

Acute and sub-acute toxicity profile of methanol extract of *Hura crepitans* leaf on Wistar rats

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Abstract

This study investigated the toxicity profile of methanol extract of *Hura crepitans* leaf on albino rats. The up-and-down method at a dose limit of 2000 mg/kg was used in the oral acute toxicity test. Twenty-four (24) albino Wistar rats were randomly assigned to 4 groups (A –D, n = 6). Group A (control) received distilled water, while groups B-D received 100, 200 and 400 mg/kg of the extract, respectively. The rats were dosed once daily for 21 consecutive days and weighed weekly. Twenty-four hours after the last treatment on day 21, the rats were fasted overnight and blood was collected into EDTA and plain bottles for hematological evaluation and serum preparation respectively. A manual method was used to determine the full blood cell count, while Randox kit was used to estimate the serum markers of liver and kidney functions. The extract was tolerated by the rat; the LD₅₀ was greater than 2000 mg/kg. At 21 days of treatment, the extract (100, 200 and 400 mg/kg) treated groups had 27.53, 25.98 and 25.33% weight gain respectively, while the distilled water treated group had 8.38% weight gain. The extract (200 and 400 mg/kg) reduced (p < 0.05) the packed cell volume, hemoglobin concentration and red cell count, serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities, but increased the total white blood cell in the treated groups when compared with the control group. This study suggests that methanol extract of *Hura crepitans* has hepatoprotective activity, promotes weight gain and could precipitate anemia when taken for a long period.

Keywords: hematology; *Hura crepitans*; hepatoprotective; toxicity; weight gain

Introduction

There is a growing interest in the use of herbal preparations in the management of disease conditions. About 80% of the world populace have used herbal preparation either as a supplement or a therapeutic agent (Ezuruike and Prieto, 2014; Onoja *et al.*, 2018). Herbal preparations are perceived to be devoid of side effects, but this is not true especially when used for a prolonged time (Kunle *et al.*, 2012; Ezeja *et al.*, 2014). Despite the soaring interest in herbal preparation, it is rarely or not prescribed by orthodox medical personnel and this could be due to the possibility of toxicity or incompatibility/interaction with other medications. Considering this, World Health Organization has recommended the safety assessment of herbal preparation (Jordan *et al.*, 2010).

Hura crepitans is a member of the family Euphorbiaceae and commonly known as “Sandbox tree”, “possumwood”, “monkey’s dinner bell” and “monkey’s pistol”. It is a tree that grows up to 40 meters high. It is native to tropical America and presently found in tropical rainforest regions. It is recognized by many dark, pointed (conical) spines and papery thin, heart-shaped leaves (Oloyede *et al.*, 2016; Owojuyigbe *et al.*, 2020). In ethnomedicinal practices, the plant is used as an emetic, purgative, antimicrobial, anti-inflammatory and in the management of leprosy (Oloyede and Olatinwo, 2011). The latex is used as an arrow poison, while the milky sap is poisonous to fish, due to the presence of huratoxine and hexahydrohuratoxin, which causes hemagglutination and inhibits protein synthesis (Vassallo *et al.*, 2020). Phytochemical evaluations have revealed the presence of alkaloids, tannins, saponins, phytate, oxalate, cyanide and trypsin in the seed, leaf and stem-bark of *H. crepitans* (Fowomola and Akindahunsi, 2007; Vassallo *et al.*, 2020). The *in vitro* cytotoxic and anti-viral effects of daphnanes diterpenes (huratoxin, 2,3-dihydrohuratoxin, 6'-oxohuratoxin, prohuratoxin, and stelleralide J) isolated the latex of *Hura crepitans* have been reported (Trinel *et al.*, 2020), but there is a dearth of information on the *in vivo* toxicity profile of *H. crepitans* despite its use for a prolonged period in ethnomedicine. Hence, this study was designed to investigate the *in vivo* hepatic, hematological and nephrological toxicity profiles of *H. crepitans* in Wistar rats.

Materials and Methods

Plant identification/collection

Fresh leaves of *Hura crepitans* were obtained from Michael University of Agriculture Umudike environment in June, 2017 and authenticated by a taxonomist, Prof. Michael C. Dike. A voucher specimen (MOUAU/CVM/VPP/2017/4) was kept in the departmental herbarium for reference.

Extract preparation

The leaves were washed and air-dried to constant weight and then pulverized into a coarse powder using an electric hammer mill (Jiangxi, China). The pulverized leaf was macerated in 80% methanol (JHD, China) for 48 h. The filtrate obtained was concentrated *in vacuo* using a rotary evaporator (Cole-Parmer type N-1110, China). The *Hura crepitans* extract (HCE) was preserved at 4 °C.

Experimental animal

Twenty-four (24) albino Wistar rats of both sexes were used for the study. The animals were housed in wire-mesh cages, fed *ad libitum* with pelleted feed (Vital Feed, Nigeria) and water except when fasting was required. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were handled regarding the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Research Council, 2010).

Acute toxicity test

The acute toxicity test of HCE was conducted using the “Up and Down” method at a limit dose of 2000 mg/kg in rats (OECD, 2008).

Experimental design

Twenty-four (24) rats were randomly assigned to four groups (A-D, n = 6). Group A received distilled water 5 ml/kg, while groups B-D received HCE 100, 200 and 400 mg/kg, respectively. The rats were dosed once daily for 3 weeks via oral gavage. The weight was taken on day zero, 7, 14 and 21. After the last treatment on week 3, the rats fasted for 16 h and the blood sample was collected afterward (into plain and EDTA sample bottles). Thereafter, the rats were sacrificed and the visceral organs (liver, kidney, spleen, and heart) were excised after laparotomy for the determination of organosomatic indices. The percentage weight gain was calculated as follows:

$$\% \text{ weight gain} = \frac{\text{weight B} - \text{weight A}}{\text{Weight A}} \times \frac{100}{1}$$

Where: Weight A = weight on week zero, while weight B = weight on days 7, 14 or 21.

$$\text{Organosomatic index} = \frac{\text{weight of organ}}{\text{body weight of animal}} \times \frac{100}{1}$$

Hematological analysis

The red blood cell (RBC), platelet and white blood cell (WBC) count, packed cell volume (PCV) and hemoglobin (HB) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using automated hematology analyzer (OX-360, Balio diagnostics, France).

Determination of biochemical parameters

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin (total and direct), urea and creatinine levels were determined in the serum using a commercially available Randox reagent kit (Randox Diagnostics, UK).

Statistical analysis

Data obtained were presented as mean \pm SEM and analysed using one-way-analysis of variance (ANOVA) and posthoc comparisons were carried out using Dunnett's t-test on SPSS version 20. Values of $p < 0.05$ were considered significant in the study.

Results*Acute toxicity test*

No signs of toxicity and death were recorded in the treated rats. The LD₅₀ was greater than 2000 mg/kg. The extract is relatively safe for consumption.

Effects of HCE on percentage body weight gain of treated rats

The HCE produced a significant ($p < 0.05$) time-dependent (but not dose-dependent) increase in weight gain in the treated rats when compared with the rats in the distilled water treated group (Table 1). At 21 days of treatment, the HCE 100, 200 and 400 mg/kg treated groups had 27.53, 25.98 and 25.33 % weight gain, while the distilled water treated group had 8.38 % weight gain.

Effects of HCE on organosomatic indices of treated rats

There was no significant ($p > 0.05$) change in the organosomatic indices of the HCE-treated groups when compared with the distilled water-treated group (Table 2).

Effects of HCE on hematological parameters of treated rats

The HB, RBC and PCV levels of the HCE-treated groups were significantly ($p < 0.05$) reduced when compared with the distilled water treated group (Table 3). There was no significant ($p > 0.05$) difference in the WBC and platelet counts as well as MCV, MCH, and MCHC levels of HCE-treated groups when compared with the distilled water treated group.

Table 1. Effects of HCE on percentage body weight gain of treated rats

Duration (day)	Weight gain (%)			
	Distilled water 5 mg/kg	HCE 100 mg/kg	HCE 200 mg/kg	HCE 400 mg/kg
7	-0.08 ± 1.29	-0.62 ± 1.05	1.56 ± 0.65	1.08 ± 0.34
14	7.78 ± 1.98	17.60 ± 2.45*	16.38 ± 2.02*	14.49 ± 2.21*
21	8.38 ± 3.09	27.53 ± 3.19*	25.98 ± 3.28*	25.33 ± 3.94*

* $p < 0.05$ when compared with the distilled water treated group, HCE = *Hura crepitans* extract

Table 2. Effects of HCE on organosomatic indices of treated rats

Organs	Distilled water 5 mg/kg	HCE 100 mg/kg	HCE 200 mg/kg	HCE 400 mg/kg
Kidney	0.72 ± 0.04	0.68 ± 0.04	0.71 ± 0.01	0.61 ± 0.03
Liver	3.31 ± 0.10	3.53 ± 0.13	3.77 ± 0.17	3.46 ± 0.12
Heart	0.37 ± 0.03	0.43 ± 0.04	0.43 ± 0.02	0.43 ± 0.05
Spleen	0.43 ± 0.02	0.58 ± 0.14	0.58 ± 0.14	0.52 ± 0.05

* $p < 0.05$ when compared with the distilled water treated group, HCE = *Hura crepitans* extract

Table 3. Effects of HCE on hematological parameters of treated rats

Parameters	Distilled water 5 mg/kg	HCE 100 mg/kg	HCE 200 mg/kg	HCE 400 mg/kg
WBC ($\times 10^3/\mu\text{l}$)	16.10 ± 2.45	14.17 ± 1.91	19.13 ± 3.80	20.50 ± 1.07
HB (g/dL)	15.23 ± 0.61	13.97 ± 0.54	12.47 ± 0.29*	12.27 ± 0.15*
RBC ($\times 10^6/\mu\text{l}$)	8.29 ± 0.33	7.51 ± 0.22	6.93 ± 0.24*	6.54 ± 0.28*
PCV (%)	56.13 ± 2.80	52.30 ± 1.51	47.47 ± 1.18*	46.37 ± 0.22*
MCV (fL)	67.73 ± 0.98	69.70 ± 1.17	67.40 ± 0.26	68.07 ± 0.18
MCH (Pg)	18.37 ± 0.13	18.53 ± 0.26	17.70 ± 0.06	17.53 ± 0.29
MCHC (g/L)	271.33 ± 5.55	266.67 ± 7.80	264.00 ± 1.53	262.00 ± 2.00
Platelet ($\times 10^3/\mu\text{l}$)	647.67 ± 16.33	621.33 ± 110.48	670.00 ± 45.09	624.67 ± 10.17

* $p < 0.05$ when compared with the distilled water treated group, HCE = *Hura crepitans* extract, RBC = red blood cell, WBC = white blood cell, PCV = packed cell volume, HB = hemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration

Effects of HCE on serum biochemical parameters of treated rats

The effects of HCE on the serum biochemical parameters of treated Wistar rats are presented in Table 4. The serum AST activities of HCE (200 and 400 mg/kg) treated groups were significantly ($p < 0.05$) diminished when compared with the distilled water treated group. The serum ALP and ALT activities of HCE 100 and 200 mg/kg treated groups respectively, were significantly reduced when compared with the distilled water treated group. The serum total and direct bilirubin levels of HCE-treated groups were significantly ($p < 0.05$) reduced when compared with the distilled water treated group.

Table 4. Effects of HCE on serum biochemical parameters of treated rats

Parameters	Distilled water 5 mg/kg	HCE 100 mg/kg	HCE 200 mg/kg	HCE 400 mg/kg
AST (IU/L)	81.25 ± 8.36	81.43 ± 7.58	63.63 ± 4.34*	56.03 ± 5.83*
ALT (IU/L)	15.68 ± 2.56	13.25 ± 1.33	14.45 ± 1.60	8.40 ± 0.24*
ALP (IU/L)	36.96 ± 4.49	23.04 ± 2.29*	29.46 ± 1.06	28.25 ± 2.58
TP (g/dL)	7.27 ± 0.25	7.04 ± 0.24	6.69 ± 0.11	6.65 ± 0.63
ALB (g/dL)	3.53 ± 0.42	3.82 ± 0.10	3.90 ± 0.11	3.68 ± 0.06
GLB (g/dL)	3.73 ± 0.24	3.22 ± 0.29	2.80 ± 0.16	2.97 ± 0.66
TBIL (mg/dL)	0.20 ± 0.02	0.09 ± 0.01*	0.08 ± 0.00*	0.10 ± 0.00*
DBIL (mg/dL)	0.17 ± 0.00	0.06 ± 0.00*	0.05 ± 0.01*	0.05 ± 0.00*
Creatinine (mg/dL)	0.96 ± 0.04	0.78 ± 0.09	0.82 ± 0.03	0.86 ± 0.03
Urea (mg/dL)	32.15 ± 1.36	37.65 ± 1.12	29.70 ± 4.95	35.59 ± 3.36

*p < 0.05 when compared with the distilled water treated group, HCE = *Hura crepitans* extract, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, TP = total protein, ALB = albumin, TBIL = total bilirubin, DBIL = direct bilirubin

Discussion

This study assessed the acute and sub-acute toxicity profile of the methanol extract of *Hura crepitans* leaf in Wistar rats. The LD₅₀ of the extract was greater than 2000 mg/kg. The sub-acute treatment of HCE produced significant (p < 0.05) weight gain, caused anemia and hepatoprotective effects in the treated rats. The aforementioned effects could be linked to the phytoconstituents of *H. crepitans* leaf. The presence of alkaloids, tannins, saponins and flavonoids in the leaf of *H. crepitans* have been reported (Fowomola and Akindahunsi, 2007; Vassallo *et al.*, 2020).

A single dose of HCE (2000 mg/kg) was well tolerated as no signs of morbidity or mortality were recorded. The LD₅₀ was greater than 2000 mg/kg, thus the extract can be adjudged to be relatively safe (OECD, 2008).

The HCE-treated groups had more (p < 0.05) accelerated weight gain when compared with the distilled water-treated group. This could be due to micronutrients and phytochemicals present in the HCE, which may have improved feed conversion efficiency and enhanced the growth (Fowomola and Akindahunsi, 2007; Vassallo *et al.*, 2020). The organosomatic index showed no significant change in all the organs (livers, kidneys, hearts, and spleens) examined. This indicates that there was no noticeable deleterious effect on these organs as elevated organosomatic index indicates pronounced organ degeneration (Singh *et al.*, 2007).

The HCE (200 and 400 mg/kg) caused normocytic and normochromic anemia in the treated rats that could be linked to the phytoconstituents. *H. crepitans* is rich in saponins which are known to induce eryptosis and hemolysis of red blood cells (Bissinger *et al.*, 2014; Fowomola and Akindahunsi, 2007; Waheed *et al.*, 2012). Saponins stimulate cell membrane scrambling via increased cytosolic calcium ion (Ca²⁺) activity and ceramide formation at the surface of RBC, which predispose the cell to eryptosis (Bissinger *et al.*, 2014; Lang *et al.*, 2012). The stimulation of eryptosis is followed by the removal of the damaged RBC before hemolysis. Enhanced eryptosis is associated with anemia (Lupescu *et al.*, 2015). Saponins-induced hemolysis occurs as a result of cell membrane leakage due to net uptake of sodium ion (Na⁺) and chloride ion (Cl⁻) and associated cell swelling, which causes the rupture of RBC (Bissinger *et al.*, 2014; Lang *et al.*, 2012). Enhanced hemolysis also leads to anemia (Bissinger *et al.*, 2019).

The biochemical findings indicate that the kidney was not negatively impacted despite the possible saponins-induced hemolysis. This is because eryptosis precedes hemolysis and prevented the massive release of hemoglobin that would have occluded the renal tubule and predispose to nephropathy (Lang *et al.*, 2012).

Elevated serum urea and creatinine levels indicate nephropathy. But in this study, the serum urea and creatinine level did not vary significantly ($p > 0.05$) when compared with the control group.

The biochemical findings also suggest that HCE did not produce deleterious effects on the liver, rather it elicited hepatoprotective properties. It reduced the ALT, AST and ALP activities as well as diminished bilirubin levels in the treated rats when compared to the control group. This is in agreement with the report of previous studies on the hepatoprotective potential of *H. crepitans* leaf (Owojuyigbe *et al.*, 2020a). The polar extracts of *H. crepitans* leaf have been shown to possess polyphenols with validated hepatoprotective properties (Vassallo *et al.*, 2020). The hepatoprotective potentials of polyphenols are linked to their antioxidant property. Antioxidants mop up free radicals, inhibit lipid peroxidation and enhance membrane integrity. The antioxidant potential of *H. crepitans* has been documented (Owojuyigbe *et al.*, 2020b).

Conclusions

In conclusion, this study suggests that methanol extract of *Hura crepitans* is relatively safe, has hepatoprotective activity, promotes weight gain and could precipitate anaemia when taken for a long period.

Authors' Contributions

GCE, NEU and SOO were involved in the conceptualization, design, execution, draft and review of the manuscript; CAO and REE participated in the design, sourcing of material, statistical analysis and review of manuscript while YNO, AOA and MIE were involved in the supervision, review and editing of the manuscript. All the authors read and approved the final manuscript.

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Ethical approval

The experimental protocol was approved by the institutional Animal Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike.

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Conflict of Interests

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

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