Original Articles

BIOCHEMICAL EFFECTS OF MATURED STEM EXTRACT OF *OPUNTIA DILLENII* IN MALE WISTAR RATS

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Summary

The effect of aqueous matured stem extract of *Opuntia dillenii* on selected biochemical parameters in Male Wistar rats was explored. Standard analytical methods were applied. Forty Wistar rats (80-100g) were used in the animal studies, separated into four groups. The control group was solely administered normal feed and saline, group I was administered 100mgkg^{-1} of the extract, group II received 300mgkg^{-1} of the extract and group III received 500 mg/kg^{-1} of the extract. A significant increase ($p<0.05$) in the activities of alanine aminotransferase (ALT) and alkaline phosphatase was observed in group II and III rats, as compared with the controls. A significant decrease in urea and creatinine concentrations was found only in group III rats against the controls. Also, a significant ($p<0.05$) decrease in triglyceride, total cholesterol, and low density lipoprotein (LDL)-cholesterol was seen in group II and group III rats when compared with the control. The hematological evaluation revealed a significant ($p<0.05$) decrease in red blood cell and hemoglobin levels in group III rats when compared with the control. The findings showed both beneficial and toxicological effects of the plant. Hence, for optimal therapeutic benefits, a further toxicological survey could still be carried out perhaps at higher doses.

Key words: *Opuntia dillenii*; biochemical parameters; toxicity; hemoglobin; lipid profile

Introduction

It is without doubts that recently, the world's increasing interests in the health benefits of foods have transcended basic nutritional benefits of foodstuff to disease prevention. The World Health Organization recognizes the use of natural plant products as cheaper and more viable treatment for diseases. *Opuntia dillenii* are members of the *Cactaceae* family used as forage for livestock, and it is widely spread in Africa and most countries in North, Central and South America [1]. Mature stems of *Opuntia dillenil* have been known as rich in secondary metabolites of plants such as flavonoids, alkaloids, and saponins [2-4] and also, calcium, magnesium, phosphorus, vitamin A and vitamin B_{12} [5].

In spite of all these benefits, the species *Opuntia dillenii* (Ker-Gawl) Haw under scrutiny can be classified

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Received: February 01, 2018
Revision received: April 17, 2018
Accepted: June 26, 2018

as an underutilized nutritional and medicinal plant in Western Africa and especially, in Nigeria [2]. Some studies have demonstrated its analgesic, anti-ulcerogenic, anti-inflammatory and radical scavenging activities [6-8].

Consequently, clinical pharmacological curiosity in the efficacy, safety and otherwise of the biologically active compounds present in the genus *Opuntia* has surged recently because of the consciousness that most individuals adopt self-medication using this pant [9].

The wrong use of the therapeutic effect of this plant may result in severe alterations, and for this reason, the experimental investigation of the risks and benefits associated with these therapies are appropriate.

Materials and Methods

Sample collection

Fresh matured stems of *Opuntia dillenii* were obtained from farmland in Umuanuma Nguru in Aboh Mbaise Local Government Area of Imo State. The plant was grown at the herbarium unit of the Department of Plant Science and Biotechnology, University of Port Harcourt Choba.

Sample Preparation

The stems were thoroughly washed, and the spines were manually removed with clean knives, air-dried and pulverized into a powder with a well-washed manual grinder and was stored using airtight sample bottles before analysis.

Preparation of Extract

The aqueous extraction was done using the cold-water method. Precisely 400 g of the dried powdered sample was soaked in 1.5 L of distilled water in a conical flask and was vigorously shaken for 2 mins and left for 24 hrs. The mixture was filtered, and the filtrates were concentrated using rotary evaporator at 50°C and evaporated to dryness in an evaporating dish with a thermostat water-bath heater at 50°C. The stem extract was removed from the evaporating dish using a spatula and collected into a dry sterile container. Precisely 16 g and 24 g were weighed from the stem extract and dissolved in separate flasks containing 77 ml of distilled water.

Experimental and Research Design

Forty Male Wistar rats weighing (80-100 g) were acquired from the animal house of the Department of Biochemistry, University of Port Harcourt, Choba, Nigeria. The experimental animals were housed for seven days in standard cages for acclimatization. The rats were kept according to the stipulations of National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals [10]. The rats were divided into four groups of ten rats each, allocated as one control group and groups I, II and III. The experimental study lasted for twenty-eight (28) days, and the administration schedule was as follows:

- Controls: normal saline + feed + water;
- Group I: 100 mg/kg body weight of matured stems of *Opuntia dillenii* extract + feed + water; Group II: 300 mg/kg body weight of *Opuntia dillenii* extract + feed + water;
- Group III: 500 mg/kg body weight of *Opuntia dillenii* extract + feed + water.

The dosage of administration of the extract was according to the methods of Loro et al. (1999) [11] and Akacha et al. (2015) [12].

Blood Sample Collection

At the end of administration (28 days), the rats were anesthetized with diethyl ether and dissected. Blood was obtained via cardiac puncture into heparin sample bottles and Ethylene diamine tetraacetic acid (EDTA) anticoagulant tubes. Kidney and liver samples were collected for histological study. The blood samples were carefully labeled and centrifuged at 2000 rpm for 10 mins at 4˚C to obtain plasma.

Histological Assessment

The liver and kidney tissues from control and test rats were fixed in 10% buffered formalin (pH 7.3) for 24 hours, dehydrated in a mounting grade of ethanol (50%, 70%, 90% then absolute, twice) for 1 hour. To permit the tissue to be embedded in paraffin, clearing in xylene preceded paraffin wax for another 1 hour to remove water from the tissue. The tissue was sectioned at 5 µm and stained with hematoxylin and eosin before examination with a light microscope (Brian SCI Coy).

Plasma Assay

The plasma assays conducted were liver, kidney function test, and lipid profile. The concentration of alanine aminotransferase (ALT) was estimated by the method of [13] while alkaline phosphatase was determined as explored by [14]. Creatinine was estimated using the method described by [15]. Urea determination was conducted via the UreaseBerthlot method. Total cholesterol, triglyceride, and high-density lipoprotein cholesterol assessment was carried using Stein [16] method. Plasma low density lipoprotein-cholesterol (LDL-C) was evaluated using [17].

Hematological Assessment

Blood samples, stored in ethylene diamine tetraacetic acid (EDTA) anti-coagulant bottles**,** were assayed using BC 5300 Mindray Hematology Auto-Analyzer. They were blended, using a blood mixer. Blood stains were cleaned with cotton wool, and then a computer system and a hematology auto analyzer were powered. The information for each sample was keyed in according to their groups. The blood samples in the EDTA bottles were presented to the sample probe in the autoanalyzer one after the other for aspiration. The samples were analyzed by the autoanalyzer and the results were printed.

Statistical Analysis

Statistical packages of social science (SPSS) v.20.0 were used to analyze all the data obtained from this study. The results are presented as mean values (M)±SEM (Standard error of the mean). One-way with Turkey test was performed to assess significant differences between groups. The significance level was considered at 95% confidence level ($p<0.05$).

Results

The outcome of ALT and alkaline phosphatase activities presented in Figure 1 and 2 in that order, showed significant ($p<0.05$) differences in the activities of these enzymes in groups II (10.20±0.333 and 424±6.009) and group III (16.20±0.881) rats when compared with the control (4.00±0.577 and 261.00±3.522).

The observed decrease in urea and creatinine level (Figure 3 and 4) in group III (4.00±0.115 and 59.60±4.337) rats against the control (4.93±0.120 and 95.00±1.732) respectively, was significant ($p<0.05$).

A significant decrease ($p<0.05$) was observed in the triglyceride, total cholesterol, and low-density lipoprotein cholesterol levels (Figures 5-7) in group II (1.03±0.082, 2.40±0.027 and 0.96±0.121) and group III rats (1.03±0.124, 2.37±0.043 and 0.80±0.058), respectively, as compared with the controls (1.60±0.105, 3.00±0.012 and 1.50±0.166).

The hematological indices investigated exhibited significant increase ($p<0.05$) in white blood cells (WBC) values (Figure 8) in group II (13.46±0.025) and III rats (16.23±0.258) following the administration of the aqueous matured stem extract of *Opuntia dillenii* when compared with the control (5.45±0.534).

Similarly, a significant decrease in red blood cells (RBCs) and haemoglobin (HGB) levels (Figure 9 and 10) was observed in group III rats (5.71±0.033 and 11.60±0.402) when compared with the control (7.38±0.031 and 13.40±0.207).

Discussion

More often than not, hepatotoxic agents influence the release of certain enzymes such as aspartate aminotransferase, ALT, and alkaline phosphatase into the bloodstream as a consequence of damage to hepatocytes. ALT is mainly localized in the cytosol of hepatocytes [18]. Hence, ALT is a more specific liver enzyme for diagnostic use [19]. The observed increase in the activities of ALT and alkaline phosphatase could be a consequence of inflammation and disturbances of hepatocytes permeability.

Studies have established that alterations in the transport capacity of the hepatocytes attributable to hepatic injury lead to leakage of enzymes from cells to the bloodstream [20]. This outcome corroborates the findings of Saleem et al. (2005) [21] and Zouhir et al. (2015) [22] who reported similar increase in rats given methanolic and aqueous extract of *Opuntia ficus indica* and *Opuntia dillenii*, respectively, though not in line with Ncibi et al. (2008) [23] and Brahimi et al. (2011) [24].

Urea, produced by the liver and the main nitrogenous end product of amino acid breakdown remains the most often utilized clinical indices for renal function evaluation [25].

Creatinine, a catabolic product of creatinine phosphate in muscles is regularly used to assess

kidney function, and its relative production by the body mass is dependent on the mass of the muscle [26]. The observed decrease in urea and creatinine level in group (III) rats against the control suggests that aqueous matured stem extract of *Opuntia dillenii* does not confer nephrotoxic effects. El-said et al. (2011) [25] posited that significant decrease in blood urea and plasma concentrations in these rats might be attributed to the high ascorbic acid present in prickly pear (*Opuntia fruit*) peel.

Thus, this statement applies to matured stems of *Opuntia dillenii* as regards to the substantial amount of β-carotenoids and ascorbic acid present in the cladodes [5]. The observed decrease in urea and creatinine level in the present study corroborates the works of Korkmaz et al. (2009) [27].

The assessment of total cholesterol, triglyceride, high-density lipoproteins and low-density lipoproteins gives insight on the various disturbances of cholesterol and triglyceride levels associated with cardiovascular diseases [28]. A decrease that is significant ($p<0.05$) was observed in the triglyceride, total cholesterol, and low-density lipoprotein cholesterol (Figures 5-7) in group II and group III rats when compared with the control. The observed significant decrease supports the reports of [29-31] Shapiro et al. (2002) [32] demonstrated in humans the anti-hyperlipidemic effect of *Opuntia*; they illustrated a significant decrease in Total cholesterol and LDL-cholesterol and a reduced platelet protein.

The significant decrease in the triglyceride, total cholesterol and low-density lipoprotein cholesterol observed in the present study suggest hypocholesteromic potentials of the matured stems of the studied plant and this could be attributed to the high saponin content (Table 1) in the matured stems of *Opuntia dillenii* [2].

The differences in the concentration of various compounds present in the blood of an organism could be an indication of certain disease conditions in such organisms [33]. The exhibited increase that is significant ($p<0.05$) in WBC values in group II and III rats following the administration of the aqueous matured stem extract of *Opuntia dillenii* might be a consequence of the activation marker of the immune system and defense mechanism as a result of inflammation in the tissues [34].

The significant ($p<0.05$) decrease in RBCs and HGB levels in group III rats when compared with the control suggest possible alterations in the balance between erythropoiesis and erythrocyte destruction as well as inhibition of hemoglobin synthesis [35].

The results of the histological assessment of the liver (Figure 11) revealed mild and severe intraparenchymal and periportal inflammation of the liver cells in group II and group III rats. An indication that increased extract concentration exerted severe inflammatory effects on the liver cells. This outcome suggests possible hepatotoxic potentials of the aqueous extract of *Opuntia dillenii* and it agrees with the reports of Saleem et al. (2005) [21] and Zouhir et al. (2015) [22].

The absence of hemorrhage between tubules, widening of the Bowman's capsule, spaces in the glomeruli as a result of contraction, severe dilatation of Bowman's capsule and glomeruli shrinkage, which are anomalies associated with renal damage (Figure 12) possibly indicates that the aqueous extract of *Opuntia dillenii* does not confer nephrotoxicity.

Table 1. Phytochemical composition of matured stems of *Opuntia dillenii* by Njoky et al. (2017) [2]

Component	**Concentration** µg/ml
Anthocyanin	0.04 ± 0.02
Oxalate	1.07 ±0.01
Tanin	13.62 ±0.05
Rutin	12.41 ±0.26
Phenol	4.66 ±0.08
Lunamarine	34.43 ±0.35
Saponin	118.08 ±0.57
Sapogenin	11.88 ±0.09
Ribalinidine	3.75 ±0.09
Phytate	0.18 ±0.04
Kaempferol	7.90 ± 0.06
Catechin	44.90 ±0.38

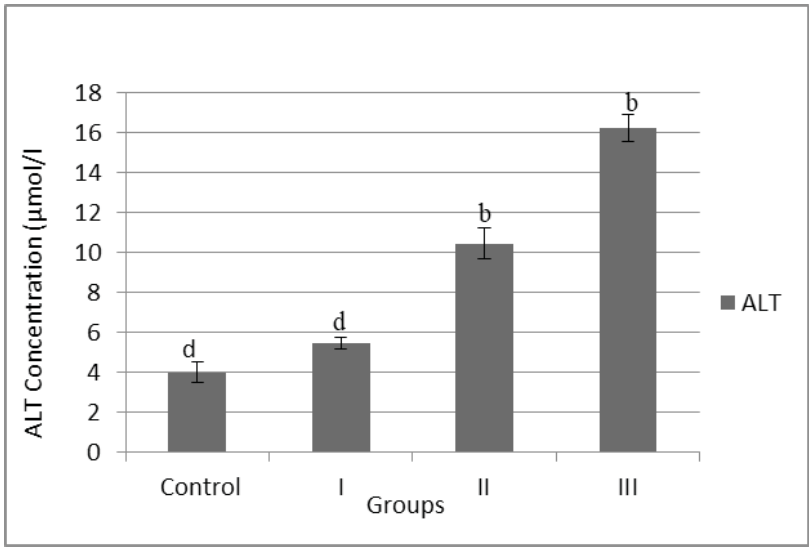

Figure 1. ALT concentration of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

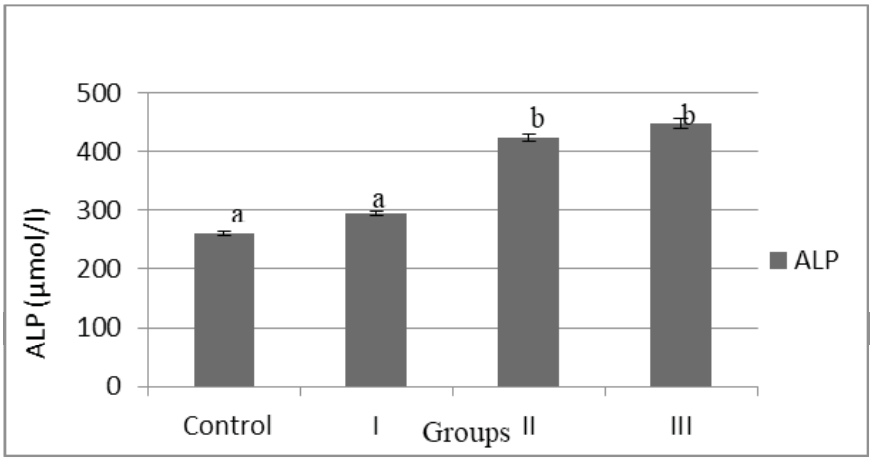

Figure 2. Alkaline phosphatase (ALP) concentration of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

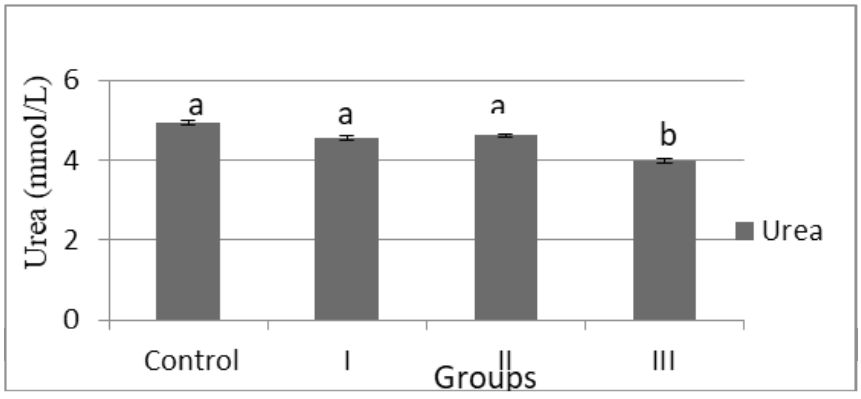

Figure 3. Urea level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

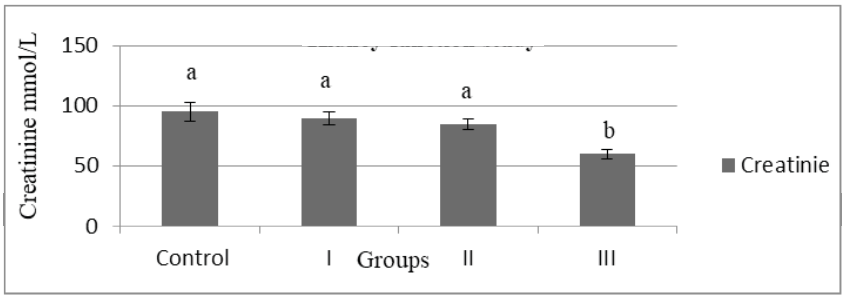

Figure 4. Creatinine level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

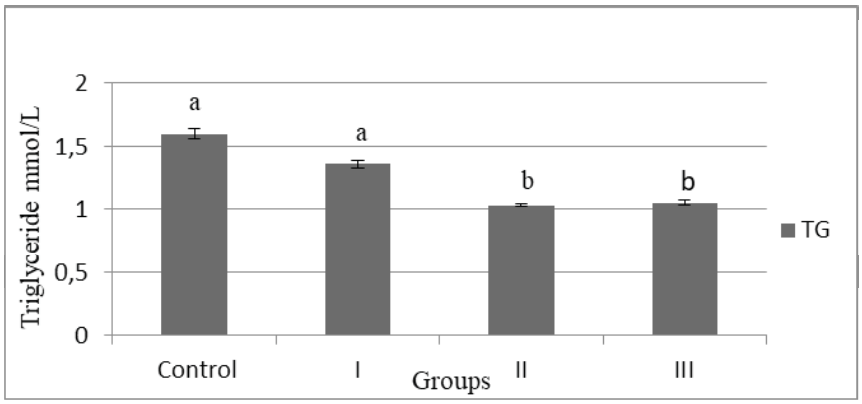

Figure 5. Triglyceride level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

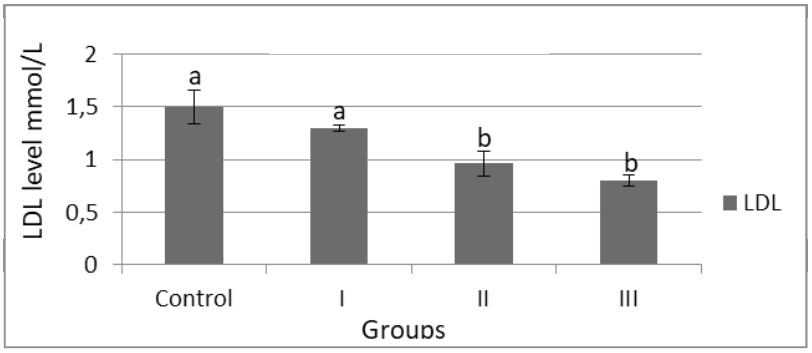

Figure 6. Total cholesterol (TC) level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

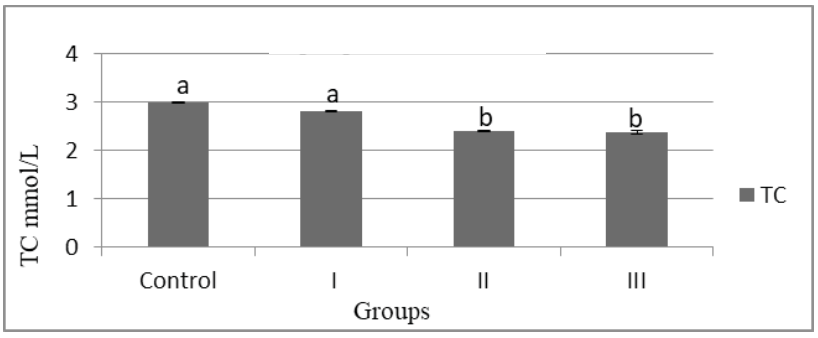

Figure 7. LDL-C level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

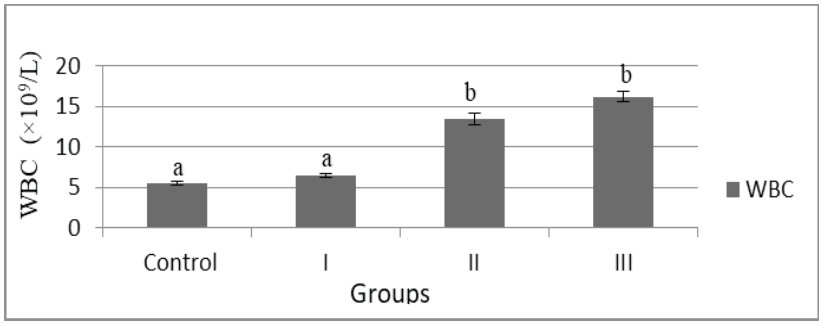

Figure 8. WBC level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

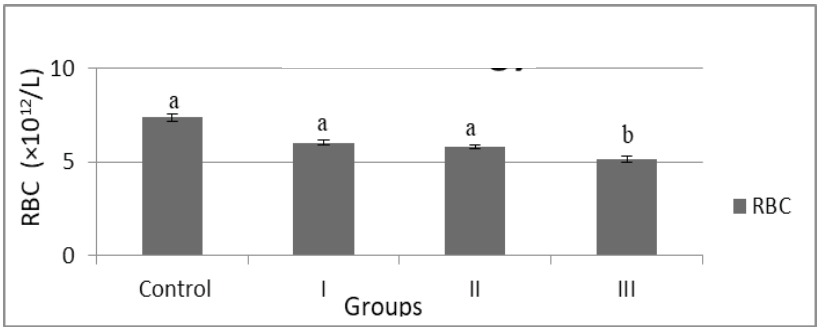

Figure 9. RBC level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

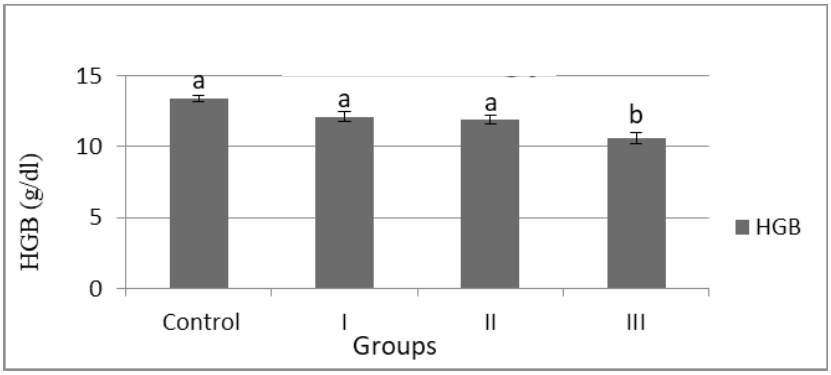

Figure 10. HGB level of rats. Bars with different letters of alphabets are statistically significant ($p<0.05$)

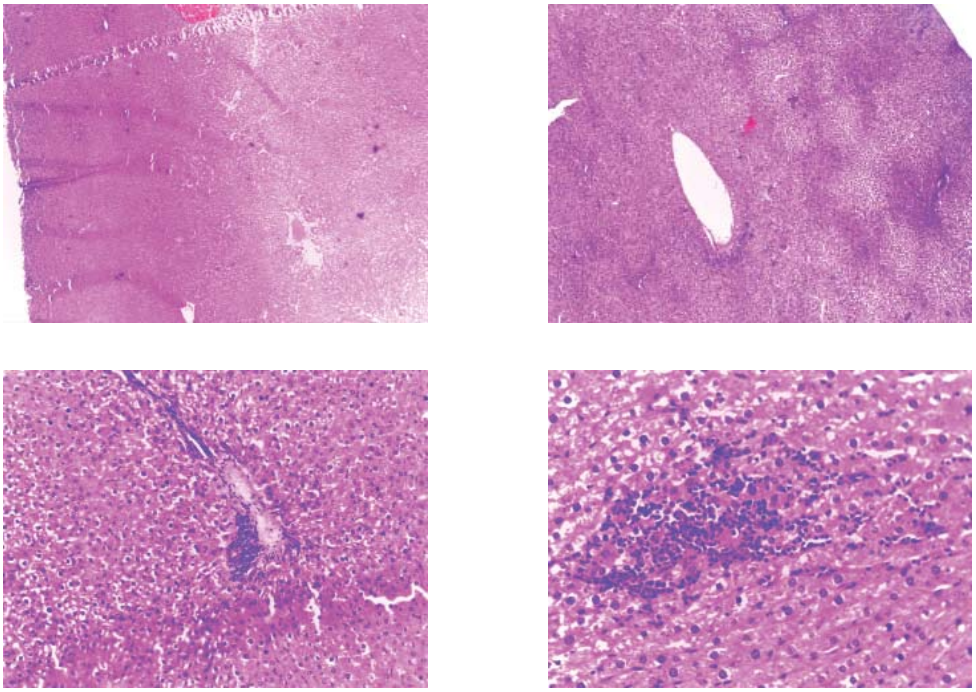

Figure 11. Microscopic view of the liver of control and the treated groups.
*A: Control rats; #B: Group I rats; §C: Group II rats; ##D: Group III rats.
MI=mild inflammation, SI=severe inflammation

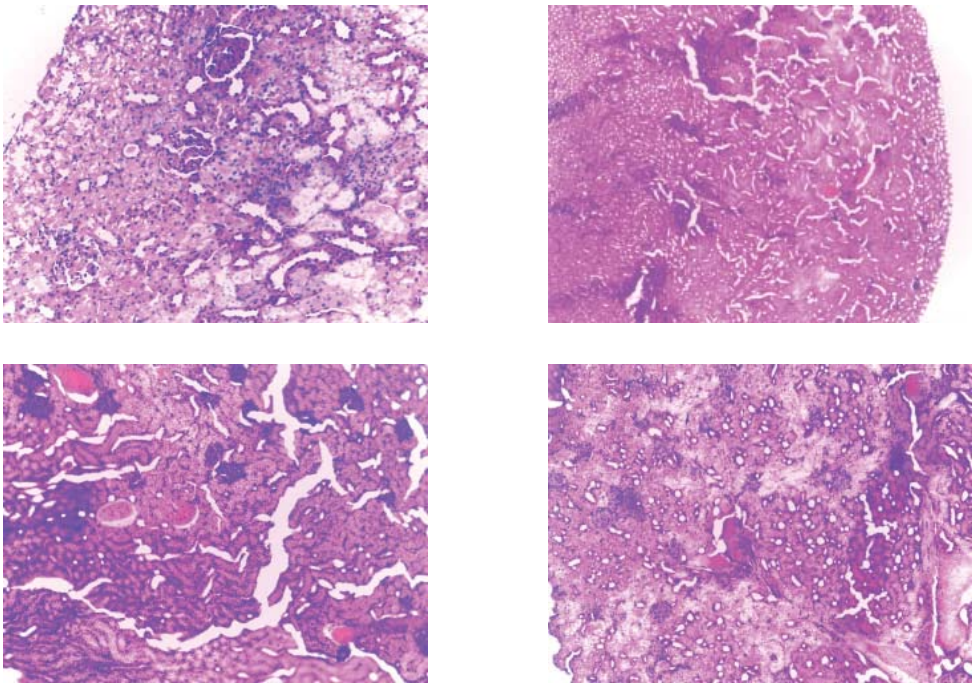

Figure 12. Microscopic view of the kidney of control and the treated groups

Conclusions

The current study demonstrated the hypocholesteromic and nephroprotective properties of the matured stems of *Opuntia dillenii*. Nevertheless, these matured stems demonstrated a profound effect on hematological indices and possible hepatotoxic effects. Consequently, further toxicological investigations may still be carried out perhaps at higher doses for optimal therapeutic benefits.

Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The study was supported and privately sponsored by the authors.

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