

Histological study of the effect of *Peganum harmala* aqueous extract on liver, liver enzymes and some blood parameters in male Albino Rats

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Abstract

Present study was carried out in physiology lab /faculty of agriculture /Kufa university .Four groups of male albino rats (*Mus musculus*) were subcutaneously administrated with normal saline(0.9 % NaCl) or *Peganum harmala* alcoholic 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. Repeated treatment with *Peganum harmala* 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 tissue and blood vessels of the liver. repeated treatment of *Peganum harmal* water extract a seeds at 3% mg/ kg dose caused severe destruction of hepatic cell nuclei and 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 were observed. Nuclear polymorph cellular infiltration and cirrhosis as well as pyknotic in hepatic cell nuclei were noticed in the 1% mg/ kg dose group.

Peganum harmala seeds aqueous extract at 1% and 2% mg/ kg caused slight to moderate histological changes in the liver manifested as degeneration and hypertrophy of tubular epithelial lining. In addition, The oral administration of extract causes maximum fall of blood glucose level to(138 and 35.5) at($p<0.01$) respectively with the normal rats Cholesterol was decreased significantly (0.01) in treated group compared with control . Lowest value 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 observed between treated and control group. The highest values were in control groups while the lowest values were in treated group Non-significant changes were observed in the values of WBC and RBC in treated rats compared to controls.

Key words, Histology, *Pegnum harmala*, rat.

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. *Peganum harmala*(locally known as harmal) belongs to the family of Zygophyllaceae 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. harmala* activities 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 of extract of *P. harmala* on 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 Wistar rats of either sex, weighing 200-350 g (121 × 45 cm) with wooden waste bedding. The cages were subjected to cleaning and disinfectant three times weekly. Animals were kept at constant conditions in regards to ventilation, light/dark cycle (14/10 hour) and temperature (22-28) °C. The animals had free access to water and standard laboratory food (Najaf poultry standard laboratory food (Najaf poultry given and *ad libitum*). The animals were divided into four groups designated as A, B, C, D. Each group consists of 16 rats divided into 4 subgroups of 4 rats, group A (control group) administered normal saline, group B administered orally with concentration 1% of *harmala*, group C administered orally with 2% from extract of *P. harmala*, group D administered orally with 3% from extract of *P. harmala*. The body weight was recorded throughout the experiment prior to dosing. Doses were adjusted to bodyweight prior to each subcutaneously administered. Animals sacrificed and specimen's evaluation after the administered period was complete, the animals were anaesthetized by diethyl ether [(C₂H₅)₂O]. The abdominal cavities of animals were opened; Liver was removed and put into formalin (10 %) for tissue fixation for 48 hours. Thereafter routine histological preparations were carried out according to reported procedures (22). Briefly, organs were washed by tap water, dehydration by series of ascending concentrations of ethyl alcohol (70%, 80 %, 90 %, and 100 %) and clearing by xylene and infiltration and embedding by paraffin wax and made up blocks, then mounting by Canada-balsam and cover slides. The histological slides examined by light microscope (Olympus, Japan).

2- Extract preparation

The dry seeds of Iraqi *Peganum harmala* (100 g) were grinded and then extracted with purified water for 24 hours in continuous (Soxhlet) apparatus. The extract was filtered, and water was removed by evaporation on a rotator evaporator under vacuum at 60°C to a small volume provided. Briefly, the active ingredients were extracted from 20 g dry seeds using Soxhlet apparatus. Thereafter the extract materials concentrated by rotatory evaporator at 40-45 °C. Thereafter the extract materials were weighted in order to prepare the stock solution, then from this solution three doses (1%, 2%, 3%) mg/kg were made up for the present study.

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

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

Blood sugar and cholesterol are decreased significantly (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 its was (138 and 35.5) respectively. The result came a similar with the results of (12) who reported that the that an ethanol extract of *P. harmala* is as effective as the known oral hypoglycemic agent metformin in reducing the blood glucose concentration after a sucrose challenge in normal and streptozotocin-induced diabetic rats. Further studies are to be conducted to find out whether long term studies would bring the fasting blood glucose level to normal levels. Further studies on the mechanism of action whether it is a pancreatic insulin release or directly on absorption and utilization of glucose are underway.

Cholesterol was decreased significantly (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 (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 these enzymes were observed between treated groups.

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

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

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

Histological results

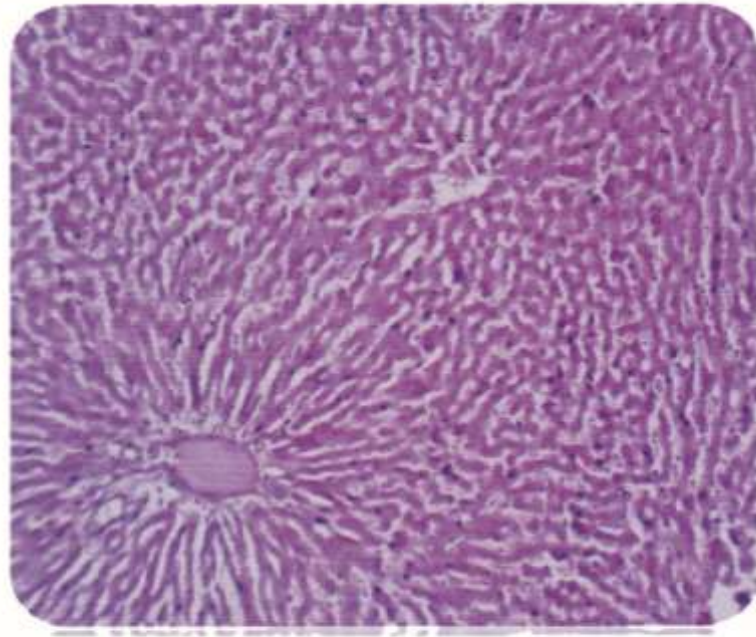
The results of this study indicated that treatment with *P. harmala* extract caused Cirrhosis (liver fibrosis) in hepatic cells , pyknotic was shown in cells and degeneration (hepatotoxic) in liver cells as Shawn in figure (2).

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* seeds extract 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 ,infiltration around 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

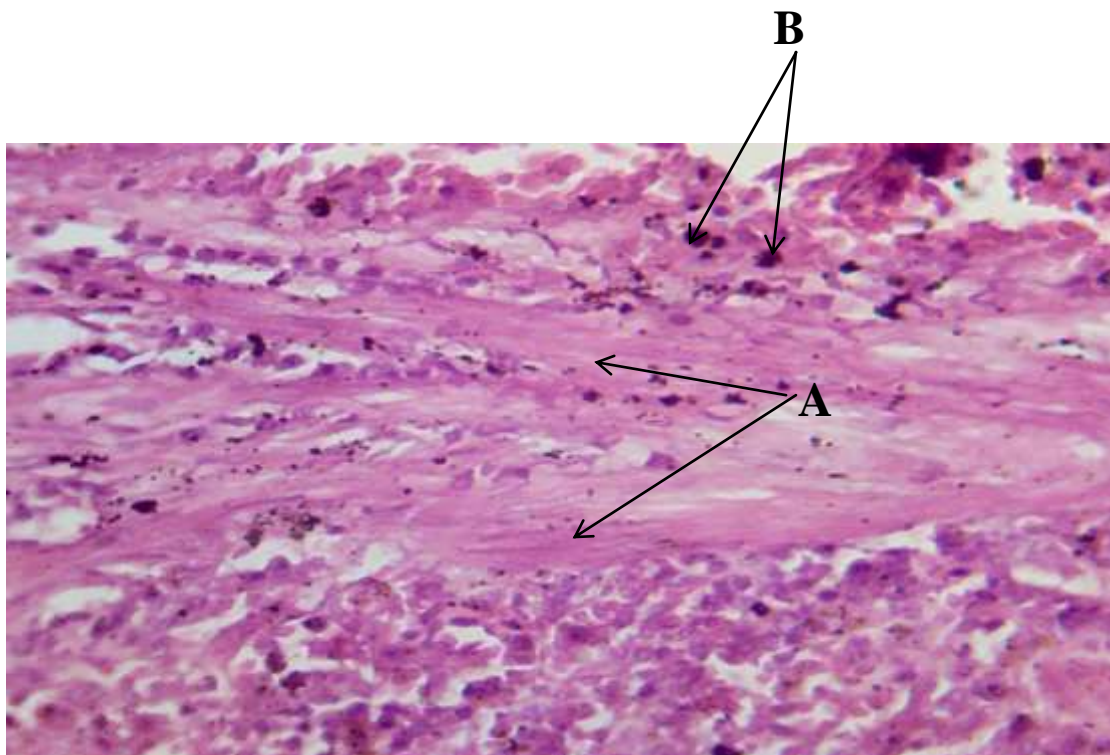


Figure 2: the effect of *peganumharmala*extract on the tissue of liver at the concentration 1% mg/ shows : A- Cirrhosis in liver cells ,B- Pyknotic in some hepatic cell . H & E 400.



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 triad (vein and artery) Occluded in bile duct B- infiltration around blood vessel . H & E 400.

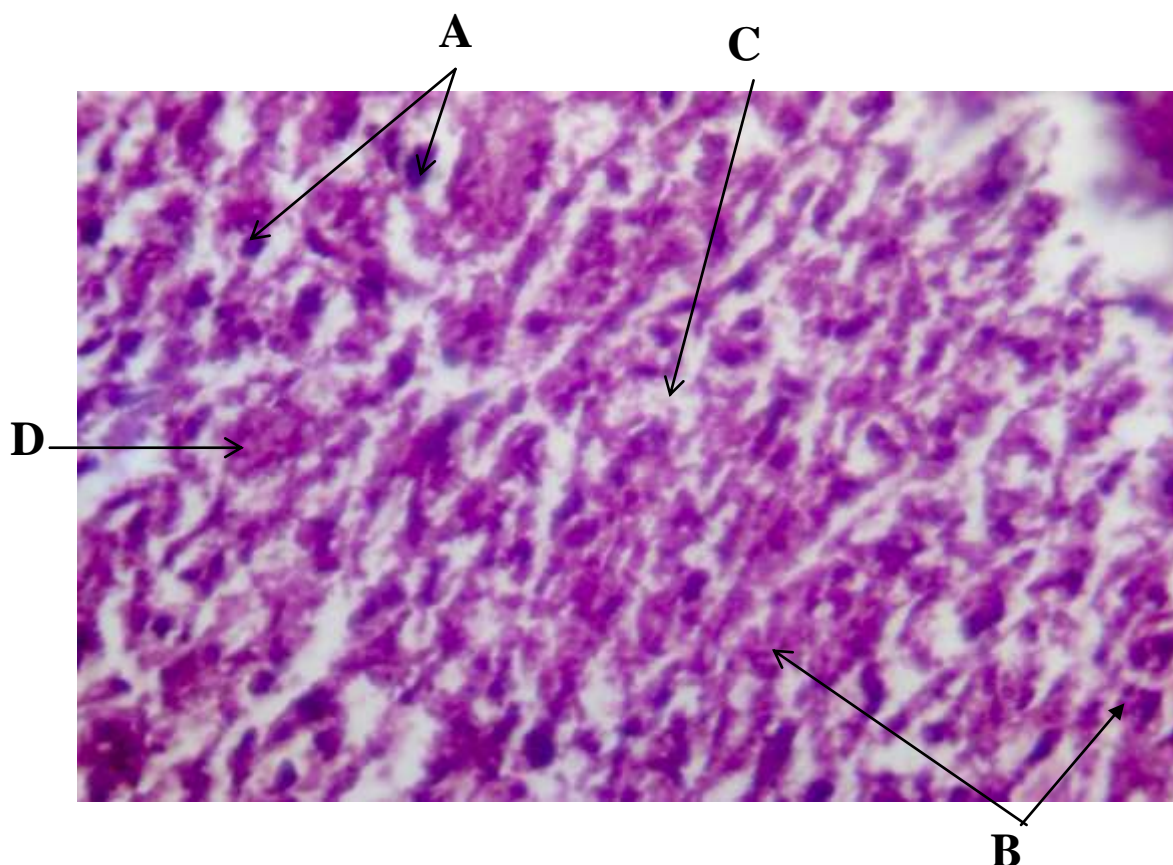


Figure 5: The effect of *peganumharmala* extract on the tissue of liver at the concentration 3% mg shows A- pyknotic nuclei .B- necrosis in some liver cells C- Dis arrangement of liver sinusoids . H&E 400

Discussion:

The present study demonstrated dose-related histological changes in the liver , there was sever changes in the liver parenchyma following different doses of water extract of *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 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 of liver , degeneration, necrosis in the epithelial cells of liver . In addition, our results revealed pathological changes in the livercells such as , infiltration and polymorph of nuclei and obstruction of bile duct . These histopathological observations are in agreement with previous studies(12,7) However,our observations were revealed histpathological changes occurred in the livers and kidneys of 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 that causes signs of intoxication due to administration of *P.harmala* extract, the present study was identical with previous findings such as studies conducted on the large animals such as sheep and horse(13) and cattle(14), in the cattle after postmortem examination of animal, no distinctive lesions were observed, rapid rigor mort has been observed, the renal and gastrointestinal system were noticed to be congested and hemorrhage in the liver has been manifested. The *P.harmala* has traditionally been in the public medicine as abortifacient and empenage agents.(20) Human toxicity has been occurred and reported in a patient with over dose of *P.harmala* plant seeds who has taken 50 gram of seeds for treatment of amenorrhea.(15)The signs of *harmala* over dose comprised of hallucinations and neuron-sensorial syndromes, bradycardia and gastrointestinal disturbances such as nausea and vomiting Para- clinical tests showed the function of liver to be normal and the patient had a normal hematological picture. A case report was recorded by(21), they mentioned a 35 year old male patient, he took around 150 gram of *P. harmala* seeds, after that vomited blood and gastrointestinal distress, endoscopy showed a 2.5 cm gastric ulcer at location of internal region. The symptoms of *P.harmala* toxicity experienced in 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 of the organs tissues such as liver , spleen especially in the epithelial cells that lined the spleen and the blood vessels, and splenic cells in white bulb , these our observations came 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 may be save in different uses, perhaps due to increase immunoglobulin or cell mediated cell response (macrophage) to produce immunoglobulin (antibody levels)

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دراسة نسجية لتأثير المستخلص المائي لنبات الحرمل على الكبد, وانزيماته وبعض معايير الدم في الجرذان البيض .

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الخلاصة

تم اجراء الدراسة الحالية في مختبر الفسلجة التابع لكلية الزراعة /جامعة الكوفة ,حيث تم تجريع اربع مجاميع من الجرذان البيض بمحلول الملح الفسيولوجي 0,09% وبمستخلص بذور نبات الحرمل بتركيز (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 والاووعية الدموية للكبد .اضافة ان التجريع المستمر للنبات بتركيز 3% سبب تحطم شديد في انوية وسايتو 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 تغلط نووي في انوية الخلايا الكبدية.

ان التجريع بتركيز 1و2% ملغرام/كيلوغرام من مستخلص بذور نبات الحرمل قد سبب تغيرات نسجية 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 سكر الدم الى (138,35.5) بمستوى احتمالية (0,01) مقارنة مع مجموعة السيطرة اضافة الى 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 معنوية في مستوى 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 .