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Evaluation of Acute Toxicity of the Ethanolic Leaf Extract of *Brachystegia eurycoma* in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author UOU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MOM and LCC managed the analyses of the study. Author LCC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The acute oral toxicity of ethanol extract of *Brachystegia eurycoma* was evaluated in Wistar albino rats.
Study Design: Sixteen male Wistar albino rats were assigned in four groups of four rats each. Group 1 served as control while groups 2, 3 and 4 were treated with a single dose of 2000, 3500 and 5000 mg/kg bd. wt of extract respectively and observed for fourteen days.
Place and Duration of Study: Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria, from 1st of April 2018 to 15th of April, 2018.
Methodology: Healthy wistar albino rats weighing 120 – 180 g were fasted over-night prior to dosing. The animals were observed physically for toxic effect for the first twenty four (24) hours and once daily for the next thirteen (13) days for delayed toxicity. On the 14th day, the rats were anesthetized, blood samples were collected for biochemical analyses while internal organs (kidney, heart, liver and spleen) were excised for histopathological examination.

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Results: No mortality was observed at all doses of the extract. 2000 and 3500 mg/kg of the extract showed no toxic effect. A significant ($p<0.05$) increase in alkaline phosphatase (240.0 ± 5.00 to 296.67 ± 7.57 IU/L units), serum albumin (19.46 ± 1.36 to 28.89 ± 2.50 g/dl units), total bilirubin (5.07 ± 0.15 to 5.73 ± 0.29 µmol/L units) and serum creatinine (68.23 ± 0.25 to 72.06 ± 1.90 µmol/L units) was observed at 5000 mg/kg of the extract with the presence of distorted tubules, lobulated glomerulus in the kidney and inflammatory cells in liver sinusoid.
Conclusion: Ethanol leaf extract *B. eurycoma* may possess nephrotoxic and hepatotoxic potentials at very high dose.

Keywords: Brachystegia eurycoma; acute; oral; toxicity; ethanol; extract.

1. INTRODUCTION

Up to 80% of the African population relies on herbal medicine to meet their health care needs due to their culture as well as the accessibility and affordability of these herbal medications [1]. Several herbal medicines have led to the production of drugs and others have shown promising potentials [2]. Although the World Health Organization update for traditional medicine (2014 – 2023) encourages the safe use of traditional medicine through regulation, evaluation and inclusion of traditional medicine products into health care systems [3], professional health-care providers still hesitate to recommend herbal products to their patients due to lack of information on the safety/toxicity profile of most herbal products [4,5].

Brachystegia eurycoma Harms is a dicotyledonous leguminous tress belonging to the family *Fabaceae.* It is found in the swamps and rainforest of south, east and western Nigeria [6]. In Nigeria, it is commonly called Achi (Igbo), Akalado or Eku (Yoruba), Okwen (Edo), Akpakpa or Taura (in Hausa). The anti-inflammatory effect of aqueous extract of leaves has been reported [7]. The leaf, bark and root of the plant are used in ethno-medicines in combination with other plant part in the treatment of various diseases including malaria, diabetes, rheumatism, hypertension, and in bone setting [8]. The leaves are used traditionally in the treatment of various inflammation-related disorders, kidney problems and as enema, [9] making it necessary to know its safety profile. Hence we deemed it fit to investigate the safety level of the ethanol extract of *B. eurycoma* using biochemical and histopathological examinations.

2. MATERIALS AND METHODS

2.1 Extract Preparation

Fresh leaves of *B. eurycoma* were collected from Abakiliki in Ebonyi State, Nigeria. The leaves were authenticated in the herbarium section of the Department of Plant Science, University of Port Harcourt, River State, Nigeria. Rinsed in distilled water to remove dust particles and allowed to dry at room temperature. After seven days, the leaves were shredded and ground into powder with an electric blender. Ethanol extractions were carried out using the cold maceration extraction method. 20 g of the powdered leaves samples were macerated in 70% ethanol (1:4 w/v) for 72hours with agitation, decanted and filtered with Whatmann No. 1 filter paper. The filtrate was concentrated using rotary evaporator at 40˚C and evaporated to dryness with a water bath at 50˚C. It was weighed and stored at 4˚C.

2.2 Experimental Animals

Sixteen healthy male Wistar albino rats weighing between 120 -150 g of about eight – ten weeks old breed in the animal house of the Department of Biochemistry, University of Port Harcourt, River state, Nigeria was used for this study. The animals were kept under standard laboratory conditions and were humanely handled as stipulated in the Guide for the Care and Use of Laboratory Animals of National Research Council. Ethical clearance on the use of laboratory animals for animal studies was granted by the Department of Biochemistry Animal Ethic Committee of the University of Port Harcourt, with ethical clearance number UPH/ BCH/AEC/2018/019.

2.3 Acute Toxicity Study

Acute toxicity test was performed according to the Organization of Economic Cooperation and Development (OECD) guideline 420 for testing of chemicals [10].

2.4 Experimental Design

Based on weight, animals were assigned into four groups of four animals per groups. The extract was dissolved in distilled water to prepare

a stock solution. Animals were fasted (food but not water was withheld) over-night prior to dosing. Group 1 served as control receiving 0.5ml of distilled, groups 2, 3 and 4 were administered a single oral dose of 2000, 3500 and 5000 mg/kg body weight of *B. eurycoma* respectively.

2.4.1 Physical study and mortality

The animals were observed for clinical signs (diarrhea, salivation, tremors, reflex, signs of toxicity in their skin, fur, eyes, mucus membrane), behavioural changes (hypoactivity, hyperactivity, restlessness), sleep, coma and death for the first thirty (30) minutes followed by hourly for eight (8) hours for the first twenty four (24) hours and once daily for the next thirteen (13) days for delayed toxicity [10].

2.4.2 Clinical biochemistry

On the 14th day, the rats were anesthetized in chloroform vapour, blood samples were collected via cardiac puncture into plain bottles, allowed to clot and centrifuged to obtain serum used for evaluating serum albumin, total protein, total bilirubin, serum urea, serum creatinine, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activity. All parameters were analyzed with an autoanalyzer using kits from Randox Laboratories Ltd, United Kingdom.

2.4.3 Histopathology study

Internal organs (kidney, heart, liver and spleen) were excised and preserved in a fixation solution of 10% formalin for histopathological examination. Slides for histological examinations were prepared as described by Ezeja et al. [11]. The slides were examined microscopically for histological changes. Photomicrographs were captured at 400 × magnifications.

2.5 Statistical Analysis

All data are presented as mean ± standard deviation (n = 4). One way analysis of variance (ANOVA) was used to analyse the data. The results were considered significant at P values of less than 0.05 *(P<0.05)*.

3. RESULTS AND DISCUSSION

3.1 Physical Study and Morality

No behavioral or physical sign of toxicity was observed with the administration of ethanol extract of *B. eurycoma* at 2000 and 3500 mg kg^{-1}. At 5000 mgkg-1 of extract, animals were lethargic with mild tremor for the first hour after dosing. No mortality was observed at all doses tested. One of the primary objectives of acute toxicity study is to determine the LD_{50}, the dose which would be lethal to 50% of the animal treated as well as clinical signs attributable to high doses, the sequence and timing of effects leading to death or recovery [12]. The result from this study indicates that the LD_{50} of ethanol extract like the aqueous extract of *B. eurycoma* is greater than 5000 mgkg^{-1} [7]. According to the chemical labeling and classification of Organization for Economic Cooperation and Development [13] and World Health Organization [14] recommended guidelines for classification, chemicals with LD_{50} > 5000 mg are categorized as class 5, low toxic product which are "Generally Regarded As safe" (GRAS).

3.2 Biochemical Parameters

No significant change (*p>0.05*) was observed in AST, ALT and ALP at 2000 and 3500 mg kg-1 of the extract. At 5000 mg kg^{-1}, a significant (*p<0.05*) increase in ALP observed (Fig. 1).

The administration of the extract at 2000 and 3500 mg kg^{-1}, produced no significant change (*p>0.05*) in total protein but at 5000 mg kg^{-1}, a significant (*p<0.05*) increase in serum albumin and total bilirubin was observed (Fig. 2).

At all doses of the extract, there was no significant difference in serum urea when compared with control. No significant change was observed in serum creatinine at 2000 and 3500 mg/kg of extract. At 5000 mg/kg of extract serum creatine significantly (p<0.05) increased (Fig. 3).

The liver and kidney are most vulnerable to toxins due to their role in detoxification hence the assessment of liver and kidney function is a very vital index in evaluating the toxicity of drugs and plant extracts. Information about the state of the liver can be obtained from the use of serum assay. Serum transaminase levels indicate the rate of enzyme release from injured cells and are used as markers for hepatocellular damage. Serum albumin and total protein describes liver functionality. Alkaline phosphatase evaluates the link between the liver and the biliary tract. Total bilirubin is a liver function biomarker, which measures the ability of liver to clear bilirubin from the blood as it circulates through the liver [15,16].

In the present study very high dose of *B. eurycoma* resulted in an increase in the serum ALP and total bilirubin, which is indicative of hepatobiliary injury altering bile homeostasis [17]. This may be due to the presence of phytochemicals which may be toxic to the liver at high dose. Urea and creatinine levels in the blood which are usually used to evaluate the kidney function [18]. The increase in serum creatinine observed in this study is suggestive of damage in the nephrons affecting nephron function [19].

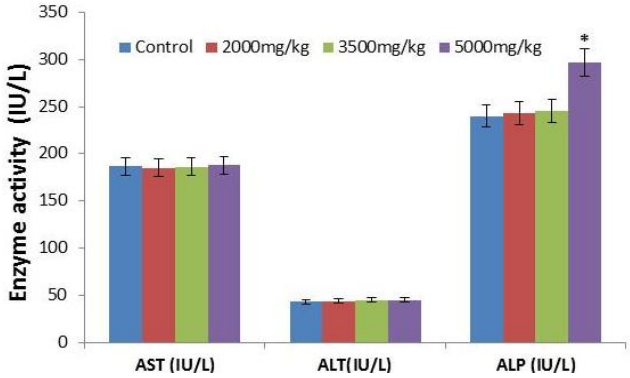

Fig. 1. The effect of ethanol extract of *B. eurycoma* on activity of serum liver enzymes
Bars with "" are significantly different from control*

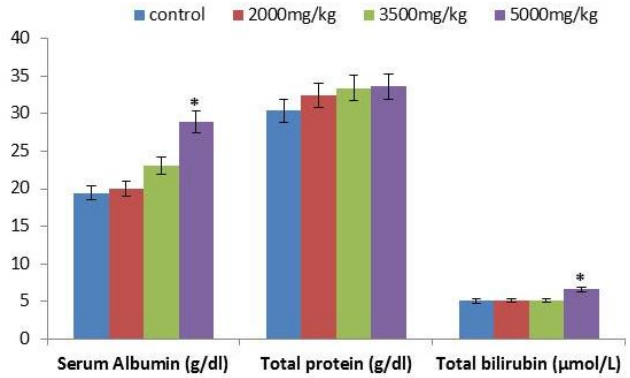

Fig. 2. The effect of ethanol extract of *B. eurycoma* on activity of serum albumin, total protein and total bilirubin
Bars with "" are significantly different from control*

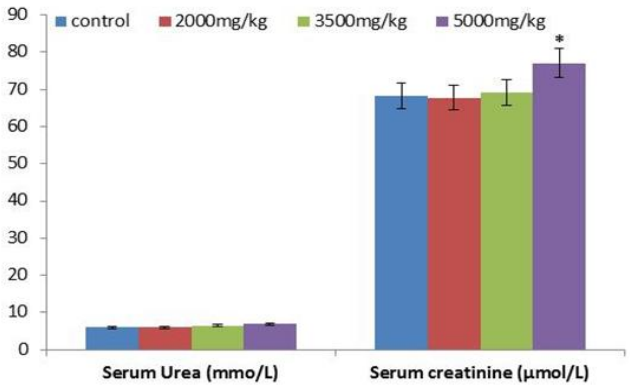

Fig. 3. The effect of ethanol extract of *B. eurycoma* on activity of serum urea and creatinine
Bars with "" are significantly different from control*

3.3 Histopathology

The photomicrograph sections of the spleens and hearts rats showed normal architecture at all doses of *B. eurycoma*. Normal liver and kidney architecture was observed at 2000 and 3500 mg/kg of *B. eurycoma.* However, treatment with 5000 mg/kg resulted in the presence of distorted tubules and lobulated glomerulus in the kidney nephron (Fig. 4) while inflammatory cells were observed in the liver sinusoid (Fig. 5). These histological changes in the liver and kidney collaborates the rise in serum ALP, bilirubin, albumin and creatinine.

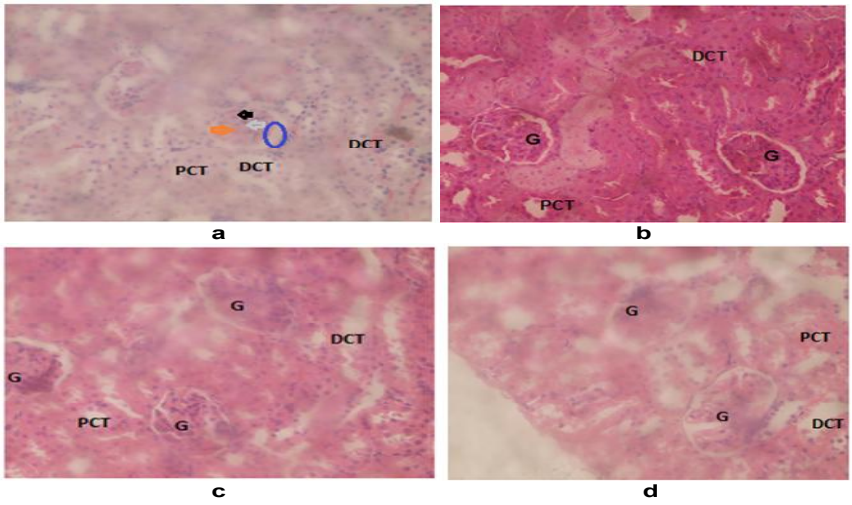

Fig. 4. Photomicrograph of kidney section (H&E, ×400) from rats 14 days post treatment with *B. eurycoma*

The 'G' indicates glomerulus, 'PCT' shows the proximal convoluted tubule and 'DCT' shows the distal convoluted tubule. a = control group; b = 2000 mg/kg B. eurycoma group; c = 3500 mg/kg B. eurycoma group; d = 5000 mg/kg B. eurycoma group

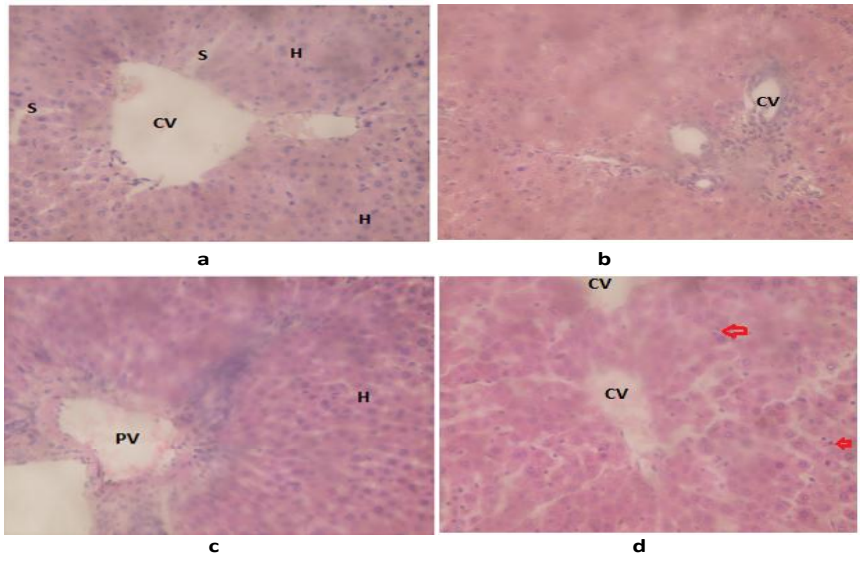

Fig. 5. Photomicrograph of liver section (H&E, ×400) from rats 14 days post treatment with *B. eurycoma*

The 'H' indicates hepatocyte, 'PV' shows the portal vein and 'CV' shows the central vein a = control group; b = 2000 mg/kg B. eurycoma group; c = 3500 mg/kg B. eurycoma group; d = 5000 mg/kg B. eurycoma group. Red arrow indicate inflamed hepatocytes

4. CONCLUSION

The LD_{50} of ethanol leaf extract of *B. eurycoma* is >5000 $mgkg^{-1}$, hence the therapeutic application of the extract appears to be quite safe. However, at very high doses, the extract may be hepatotoxic as well as nephrotoxic.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance on the use of laboratory animals for animal studies was granted by the Department of Biochemistry Animal Ethic Committee of the University of Port Harcourt, with ethical clearance number UPH/ BCH/AEC/2018/019.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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