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FORWARD

It is my pleasure to welcome you back and present you a new issue of our Journal, Volume 6, No. 3 (2011), the third 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.

We are so honored to have all the new editorial board members being joined us in 2011, to strengthen our efforts, raising the prestigious level of the journal, and share in pushing all steps toward shining scientific future in Arab World.

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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تشخيص المبيدات العضوية المكلورة في حليب الأمهات وتأثيراتها الصحية

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ENGLISH SECTION

Clinico-epidemiological trends of Visceral *Leishmaniasis* in Diyala Governorate during the period 2009-2010

Falah M. Al- Zubaidi (1),Nadhim Gh.Noaman(2)& Mehdi Sh. Al-Zuheiry (3)

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ABSTRACT

This study had been designated to evaluate the size of this public health problem in Diyala governorate and to study the clinical and epidemiological aspects of this problem. Visceral leishmaniasis (kalazar) is a disease caused by intracellular protozoa related to Leishmania affected children under five years, endemic in countries of Eastern Mediterranean Region, jackals and foxes were considered as reservoirs, sand fly act as a vector. The patients present with different symptoms and signs. The study was descriptive cross sectional study done during the period between first of October 2009 to the thirteenth of September 2010, in Al- Batul teaching hospital for maternity and pediatric in Baquba city- Diyala. The data was collected by direct interviews by researchers with the patients using special questionnaire form, in addition to the results of examination and investigations. number of patients was 243 including (49%) below one year, (34%) below two years, (52%) males and (48%) females. The patients were presented with fever for less than 15 days (58%), splenomegaly& hepatomegaly (93%), sweating (40%), bleeding (14%), chest infection (35%), diarrhea (53%) and vomiting (79%). The patients also presented with anemia (96.5%) and those who need blood transfusion were (47%), the death rate was (2%) only. The number of the patients was more in the months November and December than other months of the year and higher during the year 2009- 2010 than the previous years.
Study concluded that Visceral leishmaniasis still a major public health problem affecting mostly children under five years presented with different signs and symptoms, blood transfusion was needed in about half of the patients. Active case detection and regular surveillance by screening is needed with health education and improving the environment is important in Diyala.

Key words: Clinico- epidemiological, Visceral leishmaniasis

INTRODUCTION

Visceral leishmaniasis (Kala-azar)is a chronic systemic disease caused by an intracellular protozoa related to *Leishmania donovani* including *L. donovani* and *L. infantum*in (1), (2), (3) The disease typically affects children under five years of age in the New World *(L. chagasi)* and Mediterranean Region *(L. infantum)* and older children and young adults in Africa and Asia *(L. donovani)* (4)

The causative agent of visceral leishmaniasis (VL) in the majority of endemic countries of the Eastern Mediterranean region is *L. infantum*.(5) However, *L. donovani* is responsible for Kala azar cases in Iraq, Sudan, and some other countries (6).

In some countries wild canines(jackals and foxes) are considered to be reservoir of VL. Several species of sand fly are incriminated as vectors. *Phleptominpapatsi* more species of sand flies are coming under suspicion as potential vector in Iraq (7). Symptoms of VL usually have a gradual onset in several months after infection and include weakness, dizziness, weight loss, diarrhea, and constipation, Fever, which almost always develops, may spike twice daily and is sometimes accompanied by chills and sweating,as the disease progresses, the liver and spleen get enlarged (8) (9).

The re-emergence of leishmaniasis in some foci may be the result of interruption of previously applied methods of control, e.g.insectside spraying or early diagnosis and treatment of positive cases.It's believed that reduction in insecticides spraying for malaria control contributes to the increase in the population of synanthropic sand flies and result in the out break of the disease in some endemic foci in cutaneous and VL (10) (11)

The objectives of this study are identification of the trend of VL, investigation of the relationship between VL and certain individual variable and to throw the light on the disease as a public health problem of children in Diyala governorate.

PATIENTS AND METHODS

This cross-sectional study was conducted in Al-Batul Teaching Hospital for Maternity and Pediatrics in Baquba city (center of Diyala Governorate).

Diyala Governorate is one of Iraqi Governorates, 1.900.000 inhabitants, 60 kilometers Northeastern of Baghdad City, the capital of Iraq, which consists of five administrative sectors, Baquba, Al-Kalis, Moqdadia, Beladroze and Kanakeen.

All cases of VL admitted to the hospital, for the period from 1st October 2009 to the 30th of September 2010 were involved in well designed structured quastionnare including name, age, sex, residety & certain clinical symptom and signs (including fever, spleenomegally, hepatomegally, sweating, bleeding, diarreaha, chest infection, vomiting, lose of appetite, hemoglobin level and others). All cases were examined clinically by the researchers.

Dignosis was done by clinical findings, IFAT test, and bone marrow aspirate, with the help of specialist, the hemoglobin level was done for the patients and the need for blood transfusion was enquired. The reported cases of VL for the years 2004-2008 were collected from the records of the related units in Public Health Departement in Diayla General Health Center and were studied with its distribution among Diyala districts.

Data were presented in numbers and percentages.

RESULTS

The total number of the patients involved in this study was 243, 126 males 52% & 117 females 48%. Table (1) shows the distribution of patients according to the age group. The most affected age is in one year and below 49.5% followed by two years 34% and it was higher than other age groups and the lowest is five years.

Table (1): The distribution of patients with VL according to their ages

Age in years	No.	%
<=1	120	49.5
2	83	34.0
3	21	9.0
4	11	4.5
>= 5	8	3.0
Total	243	100

Table (2) shows the distribution according to gender, 52% and 48% in male and female respectively. Regarding clinical presentation of the patients, fever in the first 15 days was reported by 58% while in 42% of them the fever appeared more than 15 days.

Table (2): The distribution of patients with VL according to their gender.

Gender	No.	%
Male	126	52
Female	117	48
Total	243	100

The patients presented with splenomegaly and hepatomegaly were 93% and those presented with sweating were 40%, bleeding 34%, diarrhea 53%, chest infection 35%, vomiting 79% and loss of appetite in 55%.Table (3).

Table (3) : The Clinical presentation of patients with VL.

Clinical Features	No.	%
Fever 1-14 days >15 days	140 103	58 42
Splenomegaly	225	93
Hepatomegaly	225	93
Sweating	96	40
Bleeding	34	14
Diarrhea with Chest infection	33	14
Chest Infections	86	35
Diarrhea	129	53
Vomiting	193	79
Loss of appetite	130	55

The majority of patients presented with hemoglobin level below 10 mg/dl 96.5% Table (4) and 47% of them received blood transfusion.Table (5).

Table (4) : The distribution of patients according to Hemoglobin level.

Hemoglobin level (mg/dl)	**No**	**%**
> 10	**8**	**3.5**
< 10	**235**	**96.5**
Total	**243**	**100**

Table (5): The distribution of patients according to blood transfusion.

Blood transfusion	No	%
Yes	115	47
No	128	53
Total	243	100

Five patients were died (2%) from the total diagnosed cases with VL. Table (6). Figure (1) shows the distribution of patients according to the months of the year in which more cases were reported in November and December. Figure (2) shows the distribution of patients according to the districts of Diyala governorate which is more in Baquba than other districts.

Table (6): The death rate among VL patients

	No	%
Alive	238	98%
Dead	5	2%
Total	243	100%

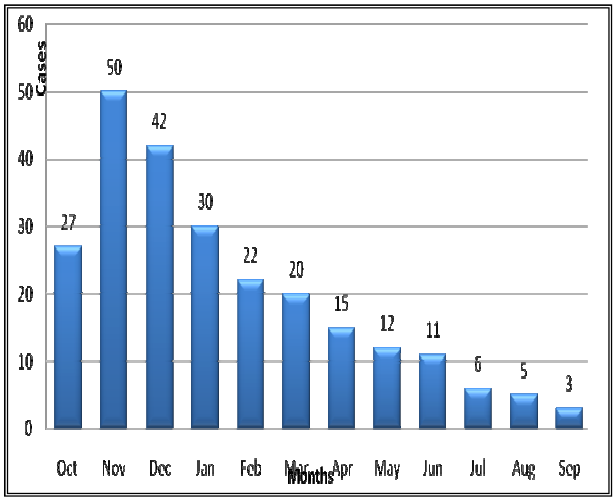

Figure (1): The distribution of patients with VL according to the months from October 2009–September 2010

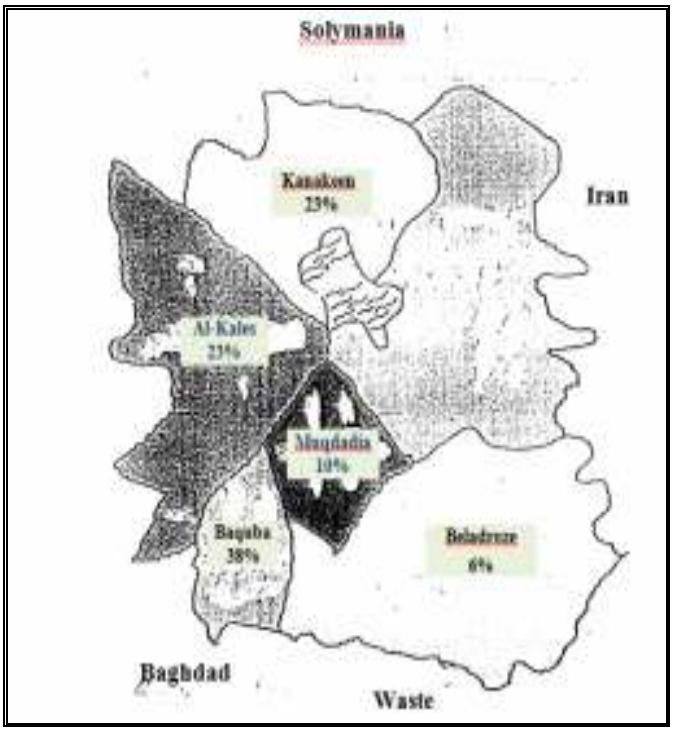

Figure (2) : The distribution of patients with VL among districts of Diyala governorate for the year 2009–2010.

The distribution of patients for the years 2004, 2005, 2006, 2007 and 2008 is shown in figure (3)

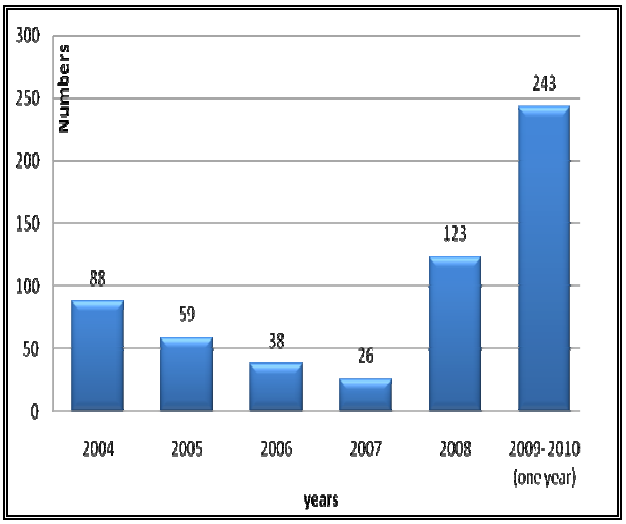

Figure (3): The distribution of patients with VL for the years 2004-2008 and (2009-2010) as one year in Diyala governorate

DISCUSSION

In the present study, children under five years of age are the susceptible risk group for the disease, children under two years of age in the study sample presented higher frequency rate. It is worth to mention that there are reported cases above five years of age (7).

Individuals with prior active disease or subclinical infection are usually immune to a subsequent clinical infection. The reported cases over 5 years may be due to unexposure of those children previously to active diseases or subclinical infection, or VL may be due to other species rather than *L. infantum* (may be *L. donovani*) that affect older children and young adults (13), (14)

There is similar number of cases in males and females. Most patients develop the insidious onset of fever most of them presented with fever of less than 15 days duration, although many of them presented with fever of more than 15 days duration which may be due to delay in seeking of medical care or may be due to deficient of diagnostic laboratories, procedures and facilities. Malaise, weight loss associated with splenomegaly, hepatomegaly, anemia, leukopenia and thrombocytopenia which lead to different type of infections and bleeding (2), (4). Also high number of patients need blood transfusion because of severe anemia (47%) and others need more than once blood transfusion. Anemia was obvious among most cases with VL although most patients had hemoglobin level less than 10 gm/dl, most common type was iron deficiency anemia,some cases had hemolytic anemia and other had mixed type of anemia (9), also some patient due to blood loss (bleeding tendency) due to marrow involvement and other due hepatic involvement, these contributed to delay in diagnosis of the diseased cases, malnutrition status of most cases, associated complication of the disease such as diarrhea and chest infection, bone marrow involvement**.**

The death rate was low (2%) among the total numberof VL (5 deaths among 243 case). The causes of death are most probably due to infections that lead to septicemia, and hepatic failure due to parasitic invasion.

The incubation period of Kala-azar ranging between 10 days–years average 2-4 months, so when the bite of sand fly occur in September, the symptom will be obvious in December, but the patient might not ask for health care during early days or weeks of theillness (15), (16), also the cases of VL were seen in all months of the year which may contributed to the changes in the climate temperature, which affect period from October 2009 to September 2010. In Diyala Governorate, the cases were more in Baquba district which is the center of Diyala governorate and this probably due to high density of population than other districts.

The lack of diagnostic facilities and drugs for treatment have been among the possible contribution factors to the epidemic of Kala-azar in Iraq after the last war (2003), the number of patients for the years 2004, 2005, 2006, 2007 and 2008 shown in the different districts means that Diyala governorate still facing this disease and the increase in the number of patients for the years 2009- 2010 is probably due to the emergence of the leishmaniasis in new focuses is the result of movement of a susceptible population into existing endemic areas, usually because of agricultural or industrial development or timber harvesting; increase in vector and/or reservoir populations as a result of agriculture development projects; increase in anthroponotic transmission owing to rapid urbanization in some focuses; and increase in sand fly density resulting from a reduction in malaria vector control programs (24), (25)

The outbreak of Leishmaniasis in existing foci may be linked to the

existence of concomitant malnutrition and other infectious diseases resulting in the aggravation of asymptomatic subclinical forms of the disease (26).

Because of poverty and difficulties in the communication between the different medical offices and the residents , low population educational level,the difficult security position, and the loss of the confidence between the population and the different level of the medical staff, absent of the medical services regarding active surveillance for the disease cases either due to poor case report or due to deficient of Lab. Facilities also the long period of the therapy, difficult in administration and monitoring all these factors make the diseases control and eradication is difficult.

In conclusion, VL still a major public health problem in Diyala governorate which affects children below five years of age and present with different signs and symptoms and need hospital care which may need blood transfusion with mortality rate about (2%) which need more attention to caught the diagnosis and treat the disease early.

It is recommended that health education of the population of Diyala governorate is of importance with improving the environment in this endemic foci with the use of advanced serodiagnostic procedures for diagnosis of VL (Enzyme linked immunosorbent assay “ElISA”, direct agglutination test “DAT” or the polymerase chain reaction “PCR” technique) for early recognition and treatment of cases. Isoenzyme studies of parasite isolates from the bone marrow is needed to confirm the types of VL species and encouraging active case detection by regular surveillance and screening by Direct agglutination test.

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Evaluating the wound healing activity of active component of *Berbaries vulgaris in* rabbits

Sundus H. Ahmed (1), Leena Shamel (2), Ishrak Abdul- Ameer(3), Mona Qaddori (4), Mohammed Moaied (5), Nuha AZ. Ramadhan (6), Shatha Z.Sakban(7), Mayyada M. Nasser (8) & Farah D. Salim (9)

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ABSTRACT

The present study was aimed to evaluate the scientific validity of berbaries plant.The fruit was subjected to determination of total phenolic content and they yielded 23.43± 0.18 mg/100 GAE in methanolic extract, ethanolic extract 11.09± 0.32 & water extract 30.63± 0.06 mg/100 GAE respectively . The berberis ointment was investigated for evaluation of its healing efficiency on excision wound model in rabbit. The effect produced by ointment, in terms of wound contracting ability, wound closure, decrease in surface area of wound, tissue regeneration at the wound site and histopathological characteristics were significant ($p < 0.01$) in treated rabbit. The present study thus provides a scientific improvement of this plant in the management of the wounds.

Key Words: Berbaries, phenolic content, histopathological

الملخص باللغة العربية

هدفت الدراسة الحالية لتقييم الاهمية العلميةلنبات البرباريس. استخدمت الثمرة لتحديد الفينولات الكلية والحصيلة اذ وجد ان الحصيلة لمستخلص الميثانول هي 23.43± 0.18 GAE 100 /mg ,ولمستخلص الايثانول 11.09± 0.32 GAE 100 /mg والمستخلص المائي 30.63± 0.06 GAE 100 /mg .
تم تقييم كفاءة مرهم البرباريس في علاج جروح الحروق في موديل الارانب من ناحية قابلية التقرن واللتحام وتقلص منطقة جرح الحرق واعادة بناء انسجة الجلد وكانت نتائج الدراسة النسيجية معنوية ($p < 0.01$) في علاج الارانب. ان دراستنا الحالية وفرت اثبات علمي لدور هذا النبات في علاج جروح الحروق.

INTRODUCTION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (1). Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues (2). Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation (0–3 days), cellular proliferation (3–12 days) and remodeling (3–6 months) (3). Healing requires the collaborative efforts of many different tissues and cell lineages (4). It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and reepithelialization. Healing is not complete until the disrupted surfaces are firmly knit by collagen (5). *Berberis vulgaris* is a shrub in the family Berberidaceae, native to central and southern Europe, northwest Africa and western Asia. The fruit is an oblong red berry 7-10 mm long and 3-5 mm broad, ripening in late summer or autumn; they are edible but very sour, and rich in vitamin C. In southwestern Asia, especially in Iran, where they are called *zereshk*, the berries are commonly used for cooking as well as for making jam so the production of fresh edible seedless barberries fruit reaches to about 22000 tons annually (3, 4). It has multiple pharmacological effects including; antimicrobial activity against 54 microorganisms (5), inhibition of intestinal ion secretion and smooth muscle contraction, inhibition of ventricular tachyarrhythmias, reduction of inflammation, stimulation of bile secretion and bilirubin discharge (6)**.** The barberry phenol compounds include anthocyanins and carotenoid pigments (7). Several pharmacological effects such as antioxidant and cytoprotective (8), inhibitory effects on capillary permeability (9) and epidermal growth factor (10), anticholinergic and antihistaminergic (11), have been demonstrated for anthocyanins and berberry fruit extract (BFE). The present study aims to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin and to investigate the medicinal use of *Berberis vulgaris* as a wound healing promoter that had been cited in folkloric literature.

MATERIALS AND METHODS

Plant Material

Plant fruit material were collected from the north of Iraq. The (100 gms) of dried fruit was exhaustively extracted with methanol, ethanol & water separately in the ratio of 1:7(w/v) for 24 h by using Soxhlet apparatus for methanol and ethanol extracts and soaking in water for 24 hours at room temperature with continuous stirring. The extract was completely evaporated to dryness using rotary evaporator at 40 Cº. Various concentrations of methanol extract were prepared for performing the in vitro antioxidant assays.

Determination of total phenolics

The total phenolic content (TPC) was determined with Folin-Ciocalteu reagent in alkaline medium using gallic acid as standard (10) with minor modifications. Briefly, 1 ml of the plant samples were oxidized with 1 ml Folin-Ciocalteu reagent. After 3 minutes 2 ml of 20% sodium carbonate was added for neutralization and adjusted to 10 ml with distilled water. The reaction mixture was then thoroughly mixed and allowed to stand for 60 minutes at ambient temperature and then cooled. The absorbance was measured at 650nm in Scanning mini spec spectrophotometer (Elico, India) against the reagent blank. The standard calibration was prepared

using Gallic acid and the content of total phenolics was calculated. The results were
Mean+ Standard Deviation (n=3) and expressed as gallic acid equivalent (mg GAE/g).

Formulation of emulsifying ointments

In preparing the anionic emulsifying ointment, the liquid paraffin, white soft paraffin and emulsifying wax were melted together in a beaker and immersed in a thermostated hot water bath. The melted ingredients were stirred until cold.

Preparation of medicated ointments

The medicated ointments were prepared according to the following formula: Anionic emulsifying ointment - 98% Methanol extract – 2%.

Induction of Burns

The skin of rabbits were shaved mechanically and leaved for 24 h, two or three drops of concentrated HCl were topically put carefully on the shaved skin.

Topical Application of Treatments

The two experimental groups were treated twice daily by the treatment materials. One of these groups treated by Berbaris ointment , while the other by antibiotic known as Flamazine. The Berbaris ointment was a product of Dr.Sundus and Dr.Lenna Science and technology& Medical herbal and plants center Ministry of health , while the antibiotic was produced by Pharco Pharmaceuticals, Egypt, under the license of Biochemie GmbH, Vienna Austria. The active ingredients were Bacitracin zinc 250 IU/gm, and Neomycin (as sulphate) 500 IU/gm. The areas of the burns were recorded daily to show the contracting ability of wounds and to determine the closure time. The granulations of tissues or rebuilt layers were observed and how the contamination of wounds was also observed. The hair growing time was recorded. The contracting ability of burns was measured by drawing the wound. This was carried out by putting clean filter paper on the wound and on other paper. The wound shape was drowning as stamped upon the filter paper. The drown wound shape was smoothly cut and weighted. Statistical student T-test was applied to measure the significant difference between the areas of wounds of burns the control and treated groups.

Measurement of wound area

The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

Histological Examination

At day 21 the experiment was terminated and the wound area was removed from the surviving animals for histological examination. The tissue was processed in the routine way for histological evaluation. Five micrometer thick sections were stained with haematoxylin and eosin, the routine stain used in the histopathology, and recommended as a general survey stain. The tissue samples were evaluated for the following histological criteria; the extent of reepithelisation, the maturation and organization of the epidermal squamous cells, the thickness of the granular cell layer, the degree of the tissue formation. The different animal groups were assessed blindly by the pathologist and results were compared with the control groups.

Statistical analysis

The relative wound area results were compared using one- way analysis of variance (ANOVA) followed by Dunnett's tests. *P* values less than 0.05 were considered as indicative of significance.

RESULTS AND DISCUSSION

The present study examined the phenolic content of the methanolic and ethanolic berberis fruits and the results are shown in table (1). The plant was found to contain high amounts of phenolics contents. The amount of phenolic content was 819.37± 0.06 and 755.32± 0.04 mg, respectively, (GAE) Gallic acid equivalent.

Table (1) : Extracts yield, total phenolic content of *Berberis vulgaris*.

Plant sample	Yield (%)	Total phenol content
Methanolic Extracts	23.43± 0.18 [a]	755.32± 0.04 [a]
Ehanolic Extracts	11.09± 0.32 [b]	819.37± 0.06 [b]
Water Extracts	30.63± 0.06 [c]	421.09 ± 0.14 [c]

Burns Wound Area

A better healing pattern with complete burns wounds closure was observed in rabbit with third degree wound treated with prepared ointment within 21 day while it took about 30- 35 days in control rabbits. Table (2) & Figure (1). There was a significant reduction in wound area from first week on wards in treated rabbit and also, on later days the closure rate was much faster than when compared with control rabbit. The table 1&2 shows the effect of Berberis ointment on excision wound model in rabbit.

Table (2):. Effect of topical application of treatments on the induced skin Burns wounds of rabbit.

Experimental groups		Average of the wound area (cm2)		
		Experimental times (weeks)		
Control group	Average Mean ± S.D. Closure time	27.04± 0.53	45.75±0.86	62.03± 0.53
Antibiotic group	Average Mean ± S.D. Closure time	49.96 ± 0.12*	63.04 ± 0.06*	89.34± 0.07*
B. vulgaris Ointment	Average Mean ± S.D. Closure time	57.01± 0.06*	72.15± 0.08*	100± 0.07*

Values are means ± S.E. for each group of six Rabbits ; * significantly different from control, $P < 0.05$

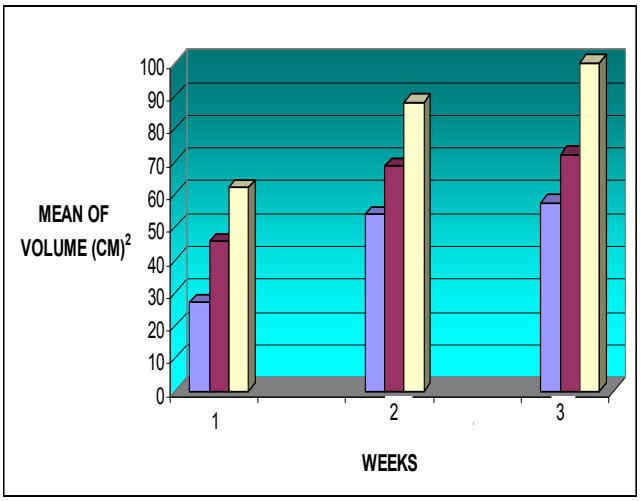

Figure (1): Changes of burns wound areas (cm²) after topical application of treatments till 3 weeks.

Control Antibiotic Berberis ointment

Histological Evaluation

Histological evaluation was carried out for the treated and untreated samples. There was a marked infiltration of the inflammatory cells, increased blood vessel formation and enhanced proliferation of cells as a result of

treatment with Berberis ointment . There was full thickness reepitheliasation, in which epidermis was thin and well organized, comparable to the normal adjacent skin which was not involved in the wound generation and healing process. The granular layer was well formed and one cell in thickness in comparable results when compared with control, there was a full thickness epidermal regeneration which covered completely the wound area. The epidermis was thick and disorganized, especially when compared with the adjacent normal skin. In all, complete epitheliasation, vasculirisation and hair follicles formation were observed in treated rabbit Figure (1) & (2). Early dermal and epidermal regeneration in treated rabbit . The treated wound after six days itself exhibit marked dryness of wound margins with tissue regeneration. However, histological evaluation showed that, increased cellular infiltration from haematoxylin and eosin staining in treated cases may be due to chemo tactic effect enhanced by the active component *of Berberis vulgaris* which might have attracted inflammatory cells towards the wound site (12).

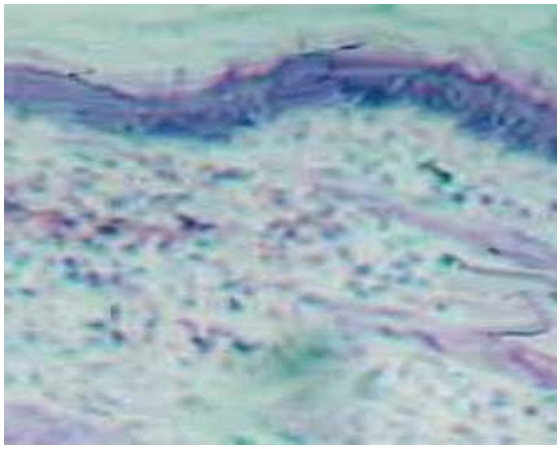

Figure (2-A) : normal skin architecture

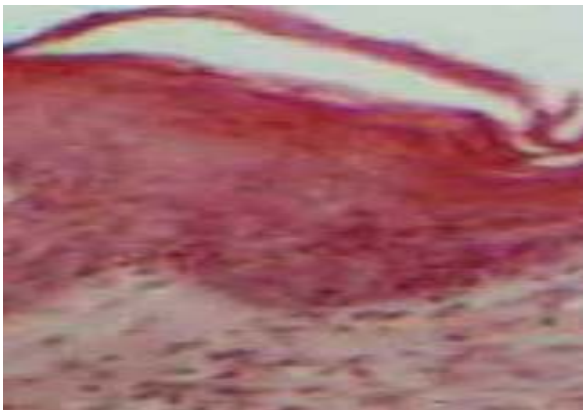

Figure (2-B): burned skin showing severe destruction of skin structure

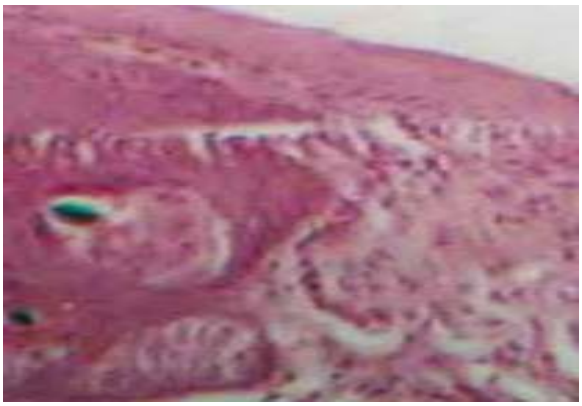

Figure (2- C): the periphery of burned skin treated by Berberis ointment after 3weeks showing a number of renewed hair roots

Figure (3) shows the effect of Berberis ointment on excision wound model in rabbit (A & B)

Figure (3-A): Wound burn induction in rabbits after two days

Figure (3-B): Wound burn induction in rabbits after 21 days

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin (13). Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The aim in these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin. Healing process, a natural body reaction to injury, initiates immediately after wounding and occurs in four stages. The first phase is coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of wound followed by re-epitheliasation which includes proliferation, migration and differentiation of squamous epithelial cells of the epidermis. In the final stage of the healing process collagen deposition and remodeling occurs within the dermis (14). The results showed wound healing and repair, accelerated by applying ointment of *Berberis vulgaris ,* which was high lighted by the full thickness coverage of the wound area by an organized epidermis. The enhanced capacity of wound healing with the plant ointment could be explained on the basis of anti-inflammatory effects of the plant that are well documented in the literature. Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epitheliasation in the animals treated with ointment.

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In terms of biofilm inhibitory concentration and minimal biofilm eradication concentrations, the role of microbial biofilm in upper versus lowers urinary tract infections

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ABSTRACT

This study has been undertaken for detection the most common microorganisms isolated from infected JJ stents and infected calculi in upper urinary tract and their representative urine samples from lower urinary tract and their ability to produce biofilm quantitatively. Furthermore, depending on Biofilm inhibitory concentration (BICs) and Minimal biofilm inhibitory concentration (MBECs) for determine the biofilm antibiogram for biofilm producer study isolates to the selected antimicrobial agents.One hundred and thirty specimens obtained from Sixty five patients admitted to Urology Department in Al-Ramadi Teaching Hospital during the period from April through December 2008. They include 94 specimens taken from 47indwelling double J ureteric stents in addition to renal stones and representative urine samples obtained from 18 patients with renal calculi. Quantitative biofilm formation assay and biofilm antimicrobial susceptibility test was achieved.Out of 47 upper end of JJ stent, 19 (40.4%) were positive for culture. Out of 24 yield microorganisms 19 (79.2%) were biofilm producer isolates. Out of 47 lower ends of JJ stent, 16 (34%) were positive for culture. Of these, 16 microorganisms were biofilm producer isolates. Also, 5 renal stones were positive for culture and all the yielded bacterial isolates were biofilm producers. The biofilm cells were required 50-100 times the MIC values for ciprofloxacin in both of JJ stent and infection stones while with cefotaxime, the study sessile were required 50-500 X, 50-100 X MICs valuefor JJ stents and infection stones respectively. Further, the biofilm producer isolates cells were required 10- 100 times the MIC values for amikacin in both of two specimens. The study concluded that *Klebsiella pneumonia* and *Candida albicans* were the most common microorganisms isolated from infected JJ stent while *Klebsiella pneumonia*was the predominant in struvite stone followed by *Proteus mirabilis*. No significant difference observed between upper and lower urinary tract infections regarding biofilm formation. Further, with the increase in duration of insertion, double J stent will be more predisposing to microbial colonization. Furthermore, the biofilm producer study isolates were required lower concentration of amikacin to remove bacterial biofilm from JJ stents and infection stones in comparison with ciprofloxacin and cefotaxime.

Key Words: Upper versus lower UTI, microbial biofilm, BICs, MBECs

الملخص باللغة العربية

أجريت الدراسة على 130 عينة مستحصلة من 65 مريض دخلوا إلى ردهة الجراحة البولية في مستشفى الرمادي التعليمي للفترة من نيسان إلى كانون الأول 2008حيث شملت 94 عينة اخذت من 47 قسطرة (JJ)بالإضافة حصى الكلى والادرار ل 18 مريض يحملون حصى الكلية.انجزت طرق انتاج الغشاء الحيوي الكمية واختبار الحساسية الدوائية لهذا الغشاء. من مجموع 47 قسطرة JJ في الجزء العلوي اظهر 19 (40,4%) من العينات نتائج موجبة للزرع وكانت 19 (79,2%) عزلة بكتيرية منتجة للغشاء الحيوي من مجموع 24 عزلة. اما في الجزء السفلي للقناة البولية فكانت 16 (34%) عينة موجبة للزرع من مجموع 47 قسطرة JJ واظهرت 16 كائن حي مجهري قدرته على انتاج الغشاء الحيوي. اظهرت خمسة حصيات كلى نتائج موجبة للزرع لبكتريا منتجة جميعها للغشاء الحيوي.احتاجت خلايا البايوفيلم من 50−100 ضعف التركيز المثبط الادنى من السبروفلوكساسين لتثبيط الغشاء الحيوي البكتيري في كلا من قسطرة JJ والحصيات المتجرثمة فين حين كانت التراكيز من 50−500 و 50−100ضعف التركيز المثبط الادنى كفيلة بإزالة الغشاء الحيوي من قسطرة JJ والحصيات المتجرثمة وعلى التوالي باستخدام مضاد السيفوتاكسيم بينما احتاج مضاد الاميكاسين الى تراكيز اقل لتثبيط وازالة الغشاء الحيوي البكتيري. تستنتج الدراسة بأن الكلبسيلا الرئوية والمبيضات المعزولة من قسطرة (JJ) الأكثر شيوعاً أما في الحصوات المتجرثمة فكانت الكلبسيلا الرئوية الأكثر سيادة يليها بكتريا المتقلبات. ان النتيجة الأكثر بروزاً كانت بأنه مع مرور الفترة الزمنية لإدخال قسطرة (JJ) الى الحالب فأن الأخيرة ستكون أكثر عرضة للاستيطان الميكروبي المؤدية بالنهاية للإصابات قسطرة (JJ) المتزامنة مع الغشاء الحيوي. استنتجت الدراسة في اختبار فحص حساسية المضادات الميكروبية بأن العزلات الدراسة المنتجة لهذا الغشاء احتاجت اقل التراكيز من الاميكاسين لازالت الغشاء الحيوي من قسطرة (JJ) والحصوات المتجرثمة بالمقارنة بمضادي السبروفلوكساسين والسيفوتاكسيم .

INTRODUCTION

It is well realized that a biofilm is defined as the accumulation of microorganisms and their extracellular products to form a structured community on a surface (1). It is well realized that device-related biofilm infection result from the multifaceted interaction of bacterial, device, and host factors. Of these three factors, bacterial factors are probably the most important inthe pathogenesis of device-associated infection, whereas device factors are the most amenable to modification with the objectiveof preventing infection(2). Microbial adhesion and biofilm formation on medical devices represent a common occurrence that can lead to serious illness and death (3).

The majority of JJ stents are used temporarily, particularly instone-forming patients. Sometimes, the stent may also be a permanent solution, especially in patients with malignant ureteral obstruction (4).The initial step in encrustation of any urinary drainage device appears to be bacterial colonization and the formation ofa layer of microorganisms that accumulates on the surface,along with their by-products (5).The presence of this layer, called “biofilm,” in combination with elevation of urinary pHand changes in electrolyte composition, is responsible for crystalformation and stent encrustation (6). The development of encrustationscan cause stent obstruction with impaired urine flow,which can compromise patient care and may lead to pyelonephritis, sepsis, and shock (7).

On the other hand, there are several types of renal stones based on the type of crystals of which they consist. The calcium oxalate stones, uric acid stones, struvite stones, the driving force behind struvite stones is infectionof the urine with urease-producing bacteria. The urease hydrolyses urea, resulting in ammoniacalurine, alkalinity and stone formation and people with certain metabolic abnormalities may produce calcium phosphate stones or cystine stones (8)

Due to importance of biofilm related infections, the treatment is performed by prolonged and high dose of antimicrobial therapy as well as elimination of infected medical device which is cornerstone of successful therapy (9).This is critical if the devices are in place for long periods of time and/or are essential for the patient's lifewith the selection of antibiotic-resistant organismsbecoming inevitable. The result is increased morbidityand mortality of patients requiring the insertion or placement of an implantable medical device (10)

One practical implication of this is that traditional diagnostic techniques that detect planktonicorganisms that generate minimum inhibitory concentrations (MIC) ofantibiotics to kill or inhibit the organisms fail to providethe clinician with data on what concentration ofantibiotic will eradicate the biofilm. Thus, new biofilm antibiogram parameters like BICs and MBECs is much more valuable (11). Thus, this study has been undertaken for detection the most common microorganisms isolated from infected JJ stents in patients with ureteric obstruction and infected calculi obtained from patients with obstructive uropathy during pyelolithotomy.

Further, quantitative determination of bacterial biofilm formed in infected renal stones and double J stents. Furthermore, depending on BICs and MBECs parameters, for detection of biofilm antimicrobial susceptibility test for biofilm producer study isolates to three selected antimicrobial agents (Ciprofloxacin, Cefotaxime, and amikacin).

PATIENTS AND METHODS

Study patients and specimen collection:

This study includes one hundred and thirty (130) specimens obtained from Sixty five (65) patients admitted to Urology Department in Al-Ramadi Teaching Hospital and carried out during the period from April through December 2008. The patients were of different sex and the mean of age was 43.68 with standard deviation (18.34). Full informative history had been taken directly from the patient or his parents or relatives, and the informations were arranged in formula sheet . All studied specimens were assigned in to upper and lower urinary tract samples. They include 94 specimens taken from 47 indwelling JJ ureteric stents with their upper end representing upper urinary tract specimen and their lower end representing the lower urinary tract specimen, 18 renal stones were obtained from the renal pelvis during pyelolithotomy (open surgery for renal stone) and at the same time a catheter collected bladder urine sample was taken for each patient.

The specimens under this study were taken by an expert urologist in the theater. The JJ stents were basically used for treatment of ureteric obstruction. The cause of ureteric obstruction and duration of JJ insertion were reviewed and included in the informative formula sheet. All the JJ stent were removed using urethrocystoscopy done under general anesthesia with complete aseptic technique with both ends stored in a sterile closed capped containers containing 10 ml of sterile normal saline and send to the laboratory for further bacteriological processing. The renal stone were removed from patients with obstructive uropathy. The stones were placed in sterile closed capped containers contained sterile normal saline or urine aspirated from renal pelvis.

Processing of samples and Laboratory investigation:

Under sterile conditions catheter collected urine samples, and JJ stent were cultured immediately into the culture media. The culture media used were nutrient agar, MacConkey agar, blood agar, sabouraud dextrose agar and chocolate agar. The streaked culture media were incubated at 37 ^{o}C for 24-48 hr.

Regarding to renal calculi, the removed stones were cultured immediately before and after crushing for the stones into the appropriate culture media mentioned previously. After crushing the stone in a sterile holder, dilution of the crushed stone were made according to criteria laid down by Baron, and associates (10). The culture was done from each dilution and the number of colonies were counted and they compared to those from original sample before dilution (10). All the study samples were bacteriologically identified and confirmed by biochemical test according to the criteria laid down by Baron, *et.al* (10)

Biofilm Study:-

Quantitative assay of biofilm formation:

Microtiter plate assay:

Adhesion and biofilm formation was determined by using a spectrophotometric method described by Mireles and associates**,** (11) as in the following: Twenty-five µl overnight bacterial growth in brain heart infusion broth added to the wells of sterile flat bottom of microtiter plates. Then, 175 µl of fresh sterile brain heart infusion broth added (medium containing 2% glucose) and incubated at 37 ^{o}C up to 24 hours or 48 hours. The planktonic cells were aspirated and the wells washed 3 times with sterile distilled water.Afterthat, the plates were inverted and allowed to dry for 30 min. at room temperature and 200 µl of (1%) crystal violet was added to each the well for30 min.The wells subsequently washed 3 times with sterile distilled water to wash off excess crystal violet. The wells were allowed to dry at room temperature for 15 minutes and the crystal violet bounded to biofilm was extracted with 200 µl of 95% ethanol .Finally, the absorbance of the extracted crystal violet was measured at (540 nm- 550nm) with automatic microplates reader.
The isolates were classified according to biofilm production depending on the criteria laid down by Christensen, *et. al*., (1985) as following: Strong producer more than 0.24; weak producer between 0.120-0.24 while non-producer less than 0.120.

Antimicrobial susceptibility for planktonic cells:

Broth macrodilution method (MIC method):

Three antimicrobial agents used for planktonic and biofilm study belonged to the following groups: Ciprofloxacin (flouroquinolones), Amikacin (aminoglycosides), and Cefotaxime (third generation Cephalosporins).Twenty eight isolates of different bacteria were included. The bacterial standardization was performed according to McFarland turbidity standard (12).

Procedure:

Antimicrobial agents stock solutions were filter sterilized and prepared at concentration (1000µg/ml). Different antibiotic concentration (0.5-32µg/ml) were prepared in 5 ml of Mueller-Hinton broth and transferred to sterile capped tubes.
At least 4-5 morphologically similar colonies were inoculated into Mueller-Hinton broth and incubated at 37^{o}C until the viable turbidity was equal to the 0.5 McFarland, (about 108 CFU/ml). After that, the suspension was diluted 1:100 and certain volumes transferred to the tubes containing antibiotic dilutions, to reach a final cell concentration of (about 105 CFU/ml). Controls were represented by two tubes; one of them contained broth only and the other contained broth plus microorganism. Then the tubes were incubated overnight at 37^{o}C.The result of MICs were interpretated as the lowest concentration of antimicrobial agents which inhibits visible bacterial growth after overnight incubation (13)

Biofilm antimicrobial susceptibility test:

Biofilm formation by study isolates on catheter segments:

The method used for bacterial biofilm formation on catheter segments was

described by Ohgaki, (14) Ishida, *et. al*.(15) Briefly, the tested bacteria incubated in brain heart infusion broth overnight at 37 oC .Then 10 µl of overnight culture was added to 500 µl of sterile media in which catheter segments (1cm2) were inoculated, and subsequently incubated overnight at 37 ^{o}C. After that washing of the segments was achieved by sterile media (3-4) times to remove weakly attached bacteria. Then segments were resuspended with sterile media and vortexed vigorously for 2 min which was considered as controls.

Bactericidal activity of antibacterials against biofilm forming sessile cells:

To determine the bactericidal activity of selected antibiotics against the sessile cells, the catheter segments were incubated with the organism as described above, were taken out, washed gently with sterile media or saline and subsequently transferred to saline containing a given antibiotic with distinct concentrations (10X, 50X, 100X, 500X and 1000X) at which X represented the MIC of mentioned antimicrobial agents against planktonic cells which was previously detected. After that the tubes were incubated for 24 hr. at 37^{o}C (15).

Extraction and quantification of biofilm bacteria:

After exposure of tested organisms to the desired concentrations of antibiotic, they were transferred to 10 ml of fresh brain heart infusion broth and stirred vigorously with a vortex mixer for 2 min. for dispersion sessile or adherent cells. Then, the suspension was diluted and plated on nutrient agar plates for bacterial colony counting and compared with original bacterial count before exposing to antimicrobial agents (15).

Detection of biofilm inhibitory concentrations (BICs) and minimal biofilm eradication concentration (MBECs):

A- Biofilm Inhibitory Concentration (BIC):

After incubation the tubes for 24hr. at 37 ^{o}C, the biofilm inhibitory concentration was detected and defined as the lowest concentration of antimicrobial agents which inhibits bacterial biofilm growth on a surface of catheter. It was represented by the clearance of broth medium consisting (1cm) catheters and the required concentrations of antimicrobial agents (16).

B- Minimal Biofilm Eradication Concentration:

After plating the diluted suspension into agar plates and counting the number of bacterial colonies, MBEC was determined. MBEC was defined as the lowest concentration of antibiotic or biocide capable of killing biofilm producer bacteria. It was represented by disappearing of colonies of biofilm producer organisms on the culture plates (16)

Statistical analysis:

All data were analyzed using the SPSS statistical program (statistical Package for the Social Science) version 14.0. Statistical significance was taken with p value < 0.05 and 0.001 . The significant differences were detected by using either the goodness fit test within chi-square test or independent sample-test. All the study graphics (bar chart, scatter diagram or dot chart) were done by using Microsoft Excel XP (17), (18), (19)

RESULTS

Out of 47 upper end of JJ stent, 19 (40.4%) were positive for culture and 28 (59.6%) were negative. Out of 24 microorganisms 19 (79.2%) were biofilm producer isolates while 5 (20.8%) were non biofilm producers. Out of 47 lower ends of JJ stent, 16 (34%) were positive for culture and 31(66%) were negative.

16 microorganisms were biofilm producer isolates while 5 were not.
Out of 94 JJ stent samples, distributed in to 47 (50%) were from upper end of the stent and 47(50%) were from lower end of stent. Among 94 JJ stents, 35 (37.2%) were infected. With regard to the duration of JJ stents used, among 35 (37.2%) infected ones, 2 (11.1%) in the duration range from (1-30) day, 8 (30.8%) were with (31-60) day, 18 (42.9 %) were with (61-90) day and 7 (87.5%) in duration of insertion more than 90 day. Figure (1).

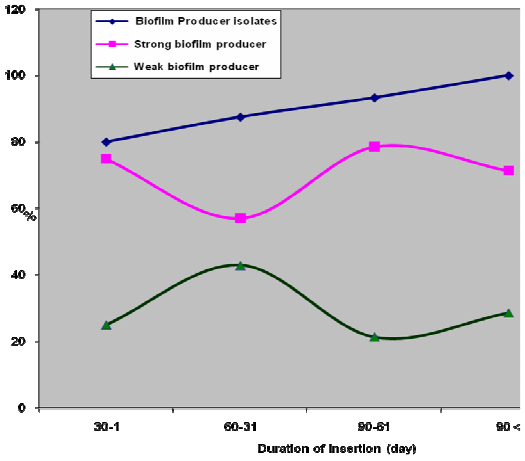

Figure (1): Biofilm production versus duration of insertion (days) for patients with infected JJ stent

Among infected JJ stent, 10 (22.2%) *Klebsiella pneumonia* and 10 (22.2%) *Candida albicans* were the most common isolated microbes from JJ stent. Out of 10 *Klebsiella pneumonia* the biofilm was produced strongly in 6 (60%) isolates, and weakly in 2 (20%), while the others were non biofilm producer isolates. Regarding to *Candida albicans*, out of 10 the biofilm was produced strongly in 6 (60%) isolates and weakly in 2 (20%) while the others were non biofilm producer isolates. Also, *Staphylococcus epidermidis* in 8 (17.9%), 5 (62.5%) isolates from upper end of stent and 3 (37.5%) isolates from lower end of stent, out of 8 *Staphylococcus epidermidis* the biofilm was produced strongly in 5 (62.5%), and weakly in 2 (25%), while the other was non biofilm producer isolates. out of 6 (13.3%) *Staphylococcus aureus* the biofilm was produced strongly in 3 (50%) and weakly in 2 (33.3%) while the others were non biofilm producer isolates. out of 6 (13.3%) *Pseudomonas aeruginosa* the biofilm was produced strongly in 2 (33.3%) and weakly in 2 (33.3%) while the others were non biofilm producer isolates. Out of 5 (11.1%) *Escherichia coli*, the biofilm was produced strongly in 2 (40%) and weakly in 1(20%) while the others were non biofilm producer isolates.
In this study, 5 renal stones were positive for culture and 13 were not and all the isolates were biofilm producers. At the same time out of 18 catheter urine sample obtained from lower urinary tract 4 (22.2%) were positive. Of these specimens, biofilm was produced in 3 (75%) isolates.
Out of 18 stones, 5 (27.8%) were infection stones while the other 13 (72.2%) were non infection stones from upper urinary tract. out of 18 urine specimens obtained from bladder with infection stones, 4 (22.2%) were infected urine with infection stones, 14 (77.8%) were non infected urine with non-infection stones and infection stones. Among infection stones *Klebsiella pneumonia* were 5 (71.4%), 3 (60%) from upper urinary tract and 2 (40%) obtained from bladder urine, followed by 4 (28.6%) *Proteus mirabilis*, were 2 (50%) from upper urinary tract and 2 (50%) obtained from bladder urine. Out of 5 Klebsiella pneumonia the biofilm was produced strongly in 3 (60%) and weakly in 1 (20%) and other non-biofilm producer, out of 4 *Proteus mirabilis* the biofilm was produced strongly in 3 (75%) and weakly in 1 (25%).
In this study, mixed microbial infection was found in ten cases in patients with obstructive uropathy due to renal stones at which the fungus, *Candida albicans* was shared with both of*Klebsiella pneumoniae* and with *Staphylococcus epidermidis* in four cases and with *Pseudomonas aeruginosa* in two cases.

Quantitative biofilm production (Spectrophotometric assay):

In the Quantitative biofilm formation assay, a spectrophotometric method was done with the presence of glucose. In the most scientific method, spectrophotometric assay with ELISA

reader our result showed that out of 20 (27.8%) isolates of *Klebsiella pneumonia*, 14 (19.4%) of *Staphylococcus aureus*, 10 (13.9%) of *Staphylococcus epidermidis*, 10 (13.9%) of *Candida albicans*, 6 (8.3%) of *Escherichia coli*, 6 (8.3%) of *Pseudomonas aeruginosa*, 5(7%) of *Proteus mirabilis* and 1 (1.4%) of *Streptococcus faecalis* biofilm phenomenon was observed in 16 (80%), 11 (78.6%), 9 (90%), 8 (80%), 3 (50%),4 (66.7%), 5 (100%) and 1 (100%) respectively.

Antimicrobial susceptibility test for planktonic cells:

Broth macrodilution technique:

In this part of study, antimicrobial susceptibility test for selected antimicrobial agents against logarithmic phase planktonic cells of study isolates obtained from patients with double J stent and infection stones was detected by detection of MICs according to the criteria laid down by National Committee for Clinical Laboratory Standard, (2001) by using an international quality isolate of *Pseudomonas aeruginosa* American Type Culture Collection (ATCC 27853) and isolate of *Staphylococcus aureus* American Type Culture Collection (ATCC 25923). Our result showed that MICs were 6.5± 7.14 , 22± 12, 6± 2.3 16± 0.0, 32± 0.0, 4± 0.0, 7.2± 6.5, 10± 4.0 and 6± 2.3 for ciprofloxacin, cefotaxime and amikacin respectively against logarithmic phase planktonic cells of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* obtained from patients with JJ stent. Furthermore, our result showed that MICs were 14.4± 3.6, 19.2± 12.1 and 8.8± 4.38 for ciprofloxacin, cefotaxime and amikacin respectively against logarithmic phase planktonic cells of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones.

Regarding biofilm antimicrobial susceptibility test, as far as ciprofloxacin is concerned, the BIC of ciprofloxacin against study isolates (A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) obtained from patients with JJ stent were 50 (50 XMIC), 800 (50 XMIC), 200 (50 XMIC), 160 (10 XMIC), 800 (50 XMIC), 80 (10 XMIC), 100 (100 XMIC), 100 (100 XMIC), 800 (50 XMIC), 400 (50 XMIC) µg/ml, respectively. Further, the MBEC of 500 (500 XMIC), 8000 (500 XMIC), 2000 (500 XMIC), 16000 (1000 XMIC), 8000 (500XMIC), 800 (100X MIC), 1000 (1000 XMIC), 1000 (1000 XMIC), 8000 (500XMIC), 4000 (500 XMIC) µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of ciprofloxacin, against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of ciprofloxacin against study isolates (A22, A24, A25, A26 and A27) were 800 (50X MIC), 1600 (100X MIC), 400 (50X MIC), 800 (50X MIC), 800 (50X MIC) µg/ml respectively. On the other hand, the MBEC of 8000 (500X MIC), 16000 (1000X MIC), 4000 (500X MIC), 8000 (500X MIC), 16000 (1000X MIC) µg/ml were enough to eradicate biofilm of above mentioned isolates respectively.

The result of third generation cephalosporins, cefotaxime against sessile cell of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* obtained from patients with JJ stents, the BIC of cefotaxime against study isolates(A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) were 800 (100X MIC), 1600 (50X MIC), 1600 (100X MIC), 3200 (100X MIC), 3200 (100X MIC), 1600 (50X MIC), 4000 (500 X MIC), 1600 (100X MIC), 400(50X MIC), 800 (100X MIC), µg/ml respectively. On the other hand, the MBEC of 4000 (500X MIC), 8000 (500X MIC), 8000 (500X MIC), 32000 (1000X MIC), 16000 (500X MIC), 32000 (1000X MIC), 4000 (500X MIC), 16000 (1000X MIC), 8000 (1000X MIC), 4000 (500X MIC), µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of third generation cephalosporins, cefotaxime against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of cefotaxime against study isolates (A22,

A24, A25, A26 and A27) were 400 (50X MIC), 3200 (100X MIC), 1600 (100X MIC), 1600 (50X MIC), 400 (50X MIC), µg/ml respectively. On the other hand, the MBEC of 8000 (1000X MIC), 32000 (1000X MIC), 8000 (500XMIC), 32000 (1000X MIC), 4000 µg/ml were enough to eradicate biofilm of above mentioned isolates respectively.

The result of amikacin against sessile cell of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* obtained from patients with JJ stents, the BIC of amikacin against study isolates (A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) were 400 (50X MIC), 200(50X MIC), 40 (10X MIC), 400(50X MIC), 200 (50X MIC), 400 (100X MIC),400 (50X MIC), 80 (10X MIC), 400 (50X MIC), 200(50X MIC), µg/ml respectively. On the other hand, the MBEC of 4000 (500X MIC), 2000 (500X MIC), 4000 (1000X MIC), 8000 (1000X MIC), 2000 (500X MIC), 4000 (1000X MIC), 4000 (500X MIC), 8000 (1000X MIC), 4000 (500X MIC), 2000 (500X MIC), µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of amikacin against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of amikacin against study isolates (A22, A24, A25, A26 and A27) were 400 (100X MIC), 800 (100X MIC), 800 (100X MIC), 160 (10X MIC), 80 (10X MIC), µg/ml respectively. On the other hand, the concentrations of 4000 (1000X MIC), 8000 (1000X MIC), 4000 (500X MIC), 16000 (1000X MIC), 8000 (1000X MIC), µg/ml were enough to eradicate biofilm of above mentioned isolates respectively. Table (1)

DISCUSSION

It is well realized that the prognosis of catheter-associated infection is complicated due to the occurrence of chronic or recurrent UTIs, complicated UTI, and pyelonephritis. If left untreated, these infections can lead to abscess formation, renal obstruction, and scarring and eventually will lead to bacteremia, sepsis, and, possibly, death. These infections are difficult to treat due to the presence of biofilms and crystals that protect uropathogens from proper treatment. Only after the complete eradication of biofilm and crystals in the urinary tract can a catheter-associated infection be eliminated (20).

It is well know that ureteric stent, is a thin tube inserted into the ureter to prevent or treat obstruction of the urine flow from the kidney. The presence of biofilm in particular with urease-producing bacteria, leads to the hydrolysis of urea, an elevation of urinary pH and the deposition of struvite and calcium phosphate encrustation on these stents. Clinically, encrustation and infection of indwelling stents are associated with pain, haematuria, blockage and sepsis (21). In this study, patients with obstructive uropathy who are submitted to JJ ureteric stent were one of the study groups. Among this group, a total of 94 JJ stent samples in 47 patients were studied, half of them from the upper end of urinary tract and the other half from the lower end.

Regarding to the infection associated with JJ stents and it's relation to the duration of insertion, our result revealed that out of 35 indwelling JJ stents studied, 2 were with (1-30) day duration of insertion, 8 were with (31-60) day duration of insertion , 18 were with (61-90) day duration of insertion and 7 were with > 90 day duration of insertion. In a study designed by Kliś and co-workers (22), these researchers documented that stent cultures were negative in all patients that had their stent kept for shorter than 30 days. There were 25% positive stent cultures in case of the stents maintained for the period longer than 30 days, but not longer than 90 days, and 45% in case of catheters kept for longer than 90 days. In another study designed by Riedl and associates (23), 93 ureteral stents from 71 patients were examined. Nine patients with permanent ureteral stenting due to malignant ureteral obstruction, and 62 patients with temporary ureteral stents. Bacteruria and bacterial stent colonization were found in all patients with permanent stents. In patients with temporary stents, colonized stents were found in 69.3%.

Kumar and associates (24) recommended that early removal at 2 weeks is advisable and the short duration of stenting is effective and saves the cost of repeat hospitalization at a later date. Laube and associates[25] mentioned that the stent is a double-edged weapon and it may behave as a friend or an enemy.

Table (1): The biofilm inhibitory concentrationsand minimal biofilm eradication concentration for amikacin against biofilm producing isolates among study specimens.

Isolate no.	Colony count for control (CFU/ml)	Biofilm Inhibitory Concentration (BIC)			Minimal Biofilm Eradication Concentration (MBEC)		
		No. of folds higher than MIC	Conc. µg/ml	Colony count CFU/ml	No. of folds higher than MIC	Conc. µg/ml	Colony count CFU/ml
The result of JJ stent specimen							
***Staphylococcus epidermidis*(A1, A7, A13) &*Staphylococcus aureus*(A15)**							
A1	66 X10^5	50X	400	34 X10^2	500X	4000	7
A7	80X10^5	50X	200	57X10^2	500X	2000	2
A13	91X10^5	100X	400	33	1000X	4000	Zero
A15	46 X10^5	10X	80	39	1000X	8000	Zero
***Pseudomonas aeruginosa* (A10 ,A14) , *Escherichia coli* (A11,A12)&*Klebsiella pneumonia* (A20, A22)**							
A11	33 X10^6	50X	400	31	1000X	8000	Zero
A12	57X10^5	50X	200	43	500X	2000	Zero
A10	30X10^5	10X	40	33	1000X	4000	Zero
A14	61 X10^6	50X	400	50 X10^2	500X	4000	Zero
A20	80X10^5	50X	400	40X10^2	500X	4000	2
A22	91X10^5	50X	200	34X10^2	500X	2000	3
The result of infection stones							
pneumonia* (A24,A25,A26) *Proteus mirabilis* (A22, A27)*Klebsiella							
A22	77X10^5	100X	400	33	1000X	4000	Zero
A24	90X10^6	100X	800	40X10^2	1000X	8000	2
A25	80X10^5	100X	800	34X10^2	500X	4000	1
A26	57X10^5	10X	160	33	1000X	16000	Zero
A27	66X10^5	10X	80	43	1000X	8000	Zero

The organisms commonly contaminating these devices and developing biofilms are *Escherichia. Coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and other gram-negative organism. In this study, out of 94 JJ stent sample, 35 (37.2%) were infected. Among infected JJ stent, the most commonly isolated microorganisms were *Klebsiella pneumonia* and *Candida albicans* followed by *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. In other study designed by Yeniyol and co-workers (26) these researchers revealed that out of 11 patients, bacterial colonization of JJ stents were observed. In 10 of them both stent and urinary cultures were positive, and showed

identical microorganisms; 80% *Escherichia coli*, 10% *Escherichia coli* + *Candida*, 10% *Klebsiella*. Kehinde and associates, (27) showed that of 250 patients requiring JJ stent insertion was investigated microbiologically prior to stent insertion and on the day of stent removal. One hundred four stents (41.6%) were culture positive and 146 (58.4%) were culture negative. The commonest isolates were *Escherichia coli, Enterococcus* spp., *Staphylococcus* spp., *Pseudomonas*, and *Candida* spp. Also, Meir and co-workers, (28) showed that out of 82, 58 (70.7%) were culture positive and 24 (29.3%) were culture negative .The commonest isolates were *Staphylococcus* (coagulase negative and positive), *Enterococci* and *Proteus*.

With regard to other study specimen group, renal calculi, Margel and associates (29) documented that renal calculi pathogens are one of the predisposing factors for infectious events. Intra-operative stone culture may be essential in directing the antibiotic regimen postoperatively and should be routinely used. Infected stones function as a sanctuary for organisms and may attenuate the effects of antibiotics against them causing persistent infection (30). Incorporation of urea splitting bacteria within the developing struvite stones as well as calcium oxalate stones that had become secondarily infected results in a focus of infection that is resistant to conventional antimicrobial therapy and manifested clinically by repeated urinary tract infections caused by the infecting organism, therefore complete removal of all the infected stones material is considered to be essential for the eradication of persistent Bacteriuria associated with the infected renal calculi (31).

Margel and associates (29) revealed that out of 75 patients, 33 (44%) had sterile urine and stone cultures while both urine and renal stones were colonized in 23 patients (30.7%). Also, a colonized stone culture associated with a sterile urine culture was found in 19 patients.

Our study showed that out of 18 patients, 5 (27.8%) had infected stones cultures while 13 (72.2%) patients were with sterile urine and stones cultures. Among infection stones, *Klebsiella pneumonia* was the most predominant which was isolated from 5 (71.4%) cases, 3 (60%) of them from upper urinary tract and 2 (40%) obtained from bladder urine followed by *Proteus mirabilis* 4 (28.6%), half of them from upper urinary tract and 2 (50%) obtained from bladder urine. In 4 (80%) of them, the urine and stone cultures showed similar pathogens. A colonized stone culture associated with a sterile urine culture was found in 1 (20%) stone.

Studies of biofilm formationhave primarily focused on biofilms formed by a single speciesof microorganism (32). However,biofilms are thought to be composed of more than one species.Although implant-associated infections involving both bacteria and fungi are not uncommon (33), mixed species biofilms of this type have been studied relatively little. In this study, mixed microbial infection was found in ten cases in patients with obstructive uropathy at which the fungus, *Candida albicans* was shared with both *Klebsiella pneumoniae* and *Staphylococcusepidermidi*s in four cases and with *Pseudomonas aeruginosa* in two cases.

Adam and associates (34) showed that extensive interactions between *Staphylococcusepidermidi*s and *Candida albicans* have been demonstrated in biofilms containing both organisms. In mixed fungal-bacterial biofilms, both staphylococcal strains showed extensive interactions with *C. albicans*. The researchers concluded that the extracellular polymer produced by *S. epidermidis* RP62A could inhibit fluconazole penetration in mixed fungal-bacterial biofilms. Conversely the presence of *C. albicans* in a biofilm appeared to protect the slime-negative staphylococcus against vancomycin. Overall, the findings suggest that fungal cells can modulate the action of antibiotics, and that bacteria can affect antifungal activity in mixed fungal-bacterial biofilms. *Pseudomonas aeruginosa* and *Candida albicans* are consistently identified as some of the more important agents of nosocomial infections. In light of the recent information regarding device-associated nosocomial infections, understanding

the nature of *P. aeruginosa* and *C. albicans* infections is increasingly important. These two microorganisms demonstrate: (1) an ability to form biofilms on the majority of devices employed currently, (2) increased resistance/tolerance to antibiotics when associated with biofilms, (3) documented infections noted for virtually all indwelling devices, (4) opportunistic pathogenicity, and (5) persistence in the hospital environment (35).

Regarding to quantitative biofilm production, the study results showed that *Proteus mirabilis* was the most common microbial isolate produced biofilm phenomenon (crystalline biofilm)5 (100%), followed by *Staphylococcus epidermidis*, *Candida albicans*, *Klebsiella pneumonia, Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* respectively. The tissue culture plate method was found to be most sensitive, accurate and reproducible screening method for detection of biofilm formation by microorganisms and has the advantage of being a quantitative model to study the adherence of microorganisms on biomedical device (36).

With regard to antimicrobial susceptibility test for planktonic cells, numerousstudies have now demonstrated that biofilm-grown microorganismshave an inherent lack of susceptibility to antibiotics, whereasplanktonic cultures of this same organism do not(37). This resistance is lost once thebiofilm is reverted to conditions that permit planktonic growth. The innate tolerance of microbial biofilms to antibiotictherapy has led to problems in their eradication and inthe management of patients with device-related infections. This difference in antibiotic susceptibility betweenplanktonic and biofilm populations of the same organism may resultfrom differences in the diffusion of antibiotics or muchmore complex changes in the microbial physiology of the biofilm (38). The slow growth of biofilm bacteria and exopolysaccharide or glycocalyx acting as a barrier to the penetration of antibacterial agents are considered responsible for failure of antimicrobial therapy of these infections (39).

In the field of antimicrobial susceptibility test for sessile cells of study bacterial isolates obtained from JJ stentand renal stones, our result revealed that the biofilm cells were required 50-100 times the MIC values for ciprofloxacin obtained for the same isolates in logarithmic phase of planktonic cells in each of the two specimens. Regarding to cefotaxime, our result showed that the sessile cells of study bacterial isolates obtained from JJ stent were required 50-500 X MIC values for cefotaxime in comparison with those observed with other study specimen, infection stones which required 50-100 MIC to eliminate bacterial biofilm for the same antimicrobial agent with high statistical significant differences.

On the other hand, the biofilm cells wererequired 10- 100 times the MIC values for amikacin against bacterial isolates obtained from JJ stent and infection stones.The high concentrations of quinolones were used to treat the preformed biofilms because 1) these concentrations would be expected to reach the biofilms when therapeutic doses of quinolones are infused through the vascular catheters and, 2) using such high concentrations would minimize the exposure of very large inoculum of bacteria in the biofilms to sub-inhibitory concentrations of the quinolones (40).It is well known that flouroquinolones are indeedvery effective in stopping the growth of a biofilm (41). Atthe same time, restricted diffusion can protect the biofilm froma degradable antimicrobial. Retarded diffusion will decrease theconcentration of the antibiotic entering the biofilm, helpingan enzyme like β-lactamase destroy the incoming antibiotic (42). A restricted penetrationof this small molecule coupled to its destruction by the microbialcells was apparently responsible for resistance. It can be expectedthat any mechanism of antibiotic destruction or modification (likeacetylation of aminoglycosides) will be especially effective whencoupled with a diffusion barrier of the biofilm. The MIC for biofilm cells is higher than that for planktoniccells (42).

The administration of a minimal biofilm eradicating concentration (MBECs) greater than the MIC of planktonic cells has been stressed in order to prevent bacterial biofilm cells from expressing antibiotic-resistant factors (33).

The study concluded that *Klebsiella pneumonia* and *Candida albicans*were the most common microorganisms isolated from infected JJ stent. Also, among infection stones, *Klebsiella pneumonia* was the most predominant microorganism followed by *Proteus mirabilis*. Mixed biofilm species consist largely of *Candida albicans* and other bacteria like *Klebsiella* spp., *Staphylococcus epidermidis* and *pseudomonas aeruginosa* play an important role in the formation of biofilm on JJ stent. Further, with the passage of time (duration of insertion), double J stent will be more predisposing to microbial colonization and eventually leading to biofilm associated JJ stent infection. Furthermore, In biofilm antimicrobial susceptibility test, the biofilm producer study isolates were required lower concentration of amikacin to remove bacterial biofilm from JJ stents and infection stones in comparison to ciprofloxacin and cefotaxime.

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Serological study of *Toxoplasma gondii*, Rubella, Cytomegalo virus and Herpes simplex virus prevalence in Pregnant Women with or without a history of abortion

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ABSTRACT

Infectious agents during pregnancy have severe consequences not only threatening maternal health, but also causing fetus death and congenital disorders. It was decided to have a serological study for the determination of the role of the infectious agents Toxoplasma gondii, rubella, cytomegalo virus and herpes simplex virus that lead to abortion in women. A total of 86 women at first trimester pregnancy were included in this study, 66 patients (study group) with a history of previous abortion, preterm deliveries and congenital malformation of children and 20 clinically normal ones (control group). Blood samples were examined for IgM antibodies for Toxoplasma gondii, IgG antibodies for rubella and herpes simplex viruses (HSV), IgG and IgM for Cytomegalo virus (CMV) using ELISA. In this study a highly significant difference was found in the study group with an anti rubella IgG in correlation with previous pregnancies and number of abortion at P value < 0.01. Also a significant difference was found in the study group with anti CMV IgM in women having previous pregnancies and in number of abortion at P value 0.013 and 0.026 respectively. However a highly significant difference in the study group was noted with anti HSV IgG in relation to maternal age (P= 0.005). The comparison between study and control group shows a highly significant difference with anti rubella IgG and a highly significant difference between the two groups with anti CMV IgM only (P< 0.01).

الملخص باللغة العربية

إن العوامل المعدية خلال الحمل مهمة جدا" ليس فقط لإنها تهدد حياة وصحة الأم، ولكنها ايضا" تسبب وفاة الجنين وتشوَه لدى الأطفال. لقد قررنا عمل دراسة مصلية عن العوامل المعدية (المقوَسة الغوندية، الحصبة الألمانية، حُمة خلوية عرطل، وحمُة الحلأ البسيط)والتي تؤدي إلى الإجهاض لدى النساء. شملت هذه الدراسة 86 من النساءفي الفترة الأولى من الحمل وتضمنت هذه المجموعة 66 مريضة (مجموعة الدراسة) يعانون من اجهاض سابق، ولادات قبل الأوان، واطفال مشوهين وايضاً 20 من النساء الصحيحات سريرياً. تم فحص عينات الدم لتحديد وجود الأجسام المضادة من نوع IgM للمقوَسة الغوندية، و IgG لحُمة الحصبة الألمانية وحمة الحلأ البسيط، و IgGو IgM لحمُة خلوية عرطل (CMV). في هذه الدراسة ظهر وجود علاقة مباشرة ذات معنوية عالية في مجموعة الدراسة مع ظهور الأجسام المضادة للحصبة الألمانية نوع IgG وعدد مرات الحمل السابقة وايضاً مع عدد مرات الإجهاض (بدلالة معنوية < 0.01) وأيضاً وجود علاقة ذات معنوية في عينة الدراسة والتي لديها مضادات لحُمة خلوية عرطل CMV من نوع IgM مع عدد مرات الحمل السابقة وعدد مرات الإجهاض (بدلالة معنوية 0.013 و 0.026) على التوالي، وايضاً ظهر وجود علاقة مباشرة ذات دلالة معنوية عالية في مجموعة الدراسة والتي ظهر لديها مضادات لحمُة الحلأ البسيط نوع IgG بالعلاقة مع عمر الأم بدلالة معنوية (0.005). بمقارنة مجموعة الدراسة مع مجموعة السيطرة ظهر وجود علاقة مباشرة ذات معنوية عالية في وجود المضادات لحمُة الحصبة الألمانية نوع IgG وايضاً وجود المضادات لحمُة خلوية عرطل CMV نوع IgM فقط وبدلالة معنوية < 0.01.

INTRODUCTION

Bacterial, viral and parasitic infections with their direct and indirect effects are the important causes of fetus death (1). A number of maternal infections can lead to a single pregnancy loss as in the cases of listeriosis, toxoplasmosis, and certain viral infection (rubella, herpes simplex, measles, and cytomegalo virus) (2). Primary infections caused by *Toxoplasma gondii*, rubella and cytomegalo virus can lead to serious complications in pregnant women (3).

Toxoplasma gondii infection in pregnant women represents a risk for congenital disease, primary infection acquired during pregnancy may result in severe to the fetus (a congenital malformation is a congenital physical anomaly that is deleterious, i.e. a structural defect perceived as a problem, a typical combination of malformations affecting more than one body part is referred to as a malformation syndrome, a malformation is associated with a disorder of tissue development and often occurs in the first trimester) (4) (5). Acute and late *Toxoplasma gondii* infections during pregnancy are mostly diagnosed by serological tests including detection of anti Toxoplasma gondii specific Igm and IgG antibodies (4), (6).

Rubella has world wide prevalence, its epidemic and pandemic outbreaks occur every 6-10 years and 20-25 years respectively (7). It causes hypoplasia in neonate's and abortion in pregnant women. (7), (8). Many mothers who contract rubella within the first critical trimester will either have a miscarriage or a still born baby (9). At every epidemic of rubella there is a considerable number of artificial abortions caused by the fear of having babies with congenital disorders (10).

Women at high risk for acquiring rubella during pregnancy are those who are non immune to rubella and are exposed to the infection. More than half of the women infected with rubella do not show the classical signs and symptoms of fever and 3 day rash (11), (12).

Cytomegalo virus (CMV) causing congenital infection is the main world health concern and transmission of infection to the fetus generally occurs during the acute phase (7)

About 30-60% of women receiving obstetric care have serologic evidence of past herpes simplex virus (HSV) infection (13). Although HSV1 and HSV2 may cause neonatal herpes. (14)approximately 10% of infections are congenital, usually a consequence of the mother acquiring primary HSV infection during pregnancy and the fetus acquiring the infection transplacentally or via an ascending infection from cervix. Intrauterine infection is associated with intrauterine growth restriction, preterm labor, and miscarriage. (15), (16)

MATERIALS AND METHODS

A total of 86 women were investigated including 66 with history of abortions, preterm deliveries and congenital malformed children and 20 clinically normal with previous normal full term deliveries.

From each woman, 5 ml of venous blood was collected in a test tube with strict aseptic precautions. The serum was used for serological evaluation using ELISA for detecting IgM antibodies to *Toxoplasma gondii*, IgG antibodies to rubella and herpes, IgG and IgM antibodies to cytomegalo virus. Statistical data analysis was used in order to analyze and assess the results of the study.

RESULTS AND DISCUSSION

The results indicate that the seropositivity rate in women with previous pregnancies is highly significant in anti rubella IgG (0.004) than in normal healthy controls ($P< 0.01$) as shown in table (1), also there is a significant difference between

study group and control group with anti CMV IgM according to the number of previous deliveries (0.013) (P< 0.05). However a highly significant difference in anti rubella IgG (0.003) in study group according to the number of abortion at P value < 0.01 and a significant difference found in number of abortion in study group with anti CMV IgM (0.026) at P value < 0.05 as shown in table (1).

We did not found any study about anti rubella IgG antibodies or anti CMV IgM antibodies and their relation to number of previous pregnancies. Rubella is generally asymptomatic in healthy adults but leads to congenital rubella syndrome (CRS) in fetus, so it is an important public health problem, however in England 56%of CRS infants mothers found to have multiple children were vaccinated to prevent CRS (17).

Table (1): IgG &IgM antibodies against *Toxoplasma gondii*, rubella virus, CMV and herpes simplex virus in association with some related variables in the study (S) and control (C) groups.

Parameters	Anti Toxoplasma IgM		Anti Rubella IgG		Anti CMV IgG		Anti CMV IgM		Anti HSV IgG	
	S	C	S	C	S	C	S	C	S	C
Maternal age groups	0.700	0.656	0.102	0.343	+ ve	0.343	0.600	0.741	0.005**	+ ve
No. of previous preg.'s	0.085	0.673	0.004**	0.490	+ ve	0.094	0.013*	0.732	0.787	+ ve
Preterm deliveries	0.122	Non	0.494	Non	+ ve	Non	.223	Non	.568	Non
No. of abortions	0.032	Non	0.003**	Non	+ ve	Non	0.026*	Non	.621	Non
No. of congenital children (died)	0.353	Non	0.514	Non	+ ve	Non	.376	Non	.650	Non
Fever	0.821	-ve	0.174	– ve	+ ve	– ve	0.916	– ve	0.344	– ve

** HS: Highly Sig. at P< 0.01; * S: Sig. at P< 0.05

Our results are in agreement with the results obtained by Surpam *et al* (2006) who concluded in their study that toxoplasma, rubella, cytomegalo virus and herpes simplex virus (TORCH) infections are associated with recurrent abortion, intrauterine growth retardation, intrauterine death, preterm labor, early neonatal death, and congenital malformation (18).Also Siegel *et al* in (1971) found that infection of the mother by rubella virus during pregnancy can be serious and spontaneous abortion occurs in up to 20% of cases (19)

Hung and Weng (1995) studied the intra uterus transmission of CMV during pregnancy in women by PCR method and they found that CMV can cause fetus death and duration of pregnancy has no effect on the process of infection in fetus (20)

Our results are in agreement with the results obtained by Abdel-Fattah *et al* (2005) who found that TORCH infections can lead to severe fatal anomalies or even fetal loss (21). However Supram *et al* (2006) suggested that pregnancy may reactivate the latent cytomegalo virus leading to further reproductive wastages (18).

In our study we found a highly significant difference between study groups with anti HSV2 IgG (0.005) in relation with maternal age (P< 0.01).

This situation is similar to that described by Johnson *et al* (1989) and Cowan *et al* (1994) who found that HSV2 infection extremely rare in childhood but increase rapidly from the age of 15 to peak in the mid of 30s (22), (23). Also Whitfield (1995) and Obasi *et al* (1999) showed that the risk of genital herpes infection may vary with maternal age (24), (25). However all tested women were found to be negative for anti *Toxoplasma gondii* IgM antibodies and this may be due to the size of the sample, which may be small in comparison to other studies, Sabahattin *et al*, who found that only 9 out of 1652 pregnant women tested (0.54%) were positive for anti *Toxoplasma* IgM (26).

The results showed a highly significant difference between study and control group with anti rubella IgG (0.009) (P< 0.01), while there's no significant difference between study and control group for the other variables as shown in table (2).

Our results are in agreement with the results of Deka *et al* (2006) who indicate that women who are immune to rubella after natural infection or vaccination demonstrate lifelong IgG antibodies, hence presence of natural immunity (IgG positive) is a parameter of protection from infection during pregnancy the same as offered by vaccination (27). Hence, pregnancy screening of all women and demonstration of high immunity places a woman at relatively no risk of rubella infection during pregnancy (12), (27). However Rubella outbreaks still arise, usually in developing countries where the vaccine is not as accessible (28). IgG to rubella lasts lifelong, therefore confirmation of recently acquired rubella infection in the pregnant women which is important- either by demonstration of an IgG titer rise in two serum samples obtained within 2 weeks interval or by an IgM positive result in one serum sample (7).

Nasrolahei and Vahedi in (2006) showed in their study that 40% of women infection with rubella virus occurred 6 weeks prior to fetus death. However rubella virus causes hypoplasia in neonate and abortion in pregnant women (29).

The above mentioned agents could be the cause of abortion or fetus death which can be treatable if diagnosed at an early stage however further studies are required.

Table (2): Distribution of some studied variables between the study and control samples with causes correlation ship and comparisons significant

Studied variables	Groups	Freq.'s & Percentages	Sample		Total	C.S. P-value
			Study	Control		
Anti Rubella IgG	Positive	Count	62	14	76	FEPT P=0.009 HS
		% within Anti Rubella IgG	81.6%	18.4%	100.0%	
		% within Sample	93.9%	70.0%	88.4%	
	Negative	Count	4	6	10	CC =0.290 P=0.003 HS
		% within Anti Rubella IgG	40.0%	60.0%	100.0%	
		% within Sample	6.1%	30.0%	11.6%	
Anti Toxoplasma IgM	Positive	Count	0	0	4	FEPT P=0.339 NS
		% within Anti Toxoplasma IgM	0.0%	0.0%	0.0%	
		% within Sample	0.0%	0.0%	0.0%	
	Negative	Count	66	20	82	CC =0.121 P=0.260 NS
		% within Anti Toxoplasma IgM	76.7%	23.3%	100.0%	
		% within Sample	100%	100.0%	100%	
Anti CMV IgG	Positive	Count	66	18	84	FEPT P=0.052 NS
		% within Anti CMV IgG	78.6%	21.4%	100.0%	
		% within Sample	100.0%	90.0%	97.7%	
	Negative	Count	0	2	2	CC =0.270 P=0.009 HS
		% within Anti CMV IgG	0.0%	100.0%	100.0%	
		% within Sample	0.0%	10.0%	2.3%	
Anti CMV IgM	Positive	Count	7	4	11	FEPT P=0.229 NS
		% within Anti CMV IgM	63.6%	36.4%	100.0%	
		% within Sample	10.6%	20.0%	12.8%	
	Negative	Count	59	16	75	CC =0.118 P=0.270 NS
		% within Anti CMV IgM	78.7%	21.3%	100.0%	
		% within Sample	89.4%	80.0%	87.2%	
Anti HSV IgG	Positive	Count	64	20	84	FEPT P=0.587 NS
		% within Anti HSV IgG	76.2%	23.8%	100.0%	
		% within Sample	97.0%	100.0%	97.7%	
	Negative	Count	2	0	2	CC =0.085 P=0.431 NS
		% within Anti HSV IgG	100.0%	0.0%	100.0%	
		% within Sample	3.0%	0.0%	2.3%	
Total Count			66	20	86	—

NS: Non Significant at P>0.05; HS: Highly Significant at P<0.01

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The cytotoxic effect of Apricot Seed (*Prunusarmeniaca*) on human and animal tumor cell line

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ABSTRACT

The present study was designed to evaluate the cytotoxic effect of apricot seed (*Prunusarmeniaca)* . The cytotoxic effect ofmethanolicextract was carried on two tumor cell line (L20B and RD) . Additionally chemical detection of the flavonoids, glycosides and alkaloids was also carried out.The chemical detection revealed that the methanol extract was positive for flavonoids and glycoside and it was negative for alkaloids.Also the methanolic extract showed an inhibitory effect on L20B and RD cell line growth rate after 72 hrfor all concentrationsin comparison with control.

Key words: Herbal medicine, apricot seed,cytotoxicity,cellline,cancer

الملخص باللغة العربية

اجريت هذه الدراسة لمعرفة التأثير السمي لبذور المشمش على خطين من الخلايا السرطانية (*L20B*و*RD*) للمستخلص الميثانولي لبذور المشمش.بالإضافة الى الكشف الكيميائي للفلافينويداتوالكلايكوسيداتوالقلويدات .لقد اظهر الكشف الكيميائي للمستخلص احتوائه على الفلافونيداتوالكلايكوسيدات وخلوه من القلويدات.كما اظهر مستخلص بذور نبات المشمش فعالية سمية واضحة في خطي الخلايا السرطانية خلال مدة التعريض (72ساعة)ولجميع التراكيز المستخدمة بالمقارنة مع السيطرة.

INTRODUCTION

Herbal medicines are dietary supplements that contain herbs , either singly or in mixture . A herb also is a plant or plant part used for its scent, flavor, and/or therapeutic properties. Products made from botanicals that are used to maintain or improve health have been called herbal supplements ,botanicals, or phytomedicines (1).However, natural products provide an inexhaustible source of anticancer drugs in terms of both variety and mechanism of action(2). The use of herbal supplements by cancer patients in the preoperative period is prevalent and consistent with the substantial increase in the use of alternative medical therapies by cancer patients (3).

In recent years , nutrients as well as non-nutrient phytochemicals are being extensively explored for their potential preventive effects against cancer (4). In this regard, phytochemicals are shown to induce differentiation and apoptosis accompanied with growth inhibition in cancer cells (5).

Cancer results from disruption in the control normally exerted over production and differentiation. One key aspect of this abnormal differentiation is the greatly prolonged life spans of cancer cells compared with those of their normal counterparts. Cancer cells are essentially immortal. Another aspect is the failure of the cancer cells to develop the specialized functions of their normal counterpart, (6).Carcinogenic processes involve multiple steps (7). It can be readily appreciated that, in the body of a healthy human, there are many cells which already have genetic alterations of cancer related genes caused by various genotoxic substances, including dietary carcinogens. Moreover, genomic instability (8), frequently resulting from mutation in genes, encoding proteins related to DNA repair would be expected to be produced by mutational events. If a mutation occurs in the genes, more rapid accumulation of additional gene alterations would be yielded in other cancer-related gene. Therefore, the potential contribution of minute amounts of mutagens/ carcinogens present in the diet can not be over looked with regard to the significance for carcinogenesis (9).

However, the component of individual herbal medicines is rather complicated and its effect is usually multiple and not single. So, beside the antineoplastic effect, the other effects are also presented. And although the active antineoplastic ingredient have been isolated from some of the herbal medicines, these ingredients may exert effects other than antineoplastic. This multiple effect phenomenon is also observed in some of the western mono-component antineoplastic drugs. For example, cyclophosphamide can act on the various phases of proliferative cells causing degeneration of DNA, RNA, enzymes and protein and serves as a killer of tumor cells (10).

The herbal medicines achieves their antineoplastic effect through various ways. Moreover, some medicine can bring on several actions, for example, they may directly inhibit the growth of tumor as well as indirectly exert an antineoplastic effect by enhancing the bodily immunologic function. Generally, they elicit no significant adverse effect on the human body and this is a strong point of herbal medicine for antineoplastic treatment (11).

Apricot seed is the small kernel enclosed within the wood-like pit at the center of the apricot fruit. Also the seed of apricot contain oil, this oil contains olein,glyceride of linoleic acid and a transparent, crystalline chemical compound,amygdalin,orlaetrile. This compound is also known as vitamin B17(12).Chinese medicine

practitioners use apricot seed as a treatment in respiratory disease, including bronchitis and emphysema. There has been considerable controversy regarding apricot seed, and specifically amygdaline,one of its components. Since the 1920,in many countries around the globle it has been recognized as a possible cancer preventive and malignant cell growth inhibitor. Several studies done in the United states through out the 1970s and early 1980s demonstrated that amygdalin did not kill cancer cells(13). Also this study aimed to evaluate the cytotoxic effect of *Prunusarmeniaca* on animal and human cancer cell line .

MATERIALS AND METHODS

Extraction of Plant(Methanolic Extract):

(50 g) of plant powder was extracted with 250 ml of 70% methanol by Soxhlet apparatus for 6 hours at 40-60ºC, then the solvent was removed under reduced pressure by rotary evaporator at 40ºC, and the crude extract was kept until used (14).

Cytotoxicity of Plants Extracts on Tumor Cells:

Cell line study of the plant *Prunusarmeniaca*was carried out in Biotechnology Research Center/Al-NahrainUniversity . In this study, the preliminary screening on cytotoxic activity of *Prunusarmeniaca*was carried out .
The screening involved the investigation of cytotoxicity of methanolic extract of *Prunusarmeniaca*,then the extract were evaporated until complete dryness. The screening of cytotoxiciy was carried out on tumor cell lien L20B and RD.

The percentage of growth inhibition was calculated according to(15) ,and according to following equation :

Growth inhibition % =

$$\frac{Control - Treatment\,cell}{Control} \times 100$$

Cell line procedure:

This was applied according to the method adopted by (16) .
1- L20B,RD were cultured in culture in culture bottles (falcons) then stored at 20ºC.
2- The cultured cells were washed with phosphate buffer saline (PBS). The trypsine –versine solution was added with gentle shake , then the final mixtures werepoured to another culture bottles (Sub-culturing) and incubated at 37ºC for 1 minutes .
3- Counting of viable cells was carried out using trypan – blue dye (0.4%).Dead cells take up the dye and appear blue under microscope while living cells exclude the dye and appear white.
4- Cytotoxic assay of plants extract were done using neutral red cytotoxic assay. The plant extract was dissolved in dimethylsulfoxide, then six concentrations (75, 125,250,500,750,1000) µg/ml of each plant extract were prepared.
5- The 0.2 ml of cultured cell (L20B,RD) were transported to 96-well micro plates, so each well contained 10^5 cell, followed by addition of 0.2 ml ofprepared concentrations of plant extract, leaving some wellscontained cultured cells but without any treatment by plant extract to be considered as negative control.
6- The cells were incubated for 72 hours at 37º C ,and then washed with PBS followed by addition of neutral red solution (0.8ml) to each well re-incubated at (37ºC) for 2 hours.

7-After incubation, the medium was discarded, and the wells were washed with PBS. Viable cells would take the dye, while the dead cells would not take dye.0.1ml of phosphate and buffered-ethanol(0.1M NaH_2PO_4-ethanol; 1:1), were added to eluent the dye from the viable cells.

8-The plate was read by micro ELISA reader at an optical density of (492nm).

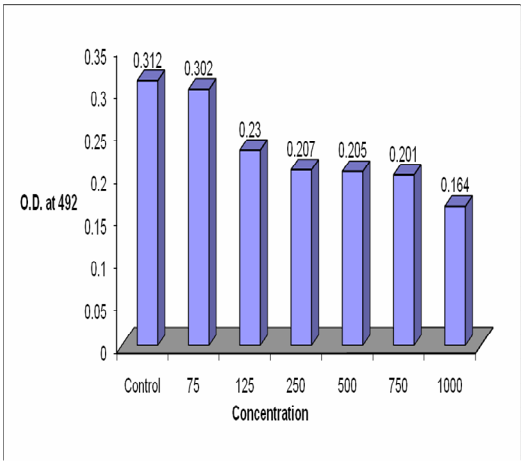
RESULTS AND DISCUSSION

The results of plant extract on their effect on both cell lines shows there are also a significant differences ($P<0.05$) between means of cell viability of each L20B and RD cultures treated with *Prunusarmeniaca*extract. Table (1). *Prunusarmeniaca*cause a significant decrease ($P<0.05$) in cell viability of L20B and RD cell line for all concentrations to reach maximum significant decrease at concentration 1000µg/ml in comparison with the negative control Fig(1) ,Fig (2) .

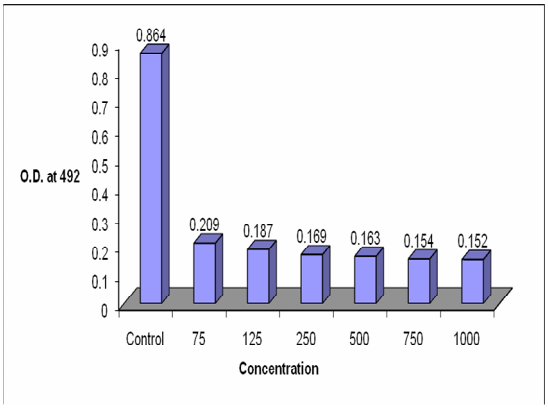
Table(1) Cytotoxic effect of *Prunusarmeniaca*extract on the growth of cancer cell line(L20B,RD).

Concentration µg/ml	Cell viability (Absorbance) % (m)	
	L20B	RD
	P. armeniaca	*P. armeniaca*
Negative control	A 0.312	a 0.864
75	B 0.302	b 0.209
125	B 0.23	c 0.187
250	B 0.207	c 0.169
500	C 0.205	d 0.163
750	D 0.201	d 0.154
1000	D 0.164	e 0.152

Differences A,B,C,D,E are significant (p<0.05) to compression column



Figure(1)Growth inhibition percentage of *P. armeniaca*on L20B after 72hr



Figure(2)Growth inhibition percentage of *P. armeniaca*on RD after 72hr

DISCUSSION

All cancer cells have sequester iron ,and the mechanism of action of flavonoids and its derivatives depended on the presence of iron in cells at high concentration,so,according to(17),it is believed to work because when these compounds and its derivatives come into contact with iron, a chemical reaction ensures spawning charged atoms that effect free radicals. Cells need iron to replicate DNA when they divide, and since cancer is characterized by out-of-control cell division, cancer cells have much higher iron concentrations than do normal cells. Pumping up cancer cells with even more iron and then introduce flavonoids selectively kill these cells(17).(18)suggested that quercetin is an anti-cancer agent arrests the cell cycle at G1 and S phase boundary. In addition, it has been found that the increase of the concentration of chemotherapeutic agents in some cell resistant cell lines and *in vitro* research demonstrates that quercetin can increase the anti-tumor activity of Cisplatin and Busulfan and can be used in conjunction with doxorubicin and etoposide without interfering with their therapeutic action (19).

Also many plant active compounds like flavonoids play an important role in reducing genotoxicity as they are considered in preventing DNA damage and scavenging the hydroxyl radicals and super oxide ions .also, flavonoids are of the compounds which increase the activity of detoxification enzymes which are important as a scavenger for free radicals (20).

Cell cycle progression is an important biological events having controlled regulation in normal cells , which almost universally becomes aberrant or deregulated transformed and neoplastic cells. In this regard, targeting deregulated cell cycle progression and its modulation by various natural and synthetic agents are gaining wide spread attention in recent years to control the unchecked growth and proliferation in cancer cells .It was show that many phytochemicals halt are uncontrolled cell cycle progression in cancer cells. Among these phytochemicals , natural flavonoids have been identified in the plant extract as a one of the major classes of natural anticancer agents exerting antineoplastic activity via cell cycle arrest as a major mechanism in various types of cancer cells (21).

Some of secondary metabolites have a selective toxicity on its effect on cancer cells, and by depending on the result, it was found that plant extract has greater cytotoxic effect on two cancer cell line(Table 1) From this, we conclude that selective toxicity may be due to the metabolic factor found in the cancer cell lines but not found in normal cells , like the angiogenic promoters and inhibitors , and associated signaling in both tumor as endothelial cells (22) ,(23). In addition to DNA of tumor cell found in relaxant shape ,and the DNA molecule was found in a unstable figure because the far away between the H-bond which connect the both strand of DNA and this make easy for compound to interfere or associated to both strands of DNA, while DNA of normal cell has a strong H-bond connect the both strands to each other and make it more stable, so the compounds cannot interfere or associated with DNA strand (24).

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The effect of *Toxoplasma gondii* on Gonadotrophic Hormones among Iraqi women with habital abortion

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ABSTRACT

Toxoplasmosis is caused by parasite ***Toxoplasma gondii*** one of the important causes in cases of spontaneous abortion or stillborns or in complete growth or may shown mental retardation of the infected mother during pregnancy
Recent studies have shown that hormones could induce anti-parasitic functions of the host immune systems.
This study aimed to determine the immuno-modulatory effect on selected hormones (LH&FSH) **in** Iraqi women having toxoplasmosis**.**
Selecting 24 women with spontaneous abortion aged rang (20-40) years old and 24 healthy women matched age as control determined their Immune globulin (IgG/IgM) & gonadotrophic hormones LH &FSH in their sera by an accurate &simple ELIFA method .
A significant decrease in LH level and non-significant decrease in FSH level was observed in the results. The relationship between the gonado hormones & IgG,IgM had also been discussed.

Keywords: Toxoplasma gondii ,LH,FSH,GnRH, Spontaneous abortion ELTA

الملخص باللغة العربية

يعتبر داء المقوسات Toxoplasmosis " داء القطط " الناتج عن طفيلي المقوسات الكونيد يه ***Toxoplasma gondii*** احد المسببات المهمة لحصول الإجهاض العضوي او ولادة جنين ميت أو غير مكتمل النمو أو متخلف عقليا نتيجة إصابة الأم خلال فترة الحمل بهذا الطفيلي .

بينت الدراسات مؤخرا أن يمكن للهرمونات أن تحث المضادات الطفيلية على وظائف النظام المناعي لجسم المضيف .

لدراسة تأثير النظام المناعي على هرمونات ال(LH&FSH) نتيجة الإصابة بطفيلي المقوسات الكوانديه ***T.gondii*** , تم اختيار (24) امرأة مصابه بالإجهاض العضوي بمدى من العمر (20-40) سنه مع 24 امرأة بنفس المدى من العمر بمجموعة السيطرة .فحص مستويات الكلوبيولينات المناعية لهن (IgG&IgM) وهرمونات المناسل LH&FSH في أمصالهن بطريقه دقيقه وبسيطة ELIFA فحص المناعي للتبلور الإنزيمي المرتبط.

لوحظ انخفاض ملحوظ في مستويات LH و غير ملحوظ لfsh في أمصال المصابات بطفيلي داء القطط مقارنة بمجموعة السيطرة النساء الأصحاء . وتم مناقشة العلاقة بين IgG&IgM وهرمونات المناسل .

إن الإصابة بطفيلي ***T.gondii*** يؤثر على النظام العصبي مسببا انخفاض FSH و LH مع علاقة خطيه عكسية سالبه مابين الهرمونات ومعاملات الكلوبيولينات المناعية .

INTRODUCTION

Toxoplasmosis ,a worldwide infection caused by the obligatory intracellular coccidian .The parasite ***toxoplasma gondii*** ;is usually acquired through the ingestion of raw or under cooked meat or by contamination by oocysts present in the faeces of cats infected with the parasite(1) . During the acute stage of the infection, tachyzoites quickly proliferate within a variety of the nucleated cells and spread through out host tissues (2).

The clinical manifestations of toxoplasmosis result from direct tissue destruction by the parasite , but inflammatory cytokine- mediated immuno pathological changes may also contribute to disease progression (3).Toxoplasmosis can cause encephalitis(inflammation of the brain) and neurological diseases , and can affect the heart ,liver ,inner ears , and eyes (4).Also it is one of the classical conditions known to have approved adverse effect on human reproductive function (5).

The spontaneous abortion is considered as one of the important clinical problems that may affect pregnant causing dangers to the mother and her fetus (6) Toxoplasmosis is one of important causes in cases of spontaneous abortion or stillbirth or incomplete growth or may show mental retardation of the infected mother during pregnancy, in addition the high rate of the infection among the population who gave a positive result of serological which proved the existence of infection (7). There are many other reasons of the spontaneous abortion. one of the causes is Maternal infections , microorganisms(like toxoplasis) , literia monocytogenes , cytomegalovirus and faravirus are known to cause sporadic pregnancy loss , but no infections agent has been proven to cause recurrent spontaneous abortion (8--15) .

Pregnancy is commonly recognized as state of physiological and temporary insulin resistance. This condition is driven by high concentrations of glycoprotein hormones i.e.,LH,FSH,PRL, and placenta derived human placental lactogen . Experimental evidence has established that mice undergo acquired hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction after chronic infection with ***Toxoplasa gondii*** (16--18).

Follicle-stimulating hormone (FSH)and lutenizing hormone (LH)are glycoprotein secreted by the anterior pituitary in response to gonadotropin hormon (GnRH),which is released by the hypothalamus .Both FSH and LH bind to its receptors presence in the testis and ovaries and regulate gonadal function by promoting sex steroid production and gametogenesis (19).

The aim of the present study was to evaluate the relation of LH and FSH levels in sera of Iraqi women with habitual abortion infected with *Toxoplasma gondii* .

MATERIALS AND METHODS

The study carried on forty eight (48) married women aged between (20-40) years during the period Feb., 2010 to Feb., 2011. Twenty four (24) of them were suffered from spontaneous abortion more than two times and they were found to be positive for mild symptoms of acute toxoplasma infection. All subjects were referred to Fattama Al-Zahraa Hospital in Baghdad/Iraq. Non of the patients had a family history of hypogondanadism .They were of normal height and weight no significant history of any drug use .The control group (no.

24)consisted of age- matched healthy women and their physical and biochemical finding were within the normal limits.

Serum Sampling:

Five milliliters of samples of venous blood were taken and left for 15 minutes at room temperature. After blood coagulation, the sera were separated by centrifugation at 4000 rpm for 10 minutes. The sera stored at -20Ċ until being used. Hemolyzed samples were discarded. All blood samples were taken after finish the menstrual (Latuel phase).

Serological Tests

Detection of Specific IgG and IgM Antibodies:

Serum concentration of antitoxoplasma IgG and IgM were measured by double antibody technique using enzyme –Linked fluorescent assay (ELFA) on an immuno serological analyzer VIDAS with a set of reagents (bio Mereieux, France).

Measurement of serum hormone concentration:

Serum FSH and LH were determined by using a commercially available microparticle enzyme immunoassay kit supplied from Bio Mereieux company,France LH and FSH were analyzed using an automated quantitative test combining immunoenzymatic detection with fluorescence (ELFA) on an immunoserological analyzer VIDAS.

Statistical Analysis:

All of the statistical analyses were performed by spss 10.0 (spss Inc.,USA) . Data were expressed as means ±standard deviation (SD). Comparisons between means were performed with the use of the students test and person's correlation coefficients.
Correlations between gonadotrophin hormones & immunological parameters were investigated by spermans p-test $p \leq 0.05$ was considered statistically significant (20). (21).

RESULTS AND DISCUSSION

Based on serological tests (ELIFA IgG/IgM) the disease toxoplasmosis was diagnosed by the presence of anti –***T. gondii*** antibodies. Table (1) shows the mean levels and comparisons of parameters in patients and control group .All patients were seropositive for ***T.gondii*** antibodies and significantly higher than control group.

Table (1): The mean levels of IgG & IgM in patients and controls

Groups/parameters	Control	Patients
Number(No.)	IgG(24) IgM(24)	IgG (20) IgM(20)
Age range (years)	20-41	19-40
Range value Mean ±SD IgG P value SE	8.0-30 15.8958±7. 6300 0.015 1.5575	8.5-127 54.200±38. 8754 0.593 8.6928
Range value Mean ±SD IgM I P value SE	0.10-0.63 0.2517±0.1 721 0.140 3.513	0.10-19.0 2.8710±6.6 670 0.069 1.4408

Usually women with Toxoplasmosis were diagnosed by the increase of anti-T.gondii (i.e.IgG&IgM) in their sera. This fact is in line with our results. Previous studies have suggested that a variety of parasitic and host factors, as well as unrecognized cofactors, may influence disease presentations in toxoplasmosis (22 , 23). The differences in immunoglobulin levels depending on methods used (24) . The wide range of *T.gondii* immunoglobulin antibodies (IgG from 8.5-127) and (IgM is from 0.1 - 19.0) may be due to various duration of infection in the patients under this study .

Data in table (2) shows a non significant decrease in LH levels in sera of women infected with toxoplasma as compared with that of healthy women (control group).

Table(2) LH Concentration in sera of patients and control group

Group	Samples NO.	Mean(Iu/ml)	SD	SE	P value
Patients	24	3.6250	1.3437	0.2743	0.009
Control	24	6.8450	3.1038	1.2459	

Our results are in agreement with Dlugouska H. and Dzibko k. (2009) study on steroid hormones and suggested that some parasites produce steroid hormones and alter host hormones levels (25).

On the other hand the FSH level had slightly decreased as shown in table (3)

Table (3) FSH Concentration in the sera of patients and control groups

Group	Samples Number	Mean(Au)	SD	SE	pvalue
Patients	24	5.6313	1.2202	0.2491	0.222
Control	24	6.8900	1.2574	0.2567	

This observation is in line with previous results of experimental studies in animals (16,17,18).Stahl et.al demonstrated that female mice undergo acquired hypogonadotrophic hypogondism secondary to hypothalamic dysfunction within a few weeks after infection with ***T.gondii*** (16,17).

In our study, we can demonstrate for the first time in Iraq that hypogonadotrophic in women is not rare in Toxoplasma infection

These results may indicate that the inhibition of gonadotrophic release may result from the release of GnRH (gonado trophic releasing Hormone) receptors either through a direct effect on the pituitary or modulating the release of GnRH form hypothalmic neurons able to induce a reduction in pituitary GnRH receptor(26).Oktenli C et al; concluded that acute toxoplasma infection in man may cause temporary hypogondatrophic gondal insufficiency regardless of the course of the disease (27).

The correlations between the immunological parameters (IgG&IgM) and the hormones feeding gonads (LH&FSH) have been studied. It shows a negative relationship with (LH r=-0.367) with IgG and(r=-0.021) with IgM and the ratio was significant ($p<0.05$). But, with FSH it was a negative with IgG r=0.252 and positive with IgM r=0.115, the ratio was non-

significant ($p<0.05$). This means that any decrease in the quantity of IgG and IgM is accompanied by decrease in the quantity of LH and FSH as it shown in table (4):

Table (4) Relation factor and p value (by T-test) for FSH &LH levels with IgG&IgM in the sera of patients

Parameters	Factors	FSH	LH
IgG	r p	-0.252 0.283	0.367- 0.012
IgM	r p	0.115 0.629	-0.021 0.031

In the current study the evaluation of data in regards to the hormones level didn't high remark able changes in correlation of immunoglobulin antibodies IgG,IgM of LH and FSH Levels .Several studies revealed a close functional relations between The immune , nervous &endocrine systems which communicate between each other using the common mediators &their receptors . the immune cells not only receive signals from the endocrine system but also produce numerous hormones , usually after stimulating with Ag including parasite Ag .(25)

In conclusion ,our study proved that T.gondii can affecte the endocrine system causing hypogondatrophic gonadal (FSH;LH)insufficiency Further more there is a linear interrelation ship between gonad hormones associated with infection with ***T. gondii*** . However , at present the following needs to be further clarified the other hormones GnRH, estradiol and progesterone involved , and their mechanism of action

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قسم الدراسات العربية

ARABIC SECTION

الامتزاز الحيوي لأيونات الكادميوم من المحاليل المائية باستخدام تقنية تقييد الخلايا البكتيرية

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الخلاصة باللغة العربية

هدفت الدراسة إلى إيجاد طريقة لمعالجة التلوث بالكادميوم وذلك باستخدام البكتيريا المقيدة على مواد ذات كلف واطئة ولا تشكل خطورة على الإنسان.
اختبرت كفاءة خمسة عزلات بكتيرية تم جلبها من كلية العلوم لامتزاز الكادميوم من المحاليل المائية وأثبتت العزلة البكتيرية Enterobacter agglomerans الأفضلية حيث بلغت نسبة الامتزاز 99,2%
صممت منظومة مختبريه لإزالة الكادميوم بتقنية البكتيريا المقيدة على مواد أساس مختلفة (الجينات الصوديوم , الرمل الأبيض) والمنظومة تعمل بنظام الجريان المستمر وأثبتت مادة الأساس (الجينات الصوديوم) أفضلية في التقييد
درست الظروف الملائمة لحصول الامتزاز وكانت 40° م ورقم هيدروجيني 4 وفترة تماس نصف ساعة
أثبتت الدراسة إمكانية استرجاع ايونات الكادميوم الممتزة باستخدام عدة أنواع من محاليل الغسل وكان محلول HCl الأفضل ويمكن استخدام المنظومة لأكثر من دورة تحميل وغسل وبكفاءة بلغت (78,5%، 76%، 69%) للدورات الأولى ،الثانية والثالثة على التوالي.

الكلمات الدالة: الامتزاز الحيوي، البكتريا المقيدة

ABSTRACT

The objective of the study was to find a way to treat the pollution by cadmium, using of immobilized bacteria on low cost materials and does not constitute a risk to human.
We Tested the efficiency of three the isolates bacterial from the Faculty of Science to adsorption of cadmium from aqueous solutions ,the isolation bacterial *Enterobacter agglomerans* proven it is the best where the percentage of adsorption 99.2%
We designed laboratory planet to remove cadmium by technology immobilized bacteria on the basis of different materials (sodium alginate, white sand) and the planet is working by continuous flow system.
The sodium alginate proved to be the best in the immobilized
We Studied the best adsorption conditions was 40°C and pH 4 and a half hour contact
The study proved the possibility of recovery of cadmium ions adsorbed by using several types of washing solvents, we find that the solution of HCl is better and can use it for more than one cycle of loading and washing an efficiency of (78.5%, 76%, 69%) for the first, second and third respectively.

المقدمة

أصبحت مشكلة تلوث البيئة بالمياه المخلفة من المنشآت الصناعية واحدة من أهم المشاكل البيئية في العالم. ونتيجة للتطور الصناعي الكبير وما ينتج عنه من نفايات ملوثة فان التقنيات والجهود المبذولة للسيطرة على هذا التلوث أصبحت غير قادرة على المضي قدما للتخلص من النفايات الملوثة الكبيرة والمتزايدة والناتجة من الصناعة . أن الإضرار البيئية الناجمة عن التلوث الصناعي لا تعد ولا تحصى، ولقد كان المفهوم السائد في الدول الصناعية إن الانتعاش الاقتصادي والتوسع الصناعي يصاحبه بالضرورة تدهور بيئي، أما الآن فان ظاهرة التلوث تعد مقياسا للتخلف التكنولوجي (1)

تلعب مياه الفضلات الصناعية فضلاً عن مياه الفضلات المنزلية والزراعية دورا رئيسا في تلوث المياه الطبيعية التي أدت جميعها إلى الإخلال بالتوازن البيئي الطبيعي لمصادر المياه الطبيعية ،من المعروف أن معظم الصناعات تتركز عادة قرب الأنهار والممرات المائية لما توفره من المياه اللازمة لتشغيل عملياتها التصنيعية من جانب، ونقل منتجاتها من جانب آخر فضلاً عن إن هذه الأنهار تشكل الأماكن المثلى في بعض دول العالم لطرح المياه الصناعية الناتجة، والتي تكون حاوية على تراكيز عالية من مواد ومركبات كيميائية معقدة وعناصر سامة فضلا عن الزيوت والشحوم ومواد التنظيف، وقد تحتوي أحيانا على المواد المشعة. وتشكل هذه الملوثات الصناعية خطرا يهدد الكائنات الحية جميعًا بشكل مباشر أو غير مباشر (2)

تعد العناصر الثقيلة من بين الملوثات غير التقليدية والسامة مثل (الزئبق والرصاص والكادميوم) ووجود هذه العناصر في المياه بتراكيز عالية تعد من المشاكل المهمة بسبب ما تمتلكه من خاصية سمية للكائنات الحية المائية جميعها ، وكذلك قابليتها على التراكم في أنسجة بعض الكائنات المائية، وانتقالها عبر السلسلة الغذائية إلى الإنسان. وبالنظر لزيادة طرح مياه الفضلات الصناعية الملوثة بهذه العناصر، فقد اعتمدت في اغلب الصناعات على استخدام المعالجات بالطرق الكيميائية أو الفيزيائية أو المختلطة لإزالة هذه العناصر الملوثة موقعيا ومن ثم طرحها إلى الأنهار،إلا انه ثبت عدم فاعلية مثل هذه المعالجات في إزالة المواد السامة في كثير من الأحيان (3) لذا اتجهت الدول الصناعية المتقدمة والكثير من دول العالم إلى اعتماد المعالجة البيولوجية المتخصصة لإزالة الملوثات من مياه الفضلات الصناعية المطروحة عن طريق استخدام أنظمة مايكرو بيولوجية انتقائية تعمل على استهلاك الملوثات وتحويلها إلى مكونات أو مركبات أخرى لا تشكل خطورة على الإنسان وما هو مسخر لحياته (4).

الكادميوم :

عنصر كيمائي نادر الوجود نوعا ما . وهو معدن ابيض- فضي يوجد على شكل كلوريدات أو اوكسيدات أو كبريتات وأملاحه جميعها سامة،وله قابلية الذوبان في الماء (5). ليس للكادميوم أية وظيفة حياتية،وهو غير ضروري لحياة النباتات والحيوانات ، يدخل الكادميوم إلى البيئة من ثلاث طرق رئيسية وهي:

تنقية واستخدام الكادميوم ، انصهار النحاس والنيكل ، احتراق الوقود .

أما المصادر الطبيعية لدخول الكادميوم إلى الغلاف الجوي فهي ذرات الغبار المنقولة عن طريق الرياح وحرائق الغابات والانبعاثات البركانية . وتقدر كمية الكادميوم في الجو من النشاطات الصناعية حوالي 3 – 10 مرات أكثر من الكادميوم المضاف من المصادر الطبيعية . وقد صنف الكادميوم من قبل وكالة حماية البيئة EPA بأنه واحد من 25 مادة خطرة فيها تهديد لحياة الإنسان . الشكل المعدني للكادميوم غير ذائب في المياه ، ويوجد بشكل كبريتات في عمليات استخراج الزنك غير النقي ، ويستخدم الكادميوم صناعيا بصورة واسعة في السبائك مع النحاس والرصاص والفضة والألمنيوم والنيكل ، ويستخدم أيضا في الصفائح الالكترونية والسيراميك والأصباغ والتفاعلات النووية. إن كلوريدات ونترات وكبريتات الكادميوم تكون ذات قابلية ذوبان عالية في المياه أما كاربونات وهيدروكسيدات الكادميوم فتكون غير ذائبة (6) يدخل الكادميوم إلى جسم الإنسان عن طريق الغذاء لاسيما بالنسبة للأشخاص غير المدخنين ، إما المدخنين فيصل إليهم عن طريق تدخين السجائر لان الكادميوم له خاصية التجمع بصورة كبيرة في نبات التبغ (7)[1]. إن الكثير من التأثيرات الصحية تعزى إلى ابتلاع الكادميوم ، مثلا فرط الضغط الذي هو مترابط مع زيادة احتباس الكادميوم في الكلى . ويسبب ابتلاع الكادميوم المغص ، والغثيان ، والتقيؤ ، والإسهال .وقد يسبب مرض فرط إفراز الكالسيوم إيتاي- إيتاي (Itai-Itai) الذي يؤدي إلى وهن العظام .

أنواع المعالجات المستخدمة لإزالة التلوث بالعناصر الثقيلة:

أ. المعادلة والترسيب على شكل هيدروكسيدات أو سلفات
ب. التنافذ بالأغشية تستعمل
ج. التبادل ألايوني

ومن أكثر الطرق المستعملة بصورة تجارية هي عملية المعادلة والترسيب ، وهذا يعتمد على حجم المواد المعالجة وكمية المواد الكيميائية المضافة ، ومن أحد مضار هذه الطريقة هو تكون الأوحال(بمعنى آخر تحول التلوث إلى مواد مترسبة) (8) هذا أدى إلى التفكير بطريقة اقل كلفة وقابلية عالية في الإزالة. ومع التطور اثبت الامتزاز الحيوي فاعليته في معالجة المياه بكلفة اقل .

الامتزاز الحيوي:

ويقصد به اخذ (Uptake) أيونات العناصر الثقيلة والنويدات المشعة بصورة رئيسة من المحاليل المائية بوساطة الأحياء المجهرية الحية والميتة (11)، (9) ، (5) .قد يكون الامتزاز معتمداً على أيض الخلية أو قد يكون عملية فيزيوكيميائية وبذلك يتضمن ارتباط ايونات العنصر الموجبة مع المجاميع السالبة مثل: الكاربوكسيل ، والهيدروكسيل ، والفوسفات الموجودة على البوليمرات الميكروبية تشمل البروتينات والسكريات المتعددة المكونة للجدار الخلوي (12). والامتزاز الحيوي يعتمد بالأساس على السعة والقابلية العالية لأنواع مختلفة من الأحياء المجهرية على الارتباط مع العناصر الثقيلة . ويمكن استخدام أنواع مختلفة من الأحياء المجهرية مثل: الطحالب، الفطريات، البكتريا والخمائر ، من أهم ميزات المعالجة باستخدام الامتزاز الحيوي هي:

- كلفة منخفضة.
- الألفة العالية لايونات العناصر الثقيلة.
- الحمأة المتكونة قليلة.
- لا تحتاج إلى إضافة مواد كيميائية أو مغذية.
- يمكن إعادة تنشيط المازات الحيوية واستعمالها مرة أخرى.
- إمكانية استرداد اغلب ايونات العناصر الثقيلة المزالة (13) .

تتضمن عمليات الامتزاز الحيوي الطور الصلب متمثلا بالمازات الحيوية (Biosorbent) والطور السائل الذي يتضمن الماء والمذيبات الحاوية على الايونات، وعملية الامتزاز تستمر حتى حصول عملية الإشباع .

تقنية تقييد الخلايا: ويقصد بها العملية التي تتم بوساطتها تحديد حركة الخلايا الميكروبية أو مشتقاتها أو الأنزيمات بامتزازها على سطوح مواد ساندة أو اقتناصها داخل المواد مثل : الألياف ، والهلامات ،أو المواد البلاستيكية وغيرها (6) . وهي إحدى الطرق التقليدية الرائدة للتقنية الحيوية .

أما المواد المستعملة في هذا المجال كثيرة فضلاً عن إنها توجد بإشكال مختلفة ويمكن تقسيم المواد المستعملة على قسمين:-

1. **المواد اللاعضوية :** إن العديد من المواد اللاعضوية يمكن استعمالها بوصفها مواداً مقيدة مثل السيراميك الذي يستخدم بكثرة في الصناعات البتروكيميائية كذلك الأطيان ، الالومينا ، البيركس ، الرمل ، الفحم الحبيبي. تستخدم لتقييد أنواع مختلفة من الأجناس البكتيرية لمعالجة المياه الملوثة بالعناصر الثقيلة .و المواد اللاعضوية المستعملة للتقييد يجب أن تكون خاملة وغير ذائبة في الماء وصلبة وغير قابلة للانضغاط ،
2. **المواد العضوية :** وهي مواد تكون ذات طبيعة بوليميرية تستعمل بصورة مباشرة أو يمكن استعمال مشتقاتها أو المواد الناتجة منها بعد إجراء بعض التحويرات عليها كي تصبح ملائمة للتقييد ومنها: السليلوز ومشتقاته ،الاكاروز، النشا الكولاجين، ومتعدد الاكريلامايد ، والالجينات ، والبوليمرات الصناعية مثل الستايرين(15).

المواد وطرائق العمل

تم اختبار كفاءة الخلايا البكتيرية المعزولة لامتزاز الكادميوم حيويا من المحاليل المائية: وزع محلول الكادميوم بتركيز 10 ملغم / لتر ورقم هيدروجيني 4 في دوارق مخروطية بواقع 20 مل في كل دورق ، ثم أضيفت الخلايا البكتيرية المحضرة إلى محلول الكادميوم بحجم يعطي تركيزا نهائيا قدره (0.5) ملغم (وزن جاف) / سم3 أغلقت الدوارق جيدا ووضعت في حاضنة هزازة عند درجة حرارة (40) °م وبسرعة هز قدرها (2000 دورة / دقيقة) لمدة ساعة واحدة، جمعت الخلايا بعد انتهاء المدة بواسطة المنبذة، واخذ الراشح لغرض قياس كمية

الكادميوم فيه بوساطة جهاز طيف الامتصاص الذري أللهبي .انتخبت الخلايا البكتيرية الأكفأ في الامتزاز للتجارب اللاحقة.

وصف عام للمنظومة:

وهي عبارة عن عمود زجاجي بطول 25 سم تسد نهايتي العمود بوساطة سدادات مطاطية تخترقها توصيلات زجاجية لتتصل من الطرف الآخر بأنابيب بلاستيكية، توضع في النهاية السفلى للعمود ورق ترشيح وذلك لمنع نزول المادة الأساس وحدوث انسداد في الأنابيب البلاستيكية. يرتبط الأنبوب البلاستيكي الأسفل عبر مضخة دافعة Peristaltic pump بالوعاء الذي يحوي على محلول الكادميوم وبوساطة المضخة الدافعة وبسرعة جريان مناسبة يتم دفع المحلول إلى داخل العمود من الأسفل إلى الأعلى ويسحب الناتج من الأعلى في دورق زجاجي لغرض قياس تركيز الكادميوم فيه.

تقييد الخلايا البكتيرية على مواد الأساس:

1. **الرمل الأبيض** :- وهو نوع من الرمل الأبيض الذي يتكون من حبيبات صغيرة من مادة السليكا SiO_2 تتراوح أقطارها بين (0.2-0.5) ملم ، واستعملت هذه المادة بعد غسلها عدة مرات بالماء المقطر ثم تجفيفها لحين ثبات الوزن. تم وزن (60) غم من مادة الرمل المجففة وتعقم داخل الفرن عند (160) °م لمدة ساعة. تبرد وتعبأ في العمود المعقم. يضاف حجم معين من عالق البكتريا إلى 20 مل من المحلول الملحي الفسيولوجي (Normal saline) ليعطي تركيزا نهائيا للخلايا قدره 0.5 ملغم/ مل. يضاف إلى العمود من الأعلى بعد أن يبرد ، ثم تترك الخلايا لمدة (24) ساعة لتستقر . يزود العمود بمحلول الكادميوم بتركيز 10 ملغم / لتر ورقم هيدروجيني (4) ، ويحضن عند درجة (40) °م وبسرعة جريان (0.5) مل / دقيقة. ثم يسحب الناتج من الأعلى لغرض قياس تركيز الكادميوم فيه.
2. **هلام الالجينات** :- يذاب (2) غم من مادة الجينات الصوديوم في (50) مل ماء مقطر مع التحريك المستمر حتى يتجانس ثم يضاف العالق البكتيري بتركيز نهائي (0.5) ملغم (وزن جاف) / مل ، وبوساطة محقنه نبيذة معقمة سعة (10) مل يقطر الجينات الصوديوم فوق محلول كلوريد الكالسيوم ($CaCl_2$) المحاط بالثلج لتتكون كرات صغيرة الحجم من الجينات الكالسيوم تترك الكرات لمدة 20 دقيقة لتتصلب جيداً عبئت في العمود الزجاجي المعقم ومرر محلول الكادميوم بتركيز 100 ملغم / لتر ورقم هيدروجيني (4) إلى الداخل عبر المضخة الدافعة وبسرعة جريان (0.5) مل / دقيقة. وحضنت المنظومة عند (40) °م وجمع الناتج من الأعلى لقياس تركيز الكادميوم فيه.
3. **استرجاع الرصاص الممتز** :- يمكن استخدام أنواع مختلفة من محاليل الغسل لاسترجاع ايونات الكادميوم الممتزة بوساطة الخلايا البكتيرية. استخدمت نوعين من محاليل الغسل وهي:

- HCl 0.2M
- NaCl 0.2M

تم الغسل باستخدام (50) مل من كل محلول على انفراد ، وتم تمرير محلول الغسل إلى داخل العمود عبر المضخة الدافعة وبسرعة جريان (0.5) مل / دقيقة. جمع الناتج من الأعلى لقياس كمية الكادميوم فيه.

دراسة بعض العوامل المؤثرة في امتزاز الكادميوم:

الغرض من هذه الدراسة هو تحديد الظروف المثلى لامتزاز الكادميوم من المحاليل المائية بوساطة الخلايا البكتيرية المقيدة.

تأثير الرقم الهيدروجيني:استخدمت محاليل الكادميوم (بتركيز 10 ملغم / لتر) ذات أرقام هيدروجينية مختلفة (2 ، 3 ، 4 ، 5) مع تثبيت درجة الحرارة عند (40) °م ومدة التماس ساعة واحدة.

تأثير درجة الحرارة: استخدمت طرق العمل المذكورة مع تغير درجة حرارة الحضن في كل مرة إذ كانت (20 ، 30 ، 40 ، 50) °م ، مع تثبيت الرقم لهيدروجيني 4 ، ومدة التماس لمدة ساعة واحدة.

تأثير مدة التماس:اعتمدت طرق العمل المذكورة مع تغير مدة التماس إذ كانت (0.5 ، 1،2 ، 3) ساعة، وتثبيت الرقم الهيدروجيني (4) ، والحضن بدرجة (40) °م.

قابلية الخلايا البكتيرية على امتزاز الكادميوم عند استعمالها أكثر من مرة:

تسعى هذه الدراسة إلى اختبار قدرة تحمل الخلايا البكتيرية المقيدة الكفؤه على الامتزاز عند تمريره على الخلايا البكترية المقيدة نفسها.وتكرر العملية (تحميل – غسل) ثلاث مرات

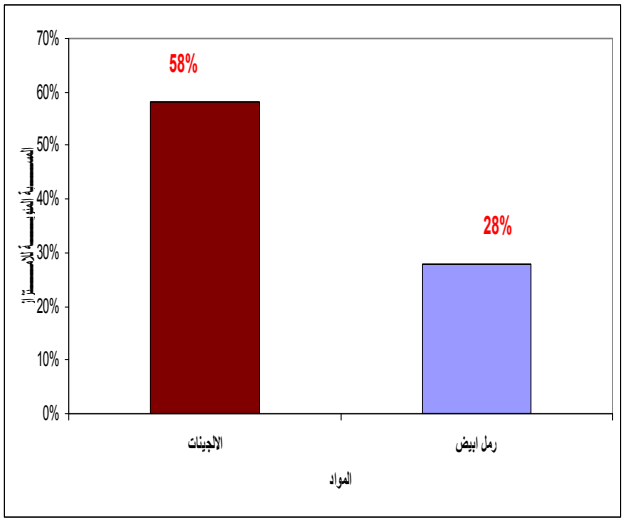
النتائج

* اختبار قابلية الخلايا البكترية المعزولة لامتزاز الكادميوم من المحاليل المائية يوضح الجدول رقم (1) أنواع البكتريا التي تم عزلها وكمية الكادميوم الممتزة لكل واحدة والنسبة المئوية للإزالة وكانت العزلة الخامسة E.agglomerans هي الأفضل من حيث الامتزاز ولهذا تـم اختيارهـا لإجـراء التجارب الأخرى.

جدول (1) كفاءة الخلايا البكتيرية المعزولة لامتزاز الكادميوم حيوياً من المحاليل المائية.

رقم العزلة	نوع البكتريا	كمية االكادميوم الممتزة (ملغم / لتر)	النسبة المئوية للإزالة (%)
1	*E. gergovia*	6.8	85.1
2	*E. sakazakii*	7.3	91.3
3	*E. agglomerans*	7.6	99.2

* اختيار المواد الأساس المستخدمة في التقييد.
يوضح الشكل رقم (1) مقارنه بين المواد الأساس المستخدمة في تقييد الخلايا البكترية وقد أظهرت مادة الالجينات كفاءة عالية في تقييد البكتريا حيث بلغت نسبة الإزالة لايونات الكادميوم 58% بينما بلغت نسبة الإزالة باستخدام الرمل الأبيض في تقييد الخلايا البكترية 28% وعلى الأساس استخدمت مادة الالجينات كمادة أساس لتقييد الخلايا البكترية لإجراء التجارب التي تخص الدراسة.

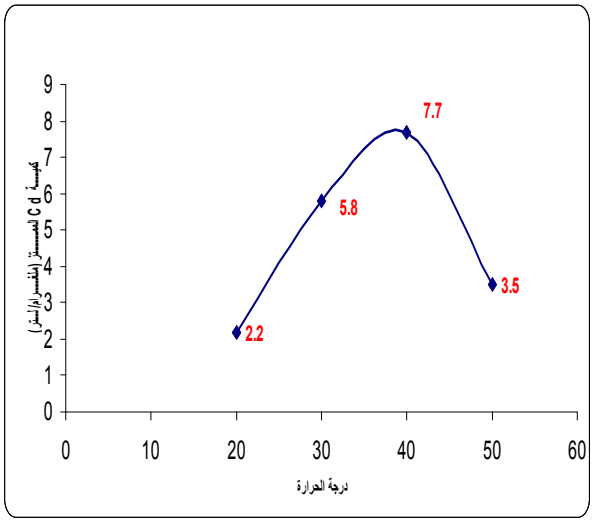


شكل رقم (1) يوضح المقارنة بين المواد المختلفة في امتزاز الكادميوم

* دراسة الظروف الملائمة للامتزاز:

1. درجة الحرارة:

يوضح الشكل رقم (2) تأثير درجة الحرارة في امتزاز ايونات الكادميوم من المحاليل المائية وفي الشكل تبين إن أفضل درجة حرارة لحدوث الامتزاز كانت عند 40°م.



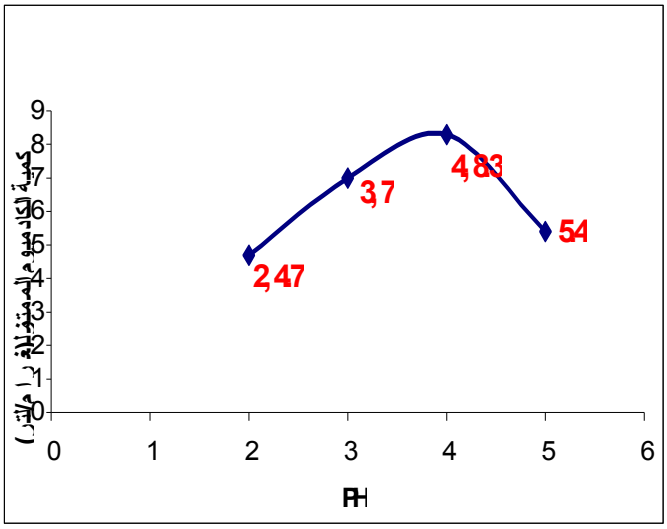
شكل رقم (2) يوضح تأثير درجة الحرارة في امتزاز الكادميوم

2. الرقم الهيدروجيني

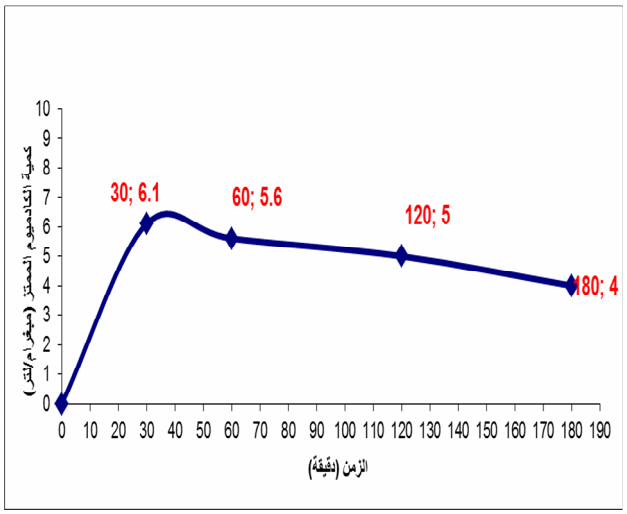
يوضح الشكل رقم (3) تأثير الرقم الهيدروجيني في امتزاز ايونات الكادميوم من المحاليل المائية وأثبتت التجارب إن أفضل قيمة للرقم الهيدروجيني كانت (4) لحدوث أعلى امتزاز للكادميوم .

3. مدة التماس

يوضح الشكل رقم (4) تأثير مدة التماس في امتزاز ايونات الكادميوم وتبين إن اعلي الامتزاز يكون في غضون نصف الساعة الأولى ولا يزداد الامتزاز بزيادة الوقت.



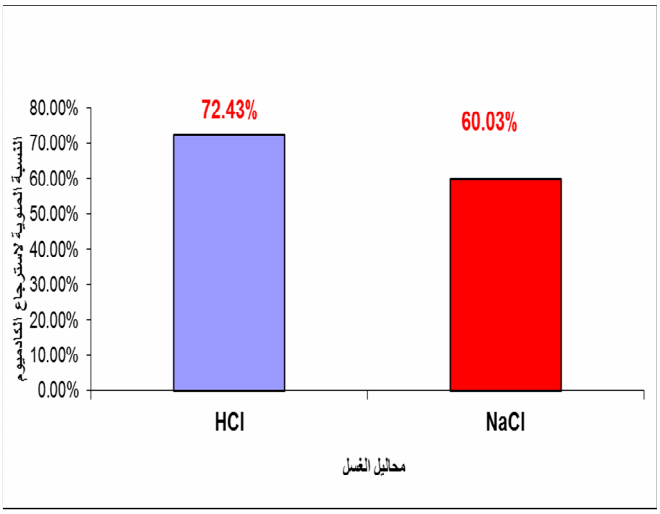
شكل رقم (3) يوضح تأثير الرقم الهيدروجيني في امتزاز الكادميوم



شكل رقم (4) يوضح تأثير مدة التماس في امتزاز الكادميوم

4. قابلية استرجاع الكادميوم الممتز باستخدام محاليل غسل مختلفة

يوضح الشكل رقم (5) قابلية محاليل الغسل المستخدمة في استرجاع الكادميوم الممتز على الجدران البكترية ، وتبين إن استخدام محلول HCL كان الأفضل في الاسترجاع حيث بلغت النسبة 72.4% مقارنه مع محلول NaCl حيث بلغت نسبة الاسترجاع 60%



شكل رقم (5) يوضح قابلية محاليل الغسل في استرجاع الكادميوم الممتز

5. إمكانية استخدام الخلايا البكترية لأكثر من دورة (تحميل ــ غسل)

يوضح الجدول المرقم (2) إمكانية امتزاز الكادميوم ولأكثر من دورة واحدة ولنفس الخلايا البكترية حيث يلاحظ من الجدول إن نسبة الإزالة في الدورة الأولى بلغت 785% وانخفضت قليلا في الدورتين الثانية والثالثة حيث بلغت 76% و69% على التوالي ومن هذا يتضح قابلية الخلايا البكترية المستخدمة في امتزاز الكادميوم ولأكثر من دورة.

جدول رقم (2) يوضح كمية الكادميوم الممتز ولأكثر من دورة (تحميل ــ غسل)

الدورات	الكادميوم المتبقي (ملغرام/لتر)	الكادميوم الممتز (ملغرام/لتر)	النسبة المئوية %
الاولى	1.02	7.98	78.5
الثانية	1.24	7.96	76
الثالثة	1.9	7.3	69

الاستنتاجات

يعد الامتزاز الحيوي من العمليات التي يمكن استخدامها في منظومات المعالجة البيئية لإزالة تلوث مياه الفضلات الصناعية بالعناصر الثقيلة وتوصلت الدراسة الحالية إلى الاستنتاجات الآتية:

1. كانت العزلة البكتيرية E. agglomerans كفؤه في امتزاز ايونات الكادميوم من المحاليل المائية القياسية حيث بلغت (99.2 %)
2. تقييد الخلايا البكتيرية الكفؤه في الامتزاز باستخدام الجينات الصوديوم بوصفه مادة أساس كانت الأفضل مقارنة مع المواد الأساس المقيدة الأخرى.
3. حددت الدراسة الظروف الملائمة للامتزاز وكانت درجة الحرارة (40) م ورقم هيدروجيني (4) ولفترة تماس نصف ساعة .
4. أثبتت الدراسة إمكانية استرجاع ايونات الكادميوم الممتزة على الجدران البكتيرية باستعمال عدة محاليل غسل ويعدّ محلول HCl الأفضل.

التوصيات:

1. إمكانية عزل الجدران الخلوية وإجراء المزيد من دراسات الامتزاز عليها لكونها الجزء المسؤول في الخلية لانجاز عملية الامتزاز الحيوي وبالتالي إمكانية فهم ميكانيكية هذه العملية.
2. استخدام أحياء مجهريه أخرى كمازات حيوية لامتزاز ايونات العناصر الثقيلة مثل الفطريات او الطحالب ومقارنة كفاءتها مع كفاءة الخلايا البكتيرية في الامتزاز.
3. إنشاء المنظومة البيولوجية لمعالجة المياه المطروحة لأي معمل تكون مياه الفضلات الناتجة منه حاوية على عناصر ثقيلة.
4. إجراء المزيد من الدراسات حول تقييد الخلايا باستخدام مواد مقيدة أخرى صناعية أو طبيعية ومقارنتها مع مادة الجينات الصوديوم.
5. دراسة تأثير العوامل والمتغيرات البيئية مثل المتطلب الحيوي للأوكسجين والمتطلب الكيميائي للأوكسجين لمعرفة الظروف المحيطة بالأحياء المجهرية الموجودة في تلك البيئة.
6. إجراء المزيد من التحري حول عمليات استرجاع ايونات العناصر المرتبطة بالجدران الخلوية وباستخدام محاليل غسل مختلفة.

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تشخيص المبيدات العضوية المكلورة في حليب الأمهات وتأثيراتها الصحية

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الخلاصة باللغة العربية

تعد مبيدات الكلور العضوية من اخطر انواع المبيدات على عناصر البيئة وعلى صحة الانسان بشكل خاص لذا برزت اهمية دراسة تلوث حليب الامهات بالمبيدات العضوية المكلورة ، حيث تم التحري على مبيـــدات DDT، Dieldrin ،Heptachlor في اثنان وعشرون نموذج من حليب الامهات جمعت من مناطق مختلفة من بغداد وضواحيها حيث حللت هذه النماذج بواسطة تقنيتين الكروماتوغرافيا الغازية GC والكروماتوغرافيا الغازية – الكتلة GC-mass حيث اظهرت النتائج تلوث ستة نماذج بمبيدات الكلور العضوية ذات التـــاثيرات الصحية على جسم الانسان كونها مواد مسرطنة وتراكمية في الانسجة الدهنية.

ABSTRACT

Organo chlorinated pesticides are high danger on environmental agent and specially human health, so that the study of the contamination of mother's milk by organic chlorinated pesticides . detection of DDT , Dieldrin and Heptaochlor pesticides in 22 samples of mother's milk around Baghdad capital and analysis of extracted samples by gas chromatography (GC) and gas chromatography-mass (GC-mass) , the results of this study indicate that 6 samples contaminated with organic chlorinated pesticides which have effects on human body by cumulated in fatty tissues and carcinogenic agents.

المقدمة

المبيدات هي مواد كيميائه تستخدم في كافة مجالات الزراعة والصحة العامة للقضاء على شتى أنواع الآفات من حشرات Insecticides وحشائش ونباتات ضارة Herbicides، وفطريات Fungicides، وقوارض Rodenticides ، وهذه المواد تساهم بقدر كبير في السيطرة على الآفات التي تصيب النباتات وتهدد إنتاج المحاصيل الزراعية كماً ونوعاً وتساهم أيضاً في القضاء على الحشرات والطفيليات التي تنقل الأمراض المختلفة للإنسان والحيوان ويصاحب ذلك زيادة كبيرة في الكميات المنتجة والمستخدمة ويؤدي الإسراف في استخدام المبيدات إلى تلوث الأراضي الزراعية (1)، (2)، (3)

وغالباً ما يتبقى جزء كبير من المبيدات في التربة لأعوام عديدة (المركبات المكلوره العضوية) حيث تمتص النباتات جزءاً من هذه الكيماويات وتخزنها في سوقها وأوراقها وثمارها(4) ، (5) ثم تنتقل إلى الحيوانات التي تتغذى بهذه النباتات وتظهر في ألبانها ولحومها (6) ، (7) .

يتأثر الإنسان بهذه المبيدات مباشرة أو غير مباشرة فهو يتغذى بالحيوانات والنباتات ويصل إليه مع هذا الغذاء كل ما يختزنه من مبيدات وتشير الدراسات إلى أن العديد من أغذية الإنسان من لحوم ودواجن وألبان وبيض وأغذية نباتية قد أصبحت ملوثة بالمبيدات (1) و(4) مما يزيد من خطورة هذه المبيدات تأثيراتها التراكمية ، وأنها تنتقل ضمن حلقات السلسلة الغذائية، ويحتوي لبن الأمهات المرضعات على أعلى نسبة من متبقيات المبيدات لكونه في نهاية السلسلة الغذائية مما يشكل خطورة كبيرة على الأطفال الرضع (2)

يهدف هذا البحث إلى معرفة مستوى تلوث حليب الأمهات بالمبيدات العضوية المكلورة نتيجة تناول مواد ملوثة بهذه المبيدات

مجموعة الـ د.د.ت ومنها [o,p.p,p - (DDT,DDE,DDD)]

مجموعة السايكلودايين ومنها Dieldrin, Aldrin, Endrin

المواد وطرائق العمل

1.النمذجة

تم جمع اثنان وعشرون عينة من نماذج حليب الأمهات من مناطق مختلفة من مدينة بغداد وضواحيها جمعت النماذج في قناني نظيفة ومعقمة سعة (100) مليلتر ونقلت تحت ظروف مبردة وتم الاستخلاص وفق الخطوات التالية:-

- أخذت عينة الحليب ووضعت في مذيب الايثل استيت
- نبذ النموذج في جهاز الطرد المركزي بسرعة 10000 دورة/دقيقة لمدة عشرة دقائق.
- أخذت طبقة الاثيل استيت وتم مزجها جيداً بالمازج
- نبذ النموذج في جهاز الطرد المركزي بسرعة 10000 دورة/دقيقة لمدة حمس دقائق.
- أخذ الراشح وحقن من هذا المحلول في عامود جهاز الكروماتوغرافي الغازية GC وجهاز الكروكاتوغرافيا غازية – الكتلة GC – Ms
- استخدام طريقة العينة القياسية الخارجية

النتائج والمناقشة

تم تحليل اثنان وعشرون مستخلص لعينات حليب الأمهات باستخدام تقنية الكروماتوغرافي الغازية GC وجهاز الكروكاتوغرافي الغازية – مطياف الكتلة GC - mass وأظهرت النتائج ما يلي:-

1- خلو 16 نموذج من مستخلصات حليب الامهات من المبيدات العضوية الكلورينية.

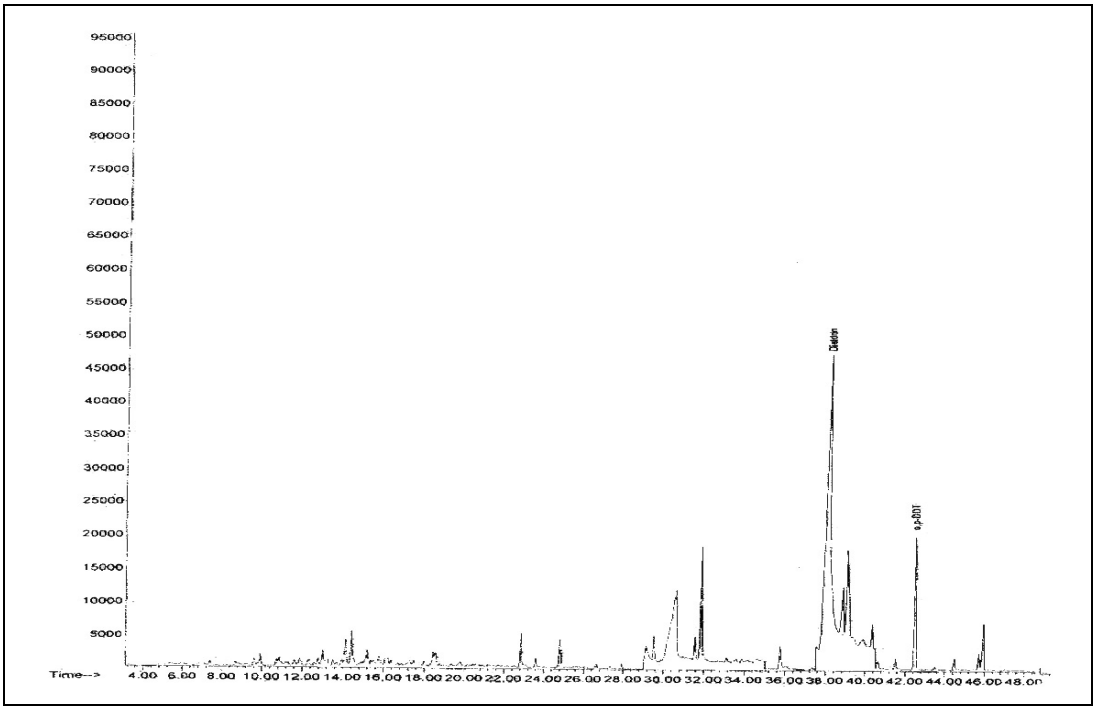
2- احتواء 6 نماذج منها على بقايا المبيدات العضوية المكلوره حيث وجد تلوث النموذج (3) ببقايا مبيد Dieldrin و o,p-DDT الشكل (1) كما أظهر النموذج (7) تلوثه ببقايا مبيدات Heptachlor و o,p-DDT و p,p-DDT الشكل (2) وأظهر النموذج (10) تلوثاً بمبيد p,p-DDT الشكل (3) أما النموذج (13) فأنه ملوث بمبيدي Dieldrin و o,p-DDT وأظهرت نتائج تحليل النموذج (16) تلوثه بمبيدي Dieldrin و

p,p-DDT أما النموذج (18) فأظهر
تلوثاً بمبيد o,p-DDT .

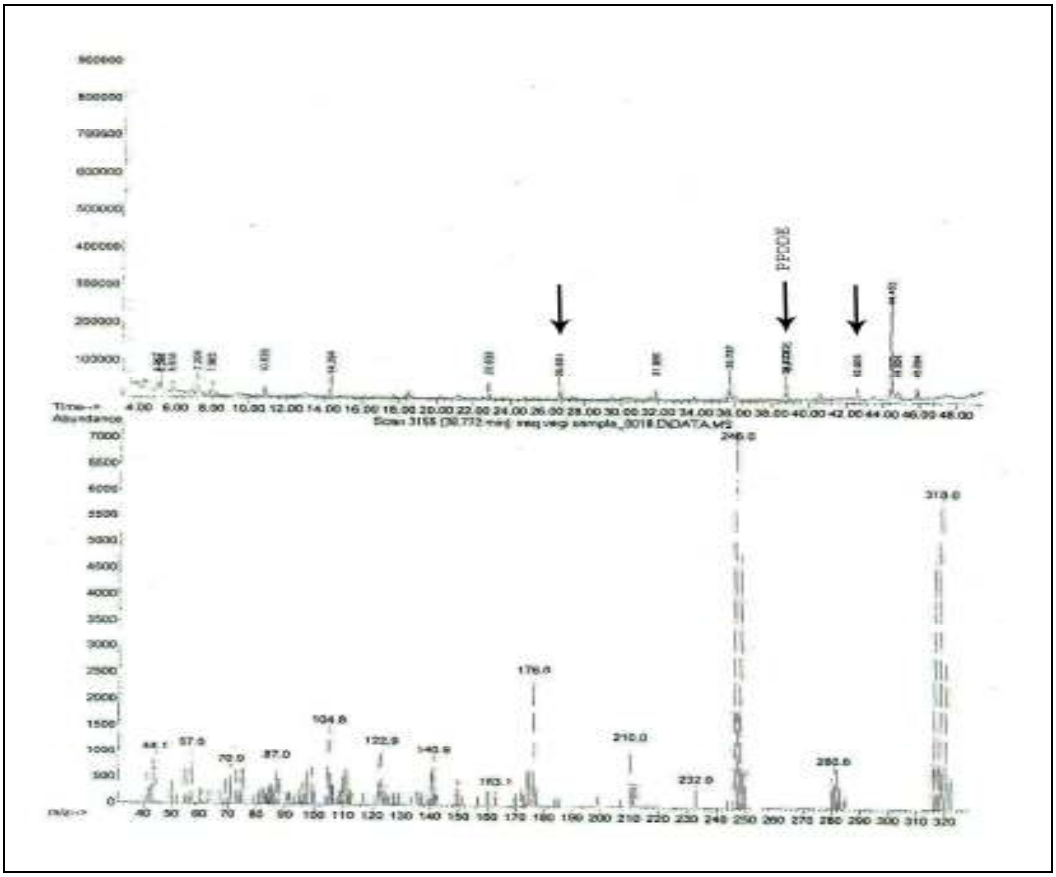
جدول –1– نتائج تحليل مستخلصات حليب الأمهات بتقنية الكروماتوغرافيا الغازية

النموذج	نوع المبيد الملوث ppm		
	Heptachlor	Dieldrin	DDT
حليب أمهات (1)	ND	ND	ND
حليب أمهات (2)	ND	ND	ND
حليب أمهات (3)	ND	3.8	o,p-DDT 1.7
حليب أمهات (4)	ND	ND	ND
حليب أمهات (5)	ND	ND	ND
حليب أمهات (6)	ND	ND	ND
حليب أمهات (7)	0.35	ND	o,p-DDT 0.41 p,p-DDT 0.62
حليب أمهات (8)	ND	ND	ND
حليب أمهات (9)	ND	ND	ND
حليب أمهات (10)	ND	ND	p,p-DDT 2.8
حليب أمهات (11)	ND	ND	ND
حليب أمهات (12)	ND	ND	ND
حليب أمهات (13)	ND	1.42	o,p-DDT 1.3
حليب أمهات (14)	ND	ND	ND
حليب أمهات (15)	ND	ND	ND
حليب أمهات (16)	ND	0.73	p,p-DDT 0.82
حليب أمهات (17)	ND	ND	ND
حليب أمهات (18)	ND	ND	o,p-DDT 1.63
حليب أمهات (19)	ND	ND	ND
حليب أمهات (20)	ND	ND	ND
حليب أمهات (21)	ND	ND	ND
حليب أمهات (22)	ND	ND	ND

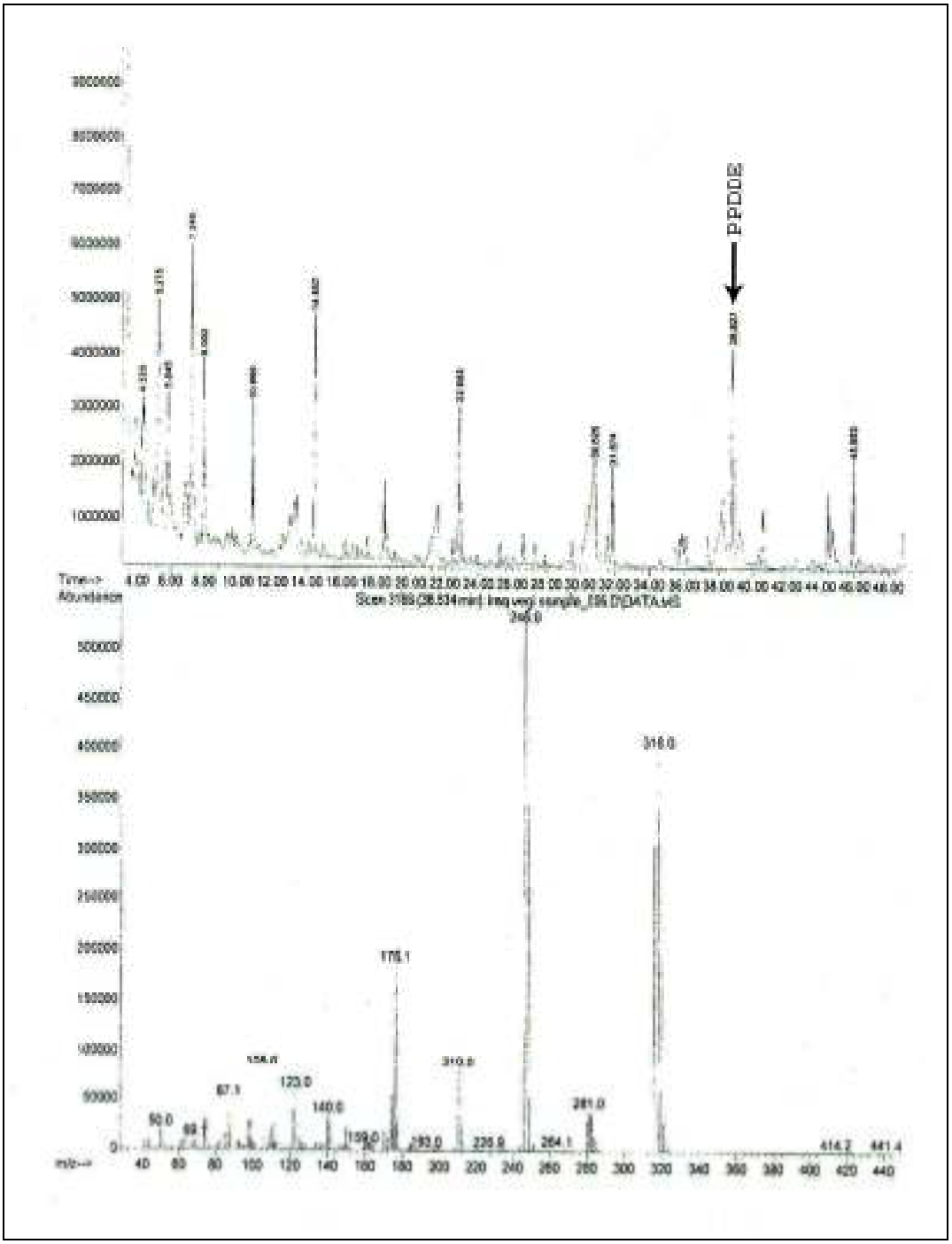
***ND (not detectable)= لايكشف**



الشكل (1) يمثل كروماتوغرام (GC) لمستخلص نموذج حليب رقم (3) ملوث بمبيدDicldrin, DDT



شكل (2) يمثل كروموتوغرام GC وطيفGC-Ms لمستخلص نموذج حليب (7)



شكل (3) يمثل كروموتوغرام GC وطيفGC-Ms لمستخلص نموذج حليب رقم (10)

مما تقدم تبين أهمية دراسة انتقال هذه المبيدات عبر السلسلة الغذائية ومدى تأثيرات هذه البقايا على صحة الطفل الرضيع سيما وان لهذه المبيدات خاصية التراكم في الأنسجة الدهنية لأعضاء الجسم المختلفة ، ومن هنا يتم اجراء دراسات مستفيضة ومسح شامل للمنتجات الغذائية المستوردة وخصوصاً من الدول التي لازالت تستخدم المبيدات العضوية الكلورينية كاللحوم الهندية وغيرها.

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Acknowledgements

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Smith PF, Patel KR, Al-Shammari AJN. 1980. An Alde hydro-Phosphoglycolipid from Acholeplasma granularum. Biochem.Biophys. Acta 617: 419-429

Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

McCarthy, AJ. 1989. Thermomonospora. In: Bergey's Manual of Systematic Bacteriology (ed. Williams ST, Sharpe ME, Holt JG), Vol. 4, pp. 2552-2572. Williams and Wilkins, Baltimore, MD.

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