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Histological Study of Effect of Pegnam Harmala Eqeuos Extract on Spleen; Liver Enzymes and Parameters of Blood in Albino Rats

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Abstract: ***Four groups of male albino rats (Mus musculus) were subcutaneously administrated with normal saline(0.9 % NaCl2) or peganum harmala alcoholic seed extract (1%,2%,3%) mg/ kg) at daily interval for one month period. Thereafter, animals were scarified and specimens from the spleen were examined under light microscope for structural changes. Repeated treatment with peganum harmala seeds equeos extract caused dose- related structural changes in the spleen treated groups. severe changes were observed following 3% mg/ kg dose that were manifested by hemorrhage in interstitial connective tissue and blood vessels of the white and red bulbe of spleen as well as degeneration and necrosis in the epithelial lining of spleen.Peganum harmala seeds Aqueous extract at 1% and 2% mg/ kg caused slight to moderate histological changes in the white and red bulb of spleen manifested as degeneration and hypertrophy of tubular epithelial lining with narrowing of germinal artery . In the spleen, repeated treatment of peganum harmala seeds aqueous extract at 3% mg/ kg dose caused severe destruction of cells, by Histological study of the effect spleen,some liver enzymes and blood parameters in Albino Rats knotic of splenic cell and vesiculation in the cytoplasm due to degeneration. In addition, a significant effect(0.05) of p.harmala on total body weight compared with control ,the hight body weight was at second concentration 2%(295)gm while,the lowest body weight was(222.5)gm. liver weight also,affect significantly (0.05) with p.harmala ,it was 9.75gm at dose 2% compared with control(3.30)gm .both kidney and spleen have no affect significantly by p.harmala treatment . 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 observed between treated and control group. The highest values were in control groups while the lowest values were in treated group No significant changes were observed in the values of WBC and RBC in treated rats compared to controls.***

Keywords: histology ,pegnum harmala ,albino rats

1. 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 (8) agents. 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 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 monoamineoxidase (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. harmala on serum lipid profile and its economic benefits in broiler chicks Present study was designed to examine the effect of harmala extract in some parameters of blood and histobathological changes in liver of albino rats .

2. Materials and Methods

2.1 Animals

The present study was carried out in the laboratory of physiology in facility of agriculture, Albino Wister rats of either sex, weighing 200-350 g were used throughout the study Rat were housed in plastic cages (20 × 21 × 45 cm)with wooden waste bedding. The cages were subjected to cleaning and disinfectant 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 andlibitum.The animals were divided into four groups designated as A, B, C,D. Each group consists of 42 rats divided to 6 subgroups of 7 rats ,group A (control group)administrated normal saline ,group B administrated orally with concentration 1% of harmala, group c administrated orally with 2% from extract of harmala ,group D administrated orally with 3% from extract of harmala .The body weight was recorded throughout theexperiment prior to dosing. Doses were adjusted to bodyweight prior to each subcutaneously admin istrated .

2.2 Animals scarified and specimen's evaluation

After the administrated period was complete, the animals were anaesthetized by diethylether [(C2H4)2O]. The abdominal cavities of animals were opened; spleen 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 washing by tap water, dehydration by series of ascending concentrations of ethyl alcohol (70 %, 80 %, 90 %, and100 %) and clearing by xylole and infiltration and embedding by paraffin wax and made up blocks, then mounting by Canada- blasm and cover slides. The histological slides were examined by light microscope (type Olympus, Japan).

2- Extract preparation

The dry seeds of Iraqi Peganum harmala (100 g) were grinded and then were extracted with purified water for 24 hours in continuous (Soxhelt) apparatus. The extract was filtered, and water was removed by evaporation on a rotator evaporator under vacuum at 60ºC to a small volume provided.25 Briefly, the active ingredients were extracted from 20 g dry seeds using soxholate apparatus. There after the extract materials were concentrated by rotatory evaporator at 40- 45 C°. There after the extract materials was 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 p. harmala extract on total body weight and weight of organs

Factors	Mean sequars				
	Total weight/gm	Liver weight /gm/km	Right kidney weight gm/km	Left kidney weight gm/km	Spleen weight gm/km
T1	283.33 a	4.16 a	0.93 a	0.93 a	0.70 a
T2	295.0 a	9.75 b	0.95 a	0.95 a	0.95 a
T3	271.66 a	7.80 b	0.93 a	1.00 a	0.86 a
T.cont.	222.5 b	3.30 a	0.91 a	0.89 a	0.82 a
Significant level	*	*	n.s	n.s	n.s

total body weight compared with control .the highest body weight was in T2 (295) gm while the lowest body weight was in control (222.5) gm .liver weight is also, affected significantly (0.05) with p.harmala ,it was (9.75)gm in T2 compared with (3.30)gm in control group . both kidney and spleen are not affected significantly by p. Harmala treatment .many studies indicated that treatment with p.harmala extract caused a significant improvement in liver and kidney function in mice ,since this extract modulated the liver enzymes and kidney function regarding both pre and post- treatment (24). Alkaloid components may be lead to activation thyroid gland to secret T3 and T4 hormones and that cause increase metabolism process in the body of rats which caused increase in body weight (23).

Table 2: Effect of pegenum Harmala on some parameters of blood

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

Blood sugar and cholesterol are decreased significantly (0.01) by P.harmala treatment (table – 2) .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 ethanolic extract of *P. harmala* is as effective asthe known oral hypoglycaemic agent metformin in reducingthe blood glucose concentration after a sucrose challenge innormal and streptozotocin-induced diabetic rats. But further studies are to be conducted to find out whether long termstudies would bring the fasting blood glucose level to normal levels. Further studies on the mechanism of action whether itis an 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 were 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 a .

Table 3: Effect of pegenum Harmala on some parameters of blood

Factors	Mean squares			
	WBC	RBC	Hb	PCV
T1	6.43	7.31	15.46 a	42.9
T2	6.6	7.52	13.23 b	41.90 a
T3	5.76	6.78	13.40 b	38.90 ab
Normal	4.4	6.9	12.00 b	34.90 b
Significant level	n.s	n.s	*	*

No significant changes were observed in the values of WBC and RBC in treated rats compared to controls.(Tabe – 3) .

Histological results

The results of this study indicated that treatment with methanol *P. harmala* extract caused Autolysis in the c.t of trabeculae (septae) ,in collagen and elastic fibers ,necrosis was shown in cells and degeneration in some cells of white bulb ,and the same changes in plasma, lymphocyte,and macrophage cells of lymphatic tissue as shown in figure (1).

Figure (2) Shows the effect of extract of pegenum harmala at 1% mg dose on the tissue of spleen ,there was sever changes such as Damage in germinal artety (branch of splenic artery) in tunica media of artery, imperment of tunica media and damage in smooth muscle of white bulbe .

Figure (3) reveals Activation of white bulbe, hemorrihage in some of red bulbe and conjestion in splenice parenchyma

Figure (4) Shows Severe histological changes at concentration of 2% mg dose in white bulbe around the germinal artery ,thereis necrosis and degeneration and hemorrihage in lymphatic ,plasma amd macrophage cells of white bulbe and congestion in red bulbe ,some splenic sinosis have conjestion and pyknitic in nuclei of cell that mediatic immmune response.

Figure (5) there was hyperplasia in trapicule of the splenic structure ,also found foci in white bulbe and conjestion in trabicule and narrowing and inculussion in germinal artery (branch of white bulbe) and enlargment in the splenic sinusis ai concentration of 3% mg dose of extract of pegnum harmala.

Figure (6) the concentration of 3% shows sever effect sush as conjestion and hemorrihage and damage in parenchyma of the spleen, it was heavy pathological changes.

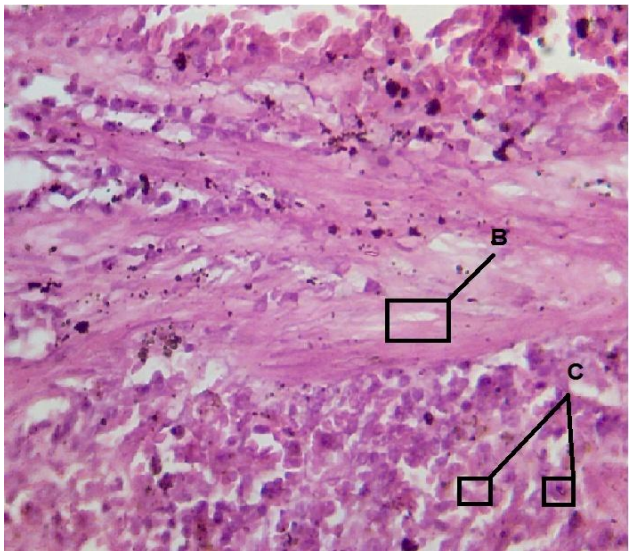

Figure 1: the effect of peganum harmala extract on the medulla of spleen of rat at the concentration 1% mg/ kg shows B- necrosis in connective tissues. C- degeneration in epithelial cells of spleen H & E 40 X.

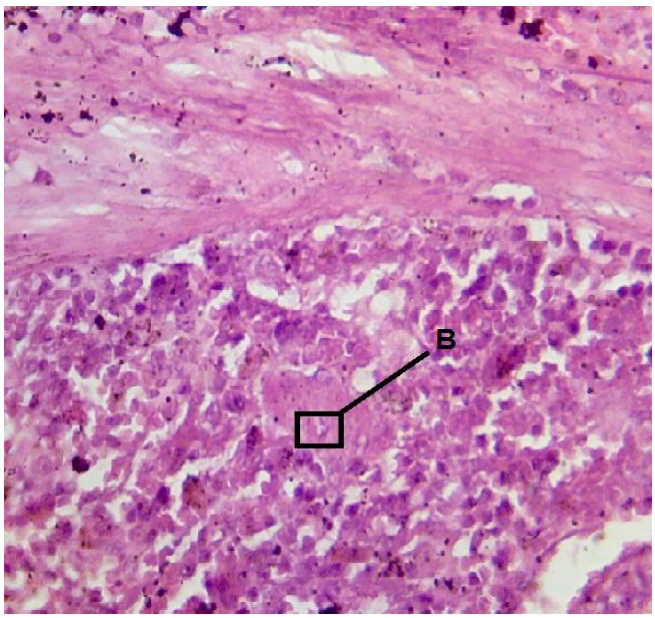

Figure 2: the effect of peganum harmala extract on the tissue of spleen of rat at the concentration 1% mg/ shows B- damage in smooth muscle in tunica media of germinal artery in the white bulbe H & E 40 X.

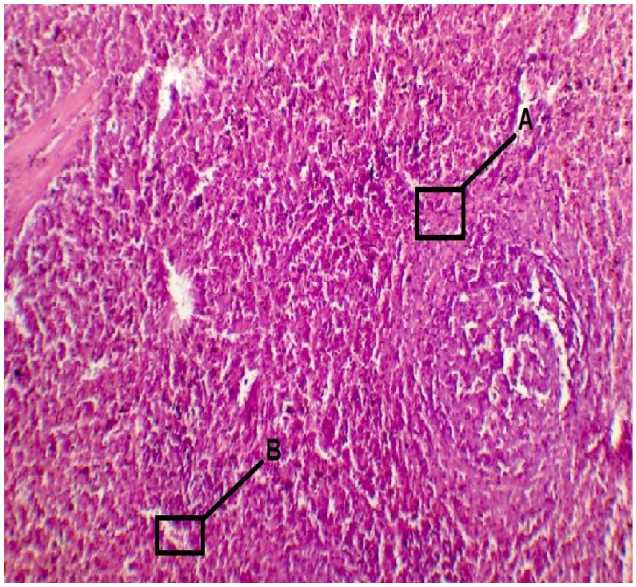

Figure 3: the effect of peganum harmala extract on the spleen of rat at the concentration 2% mg shows A-hemorrhage in red bulbe B- conjestion in splenic sinuses cells. H & E10 X.

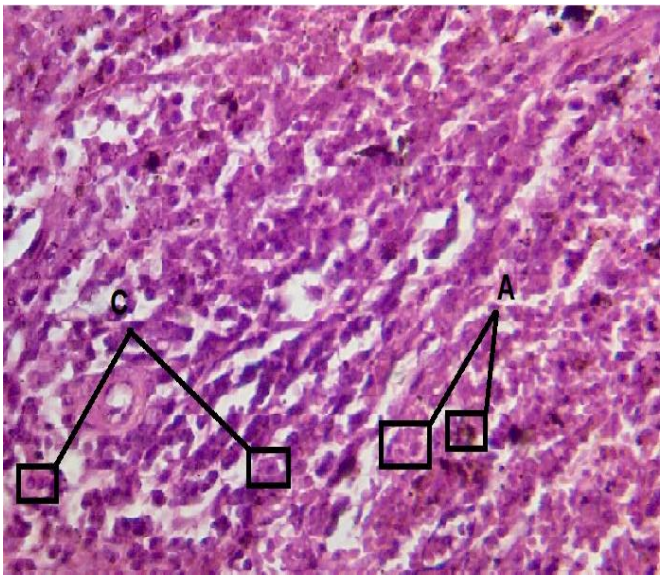

Figure 4: the effect of peganum harmala extract on the stissue of spleen of rat. at the concentration 2% mg shows . A- necrosis in some of lymphatic cells. . C- degeneration in in some cells of white bulbe H & E 40 X

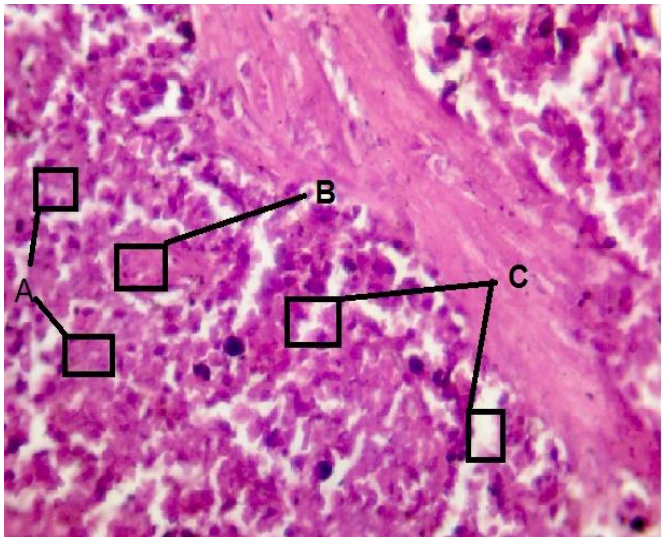

Figure 5: The effect of peganum harmala extract on the tissue of spleen at the concentration 3% mg shows A-foci in white bulbe .B- narrowing in germinal Artery C- Enlargement in the splenic of sinusoide H&E 40x.

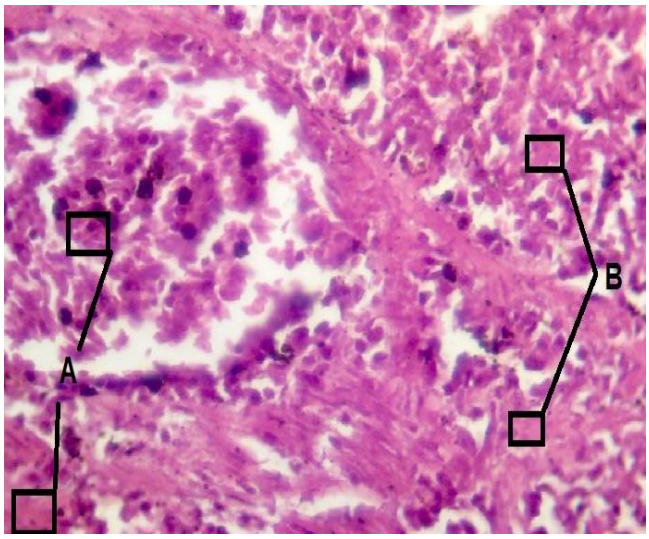

Figure 6: the effect of peganum harmala extract on the tissue at the conce of spleen of rat at concentration of 3% mg shows severe effect A- hemorrhage in some of epithelial cells B- conjestion in parenchyma . H & E 40X

3. Discussion

The present study demonstrated dose-related histological changes in the pleen, there was sever effects in red and white bulbe of spleen. because of long duration of expermant (30) days leads to sever patiological changes of the spleen especially in 2% and 3% concentration ,slight changes in in spleen at concentration of 1% mg of pegnum harmala extract .

Some recent studies in liver and kidney of mice indicated that in low concentration of harmala caused slight effects in mice (12). Peganum harmalaseed extract induced hemorrhage in the interstitial connective tissue of spleen, degeneration, necrosis in the red and white bulb of spleen. In addition, our results revealed severe changes in the spleen parenchyma following different doses of alcoholic extract of harmala seeds, which were manifested by hypertrophy of splenic cells, congestion and hemorrhage in the blood vessels. These histopathological observations are in agreement with previous studies (12, 7) However, our observations were revealed histopathological changes occurred in the livers and kidneys of mice, these changes represented, fatty degeneration ,necrosis, fibrosis, congestion of blood vessels, hemorrhage in the liver structures, from other hand, numerous histological alterations were happened in the cortex and medulla of mice kidneys, these were glomerulonephritis, interstitial hemorrhage, degeneration and necrosis in the kidney tubules have been ranged from moderate to severe signs, these our results ensure that causes signs of intoxication due to injection of harmala extract, the present study was identical with previous other findings suchas studies conducted on the large animals such as sheep and horse(13) and cattle (14), in the cattle after postmortem examination of animal, no distinctivelesions were observed, rapid rigor mort has been observed, the renal and gastrointestinal system were noticed to be congested and hemorrhage in the spleen has been manifested. The harmala has traditionally been inthe public medicineas abortifacient and emmenagogue agents.(20) Human toxicity has been occurred and reported in a patient with over dose of 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 neuro-sensorial syndromes, bradycardia and gastrointestinal disturbances such as nausea and vomiting para- clinical tests showed the function of spleen to be normal and the patient had a normal hematological picture, she was discharged from hospital few hours later after the signs of intoxication had disappeared. A case report was recorded by(21), they mentioned a 35 year old male patient, he took around 150 gram of peganum harmala seeds, after that vomited blood and gastrointestinaldistress, endoscopy showed a 2.5 cmgastric ulcer at location of internal region. The symptoms of peganum harmala toxicity experienced in the patients were similar to what had been reported for animal (16),and over dose of peganum harmala led to the damage and ulceration ofthe organs tissues such as liver , spleen especially in the epithelial cells that lined the spleen and the blood vessels, and splenic cells in white bulbe , these our observation scame to ensure the previous reports about peganumharmala intoxication.(17, 16).

In conclusion, these results, suggest that peganum harmala exerted a potent toxic effect on tissues of spleen 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 perhabs due to increase immunoglobin or cell mediated cell response (macrophage) to produce immunoglobin (antibody levels) .

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