Trop J Nat Prod Res, October 2022; 6(10):1632-1637 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of the Subacute Toxic Effects of the Alkaloids of the Seeds of *Peganum harmala* L in the Liver, Kidney and Ovary of Female Rats

Mahdeb Nadia*, Allouni Rima, Reguai Rima, Azzoug khouloud, Bouzidi Abdelouahab

Department of Biochemistry – FSNV – Ferhat Abbas Sétif1 University

ARTICLE INFO

Article history:
Received 05 June 2022
Revised 20 August 2022
Accepted 20 October 2022
Published online 01 November 2022

ABSTRACT

Peganum harmala (Harmel) is a medicinal plant used in traditional Algerian medicine to treat several diseases. The subacute total alkaloid toxicity of *Peganum harmala* seeds on the major organs of female Wistar rats treated with 5.5 mg/kg, i.p. (1/30 LD_{50}) for 30 days was assessed in this study. The treated animals revealed a significant increase in body weight after the first 10 days of treatment followed by a significant drop after the 20th day. The analysis of the blood and serum parameters showed: at $p < 0.05$, a significant elevation in relative liver mass was recorded in parallel with the significant increase in glutamate-pyruvate transaminase (TGP) in liver parameters while hematologic and renal parameters did not change significantly, the exception however was for Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and urea. Histological sections of the liver of the treated rats showed some necrosis, a slight dilation of the Centro-lobular vein, mobilization of Kupffer cells, fatty deposits, blood (abnormal accumulation of blood) and hepatocyte ballooning. Some renal histological sections revealed glomerular atrophy. Observation of histological sections of the ovaries showed abnormalities: follicles developed devoid of oocytes, and a very limited number of developed follicles. In conclusion, the alkaloids of *Peganum harmala* have a toxic effect mainly on the ovaries, kidney and liver of female Wistar rats.

Keywords: *Peganum harmala*, Alkaloids, Toxicity, Rat, Organ.

Introduction

The *Peganum harmala* belongs to the Zygophyllaceae family. It is generally known as harmel in Algeria and throughout the Arab world. Harmel contains various classes of compounds: alkaloids, flavonoids, triterpenoids and other compounds.[1] Alkaloids are considered to be the active compounds of the plant. They are found mainly in seeds and roots and belong to the class of the β-carboline (Harmaline, Harmine, harmalol, Harmol) and quinazolic alkaloids.[2, 3] They have interesting pharmacological properties, which explain the wide use of harmel in traditional medicine across the Arab, Asian, and African countries, particularly in Algeria.[4, 5] Nevertheless, hamels'toxic effects in human and animals are common. [6, 7, 8] In some countries, the plant seeds are used by pregnant women to facilitate abortion or to induce childbirth.[9] The study evaluated the sub-acute toxic effects of the total alkaloids of the seeds of *Peganum harmala* on the female Wistar rats using the hematological and biochemical parameters relateing to the structure of the hepatic, renal and ovarian function.

*Corresponding author. E mail: nmahdeb@yahoo.com
Tel: +213 664380399

Citation: Nadia M, Rima A, Rima R, khouloud A, Abdelouahab B. Evaluation of the Subacute Toxic Effects of the Alkaloids of the Seeds of *Peganum harmala* L in the Liver, Kidney and Ovary of Female Rats. Trop J Nat Prod Res. 2022; 6(10):1632-1637.
http://www.doi.org/10.26538/tjnpr/v6i10.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Plant material

The seeds and the whole plant with the fruits of the *Peganum harmala* were purchased from an herbalist in the city of Setif (east of Algeria). The seeds were collected at the end of July 2020 in the south of the city that is characterized by a semi-arid climate. The seeds and the plant were identified on the basis of their morphological characteristics. The specimen with voucher number USRB 20-7 was deposited in the department. The seeds of the plant were used in our experiments due to their high concentration of alkaloids.[10] The seeds were cleaned of all impurities, dried in the laboratory at room temperature between 25°C and 27°C, and were protected from sunlight. They were stored in an airtight container until use.

Extraction of total alkaloids from the grains of Peganum harmala

The extraction of total alkaloids was according to the method of Mahdeb *et al*.[10], 100 g of *Peganum harmala* grains were ground using an electric grinder to a fine powder. The powder was macerated in 150 ml hexane at room temperature. It was mechanically agitated for 90 minutes to ensure proper elimination of fats. After vacuum filtration, the marc was recovered. The defatted powder was alkalized for at least 8 hours with stirring with a 1.5 N ammonia solution at room temperature (the pH was checked), thus allowing the alkaloids to change from the salt form to the organic form. The chloroform solvent (immiscible with water) was added to the previously alkalized marc with stirring for 1 hour. After vacuum filtration, marc (spent powder) and an organic solution were obtained. The organic solution was then extracted with 200 ml of 1.5 N acetic acid, in which the organic alkaloids turn into the salt form. The whole solution was transferred into a separating funnel. The acidic aqueous phase rich in alkaloids (the alkaloids pass to the state of salts and become soluble in the aqueous phase) was recovered, then alkalinized with 200 ml of 2N ammonia (the alkaloids salts then return to their organic form), then washed 3 times with dichloromethane and allowed to decant again to obtain an organic fraction rich in alkaloids. The organic fraction recovered was dehydrated by filtration through filter paper containing anhydrous sodium sulfate. The extract collected in a pre-tared beaker was evaporated on a hot plate. After cooling, the beaker was weighed again. The dry residue represented the total alkaloids of the grains of *Peganum harmala* [10-12]

Qualitative analysis of total alkaloids in seeds by thin layer chromatography (TLC)

TLC is a simple, fast and inexpensive method that allows you to highlight the active ingredients. Analytical chromatography was used to verify the presence of the indole alkaloids: harmine, harmaline, harmalol and harmol in the extract of total alkaloids from the seeds of *Peganum harmala*. Ready-to-use plates of Silica gel / UV254 with MACHEREY-NAGEL brand (Germany) dimensions 20 X20 cm were used. The mobile phase used was methanol / chloroform / ammonia: 79/20/1 (V / V / V). After dissolving the extract in methanol, 10 µl of solution was added using a micropipette on the previously activated plate in an oven at 110 ° C for 3 min and 1 cm from the lower edge on the line of base; and the deposit was dried using a hair dryer. The plate was then placed in the tank containing the mobile phase. When the solvent front reached 3 cm from the top edge of the plate, the chromatogram was removed, dried and sprayed with Dragendorff's reagent until the colored spots appear.[10-13]

Animals

Female Wistar rats used for the study, were obtained from the Pasteur Institute in Algiers (Algeria). They were housed in wire mesh cages, 55 cm long, 33 cm wide and 19 cm high. The animals were given tap water and food *ad libitum*, the litter was changed three times a week. For easy identification, they were marked on their body by a solution of picric acid (2%). The animals were acclimatized to the conditions of the animal house in the research unit at Ferhat Abbas University - Setif 1, for 14 days prior to the experiment. In the absence of an ethics committee on the use of animals for scientific purposes in our University, 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 and Life Sciences-Ferhat Abbas University Setif 1.

Subacute toxicity

Two groups of 10 female rats each were weighed and then treated, one with a dose of 5.5 mg / kg for 30 days of the extract of total alkaloids of *Peganum harmala* seed, dissolved in 1 ml of a methanol and physiological solution. And the other with physiological solution containing 1 ml of methanol (control group), both groups were administered intraperitoneally**.**

Blood collection

Hematological and serum biochemical parameters

At the end of the treatment period (30 days), the rats were weighed and then anesthetized with chloroform. The blood samples were taken from the orbital vein of the eye of the rats using hematocrit tubes. Blood was collected in two types of tubes, one with EDTA (Ethylenediaminetetraacetic) and the other with heparin. The EDTA tubes were immediately used for a numerical blood count, while the heparin tubes were centrifuged at 3200 RPM for 5 min and the serum was used for biochemical analyzes.

The hematological parameters namely RBC *(Red blood* cells), WBC *(White blood* cells*)*, HCT (Hematocrit), HGB (Hemoglobin), PLT (Platelet), VGM (Average Globular Volume), MPV (Mean platelet volume), MCHC (Mean corpuscular hemoglobin concentration) were carried out using a α Swelab Coulter blood cell counter.

Serum parameters were determined with the following biochemical kits: Glutamate-oxaloacetate-transaminase TGO, Glutamate-pyruvate transaminase TGP, Alkaline phosphatase (ALP) using the Tunisian Society's Biomaghreb enzyme kits (BIOMAGHREB) - Tunis Tunisia; Glucose, Urea and Creatinine using the Spinreact clinical diagnostic reagent kits - Barcelona Spain.

Histopathology

At the end of the experiment, the animals were sacrificed and after dissection, the organs were observed macroscopically, removed, washed with a 0.9% sodium chloride (Na Cl) solution, and weighed using a weighing scale. The liver, kidneys and ovary were removed and fixed in 10% neutral formalin. Dehydration of the organs by passage in alcohol baths of increasing concentrations was done (from 50° diluted alcohol to 100° absolute alcohol). Moreover, impregnation of organs by passage through toluene and inclusion of organs in paraffin were done. The passages of the paraffin block in a microtome allowed making sections of 5 µm. After which, deparaffinization and rehydration of organs with alcohol and then with distilled water were performed. The histological sections (5 µm) of the liver, kidney and ovary tissues were assessed by hematoxylin and eosin (H and E) for evaluation through light microscopy. [14]

Statistical analysis

The results obtained were analyzed statistically using the t-test and are expressed as mean ± standard error of the mean (SEM). The statistical analysis of these results was carried out with Sigma Stat 3.5 software. P values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results and Discussions

Peganum harmala L. is a species belonging to the zygophyllaceae family, it is widespread in the steppes and arid regions. The harmel is one of the most widely used plants in traditional medicine to treat joint and rheumatic pain. Poultices of chopped leaves applied *in situ* and held by a scarf against snakebite.[7] Although it can be used for therapeutic purposes, harmel can be dangerous because it is a poisonous plant especially when taken at high doses; many cases of poisoning have already been reported in animals and humans. [8, 9]

Peganum harmala contains β-carboline alkaloids, the most important of which are harmine, harmaline, harmol and harmalol and quinazoline alkaloids are responsible for its toxicological and pharmacological effects.[7, 15] The level of β-carboline alkaloids in the seeds of the harmel are higher, and the important ones includes: harmaline, harmine and harmalol with concentrations of 3.8%, 2.93% and 0.12% respectively. [16] The liquid-liquid extraction of these alkaloids produced a brick-red extract with a yield of 1%. This yield is in agreement with that obtained by Mahdeb *et al*.[10] (1.2%) on the same species.

Thin layer chromatography of the total alkaloid extract of Peganum harmala seeds allowed the separation of three indole alkaloids which appeared as orange spots after revelation with Dragendorff reagent and in the absence of standards, it could be harmaline, harmine and harmalol[16] (Figure 1).

Subacute toxicity

Numerous published studies have reported potential toxic effects of this plant in humans and animals.[17,18] It is necessary to characterize the effects of this plant on biological systems, including its toxicological effects of alkaloids at a dose of 5.5 mg / kg (1 / 30DL_{50}) characterized by the presence of several factors: A decrease in food intake, a change in physical activity and behavior (hair straightening, difficulty and reduction in motor skills).

Figure 1: Thin layer chromatography of total alkaloids of seeds of *Peganum harmala.*
Mobile phase: methanol / chloroform / ammonia: 79/20/1 (V / V / V).
Developer: Dragendorff reagent.

The signs reported agree with those described in literature by Abbassi *et al*. Mahmoudian *et al*. and Lamchouri *et al*.[19, 8, 17] They also indicated

that all domestic animals are susceptible to poisoning by this plant. The difficulty and reduction in motor skills can be explained by a direct blockade of energetic brain GABA receptors by harmane, harmine and harmaline producing a stimulating effect which would be responsible for the elevation of muscle tone. [20] The body weight of the rats in the treated and control groups changed throughout the period of experiment. Rats treated with the extract showed a decrease in body weight during the first 10 days of treatment compared to the control group. After 10 days the body weight of both groups showed a significant increase at $p<0.05$. On the 30th day of treatment, the body weight of the treated rats showed a significant drop compared to the control group (Figure 2). According to Shamaki *et al*.[21] changes in body weight can occur through impaired growth, especially when they contain agents that modify growth hormone secretion or somatostatin, or through impaired hormonal status, for example, agents modifying the secretion of sex steroids and therefore altering the maturation pattern and food consumption, such as agents acting on the central serotoninergic or dopaminergic systems (harmine antagonists of serotonin). Kifayatullah *et al*.[22] confirmed that these changes in body weight accompanied by fat accumulation and a physiological adaptive response to plant extracts rather than toxic effects of chemicals or drugs that lead to decreased appetite hence lowering the calorie intake for the animal. Hematologic assessment in animals is of paramount interest in defining the diagnosis of many diseases. Altered parameters (white blood cells and red blood cells) are an indicator of early exposure to toxicants.[23,24] The results showed that there was no change in the normal proportions of red blood cells, hemoglobin and white blood cells during the treatment, except for MPV and PDW which show a significant increase of 8.07%, 5.88% at $p<0.05$ respectively (Table 2). So it can be said that the extract has no effect on the hematological parameters. The most widely used criteria for the toxic action of a substance in rats are the detection of gross and histological abnormalities in the organs, change in organ weights, and increased mortality rate.[25] After dissection, macroscopic examination of the organs in situ (kidney, liver, lungs, brain, spleen, heart and ovaries) of female Wistar rats treated with total alkaloids from the seeds of *Peganum harmala*, did not reveal any morphological change in comparison with that of the control. After 30 days of treatment, there was a significant increase in the relative mass of the liver of 15.05%, on the other hand the relative mass of the other organs: spleen, lung, kidney, heart, brain of the treated rats did not reveal any significant modification, compared to those in the control group (Table 1).

According to Xin *et al*.,[26] an increase in relative organ mass indicates congestion, edema or enlargement, while a decrease in relative organ mass indicates organ atrophy and other degenerative changes. The relative increase in mass could be due to Hepatomegaly which is defined as an increase in liver size. According to Blétry and Marroun,[27] liver diseases can be the cause of hepatomegaly. Steatosis (fatty deposits in the liver tissue) is one of the causes of hepatomegaly. Histological sections confirmed the presence of hepatic steatosis.

Hepatic cytolysis is conventionally studied by measuring plasma enzymes such as transaminases (TGP and TGO). The results of the hepatic parameters of the treated and sacrificed female Wistar rats after 30 days showed a non-significant increase in ALP and the TGO enzymes of 6.26%, 14.07% respectively and a significant increase in TGP of 53.82% compared to the control group (Figure 3). According to Lauda Maillen *et al*,[28] *an* elevated glutamate-pyruvate transaminase (*TGP*) can be associated with much liver pathology (hepatitis, hepatic steatosis, and biliary tract disease), the intake of toxicants, systemic diseases, infectious or blood diseases.

Microscopic observation of the liver of the treated Wistar rats showed dilation of the centrilobular veins, and often blood congestion in the hepatocyte tissue, some necrosis, and mobilization of Kupffer cells and balloonization of the hepatocytes. In addition, large fatty deposits were observed in the hepatocyte tissue (Figure 6). The results obtained are consistent with the results of Mohamed *et al*,[29] who found that the treatment of male mice with a repeated dose of 150 mg / kg of alcoholic extract from the seeds *of Peganum harmala*, twice a week for one month period subcutaneously to a toxic effect on the liver presented by:

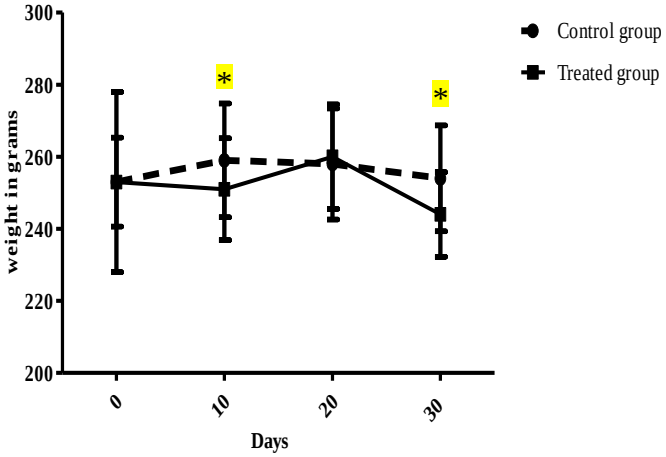

Figure 2: Change in body weight of control and rats treated with the extract of the total alkaloids of *Peganum harmala* L at a dose of 5.5 mg / kg (1 / 30 DL_{50}). The results are expressed as the mean ± SEM..

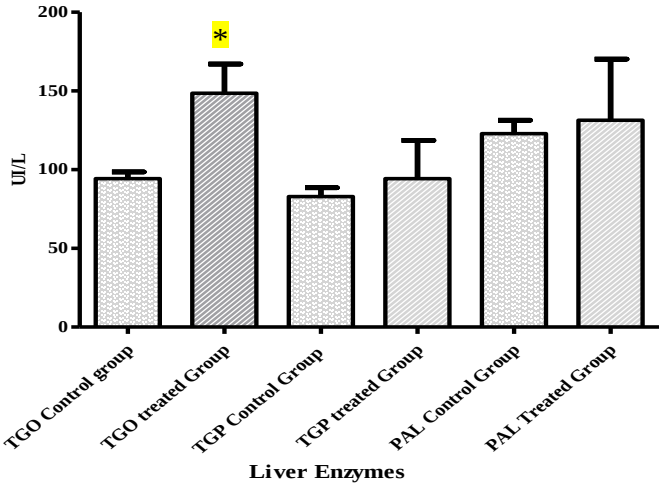

Figure 3: The hepatic parameters TGO, TGP, PAL (IU / L) of female Wistar rats in control and treated groups..

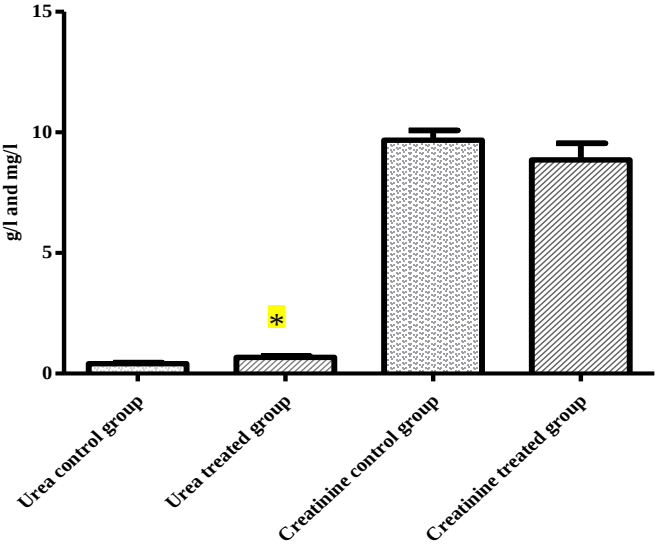

Figure 4: Serum urea (g / l) and creatinine (mg / l) level of control and treated female rats values are expressed as mean ± SEM.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Relative mass of the organs of female rats treated with a dose of 5.5 mg / kg (1 / 30DL_{50}). The results are expressed as mean ± SEM.

Organs	Spleen	lungs	Liver	Kidneys	Heart	Brain
Treated Group	0.0049 ± 6.63325E-04	0.00714 ± 3.7815 E-04	0.0372* ± 2.6832E-03	0.00806 ± 1.1059E-03	0.00334 ± 8.9443E-05	0.00788 ± 1.31225E-03
Control group	0.004283333 ± 6.21021 E-04	0.007 ± 7.3212 E-04	0.03233333 ±3.4448E-03	7.1666 E-03 ± 6.9761E-04	0.0033 ± 0.00028	7.1166E-04 ± 1.0998 E-04

Table 2: Hematological parameters of female rats treated with a dose of 5.5 mg / kg (1 / 30DL_{50}) of the total alkaloids of the seeds *of Peganum harmala*. Values are expressed as mean ± SEM.

Parameters	RBC×10^1/l	VGM	IDR %	IDRa	HCT %	PLT × 10^9/l	MPV	PDW	PTC %
Control group	7.47 ± 0.23	56.08 ±1.165	14.4 ± 0.409	39.116 ± 1.105	41.916 ± 1.808	678.333 ± 118.867	5.7 ± 0.244	9.183 ± 0.278	0.388 ± 0.074
Treated group	7.35 ± 0.59	54.98 ± 1.500	15 ± 0.494	38.56 ± 1.073	40.36 ± 2.590	654 ± 120.4	6.16* ± 0.313	9.72* ± 0.414	0.402 ± 0.075

LPCR %	WBC×10^9/l	HGB g/dl	TCMH	MCHC g/dl	LYM×10^9/l	GRAN×10^9/l	MID×10^9/l	LYM %	GRA %	MID %
3.55 ± 0.859	5.916 ± 1.515	13.4 ± 0.473	17.91 ± 0.204	31.96 ± 0.463	4.116 ± 1.022	1.166 ± 0.560	0.633 ± 0.366	70.6 ± 9.724	20.16 ± 7.256	9.233 ± 3.55
4.98 ± 1.713	5.82 ± 1.503	12.8 ± 0.554	17.56 ± 0.740	31.96 ± 0.844	4.14 ± 1.415	1.06 ± 0.378	0.58 ± 0.19	70.58 ± 7.369	20.26 ± 6.668	9.16 ± 2.17

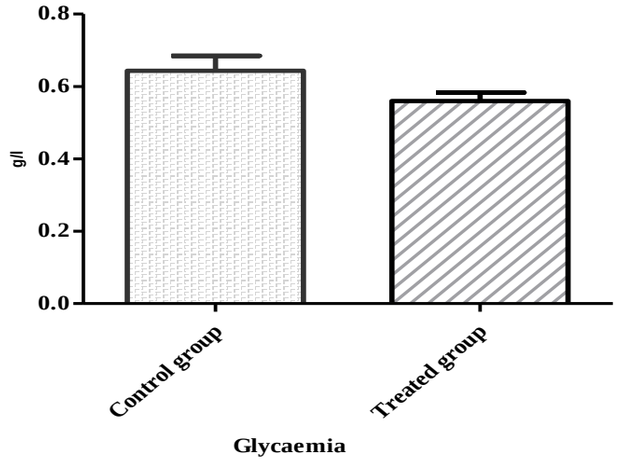

Figure 5: Serum level of "glycemia" of control and treated female rats. The values are expressed on average ± SEM.

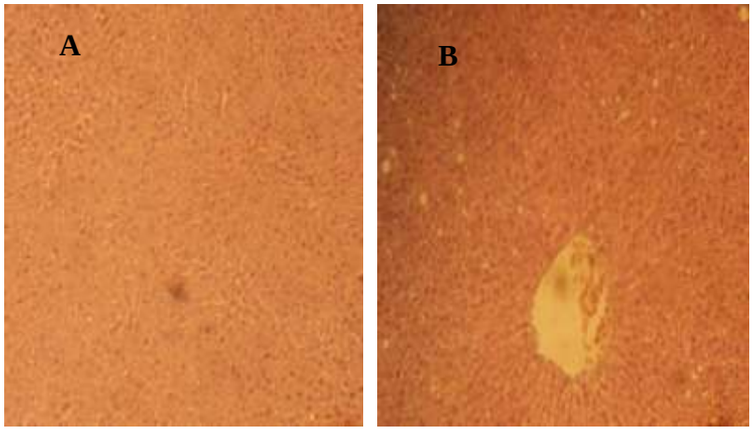

Figure 6: Histological section of the liver of female rats. B: Group treated with a dose of 5.5 mg / kg of the extract of the total alkaloids of *Peganum harmala* (× 100) and A: Control group (× 100).

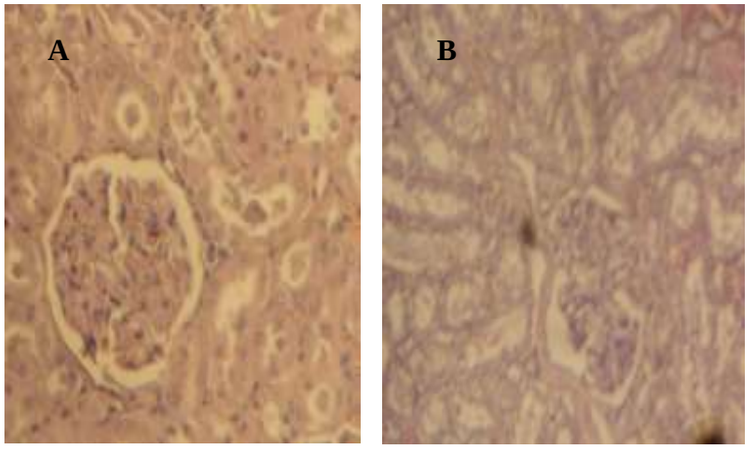

Figure 7: Histological section of the kidney of female rats. A: control group (× 100). B: group treated with a dose of 5.5 mg / kg of the extract of the total alkaloids *of Peganum harmala* (× 100).

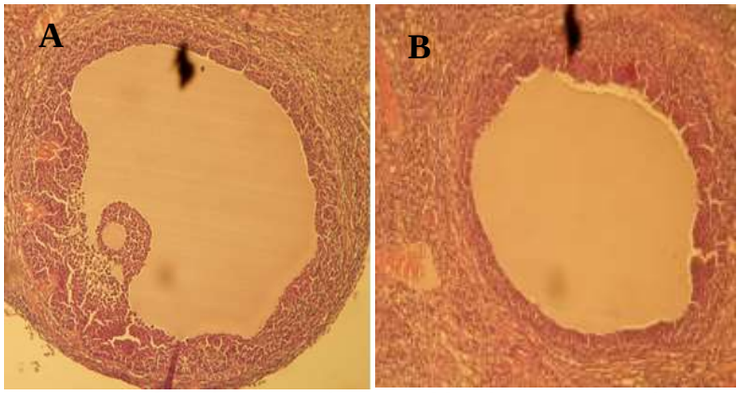

Figure 8: Histological section in the ovary of female rats. A: Control group (× 100). B: Group treated with a dose of 5.5 mg / kg of the extract of total alkaloids *of Peganum harmala* (× 100).

central vein congestion enlargement of sinusoid cells and liver cell necrosis. And with the work of Lamchouri *et al*,[17] who observed an increase in transaminases and necrosis foci in the liver of animals treated with aqueous extract of the seeds of *Peganum harmala*, under chronic conditions. Urea is a waste product formed in the liver when the protein is metabolized; it is used to assess kidney function. Urea is transported to the kidneys where it is filtered from the blood into the urine.[30] The serum urea of treated female rats shows a significant increase of 62.75% at $p<0.05$. While creatinine shows a slight non-significant decrease of 6.09% compared to that of the control animals (Figure 4). According to Higgins,[31] the increased serum urea level suggests impairment or damage to the kidneys that prevents glomerular filtration. An increase in urea level can also be due to an increase in dietary protein. On other hand, the histological section of the kidney in the treated group showed slight blood congestion, and limited glomerular atrophy of some nephrons which is reflected negatively on the glomerular filtration (figure 7). It is assumed that the kidney functions normally and that the increase in urea is due to increased protein catabolism to compensate for the lack of energy. Since there was a decrease in carbohydrate catabolism explained by an increase in serum glycaemia of 48.01% in the treated Wistar rats compared to the control group (Figure 5).

Histological sections in the ovaries have shown the existence of a disorder, such as the presence of a few developed follicles and some of them do not contain an oocyte (Figure 8). Previous studies have shown that the *Peganum harmala* plant has an effect on the fertility of Wistar rats and that it can induce abortion.[7] In addition, Shapira *et al*,[32] found that the methanolic extract of *Peganum harmala* at a dose of 2.5 g / kg / day, presented in food or in drink suspension for 30 days, significantly prolonged the 1-day diestrus. At the same time, the phytochemical study by Abbassi *et al*,[19] carried out on ethanolic extracts of *Peganum harmala*, revealed that this extract has an effect on the size of the oocytes and leads to a delay in sexual maturity. So, it is likely that this plant has an effect on oogenesis and the development of follicles.

However, the histological sections of the ovaries of the treated rats showed a few developed tertiary follicles not containing oocytes (Figure 8).

Conclusion

The intraperitoneal treatment of female Wistar rats with a dose of 5.5 mg/kg (1/30 LD_{50}) alkaloids of Harmel seeds, for 30 days, disrupted some hematological and biochemical parameters.

Histological examination showed the presence of several abnormalities in the liver, kidneys and ovary ranging from small vascular congestions to necrosis, atrophy of glomerulus, and effect on follicular evolution with absence of oocyte in some rats.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This Research was Supported By Faculty of Natural and Life Sciences - University Ferhat Abbas Setif 1 - Algeria

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