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FORWARD

With well- established ambitious steps on continuing success way, IJST is coming for you all today in its recent issue of volume eight for year 2013.

Year after year, IJST proves its strength and faithful belief in developing our scientific communities among Arab World, especially in Iraq by giving an opportunity to all researchers to present their fruitful achievements in main vital fields to let all world knows that we are still the first leaders in civilized scientific life, despite all the unfortunate situations or constraints.

It is my pleasure to welcome you and present you a new issue of our Journal, Volume 8, No. 2 (2013), the second issue of this year, with diversity of researches and elite experts of the Editorial Board and Advisory Group. The members of Editorial Board, the ICAST and TSTC teamwork and I hope you will find this collection of research articles useful and informative.

*The journal is one of the scientific contributions offered by **the International Centre for Advancement of Sciences and Technology** in cooperation with **Treasure Est. for Scientific Training and Consultations** to the science and technology community (Arab region with specific focus on Iraq and International).*

Finally, on behalf of the International centre, I would like to express my gratitude and appreciation to the efforts of the Editorial Board, Advisory group with their valuable efforts in evaluating papers and the Editorial Board Secretary for 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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علــــــــــوم الأحياء الدقيقة

علــــــــــوم الغذاء

ENGLISH SECTION

Adsorption of Malachite Green and Nile blue from Aqueous Solution onto Bentonite Clay Surface

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ABSTRACT

The adsorption process of malachite Green and Nile blue from aqueous solution onto bentonite was studied. The results showed that the adsorption capacity of bentonite for the two dyes was 9.88 and 8.82 mg/g respectively.
The figure of the adsorption isotherms indicated L4 type isotherms according to the Giles classification.
The experimental adsorption results showed good correlation with the Langmuir and Ferundlich models. Experimental data indicated that the adsorption capacity of bentonite for the Malachite-Green dye was higher in basic rather than in acidic solution, whereas the Nile-blue was higher in neutral rather than in acidic solution, this was attributed to aggregation of two dyes in solution.
Thermodynamic studies indicated that the adsorption of Malachite-Green onto bentonite was an exothermic process, while the adsorption of Nile-blue onto bentonite was an endothermic process. The adsorption enthalpy (ΔH) for Malachite-Green and Nile-blue were calculated at -3.51 and +5.03 kJ/mole, respectively.

الملخص باللغة العربية

تم دراسة عملية امتزاز صبغة الملكات الخضراء وصبغة النيل الزرقاء من المحلول المائي باستخدام سطح طين البنتونايــت، حيــث بينت النتائج أن سعة امتزاز البنتونايت للصبغتين كانت 9.88 و 8.82 ملغم / غم على التوالي.
وأوضحت النتائج أن إيزوثيرمات الامتزاز على سطح طين البنتونايت كانت من النوع L_4 وفق تصنيف جيلز(Giles).
بينت نتائج الامتزاز التجريبية وجود علاقة جيدة مع علاقات Langmuir و Ferundlich، واتضح أن ســعة امتــزاز البنتونايــت لصبغة الملكات الخضراء كانت أعلى في المحلول القاعدي مما في المحلول الحامضي، بينما كانت لصبغة النيل الزرقاء أعلى فــي المحلول المتعادل مما في المحلول الحامضي، ويعزى ذلك الى تجمع الصبغتين في المحلول.
تشير الدراسات الديناميكية الحرارية أن امتزاز الملكات الخضراء على البنتونايت كان باعثا للحـرارة، في حين كان ماصا للحــرارة بالنسبة إلى صبغة النيل الزرقاء ، وتم حساب أنثالبي الامتزاز (ΔH) للــصبغتين فكانــت 3.51- kJ/mole لــصبغة الملكــات و 5.03kJ/mole+لصبغة النيل.

INTRODUCTION

Dyes and pigments have been used in many industries for coloration. Textile industry is one of the prominent pollutants, which release high concentrated effluent into the surrounding environments (1). Dyes contain carcinogenic materials, which can pose serious hazards to aquatic life as well as to the end users of water. Therefore, it is important to remove these pollutants before their final disposal.

One of the conventional methods for removal of dyes from wastewater is adsorption (2,3). Natural clay, such as bentonite, may be an adsorbent because of their abundance in most continents of the world and its low cost (4,5). Bentonite is mainly composed of montmorillonite, consists of layers of two tetrahedral silica sheets sandwiching one octahedral alumina sheet. A number of adsorbents have been tried for treatment of wastewater such as activated carbon (6), orange peel (7), palmfruit bunch (8), rice husk (9), sugar cane dust (10), Chitin (11), fly ash (12) and wool fibers (13).

The aim of the current study is to investigate the adsorption of reactive dyes by using bentonite clay, in addition to study the effect of initial dye concentration, contact time, pH and temperature.

MATERIALS AND METHODS

The absorbance measurements were carried out on an Apel-PD-303 UV-visible recording spectrophotometer whereas spectral of λ_{max} of dyes was carried out on a Shimadzu UV-visible 1700 double beam spectrophotometer using (1 cm) glass cells. Shaking Incubator (precision scientific/GCA, Chicago, Illinois, USA), Megafuge 1.0 Hero centrifuge and a digital pH-meter Hanna were used.

Preparation of bentonite clay (14) (adsorbent):
Bentonite clay used in this study was obtained from the general company of geological survey and mining, Baghdad, Iraq. The results of chemical analysis of bentonite are listed in table (1).

Table (1): The chemical analysis of bentonite

constituent	Wt%
SiO_2	70.86
Al_2O_3	14.1
Fe_2O_3	0.90
CaO	2.33
MgO	1.63
SO_3	0.31
K_2O	6.95
Total	**97.08**

Bentonite was washed with distilled water several times to remove water-soluble impurities. Then it was dried at temperature 378k for 24 hours then placed in desiccators to be used in the adsorption experiments. Two reactive dyes were selected, namely Malachite-Green (Merck) and Nile-blue (Fluka).The chemical structures and molecular weight of the dyes are illustrated in table (2).

Standard Malachite-Green solution 100 μg/ml)
This solution was prepared by dissolving 0.01 g of Malachite-Green in 100 ml of distilled water, working standard of Malachite-Green solutions were prepared by simple dilution of the appropriate volume of the standard Malachite-Green solution (100 μg/ml) with distilled water.
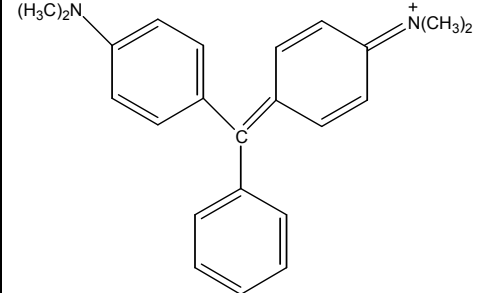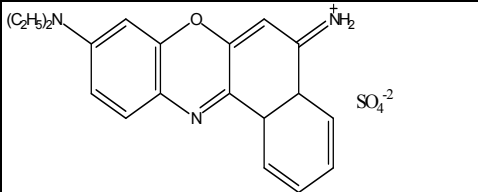
Standard Nile-blue solution (100 μg/ml)
This solution was prepared by dissolving 0.01 g of Nile-blue in 100 ml of distilled water, working standard of Nile-blue solutions were prepared by simple dilution of appropriate volume of the standard Nile-blue solution (100 μg/ml) with distilled water.

Calibration graphs of dyes
In to a series of ten calibrated flask, transfer increasing volume of Malachite-Green and Nile-blue dyes working solution 100 μg.ml^{-1}) to cover the range of the calibration curve. Linear calibration graphs of two dyes are obtained, that Beer's law is obeyed over the concentration range (2-20 ppm) with correlation coefficients (0.9951), (0.990) and intercept (0.1) and (0.04) for Malachite-Green and Nile-blue respectively.

Table (2): Structures, molecular weights and wavelengths of dyes

Dyes	Chemical Structure	Molecular mass	λmax (nm)
Malachite-Green		463	620
Nile-blue		415	631

Reactive dye adsorption isotherms procedure

Equilibrium adsorption isotherms for reactive dyes were undertaken at 25±1 C. A mass of 0.04 g of bentonite clay was added to 250 cm^3 conical flasks containing 20 cm^3 of reactive dye of varying concentrations 2-20 ppm. The flasks were placed in a thermo stated shaker for 90 minutes until equilibrium was achieved. The adsorption behavior of the dyes on the bentonite clay was studied at four temperatures (298, 303, 308 and 313 K).

Optical densities were determined at 620 nm for Malachite-Green and 631 nm for Nile-blue. Figures (1) and (2) are corresponding the maximum adsorption peaks of the two dyes.
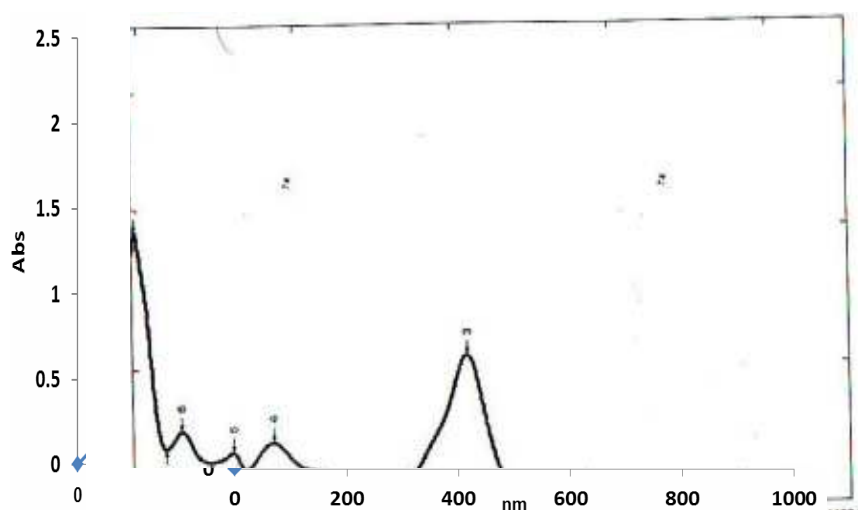
Fig. (1): Spectrum of Malachite-Green
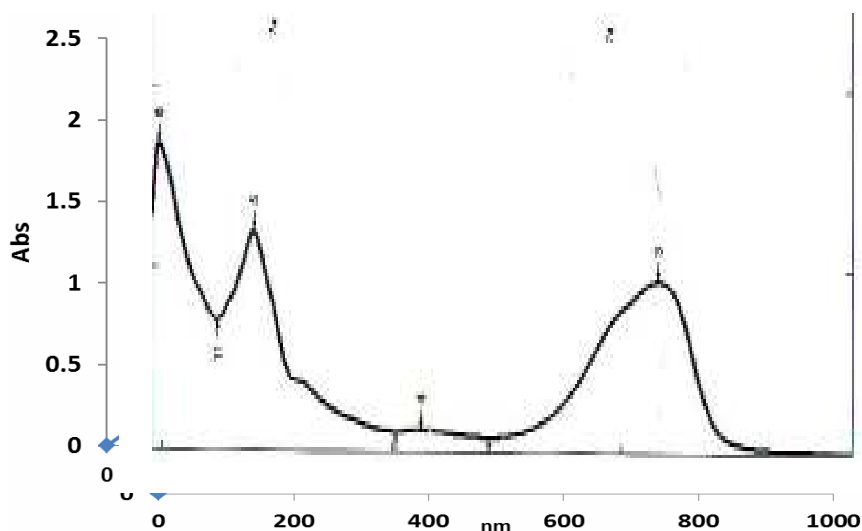
Fig. (2): Spectrum of Nile-blue

The adsorption equilibrium isotherms experiments were repeated in duplicate and the average values are reported. From the difference between initial concentration and equilibrium, the amounts of dyes adsorbed were calculated by the following relation:

$$Qe = \frac{(Co - Ce)\, V_{sol}}{M} \dots\dots\dots\dots\dots(1)$$

Where:
Qe is the adsorption capacity $(mg.g^{-1})$
Co and Ce are the initial and equilibrium concentration on $(mg.L^{-1})$ respectively.
M is the adsorbent dosage (g), V_{sol} is the solution volume (ml)

The effects of contact time, temperature, agitation rate, initial concentration of Malachite- Green and Nile- blue solutions and pH on the adsorption capacity have been determined. The equilibrium concentration and the adsorption capacity at equilibrium were determined to fit in the adsorption isotherms.

RESULTS AND DISCUSSION

Langmuir and Freundlich equilibrium isotherms models
The Freundlich and Langmuir models are the most frequently employed models. The Langmuir isotherms have been widely adopted to characterize the adsorption capacity of Malachite-Green and Nile-blue by using bentonite clay. Figure (3)
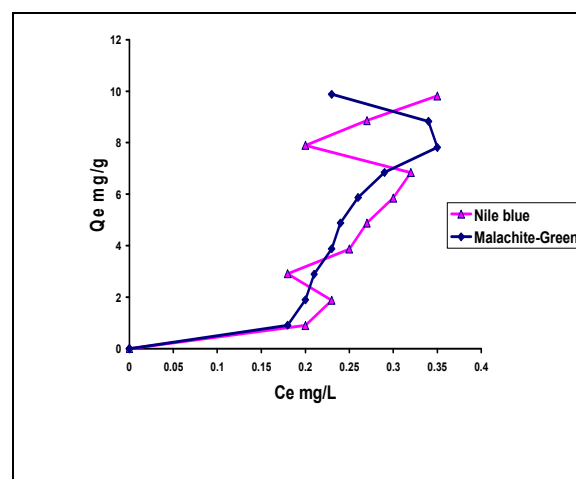
Fig. (3): Adsorption isotherms of Malachite-Green and Nile blue at pH 7and 9 respectively

The Feundlich isotherms has the general form such as (15):
$$Qe = K_f Ce^{1/n} \dots\dots\dots\dots\dots\dots\dots\dots (2)$$

This equation can be modified as:

$$Qe = \frac{Co - Ce}{M} = K_f Ce^{1/n} \dots\dots\dots (3)$$

Where: K_f is the adsorption capacity

   $1/n$ intensity of adsorption

The value of K_f and $1/n$ can be determined from the intercept and slope respectively of the logarithmic plot as shown in Table (3) and Figure (4).

Table (3): Model parameters obtained from fitting the experimental equilibrium data with isotherms model

Dyes	Freundlich isotherm				Langmuir isotherm			
	Ce (ppm)	Kf	n	r^2	Ce (ppm)	K_L *	a	r^2
Malachite-Green	2-20	0.1	0.33	0.915	2-20	7.14	12.49	0.915
Nile-blue	2-20	0.14	0.88	0.892	2-20	5.29	10	0.946

K_L represents the equilibrium adsorption constant, therefore higher values were indicative of a favorable adsorption process
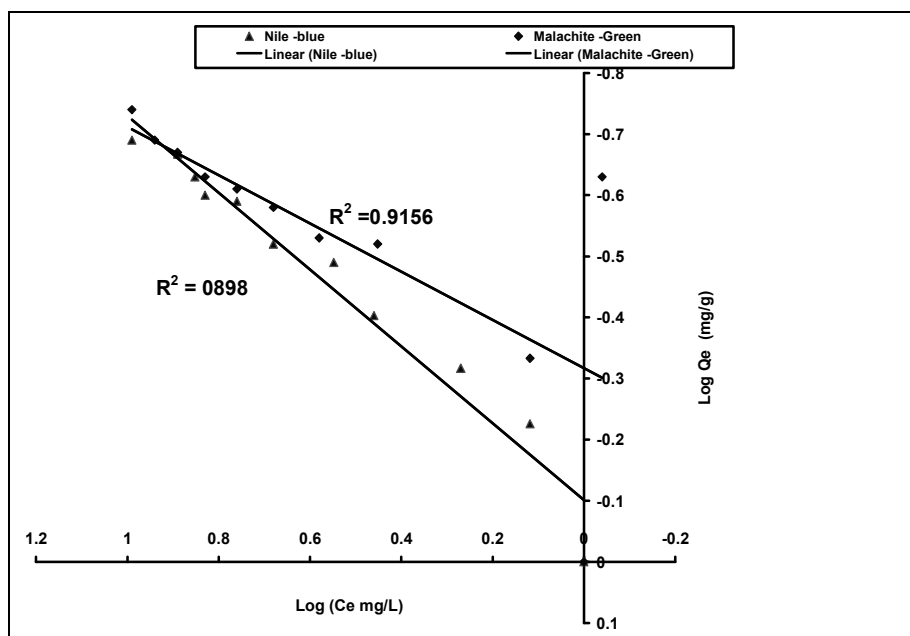
Fig. (4): Freundlich adsorption isotherm of Malachite-Green and Nile blue with bentonite at surface pH = 7 and 9 respectively

The linear Langmuir adsorption isotherms model can be represented by following relation:

$$Ce/q_e = 1/K_1 q_m + 1/q_m.Ce ……………………….. (4)$$

Where:
q_e is the amount of dye adsorbed at equilibrium $(mg.g^{-1})$

Ce is the equilibrium concentration of dye K_1 $(mg.L^{-1})$ and qm $(mg.g^{-1})$ are Langmuir constants.

The constants q_m and K_1 can be determined from the intercept and slope of the linear plot of experimental data of Ce/q_e against Ce. Table (3), Figure (5).
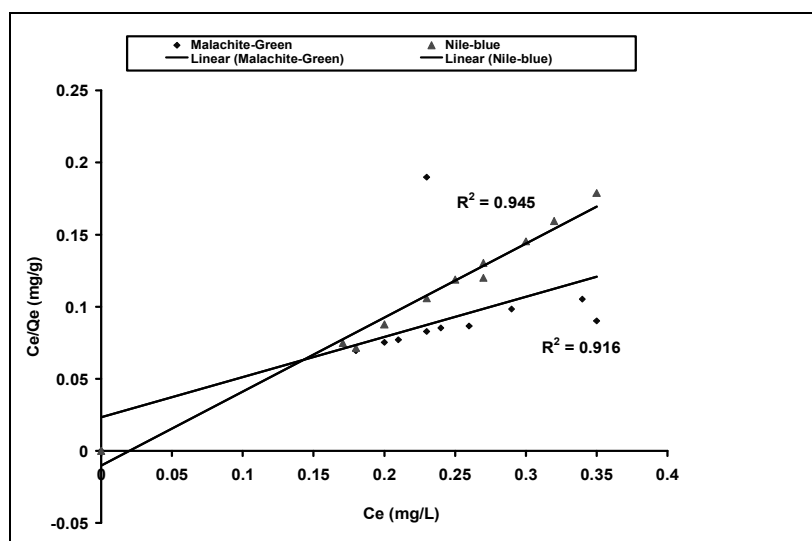
Fig. (5): Langmuir adsorption isotherm of Malachite-Green and Nile blue with bentonite at surface pH = 7 and 9 respectively

The correlation of the Malachite-Green and Nile-blue adsorption data was high, with r values of 0.9150 and 0.946 as shown in figure (5). From Table (3), the maximum adsorption values for the two dyes were 7.14 and 5.29 mmol/g for Malachite-Green and Nile-blue respectively. K_L represents the equilibrium adsorption constant, therefore higher values of K_L were indicative of a favorable adsorption process. By comparing the values of K_L, it can be concluded that adsorption of Malachite-Green was more favorable than that of Nile-blue.

Freundlich model
The r values of this model for two dyes are presented in tables (3), which indicate that this model showed lower correlation compared to the Langmuir model. Figure (4).
The n values for all studied adsorption systems were less than unit, which reflected the favorable adsorption of dyes in this study (16). Furthermore, the surface of bentonite clay highly heterogonous and the energies of active sites are highly variable, which would also tend to make the values of n less than unit (17, 18).

Effect of pH on dyes adsorption
The effect of solution pH on Malachite-Green and Nile-blue removal from solution was studied. Data are presented in figures (6) and (7), which indicate that the adsorption

behaviors of both dyes were similar from pH 4 to 9.
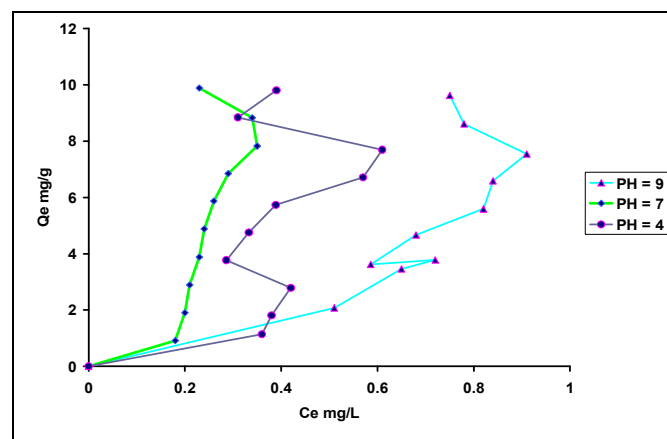
Fig. (6): Effect of solution pH of Malachite-Green adsorption on bentonite surface
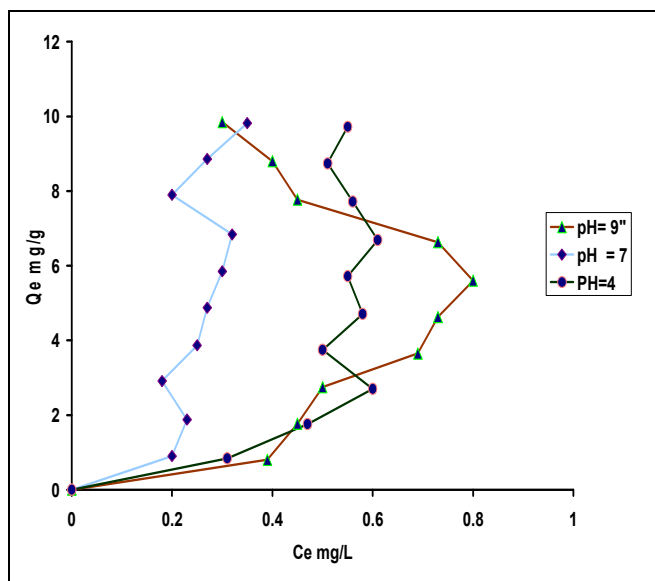
Fig. (7): Effect of solution pH of Nile blue adsorption on bentonite surface

Fig. (8): Adsorption capacity against contact time of Malachite-Green and Nile blue with bentonite surface

Similar adsorption behaviors with variations in solution pH had been reported in literatures (19).

The electrostatic interaction was the only mechanism for the dyes adsorption. The removal capacity should be at a maximum with the range of pH 7-9.

A number of intermolecular forces have been suggested to explain the aggregation; these forces include Vander Waals forces, ion-dipole forces, and dipole-dipole forces, which occur between dye molecules in solution (20).

Effect of contact time

The influence of the contact time on the adsorption of Malachite-Green and Nile blue by adsorbent bentonite clay was conducted through batch experiments to achieve the equilibrium as shown in figure (8). The mechanism of color removal can be described in migration of dye molecules from the solution to the adsorbent piratical and diffusion through surface. The results showed that the equilibrium time was reached within 90 min of operation for both adsorbates on bentonite clay. The adsorption capacity was contact thereafter for case of Malachite Green and Nile blue observed.

Adsorbent dosage

In order to study the effect of adsorbent dosage on Malachite -Green and Nile-blue removal as the adsorption capacity with fixed initial concentration of the type of dye, pH, and temperature and bentonite clay as an adsorbent. Different weight of dosage used (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11and 0.12g). The maximum removal of Malachite-Green and Nile-blue was observed with the dosage more than 0.03 g. 0.05 g used for all subsequence experiments.

Effect of temperature on dyes adsorption and thermodynamics

The adsorption of Malachite-Green and Nile-blue on bentonite clay was studied at temperatures of 298, 303, 308 and 313 K, with these adsorption isotherms being shown in figures (9) and (10).
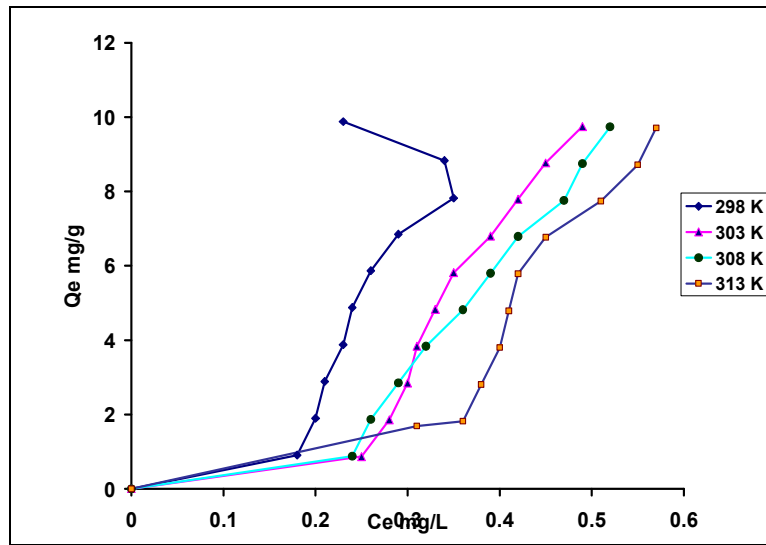
Fig. (9): Effect of temperatures on the adsorption capacity of Malachite-Green with bentonite surface at pH =7
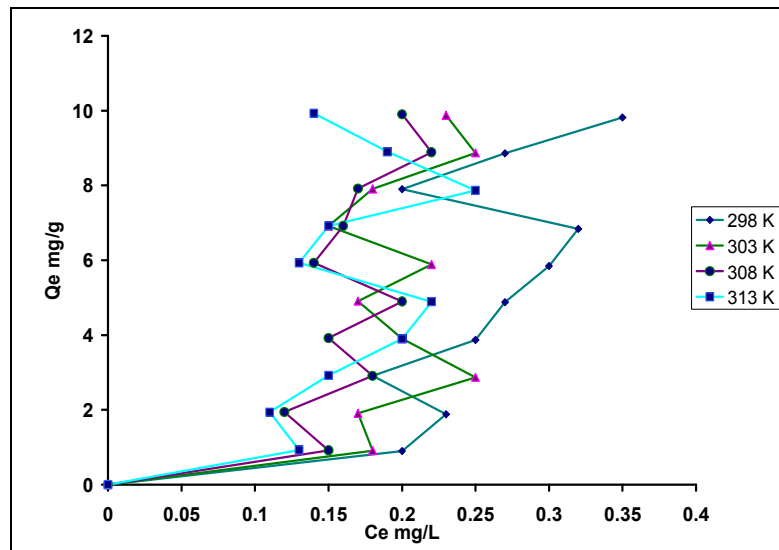
Fig. (10): Effect of temperatures on the adsorption capacity of Nile blue with bentonite surface at pH = 7

The free energy of adsorption (ΔG) was calculated from the following equations (21):

$\Delta G = -RT/\ln K_L$ ……………………………… (5)

Where:

K_L is the Langmuir constant at T; R is the gas constant (8.314 J/mol K). The apparent enthalpy of adsorption (ΔH) and entropy of adsorption, (ΔS), were calculated from adsorption data at different temperatures using the Van't Hoff equation (22):

$$\ln (K_L) = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$$ ……………….. (6)

Where:

K_L is the Langmuir constant and T is the solution temperature (K). The magnitude of ΔH and ΔS was calculated from the slope and y-intercept from the plot of $\ln K_L$ against $1/T$, with these thermodynamic parameters being given in table (4).

As shown in figures (9) and (10), the adsorption capacity of Malachite-Green dye decreased at higher temperatures, which indicate that the adsorption of dye in this system was exothermic process, while the adsorption capacity of Nile-blue dye increased at higher temperatures, which indicate that the adsorption of the dye in this system was an endothermic process. For both dyes, this may be attributed to increase penetration of reactive dyes inside micro pores at low temperatures or the creation of new active sites no at higher temperatures.

From thermodynamic parameter being given in table (4), figures (11) and (12), the adsorption of Malachite-Green and Nile-blue on bentonite clay appeared to be physical adsorption (23).

Table (4): Thermodynamic values of Malachite-Green and Nile-blue in aqueous solution using bentonite as an adsorbent

Dyes	ΔH (KJ.mol^{-1})	ΔG (KJ.mol^{-1})	ΔS (J.mol^{-1})
Malachite-Green	-3.51	-9.31	18.48
Nile-blue	+5.03	-8.25	44.57
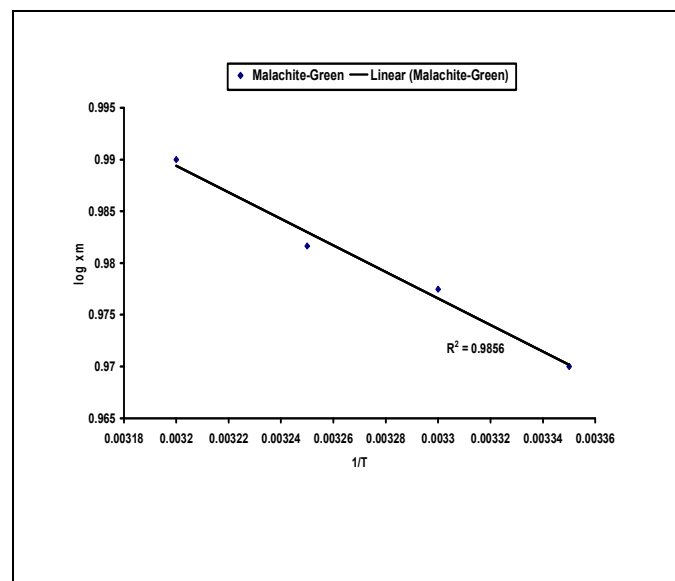
Fig. (11): Relationship between the log Xm and 1/T of Malachite-Green with bentonite surface as an absorbent
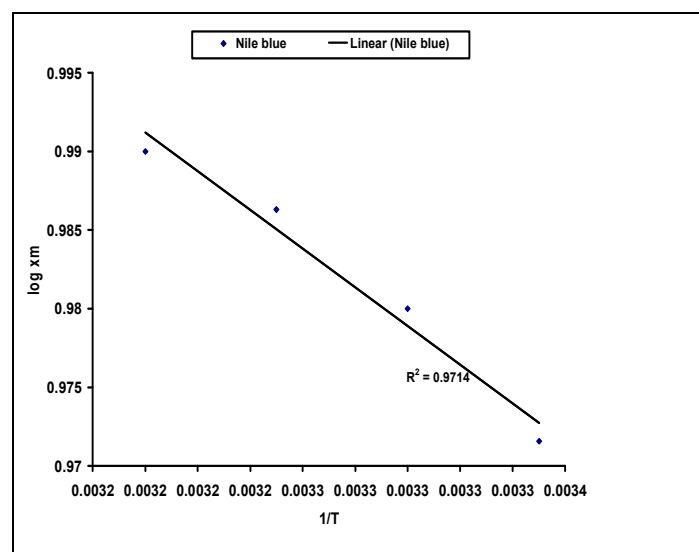
Fig. (12): Relationship between the log Xm and 1/T of Nile blue with bentonite surface as an absorbent

The positive value of Δs showed increase disorder at the solid /solution interface during the adsorption of the two dyes on bentonite clay. The adsorption increased randomness at the solid /solution interface with some structural changes in the adsorbent and an affinity of the adsorbent. The negative value of ΔG indicate the feasibility of the process and the spontaneous nature of the adsorption with a high preference of the   two dyes onto bentonite clay (24,25).

CONCLUSION

The bentonite clay is a good adsorbent for the removal of Malachite-Green and Nile-blue from a aqueous solution. Removal of two dyes are PH depends .The equilibrium adsorption is practically achieved through a time of 90 min.It was also a function of initial adsorbents dose, dye concentration and temperature of the solution. Also adsorption equilibrium data follows; Freundlich and Langmuir isotherm models. Various thermodynamic parameters such as enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) were evaluated.

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Effect of lannate pesticide and its residues in bell green pepper on human lymphocytes

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ABSTRACT

This study was conducted to detect the residual concentration of lannate pesticide and its genotoxic activity ,The results showed that the concentration of lannate in twenty grams of pepper tissue were 9750, 9100, 7450, 6500 and 5700 ppm respectively after (5hr and 5, 10, 15, 20 days respectively) of collecting samples for analysis that were previously sprayed with the recommended dose of lannate 1.5ml/L. The ability of this pesticide was studied for its possible genotoxic effects eventually in vitro, micronucleus (MN) formation and sister-chromatid exchange (SCE) induction in human lymphocytes was tested. The results of the MN analysis indicated that MN frequencies after treatment with lannate pesticide in concentrations between (0-2000) µg/ml, significantly differ from the control and its increase was between (1.3- 77.1) , even mice were used in other experiments in order to determine the formation of free radicals.

Key words: Sister-chromatid exchange (SCE); Micronucleus.

الملخص باللغة العربية

هدفت الدراسة الحالية إلى تحديد نسب متبقيات مبيد اللانيت وسميته الوراثية ، إذ أظهرت النتائج أن تركيز اللانيت في عشرين غرام من نسيج الفلفل الأخضر كانت 9750، 9100، 7450، 6500، 5700 جزء بالمليون على التوالي بعد فترات قطف الثمار لغرض التحليل (5 ساعات و 5 ، 10، 15, 20 يوم على التوالي) للفلفل الأخضر بعد الرش بالجرعة المحددة لمبيد اللانيت وهي 1.5مل/لتر. ودرست إمكانية هذا المبيد في إحداث السمية الجينية في نهاية المطاف مخبرياً، اجري فحص النويات الصغيرة (MN) micronucleus وفحص تبادل الكروماتيدات الشقيقة (SCE) المستحثة في الخلايا اللمفاوية البشرية وأظهرت النتائج بأن تكرار النويات الصغيرة (MN) بعد المعاملة بالمبيد عند التراكيز(0 – 2000) مايكروغرام / مل اختلفت معنويا عن نموذج السيطرة ومعدل الزيادة كان بين (77.1- 1.3) ، كما استعملت فئران التجارب في تجارب أخرى لغرض الكشف عن تكون الجذور الحرة.

INTRODUCTION

In recent years, pesticide toxicity has been extensively investigated. The reason for this is not only their important role in agriculture but especially findings that certain pesticides showed carcinogenic and mutagenic properties in laboratory animals and exposed humans(1). Carbamate pesticides are widely used in agriculture and home gardening. They are derivatives of carbamic acid and like organophosphates, their mechanism of action is that of inhibiting the vital enzyme acetyl cholinesterase which is reversible as compared to organophosphates which is irreversible (2). Exposure to cholinesterase inhibiting agents is considered a major health problem for the farm workers throughout the world. lannate belongs to the carbamate family and its active ingredient is methomyl, lannate is widely used throughout the world since it is effective as "contact pesticide" as well as "systemic pesticide"(3). lannate has been classified as a pesticide of category-I toxicity The present work focuses on the *in vitro* and *in vivo* analysis of genotoxic effects of Lannate using cytogenetic tests such as micronucleus assay, sister-chromatid exchange analysis in human lymphocytes in vitro and lipid peroxidation test .

MATERIALS AND METHODS

Sprying lannate*:*
Green peppers were cultured in green houses and sprayed with lannate (1.5 ml/L) when pests first appear, spraying was repeated as plants grew larger to ensure coverage and making sure that spraying should not exceed 7 days (4), samples were took for determination after 5hr, 5, 10, 15, 20 days and residues were measured by taking 20 grams from each sample of bell green peppers and gave concentrations(6500 ‹7450 ‹9100 ‹9750and 5700ppm) respectively using HPLC technique (5).

Blood Samples and Lannate:
Blood samples were obtained from two healthy nonsmokers without previous known contact with pesticides. The donors were between 18- and 22-year-old. Lannate were dissolved in sterile distilled water at defined concentrations either as pure substances or in 1:1 mixtures.

The test concentrations are below the limits of solubility for lannate (6).

Micronucleus (MN) Test in Human Lymphocytes in vitro:
Whole blood (0.5 ml) was added to 6.5 ml Ham's F-10 medium (Invitrogen), 1.5 ml foetal calf serum (Invitrogen) and 0.3 ml phytohaemagglutinin (Invitrogen) to stimulate cell division. Cultures were incubated at 37 ◦C for 72 h. The appropriate lannate were added 41 h after the start of the culture at final concentrations of 25-2000 µg/ml. Mitomycin-C (Sigma) at a final concentration of 0.5 µg/ml served as positive control. Three hours after the addition of the pesticide, i.e. at 44 h post-culture initiation, 6 µg /ml cytochalasin-B (Sigma) was added. Cells were collected by centrifugation at 72 h, fixed with freshly made methanol/acetic acid mixture (3:1 v/v) after mild hypotonic treatment, and stained with Giemsa (Fluka) (7). At least 1000 binucleate (BN) cells with preserved cytoplasm were scored, for each donor and for each case, in order to calculate the frequency of MN. Standard Criteria were used for scoring MN in at least at 2000 cells.

Sister-Chromatid Exchange (SCE) assay in Human Lymphocytes in Vitro:
Lymphocyte cultures from one healthy donor were set up as described for the MN assay. At the start of the culture, 5-bromodeoxyridine (5-BrdU) (Sigma) was added at a final concentration of 7.5 µg /ml. The appropriate chemicals were added 48 h after the initiation of the cultures at final concentrations of 25- 2000 µg/ml. Mitomycin-C (Sigma) at a final concentration of 0.1 µg /ml served as positive control. Cultures were incubated in the dark at 37 ◦C for 72 h and demecolcine (Gibco) at a final Concentration of 0.3 µg/ml was added 2 h before harvesting. Chromosome staining was performed according to the fluorescence-plus procedure, with minor modifications. Briefly, air-dried slides were immersed for 15 min in a solution of 0.5 µ g/ml (Sigma) in Sorensen's buffer, exposed for 30 min to UV radiation, washed and finally stained with Giemsa (Fluka) solution in Sorensen's buffer, pH 6.8, for 10 min. The frequency of SCE was evaluated in 50 second-division metaphases for each treatment. Calculating the replication index (RI) from 200 metaphases was the criterion for the determination of possible cytotoxic effects. The RI was given by equation: $RI = M1 + 2M2 + 3M3/N$, where M1, M2 and M3 denote those

metaphases corresponding to first, second and third or subsequent divisions, and *N* is the total number of metaphases scored (8).

Determination Of Free Radicals Through a Lipid Peroxidation Assay:

The mice were administered 4mg / kg body wt lannate for 30 days and effected. Control mice were received distilled water. All the mice were autopsied by cervical dislocation on day 31, 24 hours after the last oral dose. The liver of all mice was dissected out and processed for biochemical.

A bioassay is based on the principle of a chromogenic reaction of *N*-methyl-2-phenylindole (MPI) with malonedialdehyde (MDA) or 4-hydroxyalkenals at 45 °C, which forms a stable chromophore that can be detected by spectrophotometry at a 586 nm absorbance.

Initially, blood was removed from the tissue by immersion in a cold isotonic saline solution; then the tissue was weighed and homogenized in 0.02 M phosphate buffer, pH 7.4 (20/30% w/v). To prevent sample oxidation, 10 μL of 0.5 M butylated hydroxytoluene were added per each mL of homogenized tissue. Coarse tissue particles were removed by centrifugation (3,000 g for 10 min at 4 °C). Total protein levels were measured in a sample aliquot by the Bradford method (9), and the samples were immediately frozen at−70 °C until the assay was carried out. A volume of 650 μL of 10.3 nM MPI in a 1:3 mix of acetonitrile and methanol were added to 200 μL of sample in a microcentrifuge tube. The sample was smoothly mixed in a vortex and 150 μL of methanesulfonic acid were added, the mix was incubated at 45 °C for 1 h. Those samples showing sediment were centrifuged (15,000 g for 10 min). The supernatant was obtained and analyzed in a spectrophotometer at 586 nm.

Statistical Analysis:

The one-way ANOVA test and the Student's *t*-test of the Origin 7.0 software were applied to statistically analyze the results obtained with the different study groups.

RESULTS

This study was conducted to detect the residual concentration of lannate pesticide in 20grams of bell green pepper by collecting samples after 5hr and 5, 10, 15, 20 days after spraying

bell green peppers with the recommended dose of lannate (1.5ml/L) and its genotoxic activity. Table (1) showed that the concentration of lannate residue in twenty grams of pepper tissue were 9750, 9100, 7450, 6500 and 5700 ppm respectively.

Table (1): lannate residue until 20 days

Days	Pesticide residue (ppm)
5 hrs	9750
5	9100
10	7450
15	6500
20	5700

This pesticide was studied for its possible genotoxic effects with respect to the following cytogenetic end-points, in vitro micronucleus (MN) formation and sister-chromatid exchange (SCE). The ability of lannate to induce micronuclei in cytokinesis blocked cells is reflected in table (2). A positive influence in induced MN frequencies was attained at the highest concentration of lannate in compare with the controls. The results obtained from the SCE assays and the lymphocyte proliferation kinetics in table (3).

The results of the MN analysis indicate that MN frequencies after treatment with lannate pesticide in concentration between (0-2000) μg/ml, significantly differ from the control and its increase between (1.3- 77.1) with the increasing of lannate concentration.

Free radicals were measured by MDA synthesis in the tissue; a statistically significant difference was observed between the measurements of the exposed group and those of the vehicle control group, we found that the highest concentration of MDA was 4.78± 0.043 μm in exposed group in compare with the other groups vehicle and control group. Figure (1).

Table (2): Frequencies of sister-chromatid exchange (SCE) induced in human lymphocyte cultures *in vitro* showed statistically significant elevations of SCE at all concentrations tested (p < 0.05 to 0.001, respectively, ANOVA).

Concentration(μg/ml)	Metaphases scored	SCEs	RI
0	50	2±0.032 [a]	2.1
25	50	3±0.021 [b]	2.32
50	50	5±0.042 [c]	1.99
100	50	7±0.022 [d]	1.72
250	50	12±0.012 [e]	2.59
500	50	17± 0.033 [f]	2.39
1000	50	21±0.011 [g]	2.12
2000	50	24±0.017 [h]	2.45

Table (3): Lannate Induce Micronuclei

Concentration (μ g/ml)	BN cells scored	MN
0	2000	1.3
25	2000	7.5
50	2000	18.2
100	2000	36.5
250	2000	44.4
500	2000	59.6
1000	2000	64.4
2000	2000	77.2
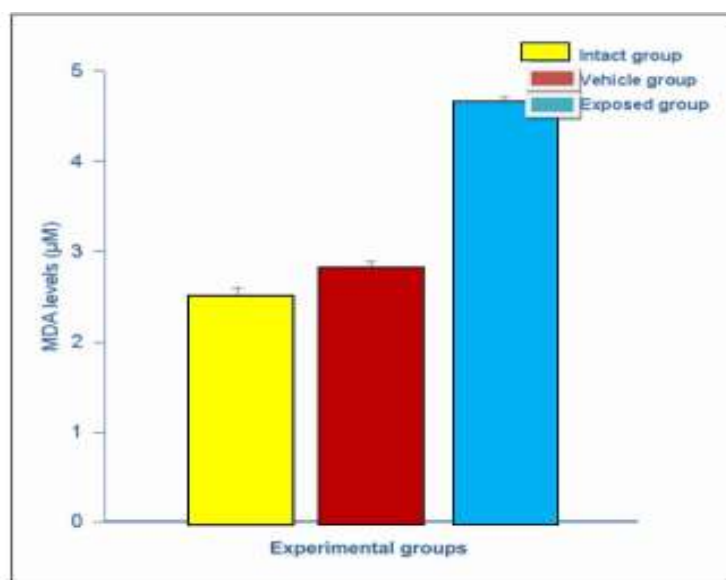
Fig (1): Lannate effects on lipid peroxidation in epithelial mammary cells. Values are significantly different from control groups at p < 0.05

DISCUSSION

Our results gave evidence that lannate residues till twenty days were very high and its ratio didn't agree with the Thai agricultural standards, 2008 which must be 0.07mg/ml. The genotoxicity of lannate in cultured human peripheral lymphocytes in the response of lymphocytes to lannate exposure was obtained in the SCE. SCEs have been commonly employed in cytogenetic studies, they are considered to be available and sensitive bioindicators or biomarkers of chemical exposure, but their prediction value for assessment of adverse health consequences has been limited (9). To our knowledge there is no information on these endpoints in human or animal cells exposed to this pesticide. The results presented here correspond to our previous finding from SCE assay with lannate treated human peripheral lymphocytes in vitro. In the SCE assay a corresponding dose dependence of induced chromosome aberrations increased with increasing lannate concentration, P < 0.05. Significant increase in MN frequencies after treatment with the highest dose of lannate (P < 0.01) was probably due to a toxicity exposure to pesticide. Lipid peroxidation is an identification of cell damage mechanism in plants and animals, and it is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and break up into diverse complex forms; peroxides from polyunsaturated fatty acids produce malondialdehyde and 4-hydroxyalkenals, which are used as lipid peroxidation indicators (10). The current study evaluates the influence of lannate on the free radicals production in mammary tissue in order to establish the induction of cellular damage caused by oxidative stress. The results showed a significant increment in the lipid peroxidation rate in mammary cell membranes on lannate exposed rats in comparison to animals from both control groups. It has been reported that due to their high persistence in the environment and their ability to accumulate in the adipose tissue, polyhalogen cyclic hydrocarbons, some organophosphate pesticides and chlorinated herbicides, produce toxic alterations (11), mainly related to lipid peroxidation processes, in which reactive oxygen species are involved. High serum MDA levels and an increment in MDA-DNA adducts formation in mammary tissue have been reported in women suffering from breast cancer (12). Chronic exposure to lannate, have shown to produce lipid peroxidation in different animal tissues, including human cells

(13). In the current work, adult rats chronically exposed to lannate showed a high lipid peroxidation rate in their mammary tissue, reflecting an oxidative stress condition. It is a well known fact that oxidative stress plays a very important role in the carcinogenesis process; also, some facts indicate that reactive oxygen species are involved in cancer early stages and in its progression (14).

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Extraction and purification of protease inhibitor from seeds of some plants and its antimicrobial activity

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ABSTRACT

The current study aimed to extraction and purification of the protease inhibitor and its antimicrobial activity. The protease inhibitor was extracted from *Lathyrus sativus* and it's inhibited the trypsin enzyme with maximum percent of inhibition (89.6%), the crude extract prepared in 0.1M potassium phosphate buffer, pH 7 with showed maximum inhibitor activity (98%). The optimum extraction ratio efficiency was founded when the extraction ratio 1:7.5 (w:v) after 1h the maximum inhibitor activity obtained was (96%). The antimicrobial activity of protease inhibitor was estimated against seven bacterial & fungal strains, the maximum inhibition zone estimated 28 mm against *Kluyveromyces marixians*. Protease inhibitor, isolated from *Lathyrus sativus* was purified by purification techniques, included ammonium sulphate percipitation 60%, followed by Ion exchange chromatography using DEAE–Cellulose column. The enzyme was purified to 2.5 times in the last step with an enzyme yield of 53.4%.

الملخص باللغة العربية

هدفت الدراسة الحالية إلى استخلاص مثبط إنزيم البروتييز وتنقيته ودراسة تأثيره ضد الميكروبي ، حيث استخلص مثبط إنزيم البروتييز من نبات الهرطمان الذي ثبط إنزيم التربسين بفعالية تثبيطية تقدر 89.6% ، استخلص المثبط باستخدام المحلول الدارئ لفوسفات البوتاسيوم بتركيز 0.1 مولر و(pH) 7، حيث كانت الفعالية التثبيطية لإنزيم التربسين 98% ، قدرت نسبة المحلول الدارئ المثلى المستخدم لاستخلاص المثبط بـ 1: 7.5 (و : ح) بعد ساعة واحدة من الاستخلاص ، حيث بلغت قيمة الفعالية التثبيطية للإنزيم 96% ، كما قدرت الفعالية الضد ميكروبية لمثبط البروتييز، إذا بلغ قطر منطقة التثبيط 28 ملم لخميرة *Kluyveromyces marixians* . تم تنقية المثبط البروتييزي بخطوتين: باستخدام التركيز بكبريتات الأمونيوم بنسبة إشباع 60% وكروماتوغرافيا التبادل الأيوني باستخدام عمود DEAE–Cellulose ، حيث بلغت عدد مرات التنقية للخطوة الأخيرة 2.5 بحصيلة إنزيمية بلغت 53.4 %.

INTRODUCTION

Protease inhibitors are proteins or peptides capable of inhibiting catalytic activities of proteolytic enzymes and are widely distributed in many plant materials used as food, especially in legumes, potato, and cereals (1). The protease inhibitors have been thought to play a vital role in the arsenal of defense mechanisms that plants use to protect against environment hazards during germination and seed growth. Pest and pathogens are major constraints to plant growth and development, resulting in heavy losses in crop yield and quality (2).

Plants are important sources of proteases and protease inhibitors. Possibly ten protease inhibitor families have been recognized in plants and mostly they are located in seeds and leaves (3). Plant protease inhibitors differ in specificities and in their ability to inhibit one or more proteases at the same time. Majority of them inhibit trypsin and many inhibit chymotrypsin. Inhibitors of elastase, kallikrein, plasmin, subtilisin and thrombin have also been found (4), legume seeds can be isolated as a source of protease inhibitor and are used in a variety of applications, such as medicine, agriculture and food technology (5).

Plants produce compounds that act as natural defenses against pests and pathogens. Anti-microbial peptides provide the first line of defense against invading microbes in both plants and animals. Peptides ranging from 15 to 40 amino acids in length, most of which are hydrophobic and cationic, are generally involved in innate immunity. Such peptides provide protection against bacteria, fungi and viruses by acting on the cell membranes of the pathogens (6,7). Protease inhibitors have recently received improved interest because of their ability to potently inhibit carcinogenesis in a wide variety of *in vivo* and *in vitro* systems (8). This phenomenon was first recorded in tomatoes infected with *Phytophthora infestans*, in which increased levels of trypsin and chymotrypsin inhibitors were found to be correlated with the plants resistance to the pathogen (9).The current study aimed to evaluate the protease inhibitor purification and application as antimicrobial activity against pathogenic microbes.

MATERIALS AND METHODS

Screening of plants for protease inhibitor

1. Plants seeds:
Plant seeds, which are locally available in market, include *Lathyrus sativus* , and *Vigna radiate*. Beans and Soybean were used as the source of material to screen for protease inhibitor activity.

2. Extraction and recovery of protease inhibitor:
Plant seeds for the study were milled by mill electrical device, a seed extract was prepared by homogenizing 1 g of each plant seed in 7.5 ml of 0.1M phosphate buffer with pH 7.0 by the mortar. The mixing was done at room temperature for 30 minutes. The slurry was centrifuged at 10,000 rpm for 15 minutes at 4°C for removing any cell debris that remains in the preparation (10). The clear supernatant obtained represented the crude extract, and was assayed for protease inhibitor activity.

3. Protease assay & Protein concentration:
The activities of the protease enzyme (Trypsin, Pepsin and Papain) were estimated according to the method described by (11) ,which depends on the decomposition soluble casein (1% of solution was prepared in 0.1 M sodium phosphate buffer, pH 7) to peptides and amino acids constituent by the enzyme. Enzymatic activity unit known as the amount of enzyme required to increase the absorbance by 0.01 per minute under standard conditions. The method of estimating protein was determined according to (12), depending on the standard curve of bovine serum albumin and using of coomassei blue G-250 at 595.

4. Remaining enzymes activity:
A crude extract of each seed plant was incubated separately with a known volume of protease enzymes (trypsin, pepsin and papain) by 1:1 ratio for 30 mins, there after the protease activity of each enzyme was estimated. The remaining activity is the percent inhibitory activity of the enzyme with respect to the percent enzyme activity without inhibitor, as shown in the following equation:

$$\text{Remaining Enzymes Activity} = \frac{\text{Enzyme activity with protease inhibitor}}{\text{Enzyme activity without protease inhibitor}} \times 100$$

Determination of optimum condition for extraction the protease inhibitor

1. Buffer Extraction:
Different types of buffers were selected for extraction of the protease inhibitors included, sodium acetate buffer pH (5,6) , potassium phosphate buffer pH (7,8) , tris base buffer pH (9) at concentration of 0.1 and 0.2 M separately.

2. Extraction ratio and optimum period for extraction:
Different ratios of potassium phosphate buffer were selected to extract inhibitor included 1:5, 1:7.5, 1:10 and 1:12.5 (w:v) with a different period included a 30 min and 1hr separately.

Determination the antimicrobial activity of the protease inhibitor against the local pathogenic isolate
The antimicrobial activity of protease inhibitor against (*E.coli, Staphyllocoocus aureus, Pseudomonas aeroginosa, Candida albicans, Candida tropicalis, Kluyveromyces marixians, Aspergillus niger*) isolates were determined according to the using agar diffusion method described by (13).

Purification of Enzyme Inhibitor
Protease inhibitor, isolated from *Lathyrus sativus* was purified by protein purification technique, included ammonium sulphate precipitation, followed by Ion exchange chromatography.

1. Ammonium sulfate concentration:
The concentration step of protease inhibitor was accomplished by addition of various concentrations ratios from ammonium sulfate to the crude extract included (30, 60 and 90) % separately, to getting better saturation ratio in precipetation and concentration of the enzyme inhibitor product from *Lathyrus sativus*.

2. Ion exchange chromatography:
Ion exchanger diethyl amino ethyl cellulose (DEAD-Cellulose) was prepared according to Whitaker (14) by addition 8 ml of concentrated extract produced from ammonium sulfate precipitation step by saturation 60% to ion exchanger column with dimensions of 3.5 x18 cm. Then the column was washed by 0.02M phosphate buffer, pH 7.6 with flow rate (30 ml / h). The fractions from column were collected with 3 ml / fraction. The elution was done by using 0.02M phosphate buffer, pH 7.6 with a linear gradual of sodium chloride between 0.1-1 M with fast flow (30 ml / h). The product solution was collected with 3ml / fraction and the absorbance was measured at 280 nm. The active parts were collected, and the protease inhibitor activity estimated as well as protein concentration and size.

RESULTS AND DISCUSSION

Screening of plants for protease inhibitor:
Results presented in (Table 1) showed that the *Lathyrus sativus* have maximum percent of inhibition (89.8%) against trypsin, followed by Bean (89.2%), Soybean (87.6%) and *Vigna radiate* (83.3%). The *Lathyrus sativus* seed had the large quantities of trypsin inhibitors. The most frequently occurring antinutritional substances in *Lathyrus sativus* are protease and amylase inhibitors, lectins, tannins, saponines (15).

Table (1): Screening of plants for protease inhibitor

Inhibitor activity %	Enzyme Activity with Inhibitor (U/ml)	Enzyme Activity without Inhibitor (U/ml)	Enzyme	Plant Source
0	2.8	1.4	Papain	*Lathyrus*
61	1.1	2.8	Pepsin	*sativus*
89.8	1.65	16.1	Trypsin	
0	4.35	1.4	Papain	Bean
36	1.8	2.8	Pepsin	
89.2	1.75	16.1	Trypsin	
0	2.25	1.4	Papain	Soybean
54	1.3	2.8	Pepsin	
87.6	2	16.1	Trypsin	
0	5.9	1.4	Papain	*Vigna radiate*
30.4	1.95	2.8	Pepsin	
83.3	2.7	16.1	Trypsin	

Determination of optimum condition for extraction protease inhibitor:

1. Buffer Extraction:
Among the various extraction buffer evaluated for recovering protease inhibitor molecules from plant sources, the crude extract prepared in 0.1M potassium phosphate buffer, pH 7 with showed maximum residual enzymes activity 2% and` followed by 0.2M tris buffer pH 9 with 3.3% (Fig.1). While trypsin inhibitor by the extract prepared in sodium acetate buffer pH (5,6) with (0.1,0.2) M and potassium phosphate buffer pH 8 with (0.1,0.2) M and tris buffer pH 9 with 0.1M showed low enzyme activity compared to that prepared in potassium phosphate buffer pH 7 with 0.1M. The plant crude extract prepared in phosphate buffer showed maximum protease inhibitor activity followed by that prepared in distilled water (10).
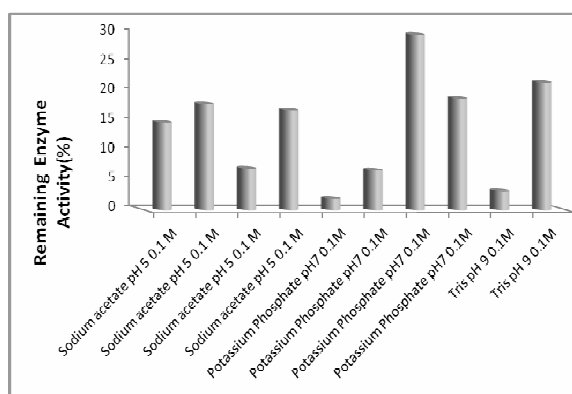
Fig. (1): Extraction of Protease Inhibitor in Different Buffers

2. Extraction ratio and optimum period for extraction:
The effect of buffer ratio for extraction and time on the recovery of trypsin inhibitor from *Lathyrus sativus* seed was showed in (Figure 2). The optimum extraction ratio efficiency was found when the extraction ratio 1:7.5 (w:v) with 1h. Maximum remaining enzymes activity obtained was (0.7%). Followed by extraction ratio 1:7.5 (w:v) with 30 min which have (2%) residual activity. It was reported that an extraction time of 1hr was found to be optimal for the extraction of trypsin inhibitor, inhibitor activity of plant decreased with decreasing the extraction time. High mechanical shear generated by shaking can cause denaturation of protein (16). An increased extraction time resulted in the protein, especially trypsin inhibitor, being more dissolved. The inhibitory activity was slightly increased for plant with 1:10 ml in (30 min and 1 hr) respectively, it have (4.4, 3.1) % of residual enzymes activity. However, a decrease in inhibitory activity was (23, 19.3)% respectively when the ratio of phosphate buffer is 1:5 ml with time of (30 min and 1 h) that indicating the lesser stability of trypsin inhibitor from *Lathyrus sativus*. This contributed to the loss of inhibitor activity (Fig.2). Also, the incorporation of air bubbles and the adsorption of protein molecules to the air–liquid interface can cause the denaturation of protein (17). Therefore, the extraction time of 1 h was selected for the extraction of trypsin inhibitor from *Lathyrus sativus*.
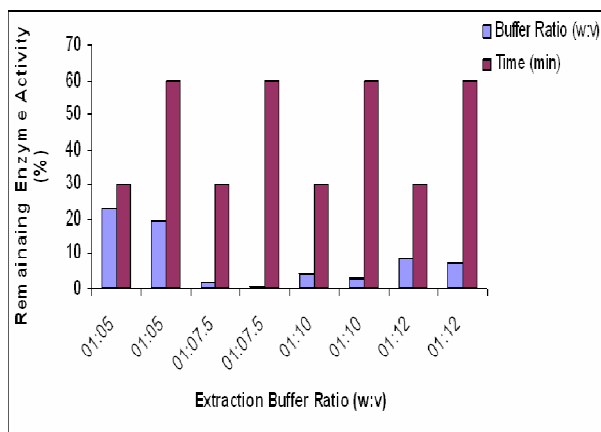
Fig. (2): Determination extraction ratio and optimum period for extraction

Table (2): Determination the antimicrobial activity of the protease inhibitor against the local pathogenic isolates

Strain	Diameter of zone of inhibition(mm)
Staphyllocoocus aureus	21
Pseudomonas aeroginosa	17.5
E.coli	18
Kluyveromyces marixians	28
Aspergillus niger	17
Candida albicans	18.5
Candida tropicalis	23.5

Determination the antimicrobial activity of the protease inhibitor against local pathogenic isolates:

The antimicrobial activity of protease inhibitor was tested against seven bacterial & fungal strains include (*E.coli, Staphyllocoocus aureus, Pseudomonas aeroginosa, Candida albicans, Candida tropicalis, Kluyveromyces marixians, Aspergillus niger*) by agar well diffusion method. After incubation of plates at 37°C for 24h the diameters of the inhibition zones were measured (Table .2). As shown in Fig.3, protease inhibitor inhibited the growth of *Kluyveromyces marixians*, *Candida tropicalis* and *Staphyllocoocus aureus* but have low activity against *Pseudomonas aeroginosa*, *E. coli* and other strains. Protease inhibitors in plant tubers and seeds are generally thought to serve as storage proteins and to act in the defense against insects and microorganisms (18). They are also known to possess potent antibiotic activity against bacteria, fungi, and even certain viruses (19 ,20).

Fig. (3): Antimicrobial activity of protease inhibitor against pathogenic isolates :(A) *Pseudomonas aeroginosa,* **(B)** *Kluyveromyces marixians*

Purification of Protease Inhibitor:
Following the protein purification technique including ammonium sulphate precipitation, ion exchange chromatography using DEAE Cellulose.

1. Ammonium sulfate concentration:
As a first step towards the purification of protease inhibitor, the crude buffer extract was subjected to ammonium sulphate precipitation and concentration of ammonium sulphate required for complete precipitation of inhibitor was standardized. The protease inhibitor precipitated at (30, 60, 90) % (w/v) saturation of ammonium sulphate. However, the fraction, obtained with 60% (w/v) saturation was found to be efficient for precipitating the protease inhibitor compared to other fractions (Table 3). The complete precipitation was done using ammonium sulphate concentration of 60% (w/v) saturation and the precipitated fractions were used for further studies. This purification step resulted in the increase specific inhibitory activity of 15.3 U/ mg protein with a purification fold up to 2 and 59.4% yeild. Ammonium sulfate was used to advantage by of high solubility and low cost compared with other organic solvents and the lack of impact on the pH and the stability of the enzyme. Concentration depends on the principle of ammonium sulfate charge equation on the surface of the protein and prejudice layer of water surrounding it, which leads to the deposition impact of salting out (21)

2. Ion exchange chromatography:
Eight ml of the ammonium sulphate fraction was loaded onto a column of DEAE-cellulose (1.75×18 cm) equilibrated with 0.02M phosphate buffer, pH 7.6 at room temperature. The column was washed with 225 ml of the equilibration buffer at a flow rate of 30 ml h-1 and the washings containing the inactive proteins were discarded. The column was then eluted with a linear gradient (0.1-1) N of NaCl formed by 0.02M of phosphate buffer pH 7.6 (22). The results presented in (Figure 4) showed that a single protein peak demonstrated in wash step without any inhibitor activity while separated five peaks in eluted step, four eluted peaks had no inhibitor activity while the last eluted peak had inhibitor activity (Fig.4). These results explain that the protease inhibitor had negative charge deposit of DEAE-column charge. Purification steps resulted in the increase in specific inhibitory activity of 19.2 U / mg protein with a purification fold up to

2.5 and 53.4% yield (Table 3), the active fractions of this peak were pooled and concentrated. Ion exchanger DEAE-Cellulose has many of the desirable properties, a broad absorption , high capability in separation , exchange capacity (High Capacity) , the possibility of re-activated and used a number of times (23).
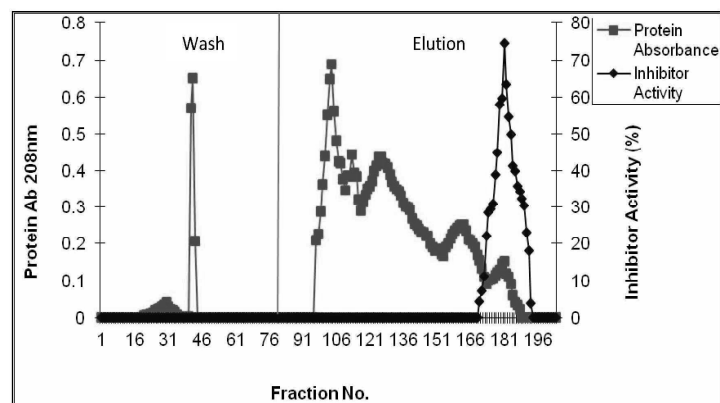
Fig .(4): Ion exchange column to purify the protease inhibitor from *Lathyrus sativus* by using DEAE-Cellulose with dimensions of 3.5 x18 cm. The column was washed by 0.02M phosphate buffer, pH 7.6. Elution was done with a linear gradient of sodium chloride between 0.1-1 M with fast flow (30 ml / h) the with 3ml / fraction

Table (3): Purification of Trypsin Inhibitor from *Lathyrus sativus*

Step	Volume ml	Activity U/ml	Protein Mg/ml	Specific Activity U/mg	Total Activity U	Purification Fold	Yield %
Crude	25	3.1	0.4	7.8	77.5	1	100
Ammonium Sulphate Saturation (60%)	5	9.2	0.6	15.3	46	2	59.4
DEAE-cellulose	18	2.3	0.12	19.2	41.4	2.5	53.4

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Inhibitory Effect of Camel Urine on Neoplastic and Transformed Cell Lines

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ABSTRACT

The cytotoxic effect of different concentrations of camel urine on two types of cell lines (RD, L20B) for different exposure time (24 , 48 and 72 hrs) was examined. Cytotoxicity assay of this study demonstrated that camel urine caused inhibitory effect on the growth of RD cell lines at high concentration (250, 500 µg/ml) for all exposure time. While the inhibitory activity was gradually reduced with decreasing of concentration. Moreover, Camel urine showed less inhibitory effect on transformed cell (L20B), compared with their effect on cancer cell line, indicating of the safety camel urine toward non malignant cells.

الملخص باللغة العربية

تم اختبار التأثير السمي لتراكيز مختلفة من بول الابل في نوعين من الخطوط الخلوية (RD , L20B) ضــمن اوقات تعريضية مختلفة (24 ، 48 ، 72) ساعة. اظهرت النتائج الاحصائية التي تم الحصول عليها التأثير السمي لبول الابل علــى خطوط خلايا RD النامية عند التراكيز العالية (250 ، 500 مايكروغرام / مل) ولاوقات التعريض جميعها ، في حين قل التـــاثير التثبيطي تدريجياً مع انخفاض التركيز المستعمل ، بينما سجل اثر تثبيطي اقل لبول الابل على الخلايــا المتحولــة (L20B) ، ممــا يؤشر التأثير غير الضار لبول الابل على الخلايا غير الخبيثة.

INTRODUCTION

Cancer is a disease where damaged cells of the patient's body do not undergo programmed cell death, but their growth is no longer controlled and their metabolism is altered (1). Next to ischemic heart disease cancer is a major cause of death in most developed countries (1, 2).

Management of cancer is one of the challenging problems in medical practice as there are no available medical modalities that can selectively kill cancer cell without adverse effect on normal living cell or the functions of vital organs (3). Conventional therapies had limited benefits as treatment of cancer due to the resistance, toxicities relapse problems, therefore researches are necessary to find alternative effective, safe and in expressive therapies (4). Natural products play an important role in our healthcare system (5). They offer a valuable source of potent compounds with wide variety of biological activities and novel structures and provide important leads for the development of novel drugs (6 , 7).

Camel urine is used in folklore medicine to treat cancer. The Bedouin in the Arab desert give camel urine to patients who where suffering from cancer (8). Camel urine can be classified as environmentally friendly inhibitor, because drinking of camel urine for the therapeutic purpose was indicated since 14 century (9). It has been shown throughout the history of medical science till today that urine has a profound medical uses (10), such as effectiveness against all ergies, psoriasis and all skin problems. Also (11) reported the effect of urine on fertility, fever, burns and tuberculosis. Microbiological studies (12, 13) on camel urine proved its high efficiency against a number of pathogenic microbes when compared with some antibiotics.

The camel urine has also been qualified as anti-inflammatory antioxidant and anti-tumor (14, 15). The best approach to evaluate the effect of a new material is *in vitro* by utilizing the growing mammalian cells at tissue culture level and not on the living organism (16 , 17). For this purpose, this study was designed to evaluate the anticancer effects of the camel urine on cancer cell lines *in vitro*, through different exposure time and different concentration of camel urine.

MATERIALS AND METHODS

Urine samples:

Urine of camel samples was collected from young male camels (one humped) in the morning before provender breakfast (foodstuff and desert plants). The samples were collected in sterile screw bottles kept in cool boxes until transported to the laboratory. Physically, the fresh extracted urine appears clear amber yellow and watery. The different concentrations of camel urine were prepared and kept at (4 ˚C) until being used.

Cell line preparation for cytotoxicity study:

The cell lines, rhabdomysarcom (RD) and murine L cells (fibroblast) expressing (L20B), that used in this study were supplied by tissue culture unit biotechnology research center / Al-Nahrin University, and maintained in RPMI-1640. when the cells in flask form a confluent monolayer, the following protocols were performed (18). The growth medium was decanted off and the cell sheet washed twice with PBS. Two to three ml of sterile trypsin-versene were added to the cells sheet and the flask rocked gently. After approximately 30 seconds most of the trypsin was poured off and the cells incubated at 37˚C until they had detached from the flask. Cells were further dispensed by pipetting in growth medium.

Afterwards, 200 µl of cells in growth medium were added to each well of a sterile 96-well micro titration plate. The plates were sealed with a self – adhesive film, lid placed on and incubated at 37˚C.

When the cells are in exponential growth at near confluence mostly after 24hrs, the medium was removed and serial dilutions of camel urine (7.81 , 15.62 , 31.25 , 62.5 , 125 , 250 and 500 µg/ml) were added to the wells. Three replicates were used for each concentration, then, the plates were re-incubated at 37˚Cfor the selected exposure times (24 , 48 and 72 hrs).

Cytotoxicity assay:

After the end of the exposure period, the medium decanted off and cells in the wells gently washed by adding and removing 0.1 ml sterile PBS two times. Finally 0.1 ml of maintenance medium added in each well and incubated for further 24 hrs.

At the end of recovery time, the maintenance medium, threw away, and replaced by 50 µl of 0.01 % crystal violet dye.

After 20 mins., the stain was washed gently with tap water for three times. The plate was left until become dry.

The optical density of each well was read by using a micro-ELISA reader at 492 nm transmitting wave length (18). The percentage of inhibition was calculated according to the following equation (19).

Inhibition % =[(optical density of control wells – optical density of test wells) / optical density of control wells] x 100.

Statistical analysis:
Experimental data for cytotoxicity assays were analyzed using statistical software SPSS 10.0 for windows. Significance between control and samples was determined using student's t-test. P value ≤ 0.05 was considered statistically significant.

RESULTS

Cytotoxic effect of different concentrations of camel urine on two types of cell lines (RD and L20B) for different exposure time (24 , 48 and 72 hrs.) was examined. The results in figure (1) showed significant effect (P<0.05) at the concentration, (250 and 500 µg/ml) during 24 , 48 and 72 hours of exposures.
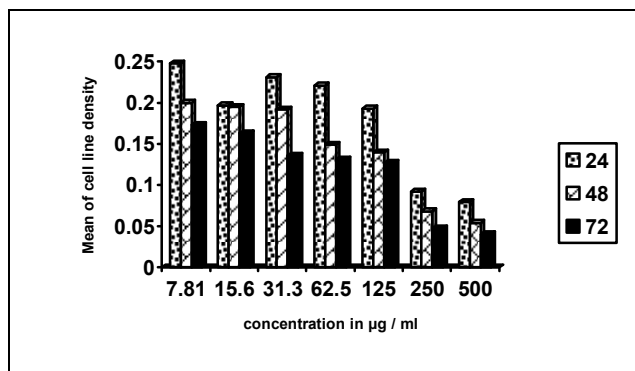
Fig.(1): Effect of different concentration of camel urine on RD cell line during different exposure period (P<0.05).

Also there is significant effect (P<0.05) for the concentration (125 µg / ml), at 24 and 48 hrs), and 62.5 µg / ml at 72 hrs., where the other concentrations showed non-significant effect during all the periods of exposures.

The effect of camel urine on L20B cell line was demonstrated in figure (2), most concentrations of camel urine had no significant effects on the growth of L20B cell line (P<0.05). Only the treatment with concentration (250 µg / ml at 72 hrs) caused significant effect (P<0.05) in cell growth.
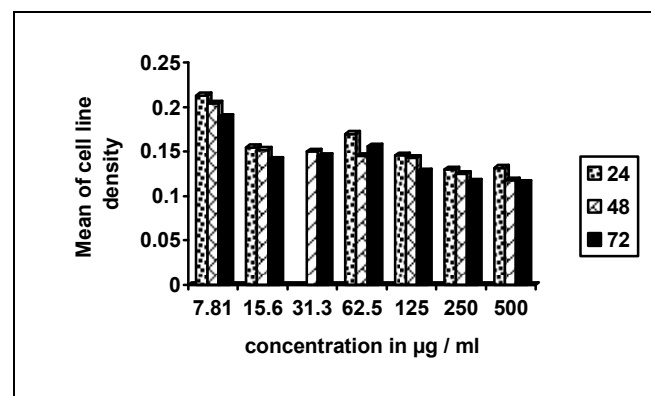
Fig.(2): Effect of different concentration of camel urine on L20B cell line during different exposure periods (P<0.05).

The results in table (1) showed that the best exposure period was 72 hrs. than the other periods (24 and 48 hrs.). The growth inhibition percentage for camel urine on RD cell line at 500 µg / ml concentrations was 67.36% , 73% and 78.02 % after 24, 48 and 72 hours respectively. The results also showed the growth inhibition percentage for camel urine on L20B cell line, by the same concentration was 14.28% , 28.48% and 25.32% after 24, 48 and 72 hours respectively.

In the other hand, the growth inhibition percentages for the camel urine on RD cell line at 250 µg / ml concentration was 61.98%, 67.62% and 74.81% after 24,48 and 72 hours respectively, while the growth inhibition percentage for camel urine on L20B cell line at the same concentration was 15.58% , 28.24% and 24.68% after 24 , 48 and 72 hours respectively.

The results in table (1) revealed that RD cell line was more sensitive to the camel urine after period of exposure.

Table (1): Percentage of inhibition of RD and L20B cell lines by using different concentrations of camel urine during three periods exposure

Type of cell line	Con. (µg / ml)	Inhibition (%)		
		24 hrs	48 hrs	72 hrs
RD	7.81	---	4.76	4.95
	15.62	12.6	7.14	10.44
	31.25	4.55	8.57	25.44
	62.5	8.68	29.05	28.02*
	125	20.45*	33.33	30.22*
	250	61.98*	67.62*	74.18*
	500	67.36*	73*	78.02*
L20B	7.81	---	---	---
	15.62	---	7.88	8.44
	31.25	0.65	9.09	5.84
	62.5	---	12.12	---
	125	5.19	13.13	16.88
	250	15.58	24.24	24.68*
	500	14.28	28.48	25.32

* Significant effect (P≤0.05)
--- No effect

DISCUSSION

The results of the present study clearly indicate the cytotoxic activity of camel urine on cancer cell line (RD). These results were in harmony with the results of Khorshid,2009 (20), which indicated that the camel urine exerted antiproliferative action and growth inhibition on various cancer cell lines including lung cancer and leukemia by inducing apoptosis without adverse effect on normal cell line.

The chemical composition of camel urine showed the presence of purine bases, hypoxanthin, sodium, potassium, creatinine. urea, uric acid and phosphates (21). Some compounds similar to the ingredients present in common urine such as purine bases, urea, creatinine and hypoxanthine are known to exhibit cytotoxic and antitumor activity in different animals and human tumors and cell lines (22 , 23). In a recent study, camel urine was reported to inhibit the formation of C-tumor in *Allium cepa* root tip cells after long treatment with colchicines (8). Camel urine treatment was found to cause a significant cytotoxic effect in the bone marrow cells of mice without induced any clastogenicity (24). Also (26 , 27) observed that the bioactive fraction has been isolated from camel urine was the most highly cytotoxic against A549 human lung cancer cells. On the other hand,

the biologically active peptides and major protein fraction of human urine were found to be cytotoxic against human leukemic cells, Ostesarcoma and Hala cells.

According to (28), the camel urine could be considered a good source of trace element essential having the ability of antioxidant and prevention of carcinogenesis.

Camel urine contain high concentration of Zing (Zn) which plays an essential role in the DNA replication, transcription and protein synthesis, influencing cell division and apoptosis (29 , 30). Also, the camel urine contain Mg , K , Ca , Na , Fe and Se, which is antioxidant activity and enhance immune function (28 , 31).

In conclusion, the results of the present study clearly indicate the cytotoxic activity of camel urine, Further researches are certainly required, and other more advanced anticancer assay must be applied.

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Apolipoprotein B as a biomarker in patients with stable and unstable *angina pectoris*

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ABSTRACT

Apolipoprotein B (Apo B) is the primary apolipoproteins of chylomicrons and low-density lipoproteins (LDL). High levels of apo B can lead to plaques that cause vascular disease (atherosclerosis), leading to heart diseases. There is considerable evidence that levels of apo B are a better indicator of heart disease risk than total cholesterol or LDL. The aim of the present study was to evaluate serum apo B and serum lipid profile in patients with stable angina pectoris (SAP) and unstable angina pectoris (USAP) and compared with their controls. This study was conducted during the period from January 2013 until the end of April 2013. The patients were admitted to Medical City Hospital and all the measurements were performed in the Medical Teaching Laboratories in Baghdad. A total of 30 patients with SAP and USAP; their age range was (40-57) years and compared with 15 healthy controls. They were studied for their body mass index (BMI), serum lipid profile including serum total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and non. HDL-C in fasting state, along with apo B levels. As a result, BMI and serum lipid profile level except HDL-C were significantly increased in patients with SAP and USAP as compared with their controls. A significant decreased was found in serum HDL-C for those patients as compared with their controls, (P=0.0001). Serum apo B and LDL associated apo B (LDL-apo B) were significantly increased in patients with SAP and USAP as compared with their controls and there was a significant difference between SAP and USAP, (P= 0.0001). It was concluded that apo B is the most frequently occurring dyslipidemia in SAP and USAP. It identifies individuals with high risk of acute coronary disease not otherwise detected on routine lipid profile

Key words: Apolipoprotein B, angina pectoris, dyslipidemia

الملخص باللغة العربية

يعد الأبوليبوبروتين B من الأبوليبوبروتينات الأساسية للدقائق الكيلوسية المايكروية والبروتينات الدهنية قليلة الكثافة. المستويات العالية من الأبوليبوبروتين B ممكن أن تؤدي لتكوين اللويحة (الصفيحة) و التي تسبب أمراض الأوعية الدموية (تصلب الشرايين) المؤدية لأمراض القلب. هنالك العديد من الأدلة التي تؤخذ بنظر الأعتبار على ان مستويات الأبوليبوبروتين B تعتبر مؤشر أفضل من الدهون الكلية أو البروتين الدهني قليل الكثافة على خطر أمراض القلب.

الهدف من الدراسة الحالية هو تقدير الأبوليبوبروتين B والدهون في مصل الدم لدى مرضى الذبحة الصدرية المستقرة وغير المستقرة ومقارنتها مع مجموعة السيطرة. تمت هذه الدراسة خلال الفترة من كانون الثاني 2013 الى نهاية نيسان 2013 على المرضى الراقدين في مستشفى مدينة الطب وجميع القياسات تم اجراؤها في المختبرات التعليمية الطبية في بغداد. يبلغ عدد المرضى الكلي 30 مريضاً مصابين بالذبحة الصدرية المستقرة وغير المستقرة والذين تتراوح أعماره ما بين (40−57) سنة ومقارنتها مع 15 شخصاً سليما. تم دراسة معدل كتلة الجسم والدهون في مصل هؤلاء المرضى والتي تتضمن الدهون الكلية, الدهون ثلاثية الكليسيرايد, البروتين الدهني عالي الكثافة, البروتين الدهني قليل الكثافة, البروتين الدهني شديد انخفاض الكثافة, والبروتين الدهني غير عالي الكثافة في حالة الصيام وعلاقتها مع مستويات الأبوليبوبروتين B. أظهرت النتائج وجود ارتفاع معنوي في معدل كتلة الجسم و مستوى الدهون في المصل باستثناء البروتين الدهني عالي الكثافة لدى المرضى المصابين بالذبحة الصدرية المستقرة وغير المستقرة, حيث يوجد انخفاض في مستوى البروتين الدهني عالي الكثافة لهؤلاء المرضى عند مقارنتها مع مجموعة السيطرة, (P= 0.0001). يوجد ارتفاع معنوي للأبوليبوبروتين B في المصل والأبوليبوبروتين B المرتبط بالبروتين الدهني قليل الكثافة لدى المرضى المصابين بالذبحة الصدرية المستقرة و غير المستقرة عند مقارنتها مع مجموعة السيطرة, و يوجد اختلاف معنوي بين المرضى المصابين بالذبحة الصدرية المستقرة وغير المستقرة, عند مستوى دلالة (P=0.0001). يمكن الأستنتاج بأن الأبوليبوبروتين B غالباً ما يظهر في حالة اضطراب الدهون في الدم لدى مرضى الذبحة الصدرية المستقرة وغير المستقرة, فهو يميز المرضى المعرضين لأمراض القلب التاجية الحادة والتي لايمكن الكشف عنها عن طريق فحص الدهون الروتيني.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in industrialized countries. Coronary artery disease (CAD) is the most common form of CVD. In CAD, atherosclerosis damages the coronary artery wall predisposing to thrombus formation. The symptoms and severity of acute coronary syndromes (unstable angina pectoris -USAP- and myocardial infarction) vary depending on the degree to which thrombi occlude the coronary arteries (1). Stable angina pectoris (SAP) is a common and sometimes disabling disorder (2). Many individual lipid and inflammatory markers have been considered as the factors playing an important role in atherogenesis and prognosis of related diseases. The atherogenic dyslipidemic profile, especially mild to marked elevation of apo-B containing lipoproteins, such as VLDL, VLDL-remnants, intermediate-density lipoproteins (IDL), and LDL (specifically small, dense LDL), and low levels of high density lipoprotein cholesterol (HDL-C) (3).

Apo B, a large amphipathic glycoprotein, plays a central role in human lipoprotein metabolism. Two forms of apo B in circulating lipoproteins: apo B48 and apo B100 (4). Apo B48 is synthesized by enterocytes and secreted in chylomicrons, and is retained in chylomicron remnants (5). Apo B100 is synthesized in the hepatocytes and its excess is degraded within cells. It resides in the VLDL, LDL and LDL particles at a ratio of one molecule per lipoprotein particle (6). VLDL, IDL, LDL, and lipoprotein each contain one molecule of apo B100. Similarly, chylomicrons and their remnants contain a single molecule of apo B48. Hence, plasma apo B concentrations reflect the total number of atherogenic particles present in the circulation (7). Apo B can be used as an effective marker for elevations in these atherogenic triacylglycerol-rich lipoproteins and a more accurate predictor of cardiovascular risk (CV) (8). However, in individuals whose LDL particles, on average, contain less cholesterol than normal, the LDL-C concentration will underestimate the number of LDL particles. In these individuals, the apo B concentration will more accurately reflect the number of LDL particles and LDL-related CVD risk (9). Also, there now appears to be less laboratory error in the determination of apo B than of LDL-C, particularly in patients with hypertriglyceridemia (10).

MATERIALS AND METHODS

This study was conducted during the period from January 2013 until the end of April 2013. The patients in this study were admitted to Medical City Hospital. All the tests were performed in the Medical Teaching Laboratories in Baghdad. About 5 milliliters of venous blood was obtained from 30 patients and 15 healthy individuals as control group after 12 hours fast. Their age range was (40-57) years. Blood samples were transferred into plain tube, allowed to stand for 15 minutes at room temperature, centrifuged at 3500 rpm for 10 minutes. Patients were categorized into two groups based on the degree of ischemic heart disease (IHD): stable angina pectoris (SAP group) and unstable angina pectoris (USAP group). The SAP group consisted of 8 male and 7 female (total 15, mean age 51.3±6.7 years); the USAP group had 8 male and 7 female (total 15, mean age 53.8±9.8 years). The patients in the SAP group are demonstrated objective evidence of ischemia on electrocardiogram (ECG). USAP was defined according to Hamm and Braunwald (11). All patients in the USAP group had chest pain of increasing frequency and severity or at rest during the last 48 hours before hospitalization associated with ST segment changes, T wave changes, or both.

Measurements:

1-Determination of Serum Total Cholesterol (TC): Serum TC is measured by cholesterol kit, using an enzymatic method (12).

2-Determination of Serum Triacylglycerol (TAG): Serum TAG is measured by TAG kit, using an enzymatic method (13).

3-Determination of Serum High Density Lipoprotein-Cholesterol (HDL-C): Serum HDL-C is measured by HDL-C kit, using an enzymatic method (14).

4-Determination of Serum Low Density Lipoprotein- Cholesterol (LDL-C): Serum LDL-C is calculated indirectly by using the Friedewald's equation (15).

LDL-C = TC − [TAG/5 + HDL-C]. This equation is only accurate when TAG levels are below 400 mg/dl.

5-Determination of Serum Non-High Density Lipoprotein Cholesterol (Non.HDL-C): Serum non HDL-C is calculated directly from the difference between serum total and high-density lipoprotein cholesterol (16).

Serum non.HDL-C= [S.TC − S.HDL-C].

6-Determination of Serum Apolipoprotein B (Apo B): Apo B antigens in sample or standard cause immunological agglutination with the anti-Apo B antibodies in the reagent. The extent of agglutination is proportional to the apo B concentration in the sample and can be measured by turbidimetry (17).

7-Determination of LDL-Associated Apolipoprotein B (LDL-Apo B): This study derived a simple equation for calculating LDL-apo B concentration in mg/dl from measurements of total apo B and TAG (18):

LDL-apo B = [apo B - 10 - TAG/32]

Statistical Analysis:

Statistical analysis system-SAS 2004 was performed to analyze of data (19). The least significant difference (LSD) test compare between the means in this study. Correlation coefficient for the variables was done. Data were expressed as means (±SE); statistical significance was set at P < 0.05.

RESULTS

Clinical characteristics of patients and control group are shown in table (1). Based on analysis of variance, BMI, apo B, LDL-apo B, TC, TAG, LDL-C, VLDL, and non.HDL-C levels were significantly higher in SAP and USAP patients than in the control group. There was a significant increase in BMI, apo B, LDL-apo B, LDL-C, and non.HDL-C in USAP as compared in SAP and control, (P= 0.0001). Also, there was a significant increase in TAG, VLDL (P=0.005) and TC, (P=0.002) in USAP as compared in SAP. While there was a significant decrease in HDL-C levels in SAP and USAP patients as compared with their controls and in USAP as compared with SAP. Correlation coefficient of the entire population showed that there was a significant positive correlation between serum apo B and BMI, TC, TAG, LDL-C, VLDL, non.HDL-C, and LDL-apo B. While there was a significant negative correlation between serum apo B and serum HDL-C, table (2).

Table (1): Clinical analysis for the study population (means ± SE)

Clinical data	SAP (n=15)	USAP (n=15)	Control (n =15)	LSD value	P value
BMI (Kg/m^2)	30.98 ± 1.31	34.32 ± 1.19	20.86 ± 0.29	2.961	0.0001
Apo B (mg/dl)	142.82 ± 3.49	164.82 ± 3.46	70.82 ± 3.46	76.435	0.0001
TC (mg/dl)	250.87 ± 32.62	290.87 ± 33.72	151.40 ± 3.23	84.268	0.002
TAG (mg/dl)	198.45 ± 34.73	250.45 ± 36.79	106.67 ± 7.43	2.860	0.005
HDL-C (mg/dl)	30.94 ± 0.79	29.94 ± 0.79	66.67 ± 1.32	63.595	0.0001
LDL-C (mg/dl)	180.24 ± 27.35	210.84 ± 26.96	63.40 ± 4.06	16.855	0.0001
VLDL-C (mg/dl)	39.69 ± 6.94	50.09 ± 7.35	21.33 ± 1.48	77.895	0.005
Non.HDL-C (mg/dl)	219.92 ± 33.30	260.93 ± 33.31	84.73 ± 4.03	9.901	0.0001
LDL-Apo B (mg/dl)	126.62 ± 2.75	146.99 ± 2.78	57.49 ± 3.52	8.675	0.0001

Table (2): Correlation coefficient between apo B and some parameters

Apo B	Correlation coefficient (r)	Apo B	Correlation coefficient (r)
BMI (Kg/m^2)	0.85**	LDL-C (mg/dl)	0.82**
T.C (mg/dl)	0.78**	VLDL-C (mg/dl)	0.68**
TAG (mg/dl)	0.68**	Non.HDL-C (mg/dl)	0.82**
HDL-C (mg/dl)	- 0.73**	LDL-Apo B (mg/dl)	0.97**

** P < 0.01

DISCUSSION AND CONCLUSION

The present study compared serum apo B and serum lipid profiles between patients with angina pectoris and control group. The results revealed that the means value of serum apo B and serum lipid profile except HDL-C were significantly higher in USAP than those with SAP and control group.

From a technical point of view there are advantages in the determination of serum apo B. Good immunochemical methods are available and easily run in conventional autoanalysers (20).

This study used apolipoprotein measurements, which present some methodological advantages when compared with LDL-C quantification. In many labs LDL-C is still calculated by the Friedewalds' equation which only provides an estimate of the LDL-C values, and depends on TC, TAG and HDL-C levels. In this manner, the estimate may include the possible analytical errors of these three parameters. This equation also presents several has several limitations and the LDL-C estimate cannot be extended to samples presenting TAG levels higher than 400 mg/dl, samples containing chylomicrons and to patients with dysbetalipoproteinemia. In addition, the homogeneous method for measuring LDL-C and the estimate of these values by the Friedewalds' equation do not show similar results. On the other hand, apolipoproteins may be measured directly in plasma through accurate and precise internationally standardized methods, by using a common reference material for apo B, which is not routinely available for measurements of LDL-C (21). The prospective value of apo B as the strongest single lipids associated risk factor has been shown in large observational studies (22,23,24). Non.HDL-C, which is calculated in the present study, provides a measure of all apo B-containing lipoproteins, all of which have the potential to deliver cholesterol into the arterial wall and contribute to atherosclerotic lesions. This measure reflects atherogenic risk not captured by LDL-C measurement alone, particularly in the context of elevated TAG (25).

Because ultracentrifugation is not readily available in most clinical laboratories, the equation which is used in the present study conveniently estimates LDL-apo B from commonly available measurements for total apo B and TAG. According to the equation of Baca and Warnick, 2008, the TAG/32 term takes into consideration the VLDL contribution to apo B and is reminiscent of the TAG/5 term in the Friedewalds' equation. Clinically; this equation can be used to estimate atherogenic apo B particle number without requiring specialized laboratory measurements.

It is therefore concluded that there was a significant difference between serum apo B, LDL-apo B, BMI, and serum lipid profiles in patients with angina pectoris as compared with their controls; therefore methodological and clinical benefits of apo B should be included in the dyslipidemic work up of angina patients to correctly assess the potential atherogenic risk.

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Synthesis, characterization and inhibitory effect on SALP of new some Schiff Bases derived from D-Erythroascorbic acid

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ABSTRACT

New five Schiff bases derived from D-erythroascorbic acid were prepared by condensing pentulosono-γ-lactone-2,3-enedianisoate (5) with 3-amino phenol, (4-iodo, 4-methoxy, 4-methyl, 4-nitro) aniline in the presence of glacial acetic acid and dry benzene. The products were characterized by spectral methods FTIR, and some by (UV-Vis), ^{1}H and ^{13}CNMR, mass spectra and measurement of its physical properties. The derivatives showed inhibitory effect on serum alkaline phosphatase (SALP) activity of healthy, hypo and hyperthyroidisum patients.

الملخص باللغة العربية

تم تحضير خمسة من قواعد شف الجديدة المشتقة من حامض الارثرواسكوربيك وذلك من تكاثف مركب (٥) (بنتيولوسونو – كامــا –لاكتون–٣،٢– ين ثنائي انيسويت) مع ٣– امينو فينول, (٤– ايودو , ٤– ميثوكسي, ٤– مثيل, ٤– نايترو) انيلين بوجود حامض الخليك الثلجي والبنزين الجاف. شخصت المركبات المحضرة بالطرق الطيفية طيف الاشعة تحت الحمراء, وبعضها بواسطة طيـف الاشعة فوق البنفسجية والمرئية, طيف الرنين النووي المغناطيسي, طيف الكتلة ,قياس بعــض خصائـــصها الفيزيائيـــة. أظهــرت المشتقات المحضرة تأثيرا" تثبيطيا" على فعالية أنزيم الفوسفاتيز القاعدي في أمصال الأصحاء والمرضى بالخلــل الـــوظيفي للغــدة الدرقية.

INTRODUCTION

The formation of carbon–nitrogen double bond plays important role in organic synthesis. This can be achieved by the reaction of aldehydes and amines in acidic medium, which leads to synthesis of Schiff bases (imines). Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, biomedical application like anticancer (1), antitumor(2), anti-inflammatory agents (3). Insecticidal (4), antibacterial (5), antituberculosis (6), antimicrobial (7), anticonvulsant activity(8), supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized (9). The Schiff bases are also used as versatile components in nucleophilic addition with organometallic reagents (10) and in cycloaddition reactions (11,12). The reaction of primary aromatic amines with aryl aldehydes is found to be catalyzed by lemon juice as natural acid under solvent-free conditions to give the corresponding Schiff bases in good yields. This eco-friendly reaction has many advantages like economical, environmental, mild reaction conditions and simple work-up with high product yield (13). The introduction of enzyme inhibitors in particular has drastically decreased the mortality and morbidity associated with many diseases (14).

 The current paper deals with the synthesis, spectral and inhibitory effect on serum alkaline phosphatase (SALP) of new five schiff bases derived from D-erythroascorbic acid.

MATERIALS AND METHODS

Melting points were determined by electrothermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on 8400s Shimadzu FT infrared spectrophotometer. ^{1}HNMR spectra were recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. ^{13}CNMR spectrum was recoded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECl 7200 England. Mass spectrum for the compound (5) was recorded on IEOLJMS-7high resolution

instrument. Thin layer chromatography (TLC) was performed on aluminum plates coated with layer of silica gel, supplied by Merck. The spots were detected by iodine vapor. All chemical were obtained from Fluka or BDH.

Synthesis of 5,6-*O*-isopropylidene-L-ascorbic acid (2)

Dry hydrogen chloride was rapidly bubbled with stirring for 20 minutes into a (250ml) flask containing (10g, 57mmol) of powdered L-ascorbic acid (1) and (100ml) of dry acetone. After addition of (80ml) n-hexane, stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with (154ml) of acetone-hexane mixture (4:7) (v/v), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (2) (95%) as a white crystalline residue (15), m.p (206-208˚C). R_f (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3240 (O-H), 2993 (C-H$_{ali.}$), 2908 (C-H$_{ace.}$), 1751 (C=O$_{lac.}$), 1662 (C=C), 1431 (-CH-$_{asym}$), 1388 (-CH-$_{sym}$), 1141-900 (C-O), 767 δ(O-H) (O.O.P.) (16).

Synthesis of 2,3-*O*-dianisoyl-5,6-*O*-isopropylidene-L-ascorbic acid (3):

To a cold solution of (2) (10g, 46mmol) in pyridine (50ml), anisoyl chloride was added as dropwise (17.5ml , 129mmol) with stirring .The resulting mixture was stirred for 2 hours , then kept in dark place at room temperature for 22 hours.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. The oil layer was extracted with chloroform (150 ml), washed with water, dilute hydrochloric acid (5%) (2 × 100ml.), saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate. Chloroform was evaporated to produce a brown syrup and purified from chloroform: petroleum ether (60-80˚C) (1:5) (v/v) to give (3) (76.5%) as a pale yellow solid (17), m.p (102-104˚C). R_f (0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3028 (C-H$_{ar.}$), 2983 (C-H$_{ali.}$), 2939 (C-H$_{ace.}$), 2843 (OC-H$_{ali.}$), 1749 (C=O$_{lac.}$), 1683 (C=O$_{est.}$), 1647 (C=C$_{ali.}$), 1604 (C=C$_{ar.}$), 1300-1107 (C-O$_{est.}$), 900-600 δ(C-H) (O.O.P.) (17).

Synthesis of 2,3-*O*-dianisoyl-L-ascorbic acid (4):

Compound (3) (10g , 23.6mmol) was dissolved in mixture (65%) acetic acid (30ml) and absolute methanol (10ml) and stirred for 48 hours at room temperature. The TLC showed that the reaction was complete (benzene: methanol, 6:4).

To the resulting solution a benzene (40ml) was added and evaporated (repeat this process four times) (17). The residue was recrystallized from chloroform and then diethyl ether to yield (4) (77.7%) as a white crystals, m.p (130-132°C), R_f (0.42). FTIR (KBr, cm^{-1}): 3444 (O-H), 3008 (C-H$_{ar.}$), 2972 (C-H$_{ali.}$), 2843 (OC-H$_{ali.}$), 1741 (C=O$_{lac.}$), 1681 (C=O$_{est.}$), 1647 (C=C$_{ali.}$), 1606 (C=C$_{ar.}$), 1319-1112 (C-O$_{est.}$), 900-600 δ(C-H$_{ar.}$) (O.O.P.) (16).

Synthesis of pentulosono-γ-lactone-2,3-enedianisoate (5):

To the stirred solution of sodium periodate (5.6g, 26mmol) in distilled water (60ml) at (0°C), a solution of (4) (10g, 26mmol) in chloroform (60ml) was added dropwise. After stirring for 15 minutes, ethylene glycol (0.5ml) was added as dropwise, stirring was continued at room temperature for 1 hour (17).

The mixture was filtered and to the filtrate water (40ml) was added then the product was extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate, then filtered and the solvent was evaporated and the residue recrystallized from benzene to yield the pure product of compound (5) (45%) as a white crystals, m.p (156-158°C). R_f (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm^{-1}): 3040 (C-H$_{ar.}$), 2983 (C-H$_{ali.}$), 2843 (OC-H$_{ali.}$), 2671, 2559 (C-H$_{ald.}$), 1782 (C=O$_{lac.}$), 1749 (C=O$_{ald.}$), 1685 (C=O$_{est.}$), 1604 (C=C$_{ar.}$), 1300-1107 (C-O$_{est.}$), 900-600 δ(C-H$_{ar.}$) (O.O.P.) (16) ^{1}HNMR (DMSO δ ppm): 12.5 (s, 1H, CHO$_.$), 7.00-7.96 (dd, 8H, aromatic), 3.82-4.10 (s, 6H, 2OCH$_3$), 3.50-3.57 (s, 1H, H$_4$), 1.23-2.72 (DMSO)[16]. ^{13}CNMR (DMSO δ ppm): 167.50 (C=O$_{lac.}$), 163.32 (C=O$_{est.}$), 131.86 (C-4), 131.83 (C-3), 131.81 (C-2), (123.44, 114.31, 114.28, 114.26) (C$_{ar.}$), 55.90 (OCH$_3$), 40.51-39.41 (DMSO)[16]. The signal of aldehydic carbonyl was disappeared due to it showed out of the scale (18). MS, (positive ion) m/z (relative intensity): 413 [M+1, (100)], UV ($λ_{max}$, nm, CHCl$_3$): 300.

Synthesis of Schiff bases (6a-e):

A mixture of primary aromatic amine (0.12mmol), aldehyde (5) (0.05g, 0.12mmol), dry benzene (10ml) and 2 drops of glacial acetic acid were refluxed for (14) hours, the TLC showed that the reaction was complete (benzene: methanol, 8:2). The solvent was evaporated under vacuum and the residue recrystallized from ethanol. The physical data of all Schiff bases are listed in Table (1). The FTIR spectra data are given in Table (2). ^{1}HNMR (DMSO δ ppm) for compound (6b): 10.16 (s, 1H, HC=N$_.$), 7.00-7.90 (dd, 12H, aromatic), 3.82 (s, 6H, 2OCH$_3$), 3.45-3.57 (s, 1H, H$_4$), 1.18-2.50 (DMSO) (16). ^{1}HNMR (CDCl$_3$ δ ppm) for compound (6c): 7.78-8.10 (dd, 8H, aromatic$_.$), 7.45 (s, 1H, HC=N), 7.28 (for impurity CDCl$_3$), 6.27-7.24 (m, 4H, aromatic), 3.78-3.89 (s, 9H, 3OCH$_3$), 3.61-3.64 (s, 1H, H$_4$) (16).

Assay of serum alkaline phosphatase (SALP):

The effect of derivatives 6a, 6b and 6e were tested on serum alkaline phosphatase (SALP) activity, by preparing a series of solutions of each derivative (10^{-2}M, 10^{-3}M, 10^{-4}M, 10^{-5}M and 10^{-6}M). The activity of SALP were assayed using a ready kit from biomerix, first without the addition of any derivative. The SALP activity was considered to be 100%. Then the activity was measured after the addition of each dilution of the derivatives above. The activities obtained were used to calculate the percentage inhibition.

RESULTS AND DISCUSSION

In the present work the synthesis of new Schiff bases were achieved from pentulosono-γ-lactone-2,3 enedianisoate (5) , scheme (1). The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L-ascorbic acid with acetal formation leading to compound (2) using dry acetone in acidic media, following Salomon method (15). This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of anisoyl chloride in dry pyridine.

The FTIR spectra for compound (2) and (3) were confirmed the formation of compound (3) by disappearance of the bands for (O-H) of compound (2) and exhibited the band at (1683) cm^{-1} for (C=O) of the ester in compound (3) spectrum.

Table (1): Physical properties of compounds (6a-e)

Comp. No.	Nomenclature	Molecular Formula	M.p˚C or dec.	R_f	Physical state	Yield %
6a	(3-Hydroxyphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{21}O_9N$	110-112	0.54	Brown solid	67
6b	(4-Iodophenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{20}O_8NI$	105 (dec)	0.68	Deep-brown solid	70
6c	(3-Methoxyphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{28}H_{23}O_9N$	124-126	0.73	Deep-brown solid	78
6d	(4-Methylphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{28}H_{23}O_8N$	125-127	0.55	Brown solid	75
6e	(4-Nitrophenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{20}O_{10}N_2$	130-133	0.58	Light-brown solid	34

Table (2): Characteristic FTIR absorption band of compounds (6a-e)

Comp. No.	υ(O-H)	υ(C-H)$_{ar.}$	υ(C-H)$_{ali.}$	υ(C=O)$_{lac.}$	υ(C=O)$_{est.}$	υ(C=N)	υ(C=C)$_{ar.}$
6a	3304	3028	2956 2845	1770	1685	1640	1602
6b	-	3028	2981 2843	1737	1685	1650	1604
6c	-	3040	2924 2860	1760	1690	1630	1604
6d	-	3028	2983 2872	1760	1685	1650	1604
6e	-	3028	2983 2843	1770	1685	1629	1604

In order to prepare aldehyde (5), the acetal moiety was cleaved under acidic condition (19), (65% acetic acid) for compound (3) to give (4) and oxidation of the product with sodium periodate to result (5), which gave a positive Tolen's test by formation a silver mirror (20). The FTIR spectra for compound (4) and (5) were confirmed the formation of compound (5) by disappearance of the bands for (O-H) of compound (4) and exhibited the band at (1749) cm^{-1} for (C=O) in compound (5) spectrum. The structure of (5) was confirmed by ^{1}HNMR which exhibited a signal at δ(12.5) ppm for (CHO) and was characterized by ^{13}CNMR and (U.V-Vis) spectrum which showed one peak at (300) nm (33333 cm^{-1}) assigned to (n- π*) transitions. Finally, the mass spectrum showed a highest mass signal at [M+1] =413 with signal intensity 100%.

In order to obtain compounds (6a-e) by condensation of primary aromatic amines such as (3-amino phenol, (4-iodo, 4-methoxy, 4-methyl, 4-nitro) aniline with aldehyde (5) using dry benzene in acidic media leading to these compounds. All FTIR spectra for these compounds exhibited disappearance of the band at (1749) cm^{-1} for (C=O) of the aldehyde (5) and displayed of the bands at (1629-1650) cm^{-1} due to (C=N) for compounds (6a-e). The structures of compounds (6b) and (6c) were confirmed by ^{1}HNMR which showed disappearance of the signal at δ(12.5) ppm for (CHO) and exhibited the signals at δ(10.16) ppm and δ(7.45) ppm for (HC=N) in compounds (6b) and (6c) spectra.
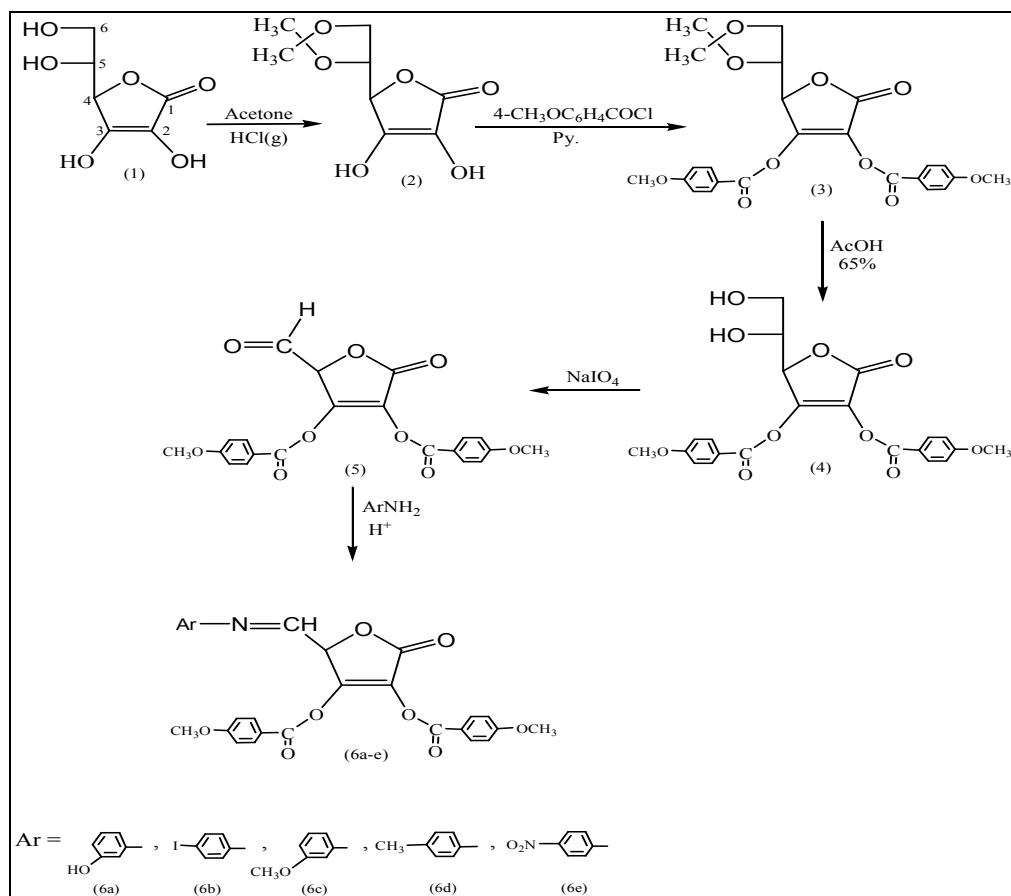
Scheme (1): scheme of prepared compounds

Enzymatic study

Figure. (1) Shows the Michaelis-Menten plot for SALP activity of healthy individuals. The maximum velocity is 7.0 IU/L, while the K_m is 0.016×10^{-2}M. Table (3) shows the percentage inhibition of derivatives 6a, 6b and 6e on SALP from healthy individuals. The highest inhibition for each derivative was obtained with the highest concentration of the derivative. Thus a linear correlation of the inhibition was obtained with the concentration.

Table (4) shows the inhibitory effect of the derivative 6a dose not correlates linearly with the concentration in both hypo and hyperthyroidism. This could be due to a variety of physiological conditions; usually the activity of enzyme is blocked by the interaction of various enzyme inhibitors. The two main reasons are either the inhibitors directed by active site (substrate-like) or altering the binding of substrate with the amino acid residues of the enzyme.
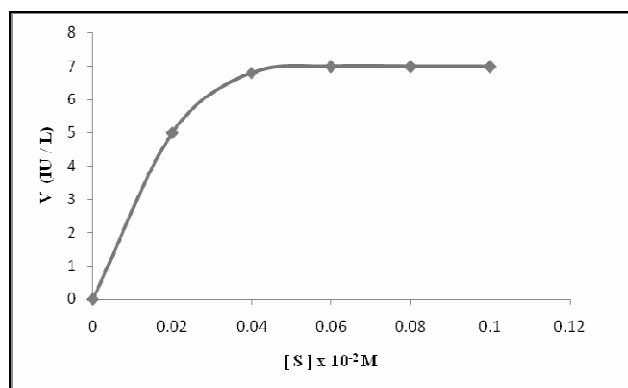
Fig.(1): Michaelis-Menten plot for SALP activity of healthy individuals

Table (3): % Inhibition of SALP from healthy individuals by 6a, 6b and 6e Schiff bases derivatives

Molar conc. of compounds	% Inhibition by 6a	% Inhibition by 6b	% Inhibition by 6e
10^{-6}	19.1	20.0	19.0
10^{-5}	24.4	21.6	26.8
10^{-4}	30.2	24.4	36.1
10^{-3}	39.6	31.0	40.4
10^{-2}	45.0	31.2	49.2

Table (4): % Inhibition on SALP from healthy, hypo and hyperthyroidism patients by compound 6a

Molar conc. of comp. 6a	Inhibition on SALP Control	Inhibition on SALP hypothyroidism (Obesity)	Inhibition on SALP hyperthyroidism (Thin)
10^{-6}	19.0	30.7	4.6
10^{-5}	21.2	27.4	8.8
10^{-4}	23.9	24.6	3.6
10^{-3}	25.0	25.2	7.4
10^{-2}	27.2	22.3	5.0

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Synthesis and anti-bacterial study of novel compounds with bis (four-, five-, and seven-membered) heterocyclic rings

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ABSTRACT

New twin compounds having four-, five-, and seven- membered heterocyclic rings were synthesized via Schiff bases (1a,b) which were obtained by the condensation of o-tolidine with two moles of 4- N,N-dimethyl benzaldehyde or 4- chloro benzaldehyde. The reaction of these Schiff bases with two moles of phenyl isothiocyanate, phenyl isocyanate or naphthyl isocyanate as in scheme(1) led to the formation of bis -1,3- diazetidin- 2- thion and bis -1,3- diazetidin -2-one derivatives (2-4 a,b). While in scheme (2) bis imidazolidin-4-one (5a,b) ,bistetrazole (6a,b) and bis thiazolidin-4-one (7a,b) derivatives were produced by reacting the mentioned Schiff bases(1a,b)with two moles of glycine, sodium azide or thioglycolic acid, respectively.

The new bis -1,3-oxazepine derivatives (8-12a,b) in scheme(3) were synthesized from various anhydrides and the same Schiff bases(1a,b).

All the synthesized compounds have been characterized by melting points , elemental analysis, FTIR and ^{1}HMNR (of some of theme) spectroscopy. The synthesized compounds have been screened for their antibacterial activities. They exhibited good antibacterial activity against *Escherichia coli* (G-) and *Staphylococus aureus* (G+) .

الملخص باللغة العربية

حضرت مركبات حلقة غير متجانسة جديده احادية على حلقتين متشابهتين رباعية وخماسية وسباعية ابتداءا من قواعد شف (1a,b)التي حضرت من مفاعلة اورثو توليد ين مع 4–ن,ن–ثنائي مثيل–بنزالديهايد او 4–كلوروبنزالديهايد. ان تفاعل قواعد شف مع مولين من ايزو ثايو –سيانات او مثيل ايزوسيانات او نفثايل ايزوسيانات كما في مخطط (1) ادى الى تكون المشتقات (2-4 a,b) . في حين تضمن المخطط (2) تحضيرمشتقات ثنائي اميدازوليدين – 4– ون (5a,b) وثنائي تترازول (6a,b) وثنائي ثايوزولدين –4– ون (7a,b) من خلال مفاعلة قواعد شف (1a,b) مع مولين من الكلايسين او ازايد الصوديوم او حامض الثايوكلايكولك على التوالي. اما مركبات (8-12a,b) في المخطط (3) حضرت من مفاعلة قواعد شف المذكورة مع انهايدريدات مختلفة. شخصت جميع المركبات الناتجة باستخدام الطرق الطيفية والفيزيائية.

كما درست الفعالية البايولوجية لهذه المركبات ضد انواع معينة من البكتريا وادرجت النتائج المستحصلة في البحث.

INTRODUCTION

Heterocyclic compounds consists of four, five and seven membered rings has attracted attention in recent times due to its importance in the field of pharmaceuticals and industries (1). 1,3-diazetidenone derivatives are one of these compounds, they are interesting kind of four membered heterocyclics because of their industrial and biological properties (2,3). Five membered hetrocycles like imidazolidinones and tetrazoles have gained attention because of their varied physiological activities. Imidazolidinones, for example, have been used as their peutic agents for Alzheimer and several diseases (4,5). Tetrazole and bis-tetrazoles derivatives exhibits anti-bacterial activity (6,7). On the other hand thiazolidinones have a wide use in medacaments as anti- convulsion (8,9) , inhibitor of polo like kinase for the treatment of various diseases and anti- cancer agents (10). Finally, 1,3-oxazepine ring (11), which constitutes a class of nitrogen and oxygen containing seven membered heterocycles have been studied for their anti-microbial and apoptosis proteins (IAPs) inhibitor activity which led to use them in the treatment of cancer (12-16). We reported in this study the synthesis , characterization and study antibacterial activity of novel heterocyclic rings derivatives of O-tolidine .

MATERIALS AND METHODS

Materials:

All chemicals were supplied from Merck , Fluka and Aldrich Chemicals Co. and used as received . FTIR spectra were recorded using potassium bromide discs on a 8400s Shimadzu spectrophotometer.
The ^{1}HNMR spectra were carried out by company : Bruker , model: ultra shield AMX-300 MHz , origin : Switzerland and are reported in ppm(δ), DMSO was used as a solvent with TMS as an internal standard . Elemental analysis (C,H,N) were carried out using a Perkin-Elmer model 2400 instrument. Uncorrected melting points (uncorrected) were determined by using hot-stage Gallen Kamp melting point apparatus.

Synthesis Procedures:

All compounds were synthesized according to Schemes 1 to 3, and the following procedures. All known compounds gave acceptable elemental analysis, FTIR and NMR spectra that matched data reported in the cited references. Compounds (Ia,b) to(4a,b) were synthesized following the Scheme (1).
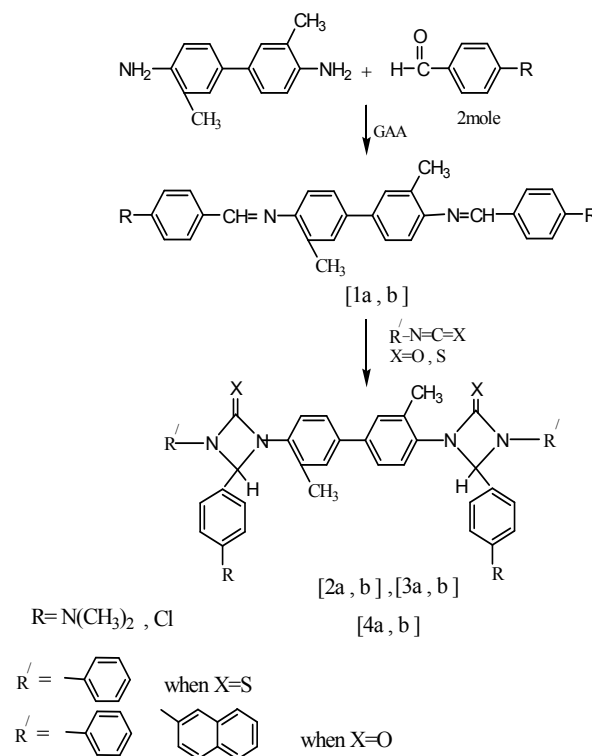
Scheme (1):The synthesis rout for Compounds(Ia,b) to(4a,b)

1. Preparation of Schiff bases (1a, b):

A mixture of 4-(4-Amino-3-methylphenyl)-2-methylaniline (o-tolidine) (0.01 mol) and 4-N,N-dimethyl benzaldehyde or 4- chloro benzaldehyde (0.02 mol) in absolute ethanol (10 mL) and 2 drops of glacial acetic acid (GAA) was refluxed for 4hrs. The solvent was evaporated under vacuum and the residue crystallized from ethanol**.** Yield (80-70%), m.p = (229-231) ^{0}C and (134-136) ^{0}C, respectively.

2. Preparation of 1,3-diazetidin-4-thion and 1,3-diazetidine-4-one derivatives (2-4a,b):

To solution of Schiff bases(1a,b) (0.01mol) and phenyl isothiocyanate , phenyl isocyanate

or naphthyl isocyanate (0.02mol) in (25 mL) toluene was refluxed for 6hrs (2). The solvent was evaporated under vacuum and the residue treated with a mixture of (1:1) ethyl acetate and petroleum ether. The resultant precipitate was filtered and dried**.**

3. Compound (2b):
Yield: 60%; m.p. = 250-252 ^oC; IR(KBr) ν cm-1: 2920-2840 (C-H), 1593(C=C), 1639(C=O), 825 (C-Cl); Anal. Calcd. for $C_{42}H_{32}N_4O_2Cl_2$: C, 72.51; H, 4.60; N, 8.05. Found: C, 71.92; H, 4.11; N, 8.45.

4. Compound (4a):
Yield: 55%; m.p. = 122-124 ^oC ; IR(KBr) ν cm^{-1}: 2924-2848 (C-H), 1525(C=C), 1313(C=S), 1168 (C-N(CH_3)_2); 1H NMR (TMS) δ ppm: 3.40 (s, 2H, CH-N), 6.97-8.611 (m, 26H, ArH), 2.723 (s, 12 H, N-CH3), 2.33 (s, 6 H, CH3); Anal. Calcd. for $C_{46}H_{44}N_6S_2$: C, 74.19; H, 5.91; N, 11.29. Found: C, 73.82; H, 5.22; N, 10.45.

5.   **Preparation   of   limidazolidinone**

derivatives (5a,b):

Compounds (5a,b) to(7a,b) were synthesized following the Scheme (2).

A mixture of Schiff bases(1a,b) (0.01mol) , glycine (0.02 mol) and triethylamine (1mL) in ethanol(15mL) was refluxed for 9hrs (16). The reaction mixture was neutralized with diluted HCl and then poured into ice-cold water. The precipitate was filtered off, washed with water and recrystallized from ethanol to give (5a,b).

6. Compound (5b):
Yield : 62% m.p = 205-208 C; IR(KBr) ν cm^{-1}: 3380(NH), 2825-2717 **(**CH), 1660-1624 (C-N),1593 (C=C), 1678(C=O), 893 (C-Cl); ^{1}H NMR (TMS) δ ppm: 9.66 (s, 2H, NH-C), 7.07-7.91 (m, 14H, ArH), 3.36 (s, 2 H, CH-N), 2.49 (s, 6 H, CH3), 3.04(s, 4H, CH_2); Anal. Calcd. for $C_{32}H_{26}N_4O_2Cl_2$: C, 67.48; H, 4.56; N, 9.84. Found: C, 67.09; H, 4.16; N, 8.99.

7.   Preparation   of   tetrazole   derivatives (6a,b):

Sodium azide (0.02mol) was added to a stirring solution of Schiff bases (1a,b) (0.01mol) in dry acetone (15 mL), and the mixture was refluxed for 2 hrs with stirring (6), then cooled at room temperature and the precipitate was filtered , washed with cold water. recrystallized from   petroleum ether (40-55)^{0}C to give compounds (6a,b).

8. Compound (6b):
Yield : 52% m.p = 186-189 C ,IR(KBr) ν cm^{-1}: 2893-2830(C-H),   1630(C=N),1589   (C=C), 1490(N=N), 890 (C-Cl); ^{1}H NMR (TMS) δ ppm: 8.92 (s, 2H, CH-N), 7.27-7.89 (m, 14H, ArH), 2.35 (s, 6 H, CH3);   Anal. Calcd. for $C_{28}H_{22}N_8Cl_2$: C, 68.81; H, 6.09; N, 25.08. Found: C, 67.89; H, 5.44; N, 23.49.

9. Preparation of thiazolidin-4-one derivatives (7a,b):
Compound of Schiff bases (1a,b) (0.01mol) and thioglycolic acid (0.001 mol) was refluxed in dry benzene (10 mL) for 8 hrs[8]. The solvent was evaporated and the reaction mixture was neutralized with sodium bicarbonate solution, the product was filtered off and recrystallized from petroleum ether (60-70)^{0}C.

[5a , b]

[1a , b]

[6a , b]   [7a , b]

R= N (CH_3)_2 , Cl

Scheme (2): The synthesis rout for Compounds(5a,b)– (7a,b)

10. Compound(7a):
Yield : 76% m.p = 160-162 C, IR(KBr) ν cm^{-1}: 2953-2912(C-H), 2980 (N-CH - S), 1715 (C=O),1577 (C=C), 1224(C-N(CH$_3$)$_2$), 903-887 (C-S); ^{1}H NMR (TMS) δ ppm: 5.06 (s, 2H, CH-N), 6.89-7.54 (d, 14H, ArH), 1.86-2.79 (s, 6 H, CH$_3$), 3.52 (s, 4H, CH$_2$); Anal. Calcd. for C$_{28}$H$_{22}$N$_8$Cl$_2$: C, 69.45; H, 6.10; N, 9.00. Found: C, 70.09; H, 5.94; N, 8.49.

11. Preparation of 1,3-Oxazepine derivatives (8-12a,b):
A mixture of equi-molar amounts (0.01mol) of Schiff bases (1a,b) and different acid anhydriedes in dry benzene was refluxed for 6 hrs (11), the solvent was removed and the resulting colored crystalline solid recrystallized from ethanol to obtained 1,3-oxazipenes (8-12a,b). Compounds (8a,b) to(12a,b) were synthesized following the Scheme (3).
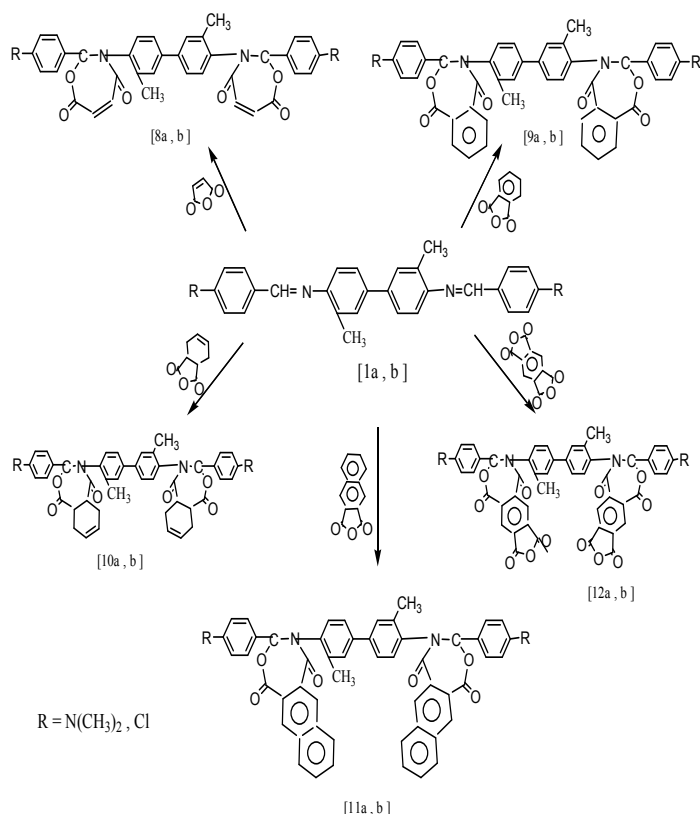
Scheme (3): The synthesis rout for Compounds(8a,b)– (12a,b)

12. Compound(10a):
Yield : 70% m.p = 260-262 C, IR(KBr) ν cm^{-1}: 2995-2845 (C-H), 1720(C=O),1200 - 1172 (C-O-C), 1599 (C=C); Anal. Calcd. for C$_{48}$H$_{50}$N$_4$O$_6$: C, 74.41; H, 5.94; N, 7.2. Found: C, 72.82; H, 5.12; N, 6.66.

13. Compound(11b):
Yield : 67% m.p = 190-192 C, IR(KBr) ν cm^{-1}: 2883-2830 (C-H), 1730 (C=O),1200 - 1172 (C-O-C), 1598 (C=C), 840(C-Cl); ^{1}H NMR (TMS) δ ppm: 8.54 (s, 2H, CH-N), 7.17-7.98 (m, 30H, ArH), 2.50 (s, 6 H, CH$_3$), 3.52 (s, 4H, CH$_2$).

RESULTS AND DISCUSSION

The novel Schiff bases(1a,b) which are required starting materials were prepared from the condensation of o-tolidine with two moles of 4-N,N-dimethyl benzaldehyde or 4- chloro benzaldehyde in dry benzene with some drops of glacial acetic acid (GAA). These Schiff bases(1a,b) were identified by their melting points and FTIR spectroscopy. FTIR absorption spectra showed the disappearance of absorption bands due to NH$_2$ and C=O groups of the starting materials together with appearance of new absorption band in the region (1607-1622) cm^{-1} which is assigned to C=N stretching .
Cycloaddition of Schiff bases (1a,b) and phenyl isothiocyanate , phenyl isocyanate or naphthyl isocyanate (0.02mol) in toluene furnishes 1,3-diazetidin-4-one and 1,3-diazetidine-4-thion derivatives (2-4a,b).
The novel limidazolidinone derivatives (5a,b) were synthesized by refluxing Schiff bases (1a,b) with two mole of glycin and tri-ethylamine in ethanol for 9hrs. Tetrazole derivatives (6a,b) were obtained by addition reaction of Schiff bases 1a and 1b with sodium azide in dry acetone.
Thiazolidin-4-one derivatives (7a,b) were synthesized by refluxing Schiff bases (1a,b) with two mole of thioglycolic acid was refluxed in dry benzene for 8 hrs. The suggested mechanism to obtain the target product is outlined (6), scheme(4):
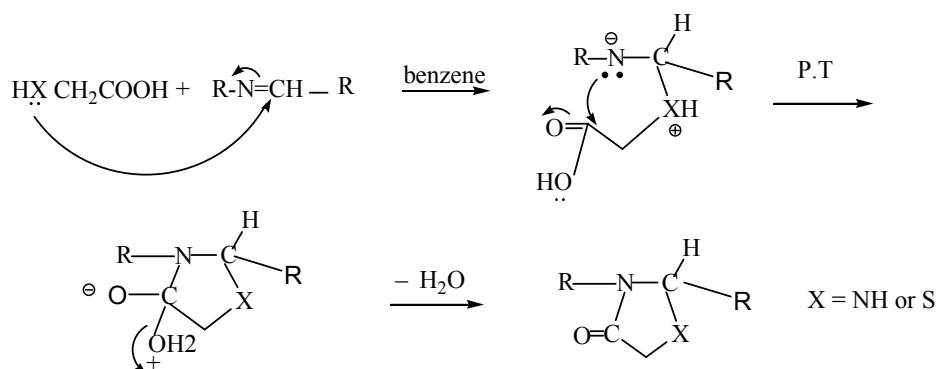
Scheme (4): Mechanism for the synthesis of compounds (5a,b) or (7a,b)

The 1,3-oxazepine derivatives (8-12) a ,b were obtained by addition reaction of Schiff bases(1a,b) with different anhydrides in dry benzene. The mechanism involves the addition of one σ- carbonyl to π-bond (C=N) to give 4-membered cyclic and 5-membered cyclic ring of anhydride in the same transition state, which opens into acid anhydrides , to give 7-membered cyclic ring 1,3-oxazepine (11). Scheme (5):
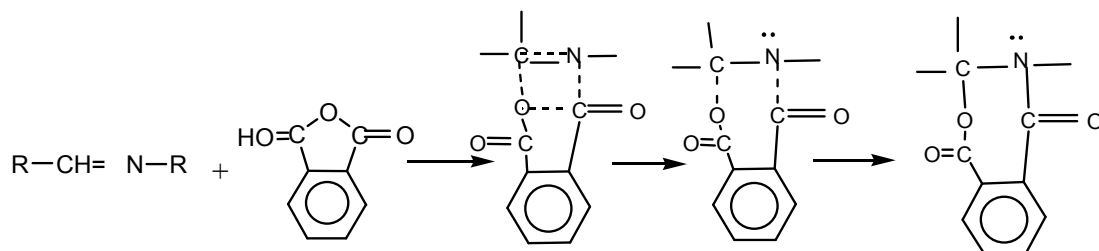
Scheme (5): Mechanism for the synthesis of compounds (8-12a,b)

Biological Activity

The antibacterial activity of the synthesized compounds was performed according to the agar diffusion method [17]. The synthesized compounds were tested against *E.coli* (G-) and *Staph. aureus* (G+). Each compounds was dissolved in DMSO to give concentration 1ppm. The plates were then incubated at 37 ^{0}C and examined after 24 hrs. The zones of inhibition formed were measured in millimeter and are represented by (-),(+),(+ +) and (+ + +) depending upon the diameter and clarity as in Table(1). Both Schiff bases (1a,b) showed no inhibition against of the two types of the bacteria. All the compounds exhibit the highest or low biological activity against *Staph. aureus* (G+) and some of them exhibit no activity against *E.coli* (G-), this could be related to the presence of the diazatine , imidazolidione, tetrazole , thiazolidin-4-one and 1,3-oxazepine rings .

Table (1) : Antibacterial activity of the synthesized compounds

Comp. No.	E.Coli (G-)	Staph. aureus (G+)	Comp. No.	E.Coli (G-)	Staph. aureus (G+)	Comp. No.	E.Coli (G-)	Staph. Aureus (G+)
2a	-	+++	6a	+++	+++	10a	-	++
2b	-	+++	6b	-	+++	10b	+++	+++
3a	-	++	7a	+++	+++	11a	-	+++
3b	++	+++	7b	-	+++	11b	-	+++
4a	-	+++	8a	+++	+++	12a	+++	+++
4b	++	+	8b	+++	+++	12b	+++	+++
5a	-	++	9a	+++	+++			
5b	+++	+++	9b	+++	+++			

Highly active = + + +(mor than)15 mm, Moderately active = + +(11-15) mm and slightly active = + (5-10)

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Synthesis and characterization of some new thiazine-4-ones containing 1,3,4-thiadiazole moiety

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ABSTRACT

2-amino-5-mercapto-1,3,4-thiadiazole [I] were prepared by the cyclization of thiosemecarbazide with carbon disulphide and anhydrous sodium carbonate in ethanol as a solvent. The reaction of compound [I] with ethyl iodide yielded 2- amino-5-thioethyl- 1,3,4- thiadiazole [II]. Compound [II] was reacted with different aromatic aldehydes to yieled 2-[(substituted benzyliden) amino] -5- thioethyl- 1,3,4-thiadiazole [III]$_{a-c}$. The Schiff ُs bases (III) were found to react with 2-mercapto benzoic acid in the triethyl amine to give 3-[5-(ethylthio) -1,3,4- thiadiazol-2-yl] -2,3- dihydro- 2- (aryl) benzo [e] [1,3] thiazine -4-one [IV]$_{a-c}$. The structures of the newly synthesized compounds were confirmed by physical properties and spectral (FT-IR, ^{1}H-NMR) analysis.

الملخص باللغة العربية

تم تحضير المركب 2- امينو – 5- مركبتو- 1و3و4- ثياديازول [I] بتحولق الثايوسميكاربازايد مع ثنائي كبريتيد الكاربون بوجود كاربونات الصوديوم اللامائية وفي مذيب كحولي. تفاعل المركب [I] مع يوديد الاثيل يعطي المركب 2- امينو –5- ثايوايثايل– 1و3و4- ثياديازول [II] . تفاعل المركب [II] مع الديهايدات اروماتية مختلفة اعطى المركب 2- [(بنزلدين معوض) امينو]–5-ثايو ايثايل –1و3و4- ثياديازول [III]a-c] . وجد ان قواعد شف تتفاعل مع 2-مركبتو حامض البنزويك بوجود ثلاثي اثيل امين لتعطي المركب 3- [5 – (ثايوايثايل) – 1و3و4 –ثياديازول –2- يل] –2و3- داي هايدرو-2-(اريل) بنزو [e] [3 و1] ثيازين –4- ون [IV]a- c] ، تم تشخيص تراكيب المركبات الجديدة المحضرة من خلال القياسات الفيزياوية واطياف الاشعة تحت الحمراء وطيف الرنين النووي المغناطيسي.

INTRODUCTION

Small ring heterocycles containing nitrogen and sulfur have been under investigation for a long time because of their important medicinal properties. Among the wide range of heterocycles explored to develop pharmaceutically important molecules, thiadiazoles had played an important role in medicinal chemistry. A survey of literature had shown that compounds having thiadiazole nucleus possess a broad range of biological activities such as anti-inflammatory (1), antibacterial (2), and antifungal activities (3).
 Thiazine-4-one and their derivatives are import classes of compounds in organic and medicinal chemistry. The thiazine-4-one ring system is a core structure in various synthetic pharmaceutical agents, displaying a broad spectrum of biological activities such as , anti-tubercular, anti bacterial , anti-HIV , anti-inflammatory , anti convulsant , anthelmintic , anticancer , anti protocol and analgesic (4,5).

MATERIALS AND METHODS

All the chemicals used in the synthesis were of analytical grades. The melting points were determined in open capillary on Digimelt MPA 161 (MSRS) electronic apparatus and are uncorrected. The IR spectra of synthesized compounds were recorded on Shimadzu FT-IR 8400 spectrophotometer using potassium bromide. To monitor the reactions , as well as , to establish the identity and purity of reactants and products , thin layer chromatography was performed on microscopic glass slides coated with silica gel , using toluene-acetone , benzene-ether and chloroform-methanol , as the solvent systems and spots were visualized under UV radiation. Nuclear magnetic resonance spectra were recorded on Bruke, model:Ultra shield 300 MH$_Z$, model spectrometer using DMSO as a solvent and TMS as internal reference (chemical shifts in δ ppm) .

General procedure for the synthesis of compounds

1. Preparation of 2-amino-5-mercapto-1,3,4-thiadiazole[I]:

A mixture of (2g, 0.02 mol.) of thiosemicarbazide and (2.33g, 0.02 mol.) of anhydrous sodium carbonate was dissolved in 25ml. abs. ethanol. To this solution (3.2g, 0.04 mol.) of carbon disulphide was added.
The resulting mixture was heated under reflux for 10 hrs. , the reaction mixture was then allowed to cool down to room temperature. Most of solvent was removed under reduced pressure and the residue was dissolved in 20ml. distilled water and then acidified with cold concentration hydrochloric acid to give pale yellow precipitate . The crude product was filtered and washed with cold water, recrystallized from water to give yellow product , yield (75%) , m.p (230-232)C^o (6).

2. Preparation of 2-amino-5-thioethyl-1,3,4-thiadiazole[II]:

Compound [I] containing (-SH) group (0.001 mol.) dissolved in (10 ml.) dioxan , which contained (0.001 mol.) potassium hydroxide. Ethyl iodide (0.001 mol.) was added using separating funnel drop wise with stirring. The reactants were refluxed for 3 hrs. then the solvent was evaporated under reduced pressure , water was added and the crude product was extracted with ethyl acetate and dried with anhydrous magnesium sulphate.
Evaporation of the organic layer gave solid products , recrystallized from dioxan to give the desired product , yield (68%) , m.p (120-121)C^o (7).

3. Preparation of 2-(substituted benzyliden amino)-5-thioethyl-1,3,4-thiadiazole[III]:

A mixture of compound [II] (0.001 mol.), absolute ethanol and appropriate aromatic aldehyde (0.01 mol.) in acidic condition was refluxed for 8 hrs. The reaction mixture was then allowed to cool at room temperature, and the precipitate was filtered and dried, recrystallized from ethanol to give colored crystals. Table (1) shows the m.p. , yield and synthesized compounds from[III]a to [III]c (8).

Table (1): Characterization data of the compounds [III] a-c

Comp. NO.	Ar	Yield(%)	M.P.(C^o)	Molecular formula
[III]a	4-N,N-(CH$_3$)$_2$NC$_6$H$_4$-	91%	160-161	C$_{13}$H$_{16}$N$_4$S$_2$
[III]b	3-NO$_2$C$_6$H$_4$-	55%	130-132	C$_{11}$H$_{10}$O$_2$N$_4$S$_2$
[III]c	4-NO$_2$C$_6$H$_4$-	75%	139-141	C$_{11}$H$_{10}$O$_2$N$_4$S$_2$

4. **Preparation of 3-(5-(ethylthio)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-2-(Aryl)benzo [e][1,3] thiazine-4-one [IV]:**

A mixture of Schiff base (0.01 mol.) and 2-mercapto benzoic acid (0.01mol.) was stirred with dry benzene (30 ml.) and 3 drops of triethyl amine or pyridine. The mixture was refluxed for 6 hrs. Then the solvent was removed and the residue washed with 10% of sodium bicarbonate then filtered and recrystallized with dioxan. Table (2) shows the m.p. , yield and synthesized compounds from[IV]a to[IV]c . scheme (1)

Table(2): Characterization data of the compounds [IV] a-c

Comp. NO.	Ar	Yield(%)	M.P.(C°)	Molecular formula
[IV]a	4-N,N-(CH$_3$)$_2$-NC$_6$H$_4$-	58%	152-154	C$_{20}$H$_{20}$ON$_4$S$_3$
[IV]b	3-NO$_2$C$_6$H$_4$-	80%	140-142	C$_{18}$H$_{14}$O$_3$N$_4$S$_3$
[IV]c	4-NO$_2$C$_6$H$_4$-	60%	155-157	C$_{18}$H$_{14}$O$_3$N$_4$S$_3$
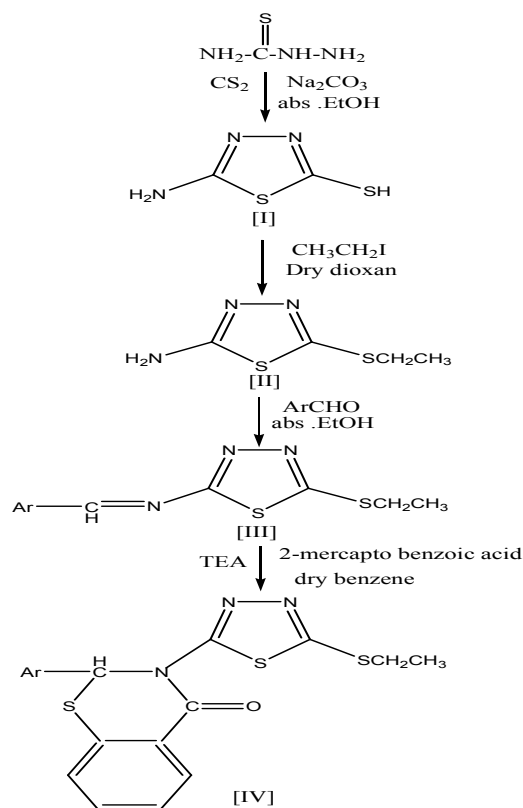
Scheme-1-

Ar =4-N,N-(CH$_3$)$_2$NC$_6$H$_4$, 3-NO$_2$C$_6$H$_4$, 4-NO$_2$-C$_6$H$_4$-

RESULTS AND DISCUSSION

Compound [I] 2- amino -5- mercapto -1,3,4-thiadiazole was prepared through the reaction of thiosemicarbazide with carbon disulphide in the presence of anhydrous sodium carbonate in absolute ethanol .

The structure of compound [I] was identified by its melting point and FT-IR spectroscopy . The FT-IR spectrum of compound [I] , figure(1) shows the following characteristic bands , two bands at 3396 cm^{-1} and 3278 cm^{-1} were due to asymmetric and symmetric stretching vibration of (-NH$_2$) group respectively , an absorption band at 3091 cm^{-1} was due to the (N-H) stretching (tautomeric form).
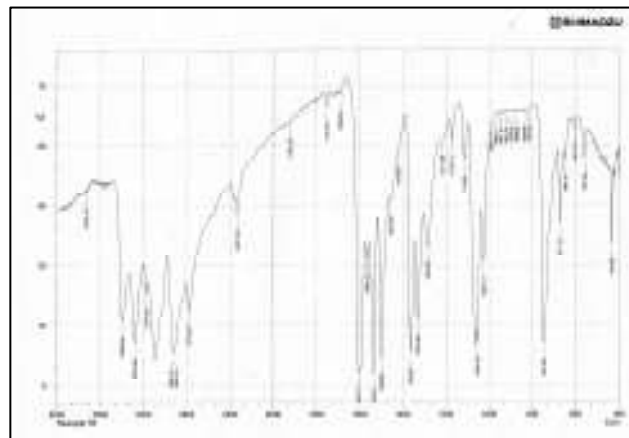
Fig. (1): FTIR- spectrum of compound [I]

The (-SH) stretching band found as very weak shoulder at 2331cm^{-1} , a band at 1604cm^{-1} was due to (C=N) stretching of the thiadiazole ring moiety . The sharp band at 1533cm^{-1} and 1330cm^{-1} are due to the (N-H) bending and (C-N) stretching vibration respectively .

Also , the absorption band at 1055cm^{-1} for the (C=S) group which gives an evidence that compound [I] can exist in two tautomeric forms , thiol (A) and thione form (B) (6) as shown in scheme (2).

(A)   (B)
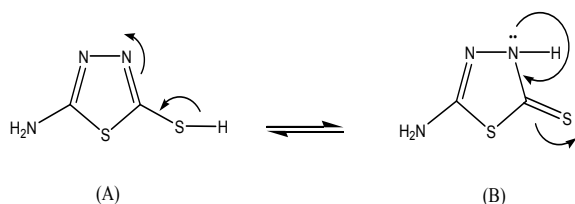
Scheme (2): Two tautomeric forms :thiol (A) and thione form (B)

Compound [II] was synthesized by the reaction of compound [I] with ethyl iodide in the presence of potassium hydroxide in dry 1,4-dioxan . The mechanism of the alkylation was explained by the nucleophilic attack of sulphide ion on the ethyl iodide and remove the iodide ion as KI ,this type of mechanism called SN^2 (9).

The FT-IR of compound [II] , figure(2) , showed disappearance band of which is due to (C=S) . Also the disappearance of very week band at (2331) cm^{-1} due to (S-H) group , and remaining of the two functional group due to (C=N) exocyclic , (C=N) endocyclic , at (1622) cm^{-1} , (1525) cm^{-1} respectively (6), and the appearance of band at (1321)cm^{-1} due to (S-CH_2CH_3) (10).
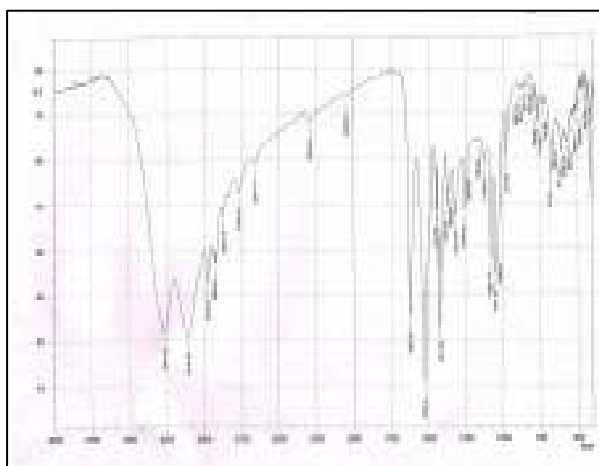
Fig. (2): FTIR- spectrum of compound [II]

The condensation reaction of equimolar quantity of primary amine with aromatic aldehydes was the major method to prepare Schiff 's bases.

FT-IR spectrum of compound [III]$_{a-c}$ figure(3) , showed the disappearance of two absorption band at (3396 ,3278)cm^{-1} due to the (-NH_2) stretching of amino group .
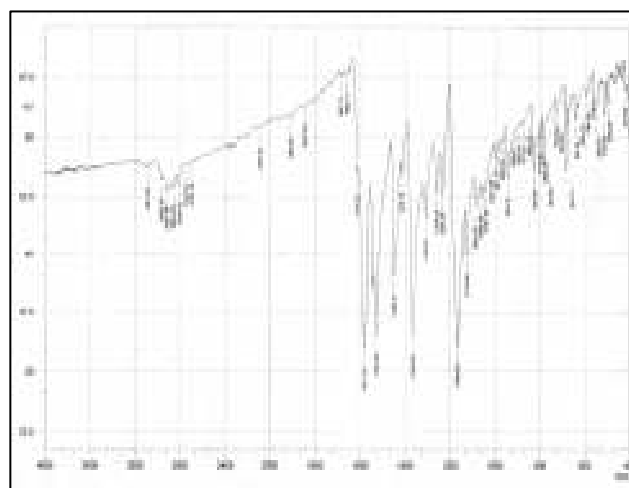
Fig. (3): FTIR- spectrum of compound [III]a

On the other hand, the FT-IR spectra showed bands for (C-H) olefinic, (C=C) aromatic, (C=N) endocyclic and exocyclic imine (C=N) group stretching vibration (6). FT-IR absorption for these compounds are shown in table (3).

The H^1-NMR data of compound [III]a , figure (4) , shows the following characteristic chemical shifts (DMSO- d6, ppm) : the aromatic ring protons appeared as multiplies at δ (6.23-7.26)ppm , single at δ(8.03) due to the (C-H) proton of imine . Furthermore, the triplet single at δ (0.81)ppm for the methyl group and the quartet signal at δ (2.67)ppm for the methylene group.

Table (3): characteristic FT-IR absorption bands (cm^{-1}) of compounds [III]a-c

Comp. NO.	Ar	C=N str Exocyclic cm^{-1}	C=N str Endocyclic cm^{-1}	C-H Arom. cm^{-1}	C=C Arom. cm^{-1}	Others cm^{-1}
[III]a	4-N,N-(CH$_3$)$_2$NC$_6$H$_4$	1616	1560	3091	1581	
[III]b	3-NO$_2$C$_6$H$_4$	1616	1527	3088	1573	C-NO$_2$ (1508-1354)
[III]c	4-NO$_2$C$_6$H$_4$	1612	1545	3109	1591	C-NO$_2$ (1525-1340)
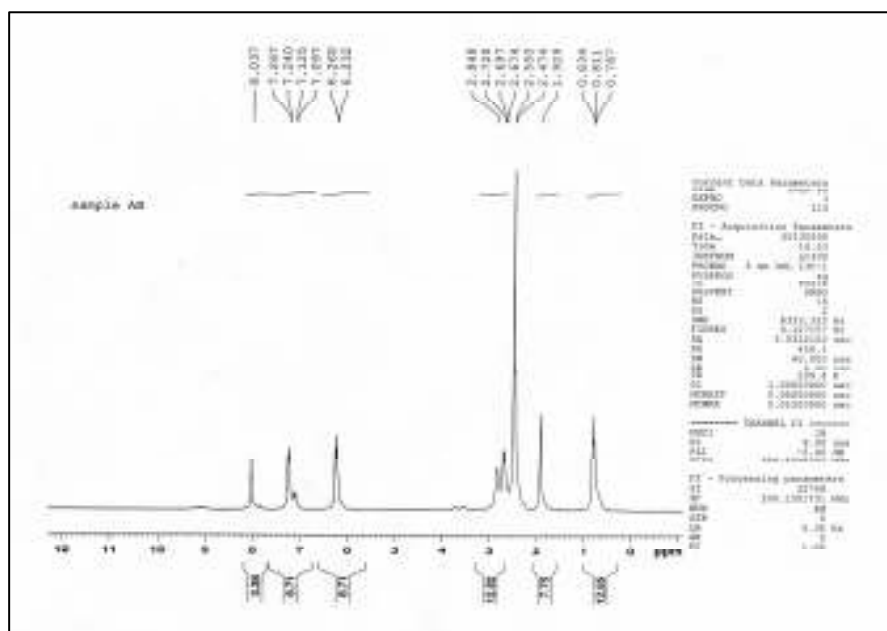
Fig. (4): ^{1}HNMR- spectrum of compound [III]a

Compounds [IV] were synthesized by the reaction of compounds containing azomethene group with 2- mercapto benzoic acid in presence of triethyl amine in dry benzene, the compounds were identified by FT-IR and ^{1}H-NMR spectra .

The FT-IR spectra of compounds [IV] a, figure (5) ,showed disappearance of the band (1616)cm^{-1} due to (C=N) exocyclic group and appearance band at (1672)cm^{-1} due to (C=O) of Lactam. Other data of functional group are shown in table (4).
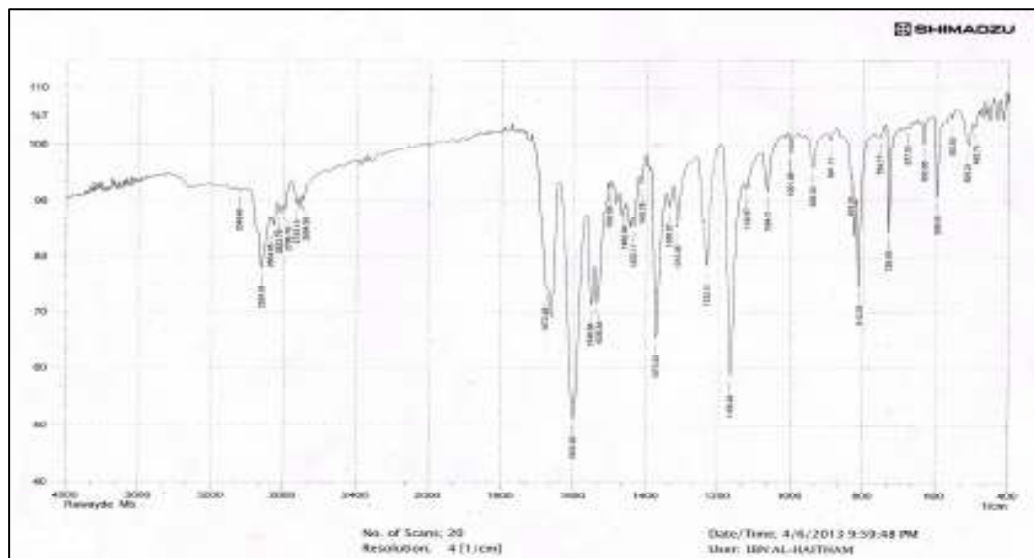
Fig. (5): FTIR- spectrum of compound [IV]a

Table (4): characteristic FT-IR absorption bands (cm⁻¹) of compounds [IV]a-c

Comp. NO.	Ar	C=N str Endocyclic cm⁻¹	C=O str Lactam cm⁻¹	C=C str. Aromatic cm⁻¹	Others cm⁻¹
[IV]a	4-N,N-(CH₃)₂NC₆H₄-	1548	1672	1600	
[IV]b	3-NO₂-C₆H₄-	1562	1683	1587	C-NO₂ (1533-1348)
[IV]c	4-NO₂-C₆H₄-	1560	1685	1590	C-NO₂ (1505-1330)

The H^1-NMR data of compound [IV]$_b$, figure (6) shows the following characteristic chemical shifts (DMSO- d6, ppm) : the aromatic ring protons appeared as multiplies at δ (6.61-8.81)ppm , single at δ(9.09) due to the (C-H) proton in thiazine ring as singlet. Furthermore, the triplet single at δ (1.36) ppm for the methyl group and the quartet signal at δ (3.7)ppm for the methylene group (11).

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Comparative study of Fracture resistance of endodotically treated teeth restored with some types of cast posts

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ABSTRACT

The current in-vitro study aimed to evaluate and compare the fracture resistance of teeth treated endodontically restored using cast posts cemented with different types of cement. Thirty intact human maxillary central incisors were selected for this study. The crowns were removed and endodontic therapy was done on the roots, which were then prepared to receive cast posts. The cast posts were cemented with zinc phosphate cement (group I), glass ionomer cement (group II),and resin cement (group III). The samples were subjected to compressive fracturing loads by a Zwick testing machine at a cross head speed of 5mm/min., with an angle of 45º to the long axis of each tooth. Results showed that the posts cemented with resin cement exhibited the highest mean value of failure load followed by those cemented with glass ionomer cement, while posts cemented with zinc phosphate cement exhibited the lowest mean value of failure load.

الملخص باللغة العربية

تهدف هذه الدراسة المختبرية على تقييم ومقارنة مقاومة الانكسار للاسنان المعالجة بحشوات الجذور والاوتاد المــصبوبة والمثبتــة بأستخدام انواع مختلفة من مادة الاسمنت. تم اختيار 30 سن بشري سليم في هذه الدراسة. ثم تم ازالــة التيجــان لجميــع الاســنان المختارة وعلاجها بحشوات الجذور والاوتاد المصبوبة. بالنسبة للمجموعة الاولى تم تثبيت الاوتــاد المــصبوبة بأســتخدام ســمنت فوسفات الزنك, اوتاد المجموعة الثانية تم تثبيتها بأستخدام السمنت الزجاجي الايوني, أما اوتاد المجموعة الثالثــة فثبتــت بأســتخدام السمنت الراتنجي. جميع النماذج عرضت الى احمال ضاغطة كاسرة بأستعمال الة اختبارية تدعى زفك وبــسرعة 5 ملمتــر فــي الدقيقة, وبزاوية 45 درجة من الطول الشاقولي لكل سن. اظهرت النتائج ان الاوتاد المصبوبة مع السمنت الراتنجي لها اعلى معــدل تحمل للكسر ثم تليها الاوتاد المصبوبة مع السمنت الزجاجي الايوني, بينما الاوتاد المصبوبة مع سمنت فوسفات الزنك اظهرت اقــل معدل تحمل للكسر.

INTRODUCTION

Contemporary endodontic therapy helps patients to retain their teeth even if severely damaged. The restoration of most endodontically treated teeth involves complex and controversial procedures. These teeth have lost significant coronal dentin because of endodontic access or previous dental caries and restoration. Several researches had been described the need for coronoradicular stabilization and numerous post systems and techniques (1).

Custom cast posts and cores have been considered the most accepted treatment mode for many years. Later on, the commercial prefabricated posts with plastic core materials have become a common and a popular method for post and core build up (2).

Several parameters such as cementing medium, length, diameter, configuration, surface roughness and matrial used in the dowel can affect teeth retention and strength (3).

The bond strength of the cementing agents plays an important role in the success of the cast restoration. Zinc phosphate cement despite its lack of adhesion property to tooth structure it is the most selected preferable for cementation of posts for many years (3). However, more new materials such as adhesive resin cements have been advocated for cementation of posts because they bond dentin to metal post and thereby achieve a stronger and more retentive restoration (4).

Nowadays, glass ionomer cement with improved mechanical properties and chemical adhesion to tooth structure are used as a cementing medium (5).

MATERIALS AND METHODS

Thirty intact human maxillary central incisors recently extracted due to periodontal reasons collected form different dental clinic, of comparable root length and width were selected, cleaned and stored in normal saline solution at room temperature through all the time of experimentation.

The anatomic crowns of the teeth were removed at the level of cementoenamel junction perpendicular to the long axis of the tooth using diamond bur rotating at a high speed under constant water spray coolant.

Endodontic therapy has been done for all teeth, and then post spaces preparation were done using pessoreamers from No.1 to No.6 successively in an increasing order of width

and for a length of 8 mm measured from the coronal end of the root with the aid of a rubber stopper. After that, 1mm gingival chamfer finishing line was done with a diamond chamfer bur on a sound tooth structure.

The external surfaces of the roots were carefully notched using diamond fissure bur approximately 3mm from the apex and at 0.5mm depth to provide adequate retention for the teeth in acrylic resin blocks. After that, teeth were embedded in individual blocks of self-curing resin to about 2mm below their coronal ends.

Wax pattern of posts (post crowns) were made directly on the prepared teeth using type II blue inlay wax and plastic posts, and then invested with a phosphate bonded investment material and the casting procedure was performed using nickel-chromium casting alloy.

After casting, the obtained cast posts were cleaned and smoothed and were carefully fitted into their canal spaces to assure proper seating and fitness, then for each post a groove was made along the side of the post with No.1/4 round bur rotating at a high speed to provide an escape vent for cement during cementation.

For all teeth in all groups, post spaces were cleaned with 1ml of 95% ethyl alcohol to remove any residual eugenol contaminantes from the sealer (6), then rinsed with normal saline solution and dried with paper points. 37% phosphoric acid was applied to post spaces for 15 seconds then rinsed thoroughly with water for 30 seconds, dried with paper points and air blower (7).

The cast posts and their respective prepared roots were randomly divided into three groups of ten each:

Group I: teeth with cast posts cemented using zinc phosphate cement.

Group II: teeth with cast posts cemented using glass ionomer cement.

Group III: teeth with cast posts cemented using resin cement.

The cementation procedure was performed by the same investigator and at a room temperature around 25ºC, and a static load of 5 Kg was used to hold each post in their canals according to the setting time of each cement.

The samples were placed in a special mounting fixture, attached to a universal testing machine (Zwick testing machine) (8). A continuously increasing compressive force was applied to the facial cusp in the axio-occlusal line angle, 45 degrees to the long axis of the tooth at a crosshead speed of 5mm/min. until failure (9,10). The data obtained were then statistically evaluated using one way analysis

of variance (ANOVA) test and Least Significant Difference (LSD) test.

RESULTS

Results were obtained for all thirty-test specimens. The means and standard deviations of the three groups are presented in table (1).

Table (1): Descriptive statistics of failure loads (in Kg) for the three groups

Groups	I	II	III
Mean	94	104	135
S.D	6.38	7.32	6.83
Min.	82	94	128
Max.	101	115	147

Statistical analysis of data by using analysis of variance "ANOVA" test revealed that there is a statistically very highly significant difference (P<0.001) between the mean forces among the three groups, as shown in table(2).

Table (2): Analysis of variance (ANOVA) test

Sources of Variance	Degree of Freedom	Sum of Squares	Mean Squares	F Value	Significance
Between Groups	2	9140.000	4570.000	97.311	Very Highly Significant
Within Groups	27	1268.000	46.963		
Total	29	10408.000			

The source of this statistically significant difference was further investigated by using Least Significant Difference (LSD) test to show where the significant difference had occurred, as shown in table (3).

Table (3): Least Significant Difference (LSD) test to compare the mean failure loads between groups.

Groups		Mean difference	Significance
I	II	-10.00	H.S
I	III	-41.00	V.H.S
II	III	-31.00	V.H.S

H.S = highly significant, V.H.S = very highly significant

The results showed that the posts cemented with resin cement (group III) exhibited the highest mean value of failure loads followed by posts cemented with glass ionomer cement (group II), while posts cemented with zinc phosphate cement (group I) exhibited the lowest mean value of failure loads.

DISCUSSION AND CONCLUSION

A cast post is considered the most retentive post with a high degree of biocompatibility.
The retention of cast post is further enhanced when a resin cement is used (11). When these posts are just cemented and not bonded to dentin, they have been shown not to strengthen the root and may actually weaken it (12). Many manufacturers today claim that their root reinforcement systems can actually strengthen the root and help prevent fracture. In this study, the effect of resin cement and glass ionomer cement was compared to determine whether bonding the post to dentin provided the root greater resistance to fracture. Zinc phosphate cement, a cement that creates no bond between the dentin in the root canal and the post.
A compressive head angle of 45 degrees to the long axis of the tooth was applied to the facial cusp in the axio-occlusal line angle of the artificial crown. This angle was used to approximate the combination of forces on teeth as opposed to purely compressive or shear forces, thus simulating the angle of occlusion of the cusps of the opposing teeth (9).
Regarding the results of the current study, the type of cement has a significant effect in root reinforcement. Posts cemented with resin cement recorded the highest mean value of failure loads than those cemented with either glass ionomer cement or zinc phosphate cement. These results were compared with findings of Mendoza et al. [12] which could be attributed to the fact that resin cement has desirable physical properties; its compressive and tensile strengths exceeded that of glass ionomer cement and zinc phosphate cement with an ability to adhere to tooth structure via dentin bonding agents which are responsible for the penetration of resin tags inside the dentinal tubules and demineralized intertubular dentin with the formation of resin reinforced hybrid dentin layer, resulting in a micromechanical bond between the adhesive cement and dentin, and also resin cement has the ability to adhere to post metal surface, such features can afford the root canal system additional resistance to fracture.

In addition, the results of this study showed that posts cemented with glass ionomer cement recorded higher mean failure loads than posts cemented with zinc phosphate cement. A possible explanation is that the values of tensile strength of glass ionomer cement exceed that of zinc phosphate cement (13) and also the adhesive nature of glass ionomer cement (chemical chelating) can afford root canal system significant additional resistance to fracture.

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Analysis Process the TFA of FH Based On Morlet CWT and SPWVD

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ABSTRACT

Analysis process of the TFA (Time-Frequency Analysis) of frequency hopping signal is presented in this paper. The algorithm that used Morlet Wavelet and SPWVD can detect the frequency hopping signal which effectively by selecting the range of the period and resolution properly, the parameters of frequency hopping signal such as hop duration, time offset and hop frequency can be estimated

Key Words: Wavelet, Frequency hopping signal, SPWVD, TFA

الملخص باللغة العربية

يقدم هذا البحث عملية تحليل لـ (تحليل التردد الزمني) (time-frequency analysis)   لترددات القفز   (Frequency Hopping)حيث كانت الخوارزمية المستخدمة هي المويجات المستمرة الموريلت (morlet CWT wavelet)   وكذلك توزيع وينكر-فايل المصقول (SPWVD) وذلك لكشف اشارة ترددات القفز بشكل فعال من خلال تحديد مجال فترة معينة والدقة بشكل صحيح او بشكل ملائم، وكذلك يمكن تخمين المؤشرات لاشارة ترددات القفز مثل فترة القفز، فترة التعديل وتردد القفز.

INTRODUCTION

The systems of Frequency Hopping spread spectrum (FHSS) have been used due to the security features of transmitted and received signals, in addition these systems used to decrease the probability of interception and detection. Also, these systems have capabilities against interferences and hostile jamming especially in military communications. As the precondition of interception and interference, estimation and tracking the parameters of unknown waveform of FH signals considered as an important tasks for information interception and radio sensing with applications to be secured in many fields, especially in military and civilian fields. Also, it's considered one of the great challenging problems as the random characteristics of hopping frequency and high levels of noise and interference. Many researchers have made many efforts and proposed all kinds of ways for estimating the time and frequency parameters of FH signals (1). The FHSS signal is typical know as non-stationary signal, and it's important to know that the characteristics of frequency domain in anytime, so, need to describing the joint time-frequency Analysis (TFA) of FH signals.

Research on parameter estimation of FH signals by using time-frequency analysis (TFA) has been carried out for at least two decades. FH signals that consist of various TFA structures are represents as a combination of components in the former type, which give important information about inner structures. These components could be obtained by using the wavelet (2). But these methods require building parametric, redundant, and well-localized TFA, which are matched with FH components difficultly. In the latter type, FH signals is described generally by a function with time and frequency variables and parameter estimation is operated on the TFA plane. Nevertheless, TFA resolution in the above methods is always losing with the reduction of interferences, where the locations of the maximal values of TFA distributions appear shift. On the other hand, hop timing can't be correctly estimated using maximum sequence as itself may be wrong and the resulting hop frequency is also a mistake, especially in low SNR noise, although difference operation is simple (3).

Based on the above analysis, we can see that all the introduced methods must estimate the maximum along time domain or frequency one. This operation is sensitive to noise due to differential operator. In this paper, we propose a new parameter estimation algorithm based on wavelet and TFA in low SNR .

Time-Frequency Analysis

In signal processing, time–frequency analysis used for characterizing and manipulating signals whose statistics vary in time and has ability to describe the distributed at time of the density of signal, and frequency consists in a set of transforms that maps a one-dimensional time domain signal into a two-dimensional representation of energy versus time and frequency (1).

TFA is a generalization of Fourier analysis which signal of frequency characteristics has varying on time. The Fourier transform obtains the frequency spectrum and requires a complete description of the signal's behavior over all time. As known, TFA working for both time and time domain, which then in order to convert time domain to frequency domain need to implement on of Fourier transformations methods especially when need to estimate the energy .

There are many forms of the TFA such as Short Time Fourier Transform (STFT), Wavelet, Wigner Ville Distribution (WVD), and Gabor-Wigner Ville Distribution (4).

In order to achieve fine simultaneous for time frequency resolution in a non-stationary signal, need to obtain an arbitrarily fine resolution simultaneously in both time and frequency domain: $\Delta t\, \Delta w \geq \frac{1}{4\pi}$, in which the selection of time resolution Δt, and frequency Δw, are not arbitrary parameters.

A trade-off between them should be considered in order to reach a "good" resolution. An overview and a brief description of the advantages/limitations of the time-frequency distributions introduced above are presented in (5).

In non-stationary signals as encountered in power networks, the research is considered a necessary to use and implement the methods of time-frequency (6).

The methods can be divided in several ways. One of the divisions is shown in the Figure (1).
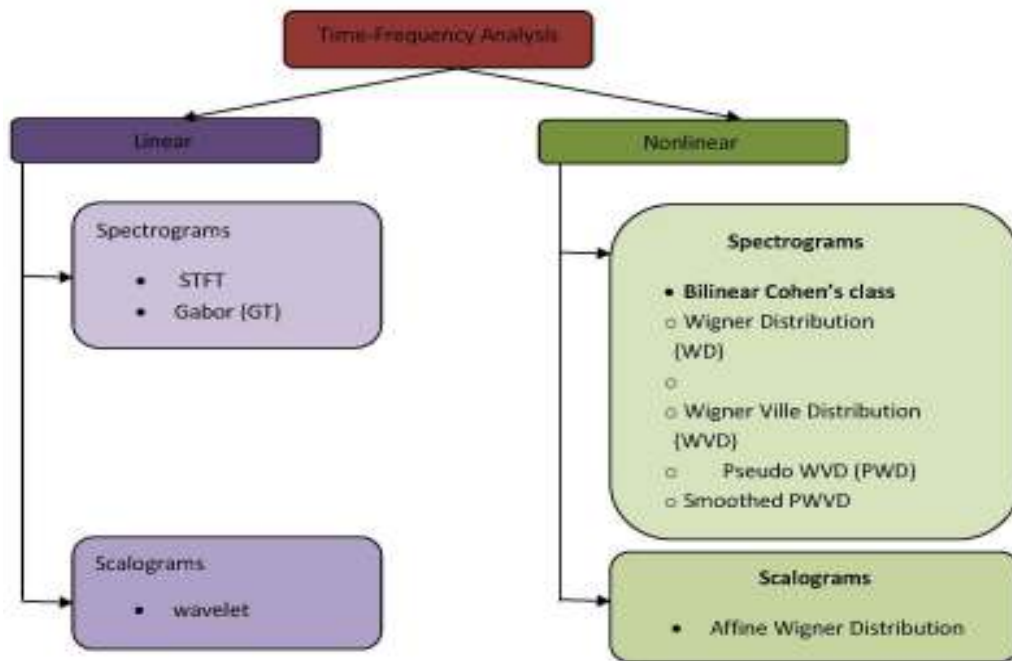
Fig. (1):One of the signal analysis classifications

As shown in figure (1), the analyzed signal in linear methods going to be compared in direct way with the elementary functions. As known a complexity in computation in these methods. In nonlinear methods are known have a significant advantages in direct energy signal projection, and this considered as an important issue especially in power signal measurements.

Wavelet Transform

The Wavelet transform is very useful tool which used to analyze the frequency components of the signal. The continuous wavelet transform (CWT) is transforming the scale structure of a signal varies with time, and this transform works when using a continuous wavelet function to find the detailed coefficients of a continuous signal. The scale structure of signal is considered as a frequency, so CWT provides a view of the frequency versus time behavior of the signal and therefore has great potential as a preliminary tool for investigating wideband, non-stationary, or other types of signals having time-dependent spectral characteristics. The results of signal analysis would then be used to construct appropriate signal processing algorithms to detect, characterize, and classify the signals. The proposed wavelet is a Morlet, has good properties for use as the kernel in a qualitative CWT. These properties are

presented, and the use of the CWT for studying wideband communications signals and for finding features that might be of significance in a seismic signal.

The CWT is defined with respect to a particular function, called a mother wavelet, that satisfies some particular properties. Not every function can qualify to be a mother wavelet. In transformation signal in kernel functions, it is important to know that the mother wavelet has been designed to give a ability for the transformation to be inverted. Then, the signal information/characteristics need to be structured in CWT (7).

Suppose that $\psi(t)$ denotes the mother wavelet, and that $\tilde{\psi}(\omega)$ denotes its Fourier transform. We use the definition (in which j denotes $\sqrt{-1}$).

$$\tilde{\psi}(\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \psi(t)e^{-j\omega t}dt \ldots \ldots \ldots \ldots (1)$$

Then the admissibility condition implies that $\tilde{\psi}(0) = 0$, that is $\int \psi(t)dt = 0$. Figure (1) illustrates this effect for the Morlet wavelet $\psi(t)$, defined by the formula:

$$\psi(t) = \sqrt{2}e^{-t^2/\alpha^2}\left(e^{j\pi t} - e^{-\pi^2\alpha^2/4}\right) \ldots \ldots \ldots \ldots (2)$$

The parameter α in Eq. 2 can be set to obtain desired time–frequency shaping in the CWT. Note that in the graph of $\psi(t)$ in Fig. (2a), there appear to be no oscillations, but this apparent absence is the result of the graph being that of the amplitude of the complex waveform—the oscillations are contributed by the phase. One can compute directly the Fourier transform of this wavelet. It is

$$\hat{\psi}(\omega) = \alpha e^{-\alpha(\pi^2+\omega^2)/4}\left(e^{\pi\alpha^2\omega}/2 - 1\right) \ldots\ldots\ldots\ldots\ldots(3)$$
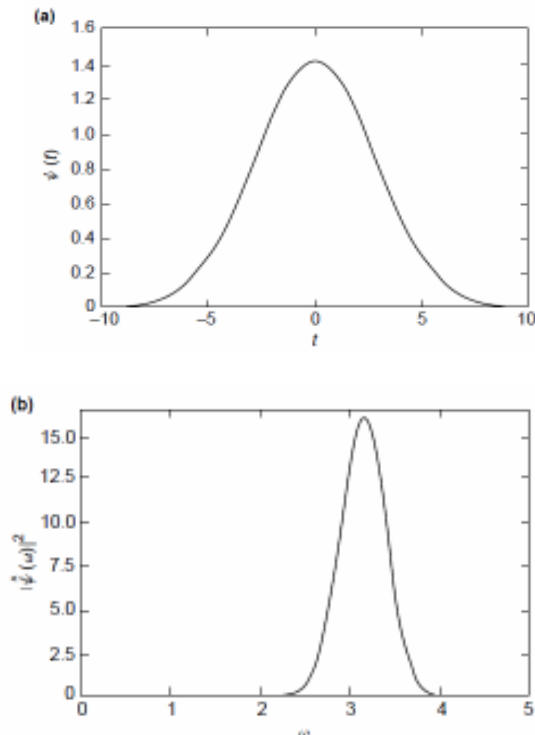
As seen in Figure (2), the Morlet wavelet, in the frequency domain, is a complex bandpass filter. Its effect as a filter would be to limit a signal to a band centered about the frequency of approximately $\pi\, rad/s$, with the center point approaching p as the parameter a gets large.

The function $WVD\{x_a(t)\}(\omega)$ presented the time-frequency of Wigner-Ville distribution (WVD) associated to a signal $x_a(t)$, of finite energy. This function depending on the temporal *(t)* and frequency *(ω)* parameters. This distribution is given by the expression

$$SPWV\{x_a(t)\}(\omega) = \int_{-\infty}^{+\infty} x_a\left(t+\frac{\tau}{2}\right) x_a^*\left(t+\frac{\tau}{2}\right) e^{-j\omega u}\, d\tau \ldots (4)$$

where $x_a^*(t)$ is the complex conjugate of the analytical signal $x_a(t)$.

In this simulation which based on a Smoothed Pseudo Wigner-Ville (SPWV) and it's implemented to attenuate the interference terms presented between the inner components figuring in Wigner-Ville representation. The SPWV using two smoothing windows which is *(h)* and *(g)*. These smoothing windows are introduced into the definition of the Wigner-Ville distribution in order to allow a separate control of the interference, either in time *(g)* or in frequency *(h)*. The expression of this representation is defined by

$$SPWV\{x_a(t)\}(\omega) = \int_{-\infty}^{+\infty} \left|h\left(\frac{\tau}{2}\right)\right|^2 \int_{-\infty}^{+\infty} g(t-u)x_a\left(u+\frac{\tau}{2}\right) x_a^*\left(u-\frac{\tau}{2}\right) e^{-j\omega u}\, du\, d\tau (5)$$

where *h(t)* is a smoothing frequency window and *g(t)* is a smoothing temporal window and (τ) is the shift of signal.

RESULTS

Two different methods of Morlet Discrete Wavelet and (SPWVD) Smoothed Pseudo Wigner-Ville Distribution were applied to analysis of (TFA) Time-frequency Analysis in frequency-hopped signal in this research, through the theory study and emulations analysis it reflects that the two methods can show frequency characteristics of frequency—hopped signal, and prove the feasibility of their application in the frequency-hopped signal analysis.

The performance of the method has been evaluated by simulation in the presence of additive Morlet wavelet. We compare the FH signal specification and results of our performance analysis with the results of SPWVD. Below the formula has been used to generate the signal of FHSS as described below.

$$s = rand(N_{samples})$$

Fig. (2): The Morlet wavelet $\psi(t)$ and its power spectrum. For simplicity, the parameter α is set to 4. (a) Amplitude of the complex Morlet wavelet. (b) Power spectrum of the Morlet wavelet.

s: is the randomized number contains the number of generated samples.
N: number of samples.

$$t = \frac{r1 * pi}{r2 * pi}$$

r1: number of samples
r2: number of samples
t: sample

$$k = \sum_{s}^{0} (cos(s)) \begin{bmatrix} if\ s = 0 & signal\ has\ a\ minus\ sign \\ if\ s = 1 & signal\ has\ a\ plus\ sign \end{bmatrix}$$

Figure (3) shows specification of the FH signal composed of 250 samples and 60 samples for each sinusoid.
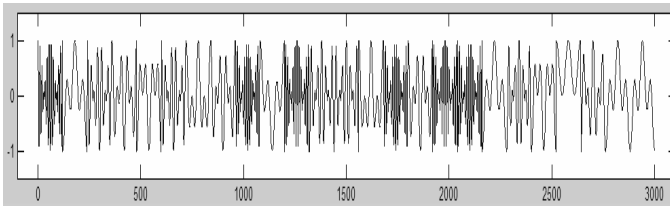
Fig. (3): Frequency Hopping Spread Spectrum Generated Signal

Then we've used the proposed identification algorithm Morlet identify the hopping frequencies, hopping rate and hopping sequence for the signal described above. The Morelet Wavelet has been applied to FHSS, and the equation be of Morelet wavelet formulated as in equation below:

$$\psi(t) = \sqrt{2} e^{-t^2/\alpha^2} \left(e^{j\pi t} - e^{-\pi^2 \alpha^2/4} \right)$$

$$\psi(k) = \sqrt{2} e^{-k^2/1.75^2} \left(e^{j\pi k} - e^{-\pi^2 1.75^2/4} \right)$$

k: determining the current signal of FHSS
α: constant (1.75)
By computing the Morlet wavelet using equation above, we get the form of wave as in Figure (4).
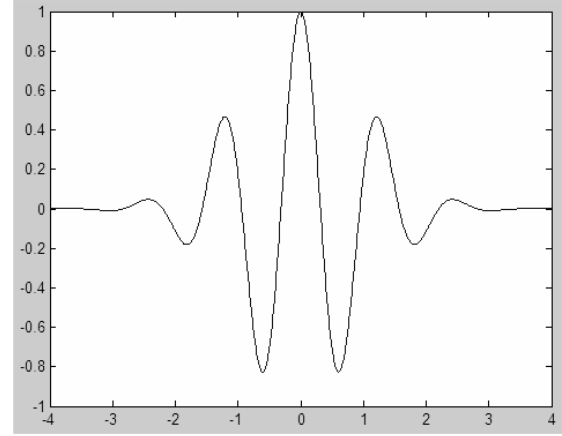
Fig. (4): Plot of Morlet Wavelet Form Signal

In this paper, we have proposed an algorithm for identifying the parameters of FH signals embedded in additive Morlet wavelet as seen in Figure (5).
Then finally, to get final wavelet by applied wavelet transform equation as in equation below:

$$\hat{\psi}(\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \psi(k) e^{-jwt} dt$$

and there are some parameters have set as below:
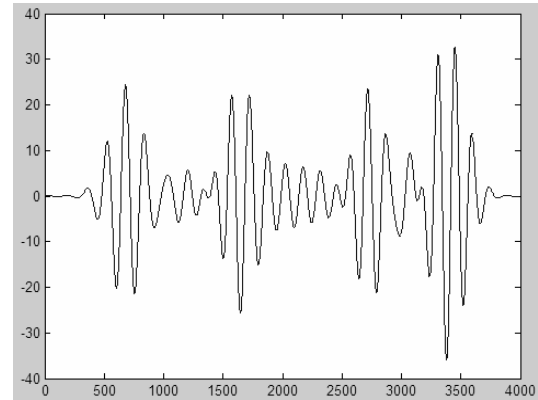
Fig. (5): Plot of Convolution Signal of Morlet Wavelet and FHSS

The SPWVD (smoothed-pseudo Wigner-Ville distribution) is applied to identify the parameters which include hopping frequencies, hopping rate and hopping sequence. The identification method does not make any assumption about the alphabet of hopping frequencies, hopping rate, or the synchronization. The simulation results are present to evaluate the performances of the proposed algorithm. Below SPWVD has generated based on equations below:

$$s = rand\big(N_{samples}\big)$$

s: is the randomized number contains the number of generated samples.
N: number of samples.

$$t = \frac{r1 * pi}{r2 * pi}$$

r1: number of samples
r2: number of samples
t: sample

$$k = \sum_{s}^{0} (cos(s)) \begin{bmatrix} if\ s <= 0 \ \ signal\ has\ a\ minus\ sign \\ if\ s >= 1 \ \ signal\ has\ a\ plus\ sign \end{bmatrix}$$

$$SPWV[x_a(t)](\omega) = \int_{-\infty}^{+\infty} x_a\left(t + \frac{\tau}{2}\right) x_a^c\left(t + \frac{\tau}{2}\right) e^{-j\omega u}\ d\tau$$

$$SPWV(k(t)) = \int_{-\infty}^{+\infty} k_a\left(t + \frac{\tau}{2}\right) k_a^*\left(t + \frac{\tau}{2}\right) e^{-j\omega u}\ d\tau$$

k: signal
t: samples
$d\tau$: specific time for current sample (delta time)

In Figure (6), A SPWVD was generated by frequency samples 350 sample.
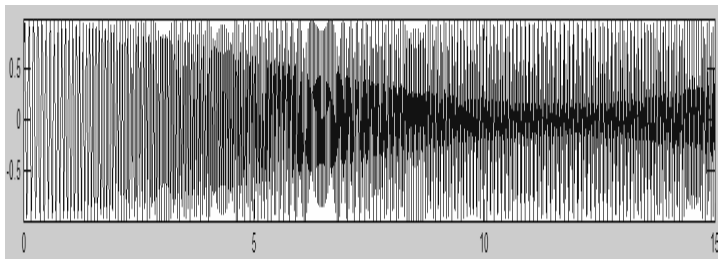
Fig. (6): Plot of Waveform Generated in SPWVD

Finally, we've convolved the first dimension of wavelet signal and, with the first dimension of SPWVD. The general equation for convolution is

$$y(k) = \sum_{n} u(n - k) * h(k)$$

$$y(k) = \sum_{n} \hat{\psi}(k(t)) * SPWV(k(t))$$

and the results is shown in plot in figure (7).
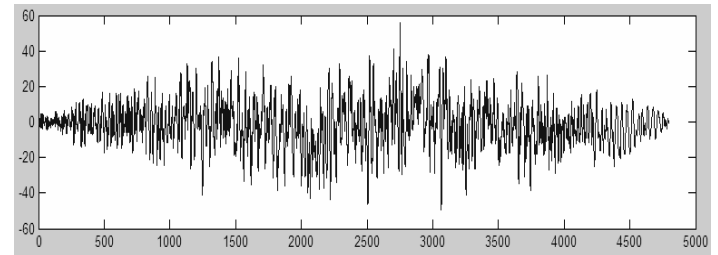
Fig.(7): Plot of Convolution Signal of SPWVD and FHSS

The Wigner distribution has been noted as one of the more useful time-frequency analysis (TFA) techniques for signal processing.
Another advantage of SPWVD is the fact that SPWVD gives direct information about time-frequency localization of signal energy. It enables application of the transformation to evaluate energy included in higher harmonics and to localize it in time domain. In general, SPWVD analyses are much more computational than standard STFT. Therefore, further research is conducted for the ways of their application in equipment that uses these analyses in on-line computation (8).

DISCUSSION

The evolution of frequency content in a signal has been focused in this paper and complete investigated based on the Smoothed Pseudo Wigner-Ville Distribution and Continues wavelet (Morlet). A variety of time-frequency analysis of frequency hopping has been depended, which provide useful information about the behavior of frequency hopping.
The method for analyzing the instantaneous spectrum of a signal is to decompose it into different bases, one such choice is the wavelet transformation. The Continuous Wavelet Transform (CWT) Morlet creates a time-scale representation of the signal, where the scale of

the wavelet bases has an intuitive association with frequency. The wavelet method has some advantages for time-frequency analysis. The result demonstrates the use of the wavelet transform on our test signal, though the frequency axis is only an approximation based on the scale of the wavelet transform.

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Comparison Study between Adam-Bohart, Thomas and Yoon Nelson Models for Adsorption of Pb(II) from Simulated Wastewater by Activated Carbon

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ABSTRACT

In this research a comparison study was made between Adam-Bohart, Thomas and Yoon-Nelson model to determine which model gives the best representation for the adsorption of Pb (II) from simulated wastewater by activated carbon. Depending on the values of (R^2) and the HYBRID error function (HEF). It is found that Adam-Bohart model describe the equilibrium isotherm data better than Thomas and Yoon-Nelson model with (R^2) value of 0.962, 0.937and 0.924 for Adam-Bohart, Thomas and Yoon-Nelson model respectively, and (HEF) of 0.281, 0.335 and 0.484 for Adam-Bohart Thomas and Yoon-Nelson model respectively. Also it found the adsorption of Pb(II) from aqueous solution by activated carbon was of favorable type.

Key Words: adsorption, activated carbon, Adam-Bohart model, Yoon-Nelson model, HYBRID error function

الملخص باللغة العربية

يهدف البحث إلى إجراء دراسة مقارنة بين نموذج Adam-Bohart ونموذج Thomas ونمـوذج Yoon-Nelson لمعرفـة أي منها الأفضل في وصف عملية امتزاز أيون الرصاص الثنائي من المياه باستخدام الكاربون المنشط .

اعتمادا على قيم R^2 وكذلك قيم دالة الخطأ الهجينة HYBRID وجد أن نموذج Adam-Bohart يصف عملية الامتـزاز لأيـون الرصاص الثنائي أفضل من نموذج Thomas و نموذج Yoon-Nelson , حيث إن قيمـة R^2 لنمـوذج Adam-Bohart كانـت 0.962 بينما لنموذج Thomas كانت 0.937 ولنموذج Yoon-Nelson كانت 0.924.

إن قيمة دالة الخطأ الهجينة كانت 0.281 لنموذج Adam-Bohart   0.335 لنموذج Thomasو0.484 لنموذج .Yoon-Nelson. وان عملية الامتزاز لأيون الرصاص الثنائي بواسطة الكاربون المنشط كانت من النوع المفضل .

INTRODUCTION

Lead and its salts consider one of the most important heavy metal elements that presented in the environment (1) As this metal has a main role in different life activities and introduced in many industrial, technological, mineral and petroleum industries (2).

Lead salts presented in the municipal wastewater effluent especially for cities and towns that near of the chemical plants and processing industries, the major risk and concern of lead salts are their presence in the waste streams or drinking water (3). The presence of lead in any form in water will add a distinguish taste to the waste even when the (Pb) concentration be around 1.2 mg/l (4).

In addition to that the accumulation of (Pb) salts within the human being body causes a chronic defects and diseases that lead in sometimes to death. Disease such as kidney failure, liver disease, heart disorder are related to the presence of lead salts in the human being body with relatively high concentration reaches to (3-5) mg/l.

The assimilation of relatively small amounts of lead over a long period of time in the human body can lead to the malfunctioning of the organs and chronic toxicity. The toxic effect of lead ions on humans, when present above the threshold level in the human body may become lethal (5).

Lead has been shown to cause brain damage in children, also, an arthritis, urethritis and conjunctivitis even when its existence is in an extremely low concentration (6).

Environmental protection Agency (EPA), the world health organization (WHO) and the Iraqi standards, all, set limits for different heavy metals concentration in a wastewater, potable water and the rivers, these limits values were presented in table (1).

Adsorption by activated carbon is widely used process for removing of different pollutant species from water and wastewater, such technique offers easy operational, economical and good efficiency for removing of these pollutants from water (7).

The use of activated carbon as an adsorbent was recorded since 500 B.C as ancient Egyptians use charcoal for water deodorizing (8).

Due to it's excellent surface properties (high surface area per unit mass), high porosity, low cost of manufacturing, these properties make activated carbon as a good adsorbent in spite of the presence of many adsorbents such as silica gel, and saw dust (9).

(10) found the adsorption of Pb (II) fitted well with Langmuir isotherm for different Pb (II) equilibrium concentration.

(11) reveals that for different pH values of Pb (II) the Frendlich isotherm model not fitted the data.

Adam-Bohart Model

Adam-Bohart model is valid for a single layer adsorption. It is based on the assumption that maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, and, the energy of adsorption is constant (12).

The Adam-Bohart isotherm is used to describe the adsorption data. The basic assumptions underlying Adam-Bohart model are:

- Solute molecules are adsorbed onto definite site on the adsorbent surface.
- Each site can be a host for only one molecule (monolayer).
- The area of each site is a fixed quantity determined the geometry of the surface.
- The adsorption energy is constant for all sites.
- The adsorbed molecules can not migrate across the surface or interact with other molecules.
- The film diffusion is the rate limiting step.

Adam-Bohart model was widely used to describe the adsorption process onto activated carbon the model can be expressed as:

$$q_e = \frac{a}{C_o} - \frac{b}{C_e} \ln(\frac{C_o}{C_e} - 1) \qquad (1)$$

Table (1), *Permissible limits of heavy metals concentration in drinking water, rivers and wastewater

Pollutants	Iraqi Standards			WHO			USEPA		
	drinking water mg/l	river mg/l	waste-water mg/l	drinking water mg/l	river mg/l	waste-water mg/l	drinking water mg/l	river mg/l	waste-water mg/l
Pb	0.005	0.05	0.05	Nil	0.03	0.05	<0.003	0.03	0.05
Cd	0.005	0.01	0.05	Nil	0.04	0.05	<0.003	0.04	0.05
Hg	0.005	<0.05	0.05	Nil	0.03	0.05	Nil	0.03	0.05
As	0.005	0.05	0.05	Nil	0.02	0.05	Nil	0.03	0.05
Cr	0.005	0.05	0.05	Nil	0.02	0.05	Nil	0.03	0.05

*Iraqi environmental legislations, Ministry of Environment (MOE), 2007.

Where;

q_e = amount of material adsorbed per amount of adsorbent (kg/kg).

C_e = equilibrium concentration (kg/m^3).

C_o = initial concentration (kg/m^3).

a, b = adsorption rate constants.

And by plotting q_e versus $\ln(C_o/C_e -1)$ the value of (b) will determined from the intersection of equation (1) with y-axis and the value of (a) may calculated.

(13) found Adam-Bohart model give the best fit for equilibrium data of Cd (II) adsorption by silica gel.

(14) investigate that Adam-Bohart model give a good match for representing the equilibrium data for adsorption of Hg(II), Pb(II) from wastewater by cocoanut shell.

Thomas Model

In 1944, Thomas developed a model for adsorption processes in which external and internal diffusion limitations are not present.

Thomas model is generally an extended isotherm from Radke-Prausnitz (table2) model and corresponds to multilayer adsorption. This model assumes that a number of layers of adsorbate accumulate at the surface, and that the Radke-Prausnitz model applies to each layer. Each additional layer of adsorbate molecules is assumed to equilibrate with the layer below it and layers of different thickness are allowed to coexist (15).

Thomas model which accounts for a multilayer adsorption and for existence of heterogeneous pore distribution in the adsorption, also this model depends on the movements of the adsorbate by the concentration difference as a driving force rather than the inter-particle and pore diffusion. This model can be expressed as:

$$\ln((C_{o}/C_t)-1) = (K\, q_e\, W /V) - K\, C_o\, t \quad (2)$$

Where K is the Thomas rate constant (ml/min.mg), qe is the equilibrium of adsorbate uptake (mg/g), C_o is the inlet concentration (mg/l), C_t is the effluent concentration at time t (mg/l), W is the mass of adsorbent (gm.), V is the volume of sample (ml) and (t) is the contact time (min.). The value of C_o/C_t is the ratio of inlet to outlet concentrations. A linear plot of $\ln [(C_o/C_t) - 1]$ against time (t) was drawn to determine the values of qe and K from the interception point and slope of the plot respectively.

Yoon-Nelson Model

Yoon and Nelson developed a model to describe the adsorption behavior. The linearized form of the Yoon-Nelson model is given in Equation (3).

$$\ln(C_{t}/(C_o - C_t)) = K_{yn}(t) - K_{yn}\,\tau \quad (3)$$

Where, k_{yn} is the rate velocity constant (l/min) and τ is the time in (min) required for 50% adsorbate breakthrough. A linear plot of $\ln [C_t / (C_o - C_t)]$ against sampling time (t) was used to determine the values of k_{yn} and τ from the slope and intercept of the plot.

In addition to above to mentioned models, there are a number of isotherm models describe the adsorption processes. The properties of these isotherms with characteristics are summarized in table (2).

Table (2): Adsorption isotherm models characteristics (16)

Isotherm	Equation	Advantages	Disadvantages
Langmuir	$q_e = \dfrac{q_m b C_e}{1 + b C_e}$	interpretable parameters	not structured, monolayer-sorption
Freundlich	$q_e = K\, C_e^{1/n}$	simple expression	not structured, not leveling off
combination (Langmuir and Freundlich)	$q_e = \dfrac{b q_m C_e^{1/n}}{1 + b C_e^{1/n}}$	combination of above	unnecessarily complicated
Radke and Prausnitz	$q_e = \dfrac{K_{RP} C_e}{1 + (\frac{K_{RP}}{F_{RP}}) C_e^{1-N_{RP}}}$	simple expression	empirical uses 3 parameters
Reddlich and Peterson	$q_e = \dfrac{A_R C_e}{1 + B_R C_e^{m_R}}$	approach Freundlich high concentration	no special advantages
Dubinin – Raduskevch	$q_e = q_{DR} \exp(-\beta \in^2)$	give a percentage filling micropors	special uses
Temkin	$q_e = \dfrac{RT}{b} \ln(K_T C_e)$	involving the binding energy	uses for a restricted isothermal adsorption process
Harkins - Henderson	$q_e = \dfrac{K^{1/n}}{C_e^{1/n}}$	multilayer adsorption and heterogeneity	not use for a general adsorption

The parameters of each model were determine from the best fitting of the equation to the measured experimental data, and the judgment on the best fitting model depends on two techniques, firstly, adopt the highest value of the correlation coefficient (R^2) resulted from

the implementation of each model, secondly, conducting the lowest value of the (HYBRID) fractional error (17).

The (HYBRID) fractional error function of non-linear regression is employed, as it compensates for low concentrations by balancing absolute deviation against fractional error and is more reliable than other error function. The (HYBRID) error function is given by:

$$\text{HYBRID} = \frac{100}{N-p} \sum \left[\frac{q_{e,exp} - q_{e,calc.}}{q_{e,exp}} \right] \quad (4)$$

Where N is the number of data points and p is the number of parameters in the isotherm model. Applying the two mentioned techniques, in this research, the model with highest (R^2) values and lowest (HYBRID) error function considered as the best fit model.

The HYBRID error function is widely used in the statistical analysis for the environmental engineering problems, since this technique provide a solid tool for assessment of any reliable process that may used for controlling any environmental process to ensure the degree of confidence of such process to be conducted and used for environmental pollution problems (18).

As Adam –Bohart model depending strongly on the film diffusion criterion, that well describes the phenomena (15),this phenomena represents the mass transfer to be controlled majorly by the film diffusion ,hence, it proposed that the greatest resistance to mass transfer will be within the film layer.

Film Diffusion

When the mass transport is assumed to be controlled by resistance of hydrodynamic boundary layer, this called film diffusion. In this case the mass transfer of the adsorbate from the bulk of the solution onto the outer surface of the particles takes place (1).

Film diffusion is also known as external mass transfer. Adsorbate molecules which migrate from the bulk solution to the adsorbed state are transferred to the outer surface of the adsorbent by liquid phase diffusion (13).

This mass transfer step occurs within the boundary layer around the adsorbent (19).

Film diffusion is governed by molecular diffusion and in turbulent flow, by eddy diffusion, which controls the effective thickness of laminar boundary layer (20) If the boundary layer is thick and the compounds need to diffuse a large distance, film diffusion may be rate limiting. The boundary layer will become thinner if the liquid is agitated, therefore the compounds will rapidly diffuse through the boundary layer. Therefore liquid agitation will reduce the influence of film diffusion. The diffusion of solute molecules through the external film around the particle may be expressed by assuming logically that concentration of solute at a point in the boundary layer immediately adjacent to the external surface of the particles is in equilibrium with average solid phase concentration on the interval surface (21).

This may be stated as:

$$\rho_p \frac{dq_i}{dt} = k_f a (C_{b_i} - C_{p_i, R=R_p}) \quad (5)$$

Where, k_f is the external mass transfer coefficient, ρ_p particle density, C_{b_i}, C_{p_i} is the fluid concentration in a bulk and particle face respectively and (a) is the surface area per unit volume of the particle.

The applicability of the film diffusion is restricted to low concentration (22). And if mass transfer is governed only by film diffusion, therefore there is no concentration gradient within the particle (23).

The mass transfer coefficient k_f is a function of hydrodynamic fluid properties and particle size, and can be correlated in terms of dimensionless correlations:

$$\text{Sh} = f(\text{Re, Sc}) \quad (6)$$

(24) had been correlated the mass transfer data in packed bed as:

$$\text{Sh} = 2 + 0.6 \, \text{Re}^{0.5} \, \text{Sc}^{0.3} \quad (7)$$

(25) revealed that when mass transfer coefficient was measured by experiments involving mass balance; the bed should include a term accounting for axial dispersion.

$$\text{Sh} = 2 + 1.1 \, \text{Re}^{0.66} \, \text{Sc}^{0.33} \quad (8)$$

MATERIALS AND METHODS

The granular activated carbon (GAC) used was brought from the local market with the following physical properties mentioned by the manufacturer and tabulated in table (3):

Table (3): physical properties of GAC

Ash content	Less than 2%
Surface area	1270-1420 m^2/gm.
Bulk density	690-720 kg/m^3
porosity	0.76

Activated carbon was firstly washed with distilled water and then put in an oven at 110 ^{o}C for 120 minutes to remove all the moist content and humidity, then a sample of (GAC) was sieved using different sieve size with sieve shaker and (GAC) with 0.5mm average particle size was used. Different weights of (GAC) of (2, 4, 6, 8, 10) gm was put in a (100) ml conical flask. The Pb (II) solution was prepared using Pb $(NO_3)_2$ solution, A (1.21) gm of Pb$(NO_3)_2$ was dissolved in 2.25 liter of distilled water to prepare a solution with (20) mg/l of Pb(II) (26). A sample of (100) ml was taken from the prepared solution and put in the conical flask of different (GAC) for (24) hr., in a rotary shaker and a sample of (10) ml was withdrawn from each flask for analysis using atomic absorption device type (Perkins 91005) the equilibrium data resulting from the analysis was tabulated in table (4).

Table (4): equilibrium concentration C_e mg/l and q_e For different mass of GAC

Sample No.	Weight of GAC, gm	C_e Pb(II) equilibrium concentration	q_e kg of adsorbed / kg of (GAC)
1	2	52.9	0.0015
2	4	11	0.0055
3	6	5.36	0.0079
4	8	4.13	0.0089
5	10	2.73	0.014

By applying equation (1), (2) and (3) for the data tabulated in table (1) the results are show in both figures (1), (2) and (3) respectively.
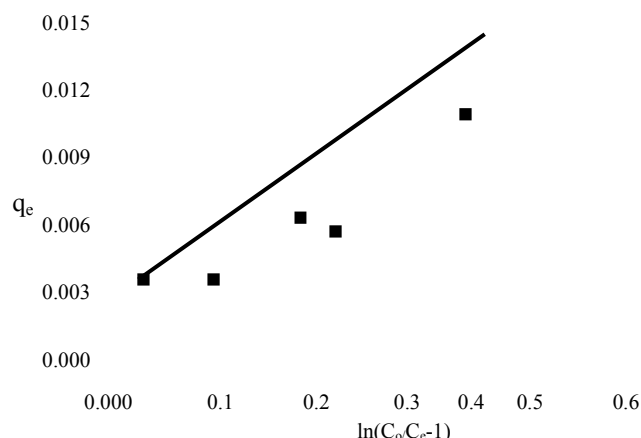

**Fig. (1): equilibrium isotherm applying Adam-Bohart model for Pb(II)**
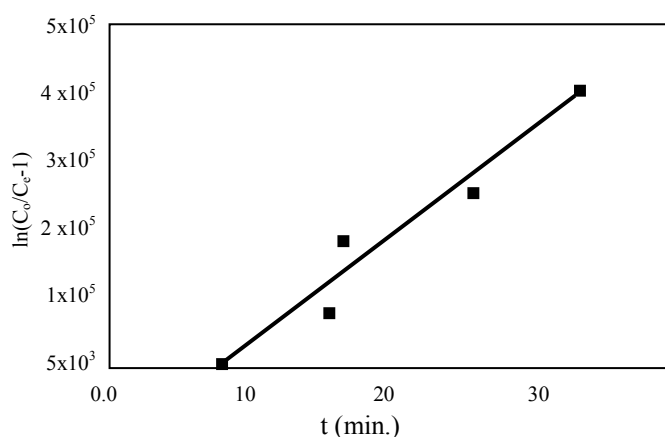
**Fig. (2): equilibrium isotherm applying Thomas model for Pb(II)**
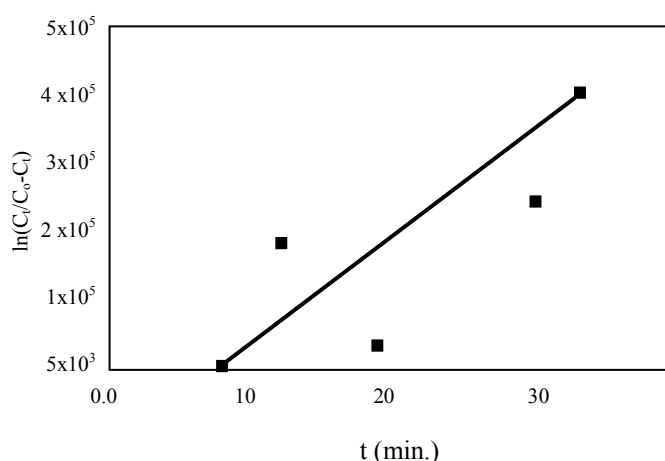
Fig. (3): equilibrium isotherm applying Yoon-Nelson model for Pb(II)

By applying equation (4) on the data mentioned in table (1) HYBRID error function of each model in found to be 0.281for Adam-Bohart model and 0.335 for Thomas model, and 0.484 for Yoon-Nelson model

RESULTS AND DISCUSSION

- The adsorption of Pb (II) by activated carbon was of a favorable type.
- An increase in the equilibrium concentration accompanied by an increasing in the adsorption uptake (sorption) of activated carbon.
- The three models can be used successfully for expressing the adsorption process of Pb (II) onto activated carbon.
- Adam-Bohart model is the best model to express the data in this research in comparison with Thomas and Yoon-Nelson model since (R^2) values of Adam-Bohart model was (0.962) and for Thomas model was (0.937), and for Yoon-Nelson model was (0.924). in addition to the (HFE) values was (0.281) for Adam-Bohart model, and (0.353) for Thomas model, and (0.484) for Yoon-Nelson model.
- Since the research appointed that Adam-Bohart model is the best model to describe the data, that may explained mass transfer of Pb (II) occurred within a multi-layers film that surrounding the (GAC) particles and the gradual concentration gradient was the main driving force of the adsorption process, rather than pore and inter-particle diffusion mechanism.
- It can be concluded that the overall kinetics in the initial part of the adsorption process were dominated by external mass transfer.

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Congenital cytomegalovirus infection in symptomatic infant in Baghdad and surrounding area

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ABSTRACT

Many viral infections are associated with significant maternal and fetal consequences during pregnancy among which cytomegalovirus is one of the most important agent, globally, both primary and recurrent infection due to this virus and its ability to result in fetal infection. Congenital human cytomegalovirus (HCMV) infection is the leading infectious cause of mental retardation and sensorineural deafness. Intrauterine transmission and adverse outcome are mainly related to primary maternal infection.

Samples from Congenital Anomalies babies are referred to CPHL from Baghdad based Government hospitals and surrounding areas for diagnosis of congenital infections like Toxoplasma, Rubella, CMV and Herpes. In the present study, accumulated data for the most common teratogenic virus-Cytomegalovirus prevalence as a causative agent for congenital infection in babies at Baghdad and surrounding areas.

Samples from 500 symptomatic babies in the age group under one year exhibiting different congenital anomalies were examined between 15th Sep/2009 to 15th Sep/2011. All the blood samples were tested for the detection of Anti-CMV (IgM) antibodies using ELISA technique. Samples from 125 babies (25%) showed positive titers for Anti-CMV-IgM antibodies. Also samples from 51 mothers (10.39%) of positive babies were found positive for Anti-CMV-IgM antibodies and all were serologically exposed to CMV virus previously as their serum samples were positive for CMV-IgG antibodies which indicated primary infection in the past or reactivation/re-infection with a different strain of CMV in the early pregnancy. This study documents symptomatic congenital CMV cases in Baghdad and surrounding Area.

Key Words: Congenital abnormalities; congenital CMV infection; herpes virus; pediatric surveillance

الملخص باللغة العربية

يمكن أن تتعرض الأم أو الجنين للعديد من الاصابات الفيروسية خلال فترة الحمل ويعتبر الفيروس المضخم للخلايا CMV من أهم تلك العوامل .. عالميا : كلا الاصابتين الاولية والاصابة الثانوية المتكررة ممكن أن تعزى اصابة الجنين لهذا الفيروس. الاصابة بفيروس المضخم للخلايا (HCMV) يسبب تخلف عقلي والعوق بالسمع وتشوهات خلقية اخرى التي غالبا تعود الى الاصابة الاولية للام بالفيروس.

ارسلت نماذج الاطفال المشوهين خلقيا نتراوح اعمارهم ما بين عدة أيام وسنة واحدة الى مختبر الصحة العامة المركزي وهم مرضى راقدون في مستشفيات بغداد والمناطق المحيطة بها لتشخيص العوامل المسببة للتشوهات الخلقية مثل داء المقوسات , الحصبة الالمانية , الفيروس المضخم للخلايا وفيروس الهيربس .. في دراستنا الحالية تم جمع المعلومات الوبائية للمرضى المصابين بفيروس المضخم للخلايا كعامل مسبب للتشوهات الخلقية في بغداد والمناطق المحيطة , حيث تم جمع خمسمائة نموذج من اطفال مشوهين خلقيا نتراوح أعمارهم دون السنة استلمت في مختبراتنا على مدى سنتين من 2009/9/15 ولغاية 2011/9/15 .. جميعها تم فحصها بتقنية الاليزا وذلك لتشخيص الاصابات الحديثة بالفيروس لمعرفة وجود مستضدات Anti-CMV-IgM ومنها (125) حالة (25%) من (500) نموذج عياريتها موجبة لوجود المستضدات Anti- CMV-IgM وكذلك اظهرت النتائج واحد وخمسون نموذجا(492/51) اخذت من أمهات الاطفال المشوهين خلقيا اي (10,39%) من الحالات لديهن اصابة موجبة بفيروس مضخم الخلايا وجميع الحالات أظهرت انها تعرضت مسبقا للاصابة بفيروس مضخم الخلايا حيث أعطت عيارية موجبة في فحص المستضدات Anti-CMV-IgG وهذا مؤشر على حدوث اصابة سابقا او حدوث اصابة ثانوية بهذا الفيروس او اصابة بسلالة جديدة من فيروس مضخم الخلايا في وقت مبكر من الحمل.

INTRODUCTION

Human cytomegalovirus (HCMV) or human herpes virus 5 (HHV-5) is a member of the *Herpesviridae* family, subfamily *Betaherpesvirinae*. HCMV is ubiquitous and highly species-specific. HCMV establishes a lifelong relationship with the infected host (once infected, always infected) resulting in primary infection, lifelong latency, and intermittent excretion following reactivation. Such a remarkable property constitutes the basis for the peculiar natural history of HCMV in pregnancy. Well known additional biological features of HCMV include the ability to infect in vivo a broad spectrum of cells (fibroblasts, epithelial and endothelial Cells, macrophages, muscle cells) (1).

Maternal infections are now being increasingly recognized as a major cause of birth defect in newborn babies. CMV infection in general is probably one of the most common infections known to human being and is characterized by mild, self limiting infection with fever in healthy individuals. In pregnant women the virus can cross placenta barriers and result in fetal infection. Nearly all symptomatic congenital infections occur when a woman sustains a primary infection with CMV either during or just before pregnancy (2). Infection appears to be associated with progressively increasing viral transmission rates by gestational age, but infections early in gestation probably result in more severe congenital disease (3,4). If infection occurs after conception, approximately 50% of fetuses will become infected, and approximately one-half of those will have symptoms at birth (3).

The prevalence of CMV infection varies from 0.3 to 2.4% and at least 90% of congenital infected infants have no clinical sign (5,6).The disease causes illness ranging from non apparent to major clinical signs disorder and death (7,8).Congenital CMV infections is described in 30000 to 40000 new born each year in the USA and approximately 9000 of these children have permanent neurological to prematurity, encephalitis, deafness and hemolytic squeal (8). The death rate of symptomatic CMV infection is approximately 30% (9). In India, serological surveys in different parts have shown the prevalence of 80-90% seropositivity (10-13) of CMV antibodies (IgG) in women of child bearing age. Studies regarding prevalence of birth defects due to congenital CMV infection are limited in Iraq. In the present study, samples of babies with signs and symptoms compatible with acute CMV infection and their mothers referred to this CPHL between 15th Sep/2009 to 15th Sep/2011. They were assayed for CMV-IgM antibodies to establish the evidence of congenital CMV infection.

MATERIALS AND METHODS

Patients:
During the period of study (15th Sep/2009 to 15th Sep/2011), Five hundred serum samples were collected from babies with various congenital anomalies and their mother's were referred to General viral infections lab/Virology Department of CPHL from Government hospitals of Baghdad and adjoining areas. Majority of the cases were reported from hospitals located in the crowded zones of Baghdad like Sader-city.

The children were in the age group of few days to 12 months. Suspected patients were with various clinical features like Lethargy (Hypotonia), Poor Suck, Jaundice, Splenomegaly, Seizures, Abnormal features…. etc. The referred samples belonged to mixed population of Urban and Rural areas but mostly from patient of lower socio-economic state.

Specimens:
Blood samples received from all the cases were clotted and centrifuged for serum separation prior to testing. All the sera were stored at $-20^{o}C$ till tested.

Serology:
The serum samples were tested for CMV-IgM antibodies using commercially available enzyme linked immunoassay (ELISA kits EuroImmune in this study). The specificity of the procedure used is 100% with no specific binding due rheumatoid or other herpetic viruses. Mothers of babies were subjected to CMV-IgG and serology using ELISA kits EuroImmune. Kit instructions were strictly adhered to while processing the samples.

Interpretation of the results was based on the controls provided with the kit. Test samples was said to be positive for IgM antibodies when it absorbance value was higher than the absorbance value of cut-off. Positivity of IgM antibodies against CMV in a sample indicates active infection of CMV.

RESULTS

One hundred and twenty five (25%) out of 500 samples from children were showed presence of IgM antibodies giving serological evidence of exposure to in-utero cytomegalovirus infection. Table (1).

Table (1): the number of suspected cases of congenital anomalies referred to Central Public Health Laboratories/ Baghdad / Iraq

Cases	Total cases tested	Positive cases with CMV-IgM	Positive cases with CMV-IgG	Number of Positive CMV-IgM In both children & their mother	Number of Positive CMV-IgG In both children & their mothers
congenital children	500	125(25%)	477(95.4%)	5(3%)	465(94.51%)
congenital children' mother	492	51(10.36%)	487(98.98%)		
Total	992	176(17.74%)	964(98.16%)		

Five (3%) out of 492 sample from mothers of positive congenital CMV infected babies were positive for CMV-IgM antibodies, where as 465 (94.51%) were positive for CMV-IgG antibodies indicating primary infection in the past and reactivation or re-infection with a different strain of the virus in early pregnancy. Table (1).

Congenital CMV related clinical manifestation when compiled in the present study, showed Lethargy as the most common clinical feature table (2) and followed by Poor suck, jaundice, seizures, Splenomegaly, Abnormal feature, Microcephaly , Hydrocephaly, impaired vision , congenital heart disease, Hearing impairment…. etc.

Table (2): Clinical Manifestation in children (Infected children may be have one or more than one signs) with serological evidence of congenital CMV Infection (15th Sep/2009 to 15th Sep/2011).

#	Clinical sign	No. of affected children(125)
1	Lethargy (Hypotonia)	45
2	Poor Suck	22
3	Jaundice	22
4	Seizures	20
5	Splenomegally	20
6	Abnormal feature	14
7	Microcephaly	12
8	Hydrocephaly	8
9	Impaired vision	8
10	Hearing Impairment	6
11	Congenital Heart Disease	7
12	Cataract	2
13	Glaucoma	1

DISCUSSION

High incidence of CMV related congenital defect in reported cases among new born was affecting (25%) of all congenital anomalies can be seen in the present study, these result similar to few studies carried out in India (14-16). In the present study false positivity for CMV-IgM antibodies was ruled out by using immune-absorption (to reduce any cross

reaction by CMV-IgG and rheumatoid factors), which lead to a highly specific and sensitive procedure

The clinical presentations in infants positive for CMV-IgM antibodies were typical of congenitally acquired CMV infection. Lethargy (Hypotonia) was the commonest clinical feature (45 cases), Next common feature was Poor Suck, Jaundice and Splenomegaly (22-20 cases), Abnormal feature & Microcephaly (14-12 cases) and other notable clinical symptoms like Hydrocephaly, Impaired vision, Congenital Heart Disease, Hearing Impairment, Cataract, Glaucoma. etc. are compatible with congenital CMV infection. This finding is in accordance with other studies from India and Poland (16,17), and the study done in Australia showed in 70 cases of congenital CMV being identified between 1999 and 2003. Nearly all of the cases were symptomatic with the most common clinical sequelae reported in infected infants being jaundice, thrombocytopaenia, hepatomegaly, petechiae, purpura and Splenomegaly. Almost half (43.5%) of the infants had central nervous system (CNS) complications, such as microcephaly, chorioretinitis, sensorineural hearing loss, intracranial calcifications, developmental delay or seizures, with over half presenting two or more CNS abnormalities. Maternal febrile illness was noted in 54.8% of the cases (18).

CMV is common in all socio-economic groups but congenital infection with significant impairment is seen at highest rate in population in which women in child bearing age have highest risk of acquired primary infection (10,12,15). In addition to the placenta route. CMV can be transmitted via the maternal genital tract, during the parturition period, breast milk and transfused blood products.

Symptomatic cytomegalovirus infection in fetus can occur after maternal recurrent infection but the incidence of these cases is still not established (19). Seropositivity of re-infected mothers by a different strain of cytomegalovirus can transmit the infection to the fetus and deliver a symptomatic child. High rate of seropositivity IgG (80-90%)was observed in child bearing age (12,13).

Delivering high number of symptomatic congenital CMV babies (as seen in present study) support the above mentioned studies.

Antenatal screening for CMV infection has been widely practiced in most developed countries which can substantially reduce the

incidence of congenital CMV infection by advising MTP in case of intrauterine symptomatic CMV infected fetus diagnosis by amniocentesis or ultrasound (20,21) rather than giving birth to infant having congenital infections with high sequel. However, mandatory testing during antenatal period is rudimentary in Iraq due to various reasons. The diagnosis is done most of the times after the mother gives birth to a baby with congenital anomalies and that is why the incidence of birth defects due to CMV virus is high in Iraq. Good hygiene and general infections control practice is, the best way to prevent transmission of CMV infection in developing countries. Though the only currently available for congenital CMV infection is Ganciclovir (22,23) yet the side effects due to this drug in most patients during the course of therapy has limited its role.

CONCLUSION

The facts presented in the present study conclude beyond doubt that maternal infections like CMV cause considerable burden on society in terms huge number of babies with birth defects.

Good hygiene, antenatal screening, antiviral therapies, development and introduction of good vaccine may achieve the goal of controlling CMV related congenital defects in the New Born.

The importance may primarily be given to introduction of antenatal screening for CMV viral infections in Iraq as done with other communicable disease , Data generated will help the health authorities to make policy against congenital CMV infection prevailing in Iraq, this also put the proper measures as listed down that contribute to decrease the burden of congenital cytomegalovirus infections, However, there are special times, such as during pregnancy, when CMV infections should be avoided, if possible. The spread of the virus can be controlled by practicing good hygiene techniques as recommended by CDC/ National Congenital CMV Disease Registry to increase health awareness in population:

1-Do not kisses young children fewer than 5 or 6 years of age on the mouth or cheek. Instead, kiss them on the forehead or the top of the head and give them a big long hug.

2-Do not share food, drinks, or items such as utensils or toothbrushes with young children.

3- Wash your hands with soap and water after diaper changes or after contact with a child's saliva. Day-care center workers also may wear gloves when changing the diapers of young children.

4-Since young children, especially toddlers, frequently put toys in their mouth, it is recommended the toys be washed with soap and water or wiped with a solution of one-part chlorine bleach to nine-part water, followed by a tap water rinse.

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Effect of Non-Thermal Plasma on Antibiotic Sensitivity and Biofilm Formation of Normal Flora *Streptococcus* spp. Isolated From Patients Exposed to X-Ray and From Healthy Persons

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ABSTRACT

This study has been explored to cope determine whether Non-Thermal Plasma was able to affect the antibiotic sensitivity and biofilm formation of the normal flora *Streptococcus spp*. isolated from healthy persons and X-ray exposed patient. The samples collected from 14persons, 7samples from patients exposed to X-ray and 7 from healthy person from different hospitals and X-ray clinic. Seven isolates from patients exposed to X-ray and seven isolates from healthy persons were obtained depending on morphological and biochemical tests and rapid ID32 system. Culture supernatant of *Streptococcus* spp isolates selected were exposed to Non-Thermal Plasma for (16,18,20 and 22) seconds . The antibiotic sensitivity and biofilm formation were determined before and after irradiation. All isolates of *Streptococcus* spp. shows that change in susceptibility to antibiotic after exposure to Non-Thermal Plasma. The *Streptococcus* spp.   exposed to X-Ray shows increase susceptibility in only one antibiotic bacitracin, while normal *Streptococcus* spp shows increase resistant to three antibiotics cephalothin, cefixime and gentamycin and   increase susceptibility to bacitracin. All isolates shows decrease in biofilm formation after exposure to Non-Thermal Plasma.

الملخص باللغة العربية

هدفت الدراسة الحالية إلى محاولة للكشف عن إمكانية تأثير البلازما غير الحرارية والأشعة السينية على الحساسية للمضادات الحيوية وتكوين الغشاء الحيوي لبكتريا spp. *Streptococcus* المعزولة من الأشخاص الطبيعيين ومن المرضى المعرضين للأشعة السينية.

جمعت 142 عينة، 122 من المرضى المعرضين للأشعة السينية و20 عينة من الأشخاص الطبيعيين من مختلف المستشفيات وعيادات الأشعة في بغداد ، وتم اختيار سبعة عزلات من المرضى المعرضين للأشعة السينية وسبعة عزلات من الأشخاص الطبيعيين اعتمادا على صفاتها المظهرية والاختبارات الكيموحيوية والتشخيص بنظام Rapid ID32. تم تعريض راسب مزروع بكتريا spp. *Streptococcus* للبلازما غير الحرارية لمدة (16، 18، 20، 22) دقيقة و قياس الحساسية للمضادات الحيوية وتكوين الغشاء الحيوي قبل وبعد التشعيع.

أظهرت جميع العزلات تغير في حساسيتها للمضادات الحيوية بعد التعرض للبلازما غير الحرارية حيث أظهرت العزلات المعزولة من المرضى المعرضين للأشعة السينية زيادة في حساسيتها لمضاد الباستراسين في حين أظهرت المعزولة من الأشخاص الطبيعيين زيادة في مقاومتها لمضادات السيفالوثين، السيفكسيم والجنتامايسين و زيادة في حساسيتها لمضاد الباستراسين. كما أظهرت جميع العزلات نقصان في تكوين الغشاء الحيوي بعد التعرض للبلازما غير الحرارية.

INTRODUCTION

The plasma system is a stainless steel apparatus consists of two electrodes isolated from each other by isolated materials. One of the electrodes was connected to the anode of the high voltage power supply (1-20)v, and other was connected to the cathode. When the circuit was switched on, the plasma was produced between the two electrodes, and the sample of bacteria was placed between the electrodes as shown in figure (1). In physics and chemistry, plasma is a state of matter similar to gas in which a certain portion of the particles is ionized. Heating a gas may ionize (reduce the number of electrons in its molecules or atoms), thus turning it into plasma, which contains charged particles: positive ions and negative electrons. Ionization can be induced by other means, such as strong electromagnetic field applied with a laser or microwave generator, and is accompanied by the dissociation of molecular bonds, if present (1). In physics plasma is considered the fourth state of matter next to solids, liquids and gases. In 1879 the British chemist and physicist William Crookes first described it as "radiant matter " (2).
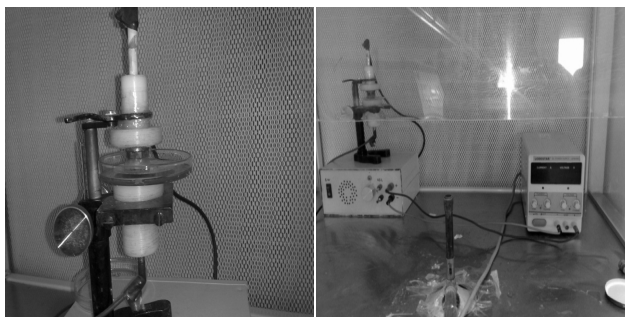

Fig. (1):The plasma system was designed by in Physics Department, College of women, Baghdad University

The presence of a non-negligible number of charge carriers makes the plasma electrically conductive so that it responds strongly to electromagnetic fields. Plasma, therefore, has properties quite unlike those of solids, liquids, or gases and is considered a distinct state of matter (3).

MATERIALS AND METHODS

Isolation and Identification of Bacteria

One hundred forty two throat samples were collected One hundred twenty two swabs from patients exposed to x-ray and twenty swabs from normal person using sterile swabs. The samples were inoculated on blood agar and *Mitis salvarius* agar plates and incubated at 37C for 24hrs. Samples were identified as *Streptococcus spp* according to Bergey's manual using different morphological and biochemical test. Rapid Id 32 (Biomerieux, France)was used to confirm *Streptococcus spp* diagnoses. These stocks were called original stocks and cultures The antibiotics sensitivity of 8 types of common antibiotics were determined by broth micro dilution in accordance with the methods of National Committee for clinical laboratory standards.

Bacterial Samples Irradiation

*Streptococcus spp. w*as cultivated in BHIB at $37°C$ for 18hrs, followed by centrifuging 10ml at 4000rpm for 10min. The cell pellet was diluted in sterile phosphate buffer PH7, and adjusted to concentration of 10^6 CFU/ml.A diluted bacterial suspension of 20μl was put in petri dish contained 10ml from *Mitis salivarius* agar medium and exposed to plasma ray at different times(6,8,10,12,14,16,18,20,22)seconds, except one of these petri dish which contains bacterial suspension was not exposed as a control. An irradiated suspension and irradiated were grown on MSA for 24 hrs at $37°C$ (4).

Antibiotic sensitivity test

Two milliliters of the diluted bacterial suspension from each group was transferred to sterile Eppendorff tube and exposed to laser light at different exposure times, another Eppendroff tube also contains one milliliter of the diluted bacterial suspension did not exposed to laser light in order to keep it as a control, then one milliliter from the irradiated and non irradiated suspension in Eppendroff tubes and cultured on plates of *mitis salvarius* agar to determined the antibiotic sensitivity aginst 8 types of common antibiotics (ampicillin, cephalothin , Bacitracin ,erythromycin, tetracycline and gentamycin) and incubated at 37C₀ for 16-18h, after incubation the antibiotic sensitivity was determined .

Bacterial biofilm detection.
The other milliliters from each Eppendroff tubes were inoculated in 5ml of Trypton soya broth(Tsb) and incubated for48hrs at37˚C, after that, the contents of the tubes were removed carefully and added the crystal violet stain 1% to each tubes for 15 minutes then rinsed the tubes and let tubes to dry at room temperature (20-25) ˚C. The result was read by notice the formation of biofilm as a layer at the internal wall of tubes by naked eye and comprise with negative control (tube contain Tsb without inoculation), thickness and color of layer consider a parameter of bacterial ability for biofilm formation (5).

RESULTS AND DISCUSSION

A total sample of one hundred forty two bacterial samples of *Streptococcus spp* isolates were identified according to their cultural characteristics microscopic and biochemical test. They were categorized as Gram positive cocci aggregates in pairs or chains The cultural and appearance of *viridans Streptococcus* was non hemolytic when grow on blood agar ,and when the bacteria grows on *mitis salvarius* agar the appearance of colonies was small blue colonies, smooth "gum drop" colonies, (1 - 5 mm) in diameter. These colonies may become easier to distinguish with longer incubation. .moreover microscopic examination demonstrated, Non- motile, Non spore forming. Often Arranged in Pairs or Chains, They prefer anaerobic environment, temperature of growth 37˚C, range of PH (6.5-7.5). The results of Rapid Id 32 system came to ensure the biochemical identification of *Streptococcus spp*. The seven isolates from patients exposed to X-ray and seven isolates from normal person of *Streptococcus spp* were tested for their sensitivity to antimicrobial agents. The results showed that several isolates of normal *Streptococcus spp* were resist to many types of antibiotics. The percentage of resistance was Amp. 92.8%, Ceph.85.7%,Bast. 50%, Eryth.64.2%,Tet.28.5% and Gent. 14.2% .(Table -1)*Streptococcus spp.* isolates of exposed to X-Rays patients were more resistant to the same antibiotics. Similar results obtained by Rozkiewicz *et al*.(2006) (6) in Poland who found that the (25.9%) viridians group *Streptococcus* resist gentamycin and (23.1%)from *Streptococcus* resist tetracycline and agreed with Brown *et al.*,(2008) (7), who found that Erythromycin resistance(40-51%).

The results agreed with Ellen *et al.(*2010) (8), who found that Microbial resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, cephalothin, amikacin, chloramphenicol and nalidixic acid was particularly high. Similar susceptibility patterns were also reported by Hiromasa *et al*.(2002) (9) who found that *Streptococcus spp* resist Bacitracin and have two gen locus involves resistant. This high resistance of *Streptococcus spp* is believed to be because of efflux systems and the ability of the organism to undergo mutation and acquire resistant genes. Table (1).
After irradiation with Non –thermal plasma slight changes in sensitivity to antibiotics was shown .While for biofilm formation decrease in the ability of bacterial for biofilm formation. Table (2)
Figure (2) shows decreased biofilm formation of *Streptococcu*s *spp*. isolated from normal person after exposed to Non –thermal plasma,While figure (3)shows decreased in biofilm formation of *Streptococcu*s *spp* isolated from patient after exposed to Non –thermal plasma.
These results agreed with Jonathan *et al,(* 2008) (10), who found the potential of plasma for biofilm inactivation and suggest that cells go through a sequential set of physiological and morphological changes before inactivation. In addition, because the plasmas contain a mixture of charged particles, chemically reactive species and UV radiation that inactivation biofilm formation.
Table(3) shows antibiotic sensitivity of *Streptococcus spp.* isolated from normal person and *Streptococcus spp.* isolated from patient after exposed to Non –thermal plasma.
These results show increase susceptibility of *Streptococcus spp.* exposed to X-Ray to bacitracin , While normal *Streptococcus spp* shows increase resistant to three antibiotics cephalothin, cefixime and gentamycin and increase susceptibility to Bacitracin .These change in antibiotic susceptibility due to the plasma is ionize gas and Ionizing radiation causes atoms and molecules to become ionized or excited. These excitations and ionizations can: Produce free radicals, Break chemical bonds, produce new chemical bonds and cross-linkage between macromolecules, Damage molecules that regulate vital cell processes (e.g. DNA, RNA, proteins).The cell can repair certain levels of cell damage. At low dose, such as that received every day from

background radiation, cellular damage is
rapidly repaired (1).

Table (1): Antibiotics sensitivity of *Streptococcus spp.* isolated from normal persons and from patients

NO. Of Isolates	Antibiotic disc(μg/ml)							
	KF(30)	**B(10)**	**AM(10)**	**CFM(5)**	**CL(30)**	**E(15)**	**TE(30)**	**CN(30)**
R1*	r	r	r	r	r	r	r	s
R2*	r	i	r	r	r	r	r	s
R3*	r	i	r	r	r	r	r	r
R4*	r	r	r	r	r	r	s	s
R5*	r	r	r	r	r	r	i	s
R6*	r	r	r	r	r	r	i	s
R7*	r	r	r	r	r	r	s	s
N1*	r	i	r	r	r	i	i	i
N2*	r	r	r	r	r	i	i	r
N3*	s	r	r	s	r	r	r	s
N4*	r	i	r	r	r	s	i	s
N5*	s	r	r	r	r	r	i	s
N6*	r	i	r	r	r	i	s	s
N7*	r	i	r	r	r	i	s	s

r: resistance , s: sensitive,   i: intermediat
KF:cephalothin, B:bacitracin, AM:ampicillin,CFM:cefixime, CL:cephalexine, E:erythromycin, TE:tetracycline, CN:gentamycin.
R(1-7): *Streptococcus spp.* from patient. *
N(1-7): *Streptococcus spp.* isolated from normal person.*

Table (2): biofilm formation after exposure to Non –thermal plasma

No. of isolates	Time of exposure(second)				
	6	10	14	18	22
N3	ve++	++ ve	+ ve	- ve	- ve
N4	ve++	++ ve	+ ve	- ve	- ve
R1	ve++	+ve	+ve	-ve	- ve
R2	ve++	+ ve	+ ve	- ve	- ve
R3	ve++	+ ve	+ ve	-ve	- ve

++ve: a thick biofilm formation.
+ve: thin biofilm formation.
-ve:no biofilm formation.
R1,R2,R3: Streptococcus spp isolated from patient.
N3,N4: Streptococcus spp isolated from normal person
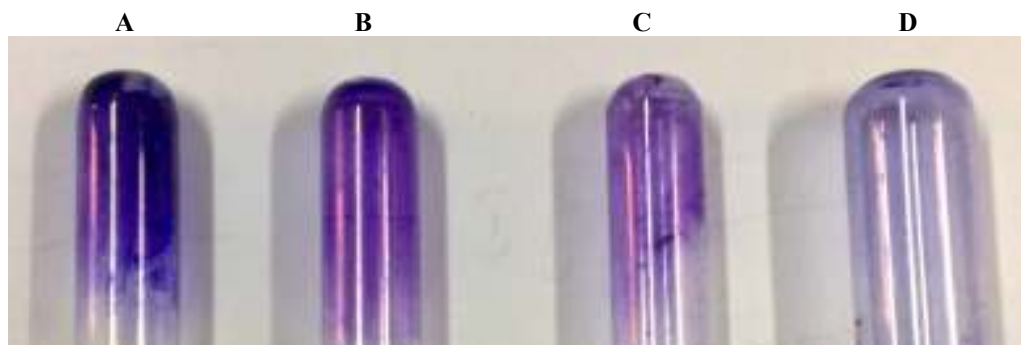
Fig.(2):Biofilm formation of *Streptococcus spp.* isolated from normal person after exposed Non thermal plasma with increased times of exposure

A: Biofilm formation after 10 sec. from exposure to non thermal plasma , **B:** Biofilm formation after 14 sec. from exposure to non thermal plasma, **C:** Biofilm formation after 18 sec. from exposure to non thermal plasma, **D:** Biofilm formation after 22 sec. from exposure to non thermal plasma
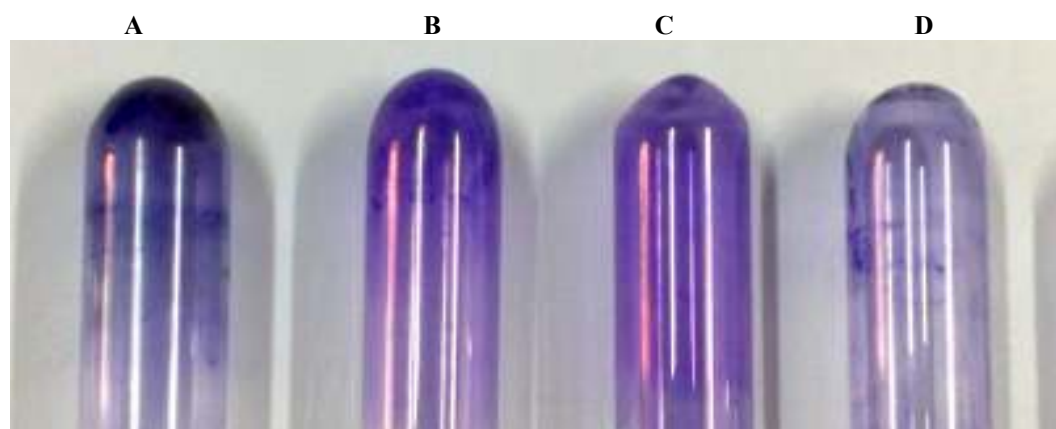
Fig. (3):Biofilm formation of *Streptococcus spp.* isolated from normal person after exposed Non thermal plasma with increased times of exposure.

A: Biofilm formation after 10 sec. from exposure to non thermal plasma, **B:** Biofilm formation after 14 sec. from exposure to non thermal plasma, **C:** Biofilm formation after 18 sec. from exposure to non thermal plasma, **D:** Biofilm formation after 22 sec. from exposure to non thermal plasma.

Table(3): Antibiotic sensitivity of normal *Streptococcus spp.* and Streptococcus spp. exposed to X-Ray after exposed to plasma ray.

NO. of isolate	Time of exposure (second)	Antibiotic disc(μg/ml)							
		KF (30)	B (10)	AM (10)	CFM (5)	CL (30)	E (15)	TE (30)	CN (30)
R3*	16	r	s	r	r	r	r	r	r
R3*	18	r	s	r	r	r	r	r	r
*R3	20	r	s	r	r	r	r	r	r
R3*	22	r	s	r	r	r	r	r	r
N3*	16	r	s	r	r	r	r	r	r
N3*	18	r	s	r	r	r	r	r	r
N3*	20	r	s	r	r	r	r	r	r
N3*	22	r	s	r	r	r	r	r	r

r :resistance,   s: sensitive
 R3: *Streptococcus spp.* isolated from patient. *
 N3: *Streptococcus spp.* isolated from normal person.*
 KF:cephalothin, B:bacitracin, AM:ampicillin,CFM:cefixime, CL:cephalexine,E:erythromycin, TE:tetracycline, CN:gentamycin.

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Isolation of *Staphylococcus aureus* from Eczema patients

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ABSTRACT

The aim of this study was to explain the effect of bacterial contamination on eczema patients with a special focus on *Staphylococcus aureus* depending on the sensitivity test , minimal inhibitory concentration (MIC) values for selected antimicrobial . Fourty four eczematic patients admitted to Ramadi General Hospital in the department of Dermatology and Venereology were included in this study . Twenty five *Staphylococcus aureus* were recovered and the antimicrobial profile was studied . Furthermore plasmid curing for resistant isolates for cephalexin antibiotic . Out of 25 isolates of *Staphylococcus aureus* (the predominant bacterial isolates) , MIC was done for three isolates which were resistant for cephalexin antibiotic . Furthermore these three isolates were still resistant to cephalexin antibiotic.
Twenty of the total patients were showing positive skin swabs cultures while thirteen patients were showing negative cultures .
It is concluded that eczema patients associated with infection showed more severe eczema than patients with eczema without infection.

Key Words: eczema patients , bacterial contamination .

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الملخص باللغة العربية

تهدف هذه الدراسة إلى توضيح تأثير التلوث البكتيري على مرضى الأكزيما ببؤرة خاصة على بكتريا المكورات العنقودية الذهبيـة اعتمادا على اختبار الحساسية وقيم التركيز المثبط الأدنى للمضادات الميكروبية المختارة، شملت عينة الدراسـة 44 شـخص فـي مستشفى الرمادي العام في قسم الجلدية والتناسلية، وأظهرت نتائج الدراسة أن 25 شخصا كانوا اكثر شدة (حـدة) للمـرض مـن اولئك المرضى الذين هم في حالة الاكزيما غير المصحوب بخمج بكتيري وذلـك بعـد أن عوملـت عينـاتهم بنظـام المـضادات الميكروبية.

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INTRODUCTION

Eczema is an acute or chronic non contagious skin disease characterized by redness , itching , blisters , crusting and scaling (1 , 2 , 3) . *Staphylococcus aureus* infection plays an important role in atopic eczema (AE) because of its ability to produce virulence factors such as superantigens. Epicutaneous application of superantigens induces eczema (4 - 6) . Regarding the causative triggering factors , eczema may be due to endogenous or exogenous factors or both (4 , 5 , 7 , 2) . So many evidences indicate the existence of different predisposing factors that influence the disease triggering and progress , among them are genetic and environmental factors (4 , 5 , 8 , 9) . Infections of different etiology were found to play a role in eczema inciation and propagation (10 -13) . The word eczema was derived from the Greek word Ekzein which means (boil out) or (to effervesce) (1 , 14) . The term eczema was often used synonymously with atopic dermatitis , though eczematous reactions in individuals are with and without other atopic findings such as allergic rhinitis , asthma (15) .

Eczema was described as a pruritic papulovesicular process which in its acute phase is associated with erythema and edema while in it´s more chronic phases is retaining some of its papulovesicular features dominated by thickening , lichenification and scaling (1, 16) .

Histologically, eczema was characterized by the presence of predominantly lymphohistiocytic infiltrate around the upper dermal blood vessels associated with varying degrees of spongiosis and acanthosis , the terms " Eczema " and " Dermatitis " are regarded as synonymous (17) .

Eczema was used as a clinical descriptive term to describe a process that is cleary superficial , erythematous , papulovesicular , oozing , crusting , red - purple , scaly , lichenified and possibly pigmented . Epithelial disruption and non - sharp margination are its characteristics (6) .

PATIENTS AND METHODS

Fourty four (44) swabs were taken from both sexes of patients admitted to Ramadi Teaching Hospital, in Ramadi city.

The age of the patients was varying between 20   years and 80 years old with the mean of 50 years.

Methods:

Swabs were cultivated on MSA, NA and incubated aerobically for 24 - 48 hrs at 37C Bacterial isolates were identified using Direct Gram stained smears and biochemical test as described by (18).
Bacterial isolates were kept frozen in glycerol brain heart infusion (10%) to be used for Antimicrobial Sensitivity test later.

Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried to study the multi - drug resistant patterns of *Staphylococcus aureus* isolates. Different antibiotics namely Rifampin (5mcg) , Cefodizime (30 mcg) , Gentamicin (10 mcg) , Cephalexin (30 mcg) , Nalidixic (30 mcg) , Trimethoprim (5 mcg) , Amoxicillin (25 mcg) and Clavulanic acid (25 mcg) (Bioanalyse) were tested . Muller Hinton agar plates were surface inoculated with 20 Ml of 24 h bacterial inoculum and then discs of different antibiotics were placed uniformly on the surface of the plate. Plates were incubated at 37C° for 24h. The sensitivity and resistance of the isolates were determined according to (19).

Preparation of antimicrobial solutions

They were prepared as described by (20) . Cephalexin 500 mg / ml were prepared as stock solution of 1000 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 concentrations (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, the last tube which shows no growth will represent the minimal inhibitory concentration (MIC) (21).

Plasmid curing

To demonstrate whether the resistances for antibiotics for the selected isolates were plasmid or chromosomal origin, plasmid curing was carried out according to (22). 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 hr. Two fold dilutions were made, after that, 10 µl was streaked over nutrient agar medium plates and incubated at 37 °C for 24 hr . The separate colonies (mutants) were resubcultured on nutrient agar plates to ensure their purity . These isolates were tested for their antibiotics sensitivity and presence of plasmids.

RESULTS

Table (1) shows that twenty five bacterial isolates were belonged to *Staphylococcus aureus*, three of these isolates were resistant for cephalexin which determined by disk diffusion test.

Table (1) : 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

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 also showed that both strains of *Staphylococcus aureus* Au 1 and *Staphylococcus aureus* Au 3 were resistant for cephalexin antibiotic and the MICs were 10 µg/ml . While the strain of *Staphylococcus aureus* Au 2 was also resistant to cephalexin and the level of resistance was 15 µg/ml.

The minimal inhibitory concentration (MIC) of curing agent used in this study was determined in brain heart infusion broth using *Staphylococcus aureus* Au 1, Au 2 and Au 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* Au 1 was not found (There is no inhibitory concentration for this strain) while the MIC for *Staphylococcus aureus* Au 2 was 5 µg/ml and sub-minimal inhibitory concentration (the highest concentration allows bacterial growth) was 10 µg/ml also the MIC for *Staphylococcus aureus* Au 3 was 10 µg/ml while the sub-minimal inhibitory concentration was 15 µg/ml.

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 (23) . 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} (24 , 23) . 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 (25 , 24 , 23) . Sodium dodecyl sulphate as a detergent is known to act on the bacterial membrane (26) . (27) postulated that 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 . (28) found that 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 . Our results on curing experiments revealed that *Staphylococcus aureus* Au 1 , Au 2 and Au 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 (23) .

(29) demonstrated that many plasmids can not be cured (refractory) . 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* Au 1 , Au 2 and Au 3 which means that these cells have not lost resistance marker for cephalexin and this resistance marker were encoded chromosomally . (30) postulated that 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 . After treatment of bacterial strains with curing agent sodium dodecyl sulphate SDS (10%) no cured cells were obtained from *Staphylococcus aureus* Au 1 , Au 2 and Au 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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قسم الدراسات العربية

ARABIC SECTION

توصيف عاثي الرايزوبيوم المعزول من العقد الجذرية لنبات الباقلاء

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الملخص باللغة العربية

تعتبر بكتريا الرايزوبيوم من بين أهم أجناس البكتريا المثبتة للنيتروجين الجوي وتتم العملية من خلال العلاقة التعايشية بين بكتريا الرايزوبيوم والنباتات البقولية ، وتصيب عاثيات الرايزوبيوم (الفيروسات) بكتيريا الرايزوبيوم حيث تؤثر على مجتمعات هذه البكتيريا في التربة.

تهدف الدراسة إلى عزل وتوصيف عاثي الرايزوبيوم الضاري من العقد الجذرية لنبات الباقلاء باستخدام عزلات محلية من بكتريا *Rhizobium leguminosarum*.

تم استخدام ستة عزلات من بكتريا *R. leguminosarum* كخلايا مضيفة للعاثي ، عزل عاثي الرايزوبيوم SA باستخدام طريقة Agar overlay ، حيث أظهر شكل العاثي بالمجهر الالكتروني احتواءه على رأس سداسي وذيل قابل للتقلص وغير مغلف وحسب المؤتمر العالمي لتصنيف الفيروسات فإن العاثي ينتمي إلى عائلة Myoviridae.

تميزت بقع Plaques العاثي بكونها واضحة ودائرية وتراوح قطرها بين (1-3) مليمتر ، لم يتأثر معيار العاثي بالمذيبات العضوية الكلوروفورم والداي اثيل الايثر في حين أظهر العاثي حساسيته للأس الهيدروجيني الحامضي في حين ظل معيار العاثي ثابتاً بين الأس الهيدروجيني 6-8.

لم يتأثر معيار العاثي عند تعرضه لدرجة حرارة 40 °م ولمدة ساعة في حين تم تثبيط 94% من جزيئاته عند تعرضه لدرجة حرارة 50 °م لمدة ساعة.

كان العاثي محددا في إصابته لبكتريا *R. leguminosarum* حيث أظهرت عزلة واحدة حساسيتها للعاثي ، تلعب عاثيات الريزوبيوم دور مهم من خلال السيطرة على أعداد بكتريا الرايزوبيوم في الترب المختلفة ، انتخاب وتحديد أنواع بكتريا الرايزوبيوم ، التعرف والتفريق بين سلالات بكتريا الرايزوبيوم وتنميط بكتريا الرايزوبيوم.

الكلمات المفتاحية: بكتيريا الرايزوبيوم ، العقد الجذرية ، عاثيات الرايزوبيوم ونبات الباقلاء

ABSTRACT

Rhizobia are the most important nitrogen fixing bacteria, which do this process during symbiosis with legumes. Rhizobiophage are viruses that infect rhizobia and affect the bacterial population of the soil.
The aim of this study was isolation and characterization of virulent rhizobiophage from root nodules of *Vicia faba* by using local isolates of *Rhizobium leguminosarum* .
Six isolates of *R. leguminosarum* used as host cells Rhizobiophage (SA) isolated by using agar overlay method , electron microscopy revealed that the phage had hexagonal head with long contractile tail , non enveloped according to International Committee on taxonomy of viruses , the phage belong to the family Myoviridae.
The plaques of the phage were clear and rounded with diameter ranging from (1-3)mm , The phage was found to be un affected by chloroform and diethyl ether more over the phage was sensitive to acidic pH and remain viable between pH 6-8.
Thermostability studies showed that the phage remained stable at 40ºC for one hour while 94% of the phage particles were inactivated after heating for one hour at 50ºC.
The host range of the phage appeared to be limited since it lysed single isolate of *R. leguminosarum.*
The major role of Rhizobiophage is controlling the population of rhizobia in different soils, selective elimination of some rhizobia , identification and differentiation of bacterial strains and phage typing of *Rhizobium* strains.

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المقدمة

تلعب النباتات البقولية دور مهم في الزراعة بسبب العلاقة التعايشية مع بكتريا الرايزوبيوم المثبتة للنيتروجين الجوي فلهذه البكتريا القدرة على تكوين العقد على جذور النباتات البقولية (1) ولبكتريا الرايزوبيوم خصوصية عالية في إصابة النبات البقولي (2) ويمكن زيادة الإنتاج وتحسين المحاصيل البقولية من خلال إضافة أنواع خاصة من بكتريا الرايزوبيوم النشطة إلى التربة الزراعية (3) وذلك من خلال استخدام هذه البكتريا كمخصبات بيولوجية Biological fertilizer بدلاً من المخصبات الكيميائية Chemical fertilizer التي من ممكن أن تشكل خطورة على صحة الإنسان بسبب تلوث البيئة بالمواد الناتجة من تحلل هذه المواد إضافة إلى أن المخصبات الكيميائية ممكن أن تؤثر على مجتمعات Population أحياء التربة من خلال تأثيرها على التركيب الفيزيائي للتربة (4).

تعتبر الباقلاء المحصول البقولي الذي يحتل المرتبة السابعة من حيث الأهمية في العالم (5) وبالرغم من وجود أكثر من 80 نوع من الباقلاء يتم زراعة ثمانية عشر نوع منها فقط بصورة واسعة (6) وتعتبر بذور الباقلاء الناضجة مصدر جيد وغني للبروتين والنشا والمعادن فهي غذاء مهم للإنسان والحيوان على حد سواء (7).

تتأثر مجتمعات بكتريا الرايزوبيوم في التربة الزراعية المكونة للعقد الجذرية لنبات الباقلاء بعوامل فيزيائية وكيميائية وبيولوجية مثل درجة حموضة التربة ، ملوحة التربة ، درجة الحرارة والمعادن الثقيلة (8). أما بخصوص العوامل البيولوجية فتعتبر عاثيات الرايزوبيوم Rhizobiophages من بين أهم العوامل البيولوجية التي تؤثر على بكتريا الرايزوبيوم في التربة (9).

تصنف عاثيات الرايزوبيوم إلى نوعين النوع الضاري او المحلل Virulent or Lytic Rhizobiophage ويحدث في هذا النوع وبعد إصابة البكتريا وانتهاء دورة تكاثر العاثي تحلل بكتريا الرايزوبيوم مؤدياً إلى تقليل أعدادها وبالتالي يؤدي إلى انخفاض الإنتاج، والنوع الثاني هي العاثيات المعتدلة Temperate Rhizobiophage ، في هذا النوع وبعد إصابة العاثي للبكتريا ودخول الحامض النووي للعاثي إلى داخل بكتريا الرايزوبيوم يرتبط مع الحامض النووي للبكتريا دون تحللها محولاً البكتريا إلى النوع اللايزوجيني Lysogeny (10). ويلعب هذا النوع من العاثي دورا مهماً في تبادل المادة الوراثية بين بكتريا الرايزوبيوم مؤدياً إلى اكتساب بكتريا الرايزوبيوم صفات جديدة.

وبالنظر لندرة وجود دراسات عن عاثيات بكتريا الرايزوبيوم لذا يهدف البحث إلى عزل وتوصيف عاثي بكتريا الرايزوبيوم من العقد الجذرية لنبات الباقلاء.

المواد وطرائق العمل

حقل الدراسة:

تم اختيار حقل لنبات الباقلاء في منطقة المدائن في محافظة بغداد زرعت بذور الباقلاء (اسبانية المنشأ) بتاريخ 2012/9/15 وتم عزل بكتريا الرايزوبيوم والعاثي من نباتات هذا الحقل التي جمعت بصورة عشوائية .

الأوساط الزرعية المستخدمة:

استخدم وسط خميرة – المانيتول الصلب والسائل Yeast Extract Mannitol (YEM) والمتكون من Mannitol 10 g ، KH2PO4 0.2 g ، K2HPO4 0.8 g ، MgSO4.7H2O 0.2 g ، NaCl 0.1 g ، Yeast Extract 1 g ، Agar 15 g ، Distilled Water 1 L ، PH=7.2-7.4 في عزل وتنمية البكتريا وكذلك استخدم (Bottom Agar) في تنمية البكتريا أثناء عزل ودراسة صفات العاثي، أما في (Top Agar) فقد تم تخفيض نسبة Mannitol 0.1% ، Yeast Extract 0.05% ، Agar 0.6% لتقليل كمية المواد السكرية المفرزة من قبل البكتريا (11).

عزل بكتريا *Rhizobium leguminosarum* من العقد الجذرية:

للتحري عن عاثي الرايزوبيوم تم استخدام عزلات محلية من بكتريا *R.leguminosarum* كخلايا مضيفة Host Cells .

اتبعت طريقة (12) حيث تم الحصول على العقد الجذرية من نباتات الباقلاء بقلع النبات بالكامل مع التربة المحيطة بجذوره ونقله إلى المختبر , أزيلت التربة العالقة بالمجموع الجذري ثم فصلت العقد الجذرية التي تميزت بكبر حجمها ولونها الوردي المحمر صورة (1) مع جزء صغير من الجذر المتصل بها غسلت العقد الجذرية جيداً بالماء المقطر المعقم لعدة مرات لإزالة التربة العالقة بالعقد وغمرت بالإيثانول بتركيز 95% لمدة 5-10 ثواني ثم غسلت بالماء المقطر المعقم 5 مرات لإزالة آثار الكحول ثم غمرت في محلول هايبوكلورايد الصوديوم (NaOCl) بتركيز 3% لمدة 4 دقائق , غسلت العقد بالماء المقطر المعقم 5 مرات لإزالة آثار المادة المعقمة ووضعت على أوراق ترشيح معقمة لإزالة الماء العالق بها ثم هرست العقد مع قليل من وسط YEM السائل لعمل معلق بكتيري نقل منه 0.1 مليلتر على وسط YEM الصلب بطريقة التخطيط للحصول على مستعمرات منفردة بعد تحضينها بالحاضنة لمدة 24-48 ساعة بدرجة حرارة 28±2 مْ .

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عزل عاثي الرايزوبيوم من العقد الجذرية:

تم اتباع طريقة (13) مع إجراء بعض التحويرات حيث أخذت عقدة جذرية فعالة (كبيرة الحجم ذو لون احمر) ووضعت في قنينة زجاجية معقمة سعة 25 مليلتر وهرست وأضيف إليها 10 مليلتر من وسط YEM السائل وتم مجانسة الخليط لمدة دقيقتين بواسطة Magnetic Stirrer وترك بدرجة حرارة الغرفة لمدة ساعة ثم نبذ الخليط في جهاز الطرد المركزي بسرعة 3000 دورة / دقيقة لمدة 10 دقائق ورشح الرائق من خلال مرشحات دقيقة بقطر 0.22 ملي مايكرون , تم إضافة 0.1 مليلتر من الراشح و 0.1 مليلتر من بكتريا الرايزوبيوم في الطور اللوغارتمي إلى 3 مليلتر من وسط YEM السائل الحاوي على 0.6% من Agar بدرجة حرارة 45 مْ (Top Agar) وتم المزج جيداً وصب المزيج على وسط YEM الصلب الحاوي على 1.5% من الاكار (Bottom Agar) وترك ليتصلب ثم حضنت الأطباق مقلوبة بدرجة حرارة 28 مْ وفحصت الأطباق بملاحظة البقع (Plaques) وتم تأكيد عزل عاثي الرايزوبيوم بنقل بقعة (Plaque) منفردة بواسطة ماصة باستور معقمة إلى أنبوب زجاجي معقم يحتوي على 1 مليلتر من وسط YEM السائل وترك الأنبوب مع الرج بهدوء في درجة حرارة الغرفة لمدة ساعتين ، نبذ المزيج بجهاز الطرد المركزي بسرعة 3000 دورة / دقيقة لمدة 5 دقائق ورشح الرائق وتم إعادة نفس الطريقة أعلاه وكررت هذه العملية لثلاث دورات للتأكد من وجود وتنقية العاثي .

تحضير عاثي الرايزوبيوم الخزين:

تم استخدام طريقة الوسط المغذي الصلب Agar Overlay Method (14) حيث اختيرت الأطباق التي أظهرت اكبر عدد من البقع، وتم إضافة 10 مليلتر من وسط YEM السائل لكل طبق، وتركت الأطباق بدرجة حرارة الغرفة لمدة 3 ساعات ثم جمع وسط YEM السائل الحاوي على العاثي ونبذ في جهاز الطرد المركزي بسرعة 3000 دورة / دقيقة لمدة 5 دقائق ورشح الرائق من خلال مرشحات دقيقة بقطر 0.22 ملي مايكرون وحفظ العاثي بدرجة حرارة 4 مْ .

معايرة عاثي الرايزوبيوم

تم معايرة عاثي الرايزوبيوم باستخدام طريقة (14)، إذ تم إضافة 0.1 مليلتر من بكتريا الرايزوبيوم في الطور اللوغارتمي و 0.1 مليلتر من عاثي الرايزوبيوم من كل تخفيف من التخفيفات العشرية إلى 3 مليلتر من وسط (Top Agar YEM) بدرجة حرارة 45 مْ ومزج جيداً وصب على سطح (Bottom Agar) وتركت الأطباق حتى تتصلب وحضنت مقلوبة بدرجة حرارة 28 مْ وتم اختيار الأطباق التي يتراوح عدد البقع فيها بين (100-200) بقعة وحساب معيار العاثي مقدراً Plaque Forming Unit (PFU) بضرب عدد البقع في مقلوب التخفيف .

دراسة شكل العاثي بالمجهر الالكتروني:

تم دراسة شكل العاثي بالمجهر الالكتروني النافذ حسب (15) وضعت قطرة من العاثي بمعيار 1×10^{12} PFU/ml على (Carbon-Coated 400-mesh copper grids) وتم التخلص من القطرة الفائضة باستخدام ورقة الترشيح , بعدها تم إضافة قطرة من صبغة PH7 , Phosphate Tingestic Acid 2% وتترك لبضعة دقائق ويتم التخلص من الصبغة الفائضة باستخدام ورقة ترشيح وتم فحص العينة بجهاز -Transmission Electron Microscope JEOL (JEM-1400 TEM).

دراسة شكل بقع العاثي:

تم دراسة شكل وحجم البقع Plaque باستخدام طريقة Agar Overlay Method (14) على وسط YEM الصلب والحضانة بدرجة حرارة 28 مْ .

حساسية عاثي الرايزوبيوم للمذيبات الدهنية:

اتبعت طريقة (16) لدراسة حساسية العاثي للمذيبات الدهنية بمزج مليلتر واحد من الكلوروفورم أو ثنائي الايثر مع مليلتر واحد من عاثي الرايزوبيوم بمعيار 1×10^{9} PFU/ml في أنابيب زجاجية معقمة محكمة الغلق حضنت بصورة مائلة لمدة ساعة عند درجة حرارة 30 مْ مع الرج المتكرر , نبذ المزيج في جهاز الطرد المركزي بسرعة 1000 دورة / دقيقة لمدة 10 دقائق , فصل الجزء العلوي في حالة الكلوروفورم والجزء السفلي في حالة ثنائي اثيل الايثر وتم معايرتها جنباً الى جنب مع ضابط التجربة المتكون من مليلتر واحد من العاثي ومليلتر واحد من وسط YEM السائل .

تأثير الأس الهيدروجيني على عاثي الرايزوبيوم:

تم دراسة تأثير الأس الهيدروجيني حسب (17) واستخدمت قيم الأس الهيدروجيني الواقعة بين 4-10 ، حيث تم إضافة 0.1 مليلتر من العاثي بمعيار 1×10^{9} PFU\ml إلى 9.9 مليلتر من الماء المقطر المعقم وبعد حضن النماذج ذات القيم المختلفة من الأس الهيدروجيني لمدة ساعة عند درجة حرارة 30 مْ ،تم معايرة العاثي المتبقي واعتبر النموذج ذو الأس الهيدروجيني 7 كضابط سيطرة للتجربة .

حساسية العاثي لدرجات الحرارة المختلفة:

تم اتباع طريقة (17) وذلك باستخدام أنابيب زجاجية رقيقة الجدران ومحكمة الغلق وضع في كل منها 9.9 مليلتر من الماء المقطر المعقم وحضنت لمدة 30 دقيقة في حمام مائي عند درجات الحرارة المرغوبة (30، 40، 50، 60) درجة مئوية ، بعدها أضيف 0.1 مليلتر من العاثي بمعيار 1×10^{9} PFU/ml لكل انبوب ، سحب نموذج في الوقت صفر وبعد ساعة واحدة وتم حساب مقدار الانخفاض الحاصل في معيار العاثي.

صورة (2): مستعمرات بكتيريا *R. legaminosarum* على وسط YEM الصلب المعزولة من العقد الجذرية مباشرة بعد فترة حضانة 24ساعة بدرجة حرارة 28 م

صورة (3): بكتيريا *R. legaminosarum* السالبة لصبغة كرام (800x)

أظهر شكل العاثي المعزول بالمجهر الالكتروني احتوائه على رأس Head الذي يتميز بشكله السداسي وبقطر (80) نانوميتر وعدم احتوائه على الغلاف Non-enveloped ويرتبط بالرأس ذيل بطول (117) نانوميتر والذي امتاز بقابليته على التقلص Contractile tail واحتواء الذيل في نهايته على Base plate صورة رقم (4) ان احتواء العاثي على ذيل قابل للتقلص وعدم احتوائه على الغلاف (Non-enveloped,contractile tail) يجعل تصنيفه ضمن عائلة Myoviridae حسب تصنيف العاثيات من قبل International Committee on Taxonomy of Viruses (ICTV) (20) ويتشابه شكل العاثي المعزول مع عاثيات بكتريا الرايزوبيوم التي تم دراستها من قبل (21) لكنه يختلف عن عاثيات *R. legaminosarum* المعزولة من قبل (17) والتي كانت تنتمي الى عائلة Siphoviridae.

حساسية عزلات بكتريا *R.leguminosarum* للعاثي:

تم اختبار حساسية عزلات بكتريا *R.leguminosarum* عدد خمسة المعزولة من نفس حقل الدراسة إضافة إلى عزلتين تم الحصول عليهما من دائرة البحوث الزراعية / وزارة العلوم والتكنولوجيا في بغداد , والمركز الوطني للزراعة العضوية / وزارة الزراعة العراقية حسب (18)، وذلك بإضافة 0.1 مليلتر من الوسط الزرعي السائل لكل عزلة من بكتريا *R.leguminosarum* في الطور اللوغارتمي إلى 3 مليلتر من (Top Agar) بدرجة حرارة 45 مْ وبعد مزجه جيداً تم صبه على (Bottom Agar) وترك ليتصلب في درجة حرارة الغرفة بعدها نقط العاثي بمعيار PFU\ml 1× 10^{12} على نقاط محددة ولكل عزلة بعدها حضنت الأطباق بدرجة حرارة 28 مْ وفحصت الأطباق لملاحظة مناطق التحلل Lysis Zone في نقاط التقطير .

النتائج والمناقشة

عزلت ستة عزلات من بكتريا *R. legaminosarum* أعطيت لها الرموز RL1– RL6 واستخدمت كخلايا مضيفة في عزل عاثي الرايزوبيوم من العقد الجذرية الفعالة لنبات الباقلاء Faba bean والتي جمعت بصورة عشوائية من حقل الدراسة، صورة رقم (1) . أظهرت البكتريا على وسط YEM الصلب بشكل مستعمرات بيضاء اللون مخاطية القوام صورة رقم (2) وأظهرت الخلايا البكتيرية تحت المجهر الضوئي على شكل عصيات سالبة لصبغة كرام (19) صورة رقم (3) ،وأظهرت إحدى العزلات RL5حساسيتها للعاثي المعزول والذي أعطي الرمز SA.

صورة (1): العقد الجذرية لنبات الباقلاء

تميزت بقع Plaques عاثي SA بكونها دائرية الشكل، أما حجم البقع فتراوحت بين 1-3 مليمتر صورة رقم (5) ، يتأثر شكل البقع وحجمها بالعديد من العوامل مثل تركيز الاكار ، مكونات الوسط الزرعي المستخدم ، درجة حرارة الحضانة وعمر البكتريا (17).

لم يتأثر العاثي بالمذيبات الدهنية الكلوروفورم وثنائي اثيل الايثر ويستدل من ذلك عدم احتواء العاثي على المركبات الدهنية في تركيبه وبخصوص تأثر العاثي بقيم الأس الهيدروجيني فقد حصل تثبيط كامل للعاثي عند الأس الهيدروجيني 4 ولم يتأثر العاثي في المدى الواقع بين 6-8 بعد حضانة دامت ساعة عند درجة حرارة 30°م مثل هذه النتائج تساعد في احتمالية بقاء العاثي في التربة فترة زمنية طويلة وقد يكون ذلك بفضل تطبع العاثي للأجواء العراقية.


صورة (4): شكل عاثي الرايزوبيوم SA بالمجهر الالكتروني النافذ (X250000)

صورة (5): بقع عاثي الرايزوبيوم SA على وسط YEM

للعاثي المعزول قابلية عالية في تحمله للحرارة حيث بقي ثابتا ولمدة ساعة عند درجة حرارة 40 مئوية في حين حدث تثبيط في فعاليته (94%) عند درجة 50 مئوية وحصل تثبيط كامل بدرجة حرارة 60 مئوية. شكل رقم (1).

شكل (1): قابلية تحمل عاثي الرايزوبيوم SA لدرجات الحرارة المختلفة

يتميز عاثي SA بكونه محددا في إصابته لبكتريا الرايزوبيوم حيث لم تظهر اي من العزلات *R. legaminosarum* حساسيتها له.

إن عزل عاثي الرايزوبيوم SA من العقد الجذرية مباشرة وبدون استخدام طريقة الاغتناء Enriched method يشير إلى احتمالية تواجد العاثي في حقل الدراسة بصورة شائعة ويمكن أن يلعب دورا مهماً في تحديد أنواع من بكتريا الرايزوبيوم الحساسة له (3).

ونظراً للأهمية الكبيرة التي تحتلها عاثيات الرايزوبيوم من خلال تأثيرها السلبي في إنتاج النباتات البقولية بتقليلها أعداد بكتريا الرايزوبيوم المثبتة للنتروجين الجوي وتحديد وانتخاب أنواع معينة منها، فإنها من جانب أخر يمكن أن تستخدم في التعرف والتفريق بين عتر بكتريا الرايزوبيوم وتنميط بكتريا الرايزوبيوم Phage typing ،كذلك فإن لعاثيات الرايزوبيوم المعتدلة Temperate Rhizobiophages دورا مهما في تبادل المادة الوراثية بين بكتريا الرايزوبيوم من خلال عملية Transduction (11، 21).لذا نوصي بإجراء دراسات مستفيضة حول تواجد عاثيات الرايزوبيوم في حقول النباتات البقولية ومن ثم المحاولة من تقليل تأثير هذه العاثيات على الإنتاج من خلال اختيار أنواع من بكتريا الرايزوبيوم المقاومة للعاثيات الضارية وفي كل حقل من حقول النباتات البقولية وإكثارها ومن ثم استخدامها كمخصب بيولوجي.

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دراسة مسحية لبعض الأحياء الميكروبية المعزولة من نوع من السلطات المحضرة محلياً في بعض مطاعم بغداد

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الملخص باللغة العربية

هدفت الدراسة إلى عزل وتشخيص أكثر الأنواع خطورة على الإنسان من البكتريا السالبة والموجبة لملون غرام والأعفان التي تشمل الفطريات والخمائر والطفيليات الملوثة والمعزولة من سلطة التبولة من أربعة عشر موقعاً في مدينة بغداد (الجادرية، البياع، السيدية، الوشاش، المنصور، الكاظمية، الشعب، الدورة، شارع فلسطين، الأعظمية، مدينة الصدر، العامل، الإعلام، بغداد الجديدة) خاصة لعمل وتحضير وبيع السلطة، تم جمع أربعة وستين عينة ، عزلت منها (61) عزلة بكتيرية، شملت أنواع بكتيرية سالبة لملون غرام وهي Escherichia coli, Citrobacter freundii Enterobacter gergoviae, وEnterobacter agglomerans فضلاً عن نوعين من البكتريا الموجبة لملون غرام Staphyllococcus aureus وBacillus subtilis ، وكذلك عزلت 116 عزلة من الأعفان التي شملت الخمائر Yeasts وفطر Gutricum candidum و Rudotorula وAlternaria alternate و Aspergillus parasticus ،أما بيوض وأكياس الطفيليات فقد تم تشخيص 40 نوع منها Trichuris trichuriaو Ascaris lambricoides و Fasciola hepatica و Entrobius vermicularis و Balantidium coli و Entamoiba histolytica و Giardia lambliaو Echinococcus granulosus و Entamoiba coli . زرعت النماذج على اوساط زرعية مختلفة بالنسبة للبكتريا والاعفان وعزلت الطفيليات بطريقة الفحص المباشر بالسلايد وقد تم تشخيص العزلات البكتيرية بوساطة بعض الاختبارات الكيموحيوية، أما الاعفان فقد شخصت بوساطة المفتاح التصنيفي للفطريات والطفيليات شخصت بوساطة الاصباغ الخاصة لها ، لقد تفاوتت أعداد هذه العزلات حسب المناطق، حيث يلاحظ ارتفاع أعداد العزلات المعزولة من سلطة التبولة في المناطق الشعبية واختلاف أنواع العزلات باختلاف المناطق مما يدل على اختلاف في طرق التعامل مع تحضير السلطات وحسب ثقافة العاملين ودرجة الوعي لديهم في التعامل مع التحضير والتلوث الاحيائي.

ABSTRACT

The study aimed to isolate and diagnose the most dangerous species on the rights of the negative and positive gram stained bacteria and molds which include fungi, yeasts and parasites isolated from contaminated salad tabulla were collected from 14 different area in Baghdad city, (Al Jadria, Al Byaa'a, Al Sydya, Al Washaash, Al Mansour, Al kaadhmya, Al Shaab, Al Dorra, Sharaa Palestine, Al A'dhamya, Madyna Al Sadr, Al Amel, AL Elam, New baghdad).), Especially for the work, preparation and sale of salads . 64 samples were obtained and isolated 61 bacterial isolate from it, it contain negative gram stain bacterial(*Citrobacter freundii, Enterobacter gergoviae, Escherichia coli,   Enterobacter   agglomerans),*and   tow   kind   of   positive   gram   stain   bacterial (*Staphyllococcus aureus, Bacillus subtilis*), As well as isolated 116 isolates of molds which included yeasts and molds (*Gutricum candidum* و *Rudotorula* و *Alternaria alternate* و *Aspergillus parasticus*) .also to parasite eggs have been diagnosed 40 type included (*Trichuris trichuria ,Ascaris lambricoides ,Fasciola hepatica , Entrobius vermicularis ,Balantidium coli , Entamoiba histolytica, Giardia lamblia ,Echinococcus granulosus, و Entamoiba coli*). the samples were cultured in different media for bacteria and molds . The parasites isolated manner direct examination. As well as bacterial isolates diagnosed by some biochemical tests. The molds were diagnosed by the taxonomic key fungi and parasites diagnosed by her own dyes . Bacterial isolates number varied in differed areas, where it notes the high numbers of isolates recorded and found in popular areas, which shows the difference in the ways of dealing authorities in the preparation. According to the culture and the degree of awareness of the workers with the preparation and biological contamination.

تتكاثر البكتريا بسرعة عند وجود مواد مغذية لها ونتيجة
للايض الناتج عنها وتحليلها للمكونات الغذائية والمواد السكرية
بالذات ومؤدية الى زيادة الحموضة في المواد الغذائية
وتحللها وظهور روائح غير مرغوبة مما يؤدي الى فقدان
صلاحيتها للأكل، وتكون لقسم منها القابلية في إفراز سموم
مضرة بصحة الإنسان وتُعد من اهم الأحياء الدقيقة التي لها
علاقة في فساد الأغذية.كما ان لبعض انواع البكتريا قابلية
تكوين السبورات وان تنمو في الاغذية وتحدث تغييرات كثيرة
كتحلل الكاربوهيدرات والبروتينات.

والتسمم بالأحياء المجهرية يقسم إلى نوعين السموم
الداخلية Endotoxine الناجم عن تلوث الغذاء ببكتريا
مسببة للتسمم الغذائي وهي لا تفرز السموم في الغذاء بل
عند تناولها مع الغذاء يحدث التسمم حيث تتحلل داخل
الجسم وتفرز سمومها وتعد سموم داخلية Endotoxine
كما في حالة التسمم بالسالمونيلا *Sallmonelosis*، ووجد
نوعان من العدوى، النوع الأول يحدث نتيجة نفوذ الأحياء
الميكروبية داخل الأمعاء حيث تتكاثر وتسبب المرض كما
في بكتريا *Shigella , Salmonella*، أو قد تنفذ من
الأمعاء إلى أنسجة أخرى بالجسم حيث تبدأ بالتكاثر كما هي
حال *Brucella*، أما النوع الثاني هو نتيجة إفراز سم من
ميكروبات العدوى في أثناء تكاثرها أو تكوينها لسبورات أو
تحللها في الأمعاء مثل *Clostridium perfringens* اما
السموم الخارجية Exotoxin: حيث تفرز البكتريا سمومها
في الغذاء وهو من نوع السموم الخارجية Exotoxine أي
ليس بالضرورة تناول البكتريا مع الغذاء وإنما السم الموجود
في الغذاء يحدث التسمم كما في *Staphylococcus*
aureas و*Clostridium botulinum* والأغذية التي
يحدث فيها التسمم الغذائي هي اللحوم والدواجن والأسماك
والمقبلات والحليب ومنتجاته . يمكن أن تسبب
الطفيليات المترافقة مع الماء والغذاء المرض للبشر. وبرغم
أن الطفيليات غالبا ما توجد في الدول النامية، فإن الدول
المتطورة قد عانت أيضا من تفشي أمراض عديدة منقولة
عن طريق الغذاء. قد تتعرض الأغذية للتلوث عن دون
قصد بسبب ممارسات معالجة غير ملائمة إما خلال
الزراعة أو خلال مرحلة معالجة الغذاء. يمكن أن توجد
الطفيليات على مستوى العالم وقد تصيب الحيوانات البرية
أو قد توجد في الماء فتتسبب في تلويث المحاصيل التي
تزرع للاستهلاك البشري؛ كما ويمكن أن يكون المرض
أكثر خطورة ومزمنا في الأفراد الذين يعانون من نقص
المناعة(6).

ينتج العفن السموم الفطرية والتي هي عبارة عن أيضات
ثانوية (Metabolites) تستطيع أن تسبب أمراضا حادة
ومزمنة في البشر عند تناولها في الطعام الملوث. وتشمل
الأمراض السرطانات والأورام التي تصيب مختلف
الأعضاء (القلب والكبد والكلية والأعصاب) والاضطرابات
الهضمية والتغيير في الجهاز المناعي ومشاكل في الإنجاب.
تنمو أنواع الاسبرجلس والاترناريا والبنيسيليوم وغيرها
في السلع الزراعية أو الأغذية وتنتج السموم الفطرية مثل
aflatoxin و Deoxynivalenol) : وochratoxin :
(Ergot Alkaloids وسم T-2 و (Zearalenone :
(Cyclopiazonic وتنتج السموم الفطرية patulin
بشكل رئيسي في الحبوب (الشعير والذرة الصفراء
والحنطة) والقهوة ومنتجات الألبان والفواكه والمكسرات
والبهارات. ركزت السيطرة على السموم الفطرية في
الأغذية على تقليل إنتاج السموم الفطرية في الحقل خلال
التخزين أو تدمير السموم بمجرد ظهورها ولا يزال

المقدمة

الغذاء عماد الحياة وفى سلامته سلامة الإنسان وهو المقوم
الأساس لاستمرار حياته ، من أجل ذلك ليس المطلوب أن
يكون الغذاء متوافراً فحسب بل الأهم هو توفير الغذاء الآمن
المتوازن والطازج الخالي من جميع الملوثات التي تضر
بصحة الإنسان كالمواد المضافة والمواد المشعة والمواد
المسرطنة والخالية من التغيرات غير المرغوبة سواء كانت
ميكروبية أو أنزيمية أو كيميائية وحيث إن الغذاء وسيلة لبقاء
الإنسان فإنه قد يحمل مخاطر نقل الأمراض إليه (1).

و تعد الأغذية ذات المصدر النباتي ومن ضمنها الخضار أحد
المصادر الأساس لغذاء البشر اليومي وباتت عصبياً في
حياة الشعوب ومدى ازدهارها وتقدمها مما دفع بالكثير من
الدول إلى توجيه اهتمام مكثف حول توفيرها والعناية
بمصادرها والسعي الجاد وراء تطوير إنتاجها والحفاظ عليها
من اجل الوصول إلى اكتفاء ذاتي حماية لاقتصادها وأمنها
الغذائي (2).

تتعرض المقبلات بكل أنواعها خلال مراحل الإنتاج
والحفظ والتسويق إلى العديد من الملوثات الكيميائية
والملوثات الإحيائية التي تؤدي إلى تلفه وفساده إذا لم تتخذ
الإجراءات والاحتياطات اللازمة للحفظ والتسويق وفق
السبل العلمية الصحيحة، فضلاً عن أن هذه المنتجات تكون
عرضة للتلوث بأحياء مرضية عدة التي قد تستل طريقها
إلى المستهلك دون علمه، ولذلك وجب دراسة طرائق العناية
والسيطرة على إنتاج وحفظ هذه المنتجات لتصل إلى
المستهلك بنوعيتها الجيدة والآمنة (3).

تعتبر جميع الأغذية الخام سريعة التلف والفساد ابتداء من
وقت الحصاد. وإن أحد العوامل الأساسية لفساد الأغذية
غير المصنعة هو مقدار ما تحتويه أنسجتها من الماء الفعال
حيوياً Biological active water لذا فان الفواكه
والخضر تفسد في أيام قليلة فضلاً عن احتوائها على المواد
الغذائية الضرورية لنمو ونشاط الأحياء الدقيقة (4).

إنّ التلف الناجم عن النمو الميكروبي في الأغذية هو عملية
نسبية يتباين تعريفها من شخص لآخر حيث لا يمكن وضع
حدود للتلف الميكروبي بصورة دقيقة إلا أن هناك تعريف
عام لتلف الأغذية يقصد به أي تغير حسي
(Organoleptic change) في الغذاء يؤدي إلى جعل
الغذاء غير مقبول من قبل المستهلك كالتغير في النكهة
Flavor والطعم Taste واللون Color والمظهر
Appearance، لذلك فإن عملية التلف تعتمد على ذوق
المستهلك والعادات الغذائية ونوع الغذاء، ويرتبط ذوق
المستهلك عادة بالعادات الغذائية للبلد المستهلك على سبيل
المثال الأسماك والأجبان واللحوم المعتقة التي أُجري تحلل
بسيط فيها تعد أغذية مرغوب فيها في بلدان مثل مصر
ودول شرق آسيا وغربها في حين تعد في بلدان أخرى تالفة
وغير مرغوب فيها، أما بالنسبة لنوع الغذاء فنشاط البكتريا
في غذاءٍ ما يعد تلفاً كما هو نشاط بكتريا حامض اللاكتيك
في اللحوم حيث تتحمض اللحوم فتعد تالفة بينما تضاف
عمداً إلى الحليب لإنتاج اللبن وهي بكتريا
Lactobacillus، ونشاط بكتريا *Acetobacter* في الخل
مرغوب به بينما وجودها في العصائر تلفاً لها بينما هناك
بعض الأغذية كالاجبان تضاف إليها الاعفان لتعطي نكهات
خاصة تكون غير مرغوبة في منطقتنا لكنها ذات قيمة
وسعر غال في اوربا (5).

الافلاتوكسين يعتبر كأهم السموم الفطرية، ويتم افرازه بواسطة بضعة أنواع من الاسبرجلس فقط حيث يعتبر فطر A. flavus وفطر A.parasiticus أكثرها إشكالا. يتأثر التعبير عن الأمراض المتعلقة بالافلاتوكسين بعدة عوامل مثل السن والتغذية والجنس والأنواع واحتمالية التعرض المتزامن للسموم الفطرية الأخرى. يعتبر الكبد العضو الهدف الرئيسي في الثدييات ولذا فإن تسمم أفلاتوكسيني هو بالأساس مرض كبدي. أما العوامل التي ترفع احتمالية هذا التسمم في البشر فتشمل توفرا محدودا للغذاء والظروف البيئية التي تحبذ نمو العفن على المواد الغذائية ونقص أنظمة التنظيم لمراقبة الافلاتوكسين والسيطرة عليه، هدفت الدراسة الحالية تقييم درجة ونوع التلوث المايكروبي (بكتريا ،فطريات وطفيليات) والخطر على صحة الإنسان لسلطة التبولة المحضرة محليًا ومدى تفاوت أعداد العزلات المايكروبية حسب المناطق ذات المستوى المعيشي المختلف (7).

مصادر التلوث الغذائي

هناك عدة مصادر لتلوث الغذاء ، حيث يختلف تواجد الكائنات الحية السطحية الطبيعية للنباتات باختلاف النبات لكنها عادة تضم أنواعا من البكتريا والخمائر والاعفان ، حيث تعتمد أعداد الكائنات على نوع النبات وبيئته وقد تتراوح من عدة مئات أو ألاف إلى ملايين في السنتمتر المربع الواحد من سطح النبات وقد وجدت أيضا داخل الأنسجة للنبات وعلى جذورها أو درناتها , أما التلوث عن طريق الحيوانات فيكون عن طريق مرور الحيوانات على النباتات التي يصنع منها الطعام وعن طريق ما تحمله من أحياء على جلودها أو أظافرها آو ريشها آو شعرها وكذلك من خلال برازها , وهناك مصدر آخر للتلوث هو مجاري الصرف الصحي من خلال استخدام مخلفات الصرف الصحي غير المعالج للتسميد الذي يؤدي إلى تلوث الأغذية النباتية الطازجة بالكائنات الممرضة للإنسان وبيوضها خاصة التي تسبب الأمراض المعوية المعدية ،أما التلوث من التربة التي تعتبر من اكبر مصادر التلوث للنبات لاحتوائها على أعداد ضخمة من الكائنات الدقيقة ذات الأهمية المرضية للإنسان وكذلك التلوث عن طريق الماء المستخدم في تصنيع الغذاء حيث يعتبر مصدرا مهما للتلوث حيث يكون ناقلا مهما للكائنات الدقيقة القادمة من غسل الأغذية أو تبريدها في المطاعم أو الثلج المستخدم لحفظ الأغذية ، ومن المصادر الأخرى للتلوث الهواء الذي يعمل على نشر الكائنات الحية الممرضة وخاصة المسببة للإصابات التنفسية أو قد يصبح المنتج الغذائي ملوثا خاصة إذا كان معرضا للجو (4).

ومن البكتريا الملوثة للسلطة والمعزولة من التبولة بكتريا القولون Escherichia coli وهي عصيات واسعة الانتشار تتواجد في التربة والمياه والنباتات والحيوانات وفي أمعاء الإنسان والحيوان وان وجودها في الغذاء يعتبر دليل تلوث الغذاء بفضلات الإنسان كما أنها لا هوائية اختيارا ،فضلا عن أن وقت تكاثرها Generation time قصير فهي تحلل السكريات إلى مجموعة كبيرة من الأحماض والغازات وتحلل البروتين إلى أحماض أمينية واستغلالها للمواد الكربوهيدراتية والعضوية الأخرى كمصادر للطاقة والنتروجين وكذلك قابليتها على تخليق اغلب الفيتامينات الضرورية لنموها ونموها بأمدية واسعة من درجات الحرارة تبلغ (10-46) م. إلا أنها تعتبر حساسة جدا للحرارة حيث تقتل في درجات حرارة التصنيع الغذائي فهي لا تكون السبورات ولذلك لا تتحمل الحرارة أو الظروف

البيئية القاسية لذا نرى وجودها وتكاثرها في الأغذية غير المطبوخة ومنها السلطات (8)، المكورات العنقودية الذهبية Staphyllococcus aureus بكتريا غير متحركة غير مكونة للسبورات اختيارية ومستعمراتها غير منتظمة وتكون مستعمراتها دائرية ومحدبة مخاطية (9). أما بكتريا Bacillus subtilis من العائلات المكونة للسبورات الداخلية Endospore مستعمراتها على الأوساط الزرعية الصلبة دائرية منتظمة وسطحا معتم غير شفاف مجعدة كريمية أو قهوائية اللون وتكون سبوراتها شديدة المقاومة لدرجات الحرارة المستخدمة في التصنيع الغذائي ولذلك تعتبر المسئولة عن تلف الأغذية المعاملة حراريا والمعلبة وكذلك مسئولة عن تلف الأغذية الطازجة في البلدان الحارة ومنها العراق . تسبب العديد من الأمراض المعدية الخطرة ولها ألفة مع درجات الحرارة العالية (محبة للحرارة) Thermophilic والنمو بدرجات حرارة تصل إلى 70 درجة مئوية (10)، أما جنس Enterobacter فهي تنتمي إلى عائلة البكتريا المعوية Enterobacteriaceae وهي عصوية غير مكونة للابواغ إلا أن قسما منها له محفظة مستعمراتها على الوسط الزرعي الصلب كبيرة ذات لون رمادي غامق مخاطية أو جافة وقسما منها متحركة وتتواجد أغلبها في أمعاء الإنسان والحيوان وقد عزلت هذه البكتريا من المستشفيات والمياه والتربة والبذور والفواكه وكان بعضها مسببا للأمراض والنباتات وعمال المطاعم ومعامل الأغذية وهي متحسسة للمضادات الحياتية، أما جنس Cetrobacter تتميز هذه البكتريا بتركيبها المستضدي حيث تحوي ثلاث أنماط من المستضدات وهي لا هوائية متحركة وغير مكونة للكبسول ولها صفات مشتركة مع بكتريا E.coli . عزلت هذه البكتريا من النباتات والحيوانات والطيور وكذلك من التربة والمجاري والأغذية وأنها عرفت كونها انتهازية مرضية (11).

والاعفان كائنات مجهرية تشبه البكتريا مع كبر حجمها عنها نسبيا، ومن مظاهر الشبه مع أغلب أنواع البكتيريا عدم القدرة على التغذية الذاتية، لذلك تنمو فوق المواد العضوية. وهناك نحو 50 ألف نوعاً من فطريات العفن منتشرة في الهواء والماء والتربة، وتتفاوت أضرارها بين إتلاف المحاصيل بأمراض النبات الفطرية وتعفن المواد الغذائية المخزونة وإتلافها . وتصل بعض أنواعها إلى حد إنتاج أصناف من السموم المسرطنة التي يبلغ عددها نحو 250 نوعاً، أو المشوهة للأجنة أو المثبطة للمناعة أو المتلفة للكبد أو التهاب الجهاز التنفسي، أو التهاب الكلى أو الجهاز العصبي ومن أشهر الفطريات ذات الطبيعة السمية والملوثة للغذاء فطر Aspergillus paraciticus المفرز لسموم أفلاتوكسين (Aflatoxineds)، والذي غالباً ما يلوث الحبوب والدرنات والبذور الزيتية وبعض الفواكه وأن معدل تناوله في الطعام يتناسب طرديا مع معدل انتشار سرطان الكبد، سواء بالنسبة للإنسان أو الحيوان .وتصنف معظم الفطريات بتكاثرها اللاجنسي، ويستطيع الفطر الواحد في ظل ظروف معينة أن يتكاثر بأعداد كبيرة. ، ونظرا لانتقال الحبوب المعرضة للتلوث بها من مكان الإنتاج إلى مكان الاستهلاك بسبب طول فترة النقل وتهيئة الظروف المسببة لتكاثرها وإفراز سمومها (12).

والطفيليات كائنات حية يمكن أن ترى بالعين المجردة منها ما تعيش على السطح الخارجي لجسم الإنسان كالقمل ، وهناك أنواع أخرى تعيش داخل جسم الإنسان في أعضائه و أحشائه الداخلية كبعض أنواع الديدان ، وعليه يمكن تقسيم

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المواد وطرائق العمل

جمعت 64 عينة لسلطة التبولة المحلية الطازجة من عدة مناطق مختلفة من مدينة بغداد خاصة لعمل وتحضير وبيع السلطة بواقع عدة نماذج من كل منطقة ومن أربعة عشر موقعاً في مدينة بغداد وهي (الجادرية، البياع، السيدية، الوشاش، المنصور، الكاظمية، الشعب، الدورة، شارع فلسطين، الأعظمية، مدينة الصدر، العامل، الإعلام، بغداد الجديدة) ويلاحظ أن هذه المناطق شملت جانبي الكرخ والرصافة وللفترة من كانون الثاني ولغاية حزيران/2012، نقلت العينات بأكياس معقمة، وأجريت عليها الفحوصات بصورة مباشرة، الفحوصات : أخذ 10 غم من كل عينة وهرست لمدة 5 دقائق مع 90 مل من المحلول الملحي المعقم Sterilized normal saline وترك لمدة نصف ساعة ثم اخذ منه 1 مل وعملت منها ثلاث تخفيفات متسلسلة وأخذ من التخفيف الثالث 0.1 مل وزرعت على أوساط اكار اكار المغذي Nutrient Agar واكار الماكونكي MacConky Agar واكار الدم Blood Agar واكار المانتول الملحي Manitol salt Agar وبواسطة الناشر الزجاجي تم فرشها على الأوساط الزرعية وبعدها حضن على درجة حرارة 37 درجة مئوية لمدة 24 ساعة . وكذلك اكار المالت malt extract agar واكار السابورويد Saboruid agar وبواسطة الناشر الزجاجي تم فرشها على الأوساط الزرعية وبعدها حضن على درجة حرارة 25 درجة مئوية لمدة 3-5 أيام بالنسبة لعزلات الفطريات ، أما الكشف عن بيوض الطفيليات وأطوارها المتكيسة فقد تم عن طريق اخذ 5مل من محلول الاصلي المهروسه فيها السلطة تم وضعها في انابيب جهاز الطرد المركزي وتدويرها بسرعة 3000دورة في الدقيقة لمدة ثلاث دقائق وبعدها اخذت قطرة من الراسب واضيف عليها اصباغ خاصة مثل الملكايت كرين وصبغة المثيل ريد وصبغة السودان بلاك وفحصت تحت المجهر الضوئي واظهر الفحص وجود بيوض واطوار كيسية لطفيليات خطرة على الصحة العامة .

أجريت الفحوصات الكيموحيوية للعزلات البكتيرية بالاعتماد على ملون كرام ومواصفات المستعمرات وإجراء الفحوصات الكيموحيوية الآتية : إنزيم الاوكسديز ، إنزيم الكاتليز، الاندول، وتخمر سكر اللاكتوز، تحلل الدم، اختبار المثيل الأحمر . أما العزلات الفطرية فقد تم تشخيصها باستخدام المجهر الضوئي وذلك بأخذ قطعة من المستعمرة الفطرية ووضعها على شريحة زجاجية وإضافة قطرة عليها من صبغة اللاكتوفينول الزرقاء ومطابقتها مع المفاتيح التصنيفية للاعفان .

التحليل الإحصائي:

تم تطبيق البرنامج SAS (2010) في التحليل الإحصائي في تحليل تأثير العوامل المدروسة، وقورنت الفروق المعنوية بين النسب المختلفة باختبار مربع كــاي (Chi-square –χ2 test) (14).

التطفل من هذه الناحية إلى تطفل داخلي (Endoparasitism) ، أو تطفل خارجي (Ectoparasitism). ويمكن كذلك تقسيم التطفل على حسب المدة أو الوقت الذي يستغله الطفيلي في علاقته بالعائل أثناء دورة حياته ، فهناك ما يعرف بالتطفل المؤقت (Temporary parasitism) حيث لا يعيش الطفيل طوال حياته متطفلاً بل يقضي إحدى مراحل دورة الحياة معتمداً على العائل كما يحدث في بعض أنواع يرقات الذباب ، وهناك ما يعرف بالتطفل الدائم (Permanent parasitism)حيث يقضي الطفيل كل دورة حياته متطفلاً على العائل لا يفارقه إلا إلى عائل آخر عن طريق العدوى بالاتصال المباشر ، حيث أنه لو ترك العائل يموت ، ومثال هذا النوع من الطفيليات هي السوطيات المهبلية التي تصيب الإناث ولا يمكن أن تغادر جسم الإنسان إلا إلى جسم إنسان آخر . وهناك أيضاً التطفل الدوري (Periodic parasitism)حيث يتردد الطفيل في هذه العلاقة على العائل من حين لآخر كالبعوض مثلاً، كما يمكن أن يصنف التطفل على حسب ظروف الطفيل التكيفية ومدى احتياجه للعائل ، فهناك التطفل الإجباري (Obligatory parasitism)وهو عدم مقدرة الطفيلي الاستغناء عن عائله أو عائلاته سواء في مرحلة معينة من دورة الحياة أو طوال حياته ، وهذا ما يحدث مع معظم الطفيليات . كما يمكن أن يكون التطفل عرضياً (Accidental parasitism) ويحدث هذا مع الطفيليات التي تتطفل على عائل غير عائلها الطبيعي الأساسي ، ومثاله بعض أنواع الديدان الشريطية التي تتطفل أساساً على القوارض وفي حالة توفر الفرصة لإصابة الإنسان فهي لا تتردد في ذلك بالرغم من أنه ليس عائلها الأساسي .كما يوجد التطفل الطارئ (Incidental parasitism) وهو وجود الطفيل في مكان غير مكانه الطبيعي أثناء تطفله ، فمثلاً نجد أن الأميبا المسببة للزحار (الدوزينتاريا) الأميبية تعيش أساساً في أمعاء الإنسان الغليظة ، ولكن في ظروف نادرة يمكن أن تصل مع تيار الدم إلى الدماغ والذي لا يعتبر مكاناً أساسياً لمعيشتها . والنوع الأخير وهو التطفل الانتهازي (Opportunistic parasitism)ونجده في بعض الكائنات التي تعيش معيشة حرة دون الاعتماد على عائل و دون أن تموت ، والتطفل غير مفروض عليها وليس ضرورياً لها ، ولكنها إذا وجدت عائلا تستطيع التطفل عليه ، ومثالها الأميبة الحرة التي تعيش في المياه العذبة حرة ولها سوطان يساعدانها على السباحة والتحرك في الماء ، وتحدث الإصابة للناس الذين يسبحون في المياه الملوثة حيث يدخل من فتحات الأنف ومن ثم تشق طريقها للمخ وذلك في حالة مصادفة الإنسان وإذا لم تصادفه لا تهلك من دونه وتكمل باقي حياتها حرة طليقة (13).

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النتائج والمناقشة

لوحظ أن المناطق التي جمعت منها النماذج مختلفــة مــن حيث الرقعة الجغرافية والمستوى المعاشي والثقافي للسكان، لذا فهي تعطي صورة قريبة من الواقـع لتمثيـل التوزيــع السكاني في مدينة بغداد بغية الحصول على صورة أوضــح لتلوث أنواع من السلطات المحضرة محلياً ومنهـا سـلطة التبولة . أظهر جدول (1) نتائج الفحوصــات الكيموحيويــة للعزلات البكتيرية التي تم عزلها مـن نمـاذج الســلطة المحضرة محلياً، وشخـصت الأنـواع البكتيريـة التاليـة: (1)*Bacillus subtilis* (2) *Staphylococcus aureus* (3) *Enterobacter agglomerans* (4) *Citrobacter freundii* (5) *Escherichia coli* (6)*Enterobacter gergoviae* وهذه النتائج تتفق مــع نتائج الطرق المرجعية للتشخيص (15).

يبين جدول (2) أعداد أنواع العزلات البكتيرية التي عزلــت من سلطة التبولة المحضرة محلياً ونسبها المئويــة وكانــت كـــالآتي: *Escherichia coli* 17/61 (27.86%), *Enterobacter gergoviae 7/61 (11.47%), agglomerans* 11/61 (18.03%), *Enterobacter Staphylococcus aureus* 10/61 (16.39%), *Bacillus subtilis* 7/61(11.47%), *Citrobacter freundii* 9/61 (14.75%). والمجمـوع الكلـي لعـدد العزلات هو 61 عزلة بكتيرية، حيث حسبت النسبة المئويــة كالآتي:النسبة المئوية = [مجموع الجزء(العزلة) / مجموع الكل (العزلات)] *100 .

تبين النتائج أن العزلة البكتيرية *Escherichia coli* هي الأكثر عدداً تليهـا كـل مــن *Staphylococcus aureus* و *Bacillus subtilis* و *Citrobacter freundii* و *Enterobacter agglomerans* و *Enterobacter gergoviae*، وتعد بكتريا القولون البرازيـة *E.coli* مــن الأنواع الخطرة وهي دليل على التلـوث البـرازي والـذي يؤدي الى تلف الغذاء ولها قابلية عالية للنمو السريع علـى المواد الغذائية بصورة عامة وتحتوي هذه البكتريـا علـى سموم داخلية Endotoxin في جدارها الخلوي الذي غالبـا ما يسبب الإسهال وقد يكون دمويا فـي بعـض الـسلالات (15)، وتعد بكتريا *Staphylococcus aureus* الملوثـة للسلطات المصنعة المحلية والمسببة للتسمم الغـذائي وذلـك لإفرازها السم المعوي Enterotoxin الذي يسبب تـسمم الأغذية عالية الكربوهيدرات ومنتجات الألبان ووجد ان هذه السموم التي تفرزها تقاوم درجة حـرارة البـسترة وحتـى درجة الغليان وكذلك تتمكن هذه البكتريا مـن العـيش فـي الأغذية المملحة التي يصل تركيز الملح فيها إلـى 10% . وتتواجد هذه البكتريا بشكل طبيعـي Normal flora فـي مناطق عديدة من جسم الإنسان منها الطبقة المخاطية للأنف وكذلك على الجلد ومنها تنتقل إلى الأغذيـة (16). وكـذلك تعتبر بكتريا *Bacillus subtilis* مـسؤولة عـن تلـف الأغذية الطازجة في البلدان الحارة ومنها العراق فهي تتألف مع درجات الحرارة في البيئة التي تتواجـد فيهـا وتعتبـر التربة المصدر الرئيسي لتواجد سلالات *Bcillus* ومـن التربة تكون السبورات الهوائية التي يحملها الهواء مـصدرا لتلوث الأغذية ،تعمل على تفكيـك المـواد ذات المـصدر الحيواني والنباتي وتتمو هذه البكتريا في الأغذية الحامضية بوجود الأوكسجين وتعمل على تحليل المركبـات النـشوية والعضوية في الأغذية وتتلفها كمـا أنهـا تنـتج سـكريات وأحماض ومركبات عضوية وغـازات فـي الأغذيـة وان ضروبا منها تسبب التسمم الغذائي والتهاب النسيج الخلـوي

بعد العمليات وأمراضا تنفسية أخـرى (17)، فـي حـين التلـوث ببكتريـا *Citrobacter freundii* الانتهازيــة المرضية الأقل خطورة على الإنسان التي تتواجـد فـي التربة والهواء وتتأثر بالحرارة ولا تتحمل الظروف البيئية القاسية (18)، تتواجد بكتريا *Enterobacter gergoviae* في أمعاء الإنسان والحيوان وتسبب له الأمـراض ومنهـا التهاب المجاري البولية وهـي مفـرزة للـسموم الخلويـة Cytotoxins التي تلعب دوراً مهماً في الالتهابات وتعد بكتريــا *Enterobacter agglomerans* إنتهازيــة ومتحسسة للمضادات الحياتية وتنتشر في كل البيئات وكافة أنواع الأغذية وعلى أيدي العاملين في مجال الأغذية (19). أما الجدول رقم (3) بين أعداد أنواع العزلات الفطرية التي عزلت من سلطة التبولة المحضرة محلياً ونسبها المئوية وحسبت كالآتي :الخمائر *Yeasts* بلغت نــسبتها 54/116 (46.5%) ، يليهـا عفـن *Geotrichum candedium* (15.5%) 18/116 ، تليهـا الخميـرة المرضـية . *Rhodotorula spp , Alternaria spp* 14/116 (12.1) ثـم العفـن *Aspergillus parasiticus* (13.7)16/116 ، حيث بلغ المجموع الكلي لعدد العـزلات الفطرية هو 116 عزلة فطرية ، حيث حسبت النسبة المئوية كما في الجدول السابق، وتعد الفطريات (الخمائر والاعفان) من الأحياء المجهرية المهمة في حياة الإنسان حيث تـسبب له الأمراض الخطيرة ومنها ما يسبب التسمم وكثير منها ما يهاجم المواد الغذائية ويعمل على إتلافها ومنها الخمائر التي تعمل بالدرجة الأساس على إتلاف المواد الغذائية وتحليـل المواد السكرية فيها ومنها تنتقل إلى داخل جـسم الإنسان مسببة أمراض الأمعاء المعوية وكـذلك خميـرة *Rudotorula* المعروفة بتسببها بالالتهابات المعوية وكذلك يعتبـر فطـر *Aspergilus parasitics* من الاعفان الشديدة الخطـورة على الإنسان وذلك لان في حالة تناول سبوراته فانه يصيب الفم والعدة والأمعاء بالالتهابات خطيرة، أما فـي حالـة استتشاقه فانه يصيب المجاري والقنوات التنفسية بالالتهابات، أما فـي حالة إصابته للجلد فيسبب قروحـا وأورامـا تـؤدي إلـى السرطان، أما إذا أصاب المواد الغذائية وخاصة الحبوب في المخازن وتوفر له البيئة المناسبة من حرارة ورطوبة فإنــه يعمل على إفراز سموم شـديدة الخطـورة تـسمى سـموم الافلاتوكسين Aflatoxin والتي تعتبر من أكثـر الـسموم الفطرية شيوعا لأنها الأكثر حدوثا والأكثـر ضـررا عنـد تناولها من قبل الإنسان أو الحيوان على حد سـواء، فهـي تدخل الجسم وتمر عبر المجرى الدموي ثم تترسـب فـي الكبد وبمرور الوقت تتراكم في الكبد مسببة له سرطان الكبد وهو من أشد الأمراض المعروفة التي يسببها هذا السم ، أما فطر *Alternaria* فمعروف أيضا بإصابته للقنوات التنفسية والمعوية، وكذلك بإفراز بعض السموم ولكنه يعتبــر أقـل خطورة مقارنة بفطر *Asp.parastics* (20).

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جدول (1): الاختبارات الكيموحيوية التشخيصية للبكتريا المعزولة من سلطة التبولة.

ت	نوع الاختبار	العزلات البكتيرية					
		1	2	3	4	5	6
1	ملون غرام	+	+	−	−	−	−
2	الاوكسديز	+	+	−	−	−	−
3	الكاتليز	+	+	−	−	+	−
4	انتاج الاندول	*	*	−	+	+	−
5	تخمر اللاكتوز	*	*	+	+	+	−
6	المثيل الاحمر	*	*	+	−	+	−
7	تحلل الدم	−	+	*	*	*	*
8	الجلاتين	−	+	*	*	*	*
9	كاشف كوفاكس	*	*	−	+	−	+
10	يوريز	+	+	*	*	*	*
11	النمو على المانتول	+	+	*	*	*	*

(1)*Bacillus subtilis* (2) *Staphylococcus aureus* (3) *Enterobacter agglomerans*
(4) *Citrobacter freundii* (5) *Escherichia coli* (6)*Enterobacter gergoviae*
+ نتيجة موجبة ، - نتيجة سالبة ، * لا يوجد فحص

جدول(2): أعداد أنواع العزلات البكتيرية التي عزلت من سلطة التبولة المحضرة محلياً ونسبها المئوية

النسبة المئوية %	المجموع	6	5	4	3	2	1	عدد النماذج	المنطقة
4.9	3	1	–	–	1	–	1	3	الجادرية
8.1	5	–	–	2	–	1	2	5	البياع
6.5	4	1	1	–	1	–	1	3	السيدية
6.5	4	–	–	1	–	1	2	4	الوشاش
3.2	2	–	1	–	1	–	–	3	المنصور
9.8	6	2	–	–	–	2	2	6	الكاظمية
6.5	4	–	–	1	1	–	2	5	الشعب
8.1	5	–	2	–	2	–	1	4	الدورة
4.9	3	1	–	2	–	–	–	6	شارع فلسطين
6.5	4	1	2	–	–	–	1	4	الاعظمية
14.7	9	–	–	1	3	2	3	7	مدينة الصدر
6.5	4	2	–	–	1	–	1	3	العامل
6.5	4	–	1	2	–	1	–	5	الاعلام
6.5	4	1	–	1	1	–	1	6	بغداد الجديدة
–––	61	9	7	10	11	7	17	64	المجموع
–––	–––	14.75	11.47	16.39	18.03	11.47	27.86	النسبة المئوية %	
*4.665	–––	5.109 *						قيمة مربع كاي	

(1) *Escherichia coli*, (2) *Enterobacter gergoviae*, (3) *Enterobacter agglomerans*, (4) *Staphylococcus aureus* , (5) *Bacillus subtilis*, (6) *Citrobacter freundii*. * (P<0.05).

<div align="center">جدول(3): أعداد أنواع العزلات الفطرية التي عزلت من سلطة التبولة المحضرة محلياً ونسبها المئوية</div>

النسبة المئوية%	المجموع	أعداد وأنواع العزلات الفطرية					عدد النماذج	المنطقة
		5	4	3	2	1		
2.5	3	–	1	–	–	2	3	الجادرية
11.2	13	3	1	2	1	6	5	البياع
4.3	5	–	2	–	2	1	3	السيدية
7.7	9	1	–	3	1	4	4	الوشاش
3.4	4	–	–	1	2	1	3	المنصور
5.1	6	1	2	–	–	3	6	الكاظمية
10.3	12	2	2	1	2	5	5	الشعب
6.03	7	1	2	–	–	4	4	الدورة
3.4	4	1	–	–	1	2	6	شارع فلسطين
3.4	4	–	–	1	–	3	4	الاعظمية
16.3	19	4	1	3	3	8	7	مدينة الصدر
10.3	12	–	2	1	4	5	3	العامل
5.1	6	1	1	–	–	4	5	الاعلام
10.3	12	2	–	2	2	6	6	بغداد الجديدة
---	116	16	14	14	18	54	64	المجموع
---	---	13.7	12.1	12.1	15.5	46.5	النسبة المئوية %	
* 4.275	---	** 8.944					قيمة مربع كاي	

(1)Yeast ssp, (2)Geotrichum.candidum, (3)Rhodotorula, (4)Alternaria ssp , (5)Aspergillus parasiticus.

*(P<0.05), ** (P<0.01).

في حين الجدول رقم (4) بين أعداد أنواع البيوض وأكياس الديدان والطفيليات التي عزلت من سلطة التبولة المحضرة محلياً ونسبها المئوية وحسبت كـالاتي : الطـور المتكـيس *Entamoiba coli* (32.5%) 13/40 وكانت نسبة كـل من بيوض ديدان *Ascaris lambricoides* و *Trichuris trichiura* هي (10%) 4/40 وكذلك نسبة كل من بيوض ديـــدان *Enterobius vermicularis* و *Fasciola hepatica* واكيـاس كـل مـن *Echinococcus granulosus* و *Balantidium coli* هـي 2/40 (5%) اما الطـور المتكـيس *Entamoiba hesolytica* (15%) 6/40 ، و الطور المتكـيس *Giardia lamblia* (12.5%) 5/40 ،حيث بلغ المجموع الكلي لعـدد انـواع بيوض والاكياس للديدان والطفيليات هو 40 نـوع ، حيـث حسبت النسبة المئوية كما في السابق، التـسممات الغذائـة بالطفيليات سببها أساسا هو تناول أطعمة ملوثة بالطفيليـات كالديدان الخيطية و الديدان الشريطية و الديدان المثقوبـة و البروتوزوا. أكثر الأطعمة تلوثـا هـي منتجـات اللحـوم والمنتجات السمكية، وما يسمى بالأطعمة الغريبـة (أرجـل الضفادع والقواقع والرخويات) وبعض الأغذية النباتية مثـل

الكرفس والخس أو البقدونس المحتوية على يرقـات بـسبب الري بمياه ملوثة (4) .وتعـد البيـوض والاكيـاس هـي الاطوار المعدية والمصيبة للكائن الحي في حالـة دخولهـا الجـسم حيـث تعتبـر دودة أو طفيلـي *Ascaris lambricoides* واسعة الانتشار خصوصا بـين صـغار السن وييلغ طولها ما بين 35-25 سم . وتعيش في الأمعاء الدقيقة وتتواجد في جماعات يتراوح عددها من 5–50 دودة في الجماعة الواحدة . تتغـذى الأسـكارس علـى الطعـام المهضوم وتسبب ضرراً كبيراً للإنسان. مـسببة إحـساس المصاب بمغص متكرر بالبطن وفقـدان الـشهية للطعـام والإرهاق وقلة النوم وخروج اللعاب من الفـم وخـصوصاً صغار السن ، وقد تخرج هذه الديدان مـن الفـم فيـصاب المريض بذعر وخوف شديدين . وقـد تـسبب الأسـكارس انسداداً في الأمعاء أو تنحشر في أعضاء هامة فـي الـبطن كالزائدة الدودية أو فتحة الكبد أو البنكرياس مسببة أخطـاراً كثيرة للمريض (10)، أمـا طفيلـي *En.vermecularis* فينتشر بين كثير من الناس خاصة الأطفال . وهي ديـدان صغيرة طول الواحدة ما بين 10-8 ملم في المتوسط وتعيش الدودة في أمعاء الإنسان . إلا أن بعـضها يفـضل الزائـدة

الدودية مسببة أعراض ضعف وفقدان الشهية لتناول الطعام وعدم الاستفادة من الطعام الذي يتناوله المصاب فينشأ عــن ذلك الأنيميا والضعف الشديدين . كما يشكو المــصاب مــن الاستحكاك حول فتحة الشرج فيهرش المــصاب بأظفاره مكان الاستحكاك مما ينتج عنه تــسلخات بفتحــة الــشرج والتهابات هذا بجانب الأرق والتشنج الذي ينتج عنها لــيلا . وقد تصيب أيضاً البالغين وكبار السن ولكن بــصورة أقــل (21) ،اما طفيلي Giardia lamblia سوطى يتكاثر في الامعاء الدقيقه مكونا مستعمرات مسببة داء الجيارديا يلتصق بالطبقة الطلائيه للامعاء بواسطة قــرص بطنــى لاصــق ويتكاثر بالانقسام البــسيط، يقتــصر وجودهــا فــي تجويف الامعاء الدقيقة. وبذلك يتمكن الطور النشط للجيارديا من امتصاص العناصر الغذائية الخاصة بــه مــن تجويــف الأمعاء الدقيقة، وهي لاهوائية التنفس. وتظهــر اعــراض المرض عند تناول اكل أو شرب ماء ملوث بالطور الساكن للطفيلي أو عن طريق وصول جزء من البراز للفم. الطــور المتكيس للجيارديا يمكنه البقاء على قيد الحياة لمدة أسابيع أو شهور في الماء البارد، وبالتالي يمكن أن تكون موجودة في الآبار الملوثة وشبكات المياه، ومصادر المياه الراكدة خاصة التي تحدث بشكل طبيعي في البرك، وأنظمة تخزين الميــاه المتدفقه، . ويمكن أن توجد أيضا في خزانات ميــاه المــدن كما توجد في مياه الصرف الــصحى المعالجــه ,والطــور المتكيس مقاوم للاساليب التقليديه لتعقيم المياه بــالكلور أو بالاوزون (21) ،اما Echinococcus granulosus هــي مــن الديــدان حلقيــات المحــاجم وتتطفل فــي الأمعــاء الدقيقة للكلبيات، المضيف المتوسط هو الماشيةوالإنسان. يعد داء الأكياس المائية من أهم الأمراض الطفيلية المشتركة بين الإنسان والحيوان .ويصاب الإنسان والحيوان وذلك بانتقــال بيوض الدودة الشريطية إليهما عن طريــق الغــذاء والمــاء الملــوث. مــسببا مــرض(الهيداتيــد) الأكيــاس المائيــة(Hydrated disease)مــرض طفيلــي يــصيب الحيوانات آكلة الأعشاب كالأغنام والماعز والأبقار والإبل، وكذلك يصيب الإنسان وتعتبر كلها عائل وسيط لهذا الطفيل. وتنتقل الإصابة عن طريق بيوض الدودة الشريطية البالغــة إلى الإنسان والحيوان كعائل وسط. تنمو اليرقات في أنسجة أعضاء الجسم وخاصة الكبد والرئتين. تعيش الدودة الكاملــة في أمعاء الكلاب والذئاب والثعالب والتــي تعتبــر عــاملا أساسيا لها   . وللدودة البالغة القدرة على البقاء فــي أمعــاء الكلاب لمدة سنة أو أكثر، مما يؤدي إلى تلوث البيئة بهــذا الداء.وتكون العدوى بواسطة براز الكلاب المصابة بالــدودة الشريطية البالغة بالإضافة إلى المراعي والأعلاف والميــاه الملوثة ببراز هذه الكلاب. ينتقل بواسطة اليد إلى الفم مــن خلال التماس مع الأدوات الملوثة ببراز الكــلاب المــصابة بالمرض. وتتناول مواد غذائية مثل الخضروات الطازجة أو الماء الملوث   ببويضات الدودة الشريطية الناتجة من بــراز الكلاب (10) ،اما Trichuris trichuri وهــي طفيليــات معوية منتشرة عالميّا ، خصوصا في الأمــاكن الإســتوائيّة والبلدان الحارّة ومعتدلة الحرارة وهي لا تؤدي غالبــا إلــى أعراض خطيرة.وتسبب مرض التريكوسـيفالوز العــدوى تكون فمويّة بالبيوض.ومن بين الأشخاص الأكثر إصابة (الأطفال والشيوخ و بعض أصناف الــشغّالين مثــل عمّــال الفلاحة) (10)، وطفيلــي Fasciola hepatica المتورقــة الكبدية طفيلي واسع الانتشار في كل أنحاء العــالم ويــصل معدل انتشارها في بعض البلدان 50% تكثر الاصــابة فــي نهاية الصيف وتصيب البالغين وبخاصة النــساء. يتوضــع

الطفيلي في الأقنية الصفراوية والمــرارة وأنــسجة الكبــد للإنــــــــــــسان والحيوانــــــــــات المــــــصابة. يصاب الانسان والحيوانات بالمتورقة الكبدية عبــر تنــاول الأعشاب والنباتات الملوثة بالمذنبات والتــي تبقى لفتــرة طويلة قادرة على العــدوى خاصة فــي الجــو الرطــب وطفيلـــــــــــــــي Entamoiba histolaitic   و Entamoibahi coli وهما أكثر الطفيليــات أهميــة للإنسان . وهو يعيش فى الأمعاء الغليظة ، وتحت ظــروف معينة يمكن أن يغزو جدار الأمعاء وذلك بإفراز الإنزيمــات التى تهاجم البطانة المعوية . وعندما يحدث هذا الغزو تنــتج الديزنتاريا الأميبية الخطيرة التى تكون مميتة أحيانــا وعــن طريق الدم يمكن أن تنتقل الطفيليات إلى الكبــد وأعــضاء أخرى ، حيث تسبب الخراجــات وتنتــشر الإصــابة بهــذا الطفيلي عن طريق تلوث الماء أو الغذاء باحتوائهمــا علــى هذه الحويصلات (21) .اما طفيلــي Balantedium coli متطفل على الانسان وينتشر فى جميع انحاء العــالم يعــيش هذا الطفيلي   فى القولون فى الامعــاء الغليظــة وبعــض الثدييات الاخرى كالخنازير القردة والكلاب يعتبر الخنزيــر العائل الاساسى لهذا الطفيلي ومنه تنتقل الاصــابة للانــسان فهو لا يسبب اى مرض للخنازير ولكــن يــسبب للانــسان مــرض الدوســنتاريا البالنتيديــة balantidiosis or balantidial dysentery دورة حياة الطفيل عبارة عــن طور متحوصــل cyst وطــور متغــذى داخــل الامعــاء trophozoite يلاحظ ان الطور المتحوصل لايحتوى علــى اهداب ويحاط الطفيلي بغلاف سميك.. يصل الطفيلي الــى الطبقة المخاطية للامعاء ويهاجمهــا ويــسبب لهــا دمامــل وخراجات (10).

جدول(4): أعداد أنواع الطفيليات المعزولة من سلطة التبولة المحضرة محلياً ونسبها المئوية.

النسبة المئوية%	المجموع	9	8	7	6	5	4	3	2	1	عدد النماذج	المنطقة
–	–	–	–	–	–	–	–	–	–	–	3	الجادرية
10	4	–	–	1	2	–	–	–	–	1	5	البياع
2.5	1	–	–	–	–	–	–	–	–	1	3	السيدية
7.5	3	–	–	1	–	–	–	–	2	–	4	الوشاش
2.5	1	1	–	–	–	–	–	–	–	–	3	المنصور
7.5	3	–	–	–	–	–	1	–	–	2	6	الكاظمية
5	2	–	–	1	–	–	–	1	–	–	5	الشعب
5	2	–	–	–	1	–	–	–	–	1	4	الدورة
7.5	3	–	1	–	–	–	–	–	–	2	6	شارع فلسطين
7.5	3	–	–	–	–	1	–	–	2	–	4	الاعظمية
15	6	–	–	2	1	–	–	1	–	2	7	مدينة الصدر
15	6	–	1	–	2	–	1	–	–	2	3	العامل
5	2	–	–	–	–	1	–	1	–	–	5	الاعلام
10	4	1	–	–	–	–	–	1	–	2	6	بغداد الجديدة
---	40	2	2	5	6	2	2	4	4	13	64	المجموع
---	---	5	5	12.5	15	5	5	10	10	32.5		النسبة المئوية %
*4.03	---					** 7.318						قيمة مربع كاي

(1)Entamoiba.coli,   (2)Trichuris   trichiura,   (3)Ascaris   lumbricoides,   (4)Fasciola.hepatica,   (5) Enterobius vermicularis,   (6)Entamoiba.hisolytica,   (7)Giadia.lamblia   ,(8)Echinococcus granulosus, (9)Balantidium coli.
* (P<0.05), ** (P<0.01).

تتعرض الخضار بشكل عام للتلوث بالأحياء المجهرية خلال تكوينها في الحقول الزراعية وبعد جنيها وجمعها على الارض مباشرة او في السلال او الصناديق خلال عمليات النقل والتسويق والخزن، كما إن استخدام السماد النباتي ومخلفات المجاري للتسميد يجعلها عرضة للتلوث بأنواع مختلفة من الأحياء المجهرية المرضية (22)، وعليه فان النتائج المبينة في الجداول   (1 ،2، 3) والتي عرضت اعداد والنسب المئوية للملوثات (بكتريا ،فطريات وطفيليات) يتضح ان حجم التلوث البكتيري كان اكبر بنسبة مئوية 14.7% في مدينة الصدر و 9.8% في منطقة الكاظمية تليها نسبة 8.1%في منطقتي الدورة والبياع وتليها نسبة 6.5% في مناطق الوشاش والشعب والاعظمية والعامل والاعلام والسيدية وبغداد الجديدة وكانت النسبة الاقل هي 4.9%في شارع فلسطين والجادرية و 3.2% في منطقة المنصور اما حجم التلوث الفطري فقد كان كبيرا بنسبة مئوية 16.3% في مدينة الصدر و 11.2%في منطقة البياع و 10.3%في مناطق الشعب والعامل وبغداد الجديدة على التوالي وبنسبة 7.7 % في منطقة الوشاش ونسبة 6%في الدورة و 5.1% في منطقتي الكاظمية والاعلام ونسبة مئوية اقل 4.3%في منطقة السيدية و 3.4%في مناطق المنصور وشارع فلسطين والاعظمية على التوالي واقل نسبة كانت 2.5%في منطقة الجادرية وكان حجم التلوث ببيوض واكياس

الطفيليات كبيرا بنسبة 15%في منطقة العامل ومدينة الصدر ونسبة 10% في بغداد الجديدة والبياع وكانت اقل بنسبة7.5% في مناطق الوشاش والكاظمية وشارع فلسطين ومنطقة الاعظمية على التوالي وبنسبة 5% في مناطق الشعب والدورة والاعلام على التوالي ونسبة 2.5% في منطقتي السيدية والمنصور وانعدام النسبة في منطقة الجادرية وهذا يعكس مقدار ضعف الشروط الصحية المطبقة في المناطق العامة والشعبية (مدينة الصدر ,العامل , بغداد الجديدة ,البياع والشعب) مقارنة مع المناطق الافضل من حيث تطبيق الشروط الصحية (الاعظمية والكاظمية ,الاعلام والدورة) وصولا الى المناطق التي هي افضل من حيث المستوى المعاشي والثقافي وتطبيق الشروط الصحية (السيدية , شارع فلسطين , المنصور والجادرية) من خلال العناية العالية بالمواد الغذائية وذلك باستخدام الاواني والادوات النظيفة في عملية صنع وعمل السلطة وتعكس هذه النتائج ان المستوى المعيشي والثقافي للمنطقة السكنية ذو تأثير كبير على انخفاض او ارتفاع نسبة التلوث بالأحياء المجهرية .

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التوصيات

لغرض السيطرة على الأحياء المنقولة بالسلطة وتطبيق السلامة الغذائية يجب :

1- التشجيع على النظافة الشخصية وخصوصا لدى العاملين في مجال التصنيع الغذائي.

2- منع التلوث العرضي عن طريق الخزن الصحيح واستخدام الادوات والاسطح المطهرة.

3- استخدام الغسل بالماء والمعقمات للخضار الكفيل بازالة الأعداد الهائلة للأحياء الملوثة .

4- استخدام طرق التبريد في حفظ المواد الغذائية.

5- شراء المواد الغذائية من مصادر موثوقة امنة ومسيطر عليها صحيا.

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دراسة تحليلية لبعض المتغيرات في أنماط التغذية لدى النساء في فترة انقطاع الطمث

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الملخص باللغة العربية

مرحلة انقطاع الطمث أو ما يعرف خطأ بسن اليأس يعد من المراحل المهمة في حياة المرأة ، ويتفاوت العمر الزمني بين امرأة وأخرى والذي يحدث عادة بعمر (45-55سنة) ، وهي مرحلة انتقالية من سن الخصوبة إلى عدم القدرة على الإنجاب والسبب هو توقف المبيض عن إنتاج هرمون الأستروجين وتصل المرأة إلى هذه السن بصورة تدريجية ، حيث يحدث عدم انتظام في الدورة الشهرية في السنة التي تسبق تلك المرحلة وتحدث بصورة متقاربة ثم تتباعد كل شهرين أو أكثر أو تصل المرأة لها بصورة فجائية ، وتعاني المرأة في هذه المرحلة من أعراض وحالات مزعجة ، فضلا عن الدور المجهول للغذاء عند غالبية النساء وأهميته في تخفيف الأعراض إذا أحسن الاختيار ، من هنا برزت أهمية البحث والحاجة إليه في كيفية إيصال المرأة في هذا السن إلى وضع برنامج غذائي لسد ثغرات المنهاج السابق لغذائها وبما يحقق راحة لها وتخفيف من وطأة أعراض تلك المرحلة العمرية ، فوضعت فقرات استبانة أجيب عنها من قبل عينات البحث واللواتي بلغ مجموعهن 320إمرأة وزعن إلى مجاميع فئات عمرية تراوحت بين (47-54 سنة) قسمت إلى مجموعتين تجريبية وضابطة وبواقع عددي بلغ 160 امرأة للفئة العمرية 47-50سنة، عدد النساء في المجموعة الأولى:40سيدة متزوجة لكل فئة من الفئات (47، 48 ، 49، 50 سنة) وزعن على مجموعتين فرعيتين الأولى تجريبية بواقع 20 سيدة لكل فئة . والثانية ضابطة وبواقع 20 سيدة أيضا لكل فئة ونفس التقسيم أجري أيضا على الفئات العمرية (51-54سنة).
وتوصل البحث إلى النتائج الآتية :
أولا : الجانب المتعلق بأعراض انقطاع الطمث وجدت أنها تزداد بتقدم العمر وبلغت 50-67% لعدم القدرة على النوم أما أعراض التعرق الليلي بلغت 40-50% وأعراض ألم الجماع بلغت 20-53 %.
ثانيا : الجانب المتعلق باستعمال الهرمونات والأعشاب في تخفيف حالة الأعراض بلغت 1-15%.
ثالثا :تحليل المنهاج الغذائي اليومي وجد أنه يعاني نقصا في بعض المواد الغذائية والعناصر المعدنية .
رابعا : أعدت أربع برامج غذائية طبقت على عينات البحث لمدة أربعة أشهر،قومت وسدت الثغرات الموجودة في المنهاج الغذائي السابق، وبعد التطبيق العملـــي للبرامج الأربعة لوحظ تخفيف أعراض انقطاع الطمث تراوح مابين 21 يوم إلى شهر ونصف لجميع الفئات العمرية .
وبينت نتائج التحليل الإحصائي للمجموعتين التجريبية والضابطة للتطبيق القبلي والبعدي للبرامج الغذائية ما يأتي :
أ/ الفئات العمرية 47سنة –50سنة بلغت القيمة المحسوبة 7،86 والجدولية 2،24 وهذا يدل على وجود دلالة إحصائية وفروق معنوية لصالح المجموعة التجريبية .
ب/ الفئات العمرية 51سنة–54سنة بلغت القيمة المحسوبة 3،175 والقيمة الجدولية 1،99،اوهذا يدل على وجود دلالة إحصائية وفروق معنوية لـــصالح المجموعــة التجريبية .
خامسا:تحليل الفقرات الخاصة بنمط الحياة لعينات البحث وبصورة نسب مئوية كانت الآتي :
1-حصلت الفقرات الخاصة بالرغبة بممارسة الرياضة والحاجة إلى التقدير والنجاح بالحياة على نسبة100%.
2-حصلت الفقرة الخاصة بالنوم لأكثر من 8 ساعات يوميا على أدنى النسب وبلغت 60-75%.
وبناء عليه يجب الاهتمام بالوسائل التي من شأنها تقليل وتخفيف أعراض حالة انقطاع الطمث والتركيز على الثقافة الصحية والغذائية والعمل على دعم الحالة النفسية للمرأة في هذه المرحلة وخاصة الدور الأسري الكبير فضلا عن دور مؤسسات المجتمع وبشكل خاص المراكز التي تكون بتماس مباشر مع المرأة .

ABSTRACT

The stage of menopause is one of the important stage in women life . The chronological age varies between one woman and another and it is usually happened between (45-55year) . Is a transitional stage from the age of fertility to the inability to conceive . The reason is that the ovary stops producing the hormone estrogen and women up to that age gradually . Where there is irregularity in the menstrual cycle in the year preceding that stage and occur close and then diverge every two months or more, sometimes it happens suddenly to women. And the suffering of women in this stage of the symptoms and troublesome situations, , As well as the anonymous role of food when the majority of women and its importance in the relief of symptoms if they get the best choice. From here emerged the importance of research and the need for it and to reach those women at this age to develop a diet program to fill gaps in the previous curriculum to their diet and to achieve her comfort and alleviate the symptoms of those aged. thus , the research has Developed a questionnaire answered paragraphs reported by the research samples by certain women about 320 ones. Their age about (47-54 year) divided into two groups : experimental and control group. Scalar rate reached 160 women their age between (47- 54 year) included 40 married women for each group distributed to (20 ladies) for each group experimental and control group and the ,
Same division has been done on the group who are about (51-54 year)
The search found the following results:
1-   for menopause symptoms found that it increases with age and 50-67% of the inability to sleep, either introduce night sweats amounted to 40-50% and intercourse pain symptoms amounted to 20-53%.
2-   For the use of hormones and herbs to alleviate symptoms amounted to 1-15%.
3-   Analysis of the daily diet curriculum found that he was suffering a shortage of some food and metal items.
4-   this research has presented four programs and applied on the research samples for four months , After the practical application of the four programs observed relieve menopause symptoms ranged between 21 days to a month and a half for all age groups.
The results of the statistic analysis , for the experimental and control group of the application before and after the food system , show the following :
a/the group of 47-50 year reach to the counted value 7.86 and table value 2.24. this refers to the existence of a statistic sense and abstract differences for the experimental group.
b/ the group of 51- 54 year reach to the counted value 3. 175 and the table value 1.99 and this refers to the existence of a statistic value for the experimental group .
5-   Analysis of paragraphs on the lifestyle of research samples as percentages were the following:
1-the items that are related to the desire of doing sport and the need for respect and succeed in life , get 100%.
2-while the items related to sleeping more than 8 hours a day , gets the minimal averages which are 60- 75% .
Accordingly, attention must be given to means that will reduce and relieve the symptoms of menopause and focus on health education, food and work to support the mental status of women in this stage, and in particular the large role of domestic as well as the role of the institutions of society, particularly centers that are in contact directly with the women..

المقدمة

إن تقسيم الحياة إلى عدة مراحل حسب النمو تعد وسيلة جيدة وسهلة ، لعدة أغراض فيما يخص التغذية منها وإن هذا التقسيم يسمح بالاهتمام بالمتطلبات الغذائية لكل مرحلة من مراحل النمو . كما أن هذا التقسيم هو بحد ذاته تقسيم نوعي الغرض منه متابعة التغيرات في المتطلبات الغذائية والاختلافات الناجمة عن النمو . ومن ناحية الأهمية الصحية والعوامل المؤثرة في الصحة ، يمكن اعتبار دورة حياة الإنسان مستمرة وإن كل مرحلة فيها تعتمد على المرحلة السابقة لها وكل مرحلة تكون أساسا للمرحلة التالية .فالانتقال من مرحلة عمرية إلى مرحلة عمرية أخرى هي ليست عملية نمو فقط ولكن كثيرا ما يتعرض فيها الأفراد إلى خصائص وصعوبات جديدة تختلف عن المرحلة السابقة وتعتمد على المرحلة والشخص ، فهناك عدة عوامل تظهر تأثيرات كبيرة والتي ترافق تطور ونمو الشخص البالغ ، ويبدأ الأشخاص يشعرون بأن العادات الغذائية والصحية المتبعة تؤثر على حياتهم وذات تأثير على الشخصية سنوات العمر الوسطى والأخيرة ، كما إن التغيرات الفسيولوجية الناجمة عن كبر السن تقلل من استفادة الجسم من الغذاء ، إذ تنخفض الإفرازات الهضمية وإنتاج حامض الهيدروكلوريك ينخفض وهذا بدوره يؤثر في امتصاص الغذاء ومنها انخفاض امتصاص فيتامين C وB_{12} وعنصر الحديد ، وانخفاض إنتاج عوامل أخرى مهمة في الجسم قد ينتج عنها فقر الدم كما وأن امتصاص الكالسيوم يقل بتقدم العمر (1). إن هذا التحول يختلف حسب العمر والجنس، فالنساء مثلا التحول والتغير في أجسامهن حسب جسم كل امرأة والوقت الذي يستغرقه يختلف ما بين (2-8 سنوات) وهذه الحالة لا تحتاج إلى علاج , أو عناية طبية ولكن كل ما هنالك ينبغي أن تلم المرأة بما تستطيع أن تفعله لتفهم تلك الحالة وتتكيف معها بشكل طبيعي ، والبداية تكون بتصحيح الخطأ المتعارف بمرحلة انقطاع الطمث والمسماة سن اليأس , وهي المرحلة العمرية في حياة المرأة تتوقف فيها الدورة الشهرية وهي علامة لنهاية الإنجاب , وتعني كذلك تغيير نمط الحياة بالنسبة لمعظم النساء ، وخاصة بوجود أعراض شائعة تتمثل بالتعرق الليلي ، والإنهاك ، وسرعة الانفعال والآم المفاصل وغيرها ، وقد بينت العديد من الأدلة والدراسات العلمية على وجود علاقة بين التغذية المثلى وتخفيف تلك الأعراض وتقصير مدتها ، (2) .من هنا تبادر إلى ذهن الباحثة إجراء تجربة عملية لعينة من النساء في تلك المرحلة العمرية ووضع برنامج غذائي مثالي يخفف من تلك الأعراض بالاستعانة بالمصادر العلمية ذات العلاقة ، ومحاولة تقويم البرنامج الغذائي اليومي بالمكملات الغذائية والتعرف على الفرق الذي يحدثه البرنامج الغذائي الخاص بالبحث من حيث تخفيف حدة الأعراض والشعور بالراحة بعد التطبيق لمدة زمنية محددة .

المواد وطرائق العمل

تم اختيار عينة عشوائية انتقائية من النساء اللـواتي يعـانين من أعراض انقطاع الطمث وبفئات عمرية (47– 54 سنة) بواقـــع (160عينـــة للفئـــة العمريـــة 47–50 ســـنة) و (160 عينة للفئة العمرية 51–54 سنة) موزعة على أساس مجموعتين تجريبية وضابطة شملت الآتي :

1- عدد النساء في المجموعة الأولى : 40سيدة متزوجة من الفئات (47 ، 48 ، 49، 50 سنة) وزعن على

مجموعتين تجريبية وضابطة بواقع عددي 20 سيدة لكل فئة .

2- عدد النساء في المجموعة الثانية : 40 سيدة متزوجة من الفئات (51، 52 ،53، 54 سنة) وزعن علــى مجموعتين تجريبية وضابطة بواقع عددي 20 سيدة لكل فئة .

ومن جانبي الكرخ والرصافة من مدينة بغداد ومـن مـدن (البياع ، الجادرية ، زيونة ، المحمودية ، التاجي ، الغزالية ، الشعلة) ووزعت أستمارات أستبانة على العينات وتضمنت معلومات خاصة بالبحث شملت الآتي :

أولا : تحليل النظام الغذائي وتضمن الفقرات التالية والإجابة عنها بنعم أو لا :

1 – إضافة السكر إلى الطعام أو الشراب كل يوم .

2–استعمال الملح بالطعام .

3–شرب الشاي لأكثر من (3 أقداح) يوميا.

4–تناول الأعشاب الطبية .

5–تناول الطعام المقلي (البطاطس,أسماك ، دجاج) لأكثـر من ثلاث مرات بالأسبوع .

6–تناول اللحوم الحمراء لأكثر من مرتين بالأسبوع .

7–الطعام اليومي ثلثه من الفواكه والخضروات الطازجة.

8–تناول الماء لأكثر من (8 أقداح باليوم) .

9–تتناولين الرز والحبوب الكاملة .

10–تتناولين لتر من الحليب أسبوعيا .

ثانيا : تحليل نمط الحياة وتضمن الفقرات التاليـة والإجابـة عنها بنعم أو لا:

1–النوم لأكثر من (8 ساعات يوميا) .

2–النهوض من النوم بشوق للانطلاق للعمل خلال 20 دقيقة

3–شرب القهوة أو الشاي للانطلاق للعمل .

4–تجنب القيام بالتمارين الرياضية لعدم امتلاك الطاقة لذلك

5–الشعور بالنعاس أو الفتور بالهمة خلال النهـار أو بعـد تناول الطعام .

6–فقدان التركيز أحيانا وانخفاض الطاقة .

7–أهدافك بالحياة واضحة .

8–الحاجة إلى التقدير والنجاح من الأمور المهمة لديك .

ثالثا: الفقرات الخاصة بأعراض انقطـاع الطمـث والـذي ذكرته العينات وبالعودة للمصادر العلمية تم توضيح الـسبب وشملت :

أ/ الجانب المتعلق بالفيتامينات فكل عارض يمثل نقص فيه

وكما يأتي :

أولا: نقص فيتامين D :

1–التهاب المفاصل أو تخلخل العظام

2–ألم الظهر .

3–تسوس الأسنان .

4–فقدان الشعر .

5–تشنج العضلات .

ثانيا : نقص فيتامين E :

1–قلة النشاط الجنسي

2–التعب والإنهاك بعد العمل الخفيف .

3–تورم العروق .

ثالثا: نقص فيتامين C :

1–زكام متكرر .

2–نقص الطاقة .

3–التئام بطئ للجروح .

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4-بثور حمراء على الجلد .

رابعا: نقص فيتامين B6 :

1-وخز باليدين .

2-سرعة الانفعال .

3-ارتعاش وتشنج .

4-احتباس الماء في الجسم .

ب/ الجانب المتعلق بالمعادن فكل عارض يمثل نقصا فيه :

أولا: نقص الكالسيوم :

1-تشنجات واختلاجات بالعضلات .

2-الأرق .

3-ارتفاع ضغط الدم .

ثانيا :المغنيسيوم :

1-ضعف العضلات .

2-نبض سريع أو غير منتظم للقلب .

3-إمساك .

4-تشوش ذهني .

ثالثا: السلينيوم :

1-عدم التركيز بالنظر .

2-اضطراب بالضغط .

رابعا:الزنك :

1-هبوط بحاستي الذوق والشم .

2-علامات بيضاء في أظافر اليد .

3-التهابات متوالية .

4-بثور دهنية .

ج/ الجانب المتعلق بالأحماض الدهنية الأساسية :

1-بشرة جافة .

2-شعر جاف .

3-عطش وعرق زائد .

4-ضعف الذاكرة .

د/ سجلت أهم الأعراض التي تعاني منها السيدات . بعد الإجابة عن الاستمارات وتطبيق البرنامج الغذائي البحثي ، تم تحليل النتائج إحصائيا وذلك بالاعتماد على الاختبار التائي ومعامل ارتباط بيرسون ، وأجري تصميم تجريبي ذا مجموعتين متكافئتين ، وهو من التصاميم ذات الضبط المحكم ، وقد خصصت إحدى المجموعتين لتكون تجريبية والأخرى لتكون ضابطة واللتان كانتا متكافئتان إحصائيا في نتائج الاختبار القبلي وكما هو موضح في الجدول(1).

جدول (1): المتوسطات الحسابية والانحرافات المعيارية لمجموعتي البحث في الاختبار القبلي في مقياس الاستطلاع العملي

المجموعة	المتوسط	الانحراف المعياري
التجريبية	12،08	3،17
الضابطة	13،75	3،31

رابعا : تم إعداد برامج غذائية بوصفات تضمنت الوجبات اليومية والتي سدت نواقص النظام الغذائي اليومي لدى عينات البحث واستغرق العمل على تطبيقه مدة أربعة أشهر (وتم التركيز فيه على إدخال الحليب وجبن الشدر الغنيان بالكالسيوم، وزيت الذرة ، السمسم والسمك الغني بفيتامينE، الفطر والكبد الغنيان بفيتامين B والحديد، المعدنوس الغني بالكالسيوم، البزاليا الغنية بالمغنيسيوم ،الطماطم ،الفلفل ، القرنبيط الغني بفيتامين C، البيض الغني بالكولين ،

الملفوف الأخضر ، البصل ، الثوم الغني بعنصر الكبريت ، الحبوب ، البقول غني بالمغنيسيوم والزنك والموليبديوم، الصويا غني بالأيسوفلافونر ، مكملات طبيعية من الأعشاب) .

النتائج والمناقشة

إن التكافؤ الحاصل للبرنامج الغذائي المعد لعينات البحث المجموعة التجريبية لم يكن هناك فروقا معنوية قد ظهرت .أما نتائج الفرز لمعلومات الأستبانة وتفريغ محتواها على وفق الإجابات ، تم التوصل إلى الآتي:

أولا : أهم أعراض حالة انقطاع الطمث كانت مدرجة في الجدول (2):

من ملاحظة الجدول نجد أن علامات وأعراض انقطاع الطمث تزداد كلما تقدم العمر ، وبشكل خاص عدم القدرة على النوم وهشاشة العظام واللتان شكلتا أعلى النسب (67 % و 63%) على التوالي ، وبهذا الصدد أشارت المصادر العلمية العديدة على ذلك وهو ما يتوافق مع نتائج البحث الحالي ، ومنها الدراسة الكندية التي بينت أن نسبة الهبات الساخنة بلغت 75% (10)، إن الشعور بالهبات الساخنة يعد من أكثر الأعراض شيوعا ويصيب أكثر من 60% من النساء والذي يعبر عنه بكونه إحساس حراري مفاجئ في الجزء العلوي من الجسم أو الجسم كله ، وقد يعقب ظهور بقع حمراء في الصدر والساعدين ، وتستمر تلك الهبات لبضعة دقائق وأحيانا لأكثر من (30 دقيقة) ، ومع تقدم العمر تتناقص شدة أعراض الهبات الساخنة وهو ما يتوافق مع نتائج المصادر العلمية (4) والتي عزت سبب حصول تلك الحالة إلى انخفاض مستوى هرمون الأستروجين في الجسم واستجابة لذلك تبدأ الغدد الصماء بالجسم بإفراز كميات كبيرة من الهرمونات الأخرى أعلى من المستوى الطبيعي مؤثرة في مراكز التحكم الحراري بالدماغ فتكون النتيجة اضطراب في الميزان الحراري للجسم . (4)

هشاشة العظام هو العرض الثاني الذي تعاني منه النساء في هذه المرحلة وقد لوحظت زيادة نسبته مع تقدم العمر وبلغت (20 %-63%) وتفسير تلك الحالة يعود إلى وجود علاقة عكسية بين كثافة العظام والعمر ، حيث تقل الكثافة بتقدم العمر ، فتصبح عملية الهدم في العظم ضعف عملية البناء ، فتشعر المرأة بألم في العظام وخاصة منطقة الرسغ وفقرات الظهر ، وعظام الحوض التي تكون عرضة للكسر بسهولة ، وأوضحت المصادر العلمية أن المرأة النحيلة أكثر عرضة للإصابة بهشاشة العظام مقارنة بالمرأة البدينة (4،3).

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جدول (2): النسب المئوية للأعراض المرافقة لانقطاع الطمث للفئات العمرية(47-54سنة)

ألم عند الجماع	التعرق الليلي	عدم القدرة على النوم	هشاشة العظام	الهبات الساخنة	الفئة العمرية
20	40	50	20	50	47
25	35	60	50	50	48
35	40	60	50	46	49
45	45	65	60	44	50
45	45	66	60	44	51
50	50	66	62	34	52
51	52	66	63	33	53
53	55	67	63	33	54

العرض الثالث كان عدم القدرة على النوم والذي تذبذبت نسبه المئوية مابين (50%-67%) وله علاقة مع العرض الرابع ألا وهو التعرق الليلي والذي تفاوتت فيه النسب المئوية متراوحة مابين (40%-55%) حيث تشعر المرأة بتعرق غزير ثم قشعريرة باردة ، وعندما يتكيف الجسم من جديد لدرجات الحرارة تتناقص شدة هذه الأعراض التي قد تكون خفيفة أو شديدة ، عند بعض النساء لدرجة تضايق المرأة لعدم قدرتها على النوم والأرق (5) كذلك يحدث تغير في مزاج المرأة و يعزى سببها إلى التغيرات الحاصلة في الهرمونات الجسمية . وتتراوح المدة الزمنية التي تتعرق فيها المرأة عدة دقائق ولعدة مرات باليوم ، وقد تصبح مرهقة ومتقلبة المزاج والميل إلى الاكتئاب ، وهو نتيجة التفاوت الحاصل في مستوى الهرمونات وتغيرها في هذه المرحلة (6). (20%-53%) هي النسبة المئوية لحالة أو عرض الشعور بألم عند الجماع وتفسير ذلك على وفق ما أوضحته المصادر العلمية (5،6) إن الغشاء المبطن للمهبل يصبح أكثر جفافا وأقل سمكا ، وأقل مرونة وأكثر عرضة للإصابة بالعدوى وتلك التغيرات تجعل عملية الجماع غير مريحة ومؤلمة أحيانا مما يؤدي إلى قلة جريان الدم في الأعضاء الجنسية والشعور بالألم .

ومن خلال تحليل نتائج الإجابة عن المعلومات التي تمتلكها النساء عن طرق علاج أعراض انقطاع الطمث بالهرمونات أو الطب البديل والأعشاب كانت النسب المئوية الموضحة بالجدول (3).

يبين الجدول من خلال ملاحظة واستعراض النسب المئوية والتي تمثل مدى ثقافة النساء بموضوع استعمال الهرمونات كعلاج في تخفيف أعراض الحالة نجد أنها تراوحت ما بين (1%-15%) وهي نسب متدنية وسبب ذلك والذي أبدته سيدات عينة البحث من خلال المناقشة هو عدم التعرف على مزايا وفوائد تلك الهرمونات في علاج الحالة التي يعانين منها ، فضلا عن قلة الضوء المسلط عليها ككيفية علاج ومزايا ، والذي كان المفترض أن تتولى تلك المهمة النشرات الصحية والتثقيفية في مراكز الرعاية الصحية الأولية ، والنتيجة بهذا المجال لا تتوافق مع استنتاجات المصدر العلمي (7) والذي وصلت نسبته المئوية 91% . أما علاج وتخفيف حالات أعراض انقطاع الطمث باستعمال الطب البديل والأعشاب واستعمال اللاصق الهرموني والمكملات والوخز بالإبر، فقد تراوحت النسب ما بين(1%-15%) وهي نسب واطئة وتعليل ذلك قلة الثقافة الصحية والغذائية حول هذه المرحلة العمرية وقلة الاهتمام بكيفية استعمال الأعشاب التي تخفف من أعراض الحالة وكذلك دور المكملات الفيتامينات والمعادن في سد النقص

الحاصل فيهما وسد الثغرة في منهاج الغذاء اليومي للنساء والذي تبين أنه منهاج منقوص المحتوى من الفيتامينات والمعادن الضرورية فضلا عن قلة الزيوت المفيدة كزيت الذرة ، وكذلك نقص العناصر المعدنية المهمة كالكالسيوم ، الزنك ، المغنيسيوم ، السلينيوم ، وقلة استعمال الصويا والمكسرات ، وتم تمثيل النتائج التي جمعت من العينة بالجدول 4: الذي يبين النسب المئوية للعناصر الغذائية المتناولة من قبل الفئات العمرية (47-54سنة).

جدول (3): النسب المئوية لمدى معرفة النساء بعلاج أعراض انقطاع الطمث بالهرمونات والطب البديل والأعشاب

الفئة العمرية	أنواع الهرمونات والأعشاب ونسبها المئوية %							
	أقراص هرمون الأستروجين والبروجسترون	أقراص هرمون الأستروجين الدوري	أقراص liviallorcibolone	هرمون لاصق تحت الجلد	هرمون كريمي أو موضعي بشكل تحاميل	الأعشاب	استعمال الإبر	استعمال مكملات الفيتامينات
47–50	10	–	–	–	–	15	5	1
51–54	10	3	4	1	–	2	5	1

جدول (4): النسب المئوية للعناصر الغذائية المتناولة وحسب الفئات العمرية (47–54سنة)

العناصر الغذائية	النسب المئوية للعناصر الغذائية المتناولة وحسب الفئات العمرية %							
إضافة السكر	100	100	100	100	100	100	100	100
استعمال الملح	100	100	90	80	80	80	70	60
شرب الشاي لأكثر من 3 أقداح يوميا	100	100	100	100	100	100	100	100
تناول الطعام المقلي لأكثر من 3 مرات	100	100	100	100	100	98	99	99
تناول اللحوم الحمراء لأكثر من مرتين أسبوعيا	100	100	90	90	80	90	90	90
ثلث الطعام اليومي من فواكه وخضروات طازجة	80	80	80	60	67	77	87	70
تناول الحليب بمعدل لتر أسبوعيا	7	5	5	4	4	3	4	3
تناول الصويا	–	–	–	–	–	–	–	–
تناول المكسرات وزيت الذرة	13	13	5	5	4	3	3	3

من خلال ملاحظة النسب المئوية في الجدول وخاصة الفقرات المتعلقة ب:(إضافة السكر ، الملح ، شرب الشاي بمعدل ثلاث أقداح وأكثر يوميا ، تناول اللحوم الحمراء بكثرة ، تناول الأطعمة المقلية بكثرة ، تناول الرز والحبوب) نجدها مرتفعة إذ تباينت ما بين (80% – 100%) , وبهذا الصدد بينت المصادر العلمية أن النظام الغذائي المرتفع المحتوى بالملح والسكر والدهون وقلة الألياف المتناولة والتي توفرها الفواكه والخضروات والتي كانت نسبها المئوية مابين (60% – 80%) وهذا بدوره يساعد باستمرار أعراض الحالة المزعجة لانقطاع الطمث ، وفسرت كذلك علاقة الدهن المرتفع بالطعام اليومي بزيادة الإصابة بأمراض القلب لأن هرمون الأستروجين الذي تقل نسبته في هذه المرحلة يؤثر في تمثيل الدهون وبالتالي في تصلب شرايين القلب (5،7) كما لوحظ أن النظام الغذائي منخفض في محتواه من الفواكه والخضروات الطازجة ، وكذلك الحليب والحبوب ، وتشير البحوث العلمية أن الهرمونات تتحكم بتوازن الكالسيوم في الجسم ، كما أن تناول الكثير من الشاي أمر جيد لاحتوائه على مضادات أكسدة ألا أن المهم في الأمر هو النوع وليس الكم فمعظم أنواعه عادية والقلة منه الشاي الأخضر ، وهذا يخل في ضبط الكالسيوم والذي يكون أسوأ بعد انقطاع الطمث والذي يعزى إلى فقدان الأستروجين ، وبشكل عام الملاحظة المهمة على المنهاج اليومي للعينات هو فقره الكبير بالعديد

من العناصر الضرورية لهذه المرحلة إذ أن النظام الغذائي ووضعية الهرمونات بالجسم لها علاقة بالتهاب المفاصل وهشاشة العظام ، فالطعام الغني بالبروتين هو مكون للأحماض ، والجسد لا يستطيع تحمل التغيرات الكبيرة في مستوى الحامض بالدم ، وهو يؤثر من خلال عاملين هما : الصوديوم والكالسيوم ، فعندما يستنفذ احتياطي الجسم من الصوديوم يأخذ الكالسيوم من العظام لذلك ، كلما كثر تناول الأطعمة البروتينيه كلما زادت الحاجة للكالسيوم وهو أمر مفقود نتيجة قلة تناول الحليب الغني بالكالسيوم وفيتامين D والذي يقلل نسبة التعرض للكسر وبمقدار 22% (5) . وهنالك عنصر غذائي مهم ولكن مستواه ضئيل ومعدوم في المنهاج اليومي ، وهو فول الصويا والذي أوضحت المصادر العلمية غناه بمادة تركيبها مشابه لهرمون الأستروجين والذي يساهم في التقليل من أعراض انقطاع الطمث ، وكما ذكرت تلك المصادر أن وجود الكمية الكافية من بروتين الصويا في الغذاء أو الأعشاب يساهم في التقليل من كوليسترول الدم وبالتالي قلة الإصابة بتصلب الشرايين وتحسين وظائفها وهو ما بينته الدراسات العلمية الأمريكية (8).

ونتيجة لذلك وبعدما وجدت الباحثة افتقار المنهج الغذائي اليومي لعينات البحث من العناصر الغذائية الضرورية في تخفيف أعراض حالة انقطاع الطمث، وبالاستعانة بالمصادر العلمية (5) تم إعداد برنامج غذائي غني بعناصر (فيتامين E ، B ، C ، D) وبالمعادن (الكالسيوم Ca،المغنيسيوم Mg، الزنك Zn، السلينيوم Se)

البرنامج الأول/ تضمن التركيز على فيتامين E، B + معدني المغنيسيوم والزنك + زيت الذرة والشمس

وتضمن الأطعمة الآتية :(فيتامين E متوفر في الخضروات الورقية الداكنة ، الكبد ، البيض ،السمك / فيتامين B متوفر في الحبوب الكاملة ، الخميرة ، اللحم ، البطاطس ، السلق /المغنيسيوم والزنك متوفران في البقول وفول الصويا والبيض، اللحوم والأغذية البحرية) .

البرنامج الثاني / تضمن التركيز على فيتامين E+ الكالسيوم + المغنيسيوم + فيتامين D+ أعشاب الزنجبيل +فيتامين C.

والأطعمة التي تغطي هذه العناصر هي (فيتامين E موجود فيما سبق / الكالسيوم يتوفر في الحليب ومنتجات الألبان وكمية معقولة في الخضروات الورقية الخضراء /

البرنامج الثالث / تضمن التركيز على فول الصويا + الحمص + الينسون + عرق السوس + الحلبة

البرنامج الرابع / تضمن التركيز على استخدام الثوم كمكمل + زيت الزيتون + زيت السمسم + مسحوق بذور دوار الشمس . وتم التطبيق العملي من خلال التركيز على اختيار الأطعمة الغنية بالعناصر المذكورة أعلاه وكما يأتي :

قسمت نساء عينة البحث البالغ عددها (160 امرأة للفئات العمرية /47-50سنة وبواقع 40سيدة لكل فئة والتي بدورها قسمت إلى مجموعتين تجريبية وأخرى ضابطة وبواقع عددي بلغ 20 سيدة لكل مجموعة الشيء ذاته طبق على الفئات العمرية 51-54 سنة) استغرق التطبيق العملي للبرامج الغذائية السابقة مدة أربعة أشهر وذلك من خلال المقابلة الشخصية للعينة وتوزيع البرامج عليها ، وتسجيل الملاحظات الخاصة بحدة أو شدة الأعراض ومدى التغير الذي طرأ عند تطبيق البرامج الغذائية المعدة ،والجدول 5: يبين الفرق الحاصل بين المجاميع التجريبية والضابطة خففت الأعراض بعد تنفيذ البرامج الغذائية وكما يأتي (البرنامج الأول خفض أعراض الحالة بعد تطبيقه لمدة شهر للفئة العمرية 47/ وشهر وعشرة أيام للفئتين 48 – 49/ وشهر و15يوم للفئتين 50-54).

البرنامج الرابع خفض الأعراض بعد تطبيقه لمدة شهر ويومان لكل الفئات) ،والأجراء الإحصائي يبين النتائج في الجدول (5).

جدول (5): نتائج الاختبار لتطبيق البرامج الغذائية بين المجموعتين التجريبية والضابطة للفئات العمرية 47 – 50سنة

| القيمة الثابتة | | الانحراف المعياري | متوسطات الفروق من الاختبارين القبلي والبعدي | العدد | المجموعة |
الجدولية	محسوبة				
		8،54	15،67	40	التجريبية
2،24	7،86	0،07	1،47	40	الضابطة

يتضح من الجدول أن متوسطات الفروق في الاختبارين القبلي والبعدي للمجموعة التجريبية هي أعلى من متوسط الفروق في الاختبارين القبلي والبعدي للمجموعة الضابطة وكانت القيمة الثابتة أعلى من الجدولية مما يدل على وجود فروق ذات دلالة لمصلحة المجموعة التجريبية .

ونفس التفسير ينطبق على الفئات 51-54 في نتائج اختبار تطبيق البرامج الغذائية وكما يبينها الجدول (6).

جدول (6): نتائج الاختبار لتطبيق البرامج الغذائية المجموعتين التجريبية والضابطة للفئات العمرية 51-54 سنة

المجوعة	العدد	متوسطات الفروق من الاختبارين القبلي والبعدي	الانحراف المعياري	القيمة الثابتة	
				المحسوبة	الجدولية
التجريبية	40	78،476	12،324		
الضابطة	40	67،324	12،461	3،175	1،99

من جدول (6)، أظهرت النتائج وجود فروق دالة إحصائيا بين متوسط درجات المجموعة التجريبية والضابطة لصالح المجموعة التجريبية ، إذ كانت القيمة الثابتة المحسوبة أعلى من القيمة الجدولية .

ثانيا : تحليل نمط الحياة والذي شمل فقرات تتم الإجابة عنها بنعم أولا ، ومثلت نسبها المئوية بالجدول (7) حيث كانت أعلى النسب للفقرات الخاصة برغبة النساء بممارسة الرياضة ألا أن المانع هو عدم توفر الظروف الموائمة لذلك , وكذلك الحاجة إلى التقدير والنجاح واللتان حصلتا على نسبة 100% ، أما أدنى النسب فقد مثلتها فقرات النوم لمدة 8 ساعات يوميا والتي تذبذبت بين(60-75) فضلا عن التذبذب بنسب الإجابة عن الفقرات الأخرى , إن الرغبة في ممارسة الرياضة والتي أبدتها نساء العينة في البحث ، تعد من الأمور المفيدة التي ينبغي تنميتها ، لكن الشيء الذي يحول دون تحقيق هذا الهدف هو عدم توفر المكان المناسب ، وكما بينت المصادر العلمية أن للرياضة فائدة كبيرة للنساء لاسيما في هذه المرحلة العمرية خاصة وأنها تؤدي إلى حفظ العظام والعضلات قوية ، ومساعدة التمارين الرياضية في حماية القلب من الأمراض التي قد تصيبه (5، 8) .

كما أن ممارسة الرياضة بانتظام يرتبط بانخفاض ضغط الدم والكوليسترول ، حيث تسهم الرياضة المنتظمة في التقليل من الكآبة وتحسن حالة النوم ، وأبسط أنواع الرياضة المشي لمدة ساعة تقلل من أمراض القلب إلى النصف ، ومن العوامل ذات العلاقة بأعراض الحالة وإنخفاضها الحالة النفسية ، حيث تميل النساء إلى الشعور بحاجتها إلى التقدير والنجاح والتي تخفف من الشد العصبي الذي له علاقة بتخفيف أعراض الحالة ، والدراسات المتعلقة بهذا الجانب أوضحت أن عدم القدرة على النوم والأرق الذي يضايق المرأة يكون سببه عوامل نفسية في الكثير من الأحيان ، فضلا عن العوامل الغذائية والذي يؤدي إلى نسبة إرتباط تصل إلى 80% وقد وجد أيضا أن ممارسة رياضة أيروبيك لمدة 20 دقيقة يوميا ولمدة 3– 5 أيام أسبوعيا تحقق أثارا أيجابية لها علاقة بتقليل الأعراض بشكل عام بمرور الوقت ، كما أن ممارسة أنواع أخرى من الرياضة يحقق الهدف ذاته (10،11)أن نتائج تحليل النمط اليومي لحياة المرأة يشير الى ضرورة زيادة الفعالية الفيزياوية والتي هي مفتاح السيطرة على الوزن والذي وجد أن الكثير من النساء تعاني زيادة فيه .

جدول (7): النسب المئوية لفقرات تحليل نمط الحياة اليومي للفئات العمرية 47-54سنة

الفقرات	النسب المئوية %لإجابات الفئات العمرية بنعم							
النوم لأكثر من 8ساعات يوميا	75	70	70	66	67	60	61	60
النهوض من النوم بشوق للانطلاق للعمل خلال 20 دقيقة	75	77	79	78	73	76	77	73
شرب الشاي قبل العمل	95	98	99	97	99	98	99	98
الشعور بالنعاس والفتور بالهمة بعد تناول الطعام	90	90	94	93	94	95	96	95
الإصابة بالدوار والانفعال عند عدم تناول الطعام خلال 6 ساعات	90	93	93	92	90	96	94	98
فقدان التركيز أحيانا	88	89	90	90	89	90	94	97
تجنب القيام بالتمارين لعدم امتلاك الطاقة	78	80	90	88	80	85	86	88
الرغبة بممارسة الرياضة لكن الظروف لا تسمح بذلك	100	100	100	100	100	100	100	100
أهدافك بالحياة واضحة	99	99	100	100	99	100	100	100
الحاجة إلى التقدير والنجاح مهمة	100	100	100	100	100	100	100	100

الاستنتاجات

أولا : الجانب المتعلق بأعراض انقطاع الطمث وجدت أنها تزداد بتقدم العمر وبلغت 50-67% لعدم القدرة على النوم أما أعراض التعرق الليلي بلغت 40-50% وأعراض ألـــم الجماع بلغت 20-53 %

ثانيا : الجانب المتعلق باستعمال الهرمونات والأعشاب فـي تخفيف حالة الأعراض بلغت 1-15%

ثالثا :تحليل المنهاج الغذائي اليومي وجد أنه يعـاني نقـصا في ما يأتي :

أ-اسـتعمال الزيـوت المفيـدة كزيـت الـذرة والـصويا والمكسرات

ب-الكالسيوم ، المغنيسيوم ، الزنك ، السيلينيوم

ج-استعمال السكر والملح بكثرة

د-كثرة تناول الشاي الأعتيادي

هـ-كثرة تناول اللحوم الحمراء والطعام المقلي والرز

رابعا : أعدت أربع برامج غذائية طبقت على عينات البحث لمدة أربعة أشهر ،قومت وسدت الثغـرات الموجـودة فـي المنهاج الغذائي السابق وكما موضح أدناه :

1-البرنــامج الأول اختيــرت أطعمــة غنيــة بفيتــامين (E،B،C،D) والمعادن (Ca الكالسيوم ، Mg المغنيسيوم ، Zn الزنك ، Se سلينيوم) وبعد التطبيق العملي لــه حقق النتائج الآتية في تخفيف أعراض انقطاع الطمث :

1-الفئة العمرية 47سنة بمدة شهر .

2-الفئات العمرية 48سنة – 49 سنة بمدة شهر وعشرة أيام

3-الفئات العمرية 50-54سنة بمدة شهر و 15يوم .

2-البرنامج الثاني أختيرت أطعمة غنية بفيتــامين(E ، D، C) ومعادن (Ca الكالسيوم ، Mg المغنيسيوم)+ الزنجبيل وبعد التطبيق العملي له حقق النتائج الآتية في تخفيف أعراض الطمث :

1-الفئة العمرية 47سنة 3 أسابيع .

2-الفئات العمرية 48سنة –49سنة شهر .

3-الفئات العمرية 50سنة –54سنة شهر وخمسة أيام .

3-البرنامج الثالث اختيرت أطعمة غنية بالصويا والحمص وعرق السوس والينسون والحلبة وبعد التطبيق العملي له حقق النتائج الآتية في تخفيف أعراض انقطاع الطمث :

1-الفئات العمرية 47سنة-51سنة بمدة 3 أسابيع .

2-الفئات العمرية 52سنة54سنة بمدة شهر .

4-البرنامج الرابع اختيرت أطعمة الثوم + زيت الزيتون + زيت السمسم + مسحوق بذور الشمس .وبعد التطبيق العملي له حقق النتائج الآتية :

1-جميع الفئات العمرية 47سنة-54سنة بمدة شهر ويومان .

وبينت نتائج التحليل الإحصائي للمجموعتين التجريبية والضابطة للتطبيق القبلي والبعدي للبرامج الغذائية ما يأتي :

أ/ الفئات العمرية 47سنة –50سنة بلغت القيمة المحسوبة 7،86 والجدولية   2،24 وهذا يدل على وجود دلالة إحصائية وفروق معنوية لصالح المجموعة التجريبية .

ب/ الفئات العمرية 51سنة-54سنة بلغت القيمة المحسوبة 3،175 والقيمة الجدولية 1،99واهذا يدل على وجود دلالة إحصائية وفروق معنوية لصالح المجموعة التجريبية .

خامسا:تحليل الفقرات الخاصة بنمط الحياة لعينات البحث وبصورة نسب مئوية كانت كالأتي:

1-حصلت الفقرات الخاصة بالرغبة بممارسة الرياضة والحاجة إلى التقدير والنجاح بالحياة على نسبة100%.

2-حصلت الفقرة الخاصة بالنوم لأكثر من 8 ساعات يوميا على أدنى النسب وبلغت 60-75%.

التوصيات

1-الإكثار من تناول السمك لغناه بالأحماض الدهنية أوميغا- 3

2-الإكثار من تناول الصويا لأنها من المصادر الغنية بالمركبات المشابهة للأستروجين والذي يمنع هشاشة العظام والتخفيف من أعراض انقطاع الطمث .

3-التأكيد على وسائل التثقيف الخاصة في مراكز الرعاية الصحية في تسليط الضوء على تلك المرحلة المهمة وكيفية العمل الطبي لتخفيف أعراضها .

4–التأكيد على دور المنظمات المعنية بالمرأة في أن يكون لها دورا في توفير أماكن أو قاعات لممارسة الرياضة في أماكن العمل أو المحلة وبذلك تتاح فرصة لممارسة الرياضة في أماكن مصممة بشكل جيد .

5–العناية الصحية والغذائية وإعداد برامج ثقافية في وسائل الأعلام عن خصائص المرحلة وأهميتها .

6– إجراء دراسات مماثلة للنساء الغير متزوجات وإجراء مقارنة بين أعراض الحالة مع النساء المتزوجات.

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