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Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats

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ABSTRACT

Objective: To evaluate the acute and sub-acute toxicity of aqueous and methanol extracts of *Nelsonia campestris* (*N. campestris*) in rats.

Methods: Acute oral toxicity study of aqueous and methanol extracts was carried out by administration of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight of *N. campestris* extracts to rats in the respective groups. Sub-acute toxicity study was conducted by oral administration of the extracts at daily doses of 100, 300 and 600 mg/kg body weight to another group of rats for 28 days, while rats in the control group received 0.5 mL of normal saline.

Results: The LD50 of the *N. campestris* extracts in rats was determined to be greater than 5000 mg/kg body weight. There was no significant difference ($P > 0.05$) between the test groups administered with aqueous and methanol extracts in relation to the control group for serum electrolytes (Na^+, K^+, Cl^-, HCO_3^-), serum albumin, total and conjugated bilirubin. Similarly, mean organ-to-body weight ratio and all haematological parameters (white blood cell, red blood cell, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, packed cell volume) evaluated were not significantly different ($P > 0.05$) from the control. There was a significant increase ($P < 0.05$) in the activity of serum liver enzymes (aspartate aminotransferase, alkaline phosphatase), serum urea and creatinine of rats administered with 300 and 600 mg/kg body weight of the aqueous extract. Methanol and aqueous extracts at 600 mg/kg body weight resulted in a significant increase ($P < 0.05$) in serum urea and total protein, respectively. The activity of serum alanine aminotransferase decreased significantly ($P < 0.05$) when the rats received 100 and 300 mg/kg body weight of both extracts. Histopathological examination revealed mild to moderate hepatic and cortical necrosis of liver and kidney respectively on administration of both extracts at 100 and 600 mg/kg body weight. A moderate dose of 300 mg/kg body weight of the aqueous and methanol extracts caused lymphocytic infiltration and portal congestion, respectively.

Conclusions: Intake of high doses of this plant extracts may exhibit mild organ toxicity.

1. Introduction

Herbs are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost[1]. There is also an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments[2]. However, herbal preparations assumed to be safe may contain contaminants such as heavy metals[3], aflatoxins and pathogenic microbes due to the manner in which they are prepared or as a result of acquisition of metals (*e.g.* cadmium) from the soil[4,5]. There is also the belief that because herbal remedies are derived from nature, they are

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devoid of adverse or toxic side effects often associated with synthetic drugs used in conventional medicine[6]. However, for proper and documented herbal medicinal products, the toxicity should be explored as in the case with conventional orthodox drugs that are properly researched and developed; the toxicity of traditional herbal medications is not often assessed[7]. As such, the users often look at the medicinal benefit of the herbal drugs and neglect their toxic effects to various organs.

Nelsonia campestris (*N. campestris*) belongs to the kingdom plantae, class Equisetopsida, subclass Magnolidae, suborder Asteranae, order Limiales brommhead, family Acanthaceae juss. and genus *Nelsonia* R.Br[8]. It grows on the margins of billabongs, creeks, and rivers and generally only near the edges of water and sometimes down into the water. Its prostrate growth habitat is maintained by high light levels and it does best in warmer water with CO_2 and pH control[9]. In the northern part of Nigeria, *N. campestris* grows in semi-arid regions and mostly after the rain has ceased. This contributes largely to the inavailability of this plant during the rainy season. Over the years, this herb has been used in traditional medicine for the treatment of respiratory and gastrointestinal complications of measles by the Nupe speaking people of Niger State, Nigeria. This study aims to evaluate the acute and sub-acute toxicity of the aqueous and methanol extracts of *N. campestris* on some renal and hepatic function indices in rats.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh samples of *N. campestris* were collected from the premises of Federal University of Technology, Minna, Bosso Campus, Niger State, Nigeria. The plant was identified and authenticated at the Herbarium Section of Department of Biological Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

2.2. Sample preparation

The plant material was washed, air-dried for four weeks and pulverized into coarse powder by using pestle and mortar. The coarse powder was further processed to fine particles with an electric blender.

2.2.1. Sample extraction

2.2.1.1. Aqueous extract

Fifty grams of *N. campestris* fine powdered sample was extracted with 800 mL distilled water by continuous refluxing for 2 h at 100 °C. The greenish liquid obtained was filtered with muslin cloth and the filtrate was further evaporated to dryness in a water bath at 100 °C.

2.2.1.2. Methanol extract

Fifty grams of *N. campestris* fine powdered sample was extracted with 250 mL methanol for 48 h by using Soxhlet apparatus at 65 °C. The extract was evaporated under reduced pressure by using a rotary evaporator and further concentrated in a water bath at 65 °C.

2.3. Experimental animals

About 64 young adult albino rats of both sexes, weighed between 180 and 250 g, were purchased from the Animal House Facility of Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria, and used for this study. They were fed with growers mash (Vital Feeds, Nigeria) and tap water *ad libitum*. The rodents were housed under standard laboratory environment, and allowed to acclimatize to the laboratory environment [temperature of (27 ± 2)°C, relative humidity and naturally illuminated environment of 12/12 h day light/dark cycles] for two weeks before the commencement of the research.

2.4. Acute oral toxicity study

The acute oral toxicity test of the aqueous and methanol extracts of *N. campestris* was evaluated in albino rats as reported by Muhammad *et al.*[10] with little modifications which involves two phases. The first phase was conducted as follows. Eighteen rats were grouped into six of three rats each. Following an overnight fast, the rats were weighed and the dose was calculated in reference to their body weight. The crude extracts were suspended in a vehicle (normal saline and dimethylsulfoxide for the aqueous and methanol extracts, respectively). The first three groups received 10, 100 and 1 000 mg/kg body weight of the aqueous extract and the other three groups were administered with 10, 100 and 1 000 mg/kg body weight of the methanol extract. The animals were observed keenly for about 30 min for any signs of toxicity or mortality, and further observations were made every 8 hours for 24 h after administration of the extracts. The absence of death of any animals in this phase necessitated the conduct of the second phase.

In the second phase, 18 rats were grouped into six of three rats each. The first three groups received 1 500, 2 900 and 5 000 mg/kg body weight of the aqueous extract while the other three groups were administered with the methanol extract at 1 500, 2 900 and 5 000 mg/kg body weight. The rats were observed for any signs of toxicity or mortality within 24 h. Further observation of all the rodents was made for a period of 14 days.

2.5. Sub-acute toxicity study

About 28 albino rats were randomly grouped into seven (A, B, C, D, E, F and G) of four rats each. Group A served as control and was administered with 0.5 mL of normal saline once daily for 28 days. Rats in groups B, C, D and groups E, F, G were orally gavaged with 100, 300 and 600 mg/kg body weight of the aqueous and methanol extracts, respectively once daily for 28 days.

The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period.

2.6. Termination of the experiment

On the 29th day of the research, following an overnight fast of 8 h, all animals in various groups were anesthetized under chloroform and blood samples were collected by cardiac

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puncture into heparinised and non-heparinised bottles for haematological and biochemical investigations respectively. Blood samples collected into clean non-heparinised bottles were allowed to clot and centrifuged according to groups; and serum was separated from the clot into clean bottles for the biochemical analyses. The liver, kidneys and heart were excised from dissected rats, immediately cleaned of blood by using physiological saline and weighed. The liver and kidneys were then fixed in 10% formalin for histopathological examination.

2.7. Calculation of organ-to-body weight ratio

Organ-to-body weight ratio was calculated by dividing the weight (g) of each organ by the weight (g) of rats before sacrifice.

2.8. Biochemical analyses

Commercial kits from Randox Laboratories Limited, United Kingdom and Agappe Diagnostics (Switzerland) were respectively used for the assay of liver and kidney indices.

2.9. Haematological analyses

White blood cell, red blood cell, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and packed cell volume were analyzed by using a Diatron Diagnostic Abacus Junior automatic hematology analyzer.

2.10. Histopathological examination

The liver and kidneys excised from all the experimental rats were fixed in 10% buffered formalin in labeled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 μm thick, stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope[11].

2.11. Statistical analysis

Data collected from the biochemical and haematological analyses were expressed as mean ± SEM. One-way ANOVA was used to test the means. Values were considered statistically significant at $P < 0.05$. All results represent as mean ± SEM ($n = 4$). Values with different superscripts are significantly different ($P < 0.05$).

3. Results

The results obtained from the acute oral toxicity study showed that aqueous and methanol extracts of *N. campestris* demonstrated high safety margin when the animals tolerated up to 5000 mg/kg body weight of the extracts orally (Table 1).

The mean organ-to-body weight ratio of rats that received the various doses of the aqueous and methanol extracts was not significantly different from the control group (Figures 1 and 2). However, 100 and 300 mg/kg body weight aqueous extract treated groups showed no significant gain in weight of liver. All doses: 100, 300 and 600 mg/kg body weight treated groups also showed no significant difference in heart and kidney (Figure 1).

Table 1
Acute oral toxicity of aqueous and methanol extracts of *N. campestris*.

Extract	Phase 1		Phase 2	
	Dose (mg/kgbw)	Mortality	Dose (mg/kgbw)	Mortality
Aqueous extract	10	0/3	1600	0/3
	100	0/3	2900	0/3
	1000	0/3	5000	0/3
Methanol extract	10	0/3	1600	0/3
	100	0/3	2900	0/3
	1000	0/3	5000	0/3

mg/kgbw: Milligram per kilogram body weight.

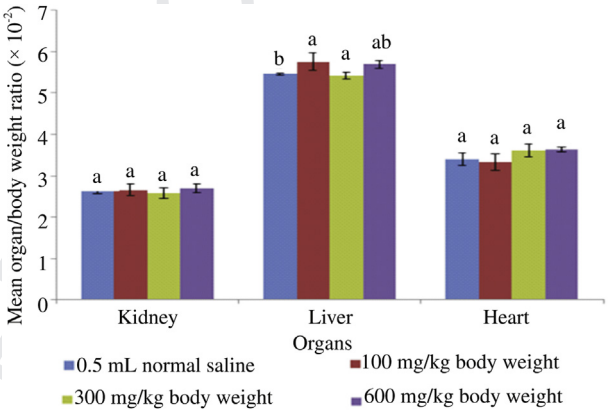

Figure 1. Mean organ/body weight ratio of rats administered with various doses of aqueous extract of *N. campestris*.
Data with different alphabets are significantly different ($P < 0.05$).

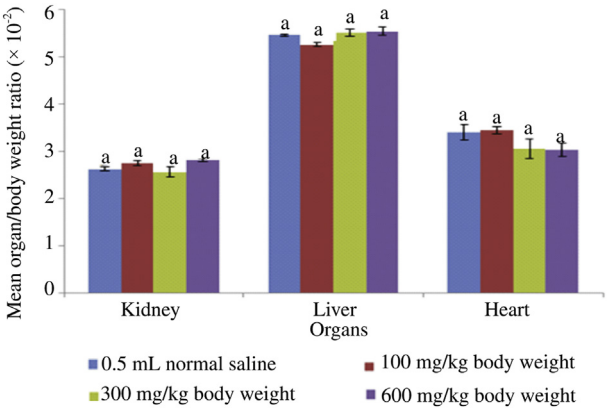

Figure 2. Mean organ/body weight ratio of rats administered various doses of methanol extract of *N. campestris*.
Data with different alphabets are significantly different ($P < 0.05$).

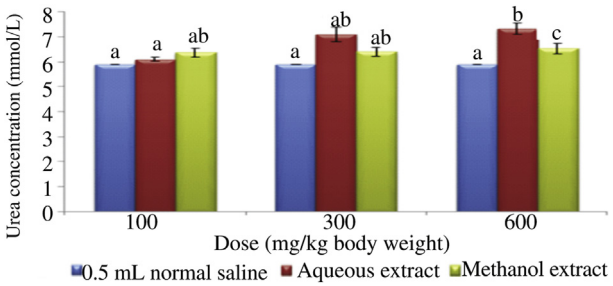

Figure 3. The effect of aqueous and methanol extracts of *N. campestris* on serum urea concentration.
Data with different alphabets are significantly different ($P < 0.05$).

However, 100, 300 and 600 mg/kg body weight of methanol extract treated group showed no significant difference in all the organs to body ratio (Figure 2).

Administration of both extracts at all doses resulted in the increased ($P < 0.05$) serum urea concentrations at 300 and 600 mg/kg body weight (Figures 3 and 4) except 100 mg/kg body weight of those receiving methanol extract which is significantly higher than both control and aqueous group. In addition the concentration of creatinine is significantly higher in aqueous extract group as compared to other groups.

Serum electrolytes (Na^+, K^+, Cl^- and HCO_3^-) were not affected by the administration of both extracts at all test doses (Figures 5 and 6). However, a significant dose-dependent increase was observed in the serum Na^+ concentration in rats that received the various doses of the aqueous extract when compared with control.

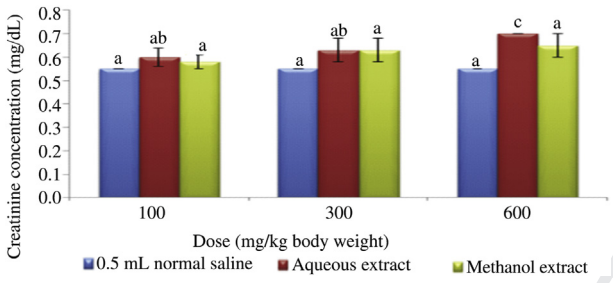

Figure 4. The effect of aqueous and methanol extracts of *N. campestris* on serum creatinine concentration.
Data with different alphabets are significantly different ($P < 0.05$).

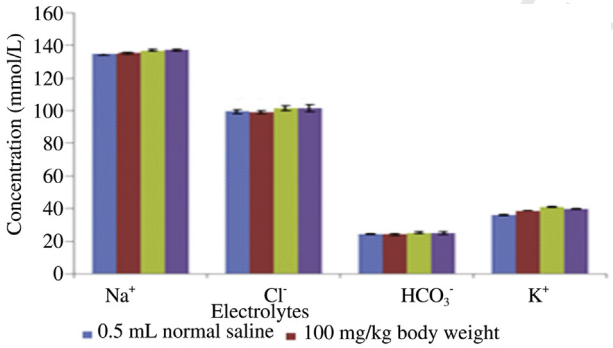

Figure 5. The effect of aqueous extract of *N. campestris* on serum electrolytes.

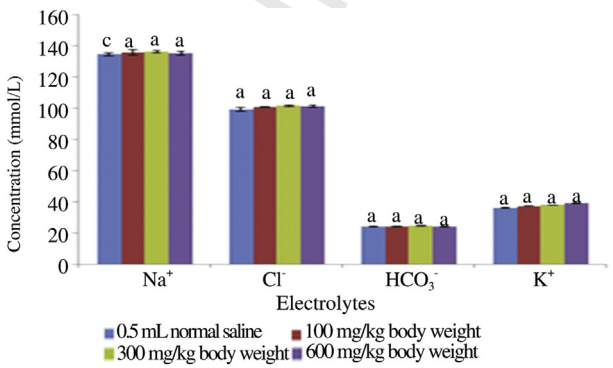

Figure 6. The effect of methanol extract of *N. campestris* on serum electrolytes.
Data with different alphabets are significantly different ($P < 0.05$).

Serum aspartate aminotransferase and alkaline phosphatase activities significantly increased ($P < 0.05$) in a dose-dependent fashion when the rats received various doses of the aqueous extract. Alanine aminotransferases activities was significantly higher in normal group when compare to others (Figure 7). Activities of aspartate aminotransferase, and alkaline phosphatase of the methanol extract groups also showed no significant difference from the control group (Figure 8) while the alanine aminotransferase of normal group showed significant higher value as compare with others.

Total protein increase significantly ($P < 0.05$) in 300 and 600 mg/kg body weight of the aqueous treated group when compared to other groups (Figure 9).

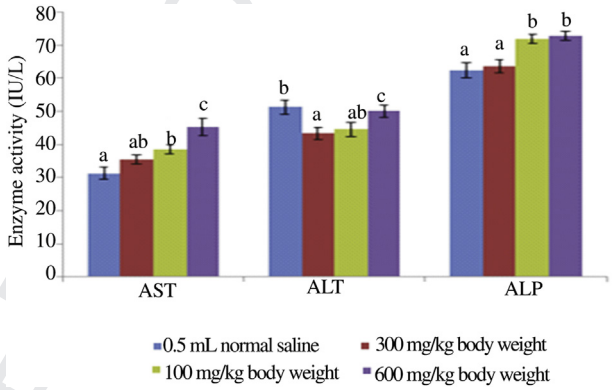

Figure 7. The effect of aqueous extract of *N. campestris* on activity of serum liver enzymes.
Data with different alphabets are significantly different ($P < 0.05$).

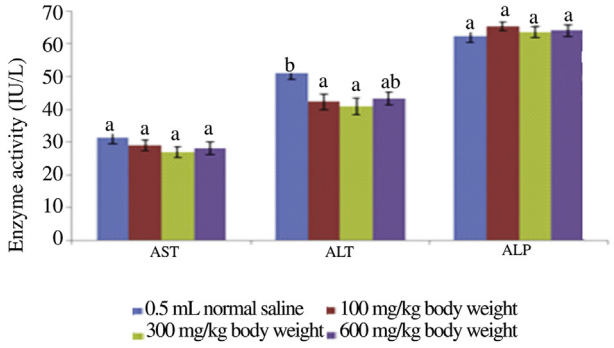

Figure 8. The effect of methanol extract of *N. campestris* on activity of serum liver enzymes.
Data with different alphabets are significantly different ($P < 0.05$).

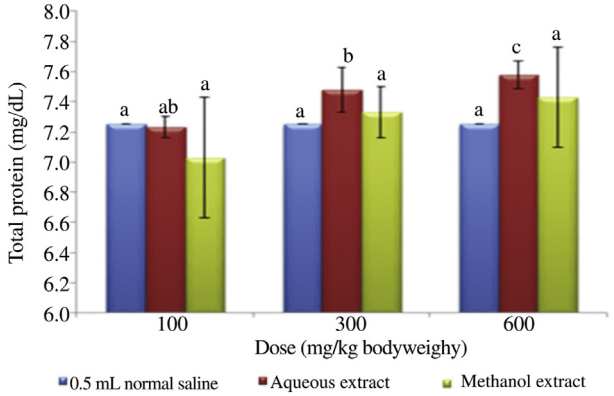

Figure 9. The effect of aqueous and methanol extracts of *N. campestris* on serum total protein.
Data with different alphabets are significantly different ($P < 0.05$).

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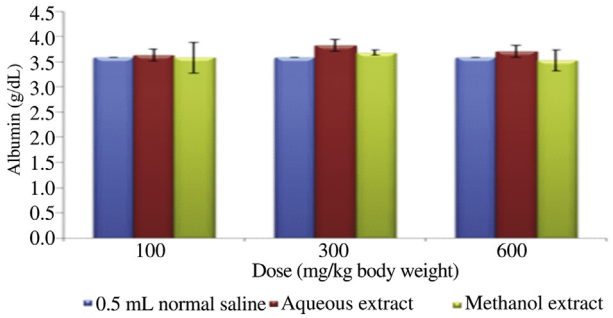

Figure 10. The effect of aqueous and methanol extracts of *N. campestris* on serum albumin.

Both aqueous and methanol extracts showed no significant differences ($P > 0.05$) in serum albumin concentration as compared to the control group (Figure 10).

The same observation as shown in the concentration of serum albumin was also observed in the serum total bilirubin concentration where there was no significant difference ($P > 0.05$) in all the treated groups (Figure 11).

No significant difference ($P > 0.05$) was observed in conjugated bilirubin of all the groups treated with aqueous, methanol and normal saline (Figure 12).

The 100, 300, and 600 mg/kg body weight of both extracts exhibited no significant difference ($P > 0.05$) in white blood cell count (Figure 13).

All extracts at all doses exhibited a significant decrease in red blood cell count when compared with control and methanol extract dose having a more significant effect (Figure 14).

Methanol extract showed reduction in haemoglobin concentration at all doses (Figure 15). However, 300 and 600 mg/kg body weight doses of both extracts demonstrated significant decrease in haemoglobin concentration in aqueous extract while

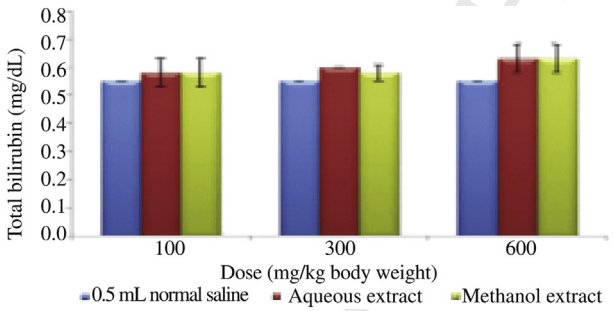

Figure 11. The effect of aqueous and methanol extracts of *N. campestris* on serum total bilirubin.

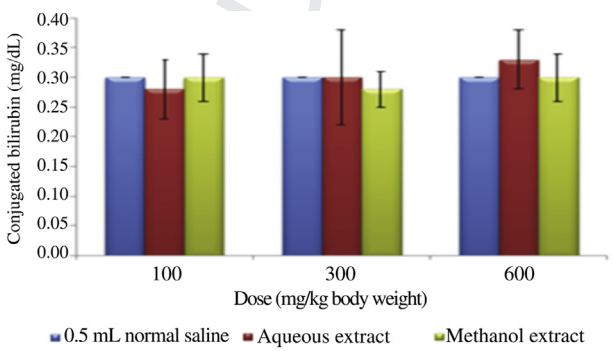

Figure 12. The effect of aqueous and methanol extracts of *N. campestris* on serum conjugated bilirubin.

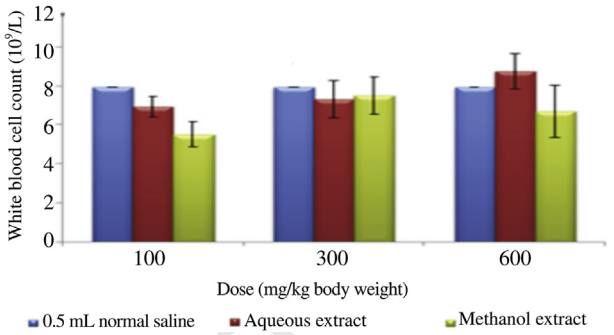

Figure 13. The effect of aqueous and methanol extracts of *N. campestris* on white blood cell count.

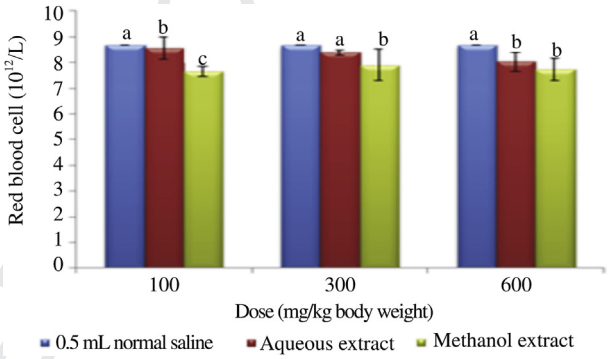

Figure 14. The effect of aqueous and methanol extracts of *N. campestris* on red blood cell count.
Data with different alphabets are significantly different ($P < 0.05$).

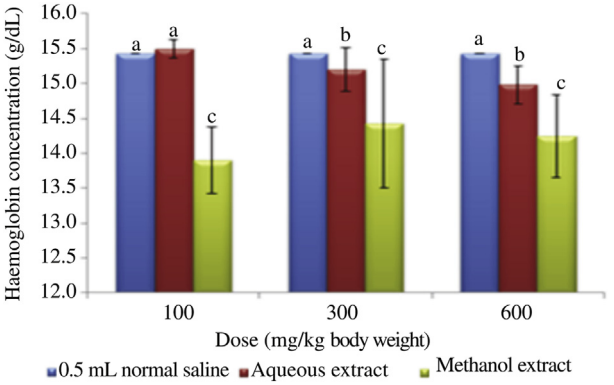

Figure 15. The effect of aqueous and methanol extracts of *N. campestris* on haemoglobin concentration.
Data with different alphabets are significantly different ($P < 0.05$).

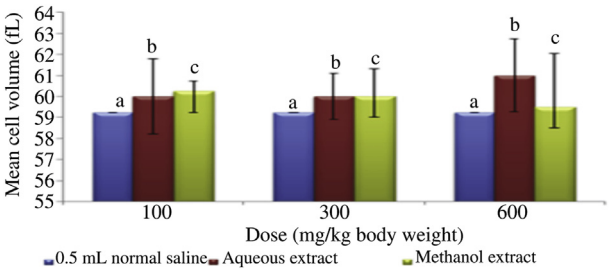

Figure 16. The effect of aqueous and methanol extracts of *N. campestris* on mean cell volume.
Data with different alphabets are significantly different ($P < 0.05$).

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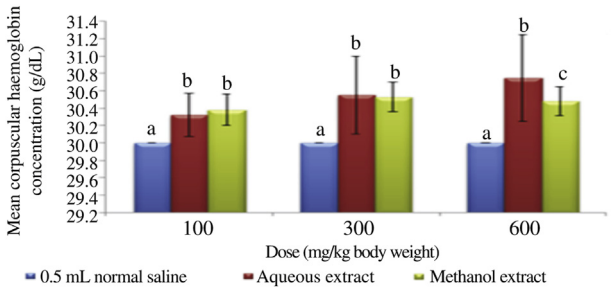

Figure 17. The effect of aqueous and methanol extracts of *N. campestris* on mean corpuscular haemoglobin.
Data with different alphabets are significantly different ($P < 0.05$).

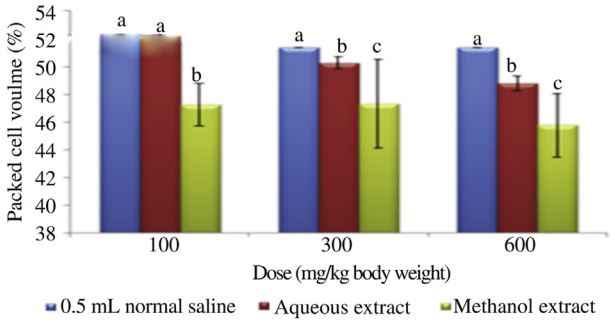

Figure 18. The effect of aqueous and methanol extracts of *N. campestris* on packed cell volume.
Data with different alphabets are significantly different ($P < 0.05$).

the control group and aqueous extract of 100 mg/kg body weight showed no significant difference ($P > 0.05$).

The control group showed significant ($P < 0.05$) lowest value of mean cell volume as compared with aqueous and methanol extract at all doses level ($P < 0.05$) (Figure 16).

Extracts at all doses significantly elevated mean corpuscular haemoglobin in all groups when compared with control group (Figure 17).

Methanol extract groups at all doses showed significant decreased ($P < 0.05$) in packed cell volume in the respective groups (Figure 18).

The results of histopathological examination of liver section in rats treated with normal saline and aqueous extracts are shown in Figure 19. The liver in rats administered with 0.5 mL normal saline for 28 days presented the normal hepatic plates (shown by long arrow) and portal vein (shown in short arrow) (Figure 19A). For the rats administered with 100 mg/kg body weight of aqueous extract of *N. campestris*, there was mild hepatic necrosis (Figure 19B), while the 300 mg/kg body weight of aqueous extract of *N. campestris* treated rats showed portal congestion (shown by long arrow) and bile lakes (shown by short arrow) in liver (Figure 19C). The moderate hepatic necrosis was presented in rats treated with 600 mg/kg body weight of aqueous extract of *N. campestris* (Figure 19D).

The results of histopathological examination of liver section in rats treated with normal saline and methanol extracts are shown in Figure 20. The liver in rats administered with 0.5

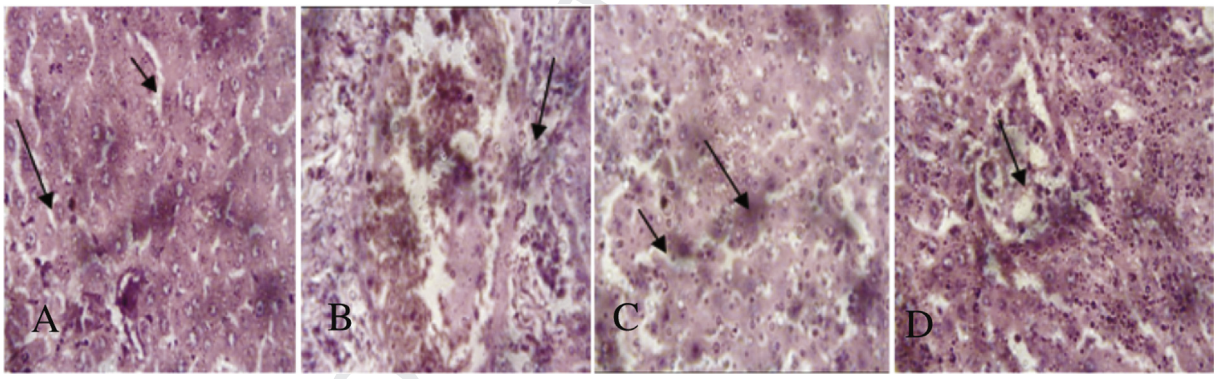

Figure 19. Photomicrograph of the liver section of rats administered with 0.5 mL normal saline and aqueous extracts of *N. campestris* for 28 days.
A: Normal saline treated liver shows normal hepatic plates (long arrow) and portal vein (short arrow); B: 100 mg/kg body weight aqueous extract of *N. campestris* treated liver shows mild hepatic necrosis; C: 300 mg/kg body weight aqueous extract of *N. campestris* treated liver shows portal congestion (long arrow) and bile lakes (short arrow); D: 600 mg/kg body weight aqueous extract of *N. campestris* treated liver shows moderate hepatic necrosis. Haematoxylin and eosin staining (H&E), magnification 40×.

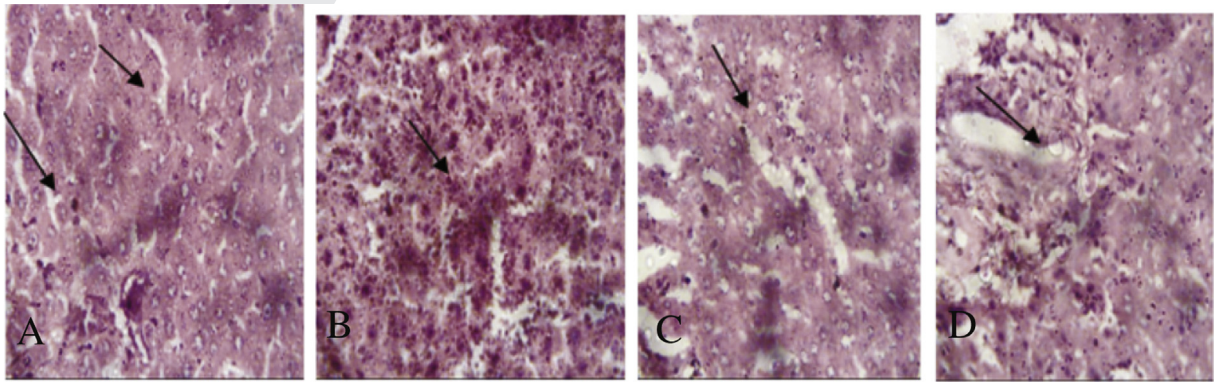

Figure 20. Photomicrograph of the liver section of rats administered with 0.5 mL normal saline and methanol extracts of *N. campestris* for 28 days.
A: Normal saline treated liver shows normal hepatic plates (long arrow) and portal vein (short arrow); B: 100 mg/kg body weight methanol extract of *N. campestris* treated liver shows mild hepatic necrosis; C: 300 mg/kg body weight methanol extract of *N. campestris* treated liver shows mild hepatic necrosis; D: 600 mg/kg body weight methanol extract of *N. campestris* treated liver shows moderate degeneration of hepatocytes. H&E, magnification 40×.

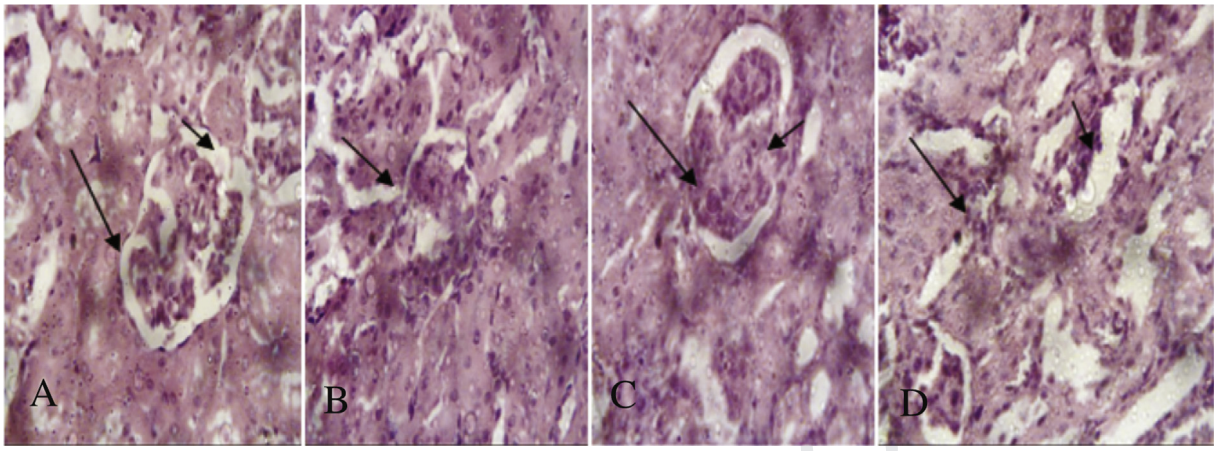

Figure 21. Photomicrograph of the kidney section of rats administered with 0.5 mL normal saline and *N. campestris* aqueous extract for 28 days. A: Normal saline treated kidney shows intact glameli (long arrow) and tubules (short arrow); B: 100 mg/kg body weight aqueous extract of *N. campestris* treated kidney shows mild tubular necrosis; C: 300 mg/kg body weight aqueous extract of *N. campestris* treated kidney shows mild tubular necrosis (long arrow) and lymphocytic infiltration (short arrow); D: 600 mg/kg body weight aqueous extract of *N. campestris* treated kidney shows mild to moderate cortical necrosis (long arrow) and tubular edema (short arrow). H&E, magnification 40×.

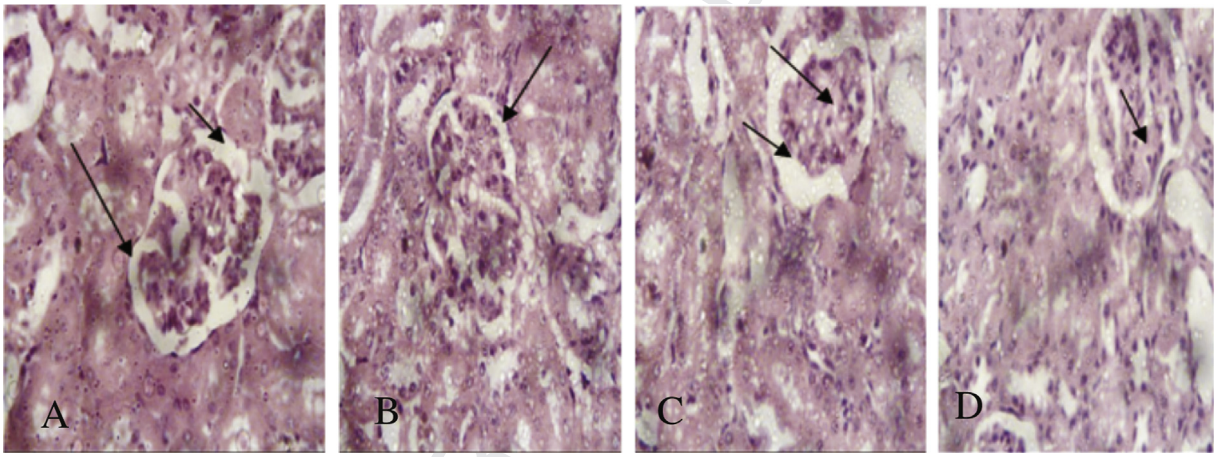

Figure 22. Photomicrograph of the kidney section of rats administered with 0.5 mL normal saline and *N. campestris* methanol extract for 28 days. A: Normal saline treated kidney shows intact glameli (long arrow) and tubules (short arrow); B: 100 mg/kg body weight methanol extract of *N. campestris* treated kidney shows mild corticomedullary necrosis; C: 300 mg/kg body weight methanol extract of *N. campestris* treated kidney shows mild cortical and tubular necrosis; D: 600 mg/kg body weight methanol extract of *N. campestris* treated kidney shows mild cortical necrosis. H&E, magnification 40×.

normal saline presented the normal hepatic plates (shown by long arrow) and portal vein (shown in short arrow) (Figure 20A). For the rats administered with 100 and 300 mg/kg body weight of methanol extract of *N. campestris*, there was mild hepatic necrosis (Figure 20B and 20C), while 600 mg/kg body weight of methanol extract of *N. campestris* treated rats showed moderate degeneration of hepatocytes (Figure 20D).

The results of histopathological examination of kidney section in rats treated with normal saline and aqueous extracts are shown in Figure 21. The kidney in rats administered with 0.5 normal saline presented the intact glameli (shown by long arrow) and tubules (shown by short arrow) (Figure 21A). For the rats administered with 100 mg/kg body weight of aqueous extract of *N. campestris*, there was mild tubular necrosis (Figure 21B), while 300 mg/kg body weight of aqueous extract of *N. campestris* treated rats showed mild tubular necrosis (shown by long arrow) and lymphocyctic infiltration (shown by short arrow) (Figure 21C). The mild to moderate cortical necrosis (shown by long arrow) and tubular edema (shown by short arrow) were presented in rats treated with 600 mg/kg body weight of methanol extract of *N. campestris* (Figure 21D).

The kidney in rats administered with 0.5 normal saline presented the intact glameli (shown by long arrow) and tubules (shown by short arrow) (Figure 22A). For the rats administered with 100 mg/kg body weight of methanol extract of *N. campestris*, there was mild corticomedullary necrosis (Figure 22B), while the 300 mg/kg body weight of methanol extract of *N. campestris* treated rats showed mild cortical and tubular necrosis (Figure 22C). The mild cortical necrosis was presented in rats treated with 600 mg/kg body weight of methanol extract of *N. campestris* (Figure 22D).

4. Discussion

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells[12]. According to the Guidance Document on Acute Oral Toxicity Testing based on oral LD50 value which were recommended by Organization for Economic Cooperation and Development[13], the crude extracts of *N. campestris* may be assigned to be class 5 (LD50 > 2 000 mg/kg body weight), which

was designated to be the lowest toxicity class (no label; unclassified).

Alteration in organ-to-body weight ratio may be as a result of organ damage[14]. The result is an indication that *N. campestris* may not elicit any deleterious effect on the weight of kidney, liver and heart, and the result is in consonance with the findings of Olorunnisola *et al.*[15]. They reported that 28-day oral administration of methanol extract of *Tulbaghia violacea* rhizomes at doses of 125, 250 and 500 mg/kg body weight was not toxic to the heart, liver, kidney and pancreas of the experimental subjects.

Assessment of liver and kidney function is a very vital index in evaluating the toxicity of drugs and plant extracts. Kidney function indices evaluated in this study were serum urea, creatinine and electrolyte concentrations. This correlates with the findings of Muhammad *et al.*[16], who carried out an investigation on the acute and sub-chronic toxicity of kernel extract of *Sclerocarya birrea* in rats. They reported that a significant increase in serum urea and creatinine was observed when the experimental rats received higher doses of the kernel extract of *Sclerocarya birrea* ranging from 3000 to 4000 mg/kg body weight. NH_3 released during deamination is removed from the blood by conversion into urea. Increase in urea may be the result of high glomerular filtration. Creatinine is not supposed to be reabsorbed but all creatinine that is filtered in the glomerular filtrate passes on through the tubular system and is excreted in the urine. In this situation, creatinine is reabsorbed rather than excreted in urine.

The values of all the electrolytes are within the normal range according the Rat Fan Club[17]. An elevation in the activity of liver enzymes (ALT, AST and ALP) is conventionally an indicator of liver injury[18]. This result is in consonance with earlier findings of Tarkang *et al.*[19], who carried out an investigation on the acute and chronic toxicity of the aqueous and ethanol leaf extracts of *Carica papaya* Linn in Wistar rats. They observed a dose-dependent increase in AST and suggested that sub-acute administration of *Carica papaya* extracts caused hepatocellular damage. The significant increase in AST observed in this study suggests that administration of higher doses of this extract may induce the destruction of the liver cells.

Administration of methanol extract at all test doses had no significant effect on the serum levels of AST and ALP in the experimental rats ($P > 0.05$). Serum ALT levels were found to decrease significantly ($P < 0.05$) in rats that received 100 mg/kg body weight of the aqueous and methanol extracts and 300 mg/kg body weight of the methanol extract when compared with the control group. This result is in agreement with the findings of earlier work of Guy *et al.*[20]. These researchers investigated an influence of age on sub-chronic toxicity of the aqueous extract of *Calotropis procera* leaves in rabbits. They observed a significant decrease in serum ALT concentrations in younger rabbits and suggested that the cause of this decrease included the decreased hepatocellular production or release of enzymes, inhibition or reduction of the enzyme's activity and interference with the enzyme assay. Wallace also postulated that since liver was also a major organ of protein synthesis, any decrease in liver synthesis can be seen as damage of hepatocytes with alteration of its production capacity[21]. In contrast, European Document for Ecotoxicology and Toxicology stated that the biological significance of the ALT enzyme decrease was unclear, it was typically dismissed as being of no toxicological importance[22].

Impaired hepatocellular function may lead to a reduction in serum concentrations of albumin, total protein and bilirubin. The insignificant change in serum concentrations of total protein, albumin and bilirubin in the treated and control groups further suggests that the synthetic functions of the liver is not altered at any of the test doses of the aqueous and methanol extracts. The significant increase in serum total protein concentration of rats that received 600 mg/kg body weight of the aqueous extract may be due to increased synthesis by the liver.

Haematopoietic system is one of the most susceptible targets of toxic compounds, especially in the bone marrow where the production of red blood cell occurs[23]. Sub-acute administration of the aqueous and methanol extracts of *N. campestris* did not cause significant changes ($P > 0.05$) in the haematological profile of rats that received the entire test doses when compared with control, suggesting that *N. campestris* may not be toxic to the blood system (Figures 11–17). Histopathological examination of the liver and kidneys in experimental rats that were administered with 100 and 600 mg/kg body weight of the aqueous and methanol extracts of *N. campestris* revealed mild to moderate hepatocellular and cortical necrosis of liver and kidney sections respectively.

Necrosis from hepatotoxic chemicals can occur within distinct zones in the liver, either distributed diffusely, or occur massively. Many chemicals produce zonal necrosis, *i.e.* necrosis confined to a specific zone of the hepatic acinus[24]. As observed in this study, significant elevations in serum AST and ALP may be due to hepatic necrosis. However, the remarkable ability of the liver to regenerate itself makes it able to withstand moderate zonal or diffuse necrosis. Over a period of several days, necrotic cells are removed and replaced with new cells; and normal hepatic architecture and function are restored[24].

Administration of aqueous and methanol extracts at 300 mg/kg body weight resulted in portal congestions and mild hepatic necrosis respectively in the liver of experimental rats. Histology of kidney section of rats that were administered with 300 mg/kg body weight of aqueous extract revealed mild tubular necrosis and lymphocytic infiltration while rats administered with 300 mg/kg body weight of the methanol extract revealed mild cortical and tubular necrosis. However, animals in the control group had intact hepatocytes, portal vein, glomeruli and intact tubules. The occurrence of lymphocytic infiltration in organs has been attributed to the presence of glycosides as reported by Adedapo *et al.*[25]. The result of this study is consistent with the findings of Builders *et al.*[26], who investigated the toxicity of *Parkia biglobosa* stem bark extracts in rats. It was reported that the toxicity of some of the herbal medications might be a result of phytochemical constituents. Muhammad *et al.* also reported that large intake of tannins may cause kidney and liver damage[23].

N. campestris at high doses caused elevation of some serum biochemical parameters and histologic changes in target organs of toxicity (liver and kidney). The plant is though a promising agent in pharmaceutics, but can cause mild organ damage at high doses.

Conflict of interest statement

The authors report no conflict of interest.

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