

Evaluation of Acute Toxicity of the Alkaloids of *Peganum harmala* L Seeds in Albino Wistar Female Rats

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Abstract The *Peganum harmala*, commonly known as syrian street, belongs to the family Zygophyllaceae. *Peganum harmala* L is widely used in traditional medicine and pharmacology. The evaluation of toxic properties of *Peganum harmala* is crucial when considering public health protection because exposure to plant extracts can result in undesirable effects on consumers. Hence, in this study the acute IP toxicity of the total alkaloids of *Peganum harmala* L seeds was investigated in female rats. The oral dose (60 mg/kg) of tested plant extract was administered to two groups of animals (group I and II) in single dose and third group III is control. The first treated group is sacrificed after 24 hours and the second treated group and control group are sacrificed after 5 days. Throughout the treatment, changes in behavioral pattern, clinical sign and body weight of rats in treatment groups are observed. Also, a significant decrease in relative liver mass and serum biochemical parameters (AST, ALT, PAL, Bil D and Bil T) were observed. An increase in red blood cells, hemoglobin and hematocrit was recorded in group II. Histological examination shows that the seed extract of *Peganum harmala* L has no remarkable toxic effect on the liver of the treated animals of the second group. The result indicates that the IP administration of the total alkaloids of *Peganum harmala* L seeds plant did not produce any significant toxic effect in albino rats.

Keywords: *Peganum harmala*, alkaloids, Acute Toxicity, Liver, Rat

1. Introduction

Medicinal plants are becoming more widely used, especially among people in the Third World, not only because of the high cost of drugs, but also because of the despair of drugs that sometimes do not give the desired therapeutic results.

The misuse of medicinal plants by patients with mostly chronic diseases leads to medical complications. Indeed,

the resemblance and the same name of many plants are the main causes and have become a source of concern for many countries. On the other hand, plants are the cause of much intoxication, especially in rural areas [1].

Among these plants, *Peganum harmala* grows in semi-arid rangeland [2].

It is locally known as Harmel, whose active ingredients are alkaloids whose chemical structure associates an indole nucleus with a pyridine nucleus: harmane, harmine, harmaline, harmalol [3]. They are serotonin antagonists [4].

The *Peganum harmala*, commonly known as syrian street and wild street, is a flowering plant and is widely distributed in central Asia, North Africa and the Middle East. It has also been introduced in America and Australia [2]. It belongs to the family Zygophyllaceae of the order Zygophyllales.

The toxic effect of this plant is attributed to four indole alkaloids: harmaline, harmine, harmalol and harmol, they are serotonin antagonists [4, 5].

Peganum harmala has been used in folk medicine and pharmacology; it is used to treat rheumatic pain, asthma and is traditionally used in the Middle East, Algeria and Morocco as an emmenagogue, abortifacient or to activate childbirth [6].

Young people consume *Peganum harmala* as hallucinogenic and sedative products; the plant is smoked or consumed as an infusion [7]. The antinociceptive effects of *Peganum harmala* alkaloids on mice have been reported [8].

There are several publications that indicate the wide variety of pharmacological and biological activities of this plant such as: antibacterial and antifungal activity [9].

Once consumed, the plant is responsible for several disorders. The intoxication symptoms manifested as blurred vision, phonophobia, floating feeling, and tinnitus ringing [9].

In this work, we tried to study the effect of the alkaloids of seeds of *Peganum harmala* on the liver and the blood of female rats (Albinos-Wistar) under conditions of acute toxicity.



Figure 1. The seeds and plants of *Peganum harmala* L.

2. Vegetal Material

The seeds and the whole plant with the seeds of the plant of *Peganum harmala* L are bought from an herbalist (market located in the center of Sétif - East-Algeria). The seeds and the plant are identified on the basis of their morphology [10] (Figure 1).

The seeds of the plant are used as a support for the biological tests because of their high concentration of alkaloids.

The ripe seeds are freed from all impurities, and are dried in the laboratory at room temperature between 25 °C and 27 °C, and protected from the sun and light.

3. Phytochemical Analysis of Total Alkaloids of Seeds

3.1. Extraction of Total Alkaloids

The extraction and identification of alkaloids have become routine methods in our laboratory.

80 g of dry seed powder are defatted with petroleum ether, and the powder is then immersed in 150 ml of NH_4OH (25%) for at least 4 hours. At alkaline pH, the alkaloids which were in the form of salts in the seeds pass to the organic form and can be dissolved in organic solvents. The alkaloids are then extracted with chloroform using the soxhlet apparatus at least for 6 hours.

The organic solution obtained containing the total alkaloids is washed three times with 150 ml of sulfuric acid (2%). Under these conditions, the alkaloids pass to the salt form.

The acid solution is treated with ammonia to pH 10. The alkaloids become organic and are extracted with dichloromethane and then dried with Na_2SO_4 and

concentrated to dryness under reduced pressure to obtain crude alkaloids [11-13].

3.2. Thin Layer Chromatography (TLC)

TLC is a simple and inexpensive method that allows to highlight the active ingredients (in this case: the alkaloids) present in our extract.

To highlight the alkaloids of *Peganum harmala* seeds, we used gel plates alugram salt G / UV 254 20 x 20, procured from Macherey-Nagel, Germany.

After several tests and modifications, we have reached this mobile phase, consisting of Methanol, chloroform, and ammonia (78.5: 20: 1.5), which remains one of the best mobile phases to separate alkaloids in a general way. After migration of the mobile phase, the TLC plate is removed, dried and revealed by Dragendorff reagents according to Kurt's method with modifications [14, 12]. Dragendorff's reagents is a solution of "heavy" iodine and bismuth complexes which gives insoluble adducts with alkaloid salts and is commonly used for the detection of alkaloids during their extraction [15].

4. Animal Material

4.1. Animal Material

The rats are used as models to study the effect of the different drugs and chemical substances. The animals of our experiments were obtained from the institute of Pasteur Algiers and weighed between 180 g to 200 g. The rats were acclimatized to the conditions of the animal house for three weeks. The rats had food (standard pellet) and ad libitum tap water. To be able to follow the animals and identify them, they were marked on their body by a solution of

picric acid (2%)

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 Sétif 1.

4.2. Evaluation of Acute Toxicity in Female Rats

Acute toxicity tests are used to evaluate toxic effects that occur in a short time. The rats are divided into 3 groups of 10, weighed and treated with the total alkaloids of the plant by simple application by the dose of 60 mg / kg intraperitoneally. 240 mg of total alkaloids are dissolved in 1 ml of methanol, then the obtained solution is diluted in 19 ml of physiological water, the solution is then well stirred with a vortex before use. And each animal receives 0.5 ml of the solution per 100 g of weight.

The dose 60 mg / kg represents $\approx 1/3$ of the LD50; it's a high dose but not lethal.

The first treated group is sacrificed after 24 hours of the application.

The second treated group is sacrificed after 5 days of the application.

The last group, considered as a control group, treated with physiological water is sacrificed after 5 days.

4.3. Blood Sample

The blood is recovered from the retro-orbital vein of the rats, in two different tubes: one contains ethylenediaminetetraacetic acid for the analysis of the different hematological parameters [RBC (red blood cells); WBC (White blood cells); HGB (hemoglobin); HCT (hematocrit); PLT(platelets)] with MEDONIC apparatus (Beckman Coulter - USA); and the other one, which, after centrifugation, the serum parameters Aspartate transaminase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP), (Kits - biosystems sa costa brava 30 Barcelona, Spain) and total bilirubin (BIL T) and direct bilirubin (BIL D) (Kits - spinreact SA / SAU Ctra Santa Colona, Spain).

After recovery of the blood, the animals are sacrificed. The major organs (liver, brain, heart, kidneys, spleen, and lung) are examined in situ, then removed and weighed to calculate their relative masses.

Pieces of the liver from all animals were fixed in 10% formalin solutions, cut into 5 μ m thin sections after being embedded in paraffin and stained with hematoxylin and eosin and examined under an optical microscope.

5. Statistical Analysis

At the end of the experiments, to calculate the significant difference between the values of the control group and those of the treated groups, the values of the various

parameters were subjected to an analysis of the variance test (ANOVA) and the TUKEY test if value $p < 0.05$ after ANOVA using SIGMA STAT 3.5. The data is expressed in: mean \pm SD.

6. Results

6.1. Extraction

Extraction of the total alkaloids from *Peganum harmala* L seeds gave a Turks red color in powder form, due to the presence of a pigment in the seed sheath of the plant, with an extraction yield of $1.3 \pm 0.448\%$. This yield is slightly below the data reported by Asgarpanah and Ramezanloo (2012).

7. Qualitative Analysis

7.1. Thin-Layer Chromatography of Total Alkaloids

Thin layer chromatography (TLC) of the alkaloid extract of *Peganum harmala* L seeds showed a presence of 4 spots after revelation by the Dragendorff reagent (Figure 2).

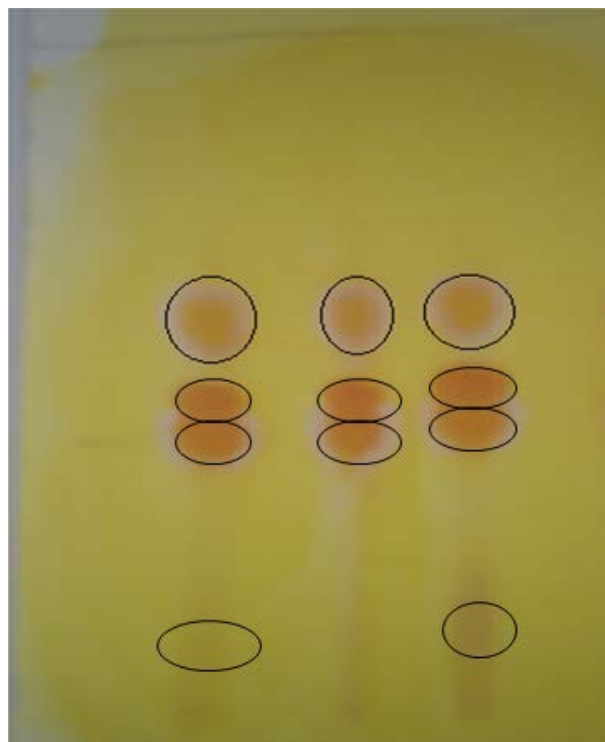


Figure 2. Thin layer chromatography of the total alkaloids of *Peganum harmala* L. Mobile phase: Methanol, chloroform, ammonia (78.5: 20: 1.5) Revealer: Dragen dorff reagent

7.2. Acute Toxicity in Female Rats (Albino Wistar)

Animals treated with the 60 mg / kg total alkaloid extract

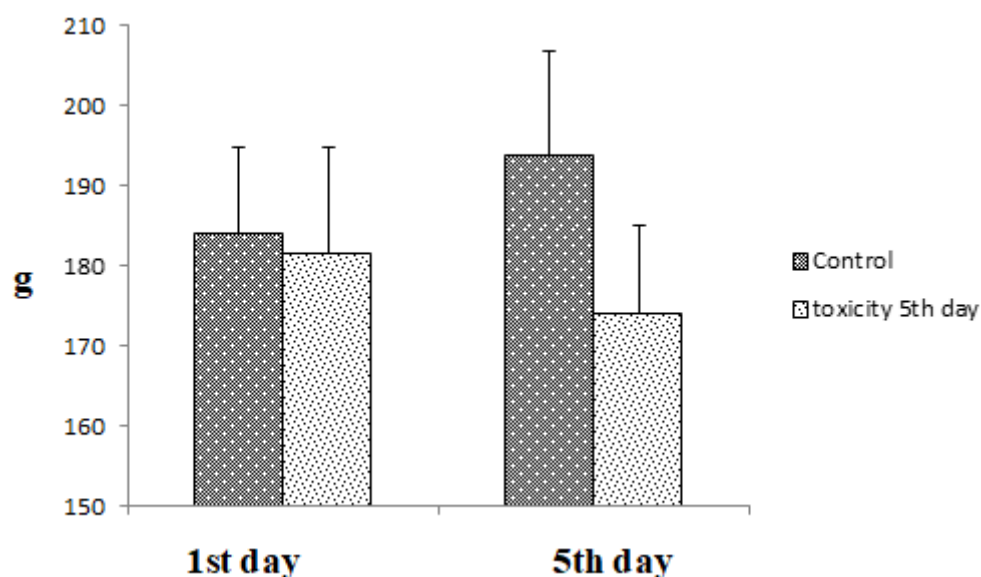
by simple application and IP showed a clinical picture with symptoms of heavy toxicity, Which consisted of convulsions, movement paralysis with the inability of the rats to stand on the hind legs, and a decrease in their general activity for a period of at least 4 hours . In addition, the treated rats gather in corners cages, with bristles erect relative to the control rats. A decrease in body weight of the day 5 treated rats was found relative to the control group (Figure 3).

The relative mass values of the organs showed changes in some organs relative to the controls. There was a significant decrease in relative liver mass of 17.10 % in rats

sacrificed after 5 days of treatment (Table 1).

The results of the parameters to evaluate the liver structure showed a significant increase in alkaline phosphatase (ALP) of 68.06% in the first day and significant decreases in the 5th of the ALAT, ASAT and Bil D respectively of 28.43%, 31.64 % and 90.05 % and there was a significant increase in the 5th day of Bil T by 13.68%.

There was a significant decrease in ALAT, ASAT, and Bil D of 5.54%, 41.13 % and 88.30 %, respectively, and a significant increase in Bil T of 8.20 % in the 5th day group compared to the 1st day group (Figure 4 and 5).



*Significantly different for ($p < 0.05$).

Figure 3. Body mass of female white rats (Albinos Wistar) controls and treated under conditions of acute toxicity by the dose of 60 mg / kg of the total alkaloids of seeds of plant *Peganum harmala* L.

Table 1. Relative masses of the organs of female white rats (Albinos Wistar) controls and treated under the conditions of acute toxicity by the dose of 60 mg / kg of the extract of the total alkaloids of the seeds of the plant *Peganum harmala* L.

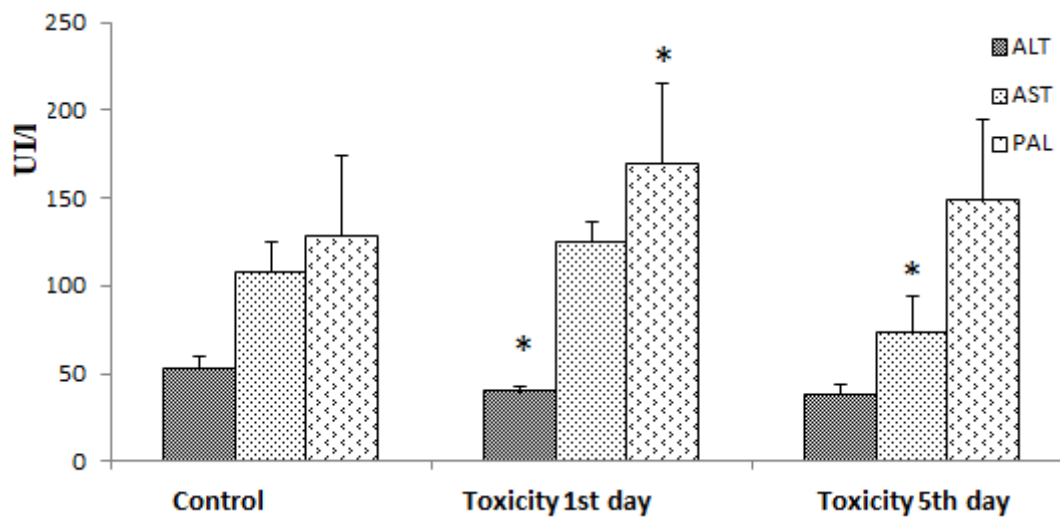
Relative mass	Lung	Heart	Kidney	Liver	Brain	Spleen
Control rats	0.00774 ± 0.00085	0.00329 ± 0.000185	0.00696 ± 0.00115	0.0421 ± 0.00628	0.00373 ± 0.000585	0.00904 ± 0.000580
Rats treated 1st day	0.0704 ± 0.000868	0.00326 ± 0.000602	0.00672 ± 0.000413	0.0433 ± 0.00305	0.00341 ± 0.000595	0.00902 ± 0.000573
Rats treated 5th day	0.00797 ± 0.00103	0.00320 ± 0.000194	0.00845 ± 0.00111	0.0349* ± 0.0109	0.00388 ± 0.000541	0.0106 ± 0.000643

The results are presented as mean ± SD. * significantly different for ($p < 0.05$).

Table 2. Hematologic parameters of female rats (Albinos Wistar) controls and treated by the dose 60mg / kg of total alkaloids seeds *Peganum harmala* L.

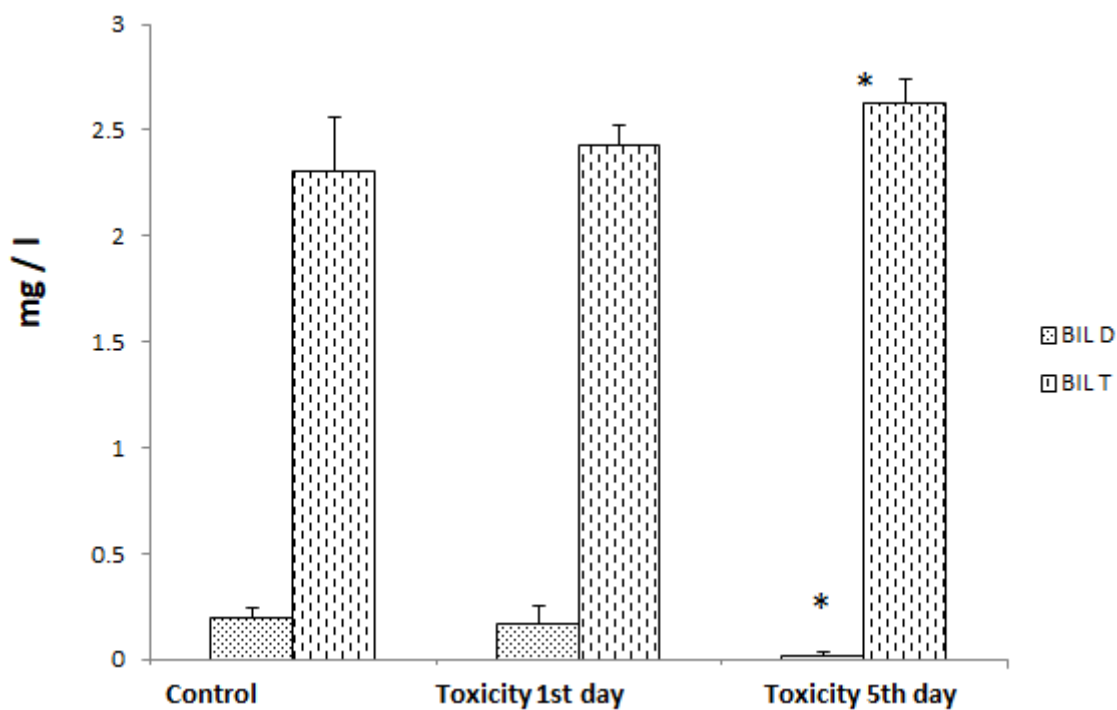
	RBC 109/ml	WBC 106/ml	HGB g/dl	HCT%	PLT 106/ml
Control rats	8.27 ± 0.32	9.26 ± 3.37	15.16 ± 0.56	43.00 ± 1.57	509.10 ± 90.69
Rats treated 1st day	8.12 ± 0.33	7.08 ± 1.54	14.94 ± 0.67	41.57 ± 1.10	528.10 ± 70.40
Rats treated 5th day	8.80* ± 0.84	7.60 ± 3.04	16.23* ± 0.92	46.08 ± 3.06	*605.70 ± 82.18

* Significantly different for $p < 0.05$.



*Significantly different for $p < 0.05$.

Figure 4. Serum parameters TGO, TGP, PAL, female rats controls and treated under conditions of acute toxicity by the dose of 60mg / kg of total alkaloids of seeds of *Peganum harmala* L.



*Significantly different for ($p < 0.05$).

Figure 5. Serum parameters of total and direct bilirubin of controls and treated rats under the conditions of acute toxicity by the dose 60mg / kg of total alkaloids seeds of the plant *Peganum harmala* L.

Histological examination shows that the seed extract of *Peganum harmala* L no remarkable toxic effect on the liver of the treated animals scarified after 5 days. The hepatic lobules keep their architecture, with moderate blood congestion in the centrolobular veins and some necrotic cells, also observed in the livers of controlled rats (Figure 6).

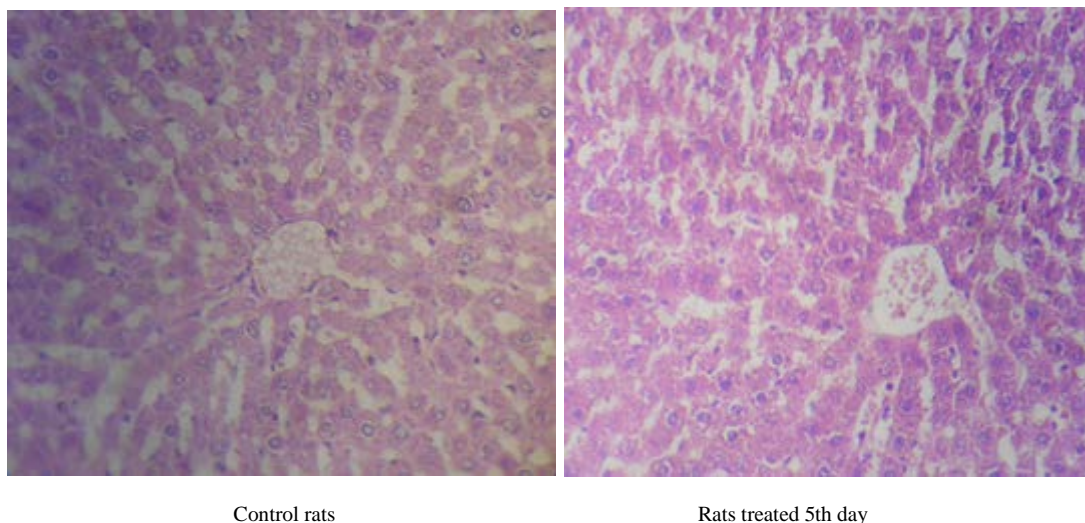


Figure 6. Photomicrographs of liver section of rats of controls treated rats under the conditions of acute toxicity by the dose 60mg / kg of total alkaloids seeds of the plant *Peganum harmala* L. (H and E, X400)

Peganum harmala L. belongs to the zygophyllaceae family. It is widely distributed in Central Asia, North Africa and the Middle East [10]. It is widely used in traditional medicine, against female sterility and diseases of the uterus [16], and is also used in tribal rituals [4].

The Pharmco-toxicological effects of the plant are due to β -carboline alkaloids which are important harmaline, harmine, harmalol and harmol [17, 5].

Thin layer chromatography (TLC) of alkaloid seeds of *Peganum harmala* L, showed the presence of 4 spots after revelation. In the absence of controls, these spots have not been identified, but it is probably: harmaline, harmine and harmalol which are alkaloids strongly present in the seeds of the plants; the fourth probably represents the harmol that is weakly present in the seeds of the *Peganum harmala* L [2, 5].

The toxic effects of a substance in animals can be fundamentally determined by physical examination, daily observation, visual examination, food and water consumption, body and organ weight, hematology, urinalysis, organ biochemical function tests, and pathology studies [18, 12, 13].

The removed organs of the animals, such as the liver, show a significant decrease in the relative mass of the 5th day relative to the control and a non-significant increase on the 1st day compared with the controls.

Our results are in disagreement with the data obtained by Mohamed *et al.* who showed that there is a hypertrophy of liver cells after repeated administration of 150 mg / kg of the alcoholic extract of the plant [19]. The toxicity observed in the liver of mice treated with the alcoholic extract of the plant could be due not to the alkaloids (found in small quantity in the extract), but to other substances of the plant soluble in the extract alcoholic.

Hematological analyses showed a significant increase in red blood cells, hematocrit and hemoglobin in animals of 5th day compared to animals of 1st day and control animals.

This significant increase could be explained by a need for oxygen to repair the damage due to the toxicity of the total alkaloids of the *Peganum harmala* L [12].

Hepatic toxicity was studied by assaying some biochemical parameters and histological analysis of the liver. Alanine transaminase (ALT) is a cytosolic enzyme secreted in liver cells from which it is released into the blood in case of hepatic cell necrosis [19]. It is a liver-specific enzyme, which is an important and very sensitive indicator of hepatotoxicity [21]. Alkaline phosphatase (ALP) is a membrane-bound glycoprotein enzyme found in different tissues such as liver. Also, it is a valuable biochemical index used in detection of hepatobiliary disorders [22, 23].

Aspartate transaminase (AST) is also an indicator of the destruction of hepatocytes (case of high lysis of hepatocytes) even if in addition to the liver it is found in the heart in cases of myocardial infarction (MI), skeletal muscle, lungs and kidneys [19]. ALT and ASAT levels rise rapidly when the liver is damaged for a variety of reasons including hepatic cell necrosis, hepatitis, cirrhosis and hepatotoxicity of some drugs [20].

In the present study, the concentration of these two enzymes (ALT and ASAT) significantly decreased with a significant increase in (PAL) in the rats treated with the dose of 60 mg / kg, thus a significant decrease of the direct bilirubin accompanied by a significant increase in total bilirubin respectively in the animals of the 5th day compared to the animals of the 1st day and the control animals. Our results show that the extract of the seeds of *Peganum harmala* L could have a hepatoprotective effect on these rats. Moreover, this hypothesis seems to be confirmed by Hamden *et al* [24]. These authors have shown that the ethanolic and chloroform extracts of the plant *Peganum harmala* L administered at a dose of 2% exert a hepatoprotective effect in the case of hepatotoxicity induced by thiourea. These observations are probably

related to the richness of this plant in β -carbolines known to exert anti-tumor activities on cultured cancer cell lines [24].

These β -carbolines are potent and specific inhibitors of cyclin-dependent kinases in cell cultures [24].

This histological observation confirms the biochemical parameters relating to these organs. This could be explained by the rapid elimination of *Peganum harmala* L alkaloids from animal organism and by their low toxic effects on the liver. These results are consistent with the work of Muhi-eldeen *et al.* [25].

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