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Subacute hepatotoxicity of alkaloids extracts of *Peganum harmala* L. seeds in Wistar albino rats

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Abstract

Peganum harmala L. is a medicinal herbal plant widely used in Algerian traditional medicine. Some reports indicated the toxicity of this plant. In this context, the present study focused on investigate the subacute toxicity of alkaloids extract of *P. harmala* seeds on histo-function of the liver in rats. Sixteen adult *Wistar albino* rats were divided equally into four groups, and treated intraperitoneally for 30 days. Group I served as control, received water. Group II, group III, and Group IV received daily a single dose of 50, 100, and 200 mg/kg b.w of crude alkaloids of *P. harmala* respectively. Blood was collected for the determination of ALT, AST and ALP. Sections of the liver were prepared for histological studies. The results showed a significant decrease in body weights of animals and a significant increase in relative weights of liver in groups III and IV. Treated groups with alkaloids extract of seeds showed a significant increase in the concentration of ALT, AST, and ALP enzymes as compared to control group. These findings were supported with histopathological examination of liver of treated rats. Liver of groups III and IV suffered from severe tissue damage, which included inflammation, cell necrosis, and increase in the volume of some hepatocytes. Some cells contained more than one nucleus and cytoplasm contained micro vacuoles, indicating the onset of steatosis. In conclusion, these biochemical and pathological changes indicate dysfunction with hepatocyte damage. Therefore, the seeds of *P. harmala* plant must be used in herbal medicine with caution to avoid toxicity to the organism.

Keywords: alkaloids; hepatotoxicity; liver histology; *Peganum harmala*; serum biochemical; Wistar rats

Introduction

Peganum harmala is a perennial herbaceous plant of the Zygophyllaceae family (Bournine *et al.*, 2017) and is widely distributed in Central Asia, North Africa, America and Australia (Mahmoudian *et al.*, 2002; El Gendyand and El-Kadi, 2009). *P. harmala* is a plant used in traditional Algerian and Maghrebian medicine to treat various disorders such as emmenagogue, abortifacient, female infertility, dermatosis (eczema), burns, purulent conjunctivitis, blepharitis, and alopecia (Hammiche *et al.*, 2013). The plant is also well known for its medicinal values including anti-diabetic and hypolipidemic activities (Komeili *et al.*, 2016), antitumor

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activities (Lamchouri, 2014), anti-leishmanial effects (Madah *et al.*, 2020), anti-inflammatory (Akhtar *et al.*, 2022) and diuretic effects (Al-Saikhanand and Ansari, 2016).

P. harmala is known as a major source of alkaloids (Abderrahman *et al.*, 2018), the most important of which are harmine, harmaline, harmol, harmalol and quinazolines which are responsible for toxicological and pharmacological effects (Herraiz *et al.*, 2010). The plant is available from all herbalists, and it is found in nature in the wild. Its use for therapeutic purposes is not without danger and exposes to the risk of overdose and many cases of poisoning have already been reported in animals and humans ((Berdai *et al.*, 2014; Ghizlane *et al.*, 2021).

Some studies *in vivo* showed that *P. hamala* alkaloids has toxic effects on the kidneys of rats, which included a decrease in the relative weight of the kidneys and lesions in the renal body represented by an increase in the area of Bowman's capsule, vascular congestion, and necrosis in some glomeruli. Congestion of the vascular cells lining the renal tubules, which may be signs of tumor appearance in the lining cells of the renal tubules (Benbott *et al.*, 2018a). The researcher also showed in another study that alkaloids from *P. harmala* seed extract effect the anti-fertility properties (Benbott *et al.*, 2018b).

Based on the above facts, it became necessary to investigate the action of alkaloids extract of *P. harmala* seeds on histo-function of the liver in rats.

Materials and Methods

Plant collection and identification

Seeds of *P. harmala* were collected from the Harmalia region (South-East of Ain M'lila, Algeria) in September 2018. The plant was identified by Prof. Y. Halis researcher in Scientific and Technical Research Center for Arid Areas (STRC, Biskra). A voucher specimen (No. 0118/HBPA) was deposited at the Herbarium of Laboratory of Bio-molecules and Plant Amelioration, Larbi Ben M'hidi University of Oum El Bouaghi, Algeria (Figures 1-2).

Figure 1. *Peganum harmala* L. plant

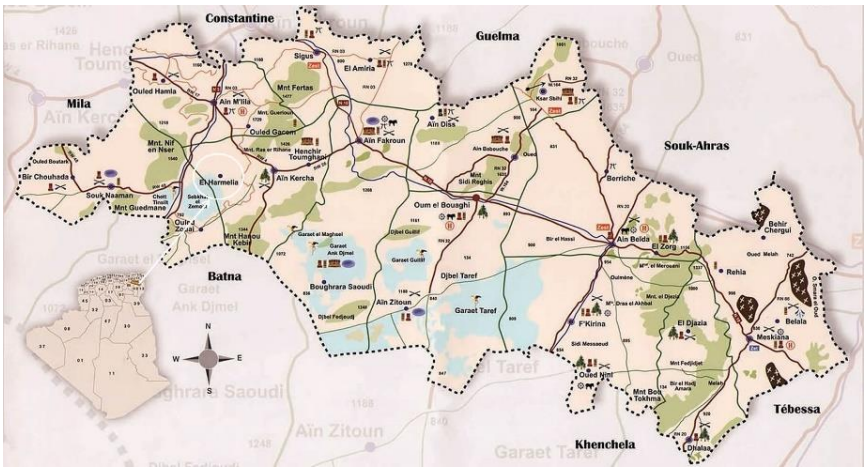

Figure 2. Geographical location of El-Haramlia region in Algeria

Identification by High performance liquid chromatography (HPLC)

The alkaloids were extracted from the seeds of the *P. harmala* plant according to Balbaa *et al.* (1981). We then identified these alkaloids by the HPLC technique. Details of this process and its results were shown in Benbott *et al.* (2018b).

Animals

Sixteen of male *Wistar albino* rats weighing about 180.56 ± 3.13 g were purchased from the breeding division of animals at Pasteur Institute located in Algiers (Algeria), and housed in plastic cages (4 animals /cage). Before experimentation, animals were acclimatized for two weeks photoperiod conditions (12 h light: 12 h dark cycle), with free access to standard pellets diet provided by National livestock food board (Bejaia, Algeria), and water *ad libitum.*

The experimental procedures were carried out according to the internationally of Health Guidelines for laboratory animal care and use (Clark *et al.*, 1997).

Experimental design

The animals were divided into four equal groups, the first group (group I) served as control. The second group (group II), the third group (group III) and the fourth group (group IV) were treated with increasing doses of the alkaloids extract 50, 100, 200 mg / kg, for 30 days intraperitoneally. Animals were weighed weekly; all the animals were euthanized and sacrificed after 24hours of the last treatment. Blood was collected to measure some biochemical parameters. The liver of each rat in each group was removed carefully and placed in normal saline to clean from unnecessary tissue remnants. The absolute weight of the liver was measured using sensitive balance. The relative weight of the organ (%) was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weights(g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Liver was prepared for histological studies.

Blood and serum biochemical

Blood samples were collected from the abdominal aorta of each animal, then blood samples were centrifuged at 3000 rpm for 10 min. Serum was separated and stored at – 20 °C until analysis of liver marker enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). All blood parameters were determined by using the kit BIOBASE BK200 for Fully Automatic Chemistry Analyzer. The assays were conducted according to the standard manufacturer's protocol.

Histological studies

For histological studies, livers were dissected immediately from sacrificed rats, cleaned from the adherent fat, weighed (absolute organ weight), fixed in Bouin solution for 24h, dehydrated in ethyl alcohol, cleared in xylol, then embedded in paraffin. Sections of 5-µm thickness were stained with hematoxylin and eosin (H&E) and examined under optical microscope. Photomicrographs of the desired sections were obtained for further observations.

Statistical analysis

All data were expressed as mean ± standard (SD). The student's "t" test was used to compare the mean values of treated and control groups, using Statistica software (Version 5.1, StatSoft France, 1997). The level of significance was set at p values less than 0.05.

Results

Effect of alkaloids extract of P. harmala on body weight

Harmine and harmaline were the components identified in total alkaloids seeds extract by using high performance liquid chromatography (HPLC) method (Benbott *et al.*, 2018b). The effect of intraperitoneal administration of alkaloid extract seeds on the body weight of male Wistar albino rats is shown in Figure 3. An increase in body weight of control group animals was recorded by 22%, while an increase in body weight was recorded in animals treated with alkaloids at doses of 50, 100 and 200 mg/kg body weight by 19.86%, 16.31%, and 9.14%, respectively, compared to pretreatment initial body weights. However, after four weeks of treatment and compared to the control group, Student's t-test yielded a high-significant decrease in body weight in animals of group III ($p< 0.01$) and group IV $p<0.001$ of animals, but showed non-significant changes in the body weight of group II ($p> 0.05$).

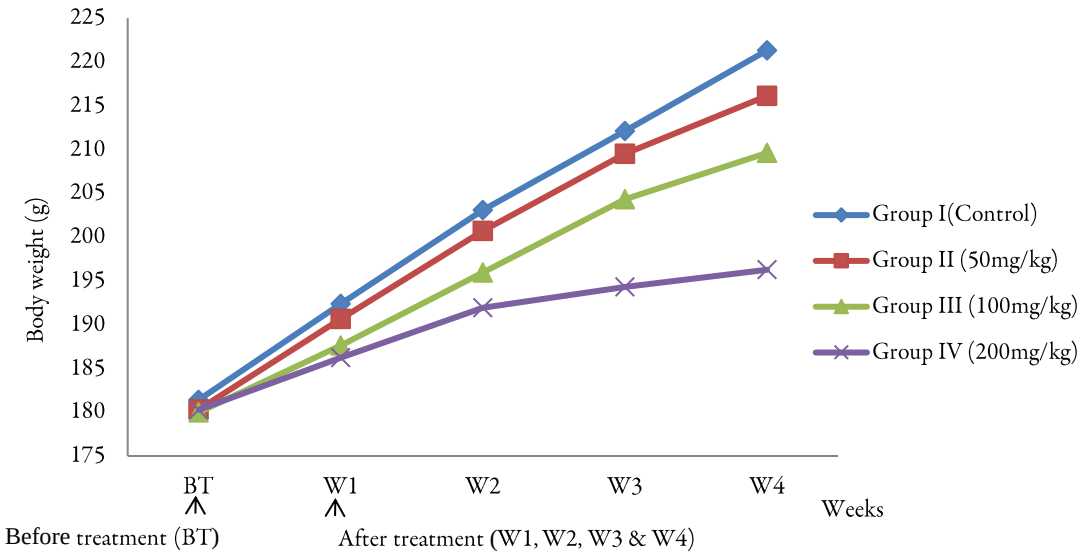

Figure 3. Mean body weight of male rats treated with alkaloids extract of *P. harmala* seeds as compared to control

Effect of alkaloid extract of P. harmala on the relative weight of the liver

As shown in Figure 4, it was observed that the relative weight of liver was significantly higher in group III and group IV ($p< 0.05$) crud alkaloids-treated rats when compared with controls. The lower dose (50 mg/kg) of crude alkaloids did not affect the relative weight of the organ.

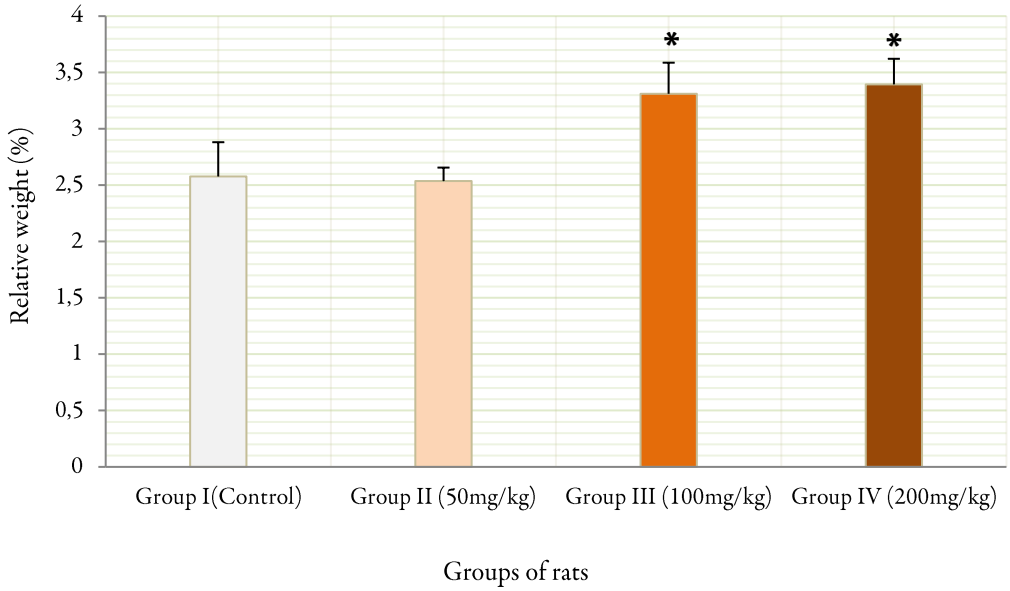

Figure 4. Relative weight (Mean ± S.D) of the liver of treated groups of different concentrations of *P. harmala* alkaloids extract compared to the control group

Values are given as mean ±SD. Significant at * p <0.05 and NS (non-significant) vs. control group

Effect of alkaloids extract of P. harmala on serum biochemical parameters

Administration of alkaloid extract at different concentrations for 30 days, induced an increase in liver enzyme activity of ALT which is significant with group II ($p<0.05$), high significant ($p< 0.001$) with group III and very high significant with group IV ($p<0.01$), but AST and ALP were significantly higher with group III ($p<0.01$), and group IV ($p<0.01$) compared to the control group (Table 1).

Table 1. Effect of different concentrations of *P. harmala* alkaloids on serum biochemical parameters

Parameters	Means ± Standard deviation			
	Group I (control)	Group II (50 mg/ kg bw)	Group III (100 mg/ kg bw)	Group IV (200 mg/ kg bw)
ALT (U/L)	63.53 ± 2.92	69.55 ± 0.52*	134.00 ± 10.06**	166.63 ± 10.54***
AST (U/L)	92.23 ± 4.31	107.99 ± 10.79^{NS}	115.46 ± 12.90*	131.43 ± 18.08*
ALP (U/L)	163.08 ± 14.46	165.16 ± 12.25^{NS}	176.12 ± 14.22*	190.63 ± 9.67*

Values are given as mean ±SD. Significant at *p <0.05, **p <0.01, ***p<0.001, and NS (non-significant) vs. control group

Histological study of liver

The liver section of the control group (Figure 5) showed normal structure, where the hepatic lobules contained hepatic cells arranged radially around the central vein (CV), and the cords of hepatic cells are separated by sinusoids (S). A portal space (PS) was a formation containing a branch of the portal vein (PV), a branch of the hepatic artery (HA), and bile ducts (BD).

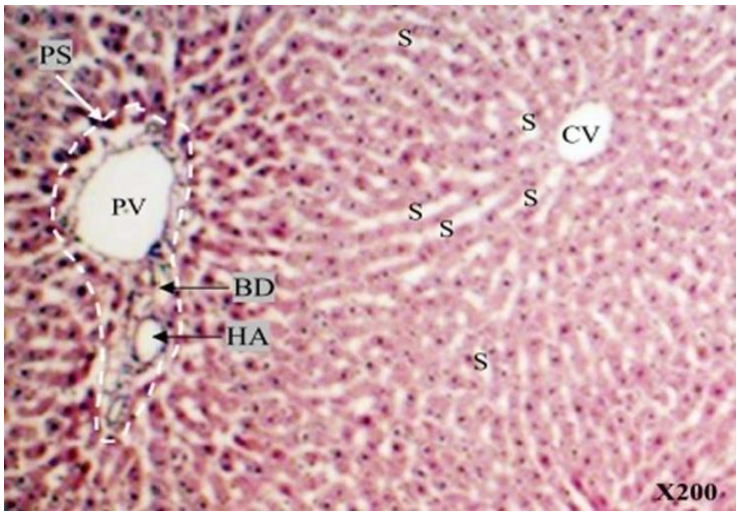

Figure 5. Microscopic section of normal rat liver with normal architecture H& E X200

The results of the effect of alkaloids extract of *P. harmala* at different concentrations (50,150,200 mg/kg) on the liver of rats are presented in Figures 6, 7, and 8.

Hematoxylin and Eosin -stained section of 30 days treated animals with 50 mg/kg alkaloids extract is presented in Figure 6. Liver sections examinations did not reveal histological changes compared to control ones, except for the appearance of a few lymphocytes (Lym) inside the portal space (PS).

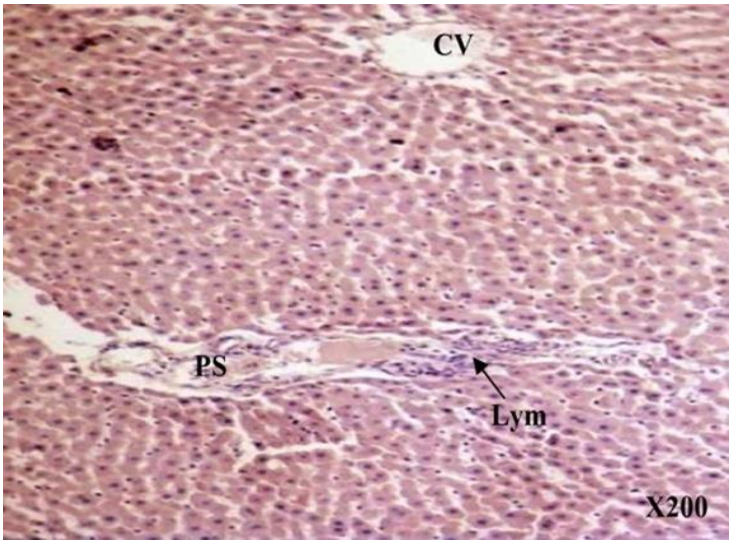

Figure 6. Liver sections for the group treated with 50 mg/kg of alkaloids extract
Lymphocytes (Lym) inside the (PS) H& E X200

While, the hepatic section of the rats administered with 100 mg /kg bw (Figure 7) showed many histopathological changes. These changes included lesion of the hepatic tissue, an infiltration of the immune cells in the portal space (Figure 7a), a focal necrosis (FN) at the level of the lobule (Figure 7b), partial necrosis (PN) (Figure 7c), and some hepatocytes were hypertrophy and binucleated (Bn) (Figure 7d)

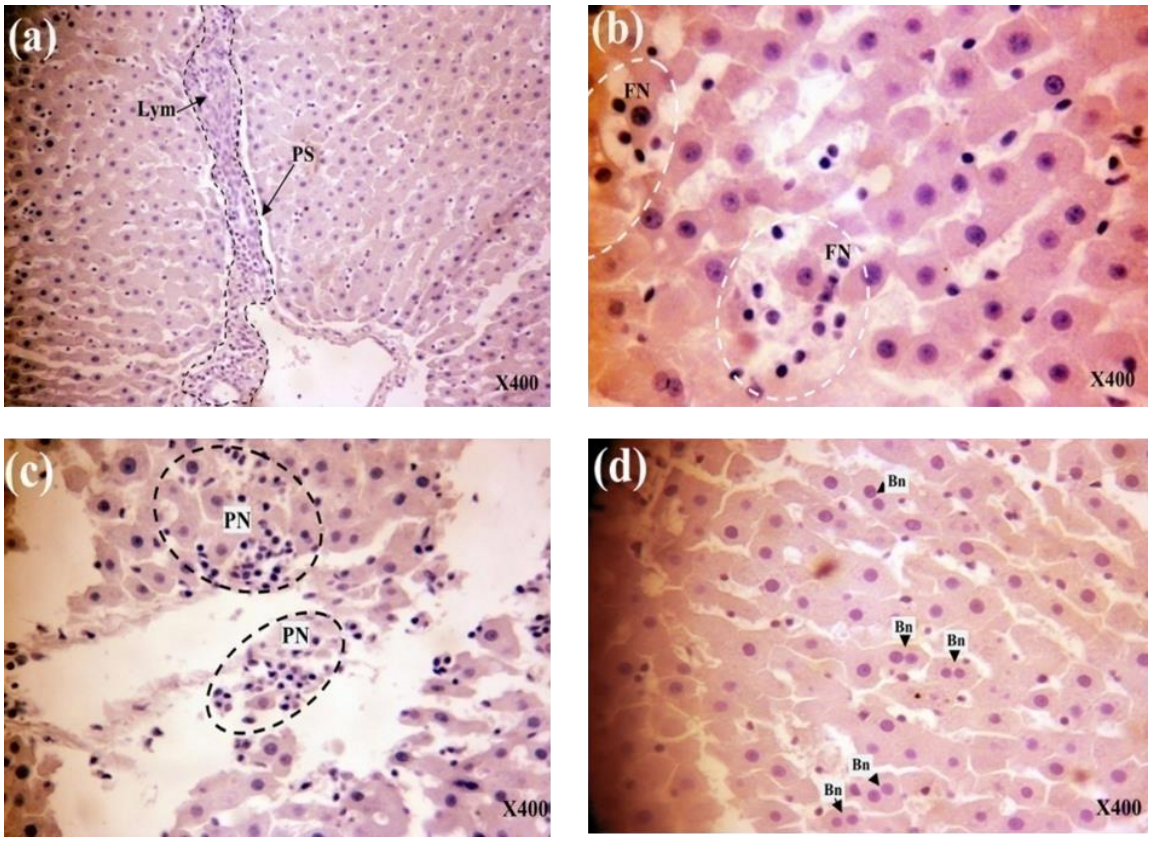

Figure 7. Liver sections for the group treated with 100 mg/kg of alkaloids extract; **(a)** Infiltration of the immune cells in the (PS) H&E X400; **(b)** and **(c)** Focal necrosis (FN) and partial necrosis (PN) in the lobule H&E X400; **(d)** Some hepatocytes were hypertrophy and binucleate (Bn) H&E X400

Microscopic examination of hepatic tissue of the rats administered with 200 mg /kg bw (Figure 8) showed acute liver damage which changed the histological structure of the hepatic with large spaces between the centrilobular hepatocytes. Some cells contained more than one nucleus, which appeared voluminous and pale (NVP), with chromatic dispersion in some nuclei and presence of immune cells (IC) close to the central vein (Figure 8a). We recorded zonal necrosis (ZN) in the central vein and enlargement of some hepatic sinusoids (S) (Figure 8b), hypertrophy in some hepatic cells, emergence of numerous vacuoles (V), and some dark nuclei that confirm apoptosis (Apo) (Figure 8c). Lobular necrosis (LN) (Figure 8d) and several types of immune cells such as lymphocytes (Lym) were present in liver sections, and the detection of histiocytes (His) in the portal space (Figure 8 d-e) and the polynuclear (Pn) and plasma cells (Pc) in the vein lumen (Figure 8f).

Discussion

The effect of increasing concentration of administered crude alkaloids of *P. harmala* into the rats for 30 days did not result in the death of the rats, but gave cases of hepatotoxicity demonstrated by a decrease in the body weight of animals, an increase in relative weights and severe histological changes in liver tissue accompanied by alterations in levels of their biochemical markers.

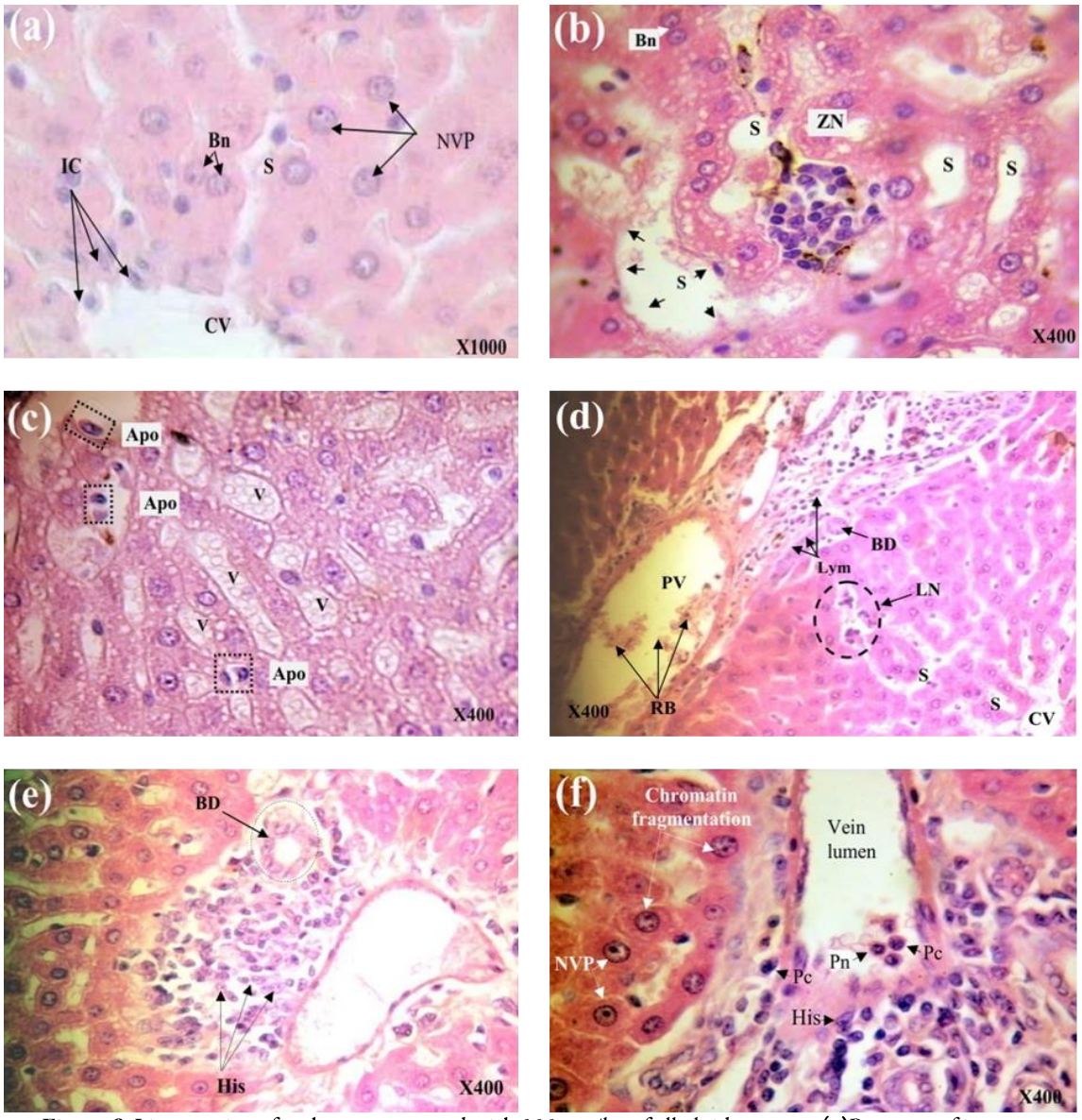

Figure 8. Liver sections for the group treated with 200 mg/kg of alkaloids extract; **(a)**Presence of immune cells (IC) and Some cells appeared voluminous and pale (NVP) H&E X1000; **(b)**Zonal necrosis (ZN) in the (CV) H&E X400; **(c)** Numerous vacuoles (V) and apoptosis (Apo) H&E X 400; **(d)** Lobular necrosis (LN) H&E X400; **(e)** Histiocytes (His) in the (PS) H&E X400; **(f)**Polynuclear (Pn) and plasma cells (Pc) in the vein lumen H&E X400

A high significant decrease ($0.001<P<0.01$) in the body weight of animals of 100 mg/kg for group III and 200 mg/kg for group IV compared to control animals, can be explained by the decrease in cholesterol, triglycerides, and low-density lipoprotein cholesterol levels in animals' rats treated with the methanol extract of harmala (Kalhor *et al.*, 2015). Moreover, a decrease in body weight could also be attributed to β-carboline, which plays a central anti-cholinergicular role, leading to digestive disorders (Salah *et al.*, 1986). β-carboline is one of the alkaloids present in *P. harmala* (Herraiz *et al.*, 2017). In line with our findings, a report from a similar study showed that administration of methanol extract of *S. occidentalis* seeds exhibited a reduction of mean body weight gain in rats (Gebrezgi *et al.*, 2020)

We recorded a significant increase ($0.01 < p < 0.05$) in the relative weight of the liver in the third and fourth group, this is due to Rasekh *et al.* (2008) that the increase in the weight of the internal organs is

associated with the appearance of blood congestion at the organ level. The increase in the relative weight of the liver also indicates the accumulation of alkaloids in this organ.

Besides, Betti *et al.* (2012) proved that the increase of the weight of the organs was due to an inflammation in the body organs. These explanations correspond to the results of our histological study of the liver, where severe blood congestion was observed in the glomeruli and renal tubules and acute inflammation in the portal space of the hepatocyte.

In a study conducted by Gebrezgi *et al.* (2020), he showed that the increase in the relative weight of the liver resulting from the treatment of S. occidentalis seeds at 400 mg/kg and 1000 mg/kg were 54% and 126%, respectively, and therefore, they can be considered as a manifestation of the hepatotoxic effect of *S. occidentalis* seeds.

Significant increase in liver enzyme values (ALT, AST and ALP) in groups III and IV indicated and were explained by the appearance of necrosis in the tissue cell and damage to the plasma membrane of liver cells, leading to the fleeing of these enzymes from the cytosol to the blood.

The results obtained by high levels of liver enzymes were in accordance with the results of Diwan (2013), who showed that the administration of the methanol extract of *P. harmala* at doses (75, 100 mg/kg BW) in mice leads to a significant increase in liver enzymes. The histological study of the liver showed hepatic lesions in groups III and IV, this deterioration being known under different types: hepatocyte necrosis caused by hypoxia (Maronpot *et al.*, 2010), as reported by Boeira *et al.* (2002) and El Gendy and El-Kadi (2009) that alkaloids have an inhibitory effect on the cytochrome P450 enzymes in mitochondria, which plays a role in metabolic reactions in the liver. The enlargement of some hepatic sinusoids due to alkaloids that release the arachidonic acid present in phospholipids, which in turn activated the biosynthetic process of the enzyme cyclo-oxygenase that activated the prostaglandin, causing the enlargement of the sinusoid's hepatic (Zhao *et al.*, 2014).

The hypertrophy of some hepatic cells is due to the accumulation of lipid droplets in the cytoplasm of the cell (Yan *et al.*, 2014). Lipid accumulation may cause group IV steatosis. Some liver cells contained pale nuclei, which can be explained by Sahapong *et al.* (1992) with the presence of a DNA related toxic substance, which leads to the degradation of ribosome's that produce proteins in liver cells, which caused these nuclei to lose their natural color. This explanation confirms the toxicity of alkaloids. Gonzalo *et al.* (2015) showed that alkaloids cause liver damage in herbivores. Some of these observations obtained were similar to the observations of the Mohamed *et al.* (2013) and AL-Jborrey and Al-Shahwany (2017) studies on the effect of alcoholic extract of *P. harmala* seeds on liver and kidney in mice. Peyrin-Biroulet's *et al.* (2004) work has also revealed that some plant species have hepatotoxic effects.

Conclusions

According to bibliographical research, many plants used in traditional medicine or used as food have shown some toxicity, among them is the *P. harmala* plant, which belongs to the Zygophyllaceae family, is one of the most important plants in Algerian flora and the most widely used by traditional healers. However, the subacute toxicity shows that alkaloids extracted from seeds decrease in mean body weight gain, increase the relative mass of the liver, and disturb some biochemical variables associated with liver function (ALT, AST and ALP). The histological observation was characterized by morphological changes of the liver. A detailed empirical analysis of chronic toxicity is needed to further support this study. In conclusion to our results, we do not recommend the use of *P. harmala* plant in human and animal nutrition.

Authors' Contributions

AB took the lead in methodology, evidence organization, resource sourcing, oversight, and statistical analysis. CM, SK, NH and DJ participated in the design and also provided critical feedback on the manuscript and assisted in its revision and editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The experimental protocol was approved by the Animal Ethics Committee of the Institute of Veterinary Sciences.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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