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Nephrotoxicity of Crude Alkaloids Extract of Peganum Harmala Seeds in Rats

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ABSTRACT

Peganum harmala plant is rich in effective compounds that have medicinal and pharmaceutical properties. The aim of this study was to investigate the effect of crude alkaloids extract of P. harmala seeds on histo-function of kidneys in rats. Sixteen adult male Wistar albino rats were divided equally into four groups, and treated intra-peritoneally 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 body weight of crude alkaloids of P. harmala; respectively. Blood biochemical markers of kidney functions were determined, and their sections were prepared for histological studies. Groups III and IV showed a significant increase in relative weights of kidneys. Both groups revealed a significant increase in the concentrations of creatinine and urea as compared to the control group. These findings were in consistency with histopathological examination of kidneys of treated rats. Lesions in the renal corpuscle included an increase in the space of the bowman capsule, vascular congestion, and necrosis in some glomeruli. Focal necrosis and vascular congestion were observed in cells lining the renal tubules, which might be the signs of beginning of the tumor in the cells lining the renal tubules. In conclusion, crude alkaloids extract of P harmala induced nephrotoxicity at higher doses in rats, and the plant cannot be used in human and animal nutrition.

Keywords: *Peganum Harmala, Crude Alkaloids, Sub-Chronic Toxicity, Serum Biochemical, Kidney Histology.*

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1. INTRODUCTION

Alkaloids are nitrogenous organic substances, most of which are synthesized from amino acids. They exist in plants in free form or bound with certain vegetable acids (Bruneton, 1999). Over 12,000 alkaloids have been identified, found in over 20% of plant species (Sarker & Nahar, 2007). Some types of alkaloids are used in medical treatments, which are also providing the commonly prescribed cholinesterase inhibitor treatments for cholinergic dysregulation of Alzheimer's disease, such as galantamine and huperzine (Mukherjee *et al.*, 2007). In contrast, many studies have shown that alkaloids are toxic substances that affect insects, herbivores and humans (Diaz et al., 2015). Such as Pyrrolizidine alkaloids, which is a large group that consists of hepatotoxic, pneumotoxic and genotoxic compounds (Chen et al., 2010). These compounds are found in many plant families (Asteraceae, Boraginaceae and Fabaceae) (Edgar et al., 2011). Another type of tropane alkaloids (scopolamine and atropine) found in Datora spp and Brugmansia spp have effects on humans. Extracts of these plants are used for criminal purposes (Uribe *et* al., 2005). *P. harmala* (zygophylacea) was chosen for this study because of its richness in indole alkaloids, especially in seeds (Benbott et al., 2013). Besides, this plant is widely distributed in the Mediterranean region (North Africa), Central Asia, America and Australia (Mahmoudian *et al*., 2002). In Algeria, it grows spontaneously on the edges of roads, in arid and rocky areas, and sandy soils (Baba-aissa, 2000). The plant is not always beneficial to human and animals. It can cause various complications, and induce toxicity if it is consumed excessively (Moshiri et al., 2013). Some *in vivo* studies showed that *P. harmala* alkaloids had toxic effects on rat embryos, leading to fetal mortality, decreasing fetal body weight, enhancing skeletal anomalies and abortion (Adaay, 2014).

The objective of this study was to investigate the sub-acute toxicity of alkaloids extract of *P. harmala* seeds on histo-function of kidneys in rats.

2. 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 2016. The plant was identified by Dr. Y. Halis Researcher in Scientific and Technical Research Center for Arid Areas (Touggourt) Algeria.

Extraction of crude alkaloids

The crude alkaloid extracts from the *P. harmala* seeds were obtained according to the method of Balbaa et al. (1981). The samples of 100g Seeds were macerated in ethanol (70%, v/v) , evaporated to one fifth of the initial volume by a rotary evaporator at the Laboratory of Biomolecules and Plant Amelioration, Larbi Ben M'hidi University of Oum El Bouaghi, Algeria. The ethanol extract was subsequently dissolved in 20 ml of hydrochloric acid (0.1N) and filtered. Then, it was extracted twice with 20 ml of chloroform, and then treated twice with 10 ml of hydrochloric acid (0.1 N). The aqueous layer was adjusted to pH= 9 with ammonia (NH_3; 0.1 N), and extracted three times with chloroform. Finally, total alkaloids were obtained by evaporation of chloroform extract.

Animals

Sixteen 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, the animals were acclimatized for two weeks to 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 *adlibitum*. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal.

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 intra-peritoneally. The animals are weighed weekly; all the animals were euthanized by chloroform and sacrificed after 24h of the last treatment. The blood was collected to measure some biochemical parameters. The relative weights of the kidneys were calculated and studied histologically.

Blood and serum biochemical

The blood samples were collected from abdominal aorta of each animal, then they were centrifuged at 3000 rpm for 10 min. The serums were separated and stored at - 20°C until they were used. Serum urea, uric acid, and creatinine were estimated by the kit BIOBASE BK200 Fully Automatic Chemistry Analyzer**.** The assays were conducted according to the standards of the manufacturer's protocol.

Histological studies

For histological studies, kidneys 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, and then embedded in paraffin. Sections of 5-µm thickness were stained with hematoxylin and eosin (H&E), and examined under light microscope. Photomicrographs of the desired sections were obtained for further observations.

Statistical analysis

All the data were expressed as mean ± standard (SD). 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.

3. RESULTS

The effect of crude alkaloids of *P. harmala* on the relative weight of the kidneys

As shown in Figure 1, it was observed that the relative weights of kidneys were significantly higher in group III (P< 0.05) and group IV (P< 0.01) of crude alkaloids treated rats when compared with controls. The lower dose (50 mg/ kg) of crude alkaloids did not affect the relative weights of kidneys.

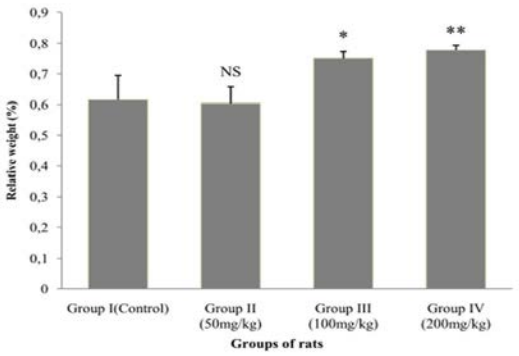

Figure 1. The effect of different concentrations of *P. harmala* alkaloids seeds extract on the relative weights of the kidneys after 30 days of treatment. Values are given as mean ±SD. Significant at **P* <0.05, ***P* <0.01 and NS (non-significant) vs. control group.

The effect of crude alkaloids of *P. harmala* on serum biochemical parameters

(T.test) showed a very significant increase in creatinine and urea values with the group III (P<0.01) and group IV (P< 0.01) compared to the control group. Slight increase in uric acid concentrations did not result in significant changes with group II, group III and group IV compared to the control group.

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

Parameters	Group I (control)	Group II (50 mg/ kg bw)	Group III (100 mg/ kg bw)	Group IV (200 mg/ kg bw)
Creatinine (mg/dL)	0.6± 0.01	0.63± 0.06^{NS}	0.67± 0.011**	0.68± 0.012**
Urea (mg/ dL)	28.17± 0.10	28.76± 0.55^{NS}	30.64± 1.17**	32.34± 1.23**
Uric acid (mg/ dL)	4.56± 0.25	4.53± 0.30^{NS}	4.93± 0.55^{NS}	5.03± 0.51^{NS}

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

Histopathology of kidney

Figure (2. a-b) shows a microscopic examination of a section in the kidney of the control group that contained a normal structure of the renal corpuscle (RC) and the convoluted proximal tubules (CPT) appeared in different sizes, and had small internal cavities, while the convoluted distal tubules (CDT) had much larger internal cavities and contained nuclei that were often located at the top.

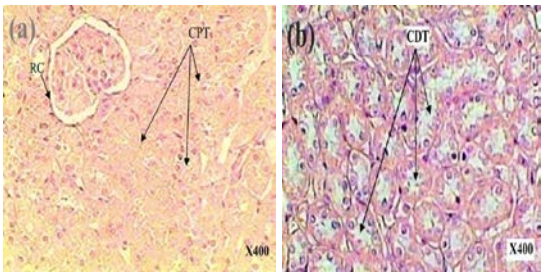

Figure 2: Kidney section of the control rats with normal architecture

However, microscopic examination of the kidney tissue structure of rats treated with 50 mg / kg alkaloid extract did not show changes in proximal and distal convoluted of renal tubules (RT) structure, but dilation and congestion were observed in a glomerular tuft (figure 3.a-b).

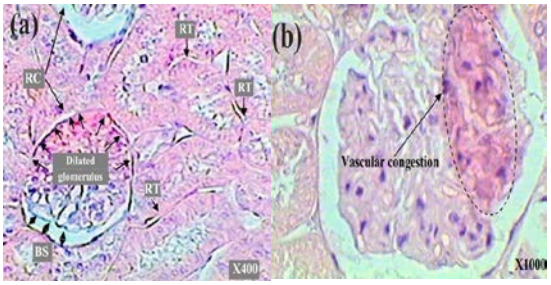

Figure 3: Kidney section of the group treated with 50 mg/kg of alkaloids extract showing congestion of glomerulur tuft

As shown in figure 4, there was an increase in the severity of structural changes in the kidney of group IV, especially in the urinary glomeruli. Most glomeruli appeared in an abnormal form where the researchers recorded mainly: fragmented glomeruli, narrowing of the glomeruli accompanied by a widening of the bowman's space, and atrophy of the glomerulus, resulting in the loss of the rounded form of the urinary glomeruli. In addition, the dilatation of distal tubules was observed.

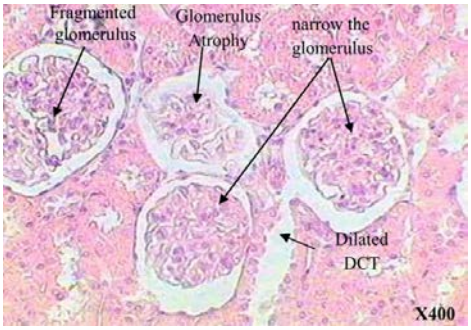

Figure 4: Microscopic section of rat kidney treated with 100 mg/kg of alkaloids extract showing damage of urinary glomeruli and dilatation of distal tubules.

Interestingly, the examination of kidney tissues in rats treated with higher doses of alkaloids extract (200 mg / kg bw) showed several striking histological changes (Figure 5). The proximal tubules were damaged by the loss of their shape, with the disappearance of some nuclei from their cells, and others fell into the cavities (Figure 5.a) The atrophy of the distal tubes resulted in the appearance of pale nuclei, while prominent nucleoli appeared, and some nuclei contained two nucleoli (Figure 5. b). The enlargement and congestion of some glomeruli, focal necrosis (Figure 6.c) and glomerular necrosis (Figure 5.d) as well as the dilation and congestion of blood vessels (Figure 7.e) were noticed.

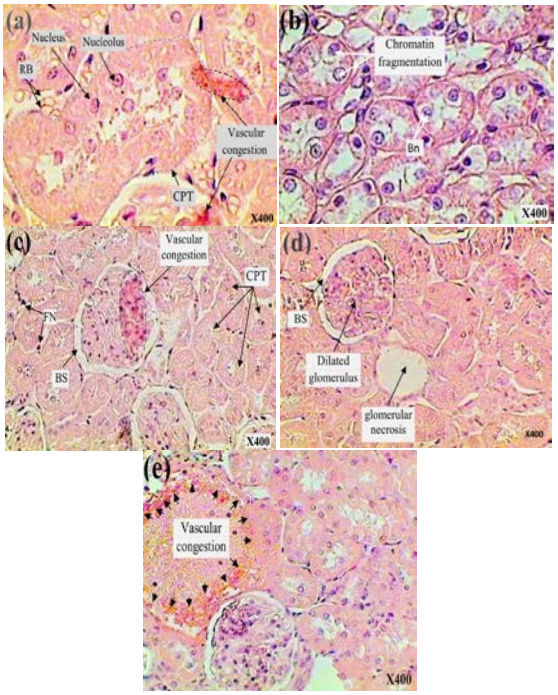

Figure 5: Microscopic section of kidneys of the treated group with 200 mg/kg of alkaloids extract

4. DISCUSSION:

The effect of increasing concentration of administered crude alkaloids of *P. harmala* in the rats for 30 days did not result in death of the rats, but gave cases of nephrotoxicity demonstrated by the increase in relative weights and severe histological changes in kidney tissues accompanied by alterations in the levels of their biochemical markers.

The registration of a significant increase in the relative weight of kidneys in group III and IV, was due to the inflammation or decrease in the activity of these organs, the accumulation of toxic substances (alkaloids) and/or, the products of their reactions, which were confirming by the damage to the liver and kidneys tissues. Rasekh et al. (2008) also explained that the increase in the weight of the internal organs was linked to the appearance of blood congestion in these organs.

Significant increase in serum creatinine and urea concentrations in groups III and IV was observed compared to the control group. This increase could be explained by the increased protein degradation and renal dysfunction (Mitch, 1991). Serum creatinine concentration and urea are two criteria demonstrating the security of renal function, which should be determined.

Histological examination of the kidneys for group III and group IV confirmed the biochemical analysis results, very significant pathological changes were observed, and anomalies appeared in most glomeruli. The glomérulolyse was due to the dissolution of cells' mesangial, which protected the walls of the blood capillaries inside the glomerulus. Two types of necrosis in the kidneys of group IV animals were observed one glomerular, and the other focal.

This necrosis was due to the direct effects of toxic substances on the disruption of metabolic reactions, resulting in a uremia that ends in death. The presence of lymphocytes in the interstitial tissue was observed, which explained that the damaged glomeruli and renal tubules produced intermediate chemical compounds that attracted inflammatory cells to help in repairing the damaged parts. Congestion observed in some renal histological sections could be attributed to the changes in the permeability of capillaries that were influenced by alkaloids. These results were confirmed the study of Benouadah *et al*., (2016), which showed the effect of alkaloids from *Datura stramonium* on the kidneys of mice.

5. CONCLUSION

In conclusion, these results indicated that the alkaloids of *P. harmala* had a toxic effect on the biochemical parameters and kidney tissues that were treated with very high doses (over 100 mg / kg). Therefore, it cannot be used in human and animal nutrition

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