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

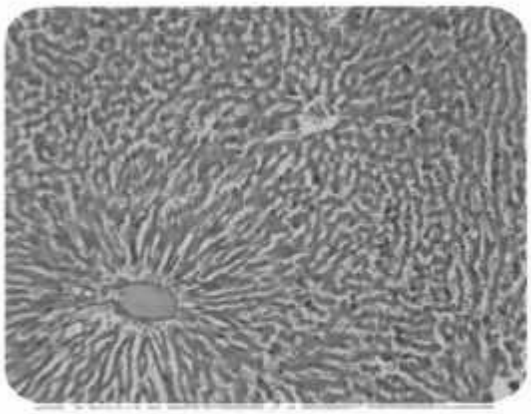
Table :(2) effect of *Pegenumharmala* on some parameters of blood

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

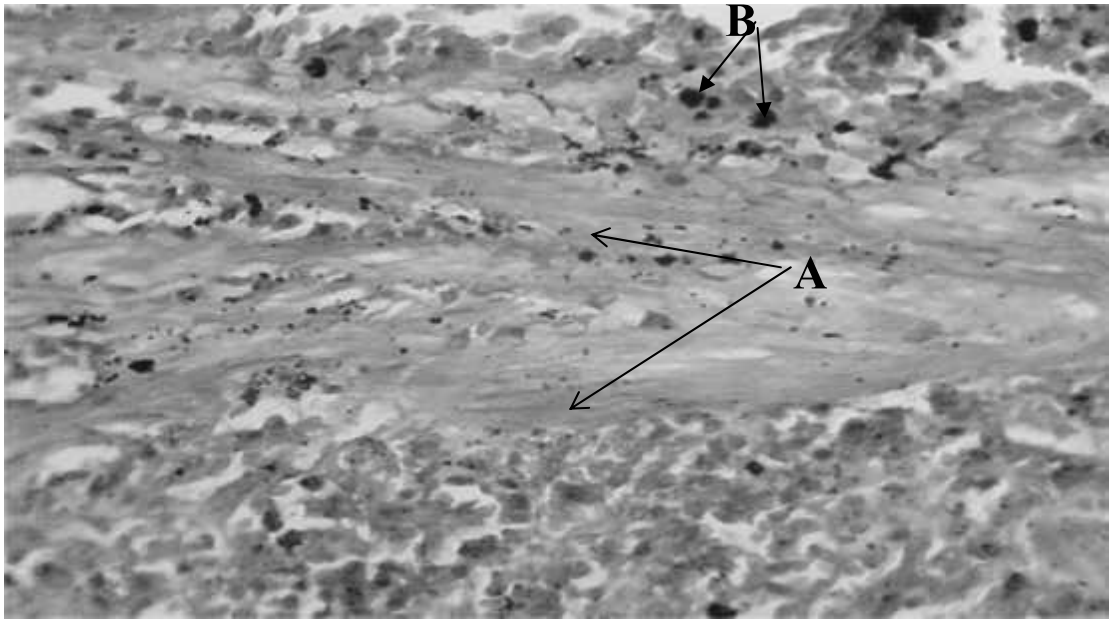


Figure 2: the effect of *peganumharmala* extract on the tissue of liver at the concentration 1% mg/ shows : A- Cirrhosis in liver cells ,B- Pyknotic in some hepatic cell . H & E 400.

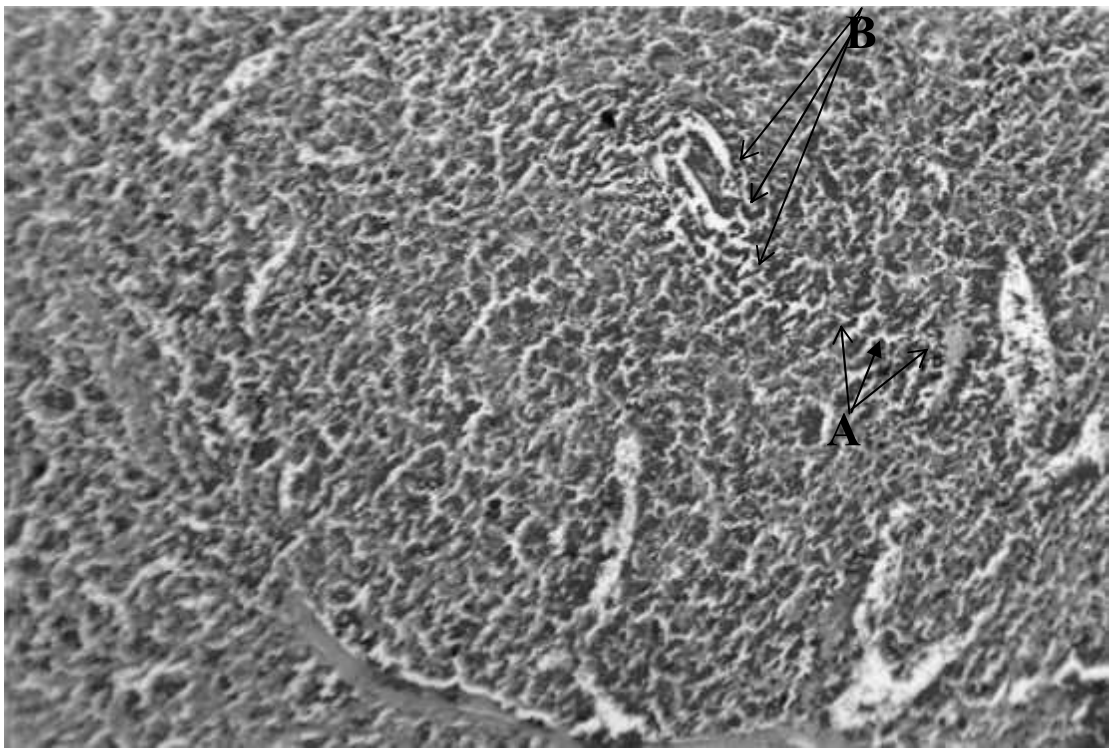
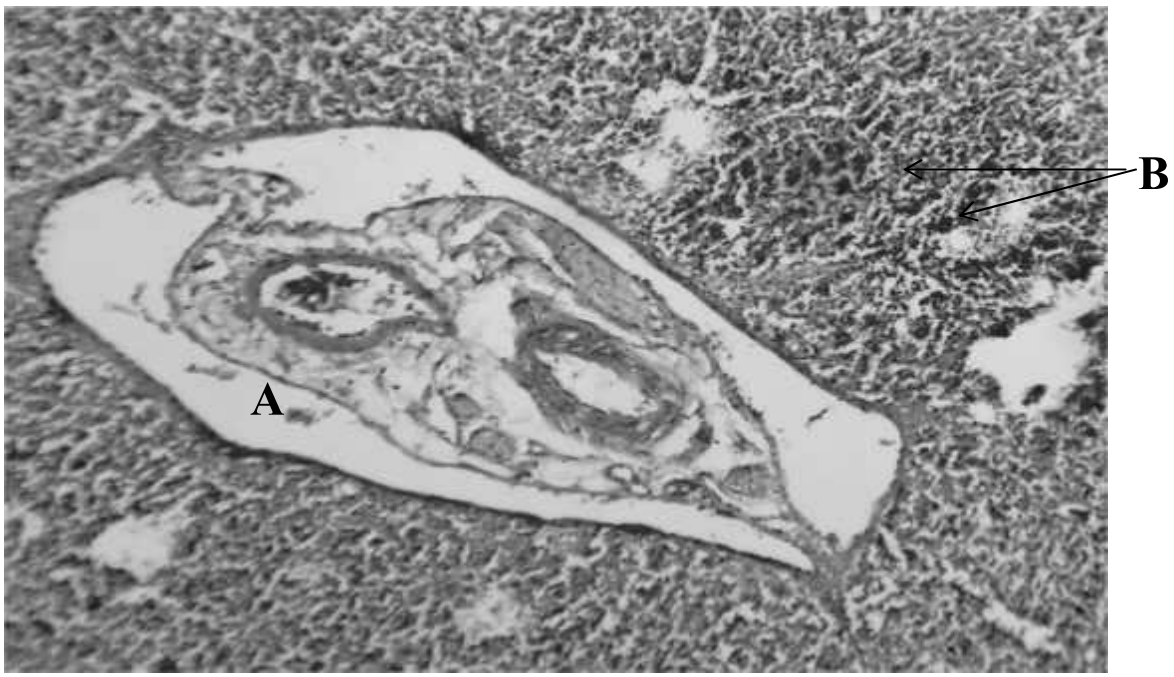


Figure 3: the effect of *peganumharmala* extract on the liver at the concentration 2% mg shows: A-necrosis in liver cells B- infiltration around blood vessel . H & E 100 X.



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.

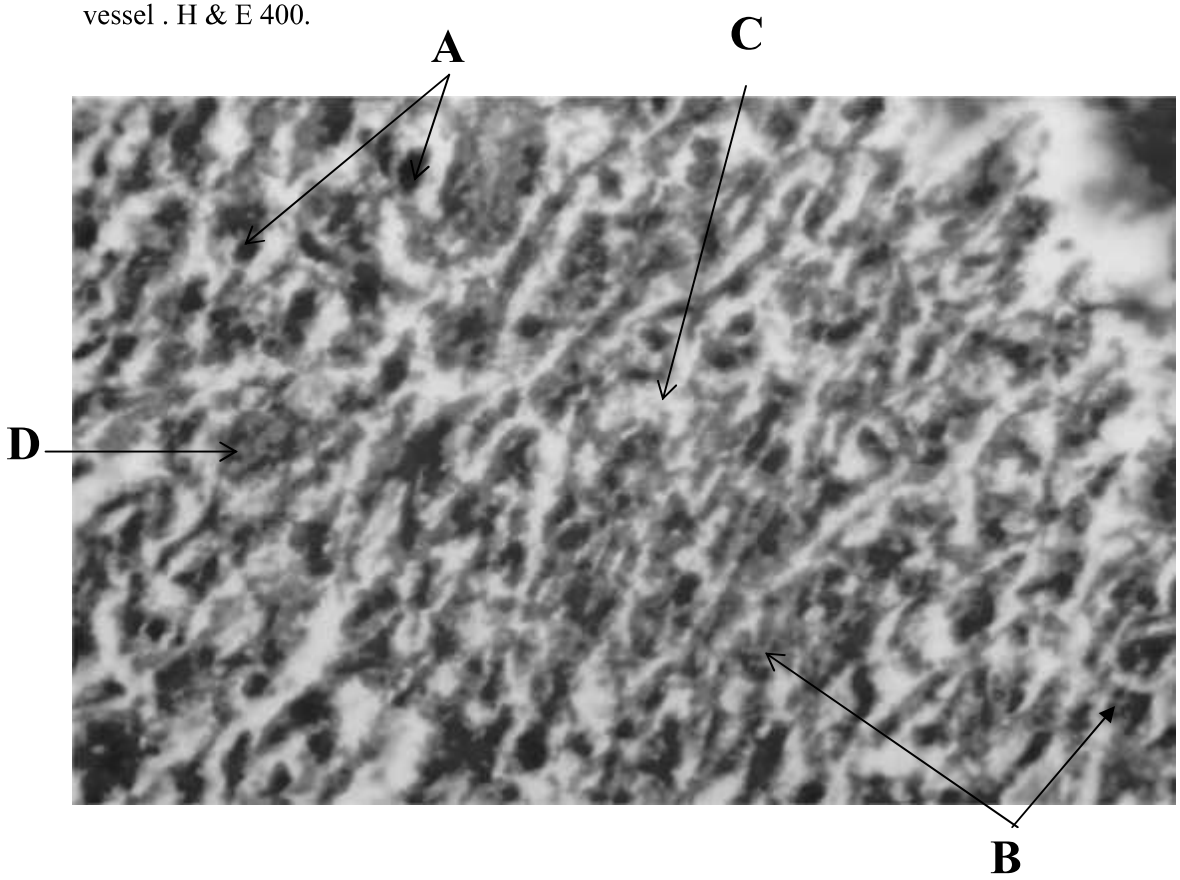


Figure 5: The effect of *peganumharmala* extract on the tissue of liver at the concentration 3% mg shows A- pyknotic nuclei .B- necrosis in some liver cells C- D dis arrangement of liver sinusoids . H&E 400

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 .