



The Phytochemical Analysis and Safety Evaluation of Ethanol Leaf extract of *Grewia carpinifolia* in Wistar Rat

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SUMMARY

The traditional use of plants is a common practice globally. *Grewia carpinifolia* is used in treatment of gastrointestinal disorders and as anti-parasitic. However its toxicity profiles are not well documented. Thus there is a need to identify the phytochemical composition of the plant as well as its toxicity following acute and sub-chronic oral administration. The phytochemical analysis, acute toxicity profile as well as daily feed and water intake, body weight changes, serum biochemical and haematological parameters and histology of vital organs were assessed following a daily single oral administration in a 28-day study. Tannins, saponins, flavonoids, alkaloids, phlobatannins, terpenoids, cardiac glycosides and anthraquinones were present in the extract. The lethal dose (LD₅₀) was greater than 4000 mg/kg. The extract caused significant decrease in water intake in a dose dependent manner, significant increases were observed in liver enzymes analysed (AST, ALP, ALT) at 800 mg/kg after 28-day administration. In addition, congestion of central nucleus and sinusoids were observed in the liver at 800 mg/kg. *G. carpinifolia* leaf extract appear to be safe at doses below 800 mg/kg body weight. Preliminary results suggest promising alternatives for exploring therapeutic and pharmaceutical interest in *G. carpinifolia* leaf extract.

Key words: *Grewia carpinifolia*, acute toxicity, phytochemical composition, histology.

INTRODUCTION

Plants have been used in the management of human and animal health over the years (Adebiyi and Abatan, 2013) The World Health Organisation (WHO) has also stated that the use of plant extracts in traditional medicine is either the mainstay of health care delivery or serves as a complement to it especially in developing countries (WHO, 2014). However for clinical purposes the safety and the potential toxic effects of the

plant must be carried out to justify its use(s). Whereas the effects of some of these plants used in folk medicine have been investigated and documented in literature, the safety profile of a number of such plants remains invalidated despite their widespread use (Maphosa *et al.*, 2008).

Grewia carpinifolia Juss is a large flowering tree belonging to the family Tiliaceae. It is widely distributed in the

warmer parts of the world. Different parts of the plant are used as folk medicine throughout the globe (Goyal, 2012). The leaf is used in treatment of parasitic and viral infections, venereal diseases, gastrointestinal tract (GIT) disorders and also as analgesics (Obidah *et al.*, 2010) while the flower is eaten as food (Goyal, 2012). There is a dearth of information on the safety and toxicological profile of this plant despite its numerous uses; this study was therefore carried out to evaluate the safety profile of the plant following an acute and sub-chronic oral administration in Wistar rats.

MATERIAL AND METHODS

Plant material and authentication

G. carpinifolia leaf was collected from the Botanical Garden of the University of Ibadan, Nigeria. It was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) where herbarium specimen (voucher number FHI 109693) was deposited.

Experimental animals

Adult male and female Wistar rats weighing between 180-210 g were used for the acute and sub-chronic toxicity studies. The animals were purchased from the Experimental Animal Unit, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. The animals were housed under standard conditions of temperature, ($25 \pm 2^\circ\text{C}$) and light, (approximately 12/12 h light-dark cycle), fed on standard diet and water provided *ad libitum*. The animals were acclimatized to laboratory conditions for two weeks before the commencement of the experiment. The experimental protocol was approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan (UI-ACUREC/App/2016/025).

Extract preparation

All the samples of *G. carpinifolia* were thoroughly rinsed with distilled water before

being air-dried at room temperature for eight weeks. The plant sample was then ground to a fine powder and soaked in absolute ethanol for 4 days with frequent agitation at room temperature. The extract was filtered with Whatman filter paper (No.1) and the residue of fine powder was then re-soaked with a fresh portion of ethanol twice for four days each time at room temperature. The filtrate was concentrated to dryness *in vacuo* at 40°C using rotary evaporator to give a yield of 17% w/w of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in this study.

Qualitative phytochemical screening

Chemical tests were carried out for preliminary phytochemical screening of the leaf extract of *G. carpinifolia* using standard procedures to identify the constituents as described by Sofowora (1993).

Acute toxicity studies

The acute oral toxicity test was performed according to the Organization of Economic Co-operation and Development (OECD) revised guideline 425 for testing of chemicals (OECD, 2001). The study was carried out in two phases. In the first phase, twelve rats were randomized into four groups (A-D) of three rats each. Group A served as control and only received distilled water, groups B, C and D were administered extract at 50, 100 and 1000 mg/kg orally respectively. The rats were kept under the same conditions and observed for signs of toxicity which include (but not limited to) paw-licking, stretching, respiratory distress, motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, righting reflex, sensations, pilo-erection, ptosis, lacrimation, exophthalmos, salivation, diarrhoea, writhing, skin colour and mortality. Animals were observed at 0, 1, 2, 4, 6, 8, 12, and at the 24th hour and subsequently once daily for 7 days. In the second phase of the study, 2000 and 4000 mg/kg body weight respectively were orally

administered to another fresh set of two groups (E and F) of three rats each. These rats were also observed for signs of toxicity and mortality for the first critical 4 hour and thereafter once daily for 7 days. The dose level that killed 50% of the members of the test group was taken as the approximate LD₅₀.

Sub-chronic toxicity study

The sub-chronic toxicity test was performed following the protocol described by the OECD guideline 408 for testing chemicals (OECD, 1998). Twenty-five rats of both sexes were divided into five groups of five rats each. Group A, received normal saline and served as the control while rats in groups B, C, D and E were given 100, 200, 400 and 800 mg/kg respectively in a single oral dose daily for 28 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality.

Feed and water intake

The quantity of feed (g) and volume of water (ml) intake by rats in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 hour respectively.

Body weight change

Rats in all the groups were weighed on the first day of the experiment and thereafter weekly during the period of extract administration and on the last day of the study. Doses of the extract administered were adjusted accordingly.

Haematology and clinical chemistry

Blood samples were collected via cardiac puncture into plain and ethylene diamine tetraacetic acid (EDTA; 2 mg/ml of blood) bottles for biochemical and haematological analyses, respectively. Thereafter, the rats were euthanized by intraperitoneal injection

of a mixture containing ketamine (80 mg/kg) and xylazine (10 mg/kg).

Estimation of Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelets, white blood cell count (WBC) and differentials, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using an automated haematological analyzer (Cell-Dyn™ Abbot, USA) was done. The portion dispensed into plain bottles was allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, temporarily stored at -4 °C and used for evaluation of biochemical parameters which include, alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) levels, total protein, albumin, bilirubin and serum urea concentration using commercial kits (Randox Laboratories, UK). Globulin levels were calculated as the difference between the total protein and albumin.

Relative organ weight

The brain, heart, spleen, liver left and right kidneys were carefully excised, weighed and observed macroscopically. The relative organ weight (ROW) of the organs expressed as percentage of body weight was calculated as follows:

$$\text{ROW} = \frac{\text{absolute organ weight (g)}}{\text{body weight of rats on sacrifice day (g)}} \times 100.$$

Relative organ weight equals absolute organ weight in grammes divided by body weight of rats on sacrifice day in grammes multiplied by one hundred (100).

Histopathology

The liver and kidneys were fixed in 10% formalin and were embedded in paraffin wax by conventional methods (Sidhu and Nehru, 2004). Sections of 5 µm in thickness were prepared with rotary microtome and then stained with hematoxylin-eosin (HE).

The sections were then observed under the microscope for histopathological changes, and their photomicrographs were captured using a high resolution digital camera (Sony® Cybershot DSC-W53 digital camera).

Statistical analysis

Results were analysed using one way analysis of variance (ANOVA) with Graph Pad Prism Version 5.0 software (San Diego, USA). The comparison between control and treated groups were made using unpaired Student's t-test while comparison within groups was done with the paired Student's t-test. $P \leq 0.05$ was considered to be significant. Data were expressed as mean \pm standard error of mean.

RESULTS

Phytochemical screening

Tannins, saponins, flavonoid, alkaloids, phlobatannins, terpenoids, cardiac glycosides and anthraquinones were present in the ethanol leaf extract of *G. carpinifolia*; however coumarin was not detected (Table I).

Acute toxicity studies

Decreased locomotion was observed in all test groups 1 hour following extract administration. No death was recorded at

doses between 50 and 4000 mg/kg (Table II).

Sub-chronic toxicity studies

Effect of ethanol leaf extract of Grewia carpinifolia on average daily feed and water intake of Wistar rats treated for 28 days

The effect of ethanol leaf extract of *G. carpinifolia* on average daily feed and water intake of Wistar rats treated for 28 days is presented in Table III. There was a significant ($P \leq 0.05$) reduction in water intake at 200, 400 and 800 mg/kg when compared with the control and the 100 mg/kg group. The result also showed a decrease in the average daily feed consumed in all the test groups when compared with the control; this decrease was however not significant ($P \geq 0.05$) and non-dose dependent.

Effect of ethanol leaf extract of Grewia carpinifolia on body weight of Wistar rats treated for 28 days

There were increases in the mean body weight in all the groups throughout the experiment. The final mean body weight difference at the onset of the study and point of autopsy did not vary significantly in *G. carpinifolia* treated rats when compared with control group (Table IV).

TABLE I: Qualitative phytochemical analysis of ethanol leaf extract of *Grewia carpinifolia*

Phytochemical	Result
Tannins	++
Phlobatannins	+
Saponins	++
Flavonoids	++
Terpenoids	+
Cardiac glycosides	+
Coumarin	-
Alkaloids	++
Anthraquinones	+

++ = strongly positive; + = trace; - = not detected

TABLE II: The dose levels used in the determination of LD₅₀ of ethanol extract of *Grewia carpinifolia*

Dosage (mg/kg)	Dead/total	Dead (%)
Control	0/3	0
50	0/3	0
100	0/3	0
1000	0/3	0
2000	0/3	0
4000	0/3	0

Effect of sub-chronic administration of ethanol leaf extract of Grewia carpinifolia on haematology and clinical chemistry

The effect of ethanol leaf extract of *G. carpinifolia* on haematology and clinical chemistry of Wistar rats treated for 28 days is shown in Tables V and VI.

There was no significant difference ($P \geq 0.05$) in the PCV, RBC, WBC, platelets, MCV, MCH and MCHC values. A significant ($P \leq 0.05$) decrease in relative neutrophils count was observed at 100mg/kg following differential count. A significant increase ($P \leq 0.05$) was observed in the levels of ALT, AST and ALP in the 800 mg/kg group when compared with other test doses and the control group.

Effect of ethanol leaf extract of Grewia carpinifolia on relative organ weight of Wistar rats treated for 28 days

There was no significant difference ($P \leq 0.05$) between the relative organ weights (Table

VII) in all tested doses; there was similarity when compared with the control.

Histopathology

The result of the histopathology of rats treated with the ethanol leaf extract of *G. carpinifolia* on the liver, heart and kidney of rats is shown in Figures I and II. No visible lesion was observed in the liver of control animals and groups administered with 100, 200 and 400 mg/kg bw of the extract (Figure IA and B), however congestion of central nucleus and sinusoids were observed in the liver section of rats in group E administered with 800 mg/kg of the leaf extract (Figure IC).

No visible lesion was observed in the kidney section of the control and treated groups (Figure IIA and B). Moderate coronary congestion was observed in the heart of treated rats at 400 mg/kg dosage (Figure IIC).

TABLE III: Effect of *Grewia carpinifolia* leaf ethanol extract on average daily feed and water intake of Wistar rats treated for 28 days

Doses (mg/kg)	Feed Consumed (g)	Water intake (ml)
Control	78.89 ± 2.02	180.10 ± 3.79 ^a
100	75.18 ± 1.11	163.70 ± 4.91 ^a
200	57.47 ± 1.97	141.20 ± 8.90 ^b
400	62.47 ± 2.12	144.30 ± 10.52 ^b
800	56.00 ± 3.66	139.50 ± 8.68 ^b

n=5; Mean ± S.E.M (Standard Error of Mean); Means with different superscripts within rows are significantly different at $p < 0.05$

TABLE IV: Effect of graded doses of ethanol extract of *Grewia carpinifolia* leaf on body weights of rats

WEIGHT (g)	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Week 1	180.40 ± 15.10	187.8 ± 12.88	207.00 ± 10.91	196.40 ± 19.79	193.40 ± 13.42
Week 2	196.80 ± 20.29	216.00 ± 10.65	221.30 ± 14.77	192.90 ± 12.28	198.00 ± 6.04
Week 3	208.40 ± 20.97	212.00 ± 12.43	221.30 ± 14.01	200.00 ± 13.42	205.80 ± 11.54
Week 4	215.76 ± 18.54	217.43 ± 12.54	223.65 ± 15.78	205.00 ± 11.65	209.34 ± 11.87

n=5; Mean ± S.E.M (Standard Error of Mean)

TABLE V: Effect of *Grewia carpinifolia* leaf on blood parameters of Wistar rats treated for 28 days

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
PCV (%)	44.20±0.58	45.60±1.60	42.00±0.58	44.80±1.91	42.50±1.20
Hb (g/dl)	14.00±0.26	14.24±0.39	13.60±0.17	14.40±0.59	13.88±0.43
RBC (×10 ¹² /L)	7.96± 0.43	8.18 ± 0.63	8.03 ± 0.81	8.14 ± 0.46	8.01 ± 0.29
WBC (10 ³ μL ⁻¹)	9.78±0.87	15.14 ± 2.25	14.43 ± 2.98	11.82 ± 2.78	13.38 ± 1.00
Neutrophils (10 ³ μL ⁻¹)	4.30±0.07 (44.00±4.66) ^a	3.85±1.46 (25.40±2.54) ^b	7.31±2.82 (50.67±3.48) ^a	4.07±1.02 (34.40±1.17) ^a	5.75±1.13 (43.00±2.86) ^a
Monocytes (10 ³ μL ⁻¹)	0.63±0.02 (6.40±1.63)	1.36±0.09 (9.00±0.45)	1.25±0.04 (8.67±0.67)	0.95±0.02 (8.00±0.32)	1.04±0.69 (7.75±0.25)
Eosinophils (10 ³ μL ⁻¹)	0.04±0.01 (0.40±0.40)	0.24±0.01 (1.60±1.17)	0.24±0.02 (1.67±0.88)	0.00±0.00	0.37±0.04 (2.75±0.25)
Platelets (x10 ³)	585.80±43.72	899.00±143.80	521.30±18.56	684.20±83.15	618.00±145.00
MCV (fl)	56.00±0.55	58.00±0.84	57.33±0.88	58.20±1.63	57.50±1.71
MCH (pg)	18.00±0.32	18.80±0.20	18.67±0.33	18.80±0.49	19.00±0.71
MCHC (%)	31.60±0.40	32.00±0.55	32.33±0.33	32.20±0.20	32.75±0.25

n=5; Mean ± S.E.M (Standard Error of Mean); Means with different superscripts within rows are significantly different at $p<0.05$; absolute values of granulocytes are in parenthesis; PCV: Packed cell volume; Hb: haemoglobin; WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb concentration

TABLE VI: Effect of *Grewia carpinifolia* leaf on serum biochemistry of Wistar rats treated for 28 days

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
ALT (U/L)	54.40±3.49 ^a	48.00± 5.48 ^a	49.67 ± 1.45 ^a	55.75±3.12 ^a	113.67 ± 8.67 ^b
ALP(U/L)	44.8 ±9.78 ^a	52.45±24.8 ^a	53.72±20.49 ^a	52.45± 17.51 ^a	101.40± 4.56 ^b
GGT(U/L)	10.89± 4.51	9.25 ± 5.06	14.46 ± 13.34	6.60 ± 0.56	13.00 ± 2.79
AST (U/L)	36.50±0.63 ^a	39.00±3.79 ^a	41.67±3.93 ^a	36.67±6.67 ^a	98.8±1.88 ^b
UREA (mg/dl)	33.78± 6.56	24.32± 13.74	41.48 ± 15.41	24.76 ± 1.07	34.94 ± 8.16
TP (g/dL)	5.48 ± 0.86 ^a	4.96 ± 0.65 ^a	5.92 ± 0.16 ^a	5.89 ± 0.23 ^a	2.03 ± 0.25 ^b
ALB (g/dL)	2.81 ± 0.43	2.54 ± 0.25	2.82 ± 0.14	2.89 ± 0.66	1.47 ± 0.43
GLB (g/dL)	2.67 ± 1.24 ^a	2.43 ± 0.68 ^a	3.10 ± 0.05 ^a	3.00 ± 0.81 ^a	0.56 ± 0.27 ^b

n=5; Mean ± S.E.M (Standard Error of Mean); Means with different superscripts within rows are significantly different at $p<0.05$;

AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; GGT- Gamma Glutamyl Transferase TP = Total Protein; ALB = Albumin; GLB = globulin

DISCUSSION

An awareness of the chemical components of plants is important for the discovery of therapeutic potent bioactive compounds found in medicinal plant as well as the synthesis of new drugs (Katiyar *et al.*, 2012). The qualitative phytochemical screening of *G. carpinifolia* showed the presence of tannins, saponins, flavonoids,

alkaloids, phlobatannins, terpenoids, cardiac glycosides and anthraquinones.

The results obtained in this study thus suggest that the phytochemical components in *G. carpinifolia* leaf could account for its numerous folklore uses. Our findings are in consonance with that obtained by Arun *et al.* (2013) in their phytochemical investigation of ethanol extract of *G. microcos* Linn leaf but contrary to those of Nidhi and Vidya

TABLE VII: Effect of *Grewia carpinifolia* leaf on relative organ weights of Wistar rats treated for 28 days

Organs	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Brain ($\times 10^{-1}$)	7.6 \pm 0.08	7.72 \pm 0.08	7.96 \pm 0.02	7.85 \pm 0.05	2.88 \pm 0.02
Heart ($\times 10^{-1}$)	3.57 \pm 0.07	3.73 \pm 0.04	4.51 \pm 0.30	3.71 \pm 0.02	3.49 \pm 0.02
Left Kidney ($\times 10^{-1}$)	2.73 \pm 0.05	2.81 \pm 0.05	2.82 \pm 0.06	2.93 \pm 0.04	2.63 \pm 0.61
Spleen ($\times 10^{-1}$)	3.52 \pm 0.15	3.63 \pm 0.11	3.58 \pm 0.09	4.39 \pm 0.01	3.20 \pm 0.03
Right Kidney ($\times 10^{-1}$)	2.73 \pm 0.09	2.94 \pm 0.04	3.00 \pm 0.09	3.27 \pm 0.04	2.77 \pm 0.03
Liver ($\times 10^{-1}$)	37.54 \pm 0.92	37.85 \pm 0.59	36.80 \pm 1.63	39.76 \pm 0.46	36.26 \pm 0.36

n=5; Mean \pm S.E.M (Standard Error of Mean)

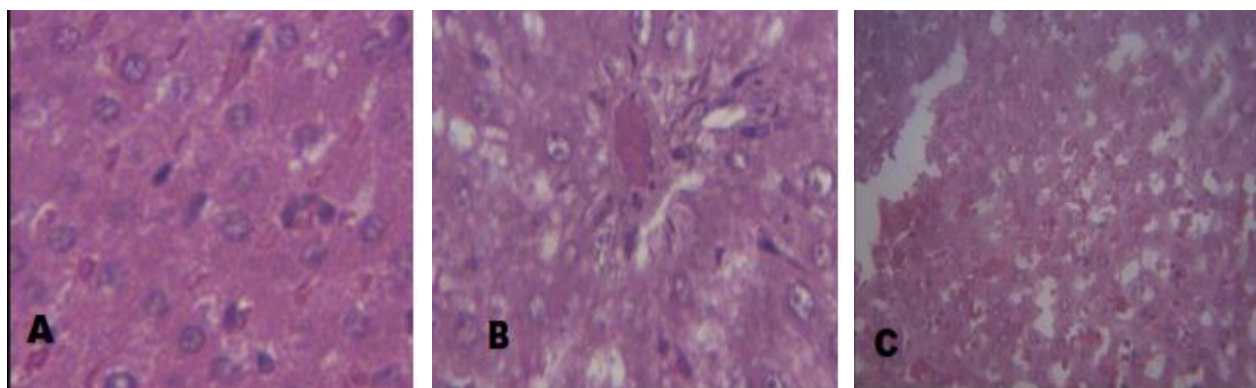


Figure I(A): The liver section of rat in *control group* showing no visible lesion (H&E stain $\times 40$). (B) The liver section of rat administered with the ethanol extract of *G. carpinifolia* (100 mg/kg, group B) leaf showing no visible lesion (H&E stain $\times 100$). (C) The liver section of rat administered with the ethanol extract of *G. carpinifolia* (800 mg/kg, group E) leaf showing central nucleus and sinusoidal congestion (H&E stain $\times 100$)

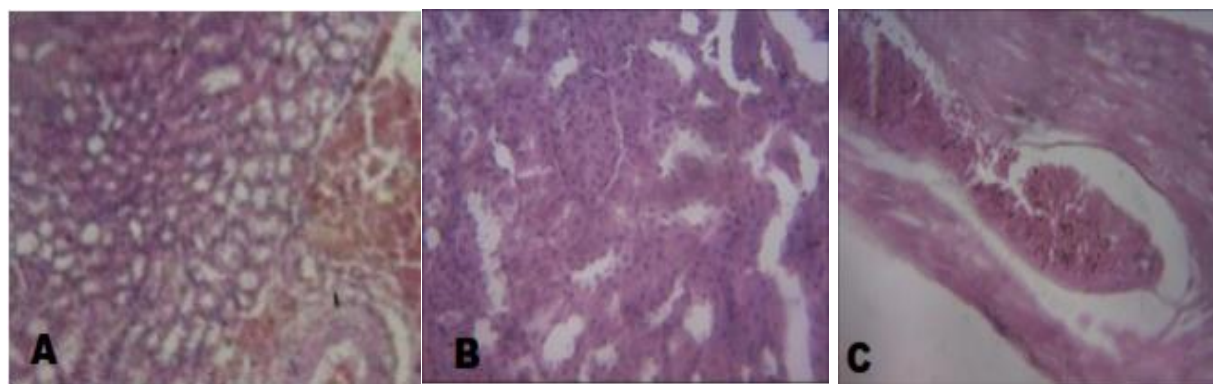


Figure II (A) The kidney section of rat in the control group showing no visible lesion (H&E stain $\times 100$) (B) The kidney section of rat in the group C (administered with 400 mg/kg of *Grewia carpinifolia*) showing no visible lesion (H&E stain $\times 100$) (C) The heart section of rat administered with 400 mg/kg (group D) of *G. carpinifolia* showing marked coronary congestion (H&E stain $\times 100$)

(2013) who reported the absence of anthraquinones, and saponin in chloroform and hexane leaf extracts of *G. damine* and *G. asiatica*. This variance may be associated with species differences as well as the effect of the environment which can affect the physiological and biosynthetic reactions of plants. Furthermore, according to Simonovska *et al.* (2003) the solvent used in the preparation of plant extracts can qualitatively and/or quantitatively affect the biologically active chemical constituents extracted.

The acute study revealed that oral administration of graded doses of ethanol extract of *G. carpinifolia* up to 4000 mg/kg body weight was tolerated by all the animals. According to the acute toxicity scale of Hodge and Sterner (1943) the crude ethanol extract of *G. carpinifolia* could be qualified as being relatively nontoxic to rats when administered orally. Our finding is in agreement with Ukwuani *et al.* (2012) who also reported that *G. crenata* has high safety margin.

The food intake was found to be unaltered during the 28-day treatment period when compared to the control group in this study suggesting the extract did not possibly metabolise to any toxic product nor cause any alterations in carbohydrate, protein or fat metabolism in these experimental animals.

The decrease in mean value of water intake at high doses may be due to some constituents in the leaf such as tannins. Tannins have been stated to reduce water intake in animals; this was attributed to its astringent property and induction of internal malaise in mammals (Jensen *et al.*, 2014).

Body weight changes serve as a sensitive indication of the general health status of animals. The findings of this study showed that Wistar rats were able to maintain growth irrespective of dose of the leaf extract of *G. carpinifolia* administered to them. According to Moore *et al.* (2008), an increase in organ-body weight ratio is an

indication of inflammation while a reduction in the same parameter can be adduced to cellular constriction. The lack of significant difference in the organ-body weight ratio of the experimental groups as compared with the control may signify that the extract did not alter the integrity of these organs at tested doses.

The absence of significant effect of the extract on the haemogram of the test rats in this study could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology of the red blood cells was altered (Davis and Bredt, 1994). Ukwuani *et al.* (2012) in their toxicological evaluation of *G. crenata* leaf extracts also observed no significant difference ($p > 0.05$) in the haematological parameter however contrary to the present study reported a reduction in platelet and increased differential blood count.

The significant increase in the levels of aminotransferases (ALT and AST) and ALP in the group administered with 800 mg/kg of the extract may suggest that the sub-chronic administration of *G. carpinifolia* leaf extract at this dose results in hepatocellular injury thereby causing the leakage of these enzymes into the blood circulation.

The observation of extract of *G. carpinifolia* leaf at all the doses investigated not altering the renal function indices in the present study may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected when compared with the control. This is attested to by Goyal *et al.* (2012), that the *Grewia sp* are used in treating several kidney and urinary tracts disorder.

There was no observed hypertrophy of organs amongst all the groups in this study, however, the congestion of central nucleus and sinusoids observed in the liver section microscopically at 800 mg/kg may indicate the response of hepatocytes to injury (Hinton and Laurén, 1990). The extract at this dose may stimulate inflammation triggered by some chemical mediators like

histamine and prostaglandin secreted from mast cells and some other inflammatory cells which caused vasodilatation and local increase flow of blood in the liver resulting in congestion (Kumar *et al.*, 2003). Hence, caution is advised at this dose following prolonged administration.

Conclusion

In light of these findings, we may conclude that *G. carpinifolia* leaf extract appear to be safe at doses below 800 mg/kg body weight and did not produce any toxic signs or evident symptoms at acute and sub-chronic oral toxicity. The preliminary results suggest promising alternatives for exploring therapeutic and pharmaceutical interest in *G. carpinifolia* leaf extract. Also, the extract is safe by oral route in relation to its folkloric practice in the administration of medicinal herbs.

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