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# Toxicological Evaluation Of *Calotropis Procera* (CALP) In Wistar Rats: Acute And Sub-Acute Studies

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**Abstract**
*Calotropis procera* (CALP), belonging to the family Apocynaceae, is a well-known medicinal plant distributed in tropical and subtropical regions. It has been extensively used in Ayurveda and folk medicine for the treatment of ailments such as fever, inflammation, asthma, skin disorders, and digestive disturbances. Despite these applications, the plant is notorious for its toxic properties, primarily attributed to the presence of cardenolides (cardiac glycosides), alkaloids, terpenoids, and other bioactive secondary metabolites. The duality of its therapeutic potential and toxic nature makes it essential to evaluate its safety profile.

The present experimental study investigates the acute and sub-acute oral toxicity of CALP root extracts in Wistar rats, adhering to OECD guidelines. In the acute toxicity study, no mortality was observed up to 2000 mg/kg; however, the $LD_{50}$ was determined to be 3000 mg/kg using the Karber method. In the sub-acute 28-day study, dose-dependent alterations were observed in hematological, biochemical, and histopathological parameters. At higher doses (1000–2000 mg/kg), CALP significantly decreased hemoglobin, red blood cell (RBC) counts, and platelet levels. Biochemical analysis revealed marked increases in glucose, serum creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and bilirubin concentrations. Histopathological studies confirmed organ-specific toxicity, including hepatocyte degeneration and necrosis in the liver, gastric mucosal erosion in the stomach, and renal tubular degeneration and necrosis in the kidney. The heart tissue, however, appeared normal across all doses.
The findings suggest that CALP is relatively safe at low doses (≤500 mg/kg), but its toxicity becomes evident at higher doses, particularly affecting the liver, kidney, and stomach. These results highlight the importance of cautious use of CALP in traditional medicine and emphasize the need for dose standardization and further mechanistic studies.

**Keywords:** *Calotropis procera*, CALP, Toxicology, Acute toxicity, Sub-acute toxicity, Histopathology, Herbal safety

## 1. Introduction

### 1.1 Background of *Calotropis procera*

*Calotropis procera* (commonly known as Sodom apple, Akra, or Madar) is a perennial shrub widely distributed across Asia, Africa, and the Middle East. In India, it is frequently encountered in wastelands, roadsides, and dry regions. The plant is recognized in Ayurveda, Unani, and Siddha systems of medicine and has been employed traditionally for its therapeutic properties. Various parts of the plant, including leaves, roots, bark, latex, and flowers, are used in remedies for skin diseases, leprosy, asthma, rheumatism, fever, digestive ailments, and as an analgesic.[1]

The ethnopharmacological significance of CALP has been validated through modern studies that report antimicrobial, anti-inflammatory, analgesic, and anticancer activities. However, the plant is also well known for its toxic properties, with reports of accidental poisoning in humans and livestock. The latex, in particular, is highly irritant to the skin and mucous membranes and has been linked to severe inflammatory responses. [2]

### 1.2 Phytochemical Composition

Phytochemical investigations of CALP have revealed the presence of a wide variety of bioactive secondary metabolites. These include cardenolides (such as calotropin, uscharin, and proceroside), alkaloids, flavonoids, terpenoids, tannins, saponins, and phenolic compounds. While many of these constituents contribute to its pharmacological actions, several are toxic. Cardiac glycosides, for instance, interfere with the $Na^+/K^+$-ATPase pump in myocardial tissue, potentially leading to arrhythmias and cardiotoxic effects. Similarly, triterpenoids and alkaloids have been associated with gastrointestinal irritation, hepatotoxicity, and nephrotoxicity. [3-5]

### 1.3 Need for Toxicological Evaluation

Despite widespread use in traditional medicine, there is limited scientific validation of the safety profile of CALP. [6] In folk practices, crude extracts are often administered without standardization of dosage, raising the risk of toxicity. Furthermore, reports of livestock poisoning after grazing on CALP highlight the plant's potential hazards. Experimental

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animal models are thus necessary to determine safe dosage levels, evaluate organ-specific toxicity, and understand dose–response relationships. [7-8]

**1.4 Previous Toxicological Studies**

Earlier studies have demonstrated the toxic potential of CALP. Acute toxicity experiments in rodents have reported $LD_{50}$ values ranging from 2000–3500 mg/kg, depending on the preparation and route of administration. Sub-acute studies have shown dose-dependent biochemical changes, such as increased liver enzymes and renal markers, along with histopathological evidence of hepatic and renal damage. However, findings vary widely, and detailed evaluations combining hematological, biochemical, and histopathological outcomes are limited. [9]

**1.5 Objectives of the Present Study**

The present study was designed to systematically evaluate the acute and sub-acute oral toxicity of CALP root extracts in Wistar rats. The objectives were:

1. To determine the acute toxicity and calculate $LD_{50}$ values.
2. To assess the impact of repeated 28-day administration on hematological, biochemical, and histopathological parameters.
3. To establish dose-dependent relationships and identify organs most vulnerable to toxicity.
4. To discuss the implications of these findings for the safe therapeutic use of CALP in traditional and modern medicine.

## 2. Materials and Methods

**2.1 Plant Material and Preparation of Extract**

Roots of *Calotropis procera* (CALP) were collected, cleaned, shade-dried, and powdered. The powdered material was subjected to successive solvent extraction using petroleum ether, chloroform, ethyl acetate, ethanol, and water. Extractive values were calculated according to standard pharmacopeial methods. [10-11]

**2.2 Experimental Animals**

Healthy adult Wistar albino rats (150–200 g) of either sex were procured and acclimatized for one week under standard laboratory conditions (temperature 22 ± 2 °C, 12 h light/dark cycle, relative humidity 50–60%). Animals were housed in polypropylene cages with free access to standard pellet diet and water. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and followed CPCSEA guidelines. [12-14]

**2.3 Acute Oral Toxicity Study (OECD 423)**

Acute toxicity was evaluated following OECD guideline 423. Groups of rats were administered CALP extract orally at doses of 500, 1000, 2000, and 5000 mg/kg. Animals were observed continuously for 4 hours and daily thereafter for 14 days for signs of toxicity and mortality. Parameters such as skin, mucous membranes, behavioral changes, salivation, tremors, convulsions, lethargy, defecation, and mortality were recorded. The $LD_{50}$ value was calculated using the Karber method. [16-19]

**2.4 Sub-Acute (28-Day) Toxicity Study (OECD 407)**

The 28-day repeated oral toxicity study was conducted as per OECD guideline 407. Rats were divided into four groups (n = 6 per group):

- Group I: Control (vehicle only, 0.5% CMC solution)
- Group II: CALP 500 mg/kg
- Group III: CALP 1000 mg/kg
- Group IV: CALP 2000 mg/kg

Doses were administered once daily by oral gavage for 28 consecutive days. Animals were observed daily for signs of toxicity, mortality, and changes in behavior. Body weight and food consumption were recorded weekly. [20-21]

**2.5 Hematological Analysis**

At the end of the experiment, blood samples were collected from retro-orbital plexus under light anesthesia. Hemoglobin (Hb), red blood cell (RBC) count, and platelet count were determined using an automated hematology analyzer.

**2.6 Biochemical Analysis**

Serum was separated and analyzed for biochemical parameters, including glucose, serum creatinine (Scr), blood urea nitrogen (BUN), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and bilirubin levels using commercial diagnostic kits. [22-24]

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### 2.7 Histopathological Examination

Following euthanasia, vital organs (liver, kidney, stomach, and heart) were excised, rinsed in normal saline, and fixed in 10% buffered formalin. After dehydration and embedding in paraffin, tissues were sectioned (5 µm thick) and stained with hematoxylin and eosin (H&E). Slides were examined microscopically for pathological changes such as congestion, necrosis, inflammation, and tissue degeneration. [25-30]

### 2.8 Statistical Analysis

All values were expressed as mean ± SEM (n=6). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. A $p$ value < 0.05 was considered statistically significant. [31]

## 3. Results

### 3.1 Organoleptic and Physicochemical Evaluation of CALP

The CALP root powder appeared whitish grey in color, with a pungent odor and bitter taste. Physicochemical analysis demonstrated a total ash content of 6.45%, acid insoluble ash of 1.60%, and water-soluble ash of 2.40%. Extractive values were highest with water (8.75%), followed by ethanol (6.70%) and ethyl acetate (4.60%). These values indicate a significant presence of polar and semi-polar phytoconstituents.

**Table 1. Physicochemical Characteristics of *Calotropis procera* (Root)**

| Parameter | CALP (Root) |
|---|---|
| Foreign organic content | 1.10% |
| Moisture content | 1.30% |
| Swelling factor | 1 |
| Foaming index | <100 |
| Total ash | 6.45% |
| Acid insoluble ash | 1.60% |
| Water soluble ash | 2.40% |
| Sulphated ash | 6.70% |
| Petroleum ether soluble matter | 2.30% |
| Chloroform soluble matter | 3.45% |
| Ethyl acetate soluble matter | 4.60% |
| Ethanol soluble matter | 6.70% |
| Water soluble matter | 8.75% |

### 3.2 Phytochemical Screening

Qualitative phytochemical analysis confirmed the presence of steroids, glycosides, tannins, flavonoids, carbohydrates, fixed oils, and alkaloids in CALP extracts. These bioactive constituents are known to possess both pharmacological and toxicological properties.

**Table 2. Phytochemical Constituents of CALP (Root extract)**

| Chemical Group | CALP |
|---|---|
| Steroids | + |
| Glycosides | + |
| Phenolic compounds & Tannins | + |
| Flavonoids | + |
| Fixed oils & fats | + |
| Carbohydrates | + |
| Alkaloids | + |

(+ indicates presence)

### 3.3 Acute Oral Toxicity

In the acute toxicity study, no mortality or severe toxic symptoms were observed up to a dose of 2000 mg/kg. At 5000 mg/kg, significant mortality was recorded (8 out of 12 animals). Using the Karber method, the $LD_{50}$ value of CALP was calculated to be **3000 mg/kg**.

Observed behavioral and clinical signs at higher doses included reduced locomotor activity, mild piloerection, and lethargy. No convulsions, salivation, or diarrhea were noted at lower doses.

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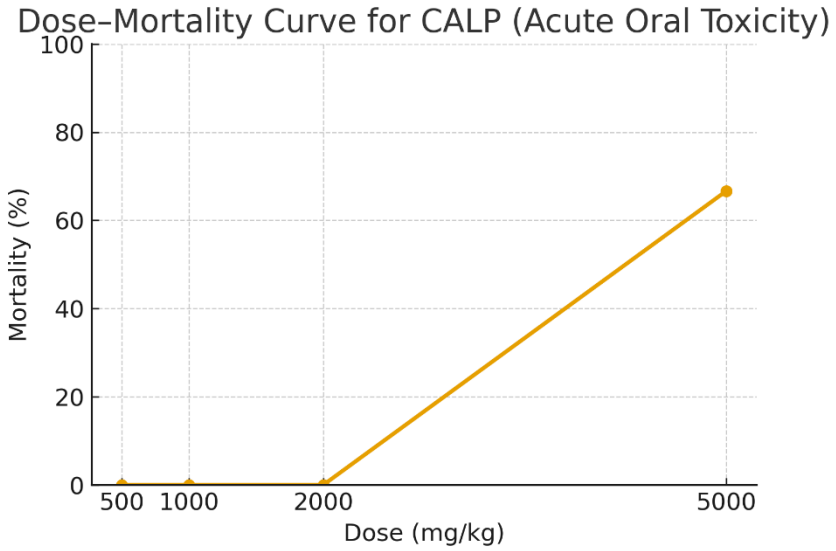


**Figure 1. Dose–Mortality Curve for CALP (Acute Oral Toxicity)**

### 3.4 Sub-Acute Toxicity (28-Day Oral Study)

#### 3.4.1 General Observations and Body Weight

Throughout the 28-day treatment period, rats in the control and low-dose group (500 mg/kg) showed no significant behavioral or physical changes. At higher doses (1000 and 2000 mg/kg), mild signs of toxicity such as reduced activity, occasional lethargy, and dullness of fur were noted after two weeks of exposure. No mortality was observed in any group. Body weight measurements demonstrated a gradual increase across all groups, but rats treated with 2000 mg/kg exhibited a relatively slower gain compared to control animals. This suggests that chronic exposure to higher doses of CALP may interfere with normal metabolic processes.

#### 3.4.2 Hematological Parameters

CALP administration produced a dose-dependent effect on hematological indices.

- At **500 mg/kg**, Hb, RBC, and platelet counts remained close to normal.
- At **1000 mg/kg**, there was a moderate reduction in RBC counts and a slight decrease in platelet levels.
- At **2000 mg/kg**, a significant reduction in Hb ($p<0.0001$), RBC ($p<0.0001$), and platelets ($p<0.05$) was observed compared to controls.

These results suggest that higher doses of CALP may induce anemia and thrombocytopenia, possibly due to the inhibitory effect of cardiac glycosides and alkaloids on hematopoietic processes.

**Table 3. Effect of CALP on Hematological Parameters (28-day study)**

| Group | Hb (g/dL) | RBC ($\times 10^6$/µL) | Platelets ($\times 10^5$/µL) |
|---|---|---|---|
| Control | 12.01 ± 0.41 | 5.11 ± 0.47 | 4.00 ± 0.19 |
| CALP 500 mg/kg | 10.35 ± 0.39 | 3.32 ± 0.06*** | 3.36 ± 0.06 |
| CALP 1000 mg/kg | 10.45 ± 0.41 | 2.71 ± 0.09**** | 2.90 ± 0.23* |
| CALP 2000 mg/kg | 8.38 ± 0.18**** | 2.42 ± 0.12**** | 2.96 ± 0.21* |

(*$p<0.05$, ***$p<0.001$, ****$p<0.0001$ compared to control, n=6)

#### 3.4.3 Biochemical Parameters

Sub-acute administration of CALP caused significant alterations in serum biochemical markers, reflecting hepatic and renal impairment.

- **Glucose:** Increased significantly in all treated groups, with maximum rise at 2000 mg/kg (307.8 mg/dL vs 116.7 mg/dL in controls).
- **Serum Creatinine (Scr):** Increased dose-dependently, indicating renal stress.
- **Blood Urea Nitrogen (BUN):** Significantly elevated at all doses, suggesting impaired renal clearance.
- **Alkaline Phosphatase (ALP) and AST:** Markedly elevated at 1000 and 2000 mg/kg, indicating hepatocellular damage.
- **Bilirubin:** Significantly elevated at higher doses, reflecting hepatic dysfunction.

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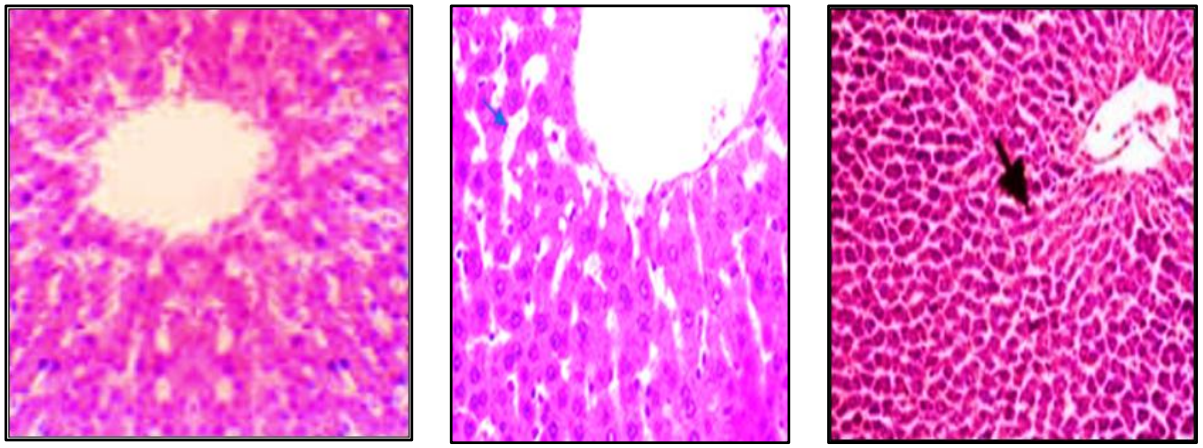
**Table 4. Effect of CALP on Biochemical Parameters (28-day study)**

| Group | Glucose (mg/dL) | Scr (mg/dL) | BUN (mg/dL) | ALP (U/L) | AST (U/L) | Bilirubin (mg/dL) |
|---|---|---|---|---|---|---|
| Control | 116.7 ± 5.62 | 0.54 ± 0.14 | 24.1 ± 1.97 | 537.8 ± 13.3 | 218.5 ± 8.26 | 0.43 ± 0.05 |
| CALP 500 mg/kg | 207.1 ± 7.05**** | 1.67 ± 0.05**** | 56.6 ± 2.32**** | 631.1 ± 15.4**** | 341.1 ± 16.9**** | 1.67 ± 0.05**** |
| CALP 1000 mg/kg | 242.3 ± 12.7**** | 1.68 ± 0.16**** | 60.3 ± 2.47**** | 680.8 ± 14.5**** | 370.0 ± 6.84**** | 1.68 ± 0.16**** |
| CALP 2000 mg/kg | 307.8 ± 5.21**** | 1.89 ± 0.23**** | 75.5 ± 2.14**** | 702.6 ± 19.1**** | 374.3 ± 7.62**** | 1.89 ± 0.21**** |

(****p<0.0001 compared to control, n=6)

### 3.4.4 Histopathological Findings

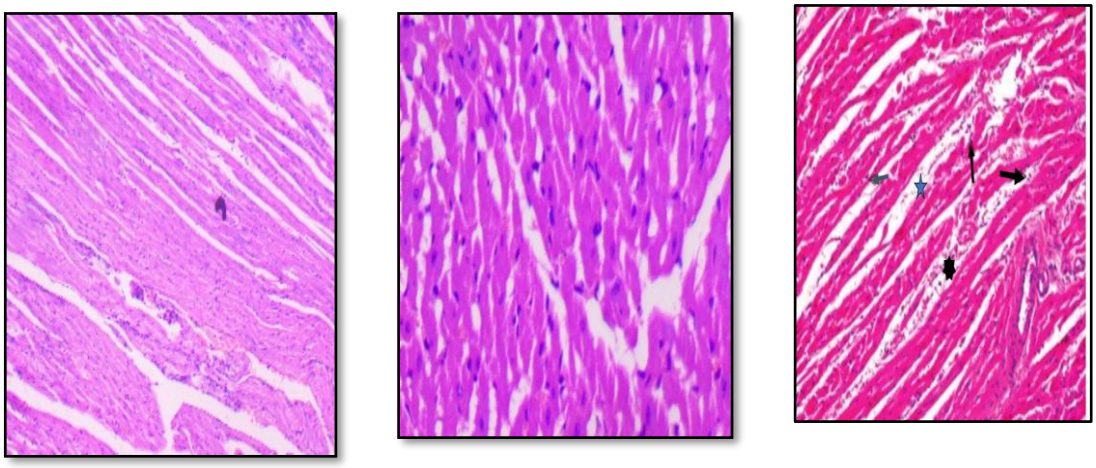
Histopathological examination of the liver following the administration of *CALP* at 500 mg/kg showed no signs of toxicity, with normal liver architecture observed. At 1000 mg/kg, mild toxicity was noted, including slight liver congestion and minor alterations in hepatocyte structure. At 2000 mg/kg, significant toxicity was evident, with moderate congestion, hepatocyte degeneration, and areas of necrosis. These findings suggest that while *CALP* is safe at 500 mg/kg, higher doses may lead to liver damage, with 2000 mg/kg being toxic. **Figure 5.4.2.12.3.1**



CALP 500mg/kg CALP 1000mg/kg CALP 2000 mg/kg

**Figure 5.4.2.12.3.1** Effect of *Calotropis procera* on liver at different doses

Histopathological examination of the stomach following the administration of CALG at 500 mg/kg showed normal gastric mucosa with no signs of toxicity. At 1000 mg/kg, mild congestion and slight epithelial cell changes were observed, indicating early signs of toxicity. At 2000 mg/kg, more pronounced damage was evident, including erosion of the gastric mucosa, epithelial cell degeneration, and moderate inflammation. These findings suggest that while CALG is safe at 500 mg/kg, higher doses may lead to gastric mucosal damage, with 2000 mg/kg being toxic. **Figure 5.4.2.12.3.2**



CALP 500 mg/kg CALP 1000 mg/kg CALP 2000 mg/kg

**Figure 5.4.2.12.3.2** Effect of *Calotropis procera* on stomach at different doses

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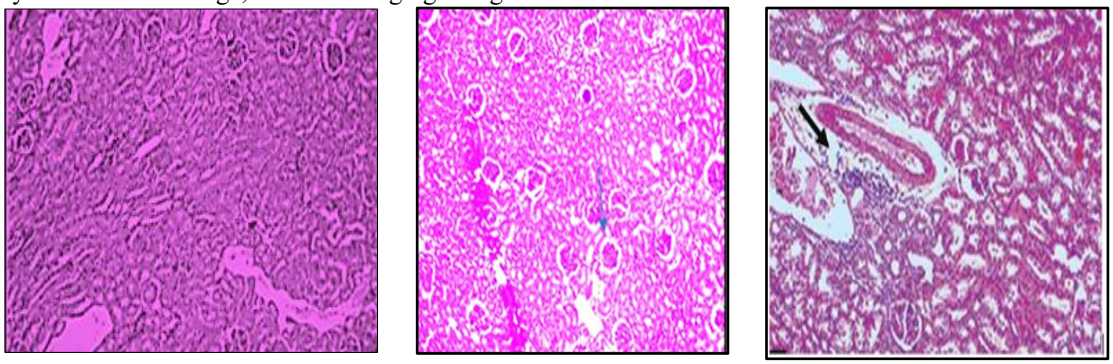
Histopathological examination of the kidneys following the administration of CALG at 500 mg/kg revealed no toxicity, with normal renal architecture observed. At 1000 mg/kg, mild signs of toxicity were noted, including slight changes in the renal tubules and minor congestion. At 2000 mg/kg, more severe renal damage was evident, including pronounced tubule degeneration, necrosis, and congestion. These findings indicate that while CALG is safe at 500 mg/kg, higher doses may cause renal damage, with 2000 mg/kg being toxic.



CALP 500 mg/kg CALP 1000 mg/kg CALP 2000 mg/kg

**Figure 5.4.2.12.3.3** Effect of *Calotropis procera* on Kidney at different doses

Histopathological examination of the heart following the administration of CALG at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg showed no signs of toxicity, with normal heart tissue observed at all doses. These findings suggest that CALG is safe for the heart at all tested doses.

## 4. Discussion

### 4.1 Overview of Findings

The present investigation provides a comprehensive toxicological profile of *Calotropis procera* (CALP) root extract based on acute and sub-acute oral administration in Wistar rats. The study demonstrated that CALP is relatively safe at lower doses (≤500 mg/kg), but prolonged exposure at moderate to high doses (1000–2000 mg/kg) resulted in significant hematological, biochemical, and histopathological alterations. The $LD_{50}$ was calculated as 3000 mg/kg, confirming a moderate level of acute toxicity. Sub-acute studies revealed that liver, kidney, and stomach are the principal target organs of toxicity, while the heart remained unaffected.

These findings align with the dual nature of CALP: while it possesses bioactive constituents with medicinal benefits, it also contains toxic principles that can lead to systemic organ damage if consumed at higher doses.

### 4.2 Hematological Effects

CALP administration significantly decreased hemoglobin levels, RBC counts, and platelet counts at 1000 and 2000 mg/kg. The observed anemia could be attributed to the inhibitory effects of cardenolides and alkaloids on bone marrow function, leading to suppression of erythropoiesis. Alternatively, increased destruction of RBCs (hemolysis) due to oxidative stress induced by CALP phytochemicals may also play a role.

Thrombocytopenia observed at higher doses suggests a possible impairment of platelet production or survival. Since platelets are critical for clotting, this effect may have clinical implications such as increased bleeding risk. These results corroborate earlier studies reporting hematological suppression in animals exposed to *Calotropis* extracts.

### 4.3 Biochemical Alterations and Organ Toxicity

#### 4.3.1 Hyperglycemia

Significant elevation in serum glucose levels was noted in all treated groups, particularly at 2000 mg/kg. This hyperglycemia may be due to β-cell damage in the pancreas or induction of insulin resistance by CALP phytochemicals. Glycosides and triterpenoids are known to alter carbohydrate metabolism, which could explain this metabolic disturbance.

#### 4.3.2 Renal Dysfunction

The dose-dependent increase in serum creatinine and blood urea nitrogen (BUN) levels indicates renal impairment. Histopathological evidence of tubular degeneration and necrosis supports this conclusion. The nephrotoxic effects of CALP could be attributed to direct cytotoxic action of glycosides or alkaloids on renal tubular epithelium, leading to compromised glomerular filtration and excretory function.

#### 4.3.3 Hepatic Damage

Significant elevations in liver enzymes (ALP, AST) and bilirubin at 1000 and 2000 mg/kg indicate hepatocellular injury. Histopathological findings of hepatocyte degeneration, congestion, and necrosis further confirm hepatic damage. These

alterations may result from oxidative stress induced by phenolic compounds and glycosides, leading to lipid peroxidation and membrane disruption in hepatocytes.

**4.3.4 Gastric Mucosal Injury**
CALP induced dose-dependent gastric mucosal damage, with epithelial desquamation and erosion at 2000 mg/kg. This effect may be related to the irritant properties of latex-derived compounds such as proteolytic enzymes (calotropain) and resinous substances. Gastric irritation is consistent with traditional warnings about the caustic nature of CALP latex.

**4.3.5 Cardiac Tissue Safety**
Interestingly, cardiac tissues did not exhibit pathological changes across all treatment groups. Despite the presence of cardiac glycosides in CALP, which are known for cardiotoxicity, histological analysis showed normal myocardial fibers. This may be due to the relatively short duration of exposure (28 days) or lower systemic availability of active glycosides in the doses tested. However, further long-term studies are warranted to exclude delayed cardiotoxic effects.

**4.4 Mechanisms of Toxicity**
The toxic effects of Calotropis procera (CALP) are believed to occur through several interrelated mechanisms. The cardiac glycosides present in the plant, such as calotropin and uscharin, inhibit $Na^+/K^+$-ATPase activity, which disrupts ion homeostasis and leads to oxidative stress and cellular toxicity. In addition, alkaloids and triterpenoids have been reported to exert cytotoxic effects by promoting oxidative stress, thereby impairing liver and kidney function. Proteolytic enzymes like calotropain contribute to local tissue irritation, gastric erosion, and inflammation, further aggravating the toxic response. Moreover, excessive generation of reactive oxygen species (ROS) induces oxidative stress, which plays a key role in hematological suppression, hepatocellular injury, and renal dysfunction. Collectively, these mechanisms highlight the multifaceted pathways through which CALP exerts its toxicological effects. [31-40]

**4.5 Comparison with Previous Studies**
The present results are consistent with earlier reports. Ali et al. (2012) reported hepatic and renal toxicity of CALP latex in rodents, with similar increases in liver enzymes and renal markers. Al-Snafi (2015) reviewed CALP toxicity and highlighted its irritant, hepatotoxic, and abortifacient effects. Our findings extend this knowledge by demonstrating clear dose-dependent relationships, supported by hematological and histopathological evidence.
While most studies emphasize latex toxicity, our work shows that root extracts also exert significant systemic toxicity when administered orally. The consistency across different plant parts underscores the need for caution in therapeutic use. [46]

**4.6 Implications for Traditional and Modern Medicine**
CALP is used in folk medicine for treating fevers, inflammation, and skin ailments, often administered as crude extracts without dosage standardization. The current findings highlight the risks of uncontrolled use, especially at higher doses or prolonged administration. [41-145]
For modern drug development, CALP phytochemicals hold promise as bioactive leads (e.g., anticancer agents). However, their therapeutic window is narrow, and careful toxicological evaluation is essential. Detoxification methods, selective extraction of beneficial compounds, and structural modifications may help reduce toxicity while retaining efficacy.

**4.7 Limitations of the Study**
The present study had certain limitations that should be acknowledged. First, only root extracts of Calotropis procera were evaluated, while other plant parts such as latex, leaves, and flowers may possess distinct toxicity profiles that remain unexplored. Second, the investigation was confined to a 28-day sub-acute period, which restricts conclusions regarding long-term safety; therefore, chronic exposure studies are necessary to better understand its toxic potential. Finally, mechanistic assays, including assessments of oxidative stress markers and apoptosis pathways, were not performed. Incorporating such analyses in future studies could provide more comprehensive insights into the underlying mechanisms of CALP-induced toxicity.

**4.8 Future Directions**
Future research directions should focus on addressing the existing gaps in knowledge regarding the safety of *Calotropis procera*. Long-term chronic toxicity and reproductive toxicity studies are essential to establish its comprehensive toxicological profile. Furthermore, isolating and evaluating specific phytochemicals responsible for toxicity would help in identifying the active compounds contributing to adverse effects. Traditional detoxification strategies, such as boiling or combining the plant with other herbs, also warrant scientific validation to assess their effectiveness in reducing toxicity. In addition, the development of standardized CALP extracts with well-defined safe dosage ranges would be a crucial step toward ensuring its safe therapeutic application

## 5. Conclusion

The present study provides a systematic evaluation of the toxicological effects of *Calotropis procera* (CALP) root extract in Wistar rats. Acute oral toxicity testing established an $LD_{50}$ of 3000 mg/kg, confirming that the plant possesses moderate acute toxicity. Sub-acute (28-day) oral administration revealed dose-dependent hematological, biochemical, and histopathological changes, with significant toxicity evident at doses of 1000 and 2000 mg/kg.

**Key findings include:**

- **Hematological alterations**: Dose-dependent reductions in hemoglobin, red blood cells, and platelets, indicating risk of anemia and thrombocytopenia at higher doses.
- **Biochemical changes**: Elevated glucose, serum creatinine, blood urea nitrogen, liver enzymes (ALP, AST), and bilirubin, suggesting metabolic stress and organ dysfunction.
- **Histopathology**: Clear evidence of hepatocellular degeneration and necrosis, renal tubular damage, and gastric mucosal erosion at higher doses, while heart tissues remained unaffected.

These results highlight that CALP is **relatively safe at lower doses (≤500 mg/kg)**, but toxic at higher concentrations, particularly affecting the liver, kidney, and stomach. Given its wide use in traditional medicine, these findings underscore the need for **cautious use, dose standardization, and further mechanistic studies**. CALP remains a plant of high pharmacological interest, but its narrow therapeutic window necessitates careful toxicological evaluation before incorporation into modern formulations.

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