Histological study of the effect of *Pegenumharmala* seed extract on liver, liver enzymes andsome blood parameters in male Albino Rats

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Abstract

Present study was carried out to find the effect of *Pegenumharmala* on liver tissue, liver function test and some blood parameters on experimental animals, thus for this purpose ,Four groups of male albino rats (*Musmusculus*) were subcutaneously administrated with normal saline(0.9 % NaCl) or *Peganumharmala* aqueous seed extract (1%,2%,3%) mg/ kg)/body weight at daily interval for one month period. Thereafter, animals were scarified and specimens from the liver were examined under light microscope for structural changes. Repeatedtreatment with*Peganumharmala* seeds aqueous extract caused dose- related structural changes in the liver treated groups. Severe changes were observed following 2 % mg/ kg dose that were manifested by fibrosis in interstitial connective tissueand blood vessels of the liver. repeated treatment of *Peganumharmal*water extract a seeds at 3% mg/ kg dose caused severe destruction of hepatic cellnucleiand vesiculation in the cytoplasm due to degeneration in hepatic cells .In addition, dis arrangement in hepatic sinusoids and destruction in the walls of central veins wereobserved. Nuclear polymorph cellular infiltration and cirrhosis as wellas pyknotic in hepatic cell nuclei were noticed in the 1% mg/ kg dose group. *Peganumharmala* seedsaqueous extract at 1% and 2% mg/ kg caused slight to moderatehistological changes in the liver manifested as degeneration and hypertrophy of tubular epithelial lining. In addition,The oral administration of extractcauses maximum fall of blood glucose level to(138 and 35.5) at($p<0.01$) respectively with the normal ratsCholesterol was decreasedsignificantly (0.01) in treatedgroup compared with control . Lowestvalue was in second dose 2% (29.0) while the highest value was in control group (148.5),significant changes (0.05) in GPT and GOT enzymes were observedbetween treated and control group. The highest values were incontrol groups while the lowest values were in treated groupNon-significant changes were observed in the values of WBC and RBC intreated rats compared to controls. In conclusions *Peganumharmala* seed extract has many histopathological effect on liver tissue as well as moderate effects on liver enzymes and some blood parameters.

Key words, Histology,*Pegnumharmala,* rat.

Zoology Classification: QL951-991

Introduction:

Medicinal plants have been used for centuries as remedies for human and animal ailments (9). They have many pharmacologically active chemical compounds, which may act as anthelmintic (2), antibacterial (3) and antifungal agents (8). Therefore, medicinal herbs have been reported to serve as safer alternative as growth promoter due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimum health hazards and environment friendliness.Peganumharmala(locally known as harmal) belongs to the family of Zygophylaceae and have been shown a diverse range of medicinal properties. Numerous beta carboline alkaloids like harmaline, harmine, harmol were present in P. harmala.

Extract exhibited great variety of pharmacological and biological extract (5,4) reported that P. harmalaactivities such as antibacterial and antifungal agents as well as monoamine oxidase (MAO) inhibition and hypothermia. Similarly analgesic, anti-inflammatory(6), disinfectant (7), growth promoting (10), cholesterol lowering and hepatoprotective effects (11) properties have also been reported. There is dearth extract of P. harmalaon serum lipid profile and its economic benefits in broiler chicks Present study was designed to examine the effect of P.harmala extract in some parameters of blood and histopathological changes in liver of albino rats .

MATERIALS AND METHODS

1- animals

The present study was carried out in the laboratory of physiology in faculty of agriculture , Albino Wister rats of either sex, weighing 200-350 121 × 45 cm)with wooden waste bedding. The cages were subjected to cleaning anddisinfectant three times weekly. Animals were kept at constantconditions in regards to ventilation, light/ dark cycle (14/ 10 hour)and temperature (22- 28) C°. The animals had free access to water andstandard laboratory food (Najaf poultry standard laboratory food (Najaf poultry given and*add libitum*.The animals were divided into four groups designated as A, B, C,D. Each group consists of 16 rats divided to 4 subgroups of 4 rats , group A (control group)administrated normal saline , group B administrated orally withconcentration 1% of *harmala* , group administrated orally with 2% from extract of *P. harmala*, group D administrated orally with 3% from extract of *P.harmala* .The body weight was recorded throughout theexperiment prior to dosing. Doses were adjusted to bodyweight prior to each subcutaneously administrated. Animals scarified and specimen's evaluation after

the administrated period was complete, the animals wereanaesthetized by diethyl ether [(C2H4)2O]. Theabdominal cavities ofanimals were opened;Liver was removed and put into formalin(10 %) fortissue fixation for 48 hours. Thereafter routinehistological preparations were carried out accordingtoreported procedures(22) Briefly, organs were washing bytap water, dehydration by series of ascendingconcentrations of ethyl alcohol (70%, 80 %, 90 %, and100 %) and clearing by xylose and infiltration and embedding by paraffin wax and made up blocks, thenmounting byCanada- balsam and cover slides. Thehistological slides examinedby light microscope(Olympus, Japan).

2- Extract preparation

The dry seeds of Iraqi *Peganumharmala* (100 g) were grinded and then extracted with purified water for 24 hours in continuous (Soxhelt) apparatus. The extract was filtered, and water was removed by evaporation on a rotator

was weighted in order to prepare the stock solution, then fromthis solution three doses （ 1%, 2% , 3% ） mg/ kg were made up for thepresent study.

evaporator under vacuum at 60ºC to a small volume provided.25 briefly, the active ingredients were extracted from 20 g dry seeds using soxholateapparatus. Thereafter the extract materials concentrated by rotatory evaporator at 40-45 C°. There after the extract materials

Table:(1)Effect of *Pegenumharmala* on some parameters of blood

Mean squares				
factors	GPT	GOT	Cholesterol	Sugar
T1	333.0 a	17.00 a	52.5 a	35.5 a
T2	262.0 a	27.50 b	29.0 b	93.5 a
T3	161.33 b	31.66 b	61.6 c	113.3 b
Normal	255.0 a	20.77 a	148.5 d	138.0 b
Significant level	*	*	**	**

mechanism of action whether itis a pancreatic insulin release or directly on absorption andutilization of glucose are underway.

Cholesterol was decreased significantly ($p< 0.01$) in treated group compared with control. Lowest value was in T2 (29.0) while the highest value was in control group (148.5).

Significant changes($p< 0.05$) in GPT and GOT enzymes observed between treated and control group. The highest values were in control groups while the lowest values were in treated group. This results is not accordance with the results of(14)who reported no significant changes in theseenzymeswere observed between treated groups.

Blood sugar and cholesterol are decreased significantly ($p<0.01$) by*P.harmala* treatment (table – 1) .The highest level of blood glucose was in control group while the lowest level of blood glucose was in T1 it was (138 and 35.5) respectively. The result came a similar with the results of (12) who reported that an ethanol extract of *P. harmala* is as effective asthe known oral hypoglycemic agent metformin in reducingthe blood glucose concentration after a sucrose challenge innormal and streptozotocin-induced diabetic rats. Further studies are to be conducted to find out whether long termstudies would bring the fasting blood glucose level to normallevels. Further studies on the

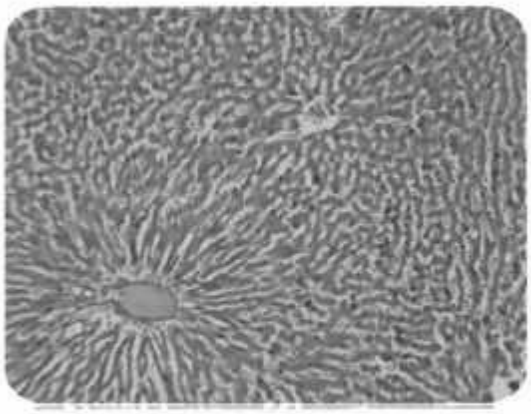
Table :(2) effect of *Pegenumharmala* on some parameters of blood

Mean squares				
factors	PCV%	Hb/gm	RBC L/m	WBC L/m
T1	42.90	15.46 a	7.31	6.43
T2	41.90 a	13.23 b	7.52	6.60
T3	38.90 ab	13.40 b	6.78	5.76
Normal	34.90 b	12.00 b	6.90	4.40
Significant level	*	*	n.s	n.s

No significant changes were observed in the values of wBCand RBC in treated rats compared to controls.(Table – 2) .

Histological results

The results of this study indicated that treatment with *P. harmala* extract caused Cirrhosis (liver fibrosis) in hepatic cells , pyknotic was shown in cells and degeneration (hepatotoxic) in liver cells as Shawn in figure (2).Figure(2)Shows the effect of extract of *P.harmala* at 2 % mg/kg dose on the tissue of liver ,there was sever changes shows infiltration or poly morph nuclear around blood vessel .Figure(3) the histological changes observed at 2% mg / kg of *P.harmala* seedsextract were appeared hepatic tried (vein and Artery) and change in blood vessel (hepatic artery) bile duct occluded or obstruction.Figure (4) hepatic tried (vein and artery and bile duct that occluded also ,infiltrationaround blood vessel . pathological changes , as Shawn in Figure 4.Figure(5) there are sever changes at third concentration such as pyknotic nuclei and necrosis in some hepatic cells as well as dis arrangement of sinusoids .



(1) normal liver tissue (control) x 400

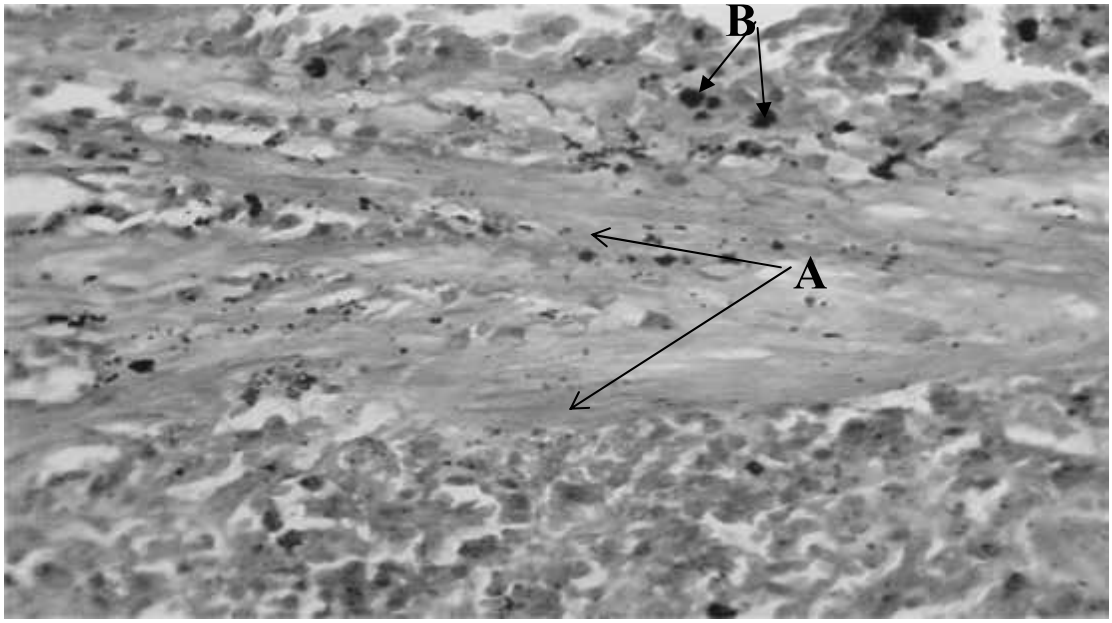


Figure 2: the effect of *peganumharmala* extract on the tissue of liver at the concentration 1% mg/ shows : A- Cirrhosis in liver cells ,B- Pyknotic in some hepatic cell . H & E 400.

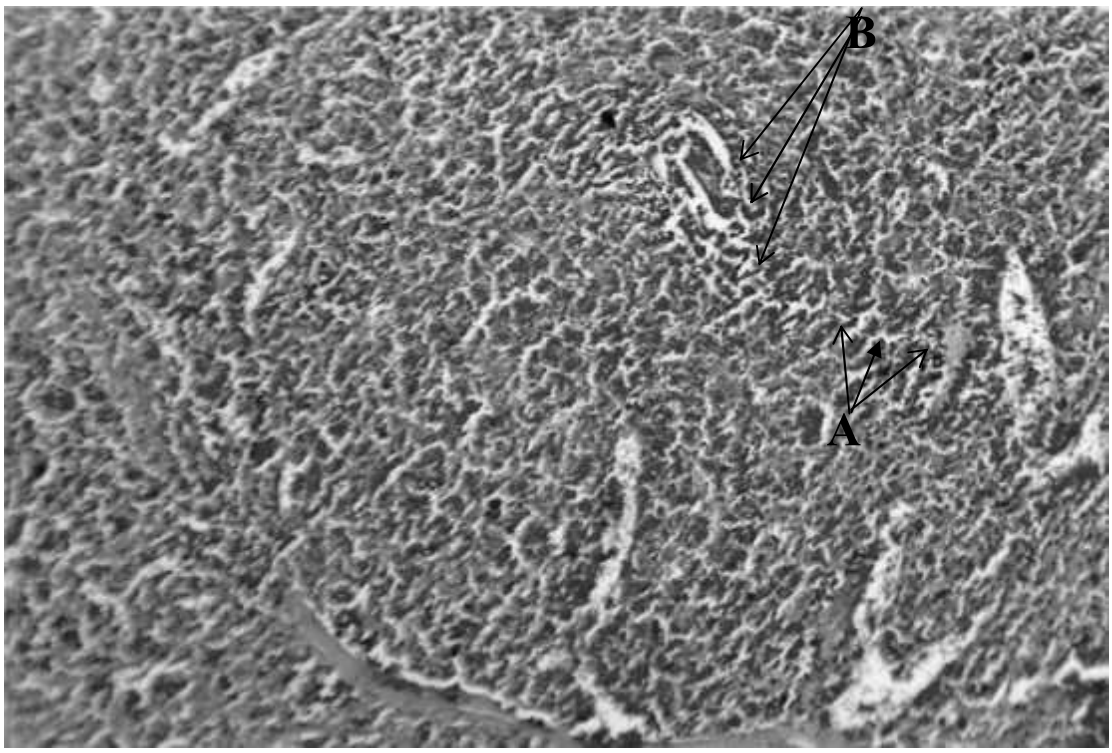
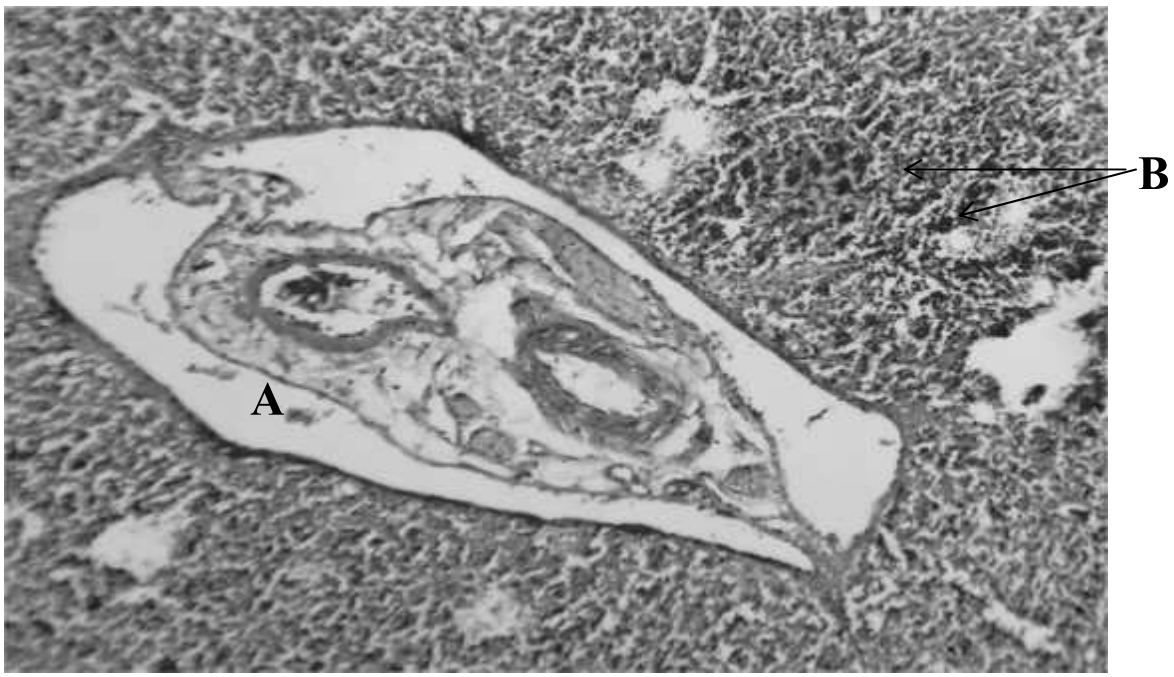


Figure 3: the effect of *peganumharmala* extract on the liver at the concentration 2% mg shows: A-necrosis in liver cells B- infiltration around blood vessel . H & E 100 X.



Figure(4)the effect of *P. harmala* extract on the tissue of liver. at the concentration 2% mg shows . A- hepatic tried (vein and artery) Occluded in bile duct B- infiltration around blood vessel . H & E 400.

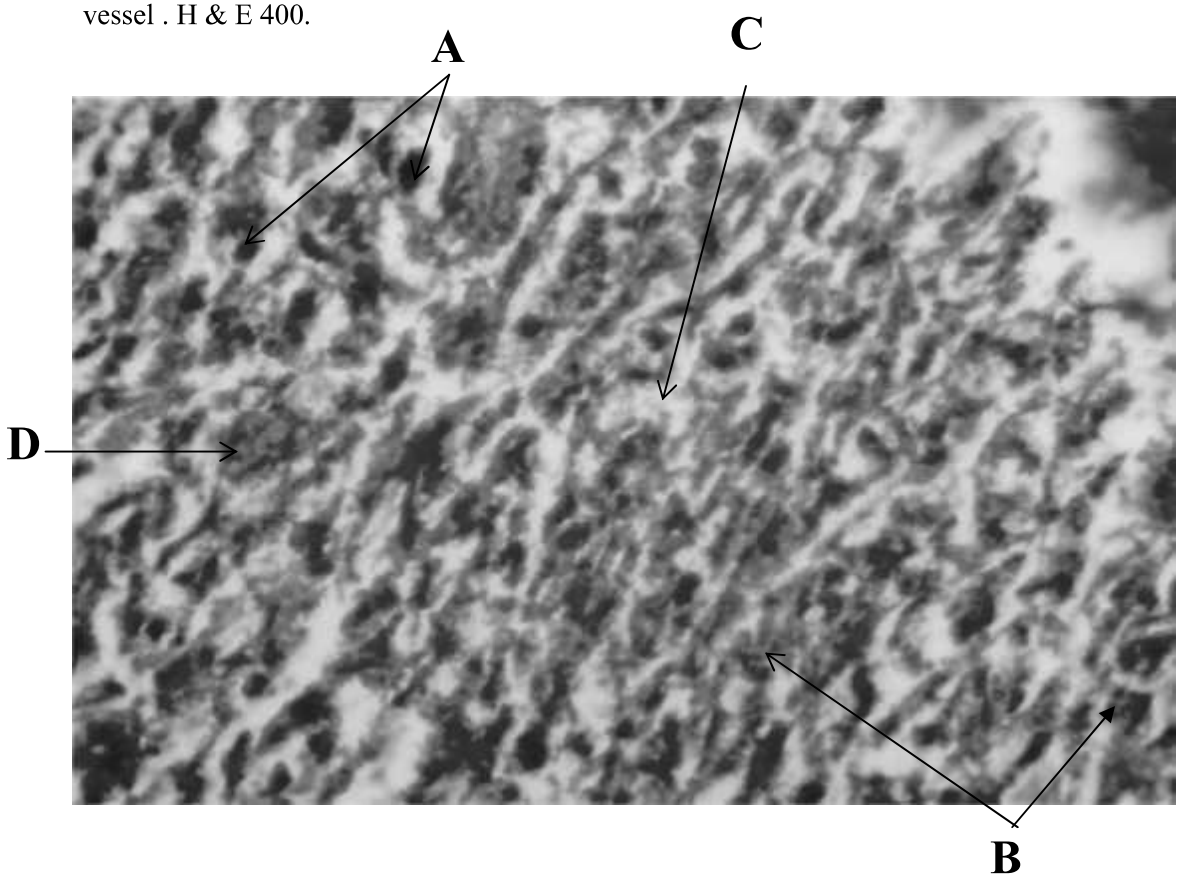


Figure 5: The effect of *peganumharmala* extract on the tissue of liver at the concentration 3% mg shows A- pyknotic nuclei .B- necrosis in some liver cells C- D dis arrangement of liver sinusoids . H&E 400

Discussion:

The present study demonstrated dose-related histological changes in theliver , there was sever changesin the liver parenchyma following different doses of water extract of *P.harmala* seeds, which were manifested by hypertrophy of hepatic cells because long duration of experiment (30) days leads to sever pathological changes of the liver especially in 2% and 3% concentration ,slight changes in liver at concentration of 1% mg of *P.harmala* extract (24).

Some recent studies in liver and kidney of mice indicated that in low concentration of *P.harmala* caused slight effects in mice (12) . *P.harmala* seed extract induced hemorrhage in the interstitial connective tissue ofliver , degeneration, necrosis in the epithelial cells of liver . Inaddition, our results revealed pathological changes in the livercells such as , infiltration and polymorph of nuclei and obstruction of bile duct . Thesehistopathological observations are in agreement with previous studies(12,7) where they noticed that their observationswere revealed histpathologicalchanges occurred in the livers and kidneysof mice, these changes represented, fatty degeneration,necrosis,fibrosis, hepatic tried which cause changes in vein and artery around the blood vessels, hemorrhage in the liver structures,

our results ensure thatcauses signs of intoxication due to administration of *P.harmala* seed extract, the present study was identical with previousfindings suchas studies conducted on the large animals such as sheep and horse(13) andcattle(14), in the cattle after postmortem examination of animal, no distinctivelesions were observed, rapid rigor mort has been observed, the renal andgastrointestinal system were noticed to be congested and hemorrhage inthe liver has been manifested. The *P.harmala* hastraditionally been inthe public medicineas abortifacient and empennage agents.(20) Human toxicity has beenoccurred and reported in a patient with over dose of *P.harmala* plant seedswho has taken 50 gram of seeds for treatment of amenorrhea.(15)The signs of *P.harmala* over dose comprised of hallucinations and neuron-sensorial syndromes,bradycardia and gastrointestinal disturbances suchas nausea andvomiting Para- clinical tests showed the function of liver to benormal and the patient had a normal hematological picture, she wasdischarged from hospital few hours later after the signs of intoxication had disappeared. A case report was recorded by(21), theymentioned a 35 year old male patient, he took around 150 gram of*P.harmala* seeds, after that vomited blood and gastrointestinaldistress, endoscopy showed a 2.5 cmgastric ulcer at location ofinternal region. The symptoms of*P.harmala* toxicity experiencedin the patients were similar to what had been reported for animal (16,17),and over dose of *P.harmala* led to the damage and ulceration ofthe organs tissues such as liver , spleen especially in the epithelial cellsthat lined the spleen and theblood vessels, and splenic cells in whitebulb , these our observationscame to ensure the previous reports about*P.harmala* intoxication.(23).

In conclusion, these results, suggest that *P.harmala* exerted a potent toxic effect on tissues of liver at dose of 3% and above. In view of its toxicity, harmaline may not be used in food of human and other animals .on the other hand ,low concentration of *harmala* extract perhaps due to increase immunoglobulin or cell mediated cell response (macrophage) to produce immunoglobulin (antibody levels) .

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دراسة نسجيه لتأثير مستخلص بذور نبات الحرمل على الكبد ,وانزيماته وبعض معايير الدم في الجرذان البيض .

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الخلاصة

تم اجراء الدراسة الحاليةلمعرفةتاثير مستخلص بذور نبات الحرمل على كل من نسيج الكبد وانزيمات وظائف الكبد وبعض معايير الدم في حيوانات التجربه لذلك,تم تجريع اربع مجاميع من الجرذان البيض بمحلول الملح الفسيولوجي 0.09% وبمستخلص بذور نبات الحرمل بتراكيز (1,2,3) % ملغرام /كيلوغرام يوميا لمدة شهر ,ومن ثم تم التضحية بالحيوانات واستئصال الكبد وفحصه بالمجهر الضوئي لتشخيص التغيرات النسيجية.ان التعرض المستمر لمستخلص نبات بذور نبات الحرمل, قد سبب تغيرات نسيجية في تركيب نسيج الكبد لمجاميع المعاملة .لوحظت تغيرات شديدة عند تركيز 3% ملغرام /كيلوغرام تمثلت بحصول تليف كبدي في الانسجة الضامة البينية والاوعية الدموية للكبد .اضافة ان التجريعتمر للنبات بتركيز3% سبب تحطم شديد في انويةوسايتو بلازم الخلايا الكبدية مما ادى الى حصول اضمحلال لتلك الخلايا . بالإضافة الى عدم انتظام الحبال الكبدية وتحطم في جدران الوريد المركزي .لقد لوحظ ان التجريع بتركيز 1% ملغرام/كيلوغرام الترشيح النووي المتعدد وتليف كبدي بالإضافة الى تغلظ نووي في انوية الخلايا الكبدية.

ان التجريع بتراكيز 1و2% ملغرام/كيلوغرام من مستخلص بذور نبات الحرمل قد سبب تغيرات نسجيه طفيفة الى متوسطة الشدة في نسيج الكبد تمثلت بحصول اضمحلال وتنسج الخلايا الطلائية ,كما ان التجريع المستمر لمستخلص بذور نبات الحرمل قد سبب انخفاض كبير في مستوى سالدم الى (138,35.5) بمستوى احتمالية(0,01) مقارنة مع مجموعة السيطرة اضافة انخفاض معنوي(0.01) في مستوى الكولسترول في المجاميع المعاملة مقارنة مع مجموعة السيطرة .كانت اقل قيمة في الجرعة الثانية 2% (29,0) بينما اقل قيمة كانت في مجموعة السيطرة (148,5) ,تغيرات معنويةلوى انزيمات الكبد وقد لوحظت ما بين المجاميع المعاملة ومجموعة السيطرة اذ كانت اعلى قيمة في مجموعة السيطرة بينما اقل قيمة في المجاميع المعاملة ,لم تحصل اي تغيرات معنوية في تعداد كريات الدم الحمر والبيض في الجرذان المعاملة مقارنة مع مجموعة السيطرة .

Identification of *cryptosporidium parvum* parasite oocyst using microscopic &PCR assay for detection GP900gene

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Abstract

The present study designed to diagnose the parasite *Cryptosporidium parvum* from patients with diarrhea arrivals to Maternity and Childhood Teaching Hospital and General Education Hospital in Al-Qadisiya Governorate , as well as to investigate and to emphasize the presence of virulence factor Glycoprotein 900 in parasite *C. parvum* using Conventional PCR Technique, and for these purposes one hundred feces samples were collected from children under 10 years old, for the period between 1 \ 5 \ 2013 - 1 \ 11 \ 2013.

The results show presence of *Cryptosporidium parvum* parasite oocyst in 28 positive samples distributed throughout the months of the study, and then, DNA was extracted from the positive samples and then after amplified using special designed for primers *C. parvum* virulence factor gene that called Glycoprotein 900 and the amplified DNA passed in electrophoresis apparatus for DNA. The results showed that the mentioned factor is present in all positive samples of *C. parvum.*

The highest percentage of infection with the parasite was in the age group (1-3 years) with (39.2%), followed by age group (less than one year) with (21.4%) and the lowest percentage of infection was in the age group (7-10 years) by 7.1%, whereas the percentages of infection with the parasite in the age groups (5-3) , (7-5) were 17.8% , 14.2%, respectively.

Also the results of the current study, showed that there are no significant differences according to sex .The number of infected males were about 15 with (53.5%) and the number of infected females were about 13 with (46.4%) , besides the results indicate that the highest percentage of infection was in rural areas rose (57.1%) and the lowest percentage of infection was in the urban (42.8 %).

Key word / *Cryptosporidium parvum* , microscopic examination, PCR , Glycoprotein 900gene

Biology Classification QP1

Introduction

Protozoan parasites cause several diseases, such as Malaria ,Leishmaniasis , and Trypanosomiasis, hampering human development worldwide. Many protozoa cause infections that often follow chronic courses, owing to coevolution between parasites and host immune system.[1]

Cryptosporidiosis is one of the commonest parasitic causes of diarrhoea and a sometimes fatal disease in the immune-suppressed [3,4].*Cryptosporidium* is a protozoan parasite of medical and veterinary importance that causes gastroenteritis in a variety of vertebrate hosts. [2]

Cryptosporidium was first described in the early 20 th century;*Cryptosporidium muris* and *C. parvum* were the first species described [5, 6]. The veterinary importance of *Cryptosporidium* spp. was highlighted by the associations of *C. meleagridis* with morbidity and mortality in turkeys in the 1950s [7] and of *C.parvum* with bovine diarrhea in the early 1970s [8]. *C. parvum* is now regarded as an economically important cause of neonatal diarrhea in calves and lambs [9, 10]. Another species, *Cryptosporidium baileyi*, is recognized as an important cause of respiratory disease in poultry and game birds [13,14].

Of some eight species which infect humans [15], *Cryptosporidium parvum* and *Cryptosporidium hominis* are the main species of public health importance, causing the majority of human cases as both sporadic and outbreak-related cases. In a survey of over 4000 isolates in the UK, *C. parvum* and *C. hominis* were responsible of 38.5% and 57.3% of the cases, respectively[16]. Although closely related *C. hominis* mainly infects humans, while *C. parvum* exhibits a broader host range including humans, livestock and rodents [17].

Cryptosporidium parvum, a coccidian protozoan of worldwide distribution, is frequently found in surface waters and is now a leading cause of waterborne outbreaks in the United States [18,19].

A number of host, environmental, and parasite-specific variables are cofactors that interplay in the prevalence of *C. parvum* in humans. It is well known that among other factors, host immunity and intensity of water contamination have an impact in disease manifestation. There is also evidence that parasiterelated factors might have an impact on intensity of infection and/or severity of clinical manifestations [20,21]. Several studies have reported different degrees of pathogenicity and virulence among *Cryptosporidium* species and isolates of the same species as well as evidence of variation in host susceptibility to infection. The identification and validation of Cryptosporidium virulence factors have been hindered by the renowned difficulties pertaining to the in vitro culture and genetic manipulation of this parasite.[2]. Nevertheless, substantial progress has been made in identifying putative virulence factors for Cryptosporidium. This progress has been accelerated since the publication of the

Cryptosporidium parvum and *C. hominis* genomes, with the characterization of over 25 putative virulence factors identified by using a variety of immunological and molecular techniques and which are proposed to be involved in aspects of host-pathogen interactions from adhesion and locomotion to invasion and proliferation.[2].

Virulence is commonly defined simply as the ability of a microorganism to cause disease [22,23].

Each of the microbiological attributes that contribute to virulence can, in general, be linked to specific structural elements or biochemical compounds within the organism; these are generally termed virulence factors. Although the terms "virulence determinants" and "virulence factors" are widely used to describe traits contributing to pathogenicity, a subtle distinction exists between the two terms. Virulence factors are "microbial traits that promote host damage" [24] and more precisely, a virulence factor is a gene product necessary but not sufficient to cause disease. In this context, virulence factors can be defined as "contributory virulence factors" [25].

Virulence factors are likely to be involved in adhesion, colonization, invasion, and host immune evasion. When characterized, most factors share one or more of the following properties: (i) they are externally exposed, either on the surface of the parasite or as secreted proteins; (ii) they are hypervariable between isolates; (iii) they are encoded telomerically or subtelomerically; (iv) they are multicopy or belong to gene families; and (v) they are glycosylated and/or lipoylated [26].

Several studies have tried to determine the factors responsible for the initiation, establishment, and perpetuation of *Cryptosporidium* infection. *Cryptosporidium* does not normally cause a systemic infection or penetrate deep tissue; rather, the parasite establishes itself in a membrane-bound compartment on the apical surface of the intestinal epithelium , Nevertheless, it causes significant abnormalities in the absorptive and secretory functions of the gut. This damage could be the result of direct injury to the host epithelial cells or could be indirect through the effect of inflammatory cells and cytokines recruited to the site of infection [27].

Glycoprotein 900 (gp900) is a large glycoprotein identified by the immunoprecipitation of sporozoite extracts with hyperimmune bovine colostrum [28]. This large mucin-like glycoprotein is located in micronemes and at the surface of invasive merozoites and sporozoites. gp900 is deposited in trails during gliding motility and is known to mediate invasion [29,30]. The deduced amino acid sequence of gp900 has a signal peptide and a transmembrane domain [29]. Specific antibodies to gp900 can competitively inhibit infection *in vitro* [29,31].

Materials & Methods

Through this study, the collection of 100 stool samples from children who suffer from intestinal disorders of children who to arrivals to Maternity and Childhood Teaching Hospital and General Education Hospital in Al-Qadisiya Governorate who are aged below 10 years old and for the period from 1 \ 5 \ 2013 until 1 \ 11 \ 2013. Where the samples collection stool in a clean and sterile with tight lid plastic containers to maintain sample moisture has been the samples using the steadfast acid dye (AFS) examination, as shown in [11] and after appoint positive samples were placed in plastic tubes and preserved in frozen for the PCR.

The first step extracted genetic material DNA samples positive by using Genomic DNA Extraction Kit Stool according to the manufacturer's instructions.

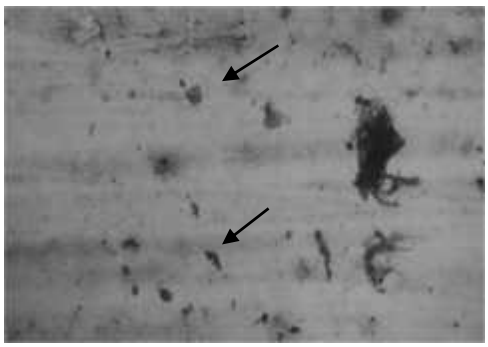
The second step used prefixes F: (AAAGAATGGCGAATGTGAGG) and R: (CCGTATGGGCTTACGTGAAT)processed Corporation (Bioneer, Korea) and these primers were designed using the NCBI Gen Bank database for the purpose of investigating the virulence factor genes private GP900 parasite *C.parvum*.

The PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Techne TC-3000. USA) . The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

Results

.Laboratory diagnosis

The microscopic diagnosis showed presence of *C. parvum* oocyst in stool samples dyed by stead fast acid in 28 of the total 100 samples distributed over the months of the study as shown in the picture s (1) and (2).



2

1

Image (1) and image (2) *Cryptosporidium parvum* oocyst

.The Relationship between age and *C.parvum* infection

Microscopic examination indicated that the highest percentage of infection was in the age group (1-3years) 39.2%, followed by the age group less than one year 21.4%, while the lowest percentage of infection (7.1%) was in the age group (7-10 years).

The statistical analysis showed that there were significant differences between the studied age groups as shown in the table no. (1).

Table (1) Prevalence of infection with *C.parvum* according to the age groups

Age groups	The number of Patients	The number of infected Patients	%	
Less than 1 year	12	6	21.4	a
1-3	35	11	39.2	b
3-5	26	5	17.8	a
5-7	20	4	14.2	ac
7-10	7	2	7.14	c

The Relationship between Sex and *C.parvum* infection

The statistical analysis showed that there were no significant differences between males and female($p>0.05$) .The number of infected males were about 15 (53.5%) and the number of infected females were about 13 (46.4%) as shown in table no.(2).

Table (2) Prevalence of infection with *C.parvum* according to the sex

Sex	The number of Patients	%
males	15	53.5 a
females	13	46.4 a

The relationship between the nature of residence and *C.parvum*infection

The statistical analysis of the results showed that the incidence of *C.parvum* in rural areas, which is (57.1%), was significantly higher than the urban areas (42.8%) ($p< 0.05$) as shown in table no.(3).

Table (3) Prevalence of infection with *C.parvum* according to the nature of residence

nature of residence	The number of Patients	%
rural areas	18	64.2 a
a Urban area	10	35.7 b

Conventional PCR technique

The results of positive samples examined showed mediated PCR that all these samples with 100% contain pathogenic factor under study which is: - Glycoprotein 900, at PCR product size 150bp where were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and examined under UV transilluminator.

Next image show PCR product analysis of *C. parvum* parasite gene GP900 in DNA from human stool samples. Where, Lane (M) DNA marker (2000-100bp), Lane (1,2,3,4,5,6,7,8,9,&10) positive as gene GP900 at PCR product size 150bp. which emphasizes accurate diagnosis of this technique where the

diagnosis method adopted for this technique to identify and amplify the gene GP900 in the DNA of parasite.

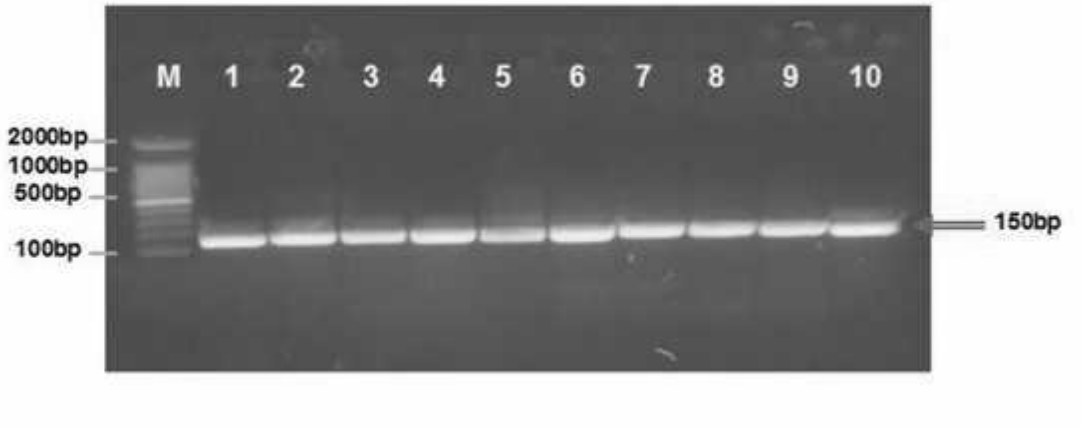


Image (3): Agarose gel electrophoresis image that show PCR product analysis of *C. parvum* parasite gene GP900. Where, Lane (M) DNA marker (2000-100bp), Lane (1,2,3,4,5,6,7,8,9,&10) positive as gene GP900 at PCR product size 150bp.

Discussion

In the developing world, the association of *Cryptosporidium* with acute and persistent diarrhea in children is striking. Several cross sectional studies in children with diarrhea suggest that cryptosporidiosis is endemic in developing world with prevalence of up to 26% in Mexican and 16.5% in Brazilian children with diarrhoea. [41] Prevalence in African countries, Central and South American countries, Asian countries is greater than in Europe and North America [42] .

The present study recorded the incidence of *C.pavum* parasite 28% by 28 sample is positive from a total of 100 stool specimens examined microscopically using the acid fast stain (AFS) and this percentage was different from some results fo studies that were conducted in Iraq, including the study conducted by AL-Alousi and Mahmood .,2012 [32], that recorded the percentage of infected stood at 18.9% after examining 92 stool specimens for children suffering from diarrhea aged (one month -12 years) in Mosul, and different from the study carried out by [43] in Ramadi province, since the results showed that the percentage of the total infection was 39.13% from total of 115 stool specimens for children suffering from diarrhea also under the age of five exclusively.

Also the study carried out by [44] in Nineveh, the incidence of the total 302 have reached 20.52% of stool specimens taken from the children ranged in age from six days to five years, and in the same province [45] recorded infection percentage with the parasite accounted to 17% of the total sample of 470 feces of children different and reconstruction.

In Diwaniyah, a study carried out by [46] recorded that the percentage of parasite prevalence has reached 18.01% of the total in 1177 stool specimens for children under the age of 12 years. all those results were using microscopic examination and using dye steadfast acid AFS.

In Zambia infection rate of 21% [39], whereas in Iran, Peru was the incidence 7.66% and 6.4% respectively [40,41].

The fluctuation of results among these studies, due to the difference in the number of tested models as well as

some these studies are epidemiological (year or more) whereas the current study was during the 6 months of the year and in the summer especially, where the frequent cases of diarrhea attributed.

There are many causes may explain the high rate of infection during the summer one of them is the favorable conditions for the growth of the parasite oocyst during summer, as well as the contaminated food and drinks , which works to move the oocyst and get the infection. Another causes may explain high rate of infection during the summer especially among children in rural areas, are swimming of children in polluted rivers and streams as a result of poor communities, and this is consistent with what was said by [42,43].

The study showed that there is inversely proportional between infection and age therefore less infection with progress of age. The study showed that the *C.parvum* parasite affects all studied age groups, but a higher proportion of cases were in the age group (1-3year), 39.2% and this may due to the high rate of babies movement in this stage and the accompanying habits such as putting any things while walking or crawling into their mouths.

The age group (7-10year) has recorded the lowest proportion of infection 7.14 % and it goes back to that kids at this age more aware than other age groups in terms of how to play abroad and the use of health facilities and follow personal hygiene methods in addition to the maturation of the immune system with the progress of the child's age .The [43] record in Ramadi, a higher incidence in the age group (1month - 12 months) 12.17% compared to other age groups, and the less of the age group (49-60 month) 4.34% of children under five years of age either in Nineveh has recorded both of [44] that the highest infection rate 40.90% greater than two months old to three months while the infection decreased in greater than two years and up to 5 years in the same province.

Also [45] recorded that, the highest infection rate in the age group (1-2 years), 28.7 % and the lowest rate in the age group (5-7years) 8.2%. In Diwaniyah Governorate (46) mentioned that the age group 1-3 years is the highest rate of infection 20.62% and the lowest rate of infection in age group (9-12 years) 10.79%, and this asymptotic to the results found in the current study, it is possible that the reason for these differences conquered in infection rates to the different age groups and the varying climatic and environmental conditions of the regions that had been carried out these studies in addition to the host's immunity.

About the relationship between the infection and sex , the results of the present study showed no significant difference between males and females . The reason for this is maybe due to the fact that male and female children at risk of being in contact with the external environment in these ages, neighborhood injury by playing occur outside the home, as well as animal husbandry and non-sterile water and food contamination the rate was consistent with [47] in the Diyala , but this study did not agree with [12] in the Basra, however [48] in the Baghdad and with [45] in the Nineveh, where he pointed out that the incidence in males higher than in females .

About infection rates depending on the nature of residence, the results were expected and logically accepted since the rural areas, that recorded the highest infection rate with *C.parvum* parasite (57.1%), are different in living style from urban areas, while the lowest percentage of infect to the

parasite in the urban areas recorded 42.8% have attributed the cause to the style of living in the countryside. The rural areas depend on animal husbandry within the housing or near which are the source of infection in addition to the lack of attention to hygiene and health awareness and the spread of rodents. The results of current study are in agreement with other studies conducted in different areas, which indicated the same as the previous results [38,43,46, 39].

The results of the present study, using the technique of PCR Conventional showed presence of gp900 in all samples (100%) and that has been documented in this study. gp900 is one of virulence factors that contribute to causing cryptosporidiosis and spread enables the parasite to invade and get the infection to hosts of mammals and causing damage to a group of hosts be prone to infection and found that the effective immune responses targeting often virulence factors . Gp900 is a unique mucin-like surface glycoprotein of *Cryptosporidium parvum* merozoites and sporozoites ,is a mediator of the invasion process [40] it is a potential target for chemotherapeutic competitive inhibitor therapy and for vaccine development, [28] confirmed on the role of GP900 as a virulence factor of *C.parvum* parasite.

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تحديد أكياس بيض طفيلي الابواغ الخبيئة باستخدام الفحص المجهري وPCR للكشف عن جين GP900

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الخلاصـــــــــــة

صممت الدراسة الحالية من اجل تشخيص طفيلي *Cryptosporidium Parvum* للأطفال الوافدين الى مستشفى الديوانية التعليمي ومستشفى النسائية والاطفال في محافظة القادسية والذين يعانون من الاسهال فضلا عن التحري والتأكيد على وجود عامل الضراوة Glycoprotein 900 في طفيلي *C. parvum* باستخدام تقنية Conventional PCR. ولهذا الغرض تم جمع 100 عينة براز للأطفال دون 10 سنة للمدة الواقعة بين 1\5\2013 – 1\11\2013 .

اشارت النتائج الى وجود اكياس بيض طفيلي *C. parvum* في العينات بواقع 28 عينة موجبة موزعة خلال اشهر الدراسة , تم استخلاص DNA للطفيلي من العينات الموجبة وبعد تضخيمه باستخدام بادئات خاصة مصممة للجين الخاص بعامل الضراوة لطفيلي *C. parvum* والمسمى Glycoprotein 900 تم تمرير نواتج الدنا المضخم في جهاز الترحيل الكهربائي لـ DNA. اظهرت النتائج بأنه العامل سابق الذكر موجود في جميع عينات *C. parvum* , ان نسبة الاصابة الأعلى للطفيلي كانت في الفئة العمرية (1-3)سنة اذ بلغت 39.2% تليها الفئة العمرية (الاقل من سنة) بنسبة 21.4% واقل نسبة في الفئة العمرية (7-10)سنة بنسبة 7.1% بينما نسبة الاصابة بالطفيلي في الفئات العمرية (3-5)و(5-7) سنة نسبة 17.8% و14.2% على التوالي .

كما بينت الدراسة الحالية عدم وجود فروق معنوية بالنسبة للجنس اذ كانت نسب الاصابة لدى الذكور 53.5% مقارنة مع الاناث والتي بلغت 46.4%,ايضا اشارت النتائج بحسب طبيعة السكن ان اعلى نسبة اصابة كانت في المناطق الريفية بنسبة 57.1% وادنى نسبة اصابة سجلت في المدينة بنسبة 42.8%.

الكلمات المفتاحية: طفيلي *Cryptosporidium Parvum* ,الفحص المجهري ,PCR, جين Glycoprotein 900