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# Safety Evaluation of Methanol Leaf Extract of *Canavalia ensiformis* (Fabaceae): Acute Oral Toxicity Assessment in Rats Using the Up-and-Down Procedure

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**ABSTRACT**

***Background***: Medicinal plants continue to serve as important therapeutic resources in traditional medicine. *Canavalia ensiformis* (L.) DC. (Fabaceae), commonly known as jack bean, is traditionally utilized in several tropical regions, including Nigeria, for nutritional and medicinal purposes. This study aimed to evaluate the acute oral toxicity profile of the methanol leaf extract of *Canavalia ensiformis* in female Sprague–Dawley rats.

***Methods***: Acute toxicity was evaluated using the Organisation for Economic Co-operation and Development (OECD) Test Guideline 425 (Up-and-Down Procedure). Ten nulliparous and non-pregnant female Sprague–Dawley rats (185 ± 7.08 g) were randomly divided into control and treated groups (n = 5). The treated group received a single oral limit dose of 2000 mg/kg of the methanol leaf extract, while the control group received distilled water. Animals were monitored for behavioural changes, signs of toxicity, and mortality for 14 days. At the end of the study, animals were sacrificed and the liver and kidneys were harvested for histopathological evaluation using haematoxylin and eosin staining.

***Results***: No mortality or overt behavioural signs of toxicity were observed during the 14-day observation period. Histopathological examination revealed portal-associated hepatic necrosis and atrophy of Bowman's corpuscles in the kidney, suggesting structural alterations in these organs at the tested dose.

***Conclusion***: These findings suggest possible organ-specific toxicity at high doses and highlight the need for further subacute and chronic toxicity studies to establish safe therapeutic dose ranges.

Keywords: Acute toxicity, *Canavalia ensiformis*, histopathology, OECD 425, safety evaluation.

## 1. INTRODUCTION

Medicinal plants remain a major source of therapeutic agents in traditional and contemporary medicine, particularly in developing countries where plant-based remedies are widely utilized for primary healthcare [1]. Among such plants, *Canavalia ensiformis* (L.) DC., commonly known as jack bean, has attracted scientific interest due to its rich phytochemical profile and diverse ethnomedicinal applications [2]. The plant belongs to the family Fabaceae and is cultivated in tropical and subtropical regions, including Nigeria, where it is used traditionally for various therapeutic purposes [3,4]. Phytochemical investigations of *C. ensiformis* have revealed the presence of bioactive constituents such as flavonoids, alkaloids, saponins, polyphenolics, and glycosides [5], and other phenolic compounds, which are associated with antioxidant, antimicrobial, and anti-inflammatory [6,7]. Despite its reported pharmacological potentials, concerns regarding the safety profile of medicinal plants remain paramount. Herbal preparations are often perceived as inherently safe; however, several plant-derived products may exert toxic effects depending on dosage [8,9], duration of exposure, extraction solvent, and route of administration. Therefore, systematic toxicological

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evaluation is essential to establish the safety margin of plant extracts intended for therapeutic use. Acute oral toxicity testing provides critical information on the potential adverse effects of a substance following a single exposure. It helps determine the median lethal dose ($LD_{50}$) or to establish a safe limit dose [10]. The Up-and-Down Procedure described in the Organisation for Economic Co-operation and Development (OECD) Test Guideline 425 [11] is widely adopted for assessing acute toxicity, as it minimizes the number of experimental animals while ensuring reliable safety assessment. In addition to clinical observations and mortality indices, histopathological evaluation of vital organs such as the liver and kidneys serves as a sensitive indicator of organ-specific toxicity, providing insight into sub-lethal cellular and structural alterations. Given the increasing interest in the pharmacological application of *C. ensiformis* and the limited data on its toxicological profile, this study was designed to evaluate the acute oral toxicity of the methanol leaf extract of *C. ensiformis* in female Sprague–Dawley rats using OECD Guideline 425.

## 2. MATERIALS AND METHODS

### 2.1 Materials

*2.1.1 Chemicals and reagents*

Methanol (Lobachem), 10% buffered formalin, gentian violet, hematoxylin and eosin, distilled water

*2.1.2 Equipment and other materials*

Light microscope (Primostar 415550-1501-000(Zeiss), USA), microtome (AM-2268 ARI), 50 mL beaker, 250 mL conical flask, microscope slides and coverslip, mortar and pestle, 1 mL syringe, Analytical balance (Ohaus PR423/E-420GX), Automated Tissue Processor (Leica ASP300 /ASP300 S), Ph meter (Hellog PH-3C), embedding cassettes, scalpels, forceps, scissors, dehydaration jars, staining jars.

### 2.2 Methods

*2.2.1 Plant collection and identification*

The leaves of *Canavalia ensiformis* (1 kg) were collected in February 2023, from Uyo Local Government Area of Akwa Ibom State, Nigeria. A taxonomist, Prof. Henry Akinibosun, authenticated the plant at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. A voucher specimen was deposited at the herbarium section of the Department with specimen number UBH-C448.

*2.2.2 Drying of plant materials*

Foreign matter was removed from the collected plant organ. The plant material was dried in the shade for 12 days. The dried plant organ was comminuted using a mortar and pestle.

*2.2.3 Extraction*

The powdered plant part (500 g) was extracted with methanol (absolute) using a Soxhlet extractor at 67ºC. The *C. ensiformis* methanol leaf extract (CEME) was concentrated *in vacuo* using a rotary evaporator. The concentrated extract was weighed, kept in a labelled bottle, and stored in the refrigerator at 4ºC until required.

*2.2.4 Experimental animals*

Ten female inbred Sprague-Dawley rats weighing 185 ± 7.08 g were obtained from the Animal House unit of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Nigeria. The National Institute of Health (NIH 45) protocols for the use and care of laboratory animals were followed. The animals were housed under standard environmental conditions. They were allowed free access to standard pellets and water *ad libitum*.

*2.2.5 Acute oral toxicity evaluation according to OECD Test Guideline 425*

OECD Test Guideline 425 (Up and Down Procedure) was adopted for the study with modifications [12]. Ten inbred nulliparous and non-pregnant female Sprague-Dawley rats, weighing 185 ± 7.08 g, were randomly assigned to two groups (n=5). Rats were fasted for 4 h before administration. The limit test was performed at 2000 mg/kg p.o. as a single dose. The animals were closely observed for 30 minutes and then for the next 4 h. Food was provided after 2 h of dosing. In the absence of mortality of the tested rats, 4 additional rats were administered the same dose under the same conditions. The normal control group of 5 rats was subjected to the same conditions as the treated group. Animals in both groups were observed closely for signs of toxicity and mortality within the first 6 h and then at regular intervals

for 14 days. The weights of animals were taken at day 0 and day 14. The animals were fasted overnight on day 14, then on day 15, were sacrificed under anaesthesia using a chloroform gas chamber.

*2.2.6 Histological examination of the animals' vital organs*

The harvested vital organs were weighed and fixed in 10% buffered formalin. They were embedded in paraffin wax and sectioned at 4 µm. The cut sections were transferred to glass slides and stained with routine haematoxylin and eosin stains. The stained sections were observed under a light microscope at a magnification of ×40. Morphological and toxicological observations were recorded. Photomicrographs of the observed sections were taken through the use of Amscope

**2.3 Statistical analysis**

The data were qualitative and none was present.

**3. RESULTS**

Table 3.1: Behavioral patterns of female Sprague-Dawley rats treated with 2000 mg/kg p.o. of CEME and the normal control group

| Parameter | 0.5 h | | 4 h | | 24 h | | 48 h | | 168 h | | 336 h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NG | CE | NG | CE | NG | CE | NG | CE | NG | CE | NG | CE |
| Piloerection | N | N | N | N | N | N | N | N | N | N | N | N |
| Eyes | N | N | N | N | N | N | N | N | N | N | N | N |
| Salivation | N | N | N | N | N | N | N | N | N | N | N | N |
| Respiration | N | N | N | N | N | N | N | N | N | N | N | N |
| Faecal consistency | N | N | N | N | N | N | N | N | N | N | N | N |
| Behavioral pattern | N | N | N | N | N | N | N | N | N | N | N | N |
| Sleep | N | N | N | N | N | N | N | N | N | N | N | N |
| Convulsions and tremors | A | A | A | A | A | A | A | A | A | A | A | A |
| Itching | A | A | A | A | A | A | A | A | A | A | A | A |
| Coma | A | A | A | A | A | A | A | A | A | A | A | A |
| Mortality | A | A | A | A | A | A | A | A | A | A | A | A |

NG=Normal control group CE=Extract treated group N=Normal A=Absent P=Present

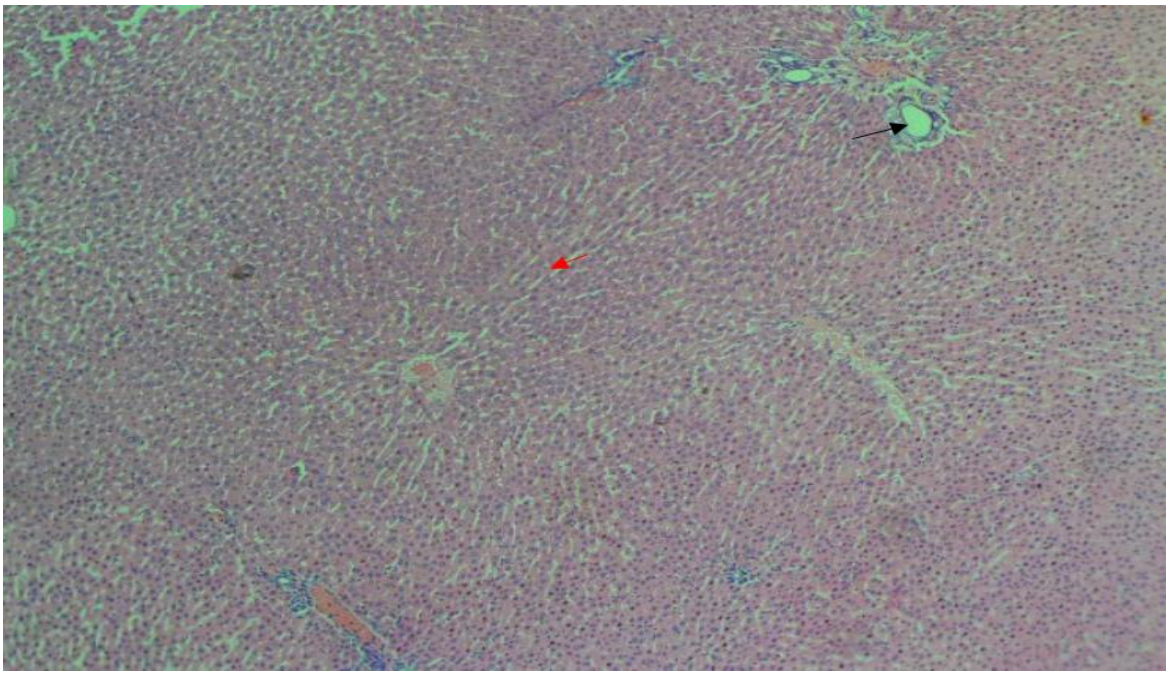

Figure 1: Photomicrograph of rat liver in the normal control group, reveals normal central vein (black arrow) and sinusoids (red arrow). X100

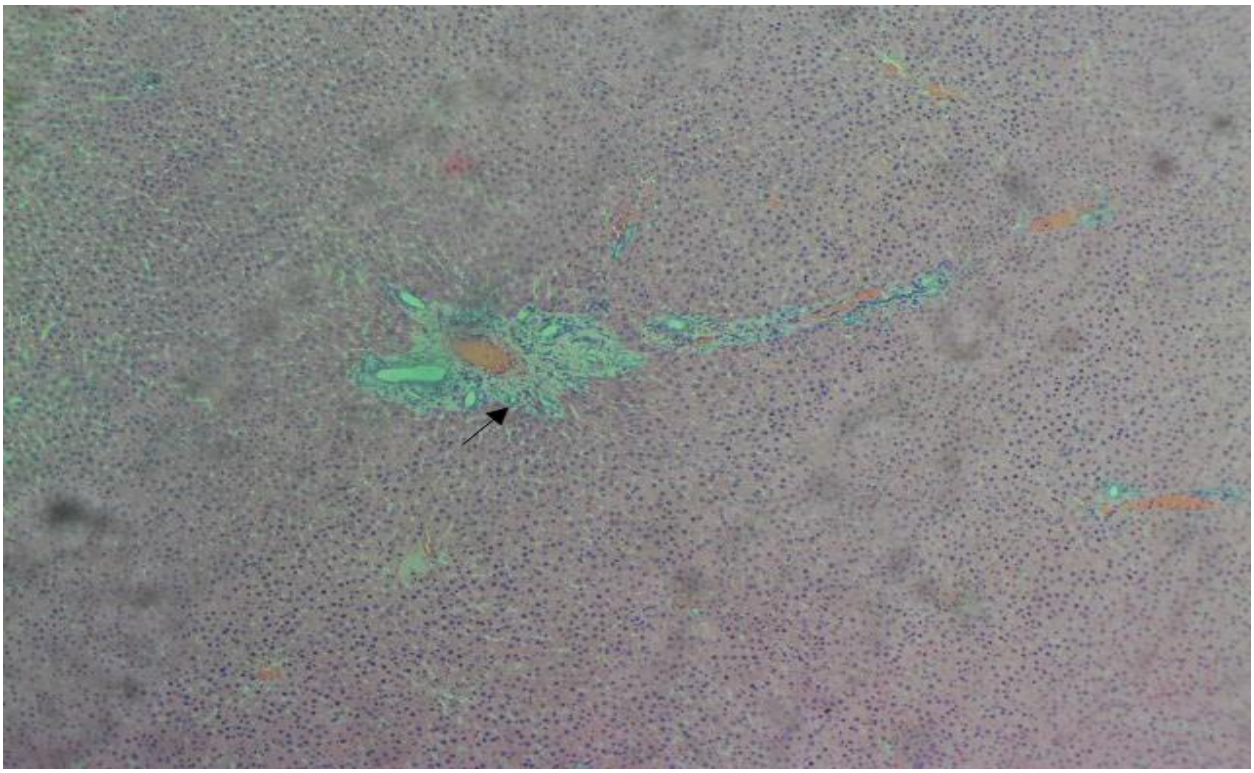

Figure 2: Photomicrograph of rat liver in the acute toxicity group, revealing necrosis of the central portal vein (black arrow) X100

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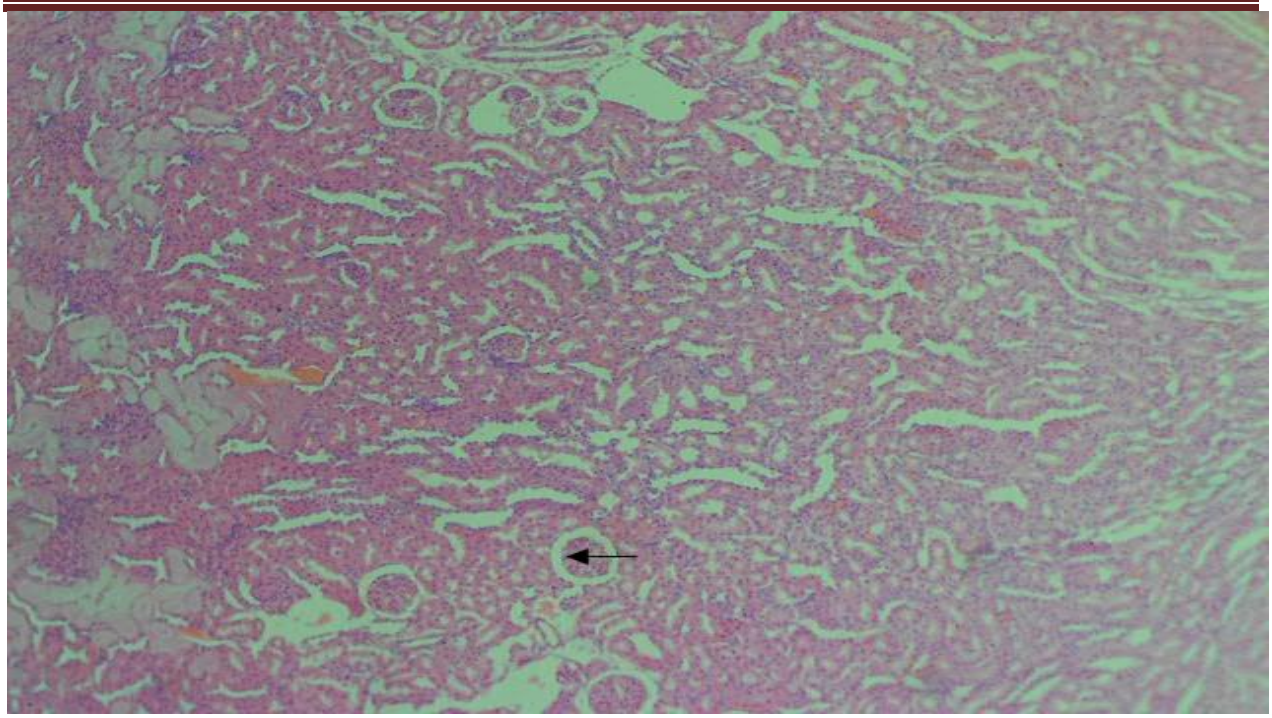

Figure 3: Photomicrograph of rat kidney in the normal control group revealing normal bowman's corpuscle (black arrow) X100

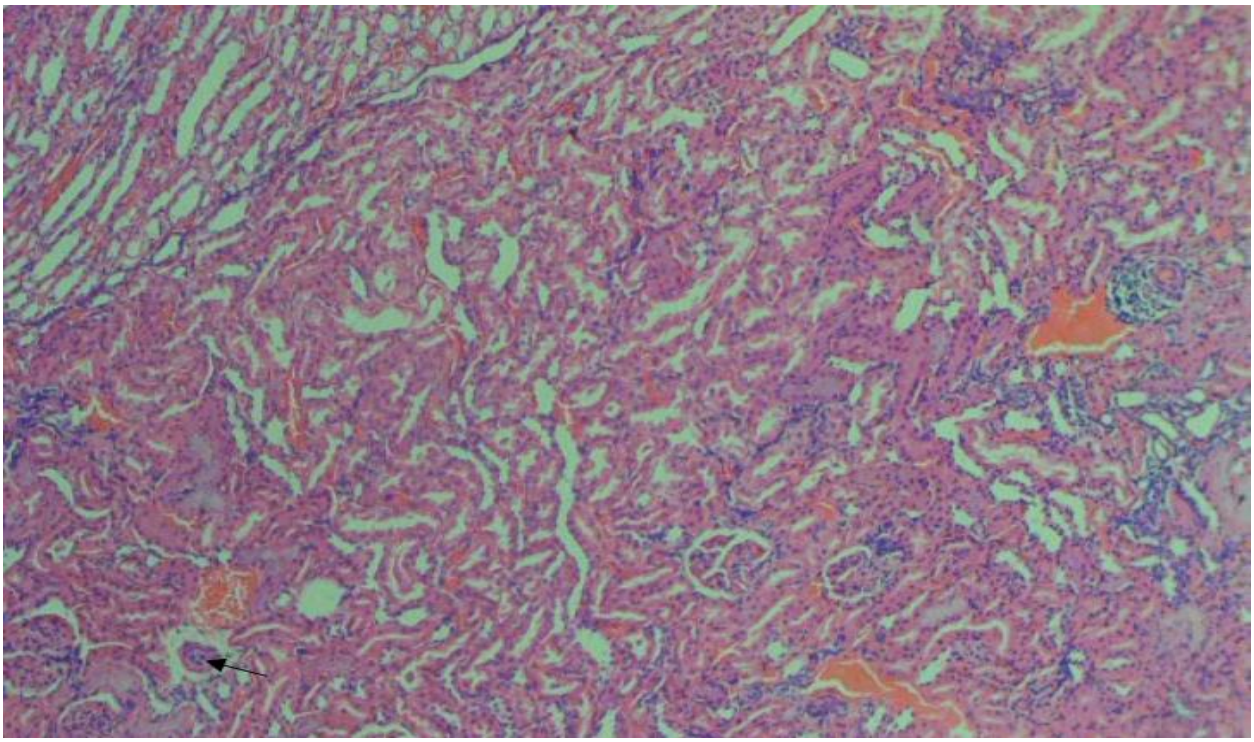

Figure 4: Photomicrograph of rat kidney in the acute toxicity group revealing atrophy of the bowman's corpuscle (black arrow) X100

## 4. DISCUSSION

In accordance with Test Guideline 425 of the Organisation for Economic Co-operation and Development, the current study assessed the acute oral toxicity profile of *C. ensiformis* extract using the Up-and-Down Procedure. This type of safety evaluation is crucial in an ethnopharmacological framework because plants that have historically been used for therapeutic purposes are frequently thought to be safe despite having little toxicological validation [13]. Our results offer significant preclinical evidence regarding the dose-dependent safety of *C. ensiformis*. Early indicators of acute toxicity are provided by the behavioral observations of animals after the administration of the test dose. The treated animals showed no obvious symptoms of toxicity during the observation period, including tremors, convulsions, piloerection, salivation, or respiratory distress (Table 3.1). The control group's histopathological evaluation verified that the hepatic (Figure 1) and renal (Figure 3) architecture were intact, suggesting that the vehicle and experimental protocols did not cause any confounding structural changes. On the other hand, significant hepatic and renal lesions were caused by the high dose of the extract. Portal-associated necrosis in the liver indicated significant vascular and hepatocellular impairment (Figure 2). Since the liver plays a key role in the biotransformation of xenobiotics, this kind of damage most likely results from metabolic excess and the production of reactive intermediates. These intermediates could lead to irreversible hepatocellular damage by encouraging oxidative stress, lipid peroxidation, and membrane instability. Additionally, vascular involvement in the portal region points to compromised perfusion, which could worsen parenchymal destruction by means of ischemic processes. Similarly, Bowman's corpuscle atrophy in treated rats as seen in the renal histology, shows structural disturbance of the glomerular filtration machinery (Figure 4). Excessive exposure may overload renal clearance mechanisms because the kidneys are in charge of eliminating both parent chemicals and their metabolites. Hemodynamic changes, the accumulation of toxic metabolites, or the direct nephrotoxic effects of bioactive components can all cause glomerular atrophy. Thus, systemic toxicity involving both metabolic and excretory pathways is suggested by the concomitant hepatic and renal abnormalities shown at the tested dose. *C. ensiformis* has been used for a number of purposes, such as medical and nutritional purposes. Nonetheless, phytochemicals found in the species, including lectins, non-protein amino acids (like canavanine), and other bioactive secondary metabolites, have been linked to biological activity that, depending on dosage, may have both beneficial and harmful effects. Crude or concentrated extracts given at large doses might not mirror traditional exposure patterns. Histopathology proof of high-dose organ damage aids in hazard identification and provides information for $LD_{50}$ estimation. Even though the extract showed noticeable toxicity at the measured limit dose, it may still have therapeutic value at lower, carefully calibrated levels [14]. To lessen toxicity, conventional preparation methods like extended soaking, fermentation, or heat treatment may be adopted. The result emphasizes the need for graded dose-response studies, subacute and chronic toxicity evaluations, and mechanistic research to establish a safe therapeutic window.

## 5. CONCLUSION

This study provides crucial preclinical safety information to support the rational development of *C. ensiformis* within an evidence-based ethnopharmacological framework. Future research should focus on identifying the specific components responsible for the observed toxicity, elucidating the underlying molecular mechanisms, and determining the no-observed-adverse-effect level (NOAEL).

## DECLARATIONS

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The authors declare no conflict of interest

**Author Contributions**

Daniel Ambe – conceived and designed the study, performed data analysis, interpretation and writing of the manuscript; Gift James – carried out the experimental work and data collection.

**Ethical Approval**

Ethical approval (AKHREC/8/11/23/197) was obtained from the Ethical Committee, Ministry of Health, Akwa Ibom State, Nigeria.

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