,clear cell sarcoma and carcinoma (46).

MPNST arising from soft tissue may mimic a varity of sarcomas clear cell sarcoma ,liposarcoma , S.S,LMS the distinction is done by limited expression of S100P . the distinction from S.S is done by prescence of pleomorphic cells that are not present in S.S though synovial sarcoma and MPNST may show glandular differentiation were the epitheloid component tend to resemble enteric epithelium with neuroendocrine differentiation while those of S.S are lined by cuboidal epithelium both S.S and MPNST may express low CK, EMA though high CK expressed in only S.S , S100P is seen in both but CD34 is not seen in synovial sarcoma (46).

The last differential diagnosis may be desmoplastic melanoma , the distinction from MPNST may be very difficult , histological features favoring melanoma include pleomorphism and strong S100P.

In conclusion, MPNST has a wide range of morphologic variability, recognition of hybride MPNST requires great availability of molecular techniques to refine the morphological diagnosis (47).

Two cases were diagnosed as ancient schwannoma:

The 1st case was 70 years female with recurrent leg mass ,the differential diagnoses by light microscopy was liposarcoma , S.S & ancient schwannoma and proved by immunostaining as ancient schwannoma where S100P strongly expressed score+++, chromograninA was mildly expressed score+, EMA and CK8 were negative. The morphological criteria revealed hypo and hyper cellular areas with hyalinized blood vessels .positivity of S100P favors diagnosis of schwannoma this is supported by (48) while the expression of s100p IS generally lost or focal in liposarcoma (3) ,and its not specific tumor marker since its expressed in both normal and neoplastic adipocytes . the morphological criteria of this particular tumor did not show lipoblasts ,focal expression of chromograninA favors neuroendocrine differentiation this support the diagnosis of schwannoma on top of liposarcoma. The negative expression of EMA &CK8 excludes the possibility of synovial sarcoma.

Representative Cases of Tumors of uncertain histogensis

Three cases out of 39 with soft tissue sarcomas were diagnosed as synovial sarcoma:

The 1st case was 51 years old female patient presented with neck swelling of the left side diagnosed as synovial sarcoma ,biphasic by H&E and immunostaining where vimentin was score+++,EMA score+++,S100P score+, and P53 and CEA were negative. EMA is the most sensitive marker for detection of epithelial component preferable to be combined with CK8or CK18 and 19 (48) , S100P is seen in 30% of Synovial Sarcoma so its not helpful in differential diagnosis of S.S from MPNST .as for CEA its expressed weakly of focally

in both biphasic or monophasic S.S (49) for P53 detection in (50), who revealed that P53 is expressed in only 10% of tumor cells and that there is no correlation between P53 expression by IHC and gene mutation detection and H ras in S.S. Vimentin is a non specific marker expressed in many tumors so its not helpful in diagnosis , the combination of morphological criteria with IHC has lead to the diagnosis of SS.

Two cases out of the 39 were diagnosed as GIST(GASTROINTESTINAL STROMAL TUMORS):

One of the two cases that were diagnosed as Extra peritoneal GIST was 25years female with retroperitoneal pelvic mass of 7 years ago , she underwent total abdominal hysterectomy &bilateral salpingio-oopherectomy 2 years ago with retroperitoneal pelvic mass clearance and diagnosed by routine stain as leiomyosarcoma of high grade , by immunostaing diagnosed as ExtraGIST ,where CD34 was score+++, actin focally positive in regards to desmine , myosin , NSE, CEA, ferritin , alfa one antitrypsin , EMA/CK & S100P all were negative. The differentiation from leiomyosarcoma depend on expression of tumor markers since the morphology showed hypercelluar pleomorphic spindle cell tumor that hardly differentiated on H.E slide , though CD34 is used to be the best indicator for GIST before the discovery of C-KIT yet its not specific the overall positivityfor this marker is 60-70% of GIST yet its expression is highest in gastric GIST is 85% (34). A small percentage GIST may show positivity for smooth muscle actin, desmin or S100P . the pattern of the results for these markers will differentiate if A non GIST diagnosis is possible tumors of smooth cell origin was LMS are usually positive for actin, desmin, while tumors of neural origin are positive for S100P ,NSE (GIST support internal group) for this case the strong diffused positivity of CD34 and focal positivity of actin with negative expression of desmin and myosin have excluded the possibility of MPNST ,neurotropic spindle cell melanoma , negative ferritin and alfa one antitrypsin have excluded MFH , negative expression of CK/EMA have excluded both the epitheloid subtype of LMS(leimyosarcoma) since its expressed in 50% of such cases (51) and possibility of secondary metastatic carcinoma (52).

Two cases were diagnosed as Extra-skeletal myxoid chondrosarcoma:

One case was 52years male diagnosed as Extra-skeletal myxoid chondrosarcomacomposed of plumpy spindle /oval cells arranged in interlacing trabecule embedded in myxoid stroma with foci of immature chondroid differentiation has been noticed the applied IHC panel showed vimentin score+++,S100P score+ correlating the IHC findings with the morphology of tumor the diagnosis was made as ESCS (extraskeletal chondrosarcoma)(51).

Three cases were diagnosed as PNET/ Extra-skeletal Ewing sarcoma:

The 1st case was 17years male with thigh mass diagnosed after immunostaining as PNET/ Extra-skeletal Ewing sarcoma where S100P was score+++,, and all other markers were negative including actin,myosin,desmin, NSE, chromograninA, LCA, and VWF. The light microscopy showed solid sheets of small hyperchromatic round cells with fine chromatin and indistinct cell cytoplasm separated by thin fibrous stroma foci of poorly differentiated pseudorosettes formation the applied IHC revealed strong expression of S100P excluded while other markers as actin,desmin, myosin, NSE chromgraninA, LCA,VWF& CD34 . differential diagnosis of small round blue tumors are numerous including lymphoma , neuroblastoma &PNET/EWS , rahbdomyosarcoma ,undifferentiated neuroendocrine tumor ,chondrosarcoma and leukemia.

Negative expression of LCA has excluded the possibility of lymphoma and leukemia .the negative expression of actine ,desmin&myosin have excluded the possibility of rahbdomyosarcomas . the negative expression of NSE& chromograninA have excluded small cell carcinoma with neuroendocrine differentiation, while the negative expression of VWF & CD34 have excluded the possibility of angisarcoma (50).

Correlating the microscopical findings with IHC findings; the case was diagnosed as PNET/EWS (primitive neuroectodermal tumor/ewing's sarcoma) (48).

PNET/EWS are now considered as a member of EWS family , they are aggressive primitive round cell tumor of uncertain histogenesis with variable degree of neural differentiation (2) traditional diagnosis of EWS family of tumors are made by exclusion but this situation has improved for last 20 years with introduction of new IHC stains CD99 (mic) , caveolin 1 (CAV 1) which are commonly expressed in EWS family of tumors and differentiated them from other small round blue cell is in addition to FLI1, which is expressed on endothelial cells and hemopoitic cells as lymphoma , its found to be specific in 92% & sensitive in 71%(53).

Neural differentiation can be demonstrated in EWS family of tumors by using antibodies against NSE,CD57,S100P according to Alfredo *etal* (53) who proposed that diagnosis of PNET can be done based on three neuronal markers NSE,CD57& S100P and that partial neural differentiation appears to be frequent even in EWS family of tumors so its hard to separate EWS family of tumors from PNET primitive neuroectodermal tumors . all previously mentioned markers should be interpreted with a panel of markers aimed to rule out other tumors with small round cells phenotype occurring specially in young individuals , panel include in addition to,CD79,VIMENTIN,ACTIN,DESMIN,MYOSIN,

KERATIN and MELANOCYTIC markers to final diagnosis (48).

CONCLUSION

Immunohistochemistry should used as complement of the morphological analysis .Antibodies must always be chosen based on the histological differential diagnosis. Wide "random" panels can be misleading.

Panels of antibodies should be used. Because of the lack of sensibility or specificity of markers, and of frequent "aberrant" immunoreactivities, the use of a single immunostain can lead to misdiagnoses.

A correct interpretation of immunohistochemical results is needed.

- Potential pitfalls must be avoided.
- "Aberrant positivities", such as the frequent expression of cytokeratins in leiomyosarcomas, Ewing sarcomas or epithelioid angiosarcomas should be known.
- The type (nuclear, cytoplasmic, membranous…) and expected extent (diffuse/ focal) of positivity should also be known.

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Maximum Power Calculation of Photovoltaic Modules at Different Irradiance Levels

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ABSTRACT

The Performance of photovoltaic (PV) solar module is widely affected by the level of solar irradiance, or in other form the angle of incident of solar radiation. PV systems are one of the next generation's renewable energy sources for our world energy demand. In this study, the we investigate the effect of angle of panel orientation on performance of PV module. The study includes one set PV module adjusted at different angles in both x and y directions. In these positions the values of current, voltage, power and solar radiation intensity were measured from the real solar radiation. The maximum power can be obtained in which maximum values of solar intensity and maximum power were registered. In photovoltaic's the actual curve of the current-voltage characteristic of a solar generator is often needed. The results are simulated with the aid of solar module analyzer.

Keywords: Solar Module Analyzer, Photovoltaic, Sun Radiation, Maximum Power

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الملخص باللغة العربية

يتاثر أداء وحدة الفولتية الضوئية الشمسية على نحو واسع بمستوى الإشعاع الشمسي، أو من زاوية سقوط الإشعاع الشمسي. تعتبر أنظمة PV إحدى مصادر الطاقة المتجدّدة القادمة لتلبية الطلب العالمي على الطاقة لدينا. في هذه الدراسة، نتحرّى تأثير زاوية توجيه اللوح على أداء وحدة PV. تتضمّن الدراسة مجموعة واحدة وحدة PV تعديلها في الزوايا المختلفة في كلتا اتجاهات x و y. في هذه المواقع تم قياس قيم التيار، الجهد(فولطية)، قدرة وشدة إلا شعاع الشمسي من الإشعاع الشمسي الحقيقي. يمكن الحصول على القدرة القصوى من القيم التي سجلت فيها حدّ أقصى للقيم شدة الإشعاع الشمسي والقدرة القصوى. في الفولتاضوئية،غالبا ما نحتاج المنحنى الفعلي لخاصية التيار–الجهد(الفولطية) من مولد الطاقة الشمسية . النتائج هي محاكاة مع المعونة من وحدة محلل الطاقة الشمسية. أداء الوحدات الكهروضوئية في ظل ظروف الإضاءة المنخفضة يمكن أن تختلف اختلافا كبيرا حتى داخل تقنية وحدة واحدة. وهذا له تأثير شديد على العائد من الأنظمة الكهروضوئية. وعي أهمية وتجانس سلوك مستوى الإشعاع من الوحدات الكهروضوئية لا يزال ضعيفا جدا.

INTRODUCTION

Photovoltaic is the process of converting sunlight directly into electricity using solar cells. It basically comprises of two steps. The first step is the absorption of solar radiation within the semiconductor. In the second step, transformation to electrical energy is made by generating current and voltage by the incident solar radiation on the solar cells that produces electrons-hole pairs. A solar module comprises of a number of solar cells that are tied in a predefined architecture to generate enough output power. Several such modules are configured in a single assembly to form a solar array. Solar powered PV systems (For e.g., charging a battery or for grid-connected systems) may either use a single module or an array depending on the total output current and output voltage requirements of the system being powered by it. Stand-alone systems usually require batteries to store power for the times when no sunlight is available while the grid-interface systems use power from the central utility whenever needed and in return supplies surplus generated power back to the utility (1). The photovoltaic (PV) system performance analysis is becoming more and more important with the aim to evaluate the quality of a photovoltaic system during operation (2).

The most important component that affects the accuracy of the simulation is the PV cell model. Modeling of PV cell involves the estimation of the *I–V* and *P–V* characteristics curves to emulate the real cell under various environmental conditions. The most popular approach is to utilize the electrical equivalent circuit, which is primarily based on diode. Many models have been proposed by various researchers; the simplest is the basic single-diode model. It comprises of a linear independent current source in parallel to a diode (3-6). The model only requires three parameters to completely characterize the *I–V* curve, namely short-circuit current (I_{sc}), open circuit voltage (V_{oc}) and diode ideality factor (a). An improvement of this model is done by the inclusion of one series resistance, *Rs* (7-12).

The performance characteristics of photovoltaic modules are needed in order to model their annual performance (13-17). Information available from manufacturers is typically limited to temperature coefficients, short circuit current I_{sc}, open circuit voltage V_{oc}, and the maximum power P_{max}, at rating conditions. This information, while useful in comparing photovoltaic module performance at rating conditions, is inadequate to predict annual performance under typical operating conditions (18–20).

In this Study, the performance of solar modules (130W (Solara PV) and 100 W (Sunworth PV)) under different conditions (i.e., solar irradiance level, temperature, series resistance, and diode ideality factor) is analyzed by a diode equivalent circuit. Next, a solar model tester is used to measure the values of currents under different irradiance (*G*) levels, and the relationship between the irradiance and current (*G–I*) is plotted. Hence, this curve can be used to calculate the irradiance values at any time without a pyrheliometer. This calculation can be achieved by simply moving the solar model at certain angles to get the current values and the corresponding values of G in the *G–I* curve.

MATERIALS AND METHODS

Site Selection

The site was made perfect for receiving maximum solar radiation and there was no shading of any structure or any object in the path of solar rays falling on the Pyranometer.

Solar Panel and Orientations and Tilting

The solar panels were oriented facing the solar analyzer (PROVA200) having seven different tilt angle of 0°,10° , 20°, 30°, 40° ,50° and 60°with the horizontal .It was done to see the effect of tilt angle on the performance of panels.

The measurement principle of a PV system is shown in Figure (1).

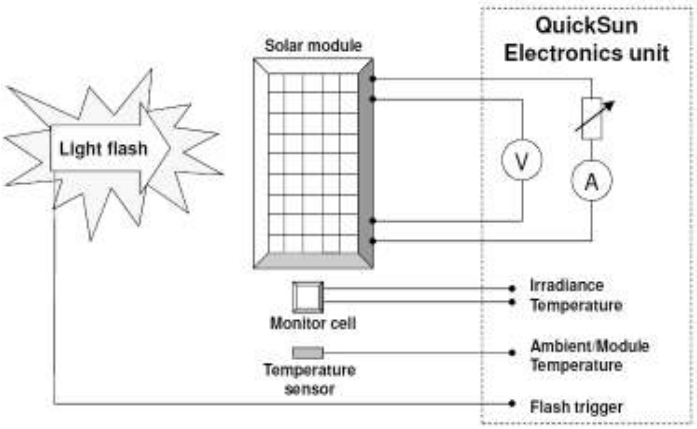

Figure (1): PV system and principls

Recording Solar Irradiance

The solar irradiance (Si) data were recorded with the help of pyrheliometer. The data recorded by pyrheliometer was in kwh.m^{-2}

Calculating Power Output

Generation of electrical power under constant solar Irradiance was achieved by the capability of the solar panel to produce voltage over an external load and current through the load at the same time. When the cell was short circuited under constant solar irradiance then the maximum current (IMpp) and the short circuit current (ISC) are generated, while under open circuit conditions no current can flow and the voltage is at its maximum, called the open

circuit voltage (VOC). The point in the IV-curve yielding maximum product of current and voltage, i.e. power, is called the maximum power point (MPP).

RESULTS AND DISCUSSION

The real solar radiation which can be measured directly during the test via pyrheliometer is used for evaluating the PV model characteristics with the aid of solar analyzer (PROVA200). In this section, the *I–V* and *P–V* characteristics of Solara-130W PV module at different angles are evaluated by using a solar module tester. The solar analyzer can measure the I_{sc}, *Voc*, I_{max}, V_{max}, and *P-V* and *I-V* curves.

From figure (2), the maximum power is at zero-angle (in x-direction, which means at the direction of sun) and begins to decrease gradually as the angle increases. The obtained voltages approximately constant while the current is more affected on the output power. At zero-angle, the power is 129.8w, while it is reduced to 46.2w when the angle increased to 60-degree.

At the y-direction, the is set to zero (x-direction), while the angle in y-direction is changed. Note that the power is also reduced to 33.2 W as the angle increased to 60 degree. From previous two figures, it can be concluded that the y-direction has more effect on the o/p power due to the shape of module which has more length in y-direction that x-direction, the width of panel is 65cm and its length in y-direction is 147cm (effective areas).

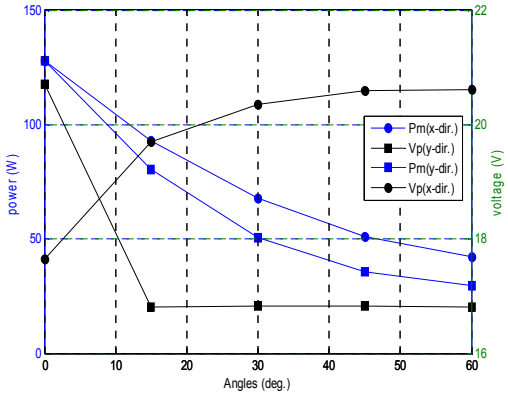

Figure (2): Comparison between P_m and V_{pm} in both directions

Figure (3) summarizes the tilt angle effect on the o/p power in both x and y-directions. The o/p power in x-direction is greater than the corresponding values in y-directions in range of angles. The percentage average difference is about 25.7%.

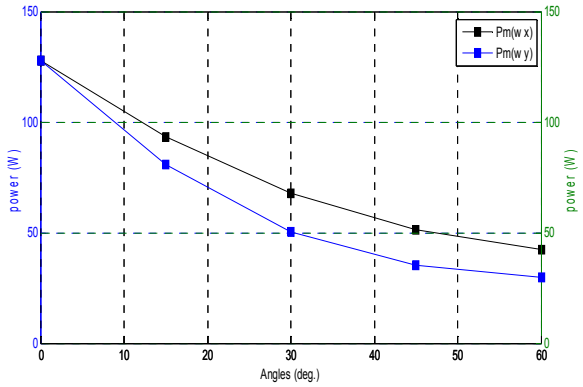

Figure (3): Comparison between maximum powers in two directions (x-y)

Figure (4) shows the tilt angle effects on the o/p voltages in x and y-directions. The obtained voltages in y-direction are greater than the corresponding values in x-direction.

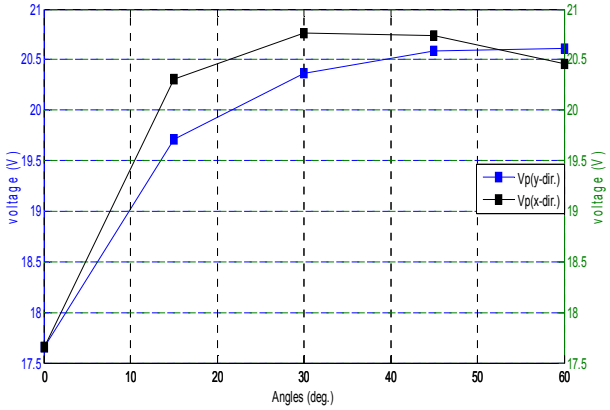

Figure (4): Comparison between voltages in both directions

Figure (5) shows the comparison of current and power performance in the x-direction. From this figure, the current and the power decreases when the tilt angle increases from zero to sixty, and the effect of tilt angle of module on the o/p current and power is shown clearly in this figure. The current and power is widely decreased as the angle increased. The differences in the current in both directions are shown in figure (6).

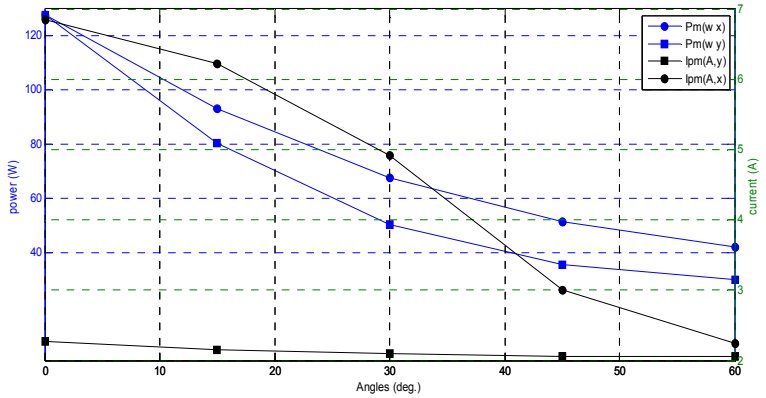

Figure (5): Comparison between $P_m(w)$ and $I_{pm}(A)$ in both directions

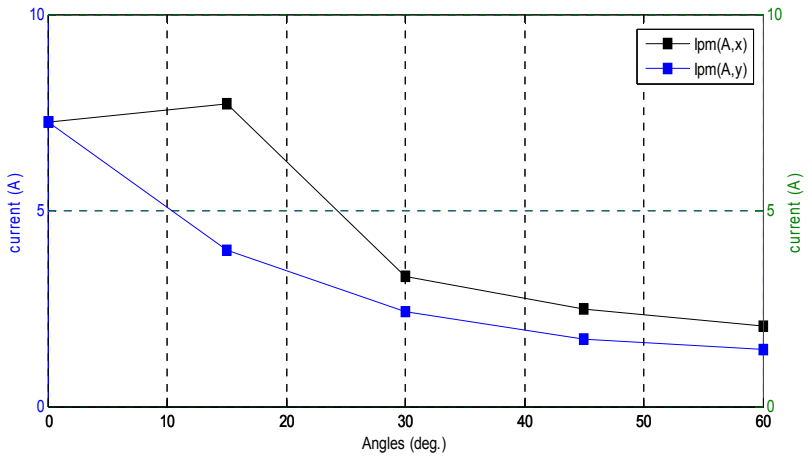

Figure (6): Comparison between currents in both directions

CONCLUSION

In this paper, the effect of installing angle with respect to the sun radiation on the performance of photovoltaic module is investigated. The incident solar radiation data on various inclined surfaces facing different orientations were calculated. The maximum power can be obtained when the sun radiation at its maximum value, or the sun radiation be perpendicular on the surface of panel. The panel can still have maximum power or more than 75% from its rated power as the angle of installation in the x-direction not more than 15 degree. So it is important to install the panels at maximum power radiation which occurs at the middle of daytime approximately.

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Theoretical simulation and design of multilayer interference filters based on MgF2/SiO2 for colored glazed thermal solar collectors

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ABSTRACT

A multilayer optical interference filter with antireflection coating in the near IR region has been designed by a theoretical simulation using matlab program designed for this purpose . The function of this filter is to increase the efficiency of the thermal solar collector. The design also includes a high colored reflectance coating at a specific wavelength in the visible region. This is to gain esthetic aspect for the thermal solar collector which can be used as building facades.

The task in this work is to determine the number of odd layers stacks required for this filter which will fulfill the most acceptable required results by employ the appropriate high refractive index (H) and low refractive index (L) dielectric materials in this design .The optical model in this design is HLH/glass employs quarter wave stacks thickness of thin films.

The proposed dielectric materials at a design wavelength ($\lambda_\circ$) of (558) nm are silicon dioxide (SiO_2) with n=1.47 and magnesium fluoride (MgF_2) with n=1.38 and the glass substrate with n = 1.52 .

The characteristics of this filter such as maximum reflection peak of reflection (R_{max}), visible reflectance ($R_{vis.}$) , solar transmission ($T_{sol.}$) , solar reflectance ($R_{sol.}$) , merit factor (M) and FWHM have been studied by simulation.

Keywords: multilayer optical, IR ergion, solar, optical model

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الملخص باللغة العربية

تم تصميم مرشح بصري تداخلي متعدد الطبقات بواسطة محاكاة نظرية و باستخدام برنامج الماتلاب صمم لهذا الغرض . يعمل هذا المرشح كطلاء مضاد للانعكاسية في منطقة الاشعة تحت الحمراء القريبة وظيفته هي زيادة كفاءة المجمع الشمسي الحراري . كما و يتضمن هذا التصميم ايضا طلاء ذو انعكاسية ملونة عالية في المنطقة المرئية و عند طول موجي معين لاكساب المجمع الشمسي الحراري سمة جمالية واستخدامها كواجهات للابنية .

المهمة في هذا العمل هي تحديد عدد رزم الطبقات الفردية المطلوبة لهذا المرشح والتي سوف تحقق النتائج المطلوبة والاكثر قبولا بتوظيف مواد عازلة ذات معامل انكسار عالي (H) ومعامل انكسار واطيء (L) في هذا التصميم . ان النموذج البصري لهذا التصميم هو HLH/Glass يستخدم رزم لاغشية رقيقة بسمك ربع طول موجي .

ان المواد العازلة المقترحة عند الطول الموجي مركزي($\lambda_\circ$) (558) نانومترهي ثنائي اوكسيد السليكون (SiO_2) ذو معامل الانكسار (1.47) و فلوريد المغنيسيوم (MgF_2) ذو معامل الانكسار (1.38) و ان معامل انكسار القاعدة الزجاجية هو (1.52) .

ان خصائص هذا المرشح و هي اعلى قمة للانعكاس (R_{max}) , الانعكاسية المرئية ($R_{vis.}$) , النفاذية الشمسية ($T_{sol.}$) , الانعكاسية الشمسية ($R_{sol.}$) , عامل الجدارة (M) و العرض عند منتصف الحزمة FWHM تم دراستها بواسطة المحاكاة .

INTRODUCTION

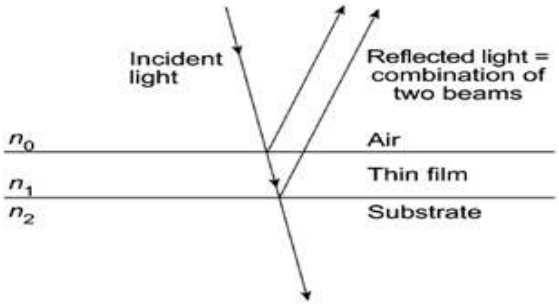
Solar thermal energy is considered as an adequate alternative energy resource for heating and cooling to replace fossil fuels . The roof areas, south facing facades also have to be used as active solar absorption surfaces. Therefore, the solar collectors have to be completely integrated into the building envelope components. Building integration is considered to be a huge barrier for their development. It concerns the overall image of the solar system in the building. From the point of view of the architects, the aesthetic aspect is the main reason for talking about building integration (1). Architectural integration of solar energy systems into buildings has become a widely recognized issue now (2) , Thermal solar collectors, typically equipped with black, optical selective absorber sheets, exhibit in general good energy conversion efficiencies (3). However, the black color, and sometimes the visibility of tubes and corrugations of the metal sheets, limit the architectural integration into buildings. One solution to this problem would be to color the absorber sheets. In this case, the absorber surface combines the functions of optical selectivity (high solar absorption/low thermal emission) and colored reflection. Alternatively, we propose to establish a colored reflection not from the absorber but from the cover glass. This approach has the advantage that the black, sometimes ugly absorber sheet is then hidden by the colored reflection . In addition to that, the functions of optical selectivity and colored reflection are separated, giving more freedom to layer optimization. No energy should be lost by absorption in the coating: all energy, which is not reflected, should be transmitted. Therefore, multilayer interference stacks of transparent materials are ideally suited for this purpose (4,5). One motivation in our work is finding a solution to the problem of black color appearance due to the black body which dominates the external aspect of buildings covered by solar thermal collectors. Until today, no satisfying economically interesting solution to increasing the architectural attractiveness of solar collectors has been found . One recent idea is the use of colored glazing of cover glass for thermal solar collectors and building faces by depositing a multilayer thin film on the glass surface. The ideal reflectivity of the glass-film system should be a narrow band of the visible light while transmitting the rest of the sunlight towards the black body to minimize energy losses (1) . To obtain colored reflected light, the cover glass of the collector should be coated on one side or both by thin films. To avoid any absorption, the thin films must be made by dielectric and transparent materials, such as SiO_2 and MgF_2 . In modern architecture, large glass planes are used as facades in commercial buildings and glazing in a residential home for day lighting. Whatever the application, structural and electronic properties of thin films depend on deposition method and growth conditions, which have to be well understood and controlled.

In this work, we study the simulation and design of multilayer interference filters based on MgF_2/SiO_2 , dielectric films deposited on microscopic glass , the design is HLH/Glass with a thickness quarter wave stacks and for a (558) nm design wavelength and for odd number of layers .

THEORY OF THIN FILMS

Thin films are fabricated by the deposition of individual atoms on a substrate. A thin film is defined as a low-dimensional material created by condensing , one-by-one, atomic/molecular/ionic species of matter. The thickness is typically less than several microns. Thin films differ from thick films. A thick film is defined as a low-dimensional material created by thinning a three-dimensional material or assembling large clusters/aggregates/ grains of atomic/molecular/ionic species (6).

In this context a thin film supports interference effects while a thick film does not. Thus the term thin implies that the film has surfaces that are sufficiently flat and parallel that when illuminated by a plane harmonic wave the infinite number of waves reflected back and forth between the two surfaces have a constant unambiguous phase relationship that does not depend on their lateral position as shown in Fig. (1) (7).



Figure(1) : A single thin film

Let another film be added to the single film so that the final interface is now denoted by c , as shown in figure (2). The characteristic matrix of the film nearest the substrate is :

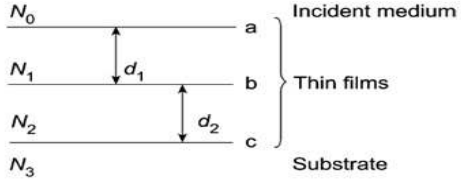


Figure (2) : Notation for two films on a surface

$$\begin{bmatrix} E_b \\ H_b \end{bmatrix} = \begin{bmatrix} \cos\delta_2 & (i\sin\delta_2)/\eta_2 \\ i\eta_2\sin\delta_2 & \cos\delta_2 \end{bmatrix} \begin{bmatrix} E_c \\ H_c \end{bmatrix} \quad (1)$$

Since the tangential components of E and H are continuous across a boundary, and since there is only a positive-going wave in the substrate, this relationship connects the tangential components of E and H at the incident interface with the tangential components of E and H, which are transmitted through the final interface. The 2 × 2 matrix on the right-hand side of equation (1) is known as the characteristic matrix of the thin film. We can apply equation (1) again to give the parameters at interface a , i.e.

$$\begin{bmatrix} E_a \\ H_a \end{bmatrix} = \begin{bmatrix} \cos\delta_1 & (i\sin\delta_1)/\eta_1 \\ i\eta_1\sin\delta_1 & \cos\delta_1 \end{bmatrix} \begin{bmatrix} \cos\delta_2 & (i\sin\delta_2)/\eta_2 \\ i\eta_2\sin\delta_2 & \cos\delta_2 \end{bmatrix} \begin{bmatrix} E_c \\ H_c \end{bmatrix} \quad (2)$$

and the characteristic matrix of the assembly is :

$$\begin{bmatrix} B \\ C \end{bmatrix} = \begin{bmatrix} \cos\delta_1 & (i\sin\delta_1)/\eta_1 \\ i\eta_1\sin\delta_1 & \cos\delta_1 \end{bmatrix} \begin{bmatrix} \cos\delta_2 & (i\sin\delta_2)/\eta_2 \\ i\eta_2\sin\delta_2 & \cos\delta_2 \end{bmatrix} \begin{bmatrix} 1 \\ \eta_3 \end{bmatrix} \quad (3)$$

and where we have now used the suffix *m* to denote the substrate or emergent medium.

$$\eta_m = \mathcal{Y} N_m \cos\vartheta_m \qquad \text{for s-polarisation (TE)}$$

$$\eta_m = \mathcal{Y} N_m / \cos\vartheta_m \qquad \text{for p-polarisation (TM)}$$

If $\vartheta 0$, the angle of incidence, is given, the values of ϑr can be found from Snell's law, i.e.

$$N_0 \sin\vartheta_0 = N_r \sin\vartheta_r = N_m \sin\vartheta_m. \quad (4)$$

A useful property of the characteristic matrix of a thin film is that the determinant is unity. This means that the determinant of the product of any number of these matrices is also unity (4, 8, 9, 10).

$$\begin{bmatrix} B \\ C \end{bmatrix} = \left\{ \prod_{r=1}^{q} \begin{bmatrix} \cos\delta_r & (i\sin\delta_r)/\eta_r \\ i\eta_r\sin\delta_r & \cos\delta_r \end{bmatrix} \right\} \begin{bmatrix} 1 \\ \eta_m \end{bmatrix} \quad (5)$$

$$\eta_r = \mathcal{Y} N_r \cos\vartheta_r \qquad \text{for s-polarisation (TE)}$$

$$\eta_r = \mathcal{Y} N_r / \cos\vartheta_r \qquad \text{for p-polarisation (TM)}$$

Color in Optical Coatings

The technology producing the desired color effect is based on thin films interference filters, by using successive layers . A large palette of colors can be obtained by varying thickness and/or number of layers (11).

Anyone who works with optical coatings knows that they can present exceedingly attractive colors. These colors originate in interference effects that enhance reflectance or transmittance in certain parts of the visible spectrum and inhibit it in others . Although colors occur with both transmitted and reflected light it has long been observed that the most vivid effects are usually to be found in reflection. In the same way that coatings can be designed to have desired spectral properties they can also be designed to present desired colors. This is a little more complicated than the usual design processes because of the subjective nature of color itself.

Color is a subjective, human, response to the spectral quality of light. The response varies with the individual observer. In order to observe the color there must be an acceptable level of reflected or transmitted light (7,12).

One recent idea is the use of colored glazing of cover glass for thermal solar collectors and building faces by depositing a multilayer thin film on the glass surface.

The ideal reflectivity of the glass-film system should be a narrow band of the visible light while transmitting the rest of the sunlight towards the black body to minimize energy losses, see on Figure (3).

In this way, one part of the solar energy in the visible spectrum is invested to make it more aesthetically pleasing and the other part of energy, most of the energy, will pass through the cover, be absorbed and converted to heat in the black surface of the absorber sheet of the solar collectors (13). Figure (4) shows the idea of reflect a narrow band in the visible range. However, a compromise has to be found between a high solar transmission and high color luminosity. For this purpose the reflecting multi-layers consisting of oxides materials have to fulfill some

requirements. Firstly, a large amount of power from solar radiation must be transmitted through the coatings. Secondly, there is a need for zero or near zero absorption materials to avoid energy loss within the coating. Another important factor is the stability of colors with respect to a varying angle of reflection. Lastly, another critical factor is a narrow peak reflectivity in the visible range fixing the desired color of the reflected light.

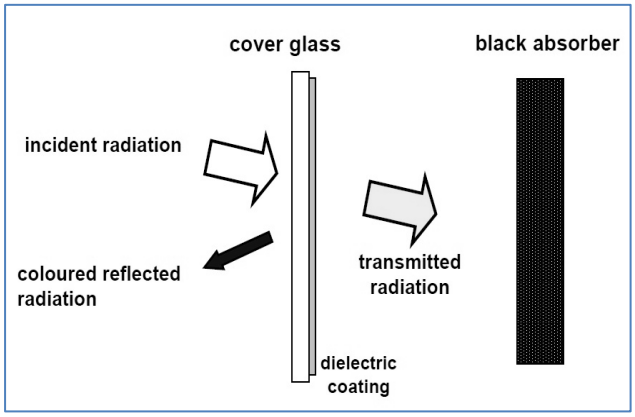


Figure (3): Principle of a colored thermal solar collector (13)

To obtain colored reflected light, the cover glass of the collector should be coated on one side or both by thin films. To avoid any absorption, the thin films must be made by dielectric and transparent materials, such as SiO_2, MgF_2 , Al_2O_3, TiO_2 or a mixture of these oxides. Such optical coatings show a large spectrum of application in every branch of science and technology due to the wide development of the physics and technology of thin films in the beginning of 1930. This includes in particular transparent dielectric coatings for optical filters such as: antireflective coatings for the visible and infrared range with one or more zeros reflectance at a specific wavelength, beam splitters , band pass filters, high reflectance coating, edge filters , broadband and narrowband pass filters , interference color-shifting films, low laser damage filters , chirped mirrors for ultra short laser pulse compression and optical sensors, hot-cold mirrors and optical waveguides.

Several criteria should be respected when choosing the material film and the film deposition process for the desired optical application:

- the deposition technique must allow good control and reproducibility of the optical properties of the film, which are strongly dependent on the preparatory conditions. In all optical film application, at least two basic materials with high n_H and low n_L refractive indexes are necessary. A large (n_H - n_L) value may help to reduce the design thickness.
- in most optical coatings application, materials are desired to be amorphous, isotropic, and scattering below 10^{-4} .
- an appropriate deposition technique is required to achieve good film thickness uniformity across the coated substrate, an acceptable deposition rate, and a good environmental stability.

The common techniques for optical filters fabrication are the physical vapor deposition methods such as evaporation and sputtering, frequently assisted by ion bombardment: ion plating, ion beam assisted deposition, unbalanced magnetron sputtering, cathodic arc deposition. Sol-gel deposition is also considered as an interesting alternative route for large-scale surface coatings (1).

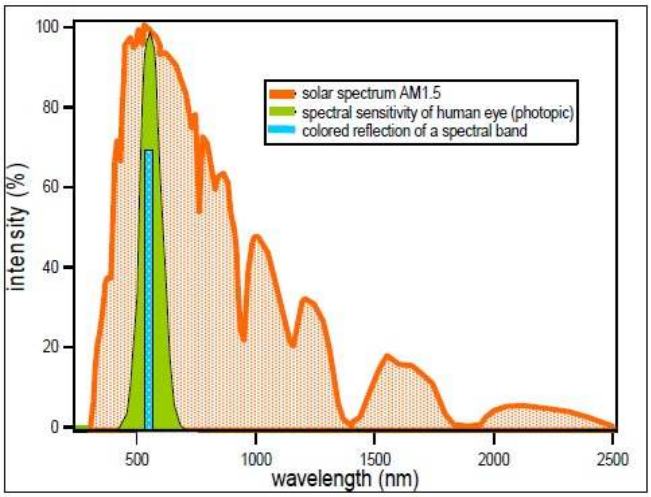


Figure (4) : The idea: Reflect a narrow band in the visible range (14)

Optical properties of multilayer films (solar reflectivity, solar transmission, visible reflectance)

As already mentioned, a large fraction of power from the solar radiation must be transmitted through the coatings. The transparency of the film permits avoiding absorption energy losses. At the same time, the multilayer films must present a narrow reflection band in the visible range fixing the color of the reflected light. To estimate if a multilayer coated glass sample is suitable to be used as a colored solar collector glass, it is characterized by its solar transmission T_{sol} and its solar reflectivity R_{sol}, defined respectively by the following relations :

$$T_{sol} = \frac{\int T(\lambda)\, I_{sol}(\lambda)\, d\lambda}{\int I_{sol}(\lambda)\, d\lambda} \qquad (6)$$

$$R_{sol} = \frac{\int R(\lambda) \cdot I_{sol}(\lambda) d\lambda}{\int I_{sol}(\lambda) d\lambda} \qquad (7)$$

T(λ) is the transmission of the film, $R(\lambda)$ the total hemispherical reflectivity and I_{sol} the intensity of the solar spectrum AM1.5. The integration range is given by the limits of the solar spectrum . The visible reflectance $R_{vis.}$ is determined from the photopic luminous efficiency function V(λ) , the standard illumination $D_{65}(\lambda)$ and the total hemispherical reflectivity R(λ):

$$R_{vis} = \frac{\int R(\lambda) \cdot D_{65}(\lambda) \cdot V(\lambda) d\lambda}{\int D_{65}(\lambda) \cdot V(\lambda) d\lambda} \quad (8)$$

The standard illuminant D_{65} closely resembles the relative spectral energy distribution of north-sky daylight and is accordingly important for color specification in northern Europe (1).

Merit factor

Merit factor *M* defined as the ratio of the visible reflectance *Rvis* and the solar reflectivity R_{sol} . *M* is then large for a high visible reflectance or low solar energy losses and consequently describes the energy efficiency of the visual perception ("brightness per energy cost") , the potential of colored thermal solar collectors can be expressed by a figure of merit M . Following this definition, we obtain (6,13):

$$M(\lambda_0) = \frac{R_{VIS}(\lambda_0)}{R_{sol}(\lambda_0)} = \frac{D_{65}(\lambda_0) \cdot V(\lambda_0)}{I_{sol}(\lambda_0)} \cdot \frac{\int I_{sol}(\lambda) d\lambda}{\int D_{65}(\lambda) \cdot V(\lambda) d\lambda}. \quad (9)$$

It is independent of the intensity of the reflection. The integrals just correspond to a normalization, the dependence on the wavelength λ_0 is simple (1, 4, 5,6,15).

Structure of Study and Computer Simulation

In our work we use the optical model air//HLH//Glass with a thickness quarter wave stacks in this case all individual layers are of optical film thicknesses n.t =$\lambda_\circ$/4 , where $\lambda_\circ$ is called the design wavelength .
Usually layers of a high index material (H) alternate with layers of a low refractive index material (L) resulting in stack of the HLHLHLH… and with odd number of layers from 3 to 39 layers , the design wavelength ($\lambda_\circ$) is (558) nm , we use the dielectric materials silicon dioxide (SiO_2) with a high refractive index (1.47) and magnesium fluoride (MgF_2) With a low refractive index (1.38) deposited on glass substrate with (1.52) refractive index .

The difference in refractive indices between the high index and the low index material governs the peak height (6). The larger the difference in the refractive indices , the larger is the spectral region of high reflection . We are interested in the opposite , a narrow reflection peak , which can in principle be created by employing a large number of layers (39 layers) , we chose the refractive indices to be very close to each other (but not identical) because the reflection at each interface is weak now, by choosing a low refractive index material such as MgF_2 the level of background oscillation in the reflectance spectra can be lowered thus gaining color saturation and some percent in solar transmission (4,10), the figure below shows the peak of the reflectance for 3 layers.

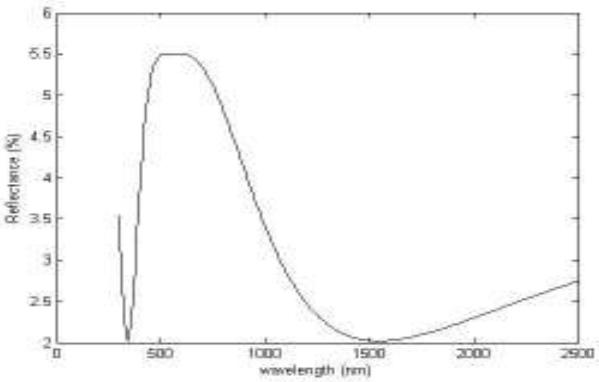
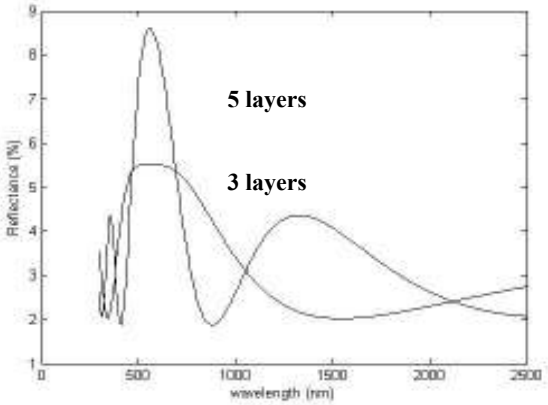


Figure (5):Reflectance spectrum curve as computed for a quarterwave stacks consisting of 3 layers

The reflectance peak is (5.51238)% , when we increase the number of layers to 5 layers , see fig.(6) we notice that the peak high is higher than once its value is (8.61905)% , this mean increasing the number of layers lead to increase the reflectance peak in the visible region this also illustrated in figures (7,8) .



Figure(6) : Reflectance spectrum curve as computed for a quarter wave stacks consisting of 3 and 5 layers

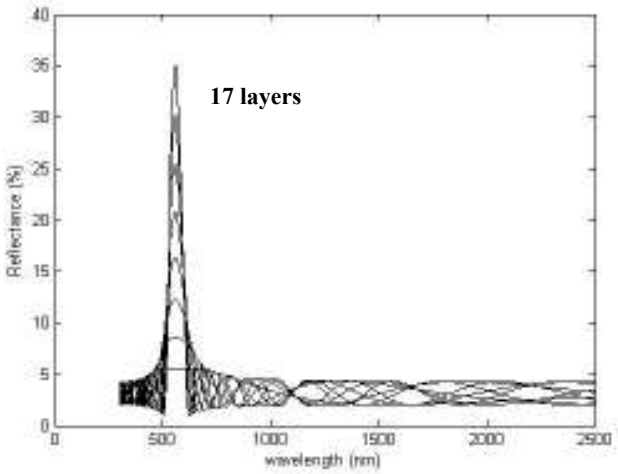


Figure (7):Reflectance spectrum curve as computed for a quarter wave stacks consisting of 17 layers

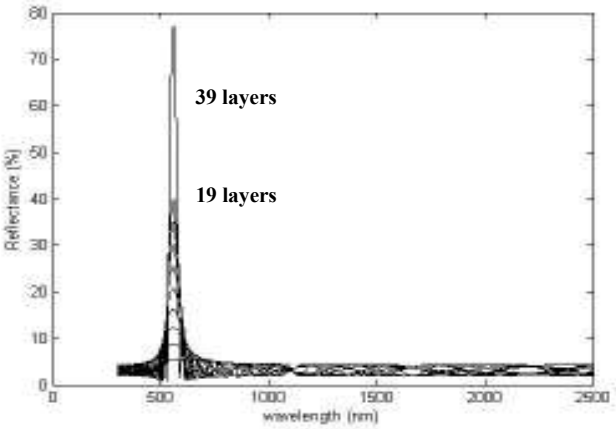


Figure (8):Reflectance spectrum curve as computed for a quarterwave stacks consisting of 39 layers

RESULTS AND DISCUSSION

Simulation results in our work show that increase the number of layers for odd number from (3-39) will produce increasing in peak high of reflectance , for 3 layers the reflectance peak is (5.51238)% , for 5 layers is (8.61905)% , the increasing is continue until reach the last layer which is 39 and has reflectance peak value (77.2571)% , this behavior was illustrated in fig.(9).

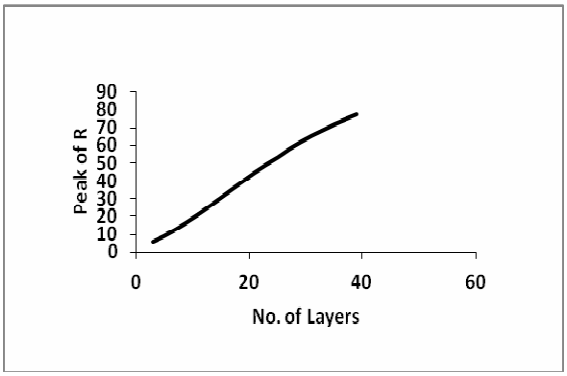


Figure (9): increase the peak value of reflectance versus increasing the number of layers

In the same time when we use matlab program and extracting the visible reflectance ($R_{vis.}$) depending on the equation (8) , we find for 3 layers its value is (5.4939) , for 5 layers is (8.0968) and for 39 layers is (28.4795) this increasing continue with increasing number of layers see fig.(10) .

A visible reflectance of 12% , which is already considerable for a color (since100% corresponds to white) (6), our coating exhibit green color reflectance which will add esthetic value to the thermal solar collectors while the near infrared region still anti-reflection region this mean the solar transmittance ($T_{sol.}$) value become high and its values vary from (95-98)% , see fig.(11) and the solar reflectance very few and varies from (2-4)% see fig. (12) , consequence the efficiency of the thermal solar collector will increase.

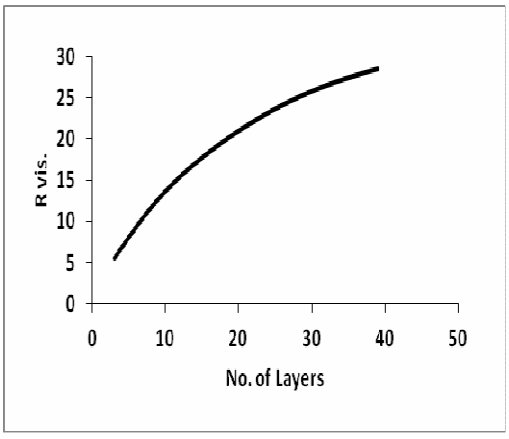


Figure (10): increase the visible reflectance value versus increasing the number of layers

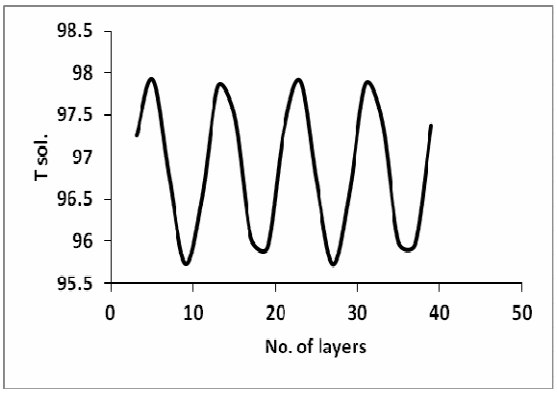


Figure (11): Solar transmittance value versus the number of layers

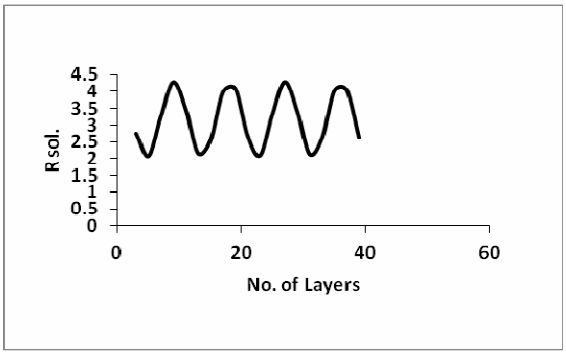


Figure (12): Solar reflectance value versus the number of layers

Figures (5,6,7,8) also show increasing the number of layers will decrease the FWHM of the curves , this will prove the color reflection idea : reflect a narrow band in the visible range , see fig.(4) (14) , fig.(13) show how increasing the number of layers from (3 to 39) layers will decrease FWHM of the reflectance curve.

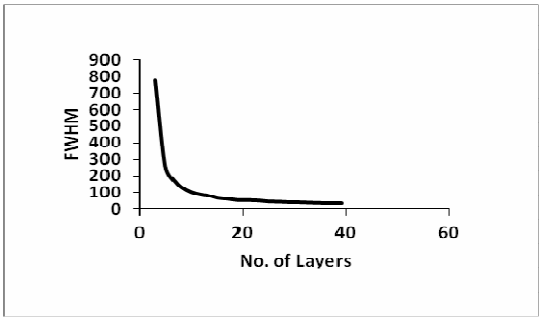
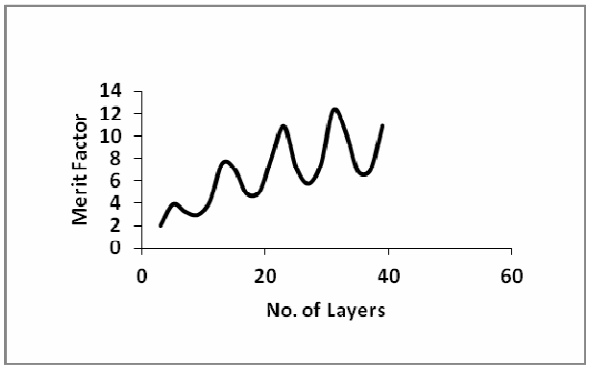


Figure (13): decrease FWHM with increasing the number of layers

The general potential of colored thermal solar collectors is promising, and can be expressed by a figure of merit M . The number of M describes the energy efficiency of the visual perception (''brightness per energy cost'') (6). Fig.(14) shows the varying merit factor with increasing number of layers , the value of M is few from (3-11) layers but it begin to increase from layer thirteen with value (7.5329) and the increasing continue until reach (12.2888) in the layer (31) .



Figure(14) : Merit factor versus increasing number of layers

CONCLUSION

Multilayer optical interference filter work as anti reflection coating in the near IR region to increase the efficiency of the thermal solar collector and as green colored reflection coating in the visible region to gain esthetic aspect for the thermal solar collector which is used as building facades has been obtained by a theoretical simulation made by using matlab program we designed it for this purpose , the structure of optical model is HLH/Glass , for quarterwave stacks and for odd number of layers from (3-39) layer . The behavior of the designed multilayers is analyzed by the computer simulation yielding the maximum peak of reflection (R_{max}), visible reflectance ($R_{vis.}$) , solar transmission ($T_{sol.}$) , solar reflectance ($R_{sol.}$) , merit factor (M) and FWHM . The proposed colored glazed solar collectors will be ideally suited for architectural integration into buildings, e.g. as solar active glass facades.

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Assessment of retrograde ureteral stenting under local anesthesia

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ABSTRACT

Ureteral stenting is one of the most common procedure done by urologist usually considered as a minimally invasive procedure that used to done under general anesthesia. Ureteral stent are of 2 type either a double J stent which is used for therapeutic purposes like ureteric obstruction by stone , or ureteric catheter which is used for diagnostic purposes like ascending pyelography, stenting of ureter is done retrogradlly by cystoscope .

The objective of our study is to assess the ability of doing retrograde ureteral stenting under local anesthesia , for patients who is unfit for general anesthesia , and comparing the success of the procedure under local anesthesia to that done under general anesthesia.

In this study thirty three patients (11 female and 22 male) with different indications of retrograde ureteral stenting were undergone the procedures under local anesthesia using rigid cystoscopy system provided with camera and screen in a private clinic in Baghdad at Al-Amyria city.

Anticholenergic ,Tamsulosin ,prophylactic antibiotic, diclofenac and tramadol were given for those patients The medications given one to two hour before procedure for good patients tolerance and to increase pain threshold.

Just before the procedure , Lidiocain gel 10cc was injected into the urethra of male patients using 10cc syringe for good lubrication and adequate anesthesia to the lining of the urethra.

Out of those 33 patients 26 (78,8%) undergone a successful stenting of the ureter under local anesthesia . Seven (21,1%) patients had a failed procedure in which stenting by double J catheter failed to bypass the obstruction . Those seven patients who failed undergone retrograde stenting under general anesthesia in Abu-gareb general hospital in the next day, six of them where failed and just one patient had succeed stenting.

We conclude that retrograde stenting of ureter can be done safely as outpatient procedure under local anesthesia with success rate similar to that done under general anesthesia

Keywords: Ureteral stenting, cystoscope, anesthesia

الملخص باللغة العربية

قسطرة الحالب عن طريق ناظور المثانه احد اكثر الاجرائات الجراحيه التي يقوم اطباء جراحة المجاري البوليه تحت التخدير العام.

ويتم قسطرة الحالب للاسباب التاليه: اما قسطره علاجيه وذلك عن طريق انبوبة الدبل جاي الخاص بفتح الانسدادات في الحالب. مثل حصاة الحالب ,او قسطره تشخيصيه عن طريق انبوبه خاصه توضع في الحالب ويتم تلوين اوعية الكليه والحالب عن طريق حقن ماده تلوين مشعه خاصه لهذا الغرض تكون القسطره في كلاتا الطريقتين عن طريق ناظور المثانه .

وتهدف هذه الدراسه الى تقييم امكانيية اجراء قسطرة الحالب العكسيه عن طريق ناظور المثانه تحت التخدير الموضعي.

الفئه المستهدفه من المرضى في هذه الدراسه هم المرضى الذيّن يعانون من انسداد الحالب ولايمكنهم تحمل مخاطر الناتجه عن التعرض للتخدير العام , حيث تمّ اجراء قسطرة حالب عكسيه عن طريق ناظور المثانه لثلاثه وثلاثون مريض تم جمع المرضى عن طريق العياده الخاصه للباحث الكائنه في بغداد مدينة العامريه .

كل مريض اجريّ له هذا التداخل تمّ ابلاغه بخطوات العمليه و اعطيّ مضادات الالام للمرضى , ومادة البسكوبان بالحقنه , وعقار التامسيوليوسين ,وذلك لغرض توسيع الحالب الامر الذي يسهل اجراء القسطرة, وحقن كل مريض بعقار الكارامايسين عن طريق الوريد لمنع الاتهابات اثنا وبعد التداخل , وقبل التداخل مباشرة تم حقن الاحليل بمادة اللايدوكين جل بسرنجه من فئة 10 مل وذلك لضمان تخدير وتزييت بطانة الاحليل .

وكانت النتائج كالاتي : حيث نجحت قسطرة الحالب تحت التخدير الموضعي عند 26 مريض(78,8%) وفشلت عند سبعة مرضى(21,1%).

كل مريض من المرضى السبعه الذين فشلو في اجراء قسطره ناجعه تحت التخدير الموضعي , ادخلو المستشفى في اليوم التالي وتم اجراء قسطره لهم تحت التخدير العام وذلك في مستشفى ابي غريب العام الكائن في بغداد .

من بين السبعة مريض واحد فقط اجرى قسطرة ناجحة تحت التخدير العام وستة فشلوا مرة اخرى.

واستنتجنا من هذه الدراسة ان القسطرة العكسية للحالب عن طريق ناظور المثانة يمكن اجراؤها تحت التخدير الموضعي ، في العيادة الخارجية بامان ، وهي ذات نتائج نجاح تضاهي التي يتم اجراؤها تحت التخدير العام.

INTRODUCTION

Ureteral stenting is one of the most common procedure done by urologist usually considered as a minimally invasive procedure that used to done under general anesthesia (1).
Ureteral stent are of 2 type either a double J stent which is used for therapeutic purposes like obstruction by stone, clots and ureteric tumors or before extracorporeal wave lithotripsy (ESWL) . Other type of ureteric catheter which is used for diagnostic purposes like ascending pyelography or urine sample retrieval from kidney for culture and sensitivity ,both catheter types are made from silicon material to avoid allergic reaction and provided with internal lumen for drainage (2). The diameter of ureteric catheter range from 3.5 French to 8 French Its introduced to ureter transurethrally via a cyst scope . These stents has a radiopaque line to be visible on X rays film for insurance of typical insertion , and follow up procedures . For the double J stent it could be remained in the ureter for six month before replacement if there is indication (3).
The complications of ureteric catheterization are either early ,like trauma resulting from forced insertion which may lead to urine extravasations , urinoma , and even urosepsis , or late complication like slipping of catheter , urinary tract infection , heamaturia , crystallization and stone formation (3).

Anatomical variations in male and female urethra

Male urethra : it's about 20 to 25 cm in length has a curve shape not in a straight canals and composed of 2 parts which anterior urethra composed of penile and bulbar urethra which has 15 to 17 cm in length and posterior urethra which is composed from membranous and prostatic urethra which has 5 to 7 cm in length .The male urethral sphincter is located at the membranous urethra and its part of lavetorani muscle, which has a voluntary control (1).
Female urethra : its about 4 to 5 cm in length has straight shape the sphincter is located at the midpart of it and its part of lavetorani muscle which under voluntary control (1).
From above variations doing cystoscopy under local anesthesia is more annoying to male due to the length and the curved course of male urethra , the most annoying part is where the scope passing the sphincter . In female because of short length straight course of urethra usually cystoscope done under local anesthesia with little discomfort (2).

PATIENTS, MATERIALS AND METHODS

As any retrograde ureteral procedure I need cystoscopy system provided with screen and camera for precise and more accurate catheterization so I use a Karl-Storz system for endourology.
The ureteric stent that used was a Double-J catheter of Urotech. Type made in Germany ,to ensure easy comfortable stenting.
Diclofenac injection 75 mg ,OR Tramadol injection 100 mg , hyosin butyl bromide injection 10mg to decrease discomfort at procedure. garamycin injections 80 mg given to prevent ascending infection as a prophylactic antibiotic. Tamsulosin tab. 0.4mg given two hours before stenting to ensure good dilatation of lower ureter pre-stenting. Finally Ldiocain jel 10cc injected to urethra just before the procedure to lubricate the male urethra and to anesthetized the mucosa.
The study is of Cross-sectional interventional type, 33 patients (22 male and 11 female) were undergo retrograde ureteral stenting under local anesthesia in my private clinic in Baghdad at Al-amyria city and in Abu-gareb general hospital in over period of 11 months from 1/4/2012 to 1/3/2013 . All patients were attended to the clinic .
All the patients were investigated for their ureteric problem by ultrasound study , IVP and some of them by CT scanner for more informative evaluation. And were investigated biochemically for their renal function , liver viral hepatitis study.
All of them had indication for retrograde ureteral stenting , 30 patients of them had indication for double J stent and the other three patients had indication for diagnostic ascending pyloureterography.
After explanation of the procedure all the patients were agreed to do the procedure under local anesthesia .
Every patients was subjected to procedure given diclofenac 75 mg IM as it reduce the episodic colicky pain (4), and buscopan 10mg IM two hour before the procedure ,Tramadol 100mg IM were given instead of diclofenac in patients who had a contraindication for NSAIDs like asthmatic patients or those with duodenal ulcer .Tamsulosin tab. 0.4mg 2 hour before procedure to induce dilatation of lower ureter as it has been demonstrated that specific adrenoceptors subtypes (alpha(1A)/alpha(1D)) are prevalent in the distal part of the ureter (5).
Garamycin 80 mg were given IV just before the procedure , Lidiocain gel is injected using 10cc syringe into urethral meatus of male patients and held for 3 minutes for good lubrication and anesthesia of urethral mucosa (2).
The patient should be placed in dorsal lithotomy position for cystoscopy, after preparations we tell the patient that we will start the manipulation and I used to tell the patient to look at the screen to see the procedure and shifting him from fair of manipulation.
In male the scope pass easily till the external urethral sphincters when a resistance s felled in introduction of the scope and the patient fell discomfort , here , the patient should be relaxed to decrease resistance as much as possible , once I pass the sphincter the scope pass easily to bladder then both ureteric orifice in bladder trigone should

be identified ,and ,canalize the target obstructed ureter.
During passage of stent into the ureter all the patients has no pain but some has discomfort, till , the site of obstruction when the patients fell some heaviness at their loin region , once the stent bypass the obstruction all the loin pain and discomfort disappear dramatically , then the patients asked to take a KUB film to evaluate a typical stent insertion. In female the procedure were easiest with less discomfort.

RESULTS

Twenty six patients (78.8%) had a successful stenting of the ureter 17 of them were male and nine were female.
Out of the 17 male patients one patients had diagnostic ascending pyelography and the others had a double J stenting , the procedure not lasting more than five minutes from beginning to termination of procedure , Of the nine females patients two patients had diagnostic ascending pyelography and the others had a double J stenting All the patients were very comfort after the procedure with no if any pain at all, instead those who had obstructed ureter by stone undergone dramatic disappearance of the pain and loin discomfort.
Immediately all of them went to the radiology clinic and took a KUB film to assess the typical insertion of the stent for double J procedure, while those who had diagnostic procedure undergone additional contrast study by doing retrograde ascending pylography .
Seven patients (21.1 %) had failed insertion of double J stent under local anesthesia they were five male and two female.
Out of those five male who failed the procedure four of them had chronic obstruction of the ureter lasting more than six weeks by impacted ureteric stones which were visible well by KUB film were impacted in mid and lower third of the ureter .
The remaining male patient who failed stenting developed nausea during doing the procedure under local anesthesia, so , it was decided to terminate it , this patient developed vasovagal attack during ureteral stenting and he became well on intravenous fluid and reassurance.
In the next day all male patients who failed the procedure undergone the same procedure but under general anesthesia, four of them failed stenting and shifted ureteroscopy at same cession, The only succeed stenting under general anesthesia was the male who developed vasovagal attack on local anesthesia.
On other hand, two females patients failed the procedure under local anesthesia. both of them had chronic obstruction by impacted stone at lower third of the ureter which diagnosed by intravenous pyelography , those two female patients shift to general anesthesia in the next day and the stent failed to bypass the obstruction and shifted to ureteroscopy. Tables (1, 2)

Table (1): The results of procedure under local anesthesia

Patients	Successful stenting	Length of procedure	Stenting indication	Failed stenting
Male (22)	17	< 5 minutes	Diagnostic 1 Therapeutic 16	5
Female (11)	9	< 5 minutes	Diagnostic 2 Therapeutic 7	2
Total (33)	26(78.8%)		Diagnostic 3 Therapeutic 30	7(21.1%)

Table (2): The criteria of stenting failure under local anesthesia and comparing it to that done under general anesthesia

Failed stenting under LA	No. of patients	Cause of failure	Stenting under GA
Male	5	4 had impacted stone > 4 weeks	All failed
		1 had (vasovagal) Attack	succeed
Female	2	2 had impacted stone >4 weeks	Both failed
Total no. failure	7	7	6 patients

DISCUSSION

Retrograde stenting of the ureter considered one of the most common minimal invasive procedure done by urologist surgeon , due to multiple indications of the procedure , This procedure used to be done under general anesthesia including routine fitness for the patient and acceptance the risk of general anesthesia especially the cardiovascular and respiratory status for those patient (1).
Keeping in mind most of the patients who need ureteral stenting could be presented with sever acute pain that need emergency stent to relive the pain episode (6).
In addition many of them may have impairment of renal function due to stone problems which make them unfit for general anesthesia either due to renal problem or due to other medical causes , and by this way we can avoid general anesthesia and associated risks**.** One of most common benefit of local anesthesia is doing ureteral stenting in patients with bilateral ureteric obstruction ,or obstructed single kidney , those patients presented with a sudden onset of ureteric colick and anuria (obstructive uropathy) which result in sudden increase of blood urea and serum creatinine in few hours and can be life threatening especially for old patients (2). Additional benefit in doing stenting under local anesthesia in the absences of continuous fluoroscopy the patient could guide us to successful stenting since his discomfort or loin pain disappear immediately once the obstruction is bypassed by

stent , Also we can assess the typical insertion of the stent immediately by taking a KUB film without hospitalzation for awaking from general anesthesia. Finally the financial benefits by lowing the cost for the patients and the medical institutions , keeping in mind the large number of patients that admitted for this purposes.

In our study 33 patients were undergone stenting under local anasthesia (22 male and 11 female) , 26 patients (78.8 %) had a successful stenting (17 male and 9 female) in term of good kidney drainage , immediate symptomatic improvement ,down grading of hydronephrosis within few minutes after stenting confirmed by ultrasound , and a KUB film for typical stent insertion.

Seven patients (21.1 %) has failed the procedure , there were five male and two female , six of them has a common feature of more than six weeks of ureteric obstruction by stone impacted in the ureter , making stenting difficult or even impossible to avoid trauma to ureter and patients discomfort , Any failed stenting patient in the clinic shifted to general hospital in the next day for stenting under general anesthesia , All those patients failed to have a successful stenting under general anesthesia , only one patient had successful stenting under general anesthesia, while ,he failed under local anesthesia . From above ; only one patient (1.33%) out of seven failed stenting under local anesthesia was succeed under general anesthesia , So there were no significant difference in both procedure as the success of by general anesthesia was no more than (1.33%) from that by local anesthesia.

By comparing this study to other which interested in ureteral stenting under local anesthesia and general anesthesia we can focus on the following studies :

A study done by Sivalingam S, Tamm-Daniels I, Nakada SY in wisconson university in 2012 to evaluate the outcomes of urgent ureteral stent placement under local anesthesia (LA) with those placed under general anesthesia (GA) for obstructing stones , They conclude that urgent ureteral stent placement for obstructing stones can be safely and effectively performed under LA in the office (6).

Some physicians used stents in 87 patients: to bypass obstruction in 57, as an adjunct to complicated upper tract surgery in 15, as initial treatment of upper urinary fistulas in 10 and for miscellaneous reasons in 5. The majority of the stents were placed endoscopically (58 %) and under local anesthesia (54 %). They concluded that stents were changed easily on an outpatient basis under local anesthesia and patient tolerance was excellent (7).

CONCLUSION

We conclude that retrograde stenting of ureter can be done safely as outpatient procedure under local anesthesia with success rate similar to that done under general anesthesia. Keeping in mind the advantages of avoiding the general anesthesia, time and cost effect of local anesthesia. Finally stenting of ureter under local anesthesia could be considered as a routine procedure other than stenting under general anesthesia

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قسم الدراسات العربية

ARABIC SECTION

الإدارة المتكاملة للمياه الجوفية بسهل صلالة في سلطنة عمان وحمايتها من تداخل مياه البحر

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الملخص باللغة العربية

يعتبر مفهوم استغلال مياه الصرف الصحي المعالجة كمصدر غير تقليدي للمياه قد أصبح شائعاً ومستخدماً في العديد من الدول خاصة تلك التي تعاني من نقص حاد في مواردها المائية الطبيعية وهو يعتبر أحد الأذرع الهامة للإدارة المتكاملة لموارد المياه. وفي سلطنة عُمان وكأحد الدول الرائدة في هذا المجال وتحت مظلة حماية البيئة ومكافحة التلوث ومن أجل تحقيق أفضل لحماية الأرض ومصادر المياه (مفهوم التنمية المستدامة) تم خلال عام 2003م استغلال مياه الصرف المعالجة ثلاثيًا والخارجة من محطة صلالة لمعالجة مياه الصرف الصحي لحقن الخزان الجوفي الساحلي بسهل صلالة وذلك لتكوين ساتر يمنع زحف مياه البحر باتجاه اليابسة هذا بالإضافة للسدين الواقعين في الأحباس السفلى لكل من وادي صحلنوت وجرزيز. ويعتبر السهل الساحلي بصلالة بتكويناته الهيدروجيولوجية من أفضل الأماكن لتواجد المياه الجوفية ، ويمتد ساحل صلالة لمسافة 40 كيلومترا على طول بحر العرب ، وبعرض يبلغ أقصى حد له عند منتصف السهل 12 كم من حافة الجبل حتى الساحل. وقد أدى النمو السكاني المتزايد والتطور المستمر في المجال الزراعي والصناعي خلال العقود الأربعة الأخيرة إلى تزايد الطلب على المياه (العذبة) . وغالبا ما تتم تغطية هذه الزيادة في الطلب بواسطة ضخ المياه الجوفية بكميات كبيرة مما يؤدي إلى انخفاض مستوى المياه الجوفية وبالتالي حدوث اختلال في التوازن القائم بين المياه العذبة والمياه المالحة وهو ما يؤدي إلى حدوث مشكلات تداخل المياه المالحة بالمناطق الساحلية. وتشير آخر الدراسات المائية لمنطقة سهل صلالة (النمذجة الرياضية) إلى وجود عجز في الميزان المائي حيث تزيد معدلات السحب من المخزون الجوفي بحوالي (21 م م^3/السنة) مقارنة بكميات المياه المستخدمة والتي تـُقدر بحوالي (92 م م^3/السنة) . ويتم تعويض هذا العجز من خلال الضخ الجائر من المخزون الجوفي ، وهو ما أدى إلى انخفاض مستويات المياه الجوفية ببعض الآبار القريبة من أماكن السحب (المزارع الكبيرة) وبالتالي تدهور نوعية وجودة المياه الجوفية بهذه الأجزاء نتيجة تحرك مخروط المياه المالحة من الأجزاء الشرقية والغربية باتجاه الجزء الأوسط من السهل. وبشكل عام يعتمد إيقاف ظاهرة تداخل مياه البحر في الأجزاء الساحلية على استمرار التغذية السنوية للخزان الجوفي من مياه الأمطار وتدفقات الأودية وبالتالي ارتفاع مستويات المياه الجوفية ، بالإضافة إلى الحد من الضخ الجائر للمياه الجوفية بتلك الأجزاء. وفي إطار إيجاد الحلول لإيقاف هذه الظاهرة والحد من تفاقمها تقوم السلطنة بتنفيذ العديد من الإجراءات والخطوات والتي من أهمها استمرار المراقبة وإجراء مسوحات الملوحة والعمل على تطويرها وتكثيفها وذلك باستخدام التقنيات المتطورة والمتاحة بالإضافة لسلسلة من عمليات وإجراءات الإدارة المتكاملة لموارد المياه منها عمليات الحقن الجوفي بمياه الصرف الصحي المعالجة معالجة ثلاثية لتغذية الحوض المائي الجوفي من خلال 40 بئرا امتدت على مسافة طولية تقدر ب 12كم على المنطقة الساحلية ، وكذلك فقد كان هناك أثرا كبيرا لسد صحلنوت والذي بدء بتشغيله عام 1993 حيث بلغت سعته التخزينية 4ر6 م م^3 وسد شمال المطار والذي تأسس عام 2009 وبلغت سعته 2ر77 م م^3 ، وستفيد هذه الدراسات والأبحاث في تحديد سُبل معالجة مشكلة تداخل المياه المالحة بالأجزاء الساحلية . بالإضافة إلى ما تقدم ، فقد اتخذت الحكومة إجراءات هامة وأساسية للسيطرة على مشكلة الملوحة كفرض الحظر على حفر أي آبار جديدة إلا في ظروف خاصة كما تم حث المزارعين على تحديث وسائل الري بإتباع وسائل الري بالتنقيط والرش.

الكلمات المفتاحية: الإدارة المتكاملة للمياه الجوفية، سهل صلالة، سلطنة عمان

ABSTRACT

The concept of using treated wastewater has become commonplace and used in many countries, especially those that suffer from a severe shortage of natural water resources. In the Sultanate of Oman, as one of the leading countries in this region and under the umbrella of integrated water resources management (IWRM) , environmental protection and pollution control in order to achieve the best to protect the land and water resources (the concept of sustainable development) during the year 2003 use the tertiary treated wastewater as a non conventional water resource which discharged from Salalah Waste Water Treatment Plant to inject the aquifer coastal plain of Salalah to stop the encroachment of sea water towards the land. The coastal plain of Salalah with its hydrogeological setting is consider one of the best places for the presence of ground water, the coast of Salalah extends for a distance of 40 km along the Arabian Sea, with a maximum width of 12 km in the middle of the plain. Increasing population growth and ongoing development in the field of agriculture and manufacturing over the last four decades has resulted in the growing demand for water (fresh). This increase in demand was often covered by over pumping underground water in large quantities, leading to a lowering of ground water table and thus an imbalance between freshwater and salt water, which leads to problems of saltwater intrusion in coastal areas. The latest studies of water for the Salalah plain (mathematical modeling) indicates a deficit in the water balance where the rates of withdrawal from the strategic reserve of about (21) million cubic meters compared to quantities of water used, estimated at about (92 million cubic meters). This amount of deficit is compensated through a withdrawal water from the storage, leading to lowering water levels within some wells near the places of intensive abstraction (large farms) and thus deterioration of the quality of groundwater in this part occurred as a result of movement of the cone of saltwater interface from the eastern and western parts towards the middle part of the plain. In general stopping of seawater intrusion in coastal parts depends on the continuation of the annual recharge from rainfall and wadi flows, and as well as to reduce over-pumping of groundwater to those parts. In the context of finding solutions to stop this phenomenon and to reduce the aggravation. The Sultanate carried out the implementation of many procedures and steps, foremost of which is the continuation of monitoring and surveys of salinity and work to develop and intensify, using advanced technology available and the artificial recharge by treated waste water to the aquifer by 40 wells stand by to this mission located for 12km belongs the coast as a part of IWRM program which contains Sahlanot Dam with a total capacity of 6.4Mm³ which operated on 1993 and north of airport Dam with a total capacity of 77.2Mm³ which operated on 2009 , the benefit of these studies and research's to identify methods to address the problem of saltwater intrusion parts of the coast. In addition to the above, the government has taken significant action and essential to control the problem of salinity such as imposing a ban on drilling any new wells, except in special circumstances were also urged farmers to modernize irrigation methods and means to follow the drip and sprinkler irrigation.

المقدمة

تقع سلطنة عُمان في الجزء الجنوبي الشرقي من شبه الجزيرة العربية ضمن حزام الدول الجافة وشبه الجافة وبسبب موقعها الجغرافي تتميز بقلة معدلات الهطول المطري (100 ملم/سنة) وارتفاع معدلات التبخر (1200 – 2300 ملم/سنة) وبالتالي محدودية الموارد المائية . وفي مدينة صلالة وهي أحد المدن الكبيرة والهامة بالسلطنة التي تقع على الساحل الجنوبي للسلطنة مطلة على بحر العرب ، تعتبر المياه الجوفية المصدر الأساسي للمياه العذبة حيث تمثل المياه الجوفية بساحل سهل صلالة أكثر من 95% من الموارد المائية المتاحة . وتعتبر هيدروجيولوجية جبال ظفار وسهل صلالة مختلفة تماما عن باقي أرجاء السلطنة كما تظهر رطوبة الجو في شكل ضباب يتساقط الرذاذ المتجمع منه ويتجمع على شكل قطرات نتيجة لاعتراض النباتات والمعالم الأرضية له ومن هنا نشأت فكرة حصاد مياه الضباب ، ويعتبر سهل صلالة ذو الكثافة السكانية العالية بأنه المنطقة الزراعية الرئيسية في جنوب البلاد حيث نجد الخزانات الجوفية للمياه العذبة والتي تغذيها المياه المتدفقة من الجبال والتي يقدر حجم الواصل منها (40 م م^3/السنة) ، حيث تزيد معدلات السحب من المخزون الجوفي والتي تبلغ (61 م م^3/السنة) حوالي (21 م م^3/السنة) مقارنة بكميات المياه المستخدمة والتي تُقدر بحوالي (92 م م^3/السنة) . ويتم تعويض هذا العجز من خلال الضخ الجائر من المخزون الجوفي والتحلية.

وفي إطار جهود الوزارة لحماية الثروة المائية وتنمية مواردها سواء من حيث الكم أو النوع وبهدف دراسة هذه المشكلة ، تقوم دائرة مراقبة الموارد المائية من خلال قسم المراقبة والدراسات بدائرة موارد المياه بمحافظة ظفار بقياس ملوحة المياه الجوفية معبراً عنها بقياس التوصيل الكهربائي في حوالي (300 بئر) بصفة دورية للتعرف على التغيرات التي قد تحدث في ملوحة المياه الجوفية.

مشكلة الدراسة

إن مشكلة الملوحة الناتجة من تداخل مياه البحر بالمياه العذبة في الخزانات الجوفية الساحلية والتي تعاني منها الكثير من بلدان العالم، وهي من أعقد المشاكل التي تواجه العديد من دول العالم خاصة بالسهول المتاخمة للبحر ، حيث تتسلل مياه البحر المالحة إلى تخوم المياه الجوفية العذبة وتؤدي إلى تملحها. وفي سلطنة عُمان ، يمثل سهل صلالة شكلاً ثلاثياً يمتد من قاعدة جبل القرا نحو البحر ومن مدينة طاقة الساحلية في الشرق وحتى ميناء ريسوت في الغرب . ويمتد على طول الساحل لمسافة (40) كيلومتر ونحو الداخل لمسافة (12) كيلومتر في أعرض نقطة له ويغطي مساحة إجمالية تبلغ (240) كيلومتر مربع تقريباً.

وتقع مدينة صلالة في الجزء الأوسط من السهل وهي تمثل المركز السكني والتجاري والزراعي الرئيسي في محافظة ظفار ، بالإضافة إلى مدينة طاقة ، حيث تمتد المساحة الزراعية التقليدية على طول شريط يصل بين الساحل والمدينة (الشكل رقم 1) وتمتاز المنطقة بالمناخ الموسمي الذي تأتي أمطاره الرذاذية في فصل الخريف والذي يمتد من شهر يوليو إلى منتصف سبتمبر وكونها أمطار رذاذية فهي تعطي تغذية جوفية بنسب مرتفعة وتقلل من الجريان السطحي. كما تمتاز هذه الفترة من السنة بدرجات حرارة معتدلة تقلل من التبخر وبسرعة رياح منخفضة تقلل من عمليات الشد التبخري.

وقد أدت زيادة معدلات السحب من المخزون الجوفي خلال العقود الأربعة الماضية إلى انخفاض مستويات المياه الجوفية وبالتالي إلى ظاهرة تداخل المياه المالحة بالمياه العذبة في بعض الأجزاء من الشريط الساحلي . ويأخذ تداخل الملوحة شكلين رئيسيين أولهما التداخل بصورة موسعة عندما تتحرك المياه المالحة نحو الداخل (اليابسة) عبر مساحة واسعة وهذه عملية بطيئة تأخذ سنوات عديدة ، والشكل الثاني فيه تأخذ التغيرات في الملوحة شكلا أسرع وتؤثر على الآبار بصورة فردية ، وبدلاً من أن تتحرك المياه المالحة إلى الداخل يتم سحبها إلى أعلى بواسطة الآبار وتُعرف هذه العملية بارتفاع مخروط الملوحة، وفي سهل صلالة نجد أن كلا من التداخل الجانبي والمخروطي قد حدثا بالفعل ببعض الأجزاء ، بل وأصبح الوضع أكثر تعقيداً حيث توجد المياه الجوفية المسوس المالحة شرقي وغربي منطقة المياه العذبة لذلك فإن دخول هذه النوعية من المياه ذات النوعية الرديئة يمكن أن يحدث من ثلاث جهات مما يجعل الخزان الجوفي عرضة للتلوث.

ومن أهم الأسباب التي أدت إلى تفاقم مشكلة تداخل الملوحة بساحل سهل صلالة (1):

1. الزيادة الكبيرة والمطردة في حفر الآبار بهذه الأجزاء خاصة خلال فترة السبعينات والثمانينات ، وما رافقه من حفر عشوائي وضخ جائر واستنزاف للخزان الجوفي وتأثير هذه السلوكيات مجتمعة على بعضها البعض.

2. تذبذب معدلات التغذية الجوفية السنوية من مياه أمطار موسم الخريف والتي تعتبر المصدر الرئيسي لتغذية المخزون الجوفي بسهل صلالة.

3. الطبيعة الجيولوجية لسهل صلالة واختلاف نوعية المياه الجوفية وتأثرها بالصخور المغلفة للحوض المائي ذات الطبيعة المالحة كبعض أنواع الصخور الجيرية وغيرها.

4. الاعتماد على المياه الجوفية لمختلف الاستخدامات وزيادة الطلب عليها سنويا.

شكل رقم (1): منطقة الدراسة بسهل صلالة ومواقع آبار حقن مياه الصرف المعالجة

الوضع المائي بسهل صلالة

1. المخزون الجوفي:

تتفاوت ملوحة المياه الجوفية بسهل صلالة ما بين المياه العذبة (أقل من 2.000 ميكروسيمنز/سم) والتي تتواجد بجميع الأجزاء الجبلية وعلى هيئة عدسة مائية بكل من وادي جرزيز ووادي صحلنوت وتمثل الجزء الأوسط من سهل صلالة والمياه المالحة (2.000–6.000 ميكروسيمنز/سم) والمياه المسوس (أكثر من 10.000 ميكروسيمنز/سم) بالأجزاء الشرقية بوادي رزات ووادي حمران والأجزاء الغربية بوادي ثمرين ووادي صريت . ويعتمد المخزون الجوفي بسهل صلالة بشكل رئيسي على التغذية السنوية من مياه أمطار موسم الخريف والأمطار الموسمية على الأجزاء الجبلية والتي تتفاوت من عام إلى آخر ، وتشير الدراسات المائية المتوفرة إلى أن معدل سريان المياه الجوفية السنوية من الأجزاء الجبلية إلى السهل تبلغ حوالي (55) مليون متر مكعب يتركز معظمها حوالي (31) مليون متر مكعب بالجزء الأوسط من السهل .

2. استخدامات المياه:

تقدر الاحتياجات المائية للأغراض الزراعية والثروة الحيوانية بحوالي (71م م^3 / السنة) وهو ما يمثل حوالي (76%) من إجمالي المياه المتوفرة ويتم الحصول على مياه الري من الآبار الآلية والمفتوحة التي تنتشر عبر السهل بالإضافة إلى ثلاث عيون رئيسية تقع تحت سفح الجبل هي عين جرزيز ، عين صحلنوت ، وعين رزات ، كما يبلغ إجمالي استخدامات المياه للأغراض التجارية والصناعية والسياحية والاستخدام المنزلي حوالي (21 م م^3 / السنة) . هذا ويتم توفير معظم إمدادات مياه الاستخدام المنزلي بواسطة الجهات الحكومية من حقول آبار توجد شمالي مدينتي صلالة وتُقدر معدلات الضخ السنوية من هذه الحقول (12–14 م م^3 / السنة) .

3. الميزان المائي:

تشير آخر الدراسات المائية لمنطقة سهل صلالة (النمذجة الرياضية) والتي قامت بها الوزارة (قسم الدراسات والبحوث) في العام 2006 إلى وجود عجز في الميزان المائي حيث تزيد معدلات السحب من المخزون الجوفي بحوالي (21 م م^3 / السنة) مقارنة بكميات المياه المستخدمة والتي تُقدر بحوالي (92 م م^3 / السنة) . ويتم تعويض هذا العجز من خلال السحب من المخزون الجوفي وهو ما أدى إلى انخفاض مستويات المياه الجوفية ببعض الآبار القريبة من أماكن السحب (المزارع الكبيرة) وبالتالي تدهور نوعية وجودة المياه الجوفية بهذه الأجزاء نتيجة تحرك مخروط المياه المالحة من الأجزاء الشرقية والغربية باتجاه الجزء الأوسط من السهل.

أهداف الدراسة

تهدف هذه الدراسة إلى مراقبة التغيرات في ملوحة المياه الجوفية في المنطقة الواقعة بسهل صلالة وأثر عمليات الحقن الجوفية المزدوجة من ساتر آبار الحقن الجوفي وسدود التغذية الجوفية (الشكل رقم 1) خلال الفترة بين عامي 1995م – 2010م استنادا إلى البيانات التي تم قياسها وتجميعها خلال تلك الفترة . حيث تم استخدام نظم المعلومات الجغرافية وإعداد الخرائط الكنتورية لملوحة المياه الجوفية لكل عام على حدة (أعوام 1995م ، 2000م ، 2005م و 2007م و 2010م) ومن ثم حساب نطاقات الملوحة ومقارنتها بعضها البعض لتحديد مدى التغير في ملوحة المياه خلال تلك الفترة . وقد غطت الدراسة الأجزاء الواقعة بين وادي حمران في الشرق من مدينة صلالة ووادي صريت بالأجزاء الغربية منها وبمساحة بلغت (692 كم^2).

كما تم الكشف عن أثر الحقن بمياه الصرف الصحي المعالجة معالجة ثلاثية والذي بدأ في العام 2003 والخارجة من محطة صلالة لمعالجة مياه الصرف الصحي على تراجع ظاهرة تداخل مياه البحر مع المياه الجوفية من خلال تكوين ساتر وعازل جوفي من المياه المختلفة كثافتها عن كثافة مياه البحر.

منطقة الدراسة

تغطي منطقة الدراسة الأجزاء الواقعة ما بين وادي حمران في الشرق ووادي صريت في الغرب من سهل صلالة والأجزاء الجبلية بطول بلغ حوالي (25) كيلومتر وبعرض يصل في المتوسط إلى (12) كيلومتر وبمساحة إجمالية تبلغ حوالي (692 كم^2) . وبلغ عدد المستجمعات المائية الواقعة بها الآبار (6) مستجمعات من الغرب إلى الشرق هي وادي صريت ، وادي ثمرين ، وادي جرزيز ، وادي صحلنوت ، وادي رزات ووادي حمران وبلغت مساحة الأجزاء التي غطتها الدراسة حوالي 33 % من إجمالي مساحة هذه المستجمعات المائية ، ويوضح الجدول رقم (1) مساحة الأجزاء التي شملتها الدراسة بكل مستجمع مائي على حدة .

جدول رقم (1) : المستجمعات المائية التي شملها المسح الميداني لعام 2010

المستجمع	المساحة المشمولة بالدراسة	المساحة الإجمالية	عدد الآبار
صريت	53 كم^2	384 كم^2	10
ثمرين	83 كم^2	515 كم^2	10
جرزيز	151 كم^2	185 كم^2	104
صحلنوت	206 كم^2	455 كم^2	157
رزات	143 كم^2	467 كم^2	15
حمران	56 كم^2	367 كم^2	6
الإجمالي	692 كم^2	2.373 كم^2	302

الأبحاث والدراسات والمسوحات السابقة للموقع

الدراسات المتعلقة بالتغذية الصناعية للخزان الجوفي من مياه الصرف الصحي المعالجة معالجة ثلاثية

بدأت الدراسات والأبحاث المتعلقة باستخدام مياه الصرف الصحي المعالجة منذ عام 1991م حيث تم إعداد دراسة عن خطة رئيسية للمياه ومياه الصرف الصحي بصلالة وقد خلُصت الدراسة إلى تقدير حجم الاستخدامات المائية ومعدلات التغذية السنوية من مياه الأمطار لمنطقة صلالة وتقدير حجم العجز المائي وحجم مياه الصرف الصحي حتى عام 2020م وأوصت بالعديد من التوصيات المتعلقة بإدارة الموارد المائية بصلالة منها ضرورة استخدام مياه الصرف الصحي المعالجة لتقليل العجز في الميزان المائي (2) . ومما لاشك فيه أن مفهوم استغلال مياه الصرف الصحي المعالجة كمصدر غير تقليدي قد أصبح شائعاً ومستخدماً في العديد من الدول خاصة تلك التي تعاني من نقص حاد في مواردها المائية الطبيعية.

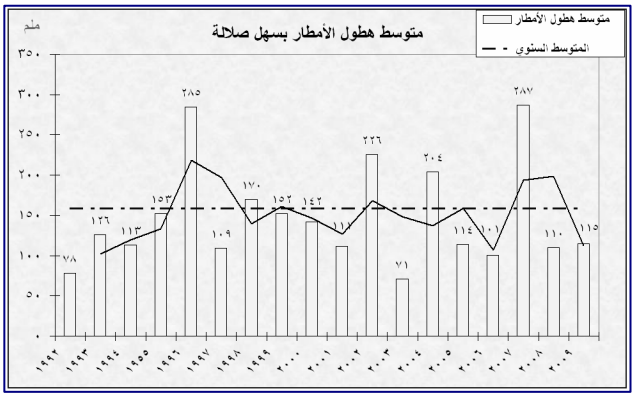
وفي سلطنة عُمان وكأحد الدول الرائدة في هذا المجال وتحت مظلة وفي إطار حماية البيئة ومكافحة التلوث ومن أجل تحقيق أفضل لحماية الأرض ومصادر المياه (مفهوم التنمية المستدامة) تم خلال عام 2003م استغلال مياه الصرف الصحي المعالجة ثلاثيا لحقن الخزان الجوفي الساحلي بسهل صلالة لإيقاف زحف مياه البحر باتجاه اليابسة. وخلال الفترة الماضية تم تنفيذ عدد من الدراسات لتقييم هذه التجربة (3)، ومن خلال تطبيق نموذج رياضي (MODFLOW and MT3DMS) لحساب حركة الحد الفاصل ما بين المياه العذبة / المياه المالحة حيث توصل إلى أن إسفين المياه المالحة سيتراجع بحلول عام 2019م بما يعادل 700 متر مع الاستمرار في ضخ معدلات الحقن الحالية (5.5 مليون م^3/سنة). كذلك تم تنفيذ دراسة بالتعاون مع وزارة البلديات الإقليمية وموارد المياه حيث تم خلال الفترة ما بين يناير 2009م ومايو 2010م أخذ عينات مياه من عدد (22) بئر مراقبة موزعة على طول امتداد خط آبار الحقن بحيث كان 11 بئرا منها واقعة شمال خط آبار الحقن و11 بئرا أخرى جنوب الخط وباتجاه الساحل ، وتم تحليل جميع العينات للأغراض الفيزيائية والكيميائية والبيولوجية والعناصر الثقيلة. وقد خلُصت نتائج الدراسة إلى أن المياه الجوفية على امتداد ساحل صلالة ليست صالحة لأغراض الشرب بدون معالجة ، إلا أنها في الوقت نفسه صالحة للاستخدامات الزراعية والحيوانية ويرجع ذلك إلى ارتفاع ملوحة المياه الجوفية مقارنة بالمواصفات القياسية العُمانية لمياه الشرب ، كما أشارت الدراسة إلى انخفاض الموصلية الكهربية للمياه الجوفية ببعض الآبار على امتداد خط آبار الحقن ، وأن معدل العناصر الثقيلة في مياه العينات يقع ضمن المعايير العُمانية للمياه غير المعبأة (4).

المسوحات السابقة لملوحة المياه بسهل صلالة

قامت الوزارة بإجراء عدة مسوحات حقلية لمتابعة ومراقبة التغير في ملوحة المياه داخل الخزان الجوفي من خلال قياس عينات المياه التي يتم تجميعها من الآبار الساحلية والتي لا يزال معظمها عاملا للآن وأضيف إليها مجموعة آبار أخرى وجميعها أصبحت ممثلة لدراستنا الحالية ، ومن ثم إعداد الخرائط الكنتورية كنتائج لهذه العينات من مئات الآبار . وقد تم تنفيذ أول مسح ميداني في منطقة سهل صلالة في عام 1974م حيث تركزت البيانات في المناطق الزراعية . وخلال الفترة من بداية وحتى منتصف الثمانينات حيث تم إعداد برنامج مراقبة رئيسي لكل منطقة سهل صلالة ، ومنذ ذلك الوقت تم التوسع في شبكة المراقبة حتى بلغ ما تقوم به الوزارة في الوقت الراهن ما يزيد على (850) قياسا منتظما في سهل صلالة منها أكثر من (400) قياسا تختص بمراقبة جودة المياه . وفي عام 1992م تم عمل مسح ميداني شامل لقياس الملوحة بساحل سهل صلالة وأشارت نتائج المسح إلى أن كلا النوعين وهما التداخل الجانبي والمخروطي قد حدثا بالفعل في سهل صلالة ، وهو ما أدى إلى تقلص حجم المياه الصالحة للزراعة بالسهل خاصة في الناحية الشرقية (منطقة الدهاريز) والغربية (منطقة عوقد) ، بالإضافة إلى ظهور جيوب مياه ذات جودة أقل في أجزاء عديدة بسبب السحب المفرط من الخزان الجوفي ببعض الآبار.

النظام الهيدرولوجي لسهل صلالة

تعتبر الأمطار المدارية الناتجة من الرياح الموسمية الصيفية التي تسبق موسم الخريف وهي العامل المؤثر الرئيسي في هطول الأمطار بالإضافة إلى أمطار الخريف خلال الفترة بين أواخر شهر يونيو إلى منتصف سبتمبر من كل عام المصدر الرئيسي لتغذية الخزانات الجوفية في كل من الجبل والسهل على حد سواء ، وتعتبر الأمطار المتساقطة على الأجزاء الجبلية ذات تأثير كبير ومباشر على تغذية الخزانات الجوفية ، في حين أن الأمطار التي تهطل على سهل صلالة تأثيرها أقل ، ويبلغ متوسط الهطول المطري خلال موسم الخريف (الشكل رقم 2 حوالي (160 ملم) وهي من أعلى معدلات الأمطار بالسلطنة والتي تبلغ بمعدلها الإجمالي لكافة مناطق السلطنة بحدود 100 ملم/سنة.

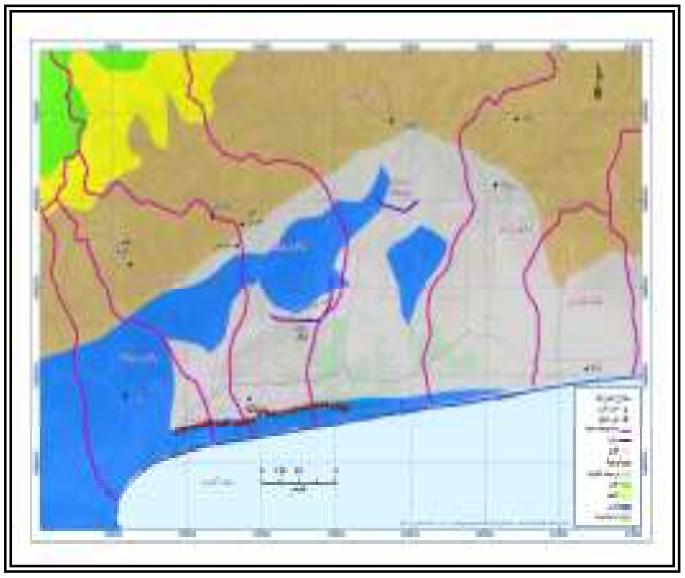


شكل رقم (2): معدلات هطول الأمطار بسهل صلالة مقارنة بالمعدل السنوي

يمثل كل من جبل القرا وسهل صلالة امتداد لنظام جوفي واحد حيث تعتبر المياه الجوفية المصدر الوحيد لإمدادات المياه للمنطقة في الماضي والحاضر ، وفي فصل الخريف بين يونيو وسبتمبر نجد أن تساقط الأمطار والرطوبة يؤدي إلى تسربها من خلال فوالق وصدوع الجبال الجيرية ثم تتدفق إلى أسفل سهل صلالة لتكون المخزون الجوفي .

تتواجد المياه الجوفية في سهل صلالة في كل من طبقة ترسبات الأودية الحصوية (اللون الرمادي الفاتح الموضح في الشكل رقم 3) والأجزاء العليا من طبقة "عدونب" وهي عبارة عن حجر جيري وترسبات حصوية حيث يتراوح سمكها ما بين (60 – 70) متر في الأجزاء الوسطى من السهل وتقل في السمك كلما اتجهنا إلى الشرق والغرب وتتميز بالنفاذية العالية والتي تتراوح ما بين (1000 – 40000 م^2/يوم) . أما في الأجزاء الجبلية ، فتتواجد المياه الجوفية في طبقة الحجر الجيري (مجموعة حضرموت) .

الجدير بالذكر أن الجزء الأوسط من سهل صلالة والممتد من الجبل وحتى الساحل يتميز بوجود طبقة مائية ذات جودة عالية "مياه عذبة" (Central Fresh Water Zone) حيث تبلغ ملوحتها أقل من 1500 ميكروسيمنز/سم ويحد هذه الطبقة من أسفلها ومن الجهة الغربية والشرقية طبقة مائية تتراوح ملوحتها ما بين 10.000 – 15.000 ميكروسيمنز/سم . ويرجع تفاوت ملوحة المياه الجوفية بهذه الطبقات إلى عدة عوامل منها تفاوت اتساع عرض السهل (من الجبل إلى البحر) ، وتفاوت حركة المياه الجوفية (التغذية) حيث أنها ليست موزعة بصورة متساوية تحت السهل ، والتراكيب الجيولوجية (منخفض صدعي – صلالة) والتغير في السحنة الجيولوجية (نوعية الصخور) واختلاف الخواص الهيدروليكية والهيدرجيولوجية لهذه الصخور (درجة النفاذية) حيث تجعل هذه العوامل جميعاً المياه المتدفقة من الجبل تتركز بالأجزاء الوسطى من السهل . بالمقابل وبسبب قلة التغذية الجوفية في الأجزاء الشرقية والغربية من السهل تتواجد طبقات المياه الضاربة في الملوحة.



شكل رقم (3): خارطة جيولوجية لسهل صلالة

منهجية الدراسة والمراحل الزمنية لتنفيذها

غطت هذه الدراسة الفترة الزمنية ما بين أعوام 1995م – 2010م حيث شارك في جمع وتحليل العينات عدد كبير من الفنيين العاملين بإدارة موارد المياه بصلالة بقسم المراقبة والدراسات ويصل عددهم إلى (17) ويعتبر هؤلاء الجنود المجهولين اللذين ندين لهم اليوم جميعا بهذا الجهد البحثي الكبير فنشكر جهودهم ونثني عليها خير الثناء لما قدموه لوطنهم . اعتمدت الدراسة على قياس الموصلية الكهربية ومستوى المياه الجوفية ل (302) بئر موزعة على مساحة (692 كم^2) ، وتم خلال هذه الدراسة زيادة مساحة المنطقة التي يغطيها المسح الميداني لتشمل بعض الأجزاء الغربية والشرقية وبعض الأجزاء الجبلية التي تمثل مناطق التغذية الرئيسية للخزانات الجوفية حيث تم إجراء الآلاف من القياسات الميدانية

ولكن دراستنا ركزت على (302 بئر) قياس ميداني مسجل ومؤرخ في كل عام من أعوام الدراسة الخمسة عشر ولكن البيانات غير متتابعة بشكل دائم وسنوي لذلك فقد تم أخذ (250) عينة من هذه الآبار أيضا في كل عام من أعوام الدراسة ولقد اعتمد الفريق البحثي هذه البيانات كونها موثقة ومحفوظة ولها تسلسل وسجل محفوظ وحافظت على صفة الاستمرارية بدون انقطاع وبناء على هذه المعطيات نعتبر أن دراستنا قد شملت 250 بئر فقط من أصل 302 بئر شملتها الدراسة ولكن لم تتمكن من تغطيتها بالكامل ، وقد أجريت لهذه العينات كافة التحاليل الفيزيائية والكيميائية في المختبر التابع للوزارة. وتم تحليل البيانات الحقلية من خلال استخدام برامج نظم المعلومات الجغرافية والخرائط الكنتورية لتحديد مساحة النطاقات المختلفة لملوحة المياه الجوفية بكل مستجمع مائي على حدة ، ونظرا لكبر حجم ملفات البيانات والتي يتعذر إرفاقها حيث تم الحصول عليها على مدار خمسة عشر عاما فهي جميعها موجودة ومحفوظة في مكتبة الوزارة وفي الأرشيف الإلكتروني للمديرية العامة لتقييم الموارد المائية ونسخة أخرى لدى مركز مختبرات الأغذية والمياه.

النتائج والمناقشة

1. بلغ عدد الآبار التي شملتها الدراسة وكانت ملوحة المياه بها أقل من (2.000 ميكروسيمنز/سم) حوالي (79 بئر) وهو ما يعادل (26%) من إجمالي عدد الآبار ، وغطت ما يمثل حوالي (62%) من إجمالي المساحة التي شملها التقرير وتركزت جميعها بالأجزاء العليا من جميع المستجمعات المائية ما عدا وادي صريت.

2. بلغ عدد الآبار التي كانت ملوحة المياه بها تتراوح ما بين (2.000–6.000 ميكروسيمنز/سم) حوالي (80 بئر) وهو ما يعادل (32% تقريبا) من إجمالي عد الآبار وتركزت بالجزء الأوسط من سهل صلالة بأودية صحلنوت وجرزيز وثمرين وتمثل حوالي(16%) من إجمالي المساحة التي شملها التقرير.

3. بلغ عدد الآبار التي كانت ملوحة المياه بها أعلى من (6.000 ميكروسيمنز/سم) حوالي (30 بئر) وتركزت بالأجزاء الشرقية من سهل صلالة بأودية حمران ورزات ، والأجزاء الغربية بأودية ثمرين وصريت حيث بلغ إجمالي مساحة الأجزاء التي تغطيها حوالي (22%) من إجمالي المساحة الكلية.

4. بلغت أقل ملوحة تم قياسها (638 ميكروسيمنز/سم) بالبئر الواقعة بوادي صحلنوت بالأجزاء الوسطى من سهل صلالة في حين بلغت أعلى ملوحة تم قياسها (63600 ميكروسيمنز/سم) بالبئر الواقعة في وادي حمران بالأجزاء الغربية من سهل صلالة .

5. تشير بيانات الملوحة إلى أن كل من الأجزاء الشرقية والغربية من سهل صلالة تتواجد بها مياه مسوس ويرجع ذلك إلى تواجدها في صخور الحجر الجيري ذات الطبيعة الملحية (أملاح كلوريد الكالسيوم) بالإضافة إلى قلة وضعف معدلات التغذية الجوفية السنوية من أمطار الخريف خاصة بأودية صريت وحمران ورزات (الجزء الساحلي) .

6. في الجزء الأوسط من سهل صلالة بوادي جرزيز والذي تتواجد به مياه جوفية ذات نوعية جيدة أقل من 2.000 ميكروسيمنز/سم وتمثل حوالي (82%) من إجمالي مساحة المياه حافظت ملوحة المياه الجوفية بالآبار على مستوياتها خلال الفترة ما بين عامي (1995– 2010م) وهو ما يعكس التوازن الحالي بين كميات السحب من المخزون الجوفي ومعدلات التغذية خاصة بتلك الأجزاء من السهل .

7. أما في وادي صحلنوت فتتواجد المياه المالحة بالأجزاء الساحلية وقد زادت مساحة المياه ذات الملوحة (2.000–6.000 ميكروسيمنز/سم) خلال الأعوام العشرة الماضية من (9%) في عام 2000م لتبلغ (12%) في عام 2010م وهو ما يعكس تدهور طفيف لملوحة المياه الجوفية ببعض الآبار خاصة القريبة من أماكن السحب من المخزون الجوفي لتغطية الاحتياجات الزراعية علما أن هناك أثرا إيجابيا كبيرا حد من تقدم مياه البحر وقلل من تفاقم المشكلة.

8. في الأجزاء الشرقية من سهل صلالة بالآبار الواقعة بكل من وادي رزات وحمران زادت ملوحة المياه الجوفية (أكبر من 10.000 ميكروسيمنز/سم) حيث بلغ مقدار الزيادة حوالي (11%) بوادي حمران و (8%) بوادي رزات خلال الفترة بين عامي 1995م–2010م مع الأخذ في الاعتبار أن ملوحة المياه الجوفية بالأجزاء الساحلية من هذه المستجمعات تتميز بالملوحة العالية بطبيعتها الهيدروجيولوجية.

9. في الأجزاء الغربية من سهل صلالة بكل من وادي ثمرين ووادي صريت تتواجد مياه جوفية عالية الملوحة (أكثر من 10.000 ميكروسيمنز/سم) وتمثل حوالي 55% من إجمالي مساحة الواديين وهي مياه بطبيعتها مالحة نظرا للظروف الجيولوجية لتكونها وتواجدها . وخلال عام 2010م يلاحظ تقلص مساحة المياه ذات الملوحة (2.000–6.000 ميكروسيمنز/سم) بوادي صريت من 30% في عام 2007م لتبلغ الصفر (0%) في عام 2010م ويرجع ذلك إلى تحرك المياه عالية الملوحة من الطبقات الجيولوجية السفلي (ذات مياه أكثر ملوحة) إلى أعلى.

10. يجب الأخذ في الاعتبار أن زيادة ملوحة المياه الجوفية ببعض الآبار بسهل صلالة لا تعود فقط إلى تداخل المياه المالحة بل كذلك إلى ضخ المياه المسوس من الطبقات الجيولوجية العميقة ، ومما لاشك فيه أن زيادة معدلات السحب من المخزون الجوفي (المياه العذبة) سيؤدي إلى تقليص حجم المياه العذبة .

11. بصفة عامة يمكن القول أن هناك تدهور طفيف في ملوحة المياه الجوفية ببعض الآبار الواقعة بالأجزاء الشرقية والغربية من سهل صلالة حيث زادت مساحة الأراضي التي تغطيها ملوحة (أكبر من 6.000 ميكروسيمنز/سم) من (110كم^2) في عام 1995م لتصل إلى (156 كم^2) في عام 2010م ، كذلك تقلصت مساحة الأراضي التي تغطيها ملوحة المياه (أقل من 6.000 ميكروسيمنز/سم) من (574 كم^2) في عام 1995م لتبلغ (537 كم^2)في عام 2010م.

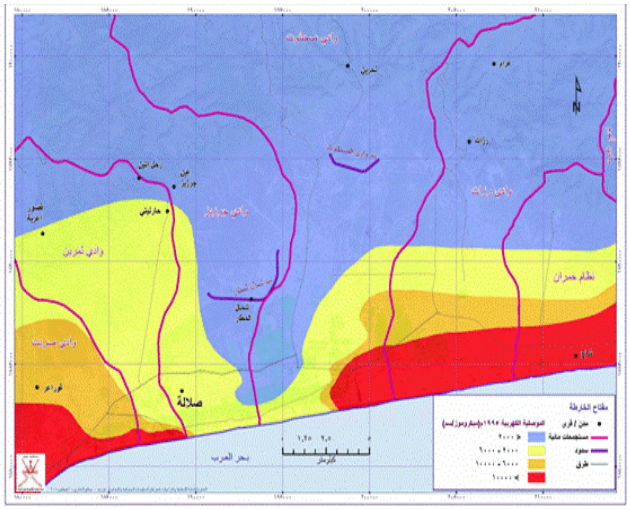
12. بالمقابل هناك تحسن طفيف في ملوحة المياه الجوفية بسهل صلالة خلال الفترة بين عامي 2000م – 2005م ويرجع ذلك إلى اعتمادها على مقدار التغذية الجوفية السنوية من مياه الأمطار وكذلك أثر سد صحلنوت وحزام آبار التغذية الجوفية بمياه الصرف الصحي المعالجة معالجة ثلاثية والتي سنأتي على تحليل وذكر نتائجهما فيما بعد خلال تلك الفترة والتي كانت أعلى من المتوسط السنوي خلال عامي 2002 و 2004م (الشكل رقم 4).

شكل رقم (4): التغيرات في ملوحة المياه الجوفية بين عامي 1995–2010 مع الإشارة إلى عامي 2002 و 2004.

النتائج التفصيلية وتحليل خرائط ملوحة المياه الجوفية

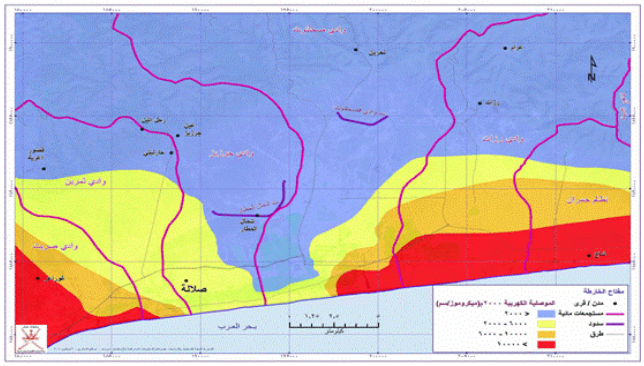
فيما يلي تحليل علمي شامل لخرائط الملوحة والأحزمة اللونية الدالة على نطاقات الملوحة للحوض المائي الجوفي وتأثرها بعمليات الحقن الصناعي في آبار التغذية الصناعية بواسطة مياه الصرف الصحي المعالجة معالجة ثلاثية والخارجة من محطة معالجة مياه الصرف الصحي بصلالة هذا بالإضافة للتغذية الجوفية التي تغذي الحوض الجوفي من خلال سد صحلنوت وسد شمال المطار حديثا وأثرها على مناسيب سطح الماء الساكن وعلى جودة مياه الحوض المائي الجوفي.

1. يلاحظ من خريطة ملوحة المياه الجوفية للعام 1995 تقدم المياه المسوس بمستوياتها المختلفة وخاصة المستوى الأعلى والمتمثل باللون الأحمر والذي تصل ملوحته إلى 10000 ميكروسيمنز/سم ، وهناك أيضا تقدم آخر في منطقة اللون الكموني من الجانب الشرقي ، وبوجه العموم فهناك تقدما شاملا للمياه المسوس باتجاه العمق البري لمنطقة صلالة (شكل رقم 5).



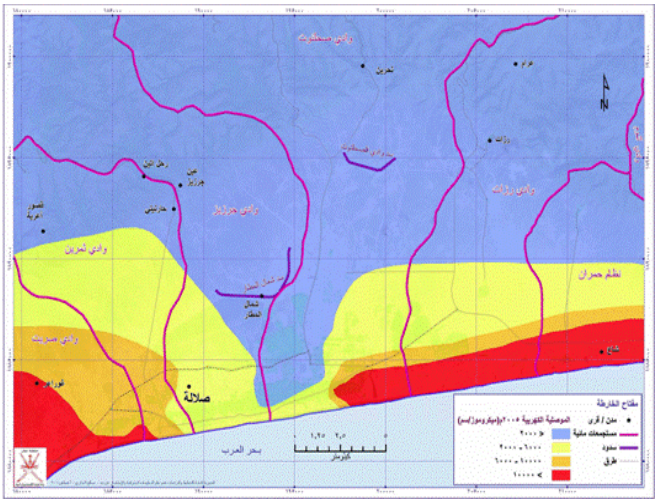
شكل رقم (5): خريطة ملوحة المياه الجوفية للعام 1995

2. لوحظ انحسار وتراجع للخلف لكل من نطاقي منطقتي اللون الأحمر في خارطة عام 2000 عنها في خارطة عام 1995 حيث نلاحظ ابتعاد فكي الكماشة التي يشكلها هذا النطاق عن بعضه وإذا ما استخدمت المسطرة تلاحظ أن هذه المسافة قد حافظت على نفسها في العام 2005 وازدادت قليلا في العام 2010 وهذا دليل قطعي على الأثر الإيجابي على إجراءات الإدارة المتكاملة للحوض الجوفي والمتمثلة في عمليات الحقن الجوفي الصناعي من خلال السدود المغذية أو ساتر حزام الآبار الجوفية الواقي (شكل رقم 6).



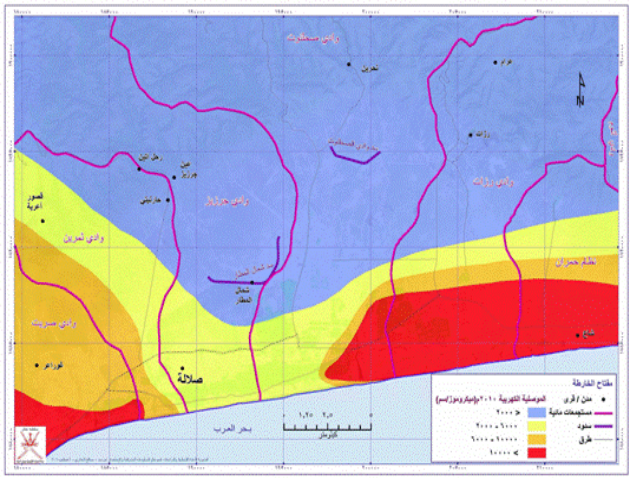
شكل رقم (6): خريطة ملوحة المياه الجوفية للعام 2000

3. في خريطة عام 2005 يظهر التراجع للمياه المسوس وإعادة تشكيل للنطاقات اللونية المالحة الألوان الأصفر والكموني والأحمر حيث يظهر التراجع بشكل واضح وللمنطقة الشرقية بالذات عن ما كان عليه في العام 2000 ، ولا تُظهر المنطقة الغربية أي تغير يذكر ولكن يظهر في خريطة عام 2010 تراجع كبير في نطاقات المياه المالحة في المنطقة الغربية وتقدم كبير في المنطقة الشرقية (شكل رقم 7).



شكل رقم (7): خريطة ملوحة المياه الجوفية للعام 2005

4. نلاحظ انحسارا واضحا في المنطقة الحمراء المواجهة لسد صحلنوت وخاصة في العام 2000 وكذلك لخرائط 2005 و 2010 (شكل رقم 8)، ونلاحظ أن هناك مناطق من المنطقة الحمراء قد انضمت للمنطقة الكمونية ويعتبر هذا دلالة إيجابية على تحسن الملوحة للنطاق الأحمر ورغم حصول امتداد لنفس النطاق الأحمر مرة أخرى في العام 2010 إلا أن الانحسار لنطاق اللون الأحمر للمنطقة المواجهة لسد شمال المطار قد أصبح واضحا بعد سنة واحدة من تشييده رغم أن نسبة التخزين كانت ضعيفة جدا ولم تتجاوز 10% من سعته الإجمالية.



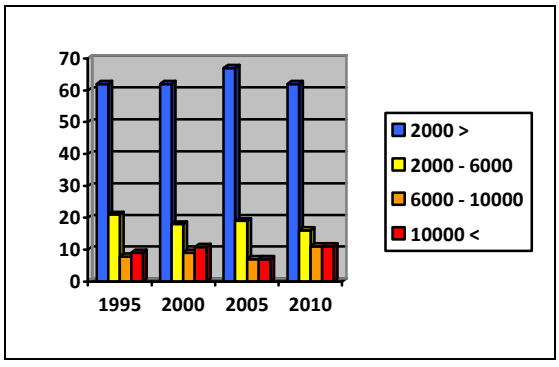
شكل رقم (8): خريطة ملوحة المياه الجوفية للعام 2010

5. بملاحظة القرن الأزرق والذي يتخذ شكلا قرنيا في خارطة عام 1995 ثم يبدأ بالانتشار عرضيا في خرائط الأعوام 2000 و 2005 ليمتد بعد ذلك بشكل عرضي في خريطة عام 2010 وينتشر بشكل أكبر حتى يزول الشكل القرني. لاحظ أيضا كيف انحسرت المنطقة الصفراء في العام 2010 عنها في العام 2005 وهذا دليل على امتداد أكثر لنطاق المنطقة الزرقاء وهو دليل قطعي على الأثر الإيجابي للتغذية الجوفية.

وبناء على ذلك يمكن استنتاج ما يلي :

1. على الرغم من عمليات الضخ الجائر من هذا الحوض وتزايدها إلا أن هناك أثرا إيجابيا للسدود الجامعة لمياه الأمطار ولحزام آبار التغذية الجوفية بمياه الصرف الصحي المعالجة معالجة ثلاثية على ردع مياه البحر المالحة من التسرب للحوض الجوفي رغم أن تأثير سد شمال المطار لا يزال محدودا.
2. أن هذه الآثار الإيجابية قد أدت إلى تحسين في نوعية مياه الخزان الجوفي أو أنها قد عملت على تأخير امتداد نطاق المياه المسوس من جانب وعلى التقليل من آثار الملوحة التي كان يتوقع لها أن تتركز بشكل أكبر بكثير من جانب آخر.
3. ضرورة اكتمال إجراءات الإدارة المتكاملة لمصادر المياه ببرنامج وطني وشامل للترشيد في استهلاك المياه وتنفيذ ذلك من خلال عمليات مراقبة الآبار وكميات المياه التي تضخ منها وبوضع تعرفة تتناسب مع كميات المياه المضخوخة منها.

والشكل رقم (9) التالي يبين الجهود التي بذلت في الحفاظ على النطاق الأزرق للملوحة تقريبا على مدار خمسة عشر عاما وينتظر أن يكون الوضع في تحسن أفضل حين يبدأ أثر سد شمال المطار يظهر بصورة جلية في السنوات القادمة كما يتضح لنا انخفاضا بنسبة 5% في نسبة نطاق اللون الأصفر وهذا يعني تقدما جيدا ومبشرا لكن كل من نطاقي اللونين الكموني والأحمر قد سجلا ارتفاعا بنسبة 3.1% و 1.9% على التوالي وهذا يعود بسبب معدلات الضخ المرتفعة في أشهر الصيف ولتذبذب أمطار فصل الخريف والتي تؤدي لتراجع نطاقي اللونين الأزرق والأصفر تراجعا كبيرا يعوض ضمن هاذين النطاقين فيما بعد ويصعب تعويضهما ضمن نطاقي اللونين الكموني والأحمر نظرا للضغط الأرتيزي الباطني الإزاحي الأفقي القوي لمياه البحر آملين أن يوفر سد شمال المطار بسعته الاستيعابية الكبيرة ضغطا مقاربا ومعاكسا يؤثر في هذه النطاقات اللونية المالحة ويسبب تراجعا أفقيا باتجاه البحر . ونلاحظ أيضا الأثر القوي للحزام الساتر لآبار التغذية الجوفية والذي أدى لتراجع أو انكماش فكي الكماشة المتكون من نطاق اللون الأحمر إلى الخلف ، وهذا يسجل تقدما جيدا لعملية الحقن.



شكل رقم (9): النسبة المئوية لنطاقات الملوحة في خرائط ملوحة المياه الجوفية للأعوام 1995 ، 2000 ، 2005 ، 2010

التوصيات

1. إعادة التوازن بين كميات المياه المستخدمة ومعدلات التغذية الجوفية السنوية عن طريق تقليل كميات المياه التي يتم استخراجها من المخزون الجوفي للأغراض الزراعية خاصة زراعات الأعلاف ونقلها إلى منطقة النجد ،علماً بأن زراعات الأعلاف تمثل حوالي 50% من مساحة المزروعات بسهل صلالة.
2. إيقاف والحد من زحف مياه البحر باتجاه المياه العذبة من خلال إعادة حقن وتغذية الخزان الجوفي بالأجزاء الساحلية بحوالي (15.000 م^3/يوم) أي ما يعادل (5.5) مليون متر مكعب في العام من مياه الصرف المعالجة معالجة ثلاثية والخارجة من محطة تنقية صلالة والتي يتم حقنها من خلال عدد (40) بئر موزعة على الأجزاء الساحلية. وقد ساهمت هذه الكمية في إيقاف زحف المياه المالحة باتجاه المياه العذبة وستساهم في تراجع الخط الفاصل بينهما 700 متر باتجاه البحر بحلول عام 2019م .
3. إنشاء محطة تحلية جديدة بطاقة إنتاجية تبلغ (20) مليون متر مكعب لتوفير مياه الشرب والاستخدامات العامة مما يساهم في تقليل كميات الضخ من حقول آبار إمدادات المياه العامة بسهل صلالة والتي تُقدر طاقتها الإنتاجية بحوالي (14) مليون متر مكعب في العام .
4. ترشيد استخدام الموارد المائية والعمل على حماية الخزانات الجوفية خاصة بالمناطق ذات الكثافة الزراعية العالية ، وتشجيع استخدام نظم الري الحديثة وزراعة المحاصيل المجدية اقتصادياً ومائياً لتقليل فاقد المياه بالري السطحي.
5. الاستمرار في مراقبة التغييرات في ملوحة المياه الجوفية لتقييم تأثير هذه الإجراءات على تحسن الوضع المائي بسهل صلالة .
6. الاستمرار في الدراسات الأخرى لموارد المياه الغير تقليدية والتي تهدف إلى استغلال مياه الضباب خلال موسم أمطار الخريف واللجوء لتقانات استعمال المياه الرمادية وطرق الحصاد المائي المتنوعة .
7. الحد من تدهور نوعية المياه وترشيدها خاصة بالمناطق الساحلية يتطلب تضافر جهود قطاعات عديدة مثل القطاع الخاص والمنظمات الغير حكومية كالجمعيات الأهلية المحلية وفي مقدمتها أصحاب المزارع للوعي بأهمية ترشيد استهلاك المياه واستخدامها الاستخدام الأمثل وضرورة الحد من استنزاف الخزان الجوفي بتلك المناطق .
8. عمل دراسة وتصميم نموذج رياضي ثلاثي الأبعاد لحساب معدلات كميات التغذية الجوفية للحوض المائي الجوفي من سدي صحلنوت وشمال المطار.

المصـــــادر

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8. وزارة التجارة والصناعة (2006). المواصفات القياسية العُمانية لمياه الشرب غير المعبأة (م ق عم رقم 2006/8) المديرية العامة للمواصفات والمقاييس ، سلطنة عُمان.

الكشف عن تراكم بعض العناصر الثقيلة في عينات التربة ومتشابهة الأقدام الأرضية Isopoda: *Armadillidium vulgare*، *porcellionides pruinosus*، *Parcellio scaber* في منطقة الجادرية- بغداد

إسراء محسن جاسم، ناديا عماد الأمين

قسم علوم الحياة / كلية العلوم للبنات / جامعة بغداد / جمهورية العراق

الملخص باللغة العربية

تناولت هذه الدراسة مشكلة تلوث تربة منطقة الجادرية ذات الزخم المروري بالعناصر الثقيلة السامة باستخدام3 انواع من متشابهة الاقدام Isopoda (*Porcellio scaber, Porcellionides pruinosus, Armadillidium vulgare*) كمؤشرات حيوية للتلوث والشائعة الانتشار في التربة اضافة الى عينات من تربة المنطقة وقد جمعت العينات في ربيع العام 2012.

ومن خلال قياس تراكيز خمس عناصر ثقيلة شائعة وهي الحديد Fe ,الزنك Zn , الرصاص Pb , النيكل Ni والزئبق Hg , اظهرت النتائج ان عنصرالحديد كان الاول بتراكيزه المرتفعة البالغة (190 ، 687، 400، 3400), تلاه عنصر الزنك بتراكيز(104 ،238، 152، 97), وسجل كل من عنصري الرصاص والنيكل تراكيز(27 ،44، 30، 50) و(20 ،40، 22، 40) على التوالي في حين سجل عنصر الزئبق اقل التراكيز والبالغة (0.16، 0.38، 0.15، 0.19) لكل من *Armadilllidium Porcellionides pruinosus vulgare,Porcellio scaber* , التربة . استخدمت وحدات جزء بالمليون لقياس تراكيز العناصر الثقيلة.

كما اظهرت النتائج ان افراد النوع *Porcellionides pruinosus* كانت هي الاعلى في تراكم العناصر الثقيلة المدروسة .

الكلمات المفتاحية: العناصر الثقيلة ، متشابهة الاقدام الارضية ،التربة

ABSTRACT

This study described the problem of soil pollution with heavy metals in Al-Jadriya district in Baghdad; which is very crowded in its traffic jam. Three types of Isopoda (*Porcellio scaber, Porcellionides pruinosus, Armadillidium vulgare*) were used as bio indicators of pollution in soil, in addition to samples from the study area soil, which were collected during the spring of 2012.

After the measurement of five concentrations of well-known heavy metals : Fe, Zn, Pb, Ni, and Hg, the results showed that Fe was the first in its high concentrations (190,687,400,3400) , followed by Zn with the concentration of (104 ,238 ,152 ,97) and for both of Pb and Ni registered the concentrations of (27 ,44 ,30 ,50),(20 ,40 ,22 ,40) respectively. Hg registered the lowest concentrations (0.16, 0.38, 0.15 ,0.19) for each of .Units of parts per million were used to measure the concentrations of heavy metals.

In addition, the results showed that the species *Porcellionides pruinosus* were the highest in the accumulation of studied heavy metals.

المقدمة

دفع التلوث البيئي بالعناصر الثقيلة في السنوات الأخيرة العديد من علماء البيئة والتلوث إلى البحث عن أساليب جديدة لتقييم الأضرار الناجمة عن العناصر الثقيلة في البيئة وإجراء التجارب والاختبارات التطبيقية المستعملة لأكثر الأحياء شيوعا من أجل تقييم الأثر البيئي للمواد الكيماوية السامة (1).

وتضم العناصر الثقيلة مجموعة كبيرة تقارب 38 عنصرا منها ماهو ضروري للفعاليات الحيوية كالحديد ومنها ماهو سام كالزئبق ،الرصاص، الكادميوم والنيكل ، وتتصف بوزنها النوعي العالي اذ تكون بحدود 5 جم\سم أو أكثر (2).

تشكل العناصر الثقيلة مكونا أساسيا من مكونات التربة الطبيعية إذ تنطلق من خلال الدورات الجيوكيميائية إلى البيئة والتي يحتاجها النبات بشكل كبير لنموه الطبيعي ولكن متى ما حدث الإفراط في تركيزها عندئذ توصف التربة بأنها ملوثة بالعناصر الثقيلة ، ويخلق هذا النوع من التلوث العديد من المشاكل الكيماوية والميكانيكية للتربة والتي تكون عادة صعبة المعالجة فضلا عن خطورتها في الكائنات الحية التي تمتلك القدرة على مراكمة هذه العناصر داخل أجسامها مما يحدث خللا في وظائفها الإحيائية فضلا عن انتقالها ضمن الشبكة الغذائية إلى الإنسان مسببة له الكثير من الأضرار الصحية (3).

نظرا للتنوع الإحيائي في مجاميع اللافقاريات وخصوصا الأنواع الأرضية ، فإن ذلك جعلها أكثر عرضة وتماسا مع المحيط الذي تعيش فيه لأن تكون محطات نهائية لتجميع العديد من الملوثات والسموم وخصوصا العناصر الثقيلة والذي جعل منها مؤشرات إحيائية مثلى لتقييم التلوث البيئي ، ومن أمثلة تلك اللافقاريات هي متشابهة الأقدام الأرضية (4).

تعد متشابهة الأقدام الأرضية Isopoda العائدة لصف القشريات Crustacea إحدى أكثر أحياء التربة شيوعا وقد يعود ذلك انتشارها الواسع إذ تمتد من الغابات حتى الصحاري القاحلة وتمتلك ما يقارب خمسة آلاف نوع تتراوح أحجامها مابين (1.2 – 30) ملم ، وتصل أقصى كثافاتها إلى 3000 فرد/م^2 وسهولة جمعها وتشخيصها وسيادتها مقارنة مع المفصليات الأخرى ، والتي لها دور في زيادة خصوبة التربة عن طريق تعزيز إنتاج ودوران المغذيات والعناصر في التربة بوساطة غذائها المعتمد بالدرجة الأساس على هضم المواد العضوية الموجودة في التربة وإنتاج العناصر المغذية مثل النترات والفوسفات وغيرها والتي يحتاجها النبات بشكل كبير لنموه الأساسي كما إنها سريعة الاستجابة لأي ضرر بيئي طارئ وخصوصا التلوث البيئي من خلال قدرتها العالية في امتصاص العناصر الثقيلة مثل النحاس والرصاص والزنك والكادميوم وغيرها ومراكمتها داخل عدة مناطق من أجسامها وبذلك فهي قادرة على إزالة العديد من العناصر السامة من التربة الملوثة مقارنة مع الحيوانات الأخرى (5، 6).

قدمت متشابهة الأقدام الأرضية لأكثر من عشرة عقود كمؤشرات إحيائية لدراسة التلوث خصوصا في البيئات الصناعية الملوثة ، إذ تمتلك هذه الحيوانات بعض الصفات الاستثنائية التي جعلتها نموذج جيد لدراسة سمية المواد الكيماوية في البيئات الملوثة ومنها حجموها المناسبة ،ووفرتها العالية ، وسهولة التعامل معها مختبريا فضلا عن قابليتها في تجميع وخزن العناصر الثقيلة السامة واستجابتها المعتمدة على مقدار الجرعة لمختلف العناصر والمبيدات (7).

تعد دراسة حياتية متشابهة الأرضية إحدى أكثر الدراسات المناسبة لتقييم التوازن البيئي لأي نظام بيئي وقد يعود ذلك لحساسيتها الهائلة لتلوث التربة بالعناصر الثقيلة والسموم والمبيدات وذلك بزيادة معدل الهلاك لأفرادها وبالتالي انخفاض الكثافة السكانية والتنوع الإحيائي بشكل خاص فضلا عن انخفاض معدل الكتلة الحية ، كما أن قدرة مراكمتها للملوثات ممكن إن تنتقل إلى مستويات عليا من السلسلة الغذائية عن طريق افتراس أفرادها من قبل أحياء أخرى (5).

ومن بين تلك الدراسات، الدراسة التي قام بها Alikhan ، حيث تمت دراسة مجموعتين من متشابهة الأقدام الأرضية النوع *P.spinicornis* في منطقتين أحداهما ملوثة والأخرى غير ملوثة في شمال اليونان ولاحظ من خلال حساب كل من معدلات النمو والتكاثر خلال ثلاث أجيال إن الأفراد في المناطق الملوثة كانت اصغر حجما ،اقل وزنا ،ضئيلة الانسلاخ ، مبكرة النضج والتكاثر،وإنتاج نسل ضئيل ،ووزن الصغار اكبر من أمهاتها ، كما إن معدلات البقاء لتلك الصغار كان عالي مقارنة مع أفراد المناطق الغير ملوثة (8)، والدراسة التي أعدها كل من Drobne و Hopkine حيث تمت دراسة تأثير سمية عنصر الزنك في أفراد نوعين من متشابهة الأقدام الأرضية *P.scaber* و *Oniscus asellus* في مستوى استهلاك الغذاء وتمثيله داخل أجسامها ، إذ استنتج كل منهما إن تركيز العناصر الثقيلة ومن ضمنها الخارصين يخضع لآليات تنظيمية داخل أجسام هذه الحيوانات والتي قد تكون تنظيم معدل الاستهلاك للغذاء أو عن طريق خزن العناصر في الغدة الهضمية بأشكال غير قابلة للذوبان والامتصاص أو التخلص من الفائض عن طريق عملية الإبراز مثل الفضلات الصلبة والفضلات النتروجينية ، وهذا ما توصل إليه أيضا Alikhan أثناء دراسته للآلية الفسلجية للعناصر الثقيلة وملوثات البيئة الأخرى في أنواع متشابهة الأقدام الأرضية من استنتاج ،إذ وجد إن تعرض أفراد هذه الحيوانات إلى جرعات مستمرة من السموم والعناصر يحفز حالة من التكيف لهذه الحيوانات والتي بدورها تنتج تطور مسارات حيوية كيماوية تستغل جزيئات المادة السامة في عمليات فسيولوجية ايضية أساسية داخل أجسامها وهذا بالتالي يعطيها القابلية في مقاومة الملوثات والسموم الموجودة والناتجة عن مصادر طبيعية أو صناعية بفعل الإنسان والمسؤلة عن إستراتيجيات البقاء والتطور والتنوع الإحيائي لهذه الحيوانات (10)، كما وجد Hobbelen *et. al* أثناء دراسة خطر التلوث بالعناصر الثقيلة في أحياء التربة الحتاتية عن طريق التحليل الكيماوي للعينات ، إن أفراد متشابهة الأقدام الأرضية راكمت مستويات عالية جدا من العناصر الثقيلة مقارنة مع أحياء التربة الأخرى الموجودة معها في مواقع الدراسة نفسها ، وان ما تم تمثيله داخل أجسامها جاء من تناول غذائها الملوث (11).

في دراسة أخرى، تمت ملاحظة تأثير نوعية المادة الغذائية ودرجة تلوثها في كفاءة سلوك التغذية لدى أفراد متشابهة الأقدام الأرضية النوع *porcellionides pruinosus* إن الاستجابة كانت متباينة بالاعتماد على مقاييس تغذية محددة مثل الاستهلاك ، التمثيل ، والهضم مع نوعية المادة الغذائية ودرجة تلوثها ، كما لاحظوا إن لدى أفراد هذا النوع القدرة على تجميع كميات هائلة من العناصر الثقيلة المستعملة في التجربة وبذلك استنتجوا أن بالإمكان استعمال هذه الحيوانات كمؤشرات إحيائية لتلوث التربة أو في تقييم المناطق الملوثة المعاد تأهيلها (12).

ان التطور الحادث في المجالات الصناعية وازدياد الكثافة البشرية سبب ابعاث هائل للمواد السامة للبيئة وانتشار هذه المواد ووصولها الى مكونات النظام البيئي الطبيعية ومنطقة الجادرية في بغداد من المناطق المعروفة بالزخم المروري اذتقع على احدى الطرق الرئيسة التي تربط جانب الكرخ بالرصافة ومع تنامي اعداد السيارات ومايبعث منها من ابخرة ضارة سببت بزيادة تلوث المنطقة بالعناصر الثقيلة ، وجاءت هذه الدراسة للتعرف على تراكيز بعض من تلك العناصر باستخدام التربة وبعض الاحياء الشائعة التي تقطنها.

المواد وطرق العمل

جمعت العينات من مواقع متفرقة ضمن منطقة الدراسة وذلك خلال شهر ايار 2012 وبواقع ثلاثة مكررات للعينة الواحدة ولكل من التربة (من عمق 10 سم) تقريبا ، كما جمعت عينات افراد انواع متشابهة الاقدام الارضية قيد الدراسة (صور أرقام 1، 2، 3) بطريقة الازالة بالمربع ووضعت العينات في حاويات بلاستكية بعد تنقيتها من الشوائب ونقلت الى المختبر لتشخيصها وعزلها حسب (13) وتم فحص تركيز العناصر الثقيلة (الحديدFe,الرصاص Pb,الزنكZn ,النيكل Ni,الزئبقHg) في العينات جميعها باستخدام

جهاز الامتصاص الذري اللهبي Atomic Spectrophotometrer في المختبر المركزي لكلية العلوم بجامعة بغداد.

صورة رقم (1): ***Porcellio scaber***

صورة رقم (2): ***porcellionides pruinosus***

صورة رقم (3): ***Armadilllidium vulgare***

هضم عينات متشابهة الاقدام الارضية

بعد ان جففت العينة بالفرن في درجة حرارة 110 م° تم اخذ 1 غم من المسحوق الحيواني ووضع في قدح زجاجي مختبري Beaker ثم اضيف اليه 5 مل من حامض الكبريتيك المركز وترك لمدة 24 ساعة بعدها سخن لمدة ساعة وترك ليبرد ، بعد ذلك اضيف اليه 2 –3 مل من حامض البيركلوريك واعيد تسخينه مرة اخرى الى ان تحول لون العينة من الاسود الى الابيض او الشفاف ، ثم اكمل الحجم الى 25 مل باستخدام الماء المقطر ثم نقلت العينة الى الفحص .

هضم عينات التربة

اتبعت الطريقة الموصوفة في (14)، اذ سحقت العينة ثم جففت في فرن درجة حرارته 110 م° ولمدة ساعتين ، ثم تم وزن 1 غم من العينة المجففة و اضيف لها بعد ذلك 15 مل من حامض الهيدروكلوريك المركز مع 5 مل من حامض النتريك المركز وسخنت العينة الى ان اختفت الابخرة عن الظهور وجف النموذج . ثم تم ترك العينة لتبرد و اضيف اليها 5 مل من حامض الهيدروكلوريك المركز وسخنت مرة اخرى لمدة 5 –10 دقيقة ، بعد ذلك تم تركها لتبرد مرة ثانية وأضيفا اليها 5 مل من حامض الهيدروكلوريك المركز و50 مل من الماء المقطر الحار ورشحت بعد ذلك تم غسل الراسب الغير ذائب بالماء المقطر وأضيف الى الراشح وأكمل الحجم الى 100 مل ثم نقلت العينة الى الفحص .

النتائج والمناقشة

يبين الجدول رقم (1) قيم المعدلات والانحراف المعياري وقيم ارتباط LSD ، ويبين الجدول (2) قيم معامل الارتباطr بين تركيز العناصر الثقيلة في حيوانات الدراسة.ويلاحظ من خلال القيم ان الارتباط معنوي ان اعلى تركيز للعناصر الثقيلة المقاسة بين انواع متشابهة الاقدام الارضية جميعها كان في النوع *porcellionides pruinosus* اذ بلغ على التوالي مقاسا بوحدات (ppm) الحديد687 ،الزنك238 ، الرصاص44 ، النيكل 40 ، و الزئبق0.38 وقد يعود ذلك الى قابلية افراد هذا النوع على امتصاص وخزن العناصر الثقيلة داخل انسجة اجسامها بشكل جيد وهذا ما اكده (15) في دراسته لافراد هذا النوع اذ اظهر مقاومة للتلوث و تنوعا جغرافيا عاليا في اشكاله وأنماط تكاثره ووجوده اذ شخصت ما يقارب 90 جماعة سكانية في شمال ووسط وجنوب تونس واليونان فقط وكانت متنوعة تكاثريا وسلوكيا.

جدول رقم (1): المعدل والانحراف المعياري لتركيز العناصر الثقيلة (ppm) لكل من عينات انواع متشابهة الاقدام الارضية والتربة وقيم LSD خلال فترة الدراسة

نوع العينة	تركيز العنصر				
	Fe	Zn	Pb	Ni	Hg
Procello. Scaber	190 ± 5.67	104 ± 7.36	27 ± 2.04	20 ± 1.58	0.02 ± 0.16
Porcellionides pruinosus	687 ± 21.66	238 ± 14.85	44 ± 3.12	40 ± 3.16	0.05± 0.38
Armadillium Vulgare	40± 16.42	152 12.6 ± 1	±30 2.29	±22 1.47	0.03±0.15
Soil	3400±87.93	97 6.28±	50 3.50±	40±3.60	0.05± 0.19
قيمة (LSD)	* 47.26	16.44 *	5.39 *	* 6.19	* 0.018

*(P<0.05) **

جدول رقم (2): قيم معامل الارتباط r مع مستوى المعنوية فيما بين مستويات تراكم العناصر الثقيلة (انواع متشابهة الاقدام الارضية والتربة خلال فترة الدراسة ((P<0.01)، * (P<0.05)، NS: غير معنوي)**

العناصر المرتبطة	معامل الارتباط (r)	مستوى المعنوية
Fe و Zn	-0.41	*
Fe و Pb	0.81	**
Fe و Ni	0.67	**
Fe و Hg	-0.06	NS
Zn و Pb	0.17	NS
Zn و Ni	0.37	*
Zn و Hg	0.86	**
Pb و Ni	0.96	**
Pb و Hg	0.51	*
Ni و Hg	0.68	**

وسجل كل من عنصري الحديد والزنك تراكيز عالية في الانواع الثلاث المدروسة وقد يعود ذلك الى حاجة افراد هذه الحيوانات الى هذين العنصرين بدرجة اكبر في بناء هيكلها الخرجي خلال النمو والانسلاخ اذ يدخل الخارصين في عملية calcification للهيكل الجديد (16).

ولوحظ من خلال البحث الحالي ان تركيز الحديد كان عاليا في اجسام متشابهة الاقدام وقد اشار (17) الى انه من العناصر المهمة في عملية البناء الضوئي للنبات ويميل الى مراكمته في الاوراق , وبما انها تعتمد الاوراق غذاءا رئيسيا فان من الطبيعي ان يكون تركيزه عاليا في اجسامها , وذكر (18) ان تراكم الزنك في اجسام هذه الكائنات نتيجة تغذيتها على البكتريا والكائنات الدقيقة الموجودة في التربة.

ومن هذا نستنتج ان لهذه الحيوانات القدرة في امتصاص وخزن العناصر الثقيلة في انسجة اجسامها وخاصة الغدة الكبدية البنكرياسية وهذا ما توصل اليه (19) اثناء دراسته في الكشف عن العناصر الثقيلة في احد انواع متشابهة الاقدام التي تعيش في الشواطئ البحرية Ligia oceanica اذ لوحظ ارتفاع كل من تراكيز الزنك والرصاص والنحاس والكادميوم بشكل معنوي في انسجة افرادها المختلفة عند مقرنته بين العينات المأخوذة من المناطق الملوثة والعينات المأخوذة من المناطق الغير ملوثة كما توصل الى ان انسجة الغدة الكبدية البنكرياسية هى الموقع الامثل لخزن العناصر الثقيلة بنسبة 50% مقارنة مع باقي انسجة الجسم .

اما بالنسبة الى التباين الذي ظهر في تراكيز العناصر في عينات الدراسة فقد يعود الى بعض الاختلافات الفسيولوجية للقناة الهضمية او لاحتواء الاوراق النباتية التي تغذت عليها هذه الحيوانات على تراكيز مختلفة من العناصر .وهذا ما توصل اليه (20) ايضا.

اما بالنسبة لمعامل الارتباط فقد اظهر جدول رقم (2) ارتباطا معنويا عاليا بين عنصري النيكل والرصاص ,والزنك والزئبق ، والحديد والرصاص ، بينما لم تظهر العناصر الحديد و الزئبق, والزنك والرصاص اية علاقة معنوية.

وقد تمت دراسة كل من فعالية التكاثر والنمو في يافعات النوع *Armadillidium vulgare* نسبة الى التباين في تراكيز العناصر الثقيلة المعرضة الى نترات الرصاص ، اذ لاحظ ان نسبة نمو الافراد المعرضة للعناصر الثقيلة بلغت 55 % مقارنة مع نسبة نمو الافراد الغير معرضة والتي بلغت 82 % لنفس فترة التعرض (22).

كما درست سمية عنصر الزنك في نظام التغذية لافراد نوعين من متشابهة الاقدام الارضية *Onioscus asellus* و *Porcellio scaber* ، اذ لاحظ اثناء دراسته ان تراكم عنصر الزنك او غيره من العناصر في بقايا الاوراق النباتية والتربة في بيئات اليابسة يحدث عندما تتجاوز تراكيزها الحدود الطبيعية وتصبح بذلك سامة لحيوانات التربة ، واشار الى ان لكل من معدل الاستهلاك والتمثيل والابراز وغيرها دورا في تنظيم تراكيز تلك العناصر داخل اجسام هذه القشريات ، اذ لاحظ انخفاض معدل كل من التكاثر والتنفس والتغذية عند زيادة عنصر الزنك داخل اجسامها (9).

اما بالنسبة لعينات التربة فقد من خلال جدول رقم (1) ايضا ان اعلى تركيز للعناصر الثقيلة كان لعنصر الحديد يليه الزنك وباقي العناصر على التوالي مقدرة بوحدات (ppm) الحديد3400 ، الزنك 97 ،الرصاص 50 ، النيكل 40 ، والزئبق0.19 ، وقد تجاوزت هذه التراكيز الحدود المسموح بها في التربة بكثير، فقد ذكر(23) ان تركيز الحديد يجب ان لايتجاوز(>0. 10) ppm وبهذا تكون عينات التربة ذات محتوى عالي لهذا العنصر واضاف ان الحديد يكو بطي التحلل في البيئة وتكون المبيدات سببا رئيسيا في تواجده في التربة، وذكر ان الزنك يعتبر عاليا اذا كان ضمن المدى(7-8) ppm ، واشار(24) الى ان تركيز الرصاص يجب ان لا يتجاوز (16)ppm .

واظهر الرصاص تركيزا عاليا في التربة فقد اشار(25) الى ان تركيز الرصاص يجب ان لايتجاوز 15ppm في التربة.

واشار(26) الى ان هواء مدينة بغداد ملوث بمستويات عالية من الدقائق العالقة اضافة الى تحديد 6 عناصر ثقيلة في الهواء هي (الرصاص ،الكوبلت ،الحديد ،النحاس ،الكروم ،الكادميوم) ، واكد (27) على وجود دقائق عالقة ورصاص في هواء مدينة بغداد وتلوث التربة ببعض العناصر الثقيلة مثل النيكل والنحاس ، ومن بين مصادر الملوثات البنزين المضاف اليه عنصر الرصاص بشكل رابع اثيلات الرصاص لتحسينه.

وسجل النيكل تركيزوهو ضمن الحدود المسموح بهاحسب تقرير لوزارة العلوم والتكنلوجيا العراقية والذي ذكر ان تركيز النيكل في الترب العراقية يتراوح بين 10−40ppm (28).

وسجل الزئبق تركيزا عاليا في تربة الدراسة بلغ 0.19ppm وهو احد المعادن الثقيلة الشديدة السمية وقد اعتبر ملوثا عاما نظرا لامكانية تحركه لمسافات بعيدة عبر الجو وتراكمه الحيوي وذكر(29) ان تركيز الزئبق الطبيعي في التربة يتراوح من0.01 −0.06 ppm ويزداد تركيزه من ملوثات المعامل التي لاتعالج نفاياتها فضلا عن البخار التصاعد من عملية احتراق الوقود الى الجو يعود ليسقط مجددا على شكل زخات من جزيئات الزئبق السامة وتستوعبها النظم الايكولوجية المائية على الارض وتسبب بتسمم الاغذية والمحاصيل الزراعية ، كما ان الزئبق لايقف عن حدود جغرافية في تلويثه للبيئة اذ انه ينتشر بسرعة لابعد المسافات من خلال الانهار والبحيرات قبل ان يلحظها احد.

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تأثير المعاملة بالكولشسين الطبيعي والصناعي في النمو الخضري والزهري وإنتاج المركبات الفعالة لنبـــات الأقحوان (*Calendula officinalis*)

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الملخص باللغة العربية

أجريت هذه الدراسة في الحقل التابع لقسم البستنة /كلية العلوم الزراعية /جامعة السليمانية للفترة من 2011/10/1 الى 2012/4/30 لدراسة تأثير الكولشسين في نمو النبات وحاصل الأزهار ومحتوى الأزهار من الكاروتينات والصابونينات لنبات الأقحوان *Calendula officinalis* وقد شملت التجربة عاملين :الأول هو الكولشسين بنوعيه الصناعي من شركة Fluka AG Chem CH – 9470 Buch والطبيعي (مستخلص بذور نبات اللحلاح البري *Colchicum autumnale*) وبخمسة تراكيز لكل منهما (0، 0.05، 0.1، 0.2، 0.4 %) .العامل الثاني: طريقة الأضافة بنوعيها، الأولى معاملة القمة النامية والثانية الرش. أستخدم تصميم القطاعات العشوائية الكاملة بعاملين حيث بلغ عدد المعاملات 20 وبثلاث مكررات وكل مكرر يحوي 5 نباتات. أهم النتائج التي توصل اليها البحث هوان المعاملة بالكولشسين الطبيعي بتركيز 0.1% بطريقة الرش ادى الى اعطاء أعلى المعدلات بالنسبة لصفة عدد الافرع /نبات أذ وصلت الى 25.37فرع/نبات قياسا بالمقارنة 12.25فرع/نبات والى زياة الوزن الجاف للمجموع الخضري بنسبة 135.4% قياسا بالمقارنة وزيادة عدد الأيام اللازمة للتزهير بالنسبة لتركيز 0.05% أذ وصل الى 125.25يوم قياسا بالمقارنة 109.يوم ، وكذلك الى زيادة الوزن الجاف للمجموع الجذري بنسبة 555.88% قياسا بالمقارنة والى زيادة عدد الأزهار على النبات 30زهرة/نبات قياسا بالمقارنة 12.50زهرة/نبات والى زيادة تركيز الكاروتينات في الأزهار 230.50 ملغم/100 غم قياسا بالمقارنة 99.25 ملغم/100غم والى زيادة تركيز الصابونينات في الازهاربنسبة 116.05% في حين ادت المعاملة بالكولشسين الى تقليل أرتفاع النبات أما أقل القيم لأغلب المعاملات كانت في معاملة الكولشسين الصناعي بتركيز 0.4%لكلا الطريقتين الرش والقمة النامية كما أدت التراكيز العالية من الكولشسين الصناعي الى حدوث تقزم وحدوث تشوهات في النبات.

الكلمات المفتاحية: الاقحوان ، الكولشسين الطبيعي ، الكولشسين الصناعي، الصابونين،الكاروتينات

ABSTRACT

This study was carried out at the field of Horticulture Department, Faculty of Agricultural Sciences, University of Sulaimani, during the period between October 1, 2011 and April 30, 2012 to study the effect of colchicine on the growth, flower yield, carotene and saponin contents of daisy plant *Calendula officinalis* flowers. The experiment included two factors, first: using two artificial types of colchicine, Fluka AG and Buch, manufactured by Chem CH-9470 Company, and the natural colchicine, extracted from the seeds of Colchicum plant *Colchicum autumnale*, each with five concentrations (0, 0.05, 0.1, 0.2 and 0.4%), second: method of addition, which included the growing tip treatment and spraying the whole plant. The experiment was laid out using a factorial RCBD design, in which 20 treatments replicated 3 times with 5 plants as a replicate. The study reached to as important results: natural colchicine treatment of 0.1% spray gave the higher mean values of number of branches per plant (25.37) compared to control (12.25), vegetative dry weight (135.4%). The required number of days to flowering (125.25 days) was increased due to 0.05% compared to control (109 days), root dry weight (555.88%) was also increased, number of flowers per plant (30) was enhanced in comparison to control (12.5) flowers per plant, carotenes contents (230.5 mg/100 g dry weight) compared to control (99.25 mg/100 g) and saponins (116.05%). However, colchicine treatment caused decreases in plant height, in which the minimum values of the majority of treatments were given by 0.4% colchicine with both addition methods, the high concentrations of artificial colchicine caused dwarfing and deformation of the plants.

المقدمة

يعد نبات الاقحوان *Calendula officinalis* العائد للعائلة Asteraceae من النباتات العشبية الحولية ذات الأهمية الاقتصادية والطبية لاحتوائها على العديد من المركبات الفعالة طبيا مثل الصابونينات، الزيوت الطيارة، الفلافونيدات، الستيرويدات، والتربينات والكاروتينات والكومارين والاحماض الأمينية (1) وهنالك عدة استعمالات طبية للاقحوان اذ يعمل كمضاد للبكتريا ومضاد للفطريات ومضاد للفايروسات ومضاد للالتهابات ومضاد للسرطان ومقوي لجهاز المناعة (2) ومضاد للأكسدة (3) وهو يعد مادة معقمة ويستعمل لعلاج الجروح والحروق والامراض الجلدية الاخرى ولعلاج حب الشباب (4) كما انه يستعمل كمسكن للالام (5) كما ان ازهار الاقحوان تستعمل كملونات طبيعية للاغذية لانها تحوي على الكاروتينات والفلافونيدات وهي تستعمل كبدائل للملونات الصناعية التي ثبت انها تشكل خطر على الصحة وتسبب بعض انواع الحساسية والسمية لها تاثيرات مسرطنة (6).

تعد عملية احداث التضاعف الكروموسومي اصطناعيا من احد الوسائل المستعملة لتحسين انواع النباتات اضافة الى تحويل الهجن العقيمة الى خصبة وهنالك نوعين من التضاعف ، التضاعف الكامل والتضاعف الناقص ويعني التضاعف الكروموسومي الكامل تضاعف لعدد الكروموسومات الاصلي حيث تحدث في الطبيعة نتيجة لتعرض النبات الى الصواعق او الاشعة او البرودة القاسية والتي تؤدي جميعها الى عدم حدوث انقسام في سايتوبلازم الخلية وعدم تكون المغزل بعد الانقسام الميتوزي الذي يحصل فيه تضاعف للكروموسومات وبدلا من ان تتكون خليتين تحوي كل منهما على العدد الاصلي للكروموسومات سوف تبقى خلية واحدة تحتوي على ضعف العدد الاصلي من الكروموسومات (7).

ويمكن احداث هذا التضاعف صناعيا باستخدام العقار قلويد الكولشسين وهو مستخلص من بذور أو كورمات نبات اللحلاح حيث تبلغ نسبته 0.04% ووزنه النوعي 399 وتركيبه الكيميائي C22H2506-N ويعمل على منع تكوين المغزل عند الانقسام الخلوي او يمنع تصنيع البروتين المكون لالياف المغزل (8) وتشير الدراسات الى اهمية التضاعف الكروموسومي ومنها التي اجراها (9) والتي تبين ان النباتات الرباعية للكالبتوس تتصف بانها اكثر قوة في نموها وذات قدرة افضل في العيش في بيئات اقل ملائمة لها وذات مقاومة اكبر للاصابات الحشرية والمرضية ولها محتوى اكبر من الكلوروفيل والبروتين وازهارها اكبر.

وقد قام (10)بمعاملة الاجزاء النباتية (اوراق،عقل ساقية ،عقل جذرية) لنبات الكارديناباالكولشسين بتراكيز (250 و 500) في المرحلة الاولية لانتاج الكالس (بتقنية زراعة الانسجة النباتية) ولفترة 2 و 4 و8 يوم قبل زراعتها على وسط لتحريض تكوين الكالس وقد وجد ان تركيز 250 ملغم / لتر تفوق معنويا على جميع التراكيز في احداث التضاعف الكروموسومي وتحسين الصفات الزهرية والخضرية ولم يحصل على اي فروقات معنوية فيما يتعلق بفترة المعاملة.كما حصل (11) على زيادة في عدد الأزهار عند معاملة القمة النامية لبادرات الأقحوان بالكولشسين بتركيز 0.04% وقد أدت معاملة نبات الثوم بالكولشسين بتركيز 0.05% الى تقليل ارتفاع النبات والى زيادة التضاعف الكروموسومي بنسبة 28.67% وزيادة عدد وحجم الثغور بزيادة التركيز (12) كذلك الحال مع نبات الفلفل البارد أذ أدت المعاملة بالكولشسين بتركيز 0.05% مع الاضافة كل 6 أيام الى زيادة في حجم الاوراق وزيادة حجم الثغور وزيادة سمك السيقان وزيادة طول الاوراق كما ادت الى زيادة التضاعف الكروموسومي (13).

في حين وجد(14)ان اضافة الكولشسين بتركيز 100 ملغم /لتر على شكل ثلاث دفعات الى مثك ازهار الحنطة ادى الى زيادة معنوية في تشكيل الأجنة وتجديد النباتات الخضراء وزيادة النباتات المخصبة والى زيادة التضاعف الكروموسومي بنسبة 84.94% قياسا بالمقارنة 55.26%.

وفي تجربة قام بها(15)على نبات dragon head اذ عامل القمة النامية لهذا النبات بالكولشسين بتركيز 0.1% وقد ادى الى حصول التضاعف الكروموسومي والى تقليل أرتفاع النبات وزيادة المساحة الورقية والوزن الجاف للنبات وزيادة حجم البذور ووزنها وزيادة محتواها من الزيوت الطيارة بنسبة 26% كما أدى رش نباتات القرنفل بالكولشسين بتركيز 0.04% الى زيادة عدد الأزهار/نبات وزيادة طول الحامل الزهري وطول البتلات وزيادة وزن 1000 بذرة .

وفي دراسة اجريت من قبل (17) تم احداث التضاعف الكروموسومي لشتلات الروبينيا من خلال نقع بذورها في المحلول المائي من قلويد الكولشسين بتركيز1000 و2000 ملغم /لتر لفترات (24 و48) ساعة فوجد ان نقع البذور بتركيز 1000ملغم / لتر ولفترة 24 ساعة ادى الى زيادة طول الساق ، قطر الساق ، طول الجهاز الثغري وعرض الجهاز الثغري، حيث ازدادت هذه الصفات معنويا بنسبة 31.31% و 35.17% و 43.75 % و54.09% على التوالي كما ادى هذا التداخل الى خفض معدلات الصفات عامل الشكل 31.25% وعدد الثغور في المليمتر المربع الواحد بنسبة 53.84% اذا ما قورن مع نقع البذور بالماء فقط.وقد وجدت (18) عند معاملتها لنباتStevia بعدة تراكيز من الكولشسين (0.25% و0.50 %و 0.75% و1%و 2.5%) قد أدى الى زيادة ارتفاع النبات وطول وعرض وسمك الورقة وعدد الثغور في وحدة المساحة .

وبسبب ما ذكر أنفا من الأهمية الطبية والأقتصادية لنبات الاقحوان وكذلك فاعلية الكولشسين في أحداث تغييرات في نمو وحاصل مجموعة كبيرة من النباتات من هنا جاءت فكرة البحث وهو استعمال نوعين من الكولشسين الطبيعي والصناعي وبطريقتين للاضافة الرش والقمة النامية وباربعة تراكيز لكل منهما لاختبار تاثيرها في نمو وحاصل نبات الاقحوان ومحتواه من بعض المركبات الفعالة.

المواد وطرق العمل

أجريت الدراسة في الحقل التابع الى قسم البستنة /فاكلتي العلوم الزراعية /جامعة السليمانية للمدة من 1/10/ 2011 وحتى 30/ 2012/4 لدراسة تأثير الكولشسين في نمو النبات وحاصل الأزهار ومحتوى الأزهار من الكاروتينات والصابونينات لنبات الأقحوان *Calendula officinalis* صنف Orange w/Black Calypso Center ذو اللون البرتقالي من شركة Benary .

وقد شملت التجربة عاملين: العامل الأول هو الكولشسين بنوعيه الصناعي والطبيعي وبخمسة تراكيز لكل منهما (0، 0.05،0.1،0.2،0.4)%، والعامل الثاني: طريقة الأضافة بنوعيها الأولى معاملة القمة النامية والثانية الرش.

استخدم تصميم القطاعات العشوائية الكاملة بعاملين حيث بلغ عدد المعاملات 20 وبثلاث مكررات وكل مكرر يحوي 5نباتات اي عدد الوحدات التجريبية هي 300 وحدة تجريبية .طريقة الزراعة:تم زراعة البذور في صواني الأنبات بتاريخ 1/10/ 2011 في صواني الأنبات في الظلة الخشبية وبعد شهر تقريبا عند وصولها الى ارتفاع 15 سم تم نقلها الى سنادين بلاستك بقطر 25 سم وبحجم 2 لتر ويحتوي الوسط على البتموس والزميج النهري والسماد العضوي بنسبة 2:1:1.

تحضير المحاليل

1. الكولشسين الصناعي من شركة Buch – 9470 CH Fluka AG Chem. Febrik: تم وزن كل واحدة من الأوزان المذكورة اعلاه بالميزان الحساس واذابتها ب20 مل من كحول الميثانول تركيز 95% واكمل الحجم الى 100 بالماء المقطر.

2. الكولشسين الطبيعي: تم جمع بذور نبات اللحلاح البري*Colchicum autumnale*. من منطقة ماوت شمال محافظة السليمانية وتم طحنها بالطاحونة الكهربائية وتم أخذ الاوزان المذكورة اعلاه ثم اضيف اليها 20 مل من الميثانول بتركيز 95% ووضعت فوق جهاز Hot plate stirrer لمدة 24 ساعة وبعد ذلك تم ترشيحها بواسطة اوراق ترشيح Whatman 1 ثم أكمل الحجم الى 100 مل بالماء المقطر .

طريقة المعاملة

1. معاملة القمة النامية: تم معاملة القمة النامية بعد ظهور الورقتين الحقيقيتين الأوليتين على شكل قطرات بواسطة قطارة وبعد مرور 24 ساعة تم غسل النباتات بالماء المقطر فقط .
2. معاملة الرش :تم رش الأوراق بالكولشسين بعد ظهور 8 أوراق على النبات وبعد مرور 24 ساعة تم غسل النباتات بالماء المقطر فقط .

وفي نهاية شهر نيسان أخذت النتائج النهائية لجميع المعاملات ودرست الصفات التالية بعد تحليل بيانات كل صفة احصائياوفق تصميم التجربة وباستخدام جهاز الكومبيوتر بواسطة برنامج SAS2001 للتحليل الاحصائي وتم مقارنة الأوساط الحسابية بأختبار أقل فرق معنوي LSDوتحت مستوى أحتمال 5% (19).

الصفات المدروسة

صفات النمو الخضري:

1- ارتفاع النبات(سم): تم قياسه بواسطة شريط القياس من منطقة أتصال الساق بالجذر الى نهاية القمة النامية للساق.
2- عدد الأفرع الخضرية :وتم حساب عدد الأفرع الخضرية على الساق الرئيسي للنبات في مرحلة اكتمال التزهير.
3 - الوزن الجاف للمجموع الخضري (غم):تم قطع الجزء الخضري في منطقة اتصاله بالجذروجفف في الفرن الكهربائي على درجة حرارة 70م وتم اخذ الوزن الجاف بعد ثبات الوزن.
4- الوزن الجاف الجذري(غم): في نهاية موسم النمو تم جمع الجذور وغسلها لأزالة الأتربة العالقة فيها ثم جففت في الفرن الكهربائي على درجة حرارة 70 م وتم أخذ الوزن الجاف بعد ثبات الوزن.

صفات النمو الزهري:

1-عدد الايام اللازمة للتزهير(يوم):وتم حسابها من اول يوم لزراعة البذور الى حين التفتح الكامل للازهار.
2- عدد الازهار/نبات: تم حسابها على النبات الواحد عندما وصلت مرحلة التزهير الى منتصفها.
3- قطر الازهار : تم قياسها بواسطة القدم.
4-الوزن الجاف للمجموع الزهري(غم):تم قطف الازهار في مرحلة التزهير الكامل وتجفيفها في الفرن الكهربائي على درجة حرارة 70 م لحين ثبات الوزن.
5- تركيز الكاروتينات في الازهار: تم قياسها وفقا الى (20).
6- تركيز الصابونين في الازهار:تم تقديرها وفقا الى (21).

النتائـــــج

يظهر من الجدول (1) أن المعاملة بالكولشسين أدت بشكل عام الى تقليل ارتفاع النبات ولكن هذا التأثير لم يصل الى درجة المعنوية عند المعاملة بالكولشسين الطبيعي عن طريق القمة النامية أو الرش أ ذ بلغ 22.75سم و 23.50سم للتركيز 0.05% و 22.63 سم و22.88 سم للتركيز 0.1% قياسا بالمقارنة 23.50 سم في حين ادت بقية التراكيز لكل من الكولشسين الصناعي والطبيعي الى تقليل أرتفاع النبات وأقل ارتفاع ظهر في معاملة الرش بالكولشسين الصناعي بتركيز 0.4% قياساً بالمقارنة 23.50 سم .

جدول رقم (1): تأثير العوامل المدروسة في صفة ارتفاع النبات (سم)

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	23.50	19.50	18.25	17.25	12.75	18.25	15.86
	رش	23.50	16.13	14.13	8.50	5.13	13.47	
طبيعي	قمة نامية	23.50	22.75	22.63	21.38	19.13	21.88	21.00
	رش	23.50	23.50	22.88	16.25	14.60	20.15	
LSD		* 4.674					*1.61	*0.505
A x T	صناعي	23.50	17.81	16.18	12.88	8.93	*2.66 = LSD	
	طبيعي	23.50	23.12	22.75	18.81	16.81		
B x T	قمة نامية	23.50	21.13	20.44	19.31	15.94	20.06	
	رش	23.50	19.81	18.50	12.38	9.81	16.80	
LSD		* 2.66					*0.505	
المعدل		23.50	20.46	19.46	15.84	12.87	*0.798 = LSD	

يظهر من الجدول (2) ان المعاملة بالكولشسين الطبيعي بتركيز 0.1% بطريقة الرش الى زيادة عدد الافرع على النبات 25.73 فرع /نبات قياسا بالمقارنة 12.25 فرع تليها المعاملة بالكولشسين الطبيعي بتركيز 0.05% بطريقة القمة النامية25فرع التي لم تختلف معنوياً عن سابقتها في حين أدت التراكيز العالية الى تقليل عدد الأفرع وأقل معدل ظهر في معاملة الرش بالكولشسين الصناعي بتركيز 0.4% أذ بلغت 2.25 فرع، و يوضح الجدول (3) أن المعاملة بالكولشسين الطبيعي بتركيزيه 0.05% عن طريق القمة النامية و0.1% عن طريق الرش الى زيادة الوزن الجاف للمجموع الخضري بنسبة 154.28% و135.4% على التوالي قياسا بالمقارنة وكذلك ادت بقية معلات الكولشسين الطبيعي والصناعي الىحدوث زيادة في الوزن الجاف باستثناء التراكيز العالية لكليهما فانها ادت الى تقليل الوزن الجاف واقل قيمة ظهرت في معاملة الكولشسين الصناعي بتركيز0.4% سواء كانت الاضافة عن طريق القمة النامية او الرش اذ بلغت 1.071.68

جدول رقم (2): تأثير العوامل المدروسة في صفة عدد الافرع الخضرية الرئيسية

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	12.25	17.00	13.75	12.50	9.50	13.00	10.77
	رش	12.25	12.25	9.50	6.50	2.25	8.55	
طبيعي	قمة نامية	12.25	25.00	20.50	16.25	10.25	16.85	16.38
	رش	12.25	19.25	25.75	14.75	7.50	15.90	
LSD		4.091 *					*1.35	*0.593
A x T	صناعي	12.25	14.62	11.62	9.50	5.87	* 2.67 = LSD	
	طبيعي	12.25	22.12	23.12	15.50	8.87		
B x T	قمة نامية	12.25	21.00	17.13	14.38	9.87	15.53	
	رش	12.25	15.75	17.62	10.63	4.87	12.22	
LSD		2.67 *					*0.593	
المعدل		12.25	18.37	17.37	12.50	7.37	*0.937 = LSD	

جدول رقم (3): تأثير العوامل المدروسة في صفة الوزن الجاف للمجموع الخضري/غم

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	3.39	5.32	6.86	4.71	1.86	4.43	4.08
	رش	3.39	6.32	4.67	3.26	1.07	3.74	
طبيعي	قمة نامية	3.39	8.62	6.76	4.78	2.18	5.15	5.15
	رش	3.39	6.57	7.98	4.88	2.88	5.14	
LSD		1.338 *					*0.436	*0.287
A x T	صناعي	3.39	5.82	5.76	3.98	1.47	* 0.877 = LSD	
	طبيعي	3.39	7.59	7.37	4.83	2.52		
B x T	قمة نامية	3.39	6.97	6.81	4.74	2.02	4.79	
	رش	3.39	6.44	6.33	4.07	1.97	4.44	
LSD		0.877 *					*0.287	
المعدل		3.39	6.70	6.57	4.40	1.99	*0.453 = LSD	

يتبين من الجدول(4) ان المعاملة بالكولشسين الطبيعي بتركيز 0.1% عن طريق الرش أدت الى أعطاء اعلى معدل للزيادة في الوزن الجاف للمجموع الجذري بلغت 555.88% قياسا بالمقارنة تليها معاملة الكولشسين الصناعي بتركيز0.1% عن طريق الرش بنسبة زيادة تقدر ب 511.03 % في حين ادت المعاملة بالتراكيز العالية لكل من الكولشسين الصناعي والطبيعي الى تقليل الوز ن واقل قيمة ظهرت في معاملة الكولشسين الصناعي بتركيز 0.4% عن طريق الرش أذ بلغت 0.70 غم قياسا بالمقارنة 1.36غم .

أدت المعاملة بالكولشسين الصناعي والطبيعي بشكل عام الى زيادة عدد الايام اللازمة للتزهير كما يظهر من الجدول (5) وأعلى معدل للزيادة ظهرفي معاملة الكولشسين الطبيعي بتركيز 0.05%بطريقة الرش أذ بلغ 125.25 يوم قياسا بالمقارنة 109.00 يوم تليها المعاملة بالكولشسين الطبيعي بتركيز 0.1% لكل من طريقة الرش ومعاملة القمة النامية اذ بلغت 123.75 و 123.50 يوم على التوالي وبدون وجود اي فروق معنوية بينها وأقل معدل ظهر في عدد الأيام اللازمة للتزهير ظهر في معاملة الكولشسين الصناعي بتركيز 0.1% أذ بلغت 110.50 يوم والتي لم تختلف معنوياً عن معاملة المقارنة.

يشير الجدول(6) الى أن المعاملة بالتراكيز المنخفضة من الكولشسين الطبيعي أو الصناعي أدت الى زيادة عدد الأزهار /نبات وأعلى معدل ظهر في معاملة الكولشسين الطبيعي بتركيز 0.1% بطريقة الرش أذ بلغ 30 زهرة / نبات قياسا بالمقارنة 12.50 زهرة/نبات تليها المعاملة بالكولشسين الطبيعي بتركيز 0.05% عن طريق القمة النامية 23.75 زهرة /نبات في حين أدت التراكيز المرتفعة من الكولشسين الى تقليل عدد الازهار/ نبات وأقل معدل ظهر في معاملة الكولشسين الصناعي بطريقة الرش بتركيز 0.4% أذ بلغت 1 زهرة /نبات وكانت مشوهة.

يشير الجدول (7) ان اعلى معدل لقطر الازهار ظهر في معاملة الكولشسين الطبيعي بتركيز 0.4%بطريقة الرش 13.83 سم على الرغم ان هذه الازهار كان عددها قليل جدا ومقزمة تليها المعاملة بالكولشسين الطبيعي بتركيز 0.1%بطريقة الرش 7.03 سم قياسا بالمقارنة4.28 أما أقل القيم فقد ظهرت في معاملة الكولشسين الصناعي بتركيز 0.4%بطريقة الرش 1.33.

يظهر من الجدول (8) أن المعاملة بالكولشسين الصناعي بتركيز 0.1% بطريقة الرش قد أدى الى زيادة الوزن الجاف للنورة الزهرية 3.25غم وبفرق غير معنوي عن معاملة الكولشسين الطبيعي بتركيز 0.05% عن طريق القمة النامية أذ بلغ 3.24 غم قياساً بالمقارنة 1.34 غم أما أقل وزن للنورة فقد ظهر في معاملة الكولشسين الصناعي بطريقة الرش بتركيز 0.4% أذ بلغت 0.49 غم.

يتبين من الجدول (9) ان تركيز الكاروتينات في أزهار الاقحوان قد أزدادت بشكل ملحوظ نتيجة المعاملة بالتراكيز المنخفضة من الكولشسين الصناعي والطبيعي وأعلى معدل للزيادة ظهر في معاملة الكولشسين الطبيعي بتركيز0.1% بطريقة الرش أذ بلغت 230.50 ملغم /100 غم تليها معاملة الكولشسين الصناعي بتركيز 0.1% بطريقة الرش أذ بلغت 225.50 ملغم/100غم وبفرق غير معنوي عن الأولى في حين أدت المعاملة بالكولشسين الصناعي بتركيز 0.4% بطريقة الرش الى تقليل تركيز الكاروتينات في الازهار اذ بلغت 78.75 ملغم /غم قياسا بالمقارنة 99.25 ملغم / غم.

أدت المعاملة بالكولشسين الطبيعي بتركيز 0.1% عن طريق الرش الى زيادة تركيز الصابونين في أزهار الأقحوان بنسبة 116.05 % قياسا بالمقارنة (جدول10) تليها المعاملة بالكولشسين الطبيعي بتركيز 0.05% عن طريق القمة النامية أذ بلغت نسبة الزيادة 99.88 % في حين أدى التركيز 0.4% لكل من الكولشسين الطبيعي والصناعي الى تقليل تركيز الصابونين في الأزهار وأقل قيمة ظهرت في معاملة الكولشسين الصناعي بتركيز 0.4% عن طريق القمة النامية اذ بلغت0.78 %.

جدول رقم (4): تأثير العوامل المدروسة في صفة الوزن الجاف للمجموع الجذري/غم

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	1.36	5.28	4.31	2.09	1.40	2.88	3.09
	رش	1.36	3.83	8.31	2.34	0.70	3.30	
طبيعي	قمة نامية	1.36	4.25	3.46	1.74	1.13	2.38	3.01
	رش	1.36	4.15	8.92	2.27	1.49	3.64	
LSD		* 1.874					*0.563	*0.225
A x T	صناعي	1.36	4.56	6.31	2.21	1.05	* 1.26 = LSD	
	طبيعي	1.36	4.21	6.19	2.01	1.31		
B x T	قمة نامية	1.36	4.76	3.89	1.92	1.26	2.64	
	رش	1.36	3.99	8.61	2.30	1.09	3.47	
LSD		* 1.26					*0.225	
المعدل		1.36	4.38	6.25	2.11	1.17	*0.356 = LSD	

جدول رقم (5): تأثير العوامل المدروسة في صفة عدد الايام اللازمة للتزهير

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	109.0	115.0	110.5	115.0	115.5	113.0	112.5
	رش	109.0	113.3	112.7	114.7	110.7	112.1	
طبيعي	قمة نامية	109.0	121.5	123.5	118.5	112.2	116.94	117.39
	رش	109.0	125.3	123.7	116.0	115.2	117.84	
LSD		* 2.463					*0.945	* 0.66
A x T	صناعي	109.0	114.1	111.6	114.9	113.1	* 1.889 = LSD	
	طبيعي	109.0	123.4	123.6	117.2	113.8		
B x T	قمة نامية	109.0	118.3	117.0	116.8	113.9	115.00	
	رش	109.0	119.3	118.3	115.4	113.0	115.00	
LSD		* 1.889					ns 0.66	
المعدل		109.0	118.7	117.6	116.1	113.4	*1.04 = LSD	

جدول رقم (6): تأثير العوامل المدروسة في صفة عدد الازهار/نبات

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	12.50	20.00	17.25	12.25	8.50	14.10	12.37
	رش	12.50	14.75	18.25	6.75	1.00	10.65	
طبيعي	قمة نامية	12.50	23.75	21.75	14.75	9.25	16.40	16.73
	رش	12.50	22.50	30.00	14.75	5.50	17.05	
LSD		* 4.291					*1.09	* 0.67
A x T	صناعي	12.50	17.38	17.75	9.50	4.75	* 2.75 = LSD	
	طبيعي	12.50	23.12	25.98	14.75	7.37		
B x T	قمة نامية	12.50	21.87	19.50	13.50	8.87	15.25	
	رش	12.50	18.63	24.13	10.75	3.25	13.85	
LSD		* 2.75					* 0.67	
المعدل		12.50	20.25	21.81	12.12	6.06	* 1.06 = LSD	

جدول رقم (7): تأثير العوامل المدروسة في صفة قطر الازهار

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	4.28	5.28	4.78	3.58	2.88	4.15	4.14
	رش	4.28	5.05	6.03	4.03	1.33	4.14	
طبيعي	قمة نامية	4.28	6.43	5.27	4.78	4.23	4.99	6.18
	رش	4.28	6.27	7.03	5.40	13.83	7.36	
LSD		* 6.299					ns 2.97	ns 1.95
A x T	صناعي	4.28	5.16	5.40	3.80	2.10	* 4.36 = LSD	
	طبيعي	4.28	6.35	6.15	5.08	9.03		
B x T	قمة نامية	4.28	5.85	5.03	4.17	3.55	4.58	
	رش	4.28	5.66	6.53	4.71	2.14	4.66	
LSD		* 4.36					ns 1.95	
المعدل		4.28	5.75	5.77	4.44	5.56	* 3.08 = LSD	

جدول رقم (8): تأثير العوامل المدروسة في صفة الوزن الجاف للنورة الزهرية (غم)

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	1.33	3.11	2.48	1.72	1.43	2.01	1.87
	رش	1.33	2.05	3.28	1.54	0.49	1.74	
طبيعي	قمة نامية	1.33	3.24	2.31	1.51	1.40	1.96	1.92
	رش	1.33	1.89	3.01	1.66	1.48	1.87	
LSD		0.752 *					*0.115	*0.062
A x T	صناعي	1.33	2.57	2.88	1.63	0.96	LSD = 0.399 *	
	طبيعي	1.33	2.56	2.66	1.58	1.44		
B x T	قمة نامية	1.33	3.17	2.39	1.61	1.42	1.98	
	رش	1.33	1.96	3.14	1.60	0.98	1.80	
LSD		0.399 *					0.062 *	
المعدل		1.33	2.57	2.76	1.60	1.20	LSD = 0.098 *	

جدول رقم (9): تأثير العوامل المدروسة في صفة تركيز الكاروتينات(ملغم /100غم)

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	99.25	216.7	206.8	175.3	106.3	160.8	154.6
	رش	99.25	210.3	225.5	128.3	78.7	148.4	
طبيعي	قمة نامية	99.25	218.7	206.0	154.7	101.7	156.07	160.21
	رش	99.25	191.5	230.5	176.0	124.5	164.35	
LSD		22.035 *					*6.83	*3.07
A x T	صناعي	99.25	213.5	216.1	151.8	92.50	LSD = 14.19*	
	طبيعي	99.25	205.1	218.3	165.4	113.1		
B x T	قمة نامية	99.25	217.7	206.4	165.0	104.0	158.47	
	رش	99.25	200.8	228.0	152.1	101.6	156.35	
LSD		14.19 *					ns 3.07	
المعدل		99.25	209.3	217.2	158.6	102.8	LSD = 4.86*	

جدول رقم (10): تأثير العوامل المدروسة في صفة تركيز الصابونين (%)

الكولشسين A	الطريقة B	المعاملة T					A x B	المعدل
		سيطرة	0.05	0.1	0.2	0.4		
صناعي	قمة نامية	5.67	7.49	6.79	5.66	0.78	5.27	5.47
	رش	5.67	7.27	9.16	4.39	1.86	5.67	
طبيعي	قمة نامية	5.67	11.22	9.57	6.37	3.94	7.35	7.66
	رش	5.67	10.44	12.25	6.78	4.71	7.97	
LSD		1.259 *					* 0.332	*0.171
A x T	صناعي	5.67	7.38	7.97	5.02	1.32	LSD = 0.779 *	
	طبيعي	5.67	10.82	10.91	6.57	4.32		
B x T	قمة نامية	5.67	9.35	8.18	6.02	2.35	6.31	
	رش	5.67	8.85	10.70	5.59	3.28	6.82	
LSD		0.779 *					* 0.171	
المعدل		5.67	9.10	9.44	5.80	2.82	LSD = 0.27*	

المناقشـــــة

ان السبب في تفوق الكثير من صفات نبات الاقحوان نتيجة المعاملة بالكولشسين قد يعود الى ان مركب الكولشسين يؤدي الى حدوث التضاعف الكروموسومي مع عدم حدوث انقسام في سايتوبلازم الخلية وعدم تكون المغزل بعد الانقسام الميتوزي الذي يحصل فيه تضاعف للكروموسومات وبدلا من ان تتكون خليتين تحوي كل منهما على العدد الاصلي للكروموسومات سوف تبقى خلية واحدة تحتوي على ضعف العدد الاصلي من الكروموسومات (7).

أما سبب تفوق الكولشسين الطبيعي على الصناعي في أغلب الصفات المدروسة ربما يعود الى كون تركيز الكولشسين في المستخلص قليل جدا 0.4% قياسابالكولشسين الصناعي النقي الذي يبلغ تركيزه 100% . أن استعمال التراكيز العالية من الكولشسين الطبيعي والصناعي أدى الى انخفاض في قيم جميع الصفات المدروسة قد يعود السبب الى التأثير السمي لهذا المركب وعدم تحمل النبات للتراكيز العالية من هذا المركب وقد ادت الى حدوث تشوهات في نمو النبات وشكل الازهار.اما سبب تفوق معاملة الرش على القمة النامية في اغلب الصفات المدروسة ربما يعود الى سهولة أختراق المحلول الى أنسجة الورقة.

النتائج والتوصيات

نستنتج من هذه الدراسة ان معاملة نبات الاقحوان بالكولشسين الطبيعي اوالصناعي بالتراكيز 0.05%و 0.1% قد ادى الى زيادة في معدل معظم الصفات المدروسة بأستثناء ارتفاع النبات وأفضل النتائج ظهرت في المعاملة بالكولشسين الطبيعي بتركيز0.1%بطريقة الرش في حين ادت المعاملة بالكولشسين الطبيعي والصناعي بالتركيز0.2%و0.4% الى تقليل معدلات معظم الصفات المدروسة وأقل القيم ظهرت في المعاملة بالكولشسين الصناعي بتركيز 0.4%. ونظرا لماتقدم لذا نوصي بأستعمال الكولشسين الطبيعي بتركيز 0.1% بطريقة الرش على نبات الاقحوان للحصول على أفضل النتائج المرجوة.

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تحضير المقترن لعدة التحليل المناعي الإنزيمي (الإليزا) بطريقة محورة

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الملخص باللغة العربية

يهدف البحث الى كشف سر المعرفة العلمي للعدد التشخيصية بالتحليل المناعي الانزيمي بتقانة الألايزا (ELISA) ، تم التركيز على تحضير كاشف مناعي انزيمي عام مهم في اغلب العدد التشخيصية لتقانة التحليل المناعي الانزيمي للكشف عن الامراض وثبتت الطريقة بعد اجراء بعض التحويرات بأستخدام مادة الكلورواي نايترو بنزين لغلق مجاميع الامين الخاصة بالانزيم والمقارنة بأستخدام الأطياف .

أستخدمت طريقة الأكسدة بمادة بيرأيوديت الصوديوم لتعليم مضاد الكلوبيولين المناعي IgG (Goat Anti-Human-IgG) بانزيم Horse-radish Peroxidase (HRP) ، واجريت دراسة بتطبيق تقانة الألايزا بالمقارنة مع المقترن المستورد ودراسة بتطبيق أطياف الأشعة تحت الحمراء والاشعة فوق البنفسجية وكانت النتائج مطابقة للمقترن المستورد وتم تطبيق المنتج وتقييمه في المختبرات التعليمية بمستشفى بغداد التعليمي.

الكلمات المفتاحية: العدد التشخيصية، ELISA، المقترن.

ABSTRACT

The study aimed to discover the secret of scientific knowledge a preparation of the diagnostic kits by using of enzyme linked immunosorbent assay (ELISA) ,that it was focused on the preparation of enzymatic immunological reagent in some kits of diagnostic by method of the enzyme linked immunosorbent assay (ELISA) to detect diseases .We were used the oxidation method with presence sodium peraiodate for labelling anti- immunoglobulin IgG (Goat-Anti-Human-IgG) with Horse-radish Peroxidase (HRP), and was applied of the conjugate in preparation ELISA - kit , and compared with the conjugate from importer kit ,was studied the application of infra – red spectroscopy and ultra-violet radiation, the results were identical between the prepared conjugate with the importer conjugate , it were evaluated of the prepared conjugate in laboratories of ministry of health/ the city of Medicine / education laboratories and also comparison with the imported kit .

المقدمة

بدأت تقانة الأليزا عام 1971 من قبل (Engvall and Perlmann) (1)، وتطورت التقانة عام 1981 لاسيما عند اكتشاف مرض الإيدز في فرنسا، وتعرف هذه التقانة الطبية بأنها التقدير الكمي والنوعي لكميات قليلة جدا من المستضدات أو الأضداد في أطباق فيها فتحات دقيقة العيارية تسمى بالصفائح الدقيقة العيارية (Microtitre Plates) ويمكن الكشف عن (5) نانوغرام من المستضد أو الضد لكل مللتر من العينة بهذه التقانة (2).عند تحضير عدة (Kit) التحليل المناعي الأنزيمي يحضر المقترن لذي يعتبر أهم كاشف بالعدة التشخيصية اذ يعلم الضد أو المسضد بالأنزيم وحسب نوع العدة (3)، ويعتمد المقترن على كفاءة ونقاوة الأنزيم ، والجزيئة المراد تعليمها بالأنزيم تكون ذات خصوصية ونقاوة عاليتين لمنع التداخلات في التحليل (4)، ويتم اختيار الأنزيم على أساس ثابت ميكاليس ومنتن (Km) للمادة الأساس يكون واطئا والأنزيم ذا استقرارية طويلة ونقاوة عالية وكلفة منخفضة (5).

ان طريقة الأليزا هي نوع من انواع التحليل المناعي الأنزيمي المتغاير (Heterogeneous) ولها أصناف حسب نوع التفاعلات: (2)

Horse-radish Peroxidase (HRP) , Alkaline Phosphatase (ALP), Urease , β-galactosidase , Glucose oxidase , Gluco amylase , Carbonic anhydrase , Acetyl cholinesterase.

وغالبا ما يستخدم أنزيم (HRP)، ويفضل على بقية الأنزيمات لعدم وجوده في مصل المريض فلا يسبب تداخلات . وله وزن جزيئي (40,000 دالتون) يتكون من متعدد الببتيد (Polypeptide) مكون من (308) حامض أميني ويحتوي على الكاربوهيدرات تربطها أربع أواصر ثنائية الكبريت (6) ، الطيف الأمثل لأنزيم HRP هو (403) نانومتر، ويمتاز بكونه واهبا للهيدروجين (H-donner) ويعمل بنظام بيرو كسيد الهيدروجين (H_2O_2) (7)، والمادة الأساس له تكون معتمدة على وجود مجموعتين من الأمين ($-NH_2$) (8).

وهنالك طرق عديدة لتحضير المقترن (Conjugate) هي (4، 5):

1- الطريقة المباشرة بالأكسدة – الاختزال باستعمال مادة البرايوديت (Periodate) المتمثلة بالصيغة ($NaIo_4$).

2- طرائق عوامل التشابك (Cross-Linking agents) ويمكن حضير المقترن بهذه الكواشف بخطوة واحدة او بخطوتين.مثل طريقة الكلوتر الديهايد (Glutareldehyde method)) والطريقة المباشرة بالأكسدة مع البيرا يوديت كما في مخطط(1)هما من الطرائق الشائعة مع الأنزيم HRP و ALP (9). أما طريقة الأكسدة – الاختزال فانها تحدث بوجود عامل مؤكسد (بيرآ يوديت الصوديوم $NaIO_4$) ثم يستعمل عامل مختزل (بوروها يدرايد الصوديوم $NaBH_4$) وينشط الأنزيم بحجب مجموعة الأمين ومنع أكسدتها باستخدام كاشف سانكر Flouro 2,3-Dinitro Benzene(FDNB) (5، 9).

وتعتمد الفعالية الأنزيمية (Enzyme activity) في طريقة الألايزا على الأنزيم والمواد الأساس لإعطاء اللون الناتج من تحليل الأنزيم (10)، نجد أن الأنزيم HRP يعمل ضمن نظام البيروكساید (H_2O_2) والمادة الأساس تكون حاوية على مجموعتين من ($-NH_2$) (11).

وتعدّ المادة الأساس (OPD) هي المفضلة لعمل أنزيم HRP لاستقراريتها العالية مقارنة بالمواد الأخرى، وتتحلل بالضوء (12)، كما أن لها مضارا لذلك تم استعمال($2,\overline{2}$ – Azino- bis – (3-ethy benzthiazoline) 6- Sulphonic) (ABTS) بدلا منها لأنها أقل سمية وذات استقرارية عالية أيضا (7). وكذلك $3,\overline{3},5,\overline{5}$ -Tetra mthylbenzidine(TMB).

وتقاس الكثافة الضوئية (Optical density) باستخدام جهاز الأليزا (ELISA-reader) هو مطياف ضوئي يقيس الكثافة الضوئية بأطوال موجية معينة (10).

المواد وطرق العمل

تحضير المقترن (Conjugate) المتمثل بمضاد الكلوبيولين المناعي IgG المعلم بأنزيم HRP

تم ربط مضاد الكلوبيولين المناعي البشري (Goat-Anti-Human-IgG) بأنزيم HRP لتحضير المقترن (Conjugate) وفقا لطريقة فولر (14) وطريقة هينك (Henning) (15) ، إذ تم دمج خطوات من الطريقيتين للتوصل الى الطريقة المثبتة. وباستخدام طريقة الأكسدة – الاختزال مع مادة ببرايوديت الصوديوم $NaIO_4$.

المحاليل المستخدمة:

1.محاليل متعددة : محلول دارئ الخلات (Acetate buffer) (0.005 مولاري، pH=4.0)، محلول بيرايوديت الصوديوم (Na-Periodate) (0.2 مولاري)، محلول بوروهايدرايد الصوديوم (Na-Borohydride) (0.1 مولاري)،محلول 1% -1 Chloro 2,4-DiNitrobenzene (CDNB) ، محلول دارئ (كاربونات – بيكاربونات) الصوديوم (0.05 مولاري ، pH=9.6) ، محلول دارئ الفوسفات الملحي(PBS), (0.15 مولاري, pH=7.2).

2.محلول دارئ الفوسفات من مادة KH_2PO_4, (3 ملي مولار ،PH=6.3) مع Ethyl (3,3-dimethyl amine proplene) carbodiimide- HCL(EDC): تمت إذابة (0.0408) غرام من KH_2PO_4 في (80) مللتر من الماء اللاأيوني وعدل الأس الهايدروجيني إلى PH=6.3 وإكمل الحجم إلى (100) مللتر بالماء اللاآيوني وأضيف (5) ملغرام من مادة EDC لكل (1) مللتر من المحلول.

3. محلول EDC / IgG: أضيف الكلوبيولين المناعي البشري IgG بتركيز (100 مايكروغرام / 1 مللتر) لمحلول (2) الحاوي (EDC) .

طريقة العمل:

1- أذيب (5) ملغرام من أنزيم HRP (المحضر محليا) (13) في (2.5) مللتر من الماء اللاآيوني.
2- أضيف (100) مايكروليتر محلولCDNB (1%) ومزج لمدة ساعة في درجة حرارة الغرفة.
3- أضيف (200) مايكروليتر من محلول (0.2 مولاري، بيرايوديت الصوديوم) ومزج بالظلام مدة 20 دقيقة في درجة حرارة الغرفة، لوحظ تحول اللون إلى الأخضر.
4- أضيف (200) مايكروليتر من محلول دارئ الخلات ومزج جيدا.
5- حضر عمود فصل PD-10 (محقنة قياس 10 مللتر) معبأ بمادة السفادكس(Sephadex G-25) وعمل له موازنة

بتحريـر (30) مللتـر مـن محلـول دارئ (كاربونـات – بيكاربونات) الصوديوم (0.05 مولاري ، pH=9.6) .
6- ترك العمود ليجف ثم أضيف محلول الانزيم الى العمود وجمع من اسفل العمود.
7- غسل العمود بـ (4.5) مللتر من محلول دارئ (كاربونـات – بيكاربونات) الصوديوم وجمع أيضا مع الأنزيم.
8- أضيف أنزيم HRP المنشط بالخطوات المـذكورة انفـا إلـى المـصل المـضاد المنقـى (Goat -Anti-Human-IgG) بوزن (10) ملغرام وتـرك بدرجة حرارة الغرفة مدة ساعة مع التحريك.
9- أضيف (100) مايكروليتر من محلول بوروهايدرايد الصوديوم وترك بدرجة حرارة الغرفة مدة ساعة ثم عمل له ديلزة مـع محلول دارئ الفوسفات الملحي لمدة 24 ساعة فـي درجـة حرارة 4 درجة مئوية.
10- أضيف الكليـسرول إلـى الحجـم النهـائي بنـسبة 50% (حجم/حجم) وحفظ في المجمدة.

مقارنة المقترن المحضر مع المقترن المستورد

المقارنة بتطبيق تقانة الأليزا:

أجريت مقارنة المقترن المحضر (Goat Anti-Human IgG) المعلم بانزيم HRP مع مقترن مستورد من عدة Anti-Cardiolipid)) والمتمثل بـ (Anti-Human-IgG) معلم بانزيم HRP.أخذت صفيحة دقيقة العيارية (صفيحة ألايزا) وأضيف (100) مايكروليتر لكل حفرة (16 حفرة بمعدل عمودين من الصفيحة) من الكلوبيولين المناعيIgG البشري بتركيز (40 مايكروغرام/مللتر) و حُضنت الصفيحة لمدة 24 ساعة في (4) درجات مئوية.و اضيف محلول EDC(محلول8) بحجم 100 مايكروليتر لكل حفرة وحضنت لمدة 24 ساعة في (4) درجات مئوية.و غسلت الحفر ثلاث مرات بمحلول الغسل الخاص بالعدة المستوردة واضيف (100) مايكروليتر من مصل الدم لمريض بالتهاب المفاصل مخفف (1/100) وحضنت الصفيحة مدة ساعة بدرجة حرارة الغرفة وغسلت ثلاث مرات بمحلول الغسل ثم اضيف (100) مايكروليتر من المقترن المحضر (لعمود فيه 8–حفر) واضيف (100) مايكروليتر من المقترن المستورد (العمود الثاني وفيه 8–حفر أيضا) وكان التخفيف للمقترن المحضر والمستورد 1/50. غسلت الحفر ثلاث مرات بمحلول الغسل لعدة مستوردة. واضيف (100) مايكروليتر من المادة الاساس (المحضرة بمحلول المادة الاساس الخاص بالعدة المستوردة) لكل حفرة وحضنت بالظلام لمدة (30) دقيقة وبدرجة حرارة الغرفة.ثم أضيف (100) مايكروليتر من محلول ايقاف التفاعل الخاص بالعدة المستوردة لكل حفرة. وتمت قراءة الكثافة الضوئية على طول موجي (492) نانوميتر باستخدام جهاز الألايزا (ELISA - Reader).

المقارنة بأستعمال الأطياف:

قورنت اطياف انتقالات فورير للأشعة تحـت الحمـراء (FTIR) للمقارنة بين المقترن المحضر والمستورد بتخفيف 1/50.
كذلك تم أستعمال أطياف الأشعة فوق البنفسجية (UV) للمقارنـة بين المقترن المحضر والمقترن المستورد بتخفيف 1/50.

النتائج والمناقشـة

تحضير المقترن (Conjugate) المتمثـل بمـضاد الكلوبيـولين المناعي IgG المعلم بأنزيم HRP:

تم تعليم مضاد الكلوبيولين المناعي IgG بانزيم HRP بطريقة الأكسدة بمادة بيرآيوديت الصوديوم $NaIO_4$ (14، 15). استخدمت مادة (CDNB) بدلا من كاشف سانكرFDNB لحجب مجموعة الامين للانزيم(HRP) (16) وبعدها استخدمت مادة بيرآيوديت الصوديوم $NaIO_4$ لغرض أكسدة مجموعة الكاربوهيدرات الموجودة بالأنزيم (17).
وعند اضافة الضد (Goat- Anti-Human IgG) تكونت قاعدة شيف (Shift base) على مجموعة الكاربوهيدرات للأنزيم أيضا مع الضد وعلى مجموعة الديهايد واحدة والمجموعة الثانية تختزل بوجود بوروهايدريد الصوديوم ($NaBH_4$) ليتكون مقترن مستقر ويمنع التحلل والزيادة من مادة $NaIO_4$ تؤدي الى تحويل مجاميع الالديهايد في الكاربوهيدرات للأنزيم الى مجاميع كاربوكسيلية تمنع ربط الضد بالأنزيم (17). وفضلت هذه الطريقة على الطرائق الاخرى لاستقرارية المقترن العالية عند تحضيره بهذه الطريقة وباستخدام مادة CDNB تم حجب مجموعة الامين وتحرر غاز HClالذي هو اقل سمية من غاز HF الذي يتحرر عند استخدام مادة FDNB ، ان تكوين قواعد شيف تعطي مجاميع غير مستقرة كمجموعة الكاربونيل لذا فأن اضافة بوروهايدرايد الصوديم تحول مجاميع الكاربونيل الى مجاميع الكاربوكسيل التي تعطي استقرارية للمقترن المحضر وقد تم دراسة استقرارية المنتج لمدة سنة والنتائج كانت جيدة مقارنة بالمستورد وأصبحت الميكانيكية كما هي مبينة في ملحق (1).

مقارنة المقترن المحضر مع المقترن المستورد

1– بتقانة الأليزا:

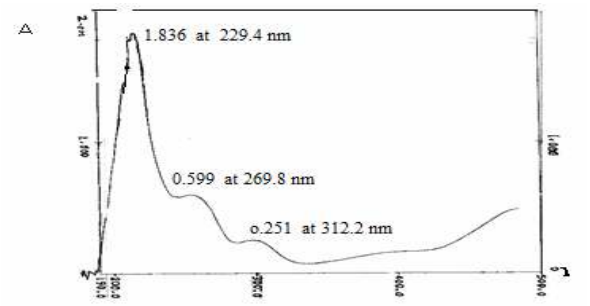
اجريت مقارنة بين المقترن المحضر والمقترن من شركة اسبانية (Biokit) لعدة Anti-Cardiolipid بتطبيق تقانة الألايزا واظهرت النتائج عدم وجود فرق معنوي (Not-significant) بالتحليل الاحصائي لاختبار (t-test) لمعدلات الكثافة الضوئية بجهاز الألايزا (ELISA-Reader) كما مبين في الجدول (1).

جدول رقم (1): التحليل الاحصائي لمعدل الكثافة الضوئية عند مقارنة المقترن المحضر مع المقترن المستورد باختبار (t-test)

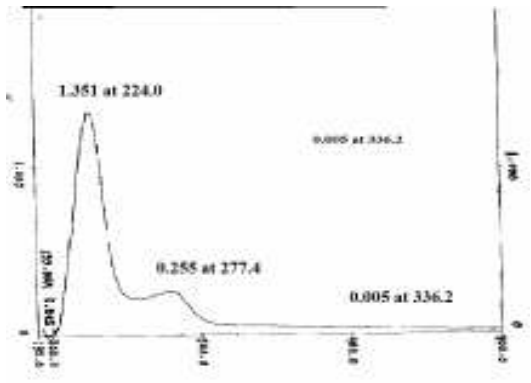
المقترن	معدل الكثافة الضوئية $\overline{X}$	الانحراف المعياريSD	الاحتمالية
المحضر	1.137	± 0.0073	0.05 < P
المستورد	1.207	± 0.00833	

2 – مقارنة المقترن المحضر مع المقترن المستورد طيفيا:

اجريت المقارنة الطيفية باستخدام اطياف الاشعة فوق البنفسجية U-V بين المقترن المحضر والمقترن المستورد من شركة (Biokit) الاسبانية لعدة (Anti-Cardiolipin) والمتمثل ب (HRP-Anti –Human IgG)واظهرت النتائج التطابق بينهما كما موضح ذلك بالشكل (1) والشكل (2).

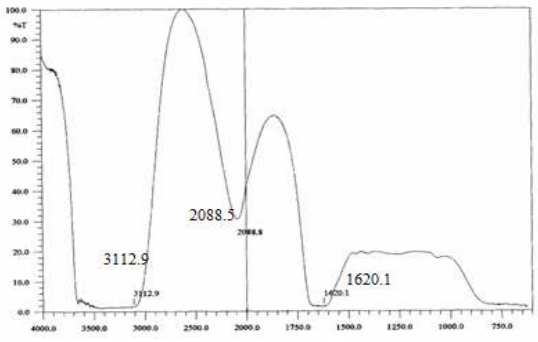


شكل رقم(1) :طيف [U-V] للمقترن المستورد (Anti - Human IgG-HRP)

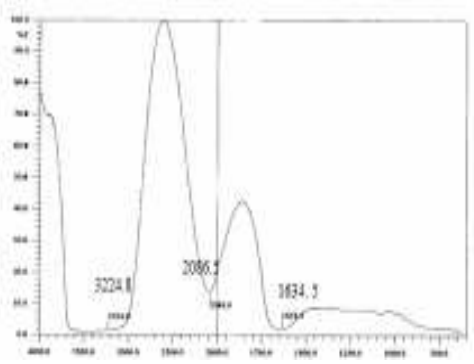


شكل رقم(2): طيف [U-V] للمقترن المحضر (Goat Anti - Human IgG-HRP)

من نتائج المقارنة بين المقترن المستورد والمقترن المحضر بالقياسات الطيفية تبين ان المقترن المحضر والمستورد لهما قمة رئيسية ضمن طيف الاشعة فوق البنفسجية اذ اعطى امتصاصية 1.836 عند طول موجي 229 نانوميتر بالنسبة للمقترن المستورد واعطى 1.351 عند طول موجي 229 نانوميتر للمقترن المحضر والفرق في الامتصاصية يعتمد على التركيز الا ان النتائج متقاربة جدا ، اضافة الى ان المقترن المحضر اعطى قمتين فقط للضد المرتبط والانزيم والمستورد اعطى اربع قمم اذ تشير القمة الثالثة الى وجود املاح لم يتم التخلص منها في المقترن المستورد واستخدمنا عملية الديلزة للتخلص من الاملاح التي استخدمت في طريقتنا لأعطاء استقرارية عالية للمقترن ، والقمة الاخيرة الرابعة التي ظهرت في المقترن المستورد والتي تظهر في المنطقة المرئية فأنها تشير الى الصبغة الحمراء المستخدمة
مع المقترن المستورد وفي المقترن المحضر اخذت القياسات الطيفية قبل اضافة الصبغة .
واظهرت نتائج اطياف انتقالات فورير للاشعة تحت الحمراء (FTIR) بين المقترن المستورد في شكل (3) والمقترن المحضر في شكل (4) انها متطابقة والمقترن المحضر اكثر نقاوة من المستورد كما توضحه صور القياسات الطيفية.



شكل رقم (3): انتقالات فورير للاشعة تحت الحمراء للمقترن المستورد



شكل رقم (4): انتقالات فورير للاشعة تحت الحمراء للمقترن المحضر

وتم تطبيق المنتج في مختبرات وزارة الصحة – مستشفى بغداد التعليمي.

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ملحق رقم (1) :ميكانيكية التحضير التي تم تطبيقها عند تحضير المقترن