



Toxicological and Histopathological Effects of Methanolic Leaf Extract of Sodom Apple (*Calotropis procera*) on Liver and Kidney of Mice (*Mus musculus*)

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Abstract

Sodom apple *Calotropis procera* is widely used in traditional medicine, yet its potential toxicity remains underexplored. This study investigates the toxicological and histopathological effects of methanolic leaf extract of Sodom apple *C. procera* on mice (*Mus musculus*). Acute and sub-acute toxicity studies were conducted using oral doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight for acute studies, and 250, 500, and 1000 mg/kg for sub-acute studies. No mortality was observed in acute toxicity tests up to 5000 mg/kg, classifying the extract as Category 5 (non-toxic) per OECD guidelines. Sub-acute studies revealed dose-dependent changes in body weight, with fatalities recorded at weeks three and four at higher doses. "Histopathological studies were conducted using standard techniques involving fixation in Formalin, paraffin embedding, microtome sectioning, and staining with Hematoxylin and Eosin stain. The prepared sections were subsequently examined under a Light microscope. The results from the experiment were expressed. Values are expressed as mean $\pm$ SEM. Statistical significance mean difference was considered at $p < 0.05$ and Bonferroni comparison test was used for post hoc analysis. The results indicate that the aqueous extract of *Calotropis procera* leaves produced the highest percentage yield (3.6%), whereas the methanolic extract recorded the lowest yield (2.5%) Histopathological analysis showed slight to moderate tubular necrosis and hyperplasia of inflammatory cells in the kidneys, and vascular calcification, slight hepatic necrosis, and Kupffer cell hyperplasia in the liver, particularly at 500 and 1000 mg/kg. These findings suggest that while Sodom apple (*C. procera*) methanolic extract is relatively safe at low doses, prolonged high-dose exposure may induce hepato- and nephrotoxicity, necessitating caution in its medicinal use.

Keywords: *Calotropis procera*, histopathology, kidney, liver, methanolic extract, mice, toxicity

1. Introduction

Calotropis procera is a soft-wooded, perennial shrub of the family Apocynaceae and subfamily Asclepiadaceae (the milkweed family). It is an evergreen xerophytic plant, generally found in arid and semi-arid habitats [1]. The word "*Calotropis*" is derived from Greek, meaning "beautiful," which refers to its flowers, whereas "*procera*" is a Latin word referring to the cuticular wax present on its leaves and stem [2]. It is known by various common names such as apple of sodom, calotrope, giant milkweed, Indian milkweed, wild cotton, rubber tree, ushar, etc., in different parts of the world.

Its subspecies, *C. procera* subsp. *procera* and *C. Procera* subsp. *hamiltonii*, vary from each other

in fruit morphology [3]. It also shares a close homology with its con-generic plant *C. gigantea* [4]. *Calotropis procera* is a multipurpose plant, which provides a wide range of provisioning ecosystem services. It has been widely used in traditional medicinal systems in North Africa, Middle East Asia, South Asia, and South-East Asia [5]. The plant has also been utilized for fiber, fuel, fodder, and timber purposes since ancient times [6]. The fruits are used for the re-gaining of memory when dementia is setting in and the leaves are worn with socks to reduce accumulation of salts and joints to prevent gout. Owing to its socio-economic importance, it has been introduced in several parts of the world outside its native range (Asia and Africa). Morpho-physiological adaptations and the ability to tolerate a wide range of environmental conditions enabled its naturalization in the

introduced habitats. Consequently, the plant has also been reported as an invasive weed of wastelands, overgrazed pastures, and poorly managed agricultural fields in several regions [4].

Generally, medicinal plants contain bioactive constituents which demonstrate both intra- and inter-species variation in type and content. Plants by virtue of their chemical compounds are potentially toxic; thus, some plants used in traditional medicinal practice are intrinsically toxic. Some of the plants well known in traditional medicine to be toxic or poisonous include *Atropa belladonna*, *Datura spp.*, *Digitalis spp.* [7].

Many plants used in traditional medicinal practice or used as food have displayed some toxicity (mutagenic and carcinogenic) effects. The issue of the possible toxic, genotoxic and/or mutagenic effects of plants used in traditional medicine has been highlighted in the review by Fennell *et al.* [8]. However, some of the toxic plants are useful to man as medicines, as poisons for hunting and for use as pesticides; for example, *Datura* (tropane alkaloids), *Digitalis* (cardiac glycosides) and *Pyrethrum* (pyrethrin insecticides). Well-known medicinal plants have demonstrated toxicity in laboratory studies and field observations. For example, *Lantana camara* used in the management of malaria and other diseases has been reported to be hepatotoxic in several animal species which could be of concern regarding its chronic use by man [9]. Likewise, *Momordica charantia*, a known anti-diabetic and antimalarial plant used in Ghana as an abortifacient, has been reported to cause deadly hypoglycemia in children [7].

The basis for the medicinal use of plants is the presence of mixtures of different biologically active plant compounds or phytochemicals (i.e., secondary metabolites) such as alkaloids, glycosides, terpenoids, and so on that may act individually, additively, or in synergy to demonstrate an effect which may be harmful or useful to health. Some of these plants have been designated as poisonous plants because of their effects in impacting biological functions in other organisms which are harmful [7].

2. Materials and Methods

2.1 Plant Collection and Extract Preparation

Fresh leaves of *Calotropis procera* were collected from the Biological Garden, Ahmadu Bello University. The plant material was authenticated by a taxonomist, and a voucher specimen was deposited under voucher number ABU0900219. The leaves were air-dried at room temperature, pulverized into a fine powder, and subjected to extraction using methanol via the Soxhlet extraction method. The resulting methanolic extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further use.

2.2 Experimental Animals

Twenty 20 Healthy adult Swiss mice (*Mus musculus*) (18–23 g) were obtained from the Ahmadu Bello University, Zaria, Nigeria animal house, Department of Pharmacology. Animals were housed under standard conditions (12-hour light/dark cycle, $25 \pm 2^\circ\text{C}$, with access to standard feed and water ad libitum). Ethical approval for the study was obtained from the Institutional Review Board of Ahmadu Bello University, Zaria, Nigeria (Reference No: ABU/IRB/2026/015)

2.3 Acute Toxicity Study

Acute toxicity was evaluated using Lorke's method [4]. In phase I, mice ($n = 3$ per group) received oral doses of 10, 100, and 1000 mg/kg of methanolic extract. In phase II, doses of 1600, 2900, and 5000 mg/kg were administered ($n = 1$ per group). Animals were observed for 24 hours for mortality, behavioral changes, and clinical signs of toxicity.

2.4 Sub-Acute Toxicity Study

Mice were divided into four groups ($n=5$ per group): control (distilled water) and treatment groups receiving 250, 500, and 1000 mg/kg of methanolic extract orally for 28 days. Body weights were recorded weekly. On day 29, mice were sacrificed, and liver and kidney weights were measured. Relative organ weights were calculated as:

$$\{\text{Relative Organ Weight}\} = \{\text{Organ Weight}\} / \{\text{Body Weight}\} \times 100 \%$$

2.5 Histopathological Analysis

Liver and kidney tissues were fixed in 10% formalin, processed, and stained with hematoxylin and eosin (H&E). The prepared sections were examined under a light microscope for histopathological alterations, including necrosis, hyperplasia, and vascular changes [10].

2.6 Statistical Analysis

Data were expressed as mean $\pm$ SEM. Body and organ weights were analyzed using one-way ANOVA, with statistical significance set at $p < 0.05$.

3. Results

3.1 Acute Toxicity

No mortality or visible toxic symptoms were observed in mice treated with methanolic extract up to 5000 mg/kg after 24 hours, indicating an estimated oral LD₅₀ of 5000 mg/kg. This classifies the extract as Category 5 (non-toxic) per OECD guidelines.

3.2 Sub-Acute Toxicity

Weekly body weight measurements showed a general increase across all groups, with no significant differences ($p > 0.05$) compared to the control (Table 1). However, one fatality was recorded in the 500 mg/kg group at week three and one in the 1000 mg/kg group at week four. Relative organ weights of the liver and kidney increased in a dose-dependent manner, suggesting possible hypertrophy or underlying pathology (Table 2).

Table 1: Body Weight of Mice During Sub-Acute Studies

Week	Control (g)	250 mg/kg (g)	500 mg/kg (g)	1000 mg/kg (g)
1	20.00 $\pm$ 0.71	19.80 $\pm$ 1.11	17.40 $\pm$ 0.25	18.80 $\pm$ 1.20
2	20.80 $\pm$ 0.74	20.60 $\pm$ 0.87	14.20 $\pm$ 3.69	18.80 $\pm$ 0.86
3	21.20 $\pm$ 0.58	17.20 $\pm$ 4.40	14.60 $\pm$ 3.82	14.60 $\pm$ 3.75
4	22.40 $\pm$ 0.93	16.20 $\pm$ 4.08	14.00 $\pm$ 3.67	14.20 $\pm$ 3.71

Values are mean $\pm$ SEM (n=5). No significant differences ($p > 0.05$) compared to control.

Table 2: Relative Organ Weights After Sub-Acute Studies

Group	Liver Weight (g)	Relative Liver Weight (%)	Kidney Weight (g)	Relative Kidney Weight (%)
Control	1.0 $\pm$ 0.1	5.0 $\pm$ 0.5	0.4 $\pm$ 0.1	2.0 $\pm$ 0.3
250 mg/kg	0.9 $\pm$ 0.2	5.5 $\pm$ 0.6	0.4 $\pm$ 0.1	2.4 $\pm$ 0.4
500 mg/kg	0.8 $\pm$ 0.2	5.7 $\pm$ 0.7	0.3 $\pm$ 0.1	2.6 $\pm$ 0.5
1000 mg/kg	1.1 $\pm$ 0.3	6.2 $\pm$ 0.8	0.3 $\pm$ 0.1	2.8 $\pm$ 0.6

3.3 Histopathological Findings

Histopathological analysis of liver and kidney sections from the control group showed normal histology of the liver, with normal hepatocytes and Kidney showing slight tubular necrosis (Plate 1). The Kidney shows slight tubular necrosis, and the liver shows vascular calcification with slight hepatic necrosis (Plate 2A and 5B). The kidney also shows moderate tubular necrosis with the liver showing normal

hepatocytes (Plate 2A and 5B). Kidney shows slight hyperplasia of inflammatory cells with slight tubular adhesion was observed in the kidney with normal hepatocytes in the liver (Plate 2B and 5A). The kidney also showed moderate tubular necrosis with the liver showing slight hepatic necrosis (Plate 3A and 7B). The liver indicated moderate hepatic necrosis with kupfer cell hyperplasia with the kidney showing slight tubular necrosis (Plate 7A and 4A). Finally, a moderate tubular adhesion was

observed in the kidney with the liver showing slight hepatic necrosis as shown in (Plate 7B and

4B).

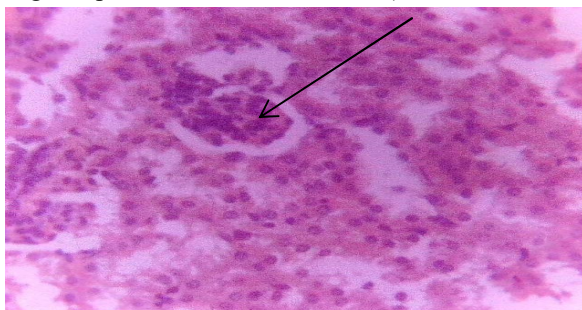
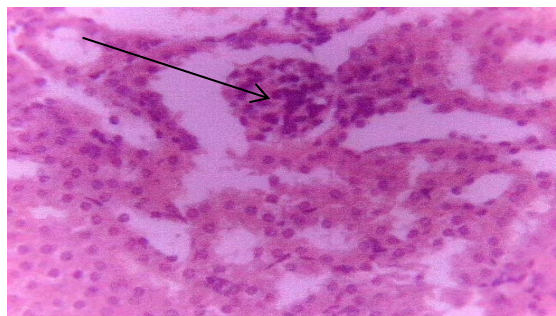


Plate 1: (A) Kidney showing slight tubular necrosis



(B) Kidney showing slight tubular necrosis

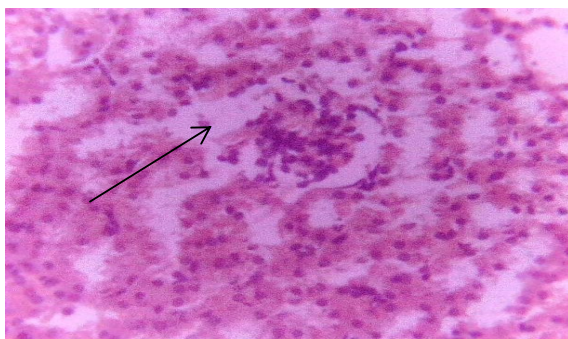
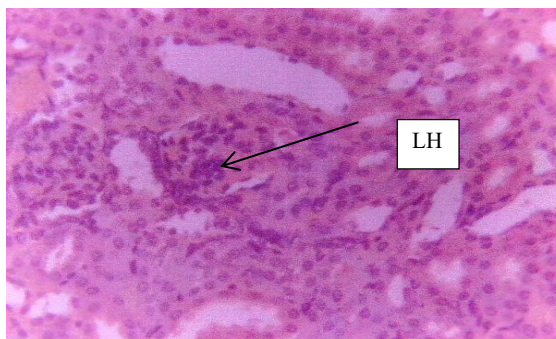


Plate 2: (A) Kidney showing moderate tubular necrosis



(B) Kidney showing slight hyperplasia of inflammatory cells with slight tubular adhesion

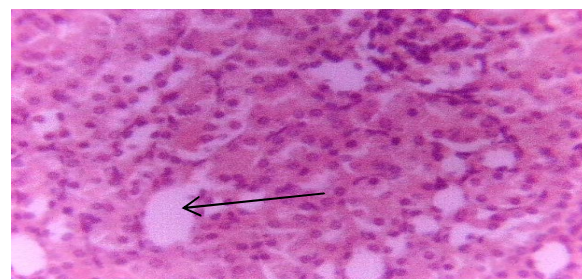
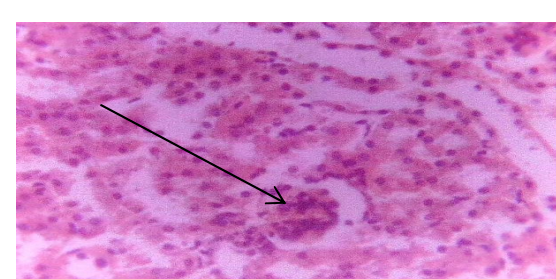


Plate 3: (A) Kidney showing moderate tubular necrosis



(B) Kidney showing hyperplasia of inflammatory cells

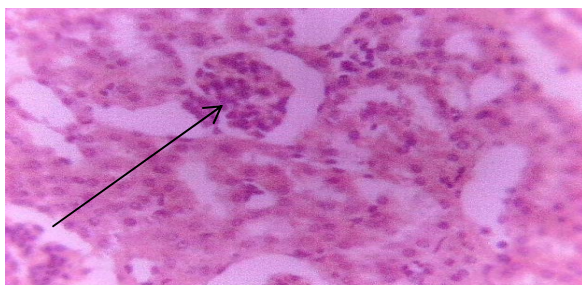
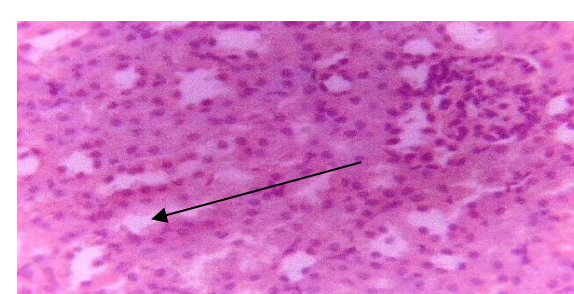


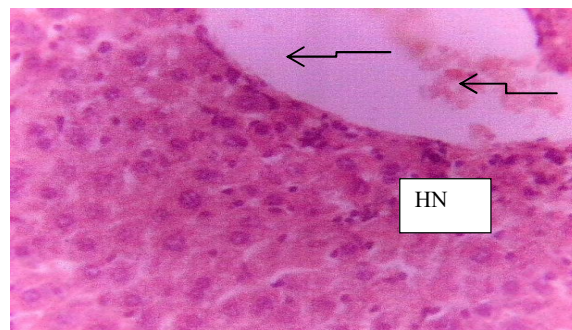
Plate 4: (A) Kidney showing slight tubular necrosis



(B) Kidney showing moderate tubular adhesion



Plate 5: (A) Liver showing normal hepatocytes



(B) Liver showing vascular calcification with slight hepatic necrosis

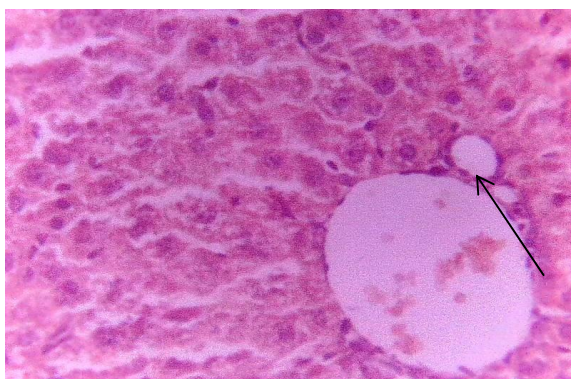
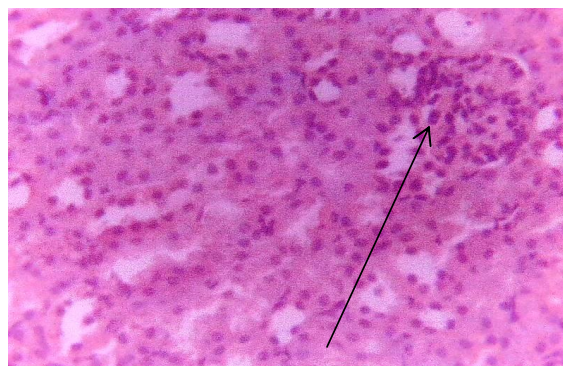


Plate 6: (A) Kidney showing moderate tubular adhesion



(B) Liver showing slight hepatic necrosis

4. Discussion

The methanolic leaf extract of *C. procera* demonstrated no acute toxicity at doses up to 5000 mg/kg, consistent with findings reported by Imosemi et al. [11], who observed no mortality at 3000 mg/kg. This supports its classification as a Category 5 substance [6]. However, sub-acute studies revealed dose-dependent effects, including fatalities at higher doses (500 and 1000 mg/kg) after prolonged exposure, suggesting potential cumulative toxicity. Histopathological findings indicate that *C. procera* extract induces hepato- and nephrotoxicity at higher doses. The observed liver changes, such as vascular calcification and hepatic necrosis, align with reports by Esther et al. [12], who observed similar vascular congestion and necrosis in mice treated with *Zingiber officinale*. Kidney alterations, including tubular necrosis and hyperplasia, corroborate findings by Ebeye et al. [13] and Noori et al. [14], suggesting that phytochemicals like terpenoids may increase membrane permeability, leading to congestion and edema [15]. The presence of cardenolides, flavonoids, and alkaloids in the extract, as

identified by GC-MS, may contribute to these effects, as these compounds have been linked to hepatotoxicity [16]. The increase in relative organ weights suggests hypertrophy or pathological changes, potentially due to enhanced metabolic activity or edema [17]. The lack of significant body weight differences indicates that the extract does not impair overall growth, but reduced food and water consumption at higher doses may reflect alkaloid- or tannin-mediated effects on gastric emptying and metabolism [18]. These findings highlight the dual nature of *C. procera* as a medicinal plant with potential toxicity. While low doses appear safe, prolonged high-dose use may pose risks to liver and kidney function, necessitating careful dosing in traditional medicine.

5. Conclusion

The methanolic leaf extract of *C. procera* is non-toxic in acute settings up to 5000 mg/kg but induces dose-dependent toxicological and histopathological changes in sub-acute studies. At 500 and 1000 mg/kg, the extract caused slight to moderate liver and kidney damage, including

necrosis and hyperplasia, suggesting potential hepato- and nephrotoxicity with prolonged use. These findings underscore the need for precise dosing and further toxicological studies to ensure the safe use of *C. procera* in herbal medicine.

6. Recommendations

1. Conduct sub-chronic and chronic toxicity studies to assess long-term effects.
2. Isolate and evaluate specific bioactive compounds responsible for toxicity.
3. Investigate the toxicity of other *C. procera* plant parts to establish a comprehensive safety profile.

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