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Phytochemical, Antioxidant and Safety Evaluation of *Hura crepitans* (L.) Stem Bark Hydroethanolic Extract in Animals

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OSO and CL conceived and designed the initial study. Authors CKF, GK, and BOE refined the study protocols. Authors OSO and CL conducted all experiments. Author OSO managed the literature search and drafted the manuscript. Authors OSO and CL managed all statistical analyses. All authors read and approved the Final manuscript.

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ABSTRACT

Background and Objectives: Medicinal plants have become popular in the search ofa cure for various hepatopathies with a considerable number of herbal products being used for the management of liver ailments. However, most, including *Hura crepitans* (HC), have not been

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toxicologically evaluated. The present study was aimed at determining the phytoconstituents of HC, their antioxidant potentials and safety in animals.
Place and Duration: The study was performed at the Department of Biochemistry and Biotechnology, KNUST, Kumasi and subacute toxicity studies lasted for 14 days.
Materials and Methods: Hydroethanolic extracts and fractions of the leaves and stem back of HC were assessed for their phytochemical constituent,DPPH and FRAP scavenging activities and total phenolic and total flavonoid content. The acute and sub acute oral toxicity of the hydroethanolic stem back of HCin animals was evaluated using standard methods to establish the LD_{50}, effect on weight, haematological and biochemical parameters.Phytochemical characterization was achieved using standard methods.
Results: The study showed that HC contained tannins, saponins, flavonoids, coumarins, glycosides, and triterpenoids. The hydroethanolic extract of the stem bark (EES) showed the greatest antioxidant potential. The LD_{50} estimate of the hydroethanolic extract of stem back was greater than 5000 mg/kg body weight/oral route, while the doses used in this study up to 1000 mg/kg body weight/oral route was safe. The extract generally did not cause any significant decrease in the weight of animals nor were there significant differences in relative organ weights in treated animals compared to the normal group. Haematology, liver function, kidney function, and serum electrolyte parameters were within the normal ranges in treated animals when compared to the control group.
Conclusion: The results showed that oral administration of hydroethanolic extract of *Hura crepitans*in animals up to 1000 mg/kg body weight is considered safe as it causes no significant derangement in clinical and haemato-biochemical parameters measured.

Keywords: Hura crepitans; toxicity; phytochemicals; antioxidants; LD_{50}.

1. INTRODUCTION

Medicinal plants have been the driving force of traditional herbal medicine amongst rural dwellers in most parts of Africa [1-4] where the cost of conventional treatment with the use of therapeutic agents is not within the reach of the populace [5]. In this poor resource setting, the aqueous and ethanolic decoctions of some medicinal plant parts is often used for cure and management of various ailments including hepatopathies. Some of the medicinal plants reported by various workers include *Byrsocarpus coccineus* (Connaraceae) leaf decoction [6], aqueous extract of *Artemisia absinthium* L. [7], aqueous extracts of root tubers of *Daucoscarota* L. [8], methanolic extract of *Helminthostachys zeylanica* L. (Hook rhizomes) [9], aqueous root extracts of *Rhoicissus tridentata* L. [10], ethanolic extracts of *Ziziphus mauritiana* Lam. Leaves [11], *Adansonia digitata* (baobab) fruit pulp [12], extracts of the seed of *Apium graveolens* L. [13], leaf extract of *Cassia occidentalis* [14], *Alchornea cordifolia* (Schum and Thonn) leaf extract [15] and several others.

In Ghana and other parts of West Africa, one of the most useful medicinal *plantsis Hura crepitans* L. (family *Euphorbiaceae*). Also called the sandbox tree, the plant is native of tropical America, and widely dispersed in tropical rain forests. It is fondly planted in the cities and villages for its shade. In Nigeria, it is known as “aroyin” by the Ijesa people. It is known as “abrokoyin” by the Akan-Fante people of Ghana, and popularly called “okafɔodidi” by most Ghanaian folks. The tree has sharp spiny stem bark which makes it also to be called “monkey no-climb”. A detailed review of the plant has previously been published [16].It has been used as purgative, emetic, antimicrobial, anti-inflammatory and hepatoprotective agents. It has also been used in the treatment of mucous diarrhoea in both dogs and humans [17]. Studies on this plant include the bactericidal properties and phytochemical screening [18,19] with a dearth of information on the toxicity of this tree in man and animals. The emergence of novel antibiotics is a very lengthy and pricy process and the success rate for clinical authorization is very low [20].

With the presence of diverse ingredients (phytochemicals), medicinal plants do not customarily aim at a particular ailment, but as a source of a holistic array of chemicals focusing on the entire health of an individual. For this reason, fewer side effects are reported as compared to conventional drugs and hence they are thought to be safe and as effective as conventional drugs. However, some allelochemicals, pathogens, agrochemical

residues, phytotoxins, fungal toxins, and heavy metals present, pose several health threats when consumed [21,22].This, therefore, raises a concern of safety, hence the need for thorough chemical evaluation of safety and efficacy of natural products. Usually, toxicants are classified based on their chemical nature, mode of action, or class (exposure class and use class). The exposure classifies toxicants as occurring in food, air, water, or soil and by use drugs are classified as drugs of abuse, therapeutic drugs, agriculture chemicals, food additives, pesticides, plant toxins (phytotoxins), and cosmetics [23].

Acute toxicity testing permits the 50% lethal dose (LD_{50}) of the investigational product to be determined. The LD_{50} was used as an indicator of acute toxicity previously. The determination of the LD_{50} involves large numbers of animals, and the mortality ratio is high. Because of these limitations, modified methods were developed: the fixed dose procedure (FDP), the acute toxic category (ATC) method, and the up-and-down (UDP) method [24].Currently the following chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values are recommended by the Organisation of Economic Co-operation and Development (OECD, Paris, France): very toxic, ≤5 mg/kg; toxic, >5≤50 mg/kg; harmful, >50≤500 mg/kg; and no label, >500≤2000 mg/kg [25].Most used medicinal plants are however considered safe based on the long duration of usage. However, the subacute and chronic toxicity profiles of these medicinal plants are useful in certifying medicinal products and making them more attractive for general use.

The present study was therefore designed to determine the phytoconstituents(hydroethanolic and aqueous leaves and stem back extracts) of Ghanaian cultivar of *H. crepitans*, the antioxidant potentials, acute and subacute oral toxicity of the hydroethanolic stem back extract in male and female animals in terms of clinical presentation, LD_{50} determination, effect on weight, haematological and biochemical parameters to support its continued use in traditional medicines.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Stem bark sheaths and leaves of *Hura crepitans* were harvested from the forest of Ahodwo, Kumasi in the Ashanti region of Ghana in the months of October 2017. The stem bark which had spines and the leaves were identified by a taxonomist (Dr. D. H. Sam, Department of Pharmacognosy, KNUST, Kumasi, Ghana) as that of *H. crepitans*. The voucher specimens were deposited at the herbarium of the department (KNUST/HM1/2017/SB001 and KNUST/HM1/2017/L003).The stem bark sheaths and the leaves were cut into small pieces, thoroughly washed under tap water and air dried for three weeks under room temperature. They were then ground separately into powdered forms with a laboratory grinding mill. Collected powdered samples were stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

2.2 Extraction of the Hydroethanolic and Aqueous Extracts of Stem Back and Leaves

A 1kg amount of plant material (stem bark andleaf) was extracted five times by percolation using 5 L of 50% of hydroethanolic solution (50:50 v/v ethanol: water). Resulting extracts were pulled together and concentrated using a rotary evaporator (Buchi R205, Switzerland) at 60°C under pressure. The extract was freeze-dried (Labconco, England) to obtain the powdered form of crude extracts. The aqueous extracts were prepared by decoction by boiling 100 g in 1 L distilled water. The decoctions were filtered, allowed to cool and freeze-dried. The crude extract was transferred into zip locks and stored in a fridge till used. The crude extracts were designated as Aqueous extract of stem bark (AES),aqueous extract of leaves (AEL), hydroethanolic extract stem bark (EES) and hydroethanolic extract of leaves (EEL). All experiments were conducted in the laboratories of the Department of Biochemistry and Biotechnology and Central Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi Ghana.

2.3 Sequential Fractionation of Crude Extract (EES)

Hydroethanolic extract of stem back (EES) was subjected to sequential fractionation with organic solvents in order of increasing polarity as follows:petroleum ether, ethyl acetate, methanol, ethanol.Twenty (20) grams of extract was weighed into a separating funnel and 200 mL of the solvent was added and shaken vigorously for proper extraction for 48 hours. The residue was taken out, dried to evaporate the solvent, and then extracted with the subsequent more polar solvent. The final residue after the various polar

solvent extractions was designated as the hydroethanolic fraction (HEE). The crude and four fractions (petroleum ether, ethyl acetate, methanolic and hydroethanolic) so obtained were freeze-dried (Labconco, England).

2.4 Preliminary Phytochemical Screening

Phytochemical tests were carried out for the plant crude extracts. The crude extracts were screened for the presence or absence of secondary metabolites which include tannins, alkaloids, phenolic compounds, saponins, terpenoids using standard procedures with minor modifications where necessary [26].

2.5 *In vitro* Antioxidant Activity

The plant extracts and fractions were examined to determine their antioxidant activities using the following assays; DPPH, FRAP, total phenolic content (TPC) and flavonoid content (FC).

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay: The effect of the *H. crepitans* extracts and fractions on DPPH radical were estimated by standard method [27]. The extract concentration ranged from 50-500 µg in 4 mL of distilled water. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm against standard Gallic acid (50 µg). The radical scavenging activity (RSA) was calculated as the percentage of DPPHdiscoloration.

$$\% RSA = \frac{Abs_{Blank} - Abs_{sample}}{Abs_{Blank}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay: The ferric reducing activities of aqueous and hydroethanolicextracts of stem back were estimated spectrophotometrically following the procedure of Benzie and Strain [28] which is based on the reduction of Fe^{3+} TPTZ complex (colourless complex) to Fe^{2+} tripyridyltriazine (blue coloured complex) formed by the action of electron donating antioxidants at low pH. The absorbance was measured at 593 nm was recorded against a reagent blank and Trolox.

Determination of Total Phenolic Content (TPC): TPC of aqueous and hydroethanolicextracts of stem back was determined using a standard method based on Folin–Ciocalteu test [29]. 0.1 g of the extract was dissolved in 5 mL of 0.3% HCl in methanol/water (60:40, v/v). The resulting solution (100 µl) was added to 2 mL of 2% Na_2CO_3. After 2 minutes, 50% Folin-Ciocalteau reagent (100 µl) was added to the mixture, which was then left for 30 minutes. Absorbance was measured at 750 nm using Gallic acid as standard.

Estimation of Total Flavonoid Content (TFC): TFC was estimated on the aqueous and hydroethanolicextracts of stem back by the aluminium chloride colorimetric method, using Gallic acid (10-100 mg/L) as a standard. The principle of this technique is the formation of complexes between $AlCl_3$ and the keto or hydroxyl groups of flavones, flavonols, and flavonoids [30]. Briefly, 500 µL of the 1:20 diluted and filtered extract (at an original concentration of 100 mg/mL in methanol) was mixed with 1.5 mL of 95% methanol, 100 µl of 10% aluminium chloride ($AlCl_3$), 100 µl of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 40 minutes and the absorbance was measured at 415 nm. The total flavonoid content was expressed as Gallic acid equivalents mg /g dry weight.

2.6 FTIR Analysis

The infrared spectra of the hydroethanolic stem bark extract was recorded to confirm the functional groups present using the Shimadzu (8400S) Fourier Transform- Infrared (FTIR) with KBr pellets. The functional groups present were determined by comparing spectra with standard IR tables.

2.7 Toxicity Assessment of the Hydroethanolic Extract of Stem (EES)

The toxicity assessment was performed on the hydroethanolic stem back extract since it had the highest antioxidant activity and to support its use in other biological assessments. The toxicity assessment was based on methods previously described by Arthur et al. [31]. Sprague Dawley rats (160-230 g) and ICR mice (22-28 g) obtained from the animal facility of the University of Ghana Medical School, Korle Bu, Ghana, were used for the study. They were, kept at the animal holding facility of the Department of Biochemistry and Biotechnology, KNUST, Kumasi. The animals were kept in aluminium cages bedded with wood shavings and at a uniform temperature of 22–25 °C, 12 h light dark cycle. Animals had free access to feed (Mash, AGRICARE, Kumasi, Ghana) and freshly prepared distilled water *ad libitum* prior to commencement of study to acclimatize to

laboratory conditions. The animals were given colour codes on their tails using permanent markers for easy identification. All animal studies were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and Guide for Care and Use of Laboratory Animals(Washington, U.S.) [32]. All animals were humanely treated and supervised by a Veterinarian on the research team.

2.8 Acute Toxicity in Mice

Healthy 10 mice (male and female) weighing 22-28 g were divided and housed by sex in groups of 5 per cage for 7 days before the start of the experiment. All animals had free access to water and food except for a 12-hour fasting period before oral administration of *H. crepitans* hydroethanolic extract (EES). EESwas dissolved in distilled water and administered by gavage at a fixed dose of 5000 mg/kg using OECD fixed dose method [24]. The general behaviour of mice was observed continuously for one hour (1 h) after the treatment and then intermittently for 4h, and thereafter over a period of 24h and then continued for 7 days for any signs of toxicity and deaths, and the latency of death [26].

2.9 Subacute Study in Rats

Treatment groups and collection of samples: *EES* at 100, 250, 500 and 1000 mg/kg *p.o.* were administered to 50 Sprague-Dawley rats, male and female (180-220 g) in 5 groups, each of 5 rats per group, daily for 14 consecutive days. Group A served as the control in either sex. The control received 1ml of distilled water daily. Group B, C, D, and E were treated with the extract (100,250,500 and 1000 mg/kg *p.o.*) respectively daily. The extracts were prepared to deliversuch that not more than 1 ml were given orally.

Animals were weighed on the first day (D0) and, thereafter, at the end of every four days using a mass balance. The percentage change in the bodyweight was calculated using the formula;

$$\%\ ChangeinBodyWeight = \frac{Weight_n - Weight_o}{Weight_o} \times 100$$

Where, $Weight_n$ is the weight on D4, D8, D12, and D14 and $Weight_o$ is the weight on the first day (D0).

Animals were monitored closely for signs of toxicity. Appearance and behaviour pattern were monitored daily and any abnormalities recorded. The rats were anaesthetized and sacrificed on the fifteenth day following an overnight fast. Blood was collected by cardiac puncture.1.5 ml of blood was collected in a vial containing 2.5 µg of EDTA for haematological assay and 2.5 ml of the blood into plain vacutainer tubes. The blood was centrifuged at 1500 g for 10 min and serum was collected and stored at – 70°C until needed for biochemical assays.

Effect of extract on organ weights in Rats: Liver, kidney, heart, stomach, spleen, lung, testes (male), and uterus (female) were excised and washed in buffered saline solution, blotted dry on tissue paper, observed grossly, and weighed to obtain the Absolute Organ Weight (AOW). The Relative OrganWeight (ROW) of each organ was calculated using the following formula.

$$ROW = \frac{AOW}{BodyWeightatSacrifice} \times 100$$

Effect of treatment on Serum Biochemistry: Levels of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) as well as determination of total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), blood urea nitrogen (BUN) and creatinine were performed using Selectra E (Vital Scientific, Japan) automated analyserand reagents from ELITECH (France).

Effect of treatment on haematological parameters of Rats: Haematological parameters including red blood cells (RBC), white blood cells (WBC), haematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and Mean corpuscular haemoglobin concentration (MCHC) were determined by an automatic analyzer (Sysmex XT-2000ICELL-DYN 1700, Abbot Diagnostics Division, Abbot Laboratories, Abbot Park, Illinois, USA).

2.10 Statistical Analyses

All numerical data were presented as mean ± SEM and examined with a test of one-way analysis of variance using GraphPad Prism for Windows version 7.04 (GraphPad Software, San Diego, CA, USA).Significant differences between pairs of groups were calculated with the Tukey's Multiple Comparison Test at the significance of $p < 0.05$.

3. RESULTS

3.1 Phytoconstituents of HC

Table 1 shows the phytoconstituents of the crude hydroethanolic extracts of HC. The presence (+) or absence (-) of phytochemical constituents of HC showed that alkaloids and sterols were absent. Tannins, saponins, flavonoids, coumarins, glycosides, and triterpenoids were constituents of HC.

3.2 *In vitro* Antioxidant Activity of Crude Extracts and Fractions

Table 2 shows the DPPH scavenging activities of crude extracts (EES and EEL) and fractions of hydroethanolic stem back extract. The crude hydroethanolic extract of stem showed the highest scavenging activity with the petroleum ether fraction of the leaf being the least.

Table 3 shows the Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and FRAP activity of the crude stem back extracts of HC.Hydroethanolic extract of stem showed the highest phenolic and flavonoid content while the aqueous extract had a better FRAP activity.

3.3 FT-IR Spectra of the EES

FT-IR spectra (% transmittance over wavenumber) of *H. crepitans*EES are as shown in Fig. 1 and Table 4. The extract was rich in phenols, carboxylic acid groups, and other compounds.

3.4 Acute Toxicity

The acute toxicity study suggests that the lethal dose is above 5000 mg/kg body weight, administered. Observation of the animals over 24 hours showed that the dose did not lead to mortality nor did it change the behavioural pattern of the mice when compared to their normal group.

3.5 Subacute Toxicity

The subacute toxicity study showed that the lethal dose is above the highest dose of 1000 mg/kg body weight. Observation of the animals over 24 hours showed that the doses ranging from 100 mg to 1000 mg/kg body weight did not lead to mortality nor did it change the behavioural pattern of the rats when compared to the normal group in both male and female rats.

Table 1. Phytoconstituents of crude extract of stem bark and leaf of HC

Test	Crude stem	Crude leaf
Tannins	++	+
Saponin glycoside	+	+
Flavonoids	+	++
Coumarins	+	+
Alkaloids	-	-
Glycosides	++	+
Triterpenoids	+	++
Plant sterols	-	-

Table 2. IC_{50}value (mg/ml) of DPPH radical (DPPH*) Scavenging abilities of extract of HC

Sample	Stem	Leaf
Crude Aqueous	0.22 ± 0.03^{b}	0.35 ± 0.03^{b}
Crude Hydroethanolic	0.17 ± 0.03^{a}	0.19 ± 0.05^{a}
Methanolic fraction	0.46 ± 0.09^{e}	0.43 ± 0.07^{c}
Ethyl Acetate fraction	0.35 ± 0.06^{d}	0.60 ± 0.04^{d}
Petroleum Ether fraction	0.45 ± 0.07^{e}	0.91 ± 0.09^{f}
Hydroethanolic fraction	0.29 ± 0.03^{c}	0.77 ± 0.02^{e}

Values represent the means of triplicate experiments. Values with the same superscript along the column are not significantly different ($p<0.05$)

Table 3. Total Phenol Content (TPC), Total Flavonoid Content (TFC) and Ferric Reducing Antioxidant Property (FRAP) of crude extracts

Extracts	TPC	TFC	FRAP
AES	24.92 ±1.09[a]	8.92±2.01[a]	31.46±4.10[a]
EES	31.19±3.53[b]	15.39±3.22[b]	44.15±2.34[b]

Values (mgGAE/g) represent the means of triplicate experiments. Values with the same superscript along the column are not significantly different p<0.05

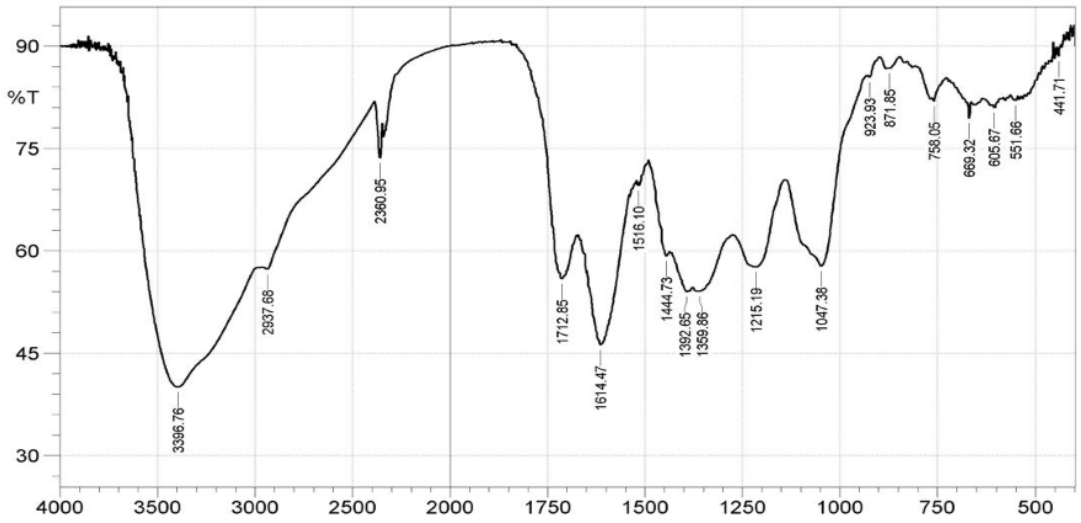

Fig. 1. FT-IR spectra of hydroethanolic extract of *H crepitans* stem back

Table 4. Functional groups present in hydroethanolic extract of *H. crepitans* stem back

	Wave Number (cm^{-1})	Y (%T)	Bond	Assignments
1	551.66	82.04	C-Br	Alkyl halide
2	605.67	80.96	C-Br	Alkyl halide
3	669.32	79.46	-C≡C-H: C-H	Alkynes
4	758.05	81.91	C-Cl stretch	Alkyl halides
5	871.85	86.72	N–H wag	1°, 2° amines
6	923.93	85.42	O–H bond	carboxylic acids
7	1047.38	57.77	C–N stretch	aliphatic amines
8	1215.19	57.62	C–N stretch	aliphatic amines
9	1359.86	54.05	C–H rock	nitro compounds
10	1392.65	53.98	Unknown	Unknown
11	1444.73	59.23	C–C stretch (in–ring)	Aromatics
12	1516.1	69.57	Unknown	Unknown
13	1614.47	46.2	N–H bend	1° amines
14	1712.85	55.91	Unknown	Unknown
15	2360.95	73.59	Unknown	Unknown
16	2937.68	57.33	C–H stretch	Alkanes
17	3396.76	40.03	O-H stretch	Alcohols, phenols

Effect of treatment on body weight: The effect of treatment on percent change in body weight of male and female rats is shown in Fig. 2. There was an increase in body weight in normal and treatment groups in both male and female except for the period between D8 and D12 when all the treatment groups except the 100mg/kg decreased in weight. However, in the females, even the normal rats decreased in weight during the D8 to D12 period. The normal group had the

greatest increases in weight followed by the 100 mg group in males and 1000 mg/kg group in females.

Effect of treatment on Relative organ weight: Table 5 shows the effect of treatment on relative organ weight in rats. There were no significant differences in the relative organ weights of the rats except in the testes of rats at 500 mg/kg b.wt.

Effect on haematological parameters: There were no significant differences between the control and treated rats with most haematological parameters except for the white blood cell count (WBC) which showed decreases in all treated male rats, whereas there were increases in WBC for female rats at higher doses of administration (Table 6).

Effect of treatment on biochemical parameters: Displayed in Table 7 are the effects of treatment on some biochemical markers to detect the state of some organs. There was a significant increase in the GGT levels in both male and female rats administered 500 mg/kg and above. Urea levels were significantly reduced in female animals in all treated groups. Most biochemical data were unaffected by extract treatment at all doses.

4. DISCUSSION

Preliminary phytochemical screening showed that *Hura crepitans* contained tannins, saponins, flavonoids, coumarins, glycosides, and triterpenoids. The presence of these phytochemicals asserts to the many applications of *H. crepitans* in traditional medicine. Tannins contain phenolics and carboxylic acids [29]. Tannin-rich plants are used in the healing of several diseases while saponins are known to have hypolipidemic and anti-cancer activities. Flavonoids are important polyphenols and severalfindings support their efficacy as free radical scavengers [33]. Coumarins are known to be useful in defence against pathogens, regulates oxidative stress and hormonal action [34]. Glycosides are bitter principles which are used as astringents, as anti-protozoan and also to reduce thyroxine and metabolism. Triterpenoids are known to have anti-inflammatory, insecticidal, sedative, and cytotoxic activities [35]. These phytoconstituents are expected to produce a synergy of therapeutic effects [36]. *Hura crepitans*phytoconstituents were supported by the FTIR spectra that indicate the various functional groups.

The percentage DPPH scavenging activities is a basis for evaluating the free radical scavenging potentials of the different extracts. This study showed EES as having the greatest free radical scavenging potential (as shown by the low IC_{50} value) and hence the greatest antioxidant capacity amongst the crude extract and fractions. The TPC, TFC and FRAP values (Table 3) further confirm these observations.Plant-derived polyphenols are potent antioxidants [37,38], having the potentials of inhibiting carcinogenesis [39].

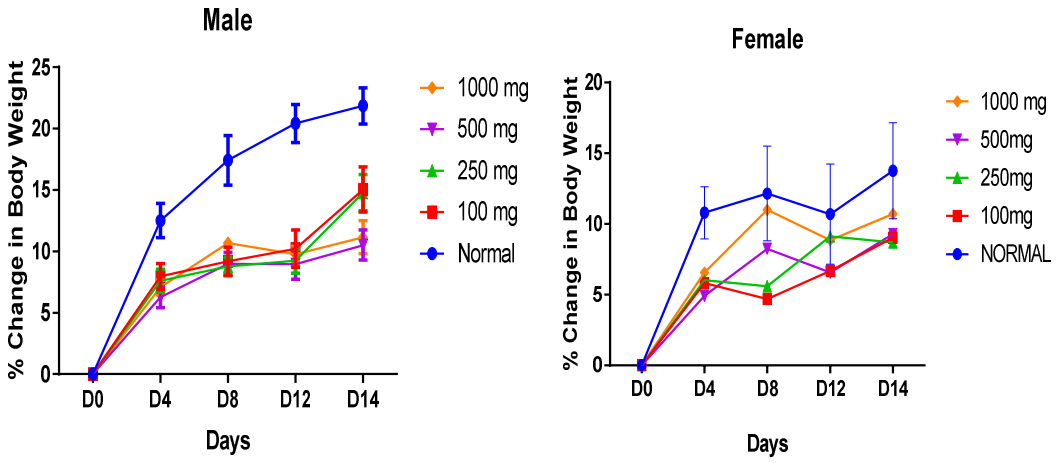

Fig. 2. Effect of treatment on percent change in body weight of male and femaleanimals

Table 5. The effect of treatment on relative organ weights in male and female animals

	Normal		100 mg		250 mg		500 mg		1000 mg	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver(%)	2.53±0.64	3.46±0.17	2.93±0.09	2.83±0.13	2.31±0.58	2.93±0.09	2.84±0.05	3.32±0.11	2.67±0.04	3.25±0.10
Lungs(%)	0.71±0.03	0.63±0.03	0.62±0.10	0.65±0.03	0.63±0.05	0.70±0.12	0.73±0.04	0.68±0.05	0.72±0.06	0.60±0.05
Kidney(%)	0.83±0.03	0.74±0.04	0.73±0.02	0.76±0.03	0.80±0.04	0.79±0.06	0.74±0.01	0.73±0.03	0.71±0.02	0.76±0.01
Stomach(%)	0.50±0.13	0.69±0.01	0.70±0.04	0.68±0.02	0.61±0.03	0.68±0.08	0.60±0.02	0.71±0.03	0.61±0.04	0.67±0.03
Heart(%)	0.33±0.01	0.34±0.01	0.31±0.01	0.31±0.02	0.34±0.01	0.34±0.01	0.32±0.01	0.34±0.02	0.31±0.01	0.36±0.02
Spleen(%)	0.18±0.01	0.17±0.02	0.22±0.01	0.21±0.02	0.22±0.01	0.21±0.01	0.23±0.02	0.26±0.02	0.21±0.01	0.22±0.01
Testes/Uterus(%)	1.19±0.11	0.31±0.03	1.21±0.03	0.31±0.04	1.21±0.06	0.24±0.02	1.02±0.07*	0.28±0.04	1.12±0.02	0.26±0.03

*Values are expressed as mean ± SEM (n=5) * values significant at P<0.05*

Table 6. Effect of treatment on haematological parameters of male rats

	Normal	100 mg	250 mg	500 mg	1000 mg
Male					
WBCx10^3/µL	11.96±0.91	9.02±0.64[a]	7.36±0.28[a]	8.12±1.21[a]	7.90±0.80[a]
RBCx10^6/µL	7.98±0.08	8.06±0.08	8.00±0.13	7.90±0.19	7.90±0.15
HGB gL	13.38±0.04	13.60±0.11	13.64±0.15	13.70±0.31	13.98±0.23
HCT%	47.82±1.34	49.36±0.42	48.30±0.54	48.10±1.41	48.06±0.97
MCV fL	59.86±1.15	61.22±0.57	60.42±0.79	60.86±0.75	60.90±1.12
MCH pg	16.74±0.36	16.88±0.14	17.06±0.18	17.34±0.25	17.74±0.29
MCHCg/dL	28.00±0.13	28,00±0.13	28.24±0.11	28.52±0.46	29.10±0.33
LYM%	69.30±2.52	75.28±1.53	72.12±2.30	68.24±1.98	70.46±3.05
NEUT%	29.44±2.34	24.72±1.53	27.88±2.30	31.76±1.98	29.54±3.05
RDW-SD fL	32.40±1.12	33.34±1.09	31.12±0.72	31.90±0.87	31.88±1.65
RDW-CV %	13.74±1.12	13.56±0.49	12.50±0.08	12.80±0.35	12.76±0.55
PDW fL	8.82±0.21	8.64±0.28	8.62±0.17	8.50±0.19	8.54±0.17
MPV fL	7.56±0.08	7.42±0.15	7.36±0.13	7.30±0.15	7.38±0.11
P-LCR %	9.08±0.54	7.60±0.87	7.28±0.58	7.72±0.89	7.72±0.89
PCT %	0.78±0.08	0.67±0.03	0.69±0.03	0.64±0.05	0.84±0.04
PLT /L	1026.20±109.21	897.40±37.80	943.40±59.60	881±65.82	1135.40±53.46
Female					
WBCx10^3/µL	8.60±1.04	8.18±0.20	8.20±2.13	11.85±2.26[a]	11.60±0.90[a]
RBCx10^6/µL	7.21±0.21	7.80±0.29	7.26±0.10	6.98±0.12	7.19±0.12
HGB gL	12.74±0.34	13.70±0.33	13.10±0.15	12.65±0.33	12.98±0.17
HCT%	43.58±1.01	48.20±1.65	45.30±0.91	43.13±0.44	43.63±0.56
MCV fL	60.52±0.45	61.86±0.52	62.40±1.04	61.88±1.28	60.70±0.63
MCH pg	17.70±0.55	17.58±0.25	18.03±0.03	18.15±0.62	18.08±0.27
MCHCg/dL	29.28±0.74	28.48±0.44	28.93±0.45	29.33±0.49	29.75±0.20
LYM%	67.98±0.94	64.16±1.73	69.43±4.40	62.80±3.32	64.05±3.25
NEUT%	31.06±1,00	35.84±1.73	30.57±4.40	37.20±3.32	35.95±3.25
RDW-SD fL	30.40±0.37	34.44±1.73	32.30±0.21	32.13±0.67	30.63±0.86
RDW-CV %	12.14±0.27	14.38±1.27	12.70±0.35	12.83±0.56	12.08±0.43
PDW fL	8.64±0.29	8.30±0.13	8.93±0.58	9.13±0.51	8.45±0.21
MPV fL	7.36±0.14	7.16±0.07	7.57±0.35	7.60±0.32	7.30±0.15
P-LCR %	7.52±0.92	6.58±0.48	9.24±2.14	9.25±1.84	7.23±0.75
PCT %	0.66±0.03	0.82±0,08	0.68±0.06	0.62±0.09	0.89±0.05
PLT/L	899.60±51.38	1146.20±105.46	909.67±116.66	823±131.94	1210.75±58.83

Values are expressed as mean ±SEM (n=5).Superscript a significant at P<0.05-0.001 compared with Normal group

Toxicity testing remains paramount as it is the only scientific basis for validating the safety of plant-based preparations in the traditional treatment of diseases. It has also been recommended that all-natural products used in therapeutics be subjected to safety tests by the same methods for new scientific drugs [40,41]. In the acute toxicity conducted, the LD_{50} ≥ 5 g/kg b.wt., thus suggesting the safety of EES for acute conditions [24]. The subacute toxicity study gave useful interpretable data on safe dosage level p.o., targeted organ toxicity and possible deleterious effects on the experimental animals. In this study effect of EES was evaluated at the doses of 100, 250, 500 and 1000 mg/kg b.wt. in rats for 14 days.

Weight loss in treated animals is an index of toxicity [42,43]. Generally, there were gains in weights in treated animals as they were fed and given water *ad libitum* showing that the extract was not toxic at the different doses of administration. Weight loss is known to occur when animals lose appetite following disturbances in the carbohydrate, protein or fat metabolism as a result of the administration of toxic material [44]. In addition, the relative organ weights did not show any significant differences compared to untreated animals, further suggesting that the extract was not toxic at the different administered doses.

Haematological parameters (Table 6) in treated animals were also not significantly different from

Table 7. Effect of treatment on biochemical parameters

	Normal	100 mg	250 mg	500 mg	1000 mg
Male					
ALT (U/L)	80.4±5.75	78.46±2.69	78.98±4.97	72.78±4.50	74.86±4.68
GGT (U/L)	2.3±0.62	3.25±0.49	3.73±0.25	5.96±0.89[a]	5.2±0.68[a]
Creatinine (mmol/L)	304.8±39.26	331.5±27.73	336.54±26.61	307.2±26.14	319.36±24.47
Urea (mmol/L)	6.08±0.65	5.38±0.21	5.86±0.24	6.72±0.15	6.48±0.38
Potassium (mmol/L)	6.81±0.56	6.60±0.10	6.75±0.17	6.70±0.08	6.83±0.05
Sodium (mmol/L)	139.95±0.39	140.18±0.31	140.36±0.38	139.74±0.75	140.04±0.49
Chloride (mmol/l)	101.93±0.95	103.23±0.67	102.56±0.41	102.16±0,52	102.96±0.60
TCHOL.(mmol/l)	1.53±0.01	1.47±0.04	1.48±0-07	1.42±0.07	1.40±0.05
HDL-C (mmol/l)	0.53±0.04	0.64±0.07	0.57±0.04	0.53±0.04	0.59±0.04
LDL-C (mmol/l)	0.69±9.10	0.58±0.10	0.69±0.07	0.67±0.05	0.61±0.04
TRIG.(mmol/l)	0.66±0.12	0.55±0.08	0.48±0.04	0.49±0.06	0.44±0.05
FBG (mmol/l)	2.67±0.35	2.69±0.14	2.94±0.19	2.74±0.22	2.72±0.13
LDH (U/l)	3607.67±290.57	3008.40±62.31	3177.10±164.33	2872.12±104.20	2592.52±176.10
Female					
ALT (U/l)	82.74±5.13	72.42±5.33	74.33±4.85	76.77±4.94	64.13±4.90
GGT (U/l)	2.14±0.17	3.25±0.49	3.73±0.25	5.96±0.89[a]	5.2±0.68[a]
Creatinine (mmol/l)	336.72±35.15	340.56±41.17	317.93±60.57	311.48±40.19	397.28±22.68
Urea (mmol/l)	9.86±0.55	7.75±0.78[a]	7.39±0.65[a]	7.98±0.45	7.53±0.68[a]
Potassium (mmol/l)	7.00±0.21	6.84±0.11	6.83±0.15	6.80±0.08	6.55±0.08
Sodium (mmol/l)	140.42±0.31	138.98±0.39	139.03±0.22	137.85±0.90	139.13±1.03
Chloride (mmol/l)	103.54±0.53	101.88±0.48	101.63±0.34	100.83±1.18	102.28±0.88
TCHOL.(mmol/l)	1.87±0.14	1.66±0.06	1.70±0.14	1.58±0.08	1.52±0.07[a]
HDL-C(mmol/l)	0.73±0.05	0.66±0.05	0.66±0.05	0.67±0.06	0.60±0.04
LDL-C (mmol/l)	0.75±0.17	0.64±0.06	0.78±0.08	0.61±0.10	0.54±0.09
TRIG.(mmol/l)	0.87±0.07	0.80±0.06	0.58±0.03	0.64±0.12	1.10±0.23
FBG (mmol/l)	3.37±0.32	3.19±0.17	3.14±0.29	2.90±0.28	3.36±0.09
LDH (U/l)	3493.84±452.46	3261.84±93.39	3143.23±156.25	3568.45±279.61	2979.53±295.40

Values are expressed as mean ± SEM (n=5). Superscript a significant at P<0.05-0.001 compared with Normal group

those of the controls establishing further that the extract at the different doses was non-toxic in animals. This is significant because the expected range of haematological parameters can be altered by the ingestion of toxic substances [45]. Haematological indices are therefore useful in monitoring toxicity in animals [46]. At higher doses of treatment (500 mg/kg and 1,000 mg/ kg body weight), the female animals displayed significant increases in white blood cell counts. Increases in WBC is a marker of stress and a defence mechanism against inflammatory dispositions [47]. It is probable that it is a situation of an adaptive response. These results generally further confirm that the ethanolic extract of *Hura crepitans* stem bark (EES) has no toxic effects on the treated animals.

The kidney plays an important role in removing toxic wastes from the body. The liver plays a vital role in the metabolism of foreign compounds, carbohydrates, fats, and proteins. It, therefore, means that both the liver and kidney are susceptible to drug-induced damage. This coupled with the continuous and global increase in liver and kidney diseases has made it expedient to check the safety of the administration of various plant extracts as it relates to these two organs. Some xenobioticscan give rise to liver and kidney damages [48]. There were no significant differences in the ALT levels in treated animals compared to the control indicating that the extract is not injurious to the liver and therefore not toxic to the rats [49]. There were also significant differences in the levels of the GGT in the treated rats and the control at higher doses which suggest the need for caution in the administration of the extract, especially at higher doses. ALT is a better parameter for assessing liver injury and the absence of significant changes at all dosessuggests that extractmay not be hepatotoxic.High levels of serum creatinine and urea is an indication of kidney dysfunction, while lowered levels of creatinine and urea indicate normal functioning of the kidneys [50]. The non-significant differences in creatinine and urea levels in treated animals and controls suggest the safety of the extract. Also, the non-significant differences in electrolyte levels is a strong pointer to probable non-deleterious effects exerted on the kidneys by the treatments. Non-significant changes in cholesterol, triglycerides, FBG, compared to controls indicate that carbohydrate and lipid metabolisms have not been disturbed and this further confirms that the plant extract is not toxic by the oral route. Elevated LDH is a factor for monitoring the treatment of cancer. In situations of tumours, LDH is released due to several cytokine activities and damage to cell membrane [51]. There were no elevated cases of LDH in the present study, thus ruling out the unusual proliferation of cells and also cell membrane damage in treated animals.

5. CONCLUSION

The study on EES has shown that it has phytoconstituents with therapeutic effects. The lethal dose, $LD_{50,}$ is estimated to be above 5000 mg/kg body weight. Based on biochemical parameters from the present study, the hydroethanolic extract of the stem bark of *Hura crepitans* (EES) is safe at the orally administered doses. It has also been demonstrated that the extract would not lead to liver or kidney dysfunction. Decreases in the WBCs in male rats may, however, be an adaptive response for male rats and this trend may have been reversed with an increase in the duration of the subacute study. Thus *H. crepitans* is safe for use in managing different diseases reported in ethnopharmacology.

SIGNIFICANCE STATEMENT

This study discovered the ethanolic extract of *Hura crepitans* had no deleterious effect on appetite and energy metabolism, organ weight, haematology and function of the liver, kidney, lipids and glucose homeostasis. This buttresses the safety of the plant and supports it continued used in traditional medical practices.

DATA AVAILABILITY

Supporting data for the findings of this study are available upon request from the corresponding author.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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