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Research Article

Acute and Subacute Toxicity Evaluation of Alkaloids of *Peganum harmala* L. in Experimental Mice

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ABSTRACT
The objective of the study was to evaluate the acute and subacute toxicity of total alkaloids seeds of *Peganum harmala* in female mice. All morphological, biochemical, hematological, histopathological changes, in addition to mortality and bodyweight changes were recorded. After acute intraperitoneal administration of dose118 mg/kg, there were no any remarkable changes in general appearance and mortality. After 24h, a significant increase in relative weights of heart and brain with no change in hematological, biochemical parameter compared to the normal group. For 5 days a significant reduction in the relative weights of the kidneys and increase of brain with significant change in hematological (MCHC, MCV) and biochemical parameter (AST, ALP and Urea). Subacute study of dose (18 mg/kg) for 28 days showed no remarkable changes in general appearance and deaths occurred in experimental group. A significant increase in relative weight of brain compared to the normal group was observed. In biochemical parameters, a significant increase was seen in both ALT and AST enzyme activities. There was no significant change in hematological parameters. Histopathological examination revealed a ground glass appearance of hepatocytes and the vascular congestion. Alkaloids seeds of *P.harmala* showed significant toxicity in female mice.

Keywords: *Peganum harmala;* acute toxicity; subacute toxicity; mice; alkaloids

INTRODUCTION

Peganum harmala L. known locally as "harmel" in Algeria[1] is a wild-growing flowering plant that belongs to the Zygophyllaceae family and is considered an important medicinal plant[2]. The pharmacologically active compounds of this plant include a number of β-carboline (such as harmine, harmaline, harman and harmalol) and quinazoline alkaloids (vasicine and vasicinone) responsible of its pharmacological and toxicological effects[3]. Its root has been used as a parasiticide to kill body lice and leaves are used for treating rheumatism and nervous conditions where as seeds are used externally in the treatment of haemorrhoids and baldness[4].

The alkaloids in the seeds have pharmacological activities which including: antibacterial effects, vasorelaxant, anticancer, antinociceptive, antitumor and finally antiprotozoal effects. In addition to the therapeutic effects, harmal also has some toxicity. There were several reports of human and animal intoxications induced by the plant[5,1]. There have been some toxic symptoms reported in different human cases following ingestions of its seed extract or infusion, such as neuro-sensorial symptoms, visual hallucinations, and cardiovascular disorders such as bradycardia and lowblood pressure, psychomotor agitation, diffuse tremors, ataxia and vomiting[6,7]. The aim of the present study was therefore, to investigate the acute and subacute toxic effects of the total alkaloids seeds of *Peganum harmala* in mice.

MATERIALS AND METHODS

Plant material and extraction

The seeds of *Peganum harmala* were collected in the month of August, in the region Bordj Bou Arreridj (north-east of Algeria). The plant was identified on the basis of its morphological characteristics.

Total alkaloids extraction

Hundred gram of Air dried powdered of seeds was defatted with petroleum ether under reflux. The defatted dry powder was alkalized with 40 ml of NH_4OH (0.5 N) for 8 hours and were extracted to exhaustion with dichloromethane using a soxhlet apparatus for 6 h. The organic extract (containing free alkaloids and lipophilic impurities) is washed three times with 150 ml aqueous sulphuric acid (0.5 N).

The solution obtained is treated with NH_4OH (0.5 N) to pH 9 to liberate the free alkaloids then are separated three times by extraction with 3 x 150 ml diethyl ether and then dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids[8] .

Animals

Female Swiss albino mice (25-30g), obtained from the animal house of the Pasteur Institute in Algiers, were used

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Figure 1: Peganum harmala.

Figure 2: Seeds of Peganum harmala.

Table 1: Relative organ's weight of female mice treated with (118 mg/kg) of total alkaloids of *Peganum harmala* seeds. Results were expressed as the mean ±S.E.M.

Organ	Control	Group 1st day	Group5th day
Liver	0.051± 0.002	0.051±0.003	0.058±0.002
Kidneys	0.0142 ± 0.002	0.0092±0.0003	0.0087±0.0004*
Spleen	0.0041±0.0002	0.0168±0.008	0.0047±0.0003
Heart	0.0049±0.0002	0.0145±0.0003***	0.0045±0.0001
Lungs	0.0095±0.001	0.139±0.006	0.0067±0.0001
Brain	0.0129± 0.0004	0.0148±0.0003*	0.149±0.0004*

***** Significantly different at P< 0.05

Table 2: Effect of acute administration of total alkaloids of *Peganum harmala* seeds (118 mg/kg) on some hematological parameters .Results were expressed as the mean ±S.E.M.

Parameters	Control	Group 1st day	Group5th day
RBC 10^6/mm	7.737 ± 0.292	7.914 ± 0.270	7.39 ± 0.180
MCV fL	45,90 ± 0,511	44,42 ± 0,760	42,05± 0,424**
HCT %	35± 1	37±2	31±0.9
WBC 10^3/mm	6,233 ± 0,6412	6,371 ± 0,8676	6,980 ± 1,877
PLT 10^3/mm	249±41.43	412.7±50.06	413.7±56.77
HGB g/dl	12,83 ± 0,306	13,65 ± 0,433	12,57 ± 0,260
MCH pg	16,68 ± 0,439	16,53 ± 0,413	17,07 ± 0,525
MCHC g/dL	36.33±0.73	37.05±1.51	40.63±1.27*

***** Significantly different at P< 0.05

for the acute and subacute toxicity study. They were housed in hanging transparent plastic cages (55 x 33x19cm) in the animal room. The litter was renewed every 3 days. They were fed with a standard pellet and tap water *ad libitium.*

All animals were kept in standard environmental. Each mouse was identified by body marks using 1% picric acid solution. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the scientific council of the Faculty of Natural Sciences and Life of the University Ferhat Abbas, Setif 1 – Algeria.

Acute toxicity

The total alkaloids of *P.harmala* to be tested is dissolved in methanol and diluted by normal saline solution (v/v 0.9%).

The mice were grouped into three groups of 10 mice each. Two groups were given single dose (118 mg/kg of total alkaloids of seeds of *Peganum harmala* by intraperitoneal route.

The control group (10 mice) received saline water. At the end of all experimental periods, the first group was sacrificed after 24 hour of treatment, the second and control groups after 5 days[9].

Subacute toxicity

The female mice (20–25 g) were divided into two groups of 15 animals each and were placed under standard

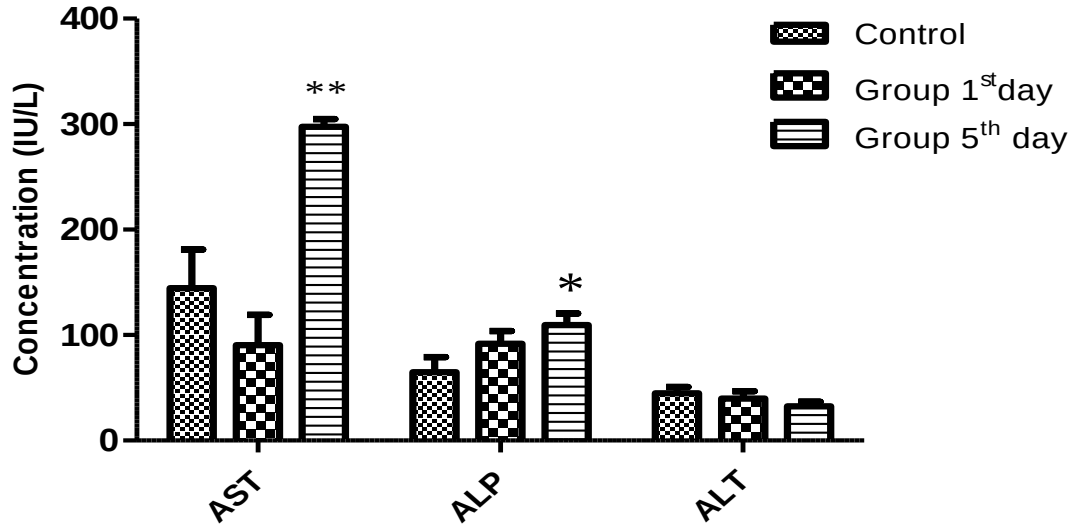

Figure 3: Effect of acute administration of total alkaloids of *Peganum harmala* seeds (118 mg/kg) on some biochemical parameters (hepatic function) of female mice. Results were expressed as the mean ±S.E.M.***** significantly different at P< 0.05.

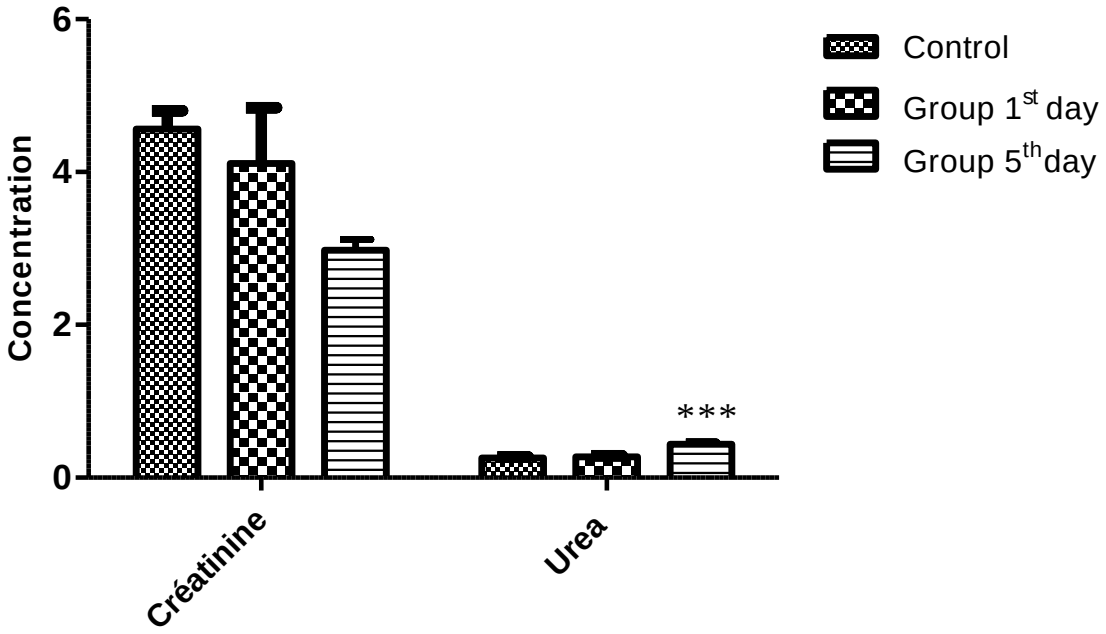

Figure 4: Effect of acute administration of total alkaloids of *Peganum harmala* seeds *(*118 mg/kg) on some biochemical parameters (renal function) of female mice. Results were expressed as the mean ±S.E.M.***** significantly different at P< 0.05.

conditions. Tested group received (18 mg/kg) of alkaloids for 28daysand control received vehicle at the same volume. The animals were weighed on the first day of the experiment and thereafter were then weighed each week, to note any weight variation.

Determination of hematological and serum biochemical parameters

The hematological and serum biochemical parameters were determined. Hematological parameters assayed included red blood cell (RBC) count, leukocyte (WBC) count, haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) ,Serum was

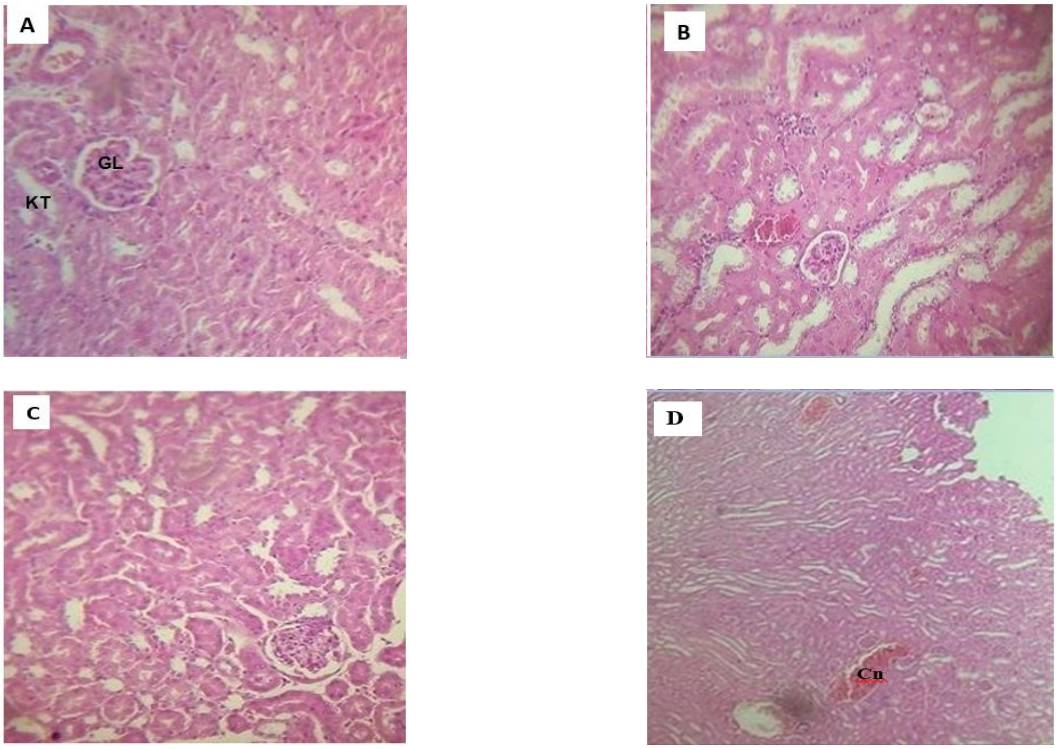

Figure 5: Histological section of kidney tissue with a single dose of (118 mg/Kg) :(**A**): control group. Treated groups; (**B):** After 24h; **C**: After 5 days; (**D**): After 28 days. (Hematoxylin/eosin stain). **GL**: glomerulus, **KT**: kidney tubes, **Cn**: Congestion. **X40.**

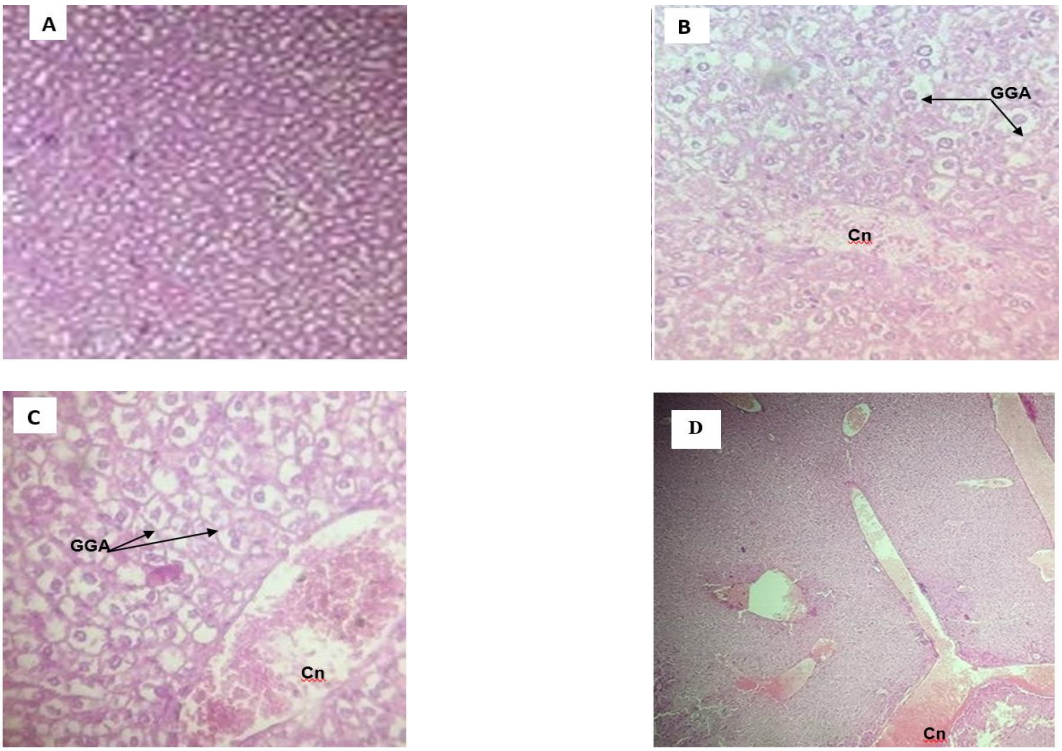

Figure 6: Histological sections of liver tissue with a single dose of 118 mg/kg: control group (**A**), treated groups; (**B)**: After 24h ;(**C)**: After 5 days; (**D**): After 28 days. **Cn**: Congestion; **GGA**: Ground Glass Appearance of hepatocytes. **X40.**

assayed for creatinine, urea, (AST),(ALT), (ALP). For the determination of hematological parameters was used medonic hematology analyzer systems (M-series), for the biochemical parameters was used an Advia 1800 Chemistry Analyzer, Siemens.

Histopathology

Immediately after collection of blood samples by cardiac puncture, animals were sacrificed. After autopsy, all tissues were examined grossly and major's organs (liver, brain, heart, kidneys, Spleen, and lung) were weighted.

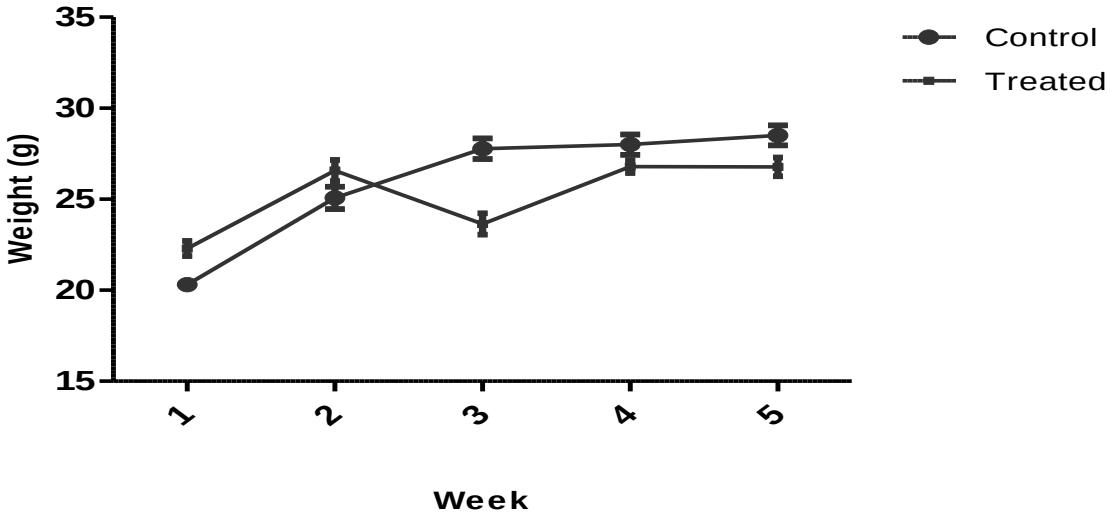

Figure 7: Effect of subacute administration of total alkaloids on body of female mice, values are Mean± SEM.

Table 3: Relative organ weights (g) of mice in the subacute toxicity study treated with 18 mg/kg of total alkaloids of *Peganum harmala* seeds. Results were expressed as the mean ±S.E.M.

Group	Control	Treated for 4 weeks
Liver	0.051± 0.0030	0.046±0.0016
Brain	0,014 ± 0,0004	0,031 ± 0,016***
Kidney	0,0107± 0,0005	0,0099 ± 0,0003
Lungs	0,0068 ± 0,0003	0,0077 ± 0,0004
Heart	0,0050 ± 0,0002	0,0047 ± 0,00018
Spleen	0,0057 ± 0,0002	0,0052 ± 0,0003

***** Significantly different at P< 0.05

Table 4: Effect of subacute administration of total alkaloids of *Peganum harmala* seeds (18 mg/kg) on some hematological parameters .Results were expressed as the mean ±S.E.M.

Parameters	Control	Treated for 4 weeks
RBC 10^6/	07,820 ± 0,2474	08,471 ± 0,2194
MCV fL	53,50 ± 0,5000	51,57 ± 0,7825
HCT %	43,78 ± 2,082	43,61 ± 1,232
WBC 10^3/mm	7,017 ± 0,9148	6,043 ± 0,9614
PLT 10^3/mm	924,8 ± 80,16	1009 ± 121,9
HGB g/dl	13,52 ± 0,2701	14,31 ± 0,2040
MCH pg	17,30 ± 0,3445	16,96 ± 0,3221
MCHC g/dL	32,35 ± 0,4759	32,97 ± 0,6171

***** Significantly different at P< 0.05

The relative organ weight of control and treated was calculated. Tissues from liver and Kidneys of all animals were fixed in 10% buffered formalin solutions then embedded in paraffin and cut with a microtome set at 5µm, stained with hematoxylin and eosin and examined by light microscopy for histopathological changes.

Statistical analysis

Comparisons among different groups were performed by One-way ANOVA and t" Student test using the software Graphpad Prism version 5.01. All data are expressed as mean± (SEM); Differences between groups were considered significant at $p<0.05$ levels.

RESULTS

Acute toxicity of total alkaloids

No signs of toxicity in general appearance observed after intraperitoneal administration of single dose tested (118mg/kg). None of the mice in all treated groups died during the course of the experiment.

The macroscopic examination of various organs *in situ* did not show any morphological changes in organs of treated animals compared with those of control.

The relative weights of heart and brain after first day were significantly increased compared to control. The group sacrificed after 5 days of treatment has presented a significant reduction in the relative weights of the kidneys and increase of brain compared to control group (Table 1).

The results of the hematological tests are summarized in (Table 2). All the tested hematological parameters were within normal limits compared to control group for the first day of treatment with total alkaloids seeds of *Peganum harmala*. No toxicologically significant differences between treated animals and control were found (Table 2).The group sacrificed after 5 days of treatment has presented significant decrease in MCHC and increase in MCV when compared with control (Table2).

The results of the various biochemical tests on the experimentally treated animals and control group are summarized in (Fig.3.4). Intraperitoneal administration of total alkaloids seeds of *Peganum harmala* did not cause significant changes in serum biochemical parameters such as Urea, Creatinine, AST, ALT and ALP levels when compared to control group for the first day of treatment, However, AST, ALP and Urea were significantly increased in treated animals when compared to control group after 5 days of treatment (Fig3.4).

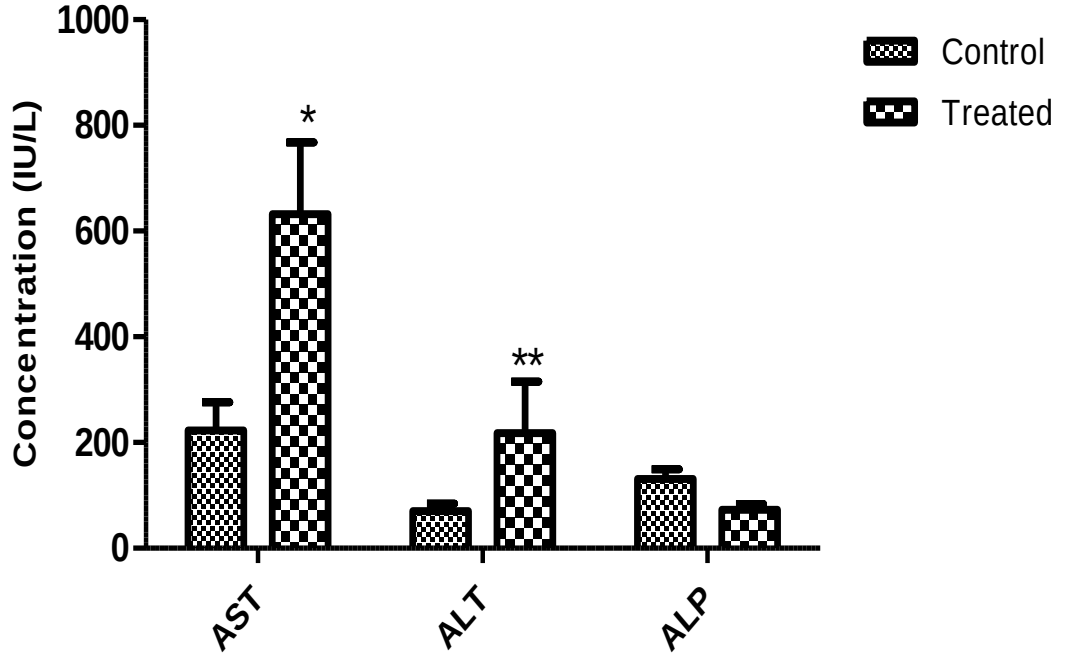

Figure 8: Effect of subacute administration of alkaloids of *Peganum harmala* seeds (18 mg/kg) on some biochemical parameters (hepatic function) of female mice. Results were expressed as the mean ±S.E.M.***** significantly different at P< 0.05.

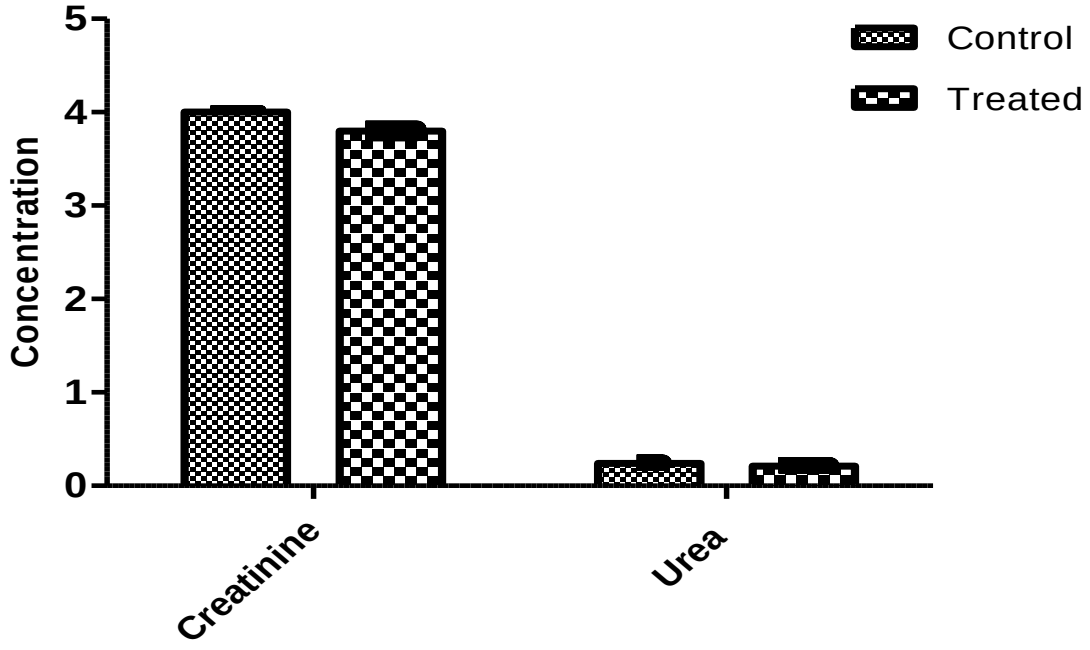

Figure 9: Effect of subacute administration of total alkaloids of *Peganum harmala* seeds (18 mg/kg) on some biochemical parameters (renal function) of female mice. Results were expressed as the mean ±S.E.M.***** significantly different at P< 0.05.

The histological examination of liver and kidneys from female mice were performed in both control and treated groups. The results of the kidneys at the dose applied (118 mg/kg) revealed no destruction architecture (Fig.5).

However, histological examination of the liver in the treated animals after 24 ^{h} and 5th days of treatment revealed a ground glass appearance of hepatocytes, and the vascular congestion (Fig.6).

Subacute toxicity

No clinical toxicity signs were observed in the treated group compared to the control group. None of the mice in the treatment or control groups died during the course of

the experiment. The saline and total alkaloids of *P.harmala* did not cause any gross morphological abnormality in various organs of the animals. There were no significant differences in the final body weight of the animals when compared with controls. The relative weight of organs showed no significant difference except of brain where an increase was observed compared to the control. The results of the hematological tests are summarized in Table 6. All the tested hematological parameters were within normal limits compared to control group.

The results of the various biochemical tests on the experimentally treated animals for 28 days and control group are summarized in (Table7). Significant differences in treated animals for AST and ALT levels when compared to control group.

DISCUSSION

In the toxicity study, organ weight is an important indicator of physiological and pathological status of animals. The relative organ weight is fundamental to confirm whether the organ weight was exposed to the injury or not. Increased in the heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant[10] .We observed a significant increase in heart (1^{st}day), brain (1^{st} and 5^{th} day) and significant decrease in kidneys (5^{th} days) of treatment at a dose 118 mg/kg, may indicate that the extract might have toxic potential on these organs. It could be argued that these changes may be toxicologically significant, as they were corroborated by the biochemical and histomorphology findings. Therefore, this study indicates that total alkaloids of *Peganum harmala* cause acute toxicity effects at the dose tested[11].

Haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals[12.] A significant decrease in mean corpuscular volume (MCV), but increase in means corpuscular hemoglobin concentration (MCHC) after 5^{th}days of treatment were observed. These parameters are useful as the RBC indices for differential diagnosis of anaemia[13].In addition the MCV has been reported to provide information on the size and status of erythrocytes, MCH and MCHC reflect the haemoglobin content of RBCs[14] .However the non-significant difference on the RBC, Hb (red blood cell counts and haemoglobin concentration) suggested that the total alkaloids of *Peganum harmala* did not affect a change in the average size of RBCs. By extension, it did not induce anemia[15].

Transaminases such as AST (SGOT), ALT (SGPT) are well known good indicators of liver function and used as biomarkers to conclude the probable toxicity of drugs and xenobiotics[16]. Significant increases in the levels of some biochemical parameters, particularly ALP, were observed in the female mice treated with total alkaloids after 5^{th} days of treatment, as compared with the control. Moreover a hepatic alkaline phosphate is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes[17]. Although elevated levels of (ALP) have been associated with bone diseases, it is also an indicator for obstructive jaundice and intra-hepatic cholestasis[18] could signal mild degeneration as observed in the histopathology part of the present study.

The total alkaloids of *Peganum harmala* seeds causes a significant increase in serum urea and AST without adversely affecting the serum levels of creatinine (non-significant reduction in creatinine).AST is mostly present in the myocardium, skeletal muscle, brain and kidney[19]. Urea, the end product of protein metabolism, and its concentration is influenced by the rate of excretion, while creatinine is the waste product of muscle metabolism. The significant increase in the serum levels of urea and the non-significant reduction in creatinine in the extract-treated mice is an indication that the kidney is able to clear the waste products from the system; this is also indicative that the extract had no deleterious effect in kidney[20]. Moreover the increase in Urea and AST levels could be explained the effect of alkaloids on the heart, brain or skeletal muscle. Studies have reported the effect of harmel on the brain and heart in humans[6, 21,22].

In the subacute toxicity study in female mice given the alkaloids, there were no significant changes in weight of body and organs except in increase of brain. All the animals exhibited a normal increment in body weight without drastic difference between both control and treated groups.

Sub-acute administration of total alkaloids of *Peganum harmala* did not cause significant changes in the haematological profile of female mice when compared with control, suggesting that the alkaloids may not be toxic to the blood system. For biochemical parameters the observed significant increase in ALT and AST activity in the test group compared to control may signify liver injury as seen in liver dysfunction, damage and liver diseases. Because the transaminases (AST and ALT) are well known enzymes used as biomarkers to predict possible toxicity, generally damage to liver cells will result in elevation of both these enzymes in the serum[16]. Increase in the activities of AST and ALT in the treated groups indicates that alkaloids of *Peganum harmala* have capacity to induce liver damage under conditions of subacute toxicity. Renal dysfunction can be assessed by concurrent measurements of urea, creatinine and uric acid and their normal levels reflect at reduced likelihood of renal problems[23].

In the present study, the insignificant changes in serum levels of urea and creatinine in female mice suggest that subacute administration of alkaloids does not affect the kidney function.

Histological changes in the kidney and liver of the animals were also examined. The histology slides of the kidneys at dose (118 mg/kg) showed no destruction to kidney architecture this observation as further confirmed by the biochemical biomarkers for renal function but on congestion was observed in subacute administration. The liver histology results revealed a ground glass appearance of hepatocytes (hepatocytes show enlarged and pale-staining cytoplasm) in acute toxicity and the vascular congestion in under conditions of acute and subacute toxicity.

CONCLUSION

In summary, acute and subacute toxicity study of *Peganum harmala* indicated that the total alkaloids seeds extract at the dose studied produce significant changes of biochemical parameters and histopathology of internal organs. Further studies to determine the chronic toxicity of this extract on animal are needed.

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