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# Acute and subacute toxicity study of ethanolic extract of *Calotropis procera* (Aiton) Dryand flower in Swiss albino mice

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ABSTRACT

*Background:* *Calotropis procera* is a large shrub which consists many medicinal properties, used in treatment of snake bite, sinus fistula, rheumatism, mumps, burn injuries, inflammation and jaundice traditionally. All the parts of *Calotropis procera* were utilized in the treatment of diseases out of which leaves and roots were investigated for its toxicity profile that showed dose dependent toxicity. Toxicity profile of flowers of *Calotropis procera* was not investigated in the previous studies. The aim of this study was to explore the acute and subacute toxicity of ethanolic extract of *Calotropis procera* flowers for the safe use of traditional medicine.

*Method:* In acute toxicity, a total of 20 female mice (Swiss albino), weighing between 23 and 32 g were randomly divided into four experimental groups: control, 300, 1000, and 2000 mg/kg groups with 5 mice each, and each received a single dose of extract at 300, 1000, or 2000 mg/kg, respectively. Animals were monitored for 14 days. In the subacute study, a total of 40 mice (23–32 g) were divided into 4 groups, each containing males and females. Group 1 (control group) received vehicle and groups 2, 3, and 4 received extract at doses of 300 mg/Kg, 1000 mg/Kg, 2000 mg/Kg of b.w., respectively, for 28 consecutive days. The study was conducted in compliance with the OECD guidelines 407 and 423.

*Results:* Acute toxicity study showed no mortality at the dose of 2000 mg/Kg. In subacute toxicity study, statistical analysis of hematological and biochemical parameters showed no significant differences compared to control group except marked increase in segmented neutrophils. Histopathological studies revealed no significant structural differences among the treated groups and in comparison to control group.

*Conclusions:* It was concluded that oral administration of doses of ethanolic extract of *Calotropis procera* flower, administered acutely, did not cause any mortality or notable changes at the dose of 2000 mg/Kg. Therefore, the approximate lethal dose (ALD) of in mice was higher than 2,000 mg/kg. In a 28-day subacute toxicity model, the extract did not cause any mortality, and no treatment-related changes were observed in body weight, organ weight, hematological and biochemical blood analysis, or histopathologic examinations at the extract dose of 2000 mg/Kg. These findings indicate that the no-observed-adverse-effect-level (NOAEL) of *Calotropis procera* flower ethanolic extract was greater than 2000 mg/kg/day.

## 1. Introduction

Medicinal plants play a significant role in our daily lives, and they are frequently utilized as therapeutic agents for a variety of diseases and maladies (Mossa et al., 1991). In many developing countries more than 80% population use some part of their medication in the form of natural sources (Basak et al., 2009). In India many texts on natural medicine are available for traditional medication known as Ayurvedic system of medication. Natural treatments have attracted much interest in the U. S. and some countries in Europe in recent years (RC et al., 2016). The whole plant or portions of the plant are used to treat a variety of diseases, each of which should be studied separately for toxicity. *Calotropis*

; EtCP, Ethanolic extract of *Calotropis procera*; b.w., body weight; ANOVA, Analysis of variance; OECD, Organisation for Economic Co-operation and Development.

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*procera* is known by a variety of names in different parts of the world, notably apple of calotrope, big milkweed, sodom, Indian milkweed, rubber tree, wild cotton, and ushar. (Dhileepan, 2014). *Calotropis procera* is native to Africa, Western Asia, Arabian Peninsula, the Indian Subcontinent etc. ("Calotropis procera (Aiton) W. T. Aiton GRIN-Global, " n.d.). *Calotropis procera* is a plant of family Apocynaceae and sub family Asclepiadaceae that has traditionally been used to medicate fever, asthma, rheumatism, digestion, cold, eczema, boils, elephantiasis, leprosy, cough, diarrhea, intestinal worms, and heal leucoderma (Silva et al., 2010) (Quazi et al., 2013). Other pharmacological activities were investigated in different research such as anti-inflammatory activity (Kumar and Basu, 1994), anthelmintic activity (Shivkar and Kumar, 2008), anticonvulsant activity (Jalalpure et al., 2009), antimicrobial activity (Nenaah, 2013), anticancer activity (Choedon et al., 2006), and antinociceptive activity (Obese et al., 2021) (Matias et al., 2005) on leaf.

*Calotropis procera* is found in dry and semi-arid regions (Al-Rowaily et al., 2020). Young stems are smooth and green in appearance, however matured stems have fissured bark (Hassan et al., 2015). The leaves are oriented in opposite phyllotaxy, are pale green, and succulent. Flowers are bisexual and have five petals. Several phytocompounds such as flavonoids, tannins, terpinoids, saponins, steroids, linolenic acid, amino acid, palmitic acid, and fatty ethyl esters were identified in *Calotropis procera* leaf extract (Kaur et al., 2021).

Leaves, stems, roots, barks and flowers of CP are used for treatment of various diseases (Raginee Verma and Microbiology, 2010). As per literature search and knowledge toxicity study of CP flower is not performed till date. In the current study, investigation of acute and subacute toxicity studies of CP flower has been performed for its use as a safe and effective treatment.

## 2. Material and method

### 2.1. Plant material

The flowers of *Calotropis procera* were collected in Varanasi area of Uttar Pradesh, India. The plant was authenticated by Assistant Professor, Ashwini Kumar Kushwaha, and a voucher specimen (no. 2019–02) was deposited in the herbarium of the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University.

### 2.2. Preparation of extracts

Flowers of *Calotropis procera* were dried and milled in powder form at room temperature. 300 g of power was macerated with 2100 mL of 95% ethanol (1:7 w/v). The extract was concentrated using rotary evaporator (Buchi R-210 Advanced, Switzerland) at 20 °C. Percentage yield was obtained 15.9% w/w. The extract was kept at room temperature until they were evaluated. (Costa et al., 2020).

### 2.3. Chemicals

Urea, creatinine, alanine amino transaminase (ALT), alanine phosphatase (ALP) and aspartate aminotransferase (AST) kits were purchased from Erba Mannheim Pvt. Ltd. Ethanol was purchased from Sisco Research Laboratories Pvt. Ltd.

### 2.4. Animals

Swiss Albino mice (25–33 g) were obtained from the animal house, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Animals were acclimatized in temperature controlled room and provided tap water and food *ad-libitum*. The study was approved by Central Animal Ethical Committee, Banaras Hindu University as per guidelines of CPCSEA (Reg. No. 542/GO/Rebi//S/02/CPCSEA dated 26.5.2017).

### 2.5. Acute oral toxicity

Acute toxicity test was performed as per guidelines of the Organization for Economic Cooperation and Development (OECD) 423 (*Test No. 423: Acute Oral toxicity - Acute Toxic Class Method*, 2002). Total 20 mice were divided in 4 groups, each group containing 5 mice. Group1 (control group) received vehicle, group 2, 3, 4 received EtCP 300 mg/kg, 1000 mg/kg and 2000 mg/Kg of b.w. respectively as single dose. The animals were monitored for mortality and other visual changes during the first 30 min, then at regular intervals over the next 24 h, with close monitoring during the first four hours. Animals were watched once a day for 14 days. Animals were provided food and water, and their daily intake was recorded. Body weight, food intake, and water consumption were all recorded and compared weekly to see if there were any changes. Deaths and other physical changes were recorded. At the end of experiment, the animals were sacrificed with anesthesia (Di-ethyl ether) and organs (liver, brain, lungs, kidney, spleen, and heart) were dissected and weighed immediately to estimate organ/body weight ratio. An organ/-body weight ratio is defined as the weight percentage of an organ to the animal's body weight (Porwal et al., 2017).

### 2.6. Sub-acute oral toxicity

Sub-acute toxicity study of EtCP was performed on mice as per OECD guideline 407 (OECD, 2008). A total of 40 animals were divided into four groups, each with both male and female animals. For 28 days, Group 1 (control group) received a vehicle, whereas Groups 2, 3, and 4 received extract dosase of 300 mg/Kg, 1000 mg/Kg, and 2000 mg/Kg of b.w., respectively. Body weight, food and water intake, and the development of any side effects were all observed during the study. Weekly weight, food, and water intake were compared. Blood samples were taken at the end of the study for hematological and biochemical analysis. After blood collection, animals were sacrificed using di-ethyl ether, and target organs (liver, brain, lungs, kidney, spleen, and heart) were dissected and weighed immediately to estimate organ/body weight ratio, and a part of the organs was kept in 10% formalin for histological examinations. The organ/body weight ratio was determined as (organ weight (g)/body weight of the animal on sacrifice day (g)) × 100 (Porwal et al., 2017).

#### 2.6.1. Histological analysis

Organs that had been kept in formalin were imbedded in paraffin. A microtome cutter was used to slice embedded tissues with a thickness of 5 μm. The samples were stained with hematoxylin and eosin, and tissue sections were examined under an inverted microscope at 400x to observe histological changes in the organs. (Calil Brondani et al., 2017).

#### 2.6.2. Hematological and biochemical analysis

$K_2$EDTA tubes were used to collect the blood for hematological analysis. Hb (g/dL), PCV (%), RBC count (mill/mm$^3$), MCV (fL), MCH (pg), RDW (%), TLC (thou/mm$^3$), segmented neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), neutrophils (thou/mm$^3$), lymphocytes (thou/mm$^3$), monocytes (thou/mm$^3$), eosinophils (thou/mm$^3$), basophils (thou/mm$^3$), platelets (thou/mm$^3$) and mean platelet volume (fL) were estimated in hematological analysis by using an automatic blood cell analyzer (arkray's auto hematology analyzer). Biochemical analysis was performed on serum, obtained from centrifugation of solidified blood samples at 4000 rpm for 10 min at temperature 4 °C. Albumin, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were analyzed by kits purchased from Erba Mannheim Pvt. Ltd (Yang et al., 2019).

### 2.7. Statistical analysis

Statistical tool one way ANOVA followed by Dunnett's multiple

comparisons test was performed. The values were considered to be significantly different compared to control group with treatment groups when $p < 0.05$. Results were expressed as Mean ± SEM (Standard error of the mean). Data analysis were performed by the software GraphPad Prism 8.0.2.

## 3. Results

### 3.1. Acute toxicity study

The body weight gain of the control and treatment groups was comparable. During the 14-day study, there was no mortality and no behavioural abnormalities were seen at EtCP dosages of 300, 1000, and 2000 mg/Kg b.w. The body weight gain of the control and treatment groups was similar, but no statistically significant difference was seen. Food and water intake were slightly lower in the treated groups than in the control groups, but no significant differences were identified in the individual groups. The organ/body weight ratio of the organs heart, liver, spleen, kidney, lung, and brain reveal no significant difference when compared to the control. During the 14-day period following EtCP treatment, no deaths were recorded. The acute toxicity test indicated that a single dose of EtCP (2000 mg/kg) administered orally did not produce morbidity or mortality in treated animals over a 14-day period.

#### 3.1.1. Body weight gain, food and water consumption of mice treated orally with EtCP

During the entire study, there was no death recorded. There were no behavioural changes seen in either the EtCP-treated or control groups. As shown in Table 1, the body weight of all groups of animals increased in the first and second weeks, but no significant changes observed. Food and water consumption did not alter considerably.

#### 3.1.2. Organ/body weight ratio

The organ/body weight ratio of the lungs, heart, liver, kidney, spleen, and brain were found to be within the normal range, and no significant difference was identified between the treatment and control groups. Table. 2.

### 3.2. Subacute toxicity study

#### 3.2.1. Mortality and behavioral observations

In the 28-day subacute toxicity study, there was no behavioural change and no mortality was recorded at the doses of 300, 1000, and 2000 mg/Kg b.w. of EtCP. There was no adverse events was recorded in any of the treated groups.

**Table 1**
Body weight gain, food and water consumption of mice treated orally with EtCP flower in acute toxicity study.

| Parameters | Control | EtCP 300 mg/kg | 1000 mg/kg | 2000 mg/kg |
|---|---|---|---|---|
| Initial wt.(g) | 31.9 ± 3.0 | 28.13 ± 1.88 | 30.66 ± 2.33 | 26.83 ± 208 |
| One week(g) | 32.43 ± 2.81 | 28.56 ± 1.97 | 31.2 ± 2.4 | 27.23 ± 20 |
| Two week(g) | 32.86 ± 2.86 | 29.43 ± 1.76 | 31.46 ± 2.48 | 27.7 ± 0.36 |
| BWG(g) | 0.96 | 1.3 | 0.8 | 0.87 |
| Food Intake(g/day) | 10.15 ± 0.37 | 8.85 ± 0.48 | 9.2 ± 0.49 | 8.9 ± 0.36 |
| Water Intake (ml/day) | 10.3 ± 1.80 | 11.5 ± 0.82 | 8.9 ± 0.42 | 9.6 ± 0.37 |

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Dunnett's multiple comparisons test was performed. There was no significant change in body weight, food intake, and water intake in the treatment groups when compared to the control group ($p > 0.05$). Body weight gain (BWG), ethanolic extract of *Calotropis procera* (EtCP).

**Table 2**
The organ/body weight ratio of mice treated orally with EtCP flower in acute toxicity study.

| Parameters | Control | EtCP 300 mg/kg | 1000 mg/kg | 2000 mg/kg |
|---|---|---|---|---|
| Heart | 0.44 ± 0.03 | 0.42 ± 0.03 | 0.53 ± 0.07 | 0.49 ± 0.02 |
| Liver | 5.19 ± 0.12 | 4.99 ± 0.69 | 4.40 ± 0.65 | 5.08 ± 0.09 |
| Spleen | 0.35 ± 0.03 | 0.68 ± 0.10 | 0.54 ± 0.10 | 0.60 ± 0.13 |
| Kidney | 0.65 ± 0.02 | 0.68 ± 0.04 | 0.69 ± 0.06 | 0.69 ± 0.08 |
| Lung | 0.84 ± 0.00 | 0.70 ± 0.07 | 0.88 ± 0.08 | 0.85 ± 0.12 |
| Brain | 1.02 ± 0.04 | 1.08 ± 0.13 | 1.11 ± 0.12 | 1.17 ± 0.10 |

Values are expressed as the mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparison test were performed. The organ/body weight ratio of the heart, liver, kidney, lungs, and brain of all the treated groups did not change significantly compared to the control group ($p > 0.05$). The organ/body weight ratio was calculated as (organ weight (g)/body weight of animal on sacrifice day (g)) × 100. Ethanolic extract of *Calotropis procera* (EtCP).

#### 3.2.2. Body weight gain, food and water consumption of mice treated orally with EtCP for 28 days

The body weight of animals in all groups was increased in the first and second weeks, but no significant change was recorded among all the groups. Food consumption and water intake were similar in the control and treated groups without any significant change. (Table.3)

#### 3.2.3. Organ/body weight ratio

After 28 days of treatment with extract dosages of 300, 1000, and 2000 mg/kg b.w., no significant changes were seen in the organ/body weight ratio (liver, kidneys, spleen, heart, lungs, brain) of the experimental groups compared to the control group. (Table 4)

#### 3.2.4. Hematological parameters

All hematological parameters were estimated and found to be within normal limits, with no significant differences between the control and EtCP-treated groups ($p > 0.05$). At higher dosages, all experimental groups showed an increase in Segmented N (%) and Neutrophils (thou/$mm^3$) levels when compared to the control group. (Table. 5).

#### 3.2.5. Serum biochemical analysis

The results of biochemical analysis of all EtCP treatment groups (300

**Table 3**
Body weight gain, food and water consumption of mice treated orally with EtCP flower in the subacute toxicity study.

| Parameters | Control | EtCP 300 mg/kg | 1000 mg/kg | 2000 mg/kg |
|---|---|---|---|---|
| Initial wt. (g) | 28.63±1.48 | 25.36 ± 3 | 26.53 ± 0.78 | 28.9 ± 2.20 |
| One week (g) | 29.1 ± 1.44 | 25.86 ± 2.9 | 26.93 ± 0.63 | 29.6 ± 1.86 |
| Two week (g) | 29.53 ± 1.40 | 26.33 ± 2.75 | 27.36 ± 0.6 | 30.16 ± 1.84 |
| Three week (g) | 29.96 ± 1.37 | 26.73 ± 2.46 | 27.76 ± 0.54 | 30.7 ± 1.76 |
| Final weight (g) | 30.33 ± 1.44 | 27.16 ± 2.5 | 28.26 ± 0.46 | 31.2 ± 1.81 |
| BWG (g) | 1.7 | 1.8 | 1.73 | 2.3 |
| Food Intake (g/day) | 8.86 ± 0.20 | 8.22 ± 0.32 | 9.4 ± 0.49 | 8.6 ± 0.45 |
| Water Intake (ml/day) | 10.2 ± 0.72 | 9.1 ± 0.68 | 10.2 ± 0.49 | 8.9 ± 0.57 |

Values are expressed as mean ± SEM. Statistical tool one way ANOVA followed by Dunnett's multiple comparisons test was performed. The values were considered significantly different compared to control when p value <0.05. There was no significant change in the parameters during the treatment. Body weight gain (BWG), ethanolic extract of *Calotropis procera* (EtCP).

**Table 4**
The organ/body weight ratio of mice treated orally with EtCP flower in subacute toxicity study.

| Parameters | Control | EtCP 300 mg/kg | 1000 mg/kg | 2000 mg/kg |
|---|---|---|---|---|
| Heart | 0.36 ± 0.03 | 0.43 ± 0.06 | 0.46 ± 0.06 | 0.42 ± 0.05 |
| Liver | 3.44 ± 0.20 | 5.01 ± 1.01 | 4.54 ± 0.14 | 3.79 ± 0.30 |
| Spleen | 0.40 ± 0.01 | 0.62 ± 0.11 | 0.35 ± 0.01 | 0.31 ± 0.03 |
| Kidney | 0.49 ± 0.03 | 0.60 ± 0.05 | 0.57 ± 0.01 | 0.52 ± 0.02 |
| Lung | 0.66 ± 0.01 | 0.72 ± 0.07 | 0.71 ± 0.06 | 0.72 ± 0.04 |
| Brain | 0.89 ± 0.06 | 1.02 ± 0.10 | 0.97 ± 0.04 | 0.86 ± 0.07 |

Values are expressed as mean± SEM. Statistical tool one way ANOVA followed by Dunnett's multiple comparison test was performed. The organ/body weight ratio of the heart, liver, kidney, lungs, and brain of all the treated groups did not change significantly compared to the control group ($p > 0.05$) The organ/body weight ratio was calculated as (organ weight (g)/body weight of animal on sacrifice day (g)) × 100. Ethanolic extract of *Calotropis procera* (EtCP).

**Table 5**
Hematological analysis of mice treated orally with EtCP for 28 days.

| Parameters | | Control | EtCP 300 mg/Kg | 1000 mg/Kg | 2000 mg/Kg |
|---|---|---|---|---|---|
| Hb (g/dL) | | 14.09 ± 0.36 | 14.70 ± 0.15 | 15.20 ± 0.40 | 14.23 ± 0.09 |
| PCV (%) | | 55.90 ± 0.72 | 53.67 ± 0.69 | 57.97 ± 0.84 | 56.30 ± 0.52 |
| RBC COUNT (mill/mm$^3$) | | 9.26 ± 0.44 | 9.40 ± 0.74 | 10.25 ± 0.09 | 9.64 ± 0.11 |
| MCV (fL) | | 59.63 ± 1.72 | 54.63 ± 2.20 | 57.67 ± 0.41 | 57.10 ± 0.76 |
| MCH (pg) | | 15.10 ± 0.61 | 14.63 ± 0.20 | 15.07 ± 0.09 | 15.20 ± 0.06 |
| MCHC (g/dL) | | 25.13 ± 1.27 | 26.80 ± 0.21 | 26.13 ± 0.03 | 26.03 ± 0.09 |
| RDW(%) | | 25.30 ± 0.57 | 22.20 ± 0.12 | 20.37 ± 1.16 | 20.53 ± 0.29 |
| TLC (thou/mm$^3$) | | 3.95 ± 0.99 | 6.10 ± 0.17[a] | 5.45 ± 0.28 | 5.30 ± 0.12 |
| DLC | Segmented Neutrophils (%) | 11.90 ± 0.35 | 22.90 ± 0.38[d] | 29.87 ± 0.34[d] | 45.63 ± 2.43[d] |
| | Lymphocytes (%) | 87.30 ± 8.51 | 70.77 ± 5.55 | 62.77 ± 7.19 | 60.77 ± 9.23 |
| | Monocytes (%) | 0.80 ± 0.06 | 0.90 ± 0.05 | 0.97 ± 0.18 | 0.87 ± 0.18 |
| | Eosinophils (%) | 0.03 ± 0.03 | 1.23 ± 0.73 | 1.27 ± 0.49 | 2.03 ± 0.82 |
| | Basophils (%) | 0.00 ± 0.00 | 0.17 ± 0.07[b] | 0.00 ± 0.00 | 0.00 ± 0.00 |
| ALC | Neutrophils (thou/mm$^3$) | 0.48 ± 0.05 | 1.43 ± 0.09[d] | 1.70 ± 0.06[d] | 2.17 ± 0.14[d] |
| | Lymphocytes (thou/mm$^3$) | 3.48 ± 0.22 | 4.22 ± 0.17 | 3.73 ± 0.29 | 2.85 ± 0.34 |
| | Monocytes (thou/mm$^3$) | 0.04 ± 0.01 | 0.17 ± 0.07 | 0.28 ± 0.12 | 0.11 ± 0.01 |
| | Eosinophils (thou/mm$^3$) | 0.00 ± 0.00 | 0.11 ± 0.05 | 0.05 ± 0.04 | 0.12 ± 0.04 |
| | Basophils (thou/mm$^3$) | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Platelets (thou/mm$^3$) | | 726.33 ± 97.50 | 1146 ± 182.63 | 863.33 ± 79.65 | 926.67 ± 66.92 |
| Mean Platelet Volume (fL) | | 6.53 ± 0.19 | 7.23 ± 0.18 | 6.97 ± 0.28 | 7.13 ± 0.20 |

Data are expressed as mean ± SEM. One way ANOVA followed by Dunnett's multiple comparison test was performed. Hemoglobin (Hb), packed cell volume (PCV), red blood cells counts (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cells distribution width (RDW), total leucocyte count (TLC), differential leucocyte count (DLC), absolute leucocyte count (ALC), ethanolic extract of *Calotropis procera* (EtCP). The values were considered to be significantly different compared to control when $p < 0.05$. ($^d = p < 0.0001$, $^c = p < 0.001$, $^b = p < 0.01$, $^a = p < 0.05$).

mg/Kg, 1000 mg/Kg, and 2000 mg/Kg) were compared to the control group, and no significant difference was detected ($p > 0.05$). The data were expressed using the mean ± SEM ($p > 0.05$). (Fig. 1).

### 3.2.6. Histological analysis

The liver, heart, kidney, spleen, lungs, and brain were sectioned for histological study to observe the changes in organs at the dosages (EtCP) of 300 mg, 1000 mg, and 2000 mg/kg. Observation by the microscope showed no remarkable histological changes. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. Spleen, brain, heart, and lungs showed normal architecture. (Fig. 2).

## 4. Discussion

There is no scientific data on the toxicity evaluation of CP flowers available till date, whereas roots and leaves have been studied for their toxicological effects. The present study has investigated the acute and subacute toxicity of EtCP flowers on mice. CP has been used traditionally for the treatment and prevention of some diseases, as mentioned in the introduction. Many pharmacological properties of the leaf, root, and flowers have been discovered, including antiepileptic, antidepressant, cognition enhancer, anti-inflammatory, analgesic, and so on.

In the acute and subacute toxicity studies, biochemical tests such as serum enzyme labeling did not differ significantly ($p > 0.05$) from the control group. (Dada et al., 2002). The variations in enzyme labels were not proportional to the administered dose, and there was no statistically significant difference between the treatment and control groups. Low enzyme label differences in the blood showed that the liver was not considerably affected; the number of injured liver cells is directly proportional to the amount of enzyme released into the blood (Mbako et al., 2009). There were no significant variation was recorded in organ/body weight ratio of different groups (300, 1000, 2000 mg/Kg) in compare to control group). An increase in serum urea and creatinine concentration may result because of dysfunction of kidney tubules and low glomerular filtration of the kidney by the toxic dosages of test drug (Eissa and Zidan, 2010). High doses of aqueous extract of Calotropis procera leaves

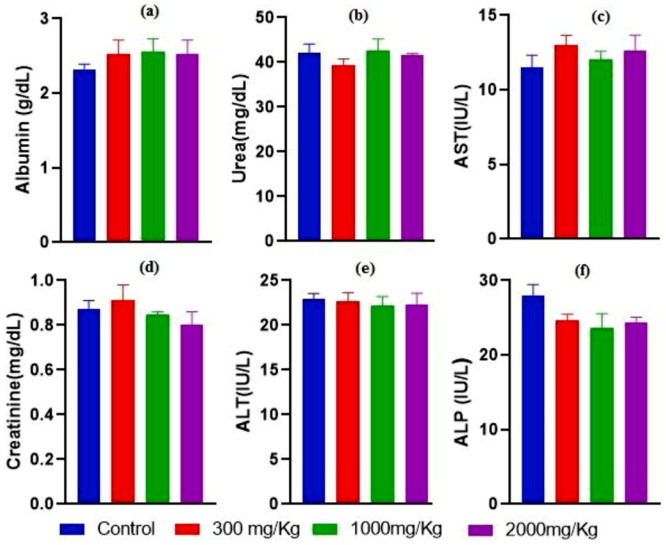


**Fig. 1.** Serum biochemical results of mice treated with Ethanolic extract of *Calotropis procera* flower for 28 days. Data are expressed as mean± SEM. Statistical tool one way ANOVA followed by Dunnett's multiple comparisons test was performed. The values were considered significantly different compared to control when $p < 0.05$. There was no significant change was found in biochemical parameters. (a) Albumin (b) Urea (c) Aspartate aminotransferase (AST) (d) Creatinine (e) Alanine transaminase (ALT) (f) Alkaline phosphatase (ALP).

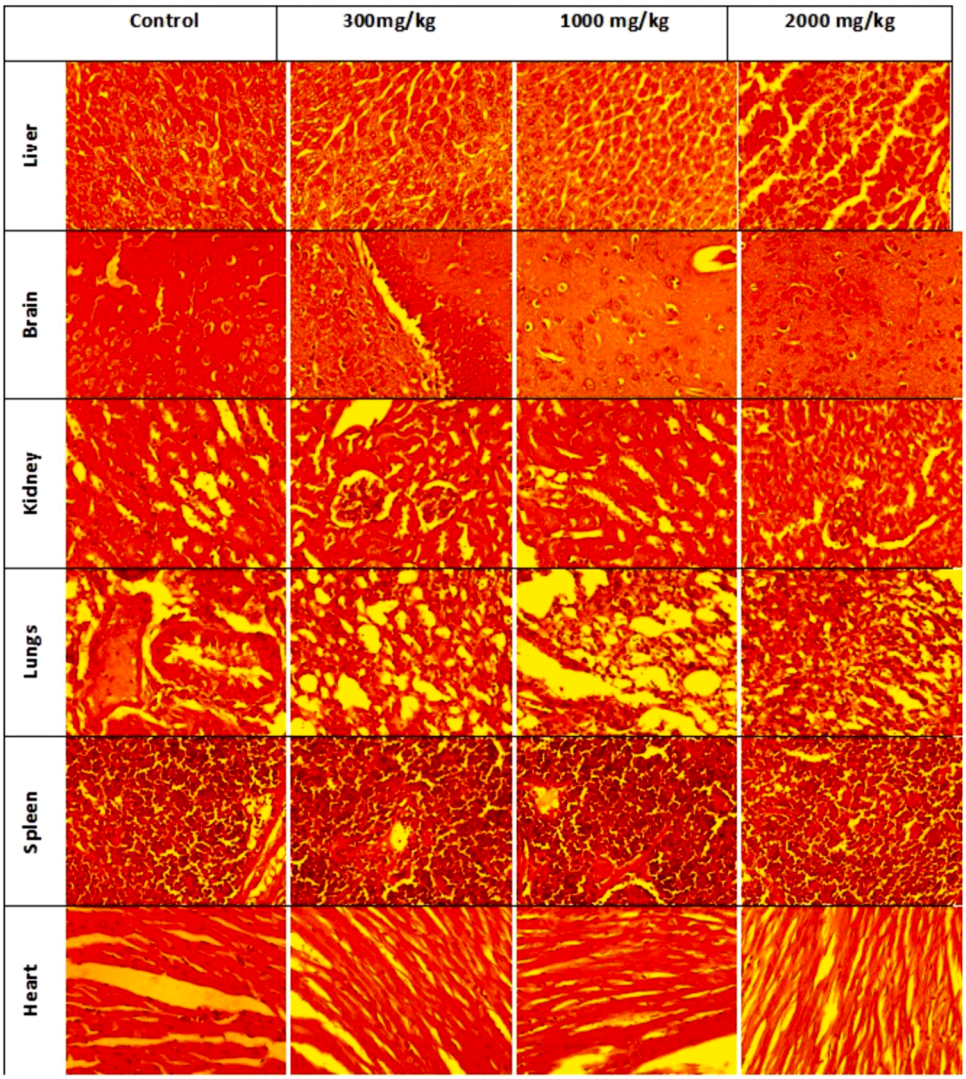


**Fig. 2.** Histopathological images of six organs (Liver, Brain, Kideny, Lungs, Spleen and Heart) in mice after oral administration of Ethanolic extract of *Calotropis Procera* for 28days at the doses of 300 mg, 1000 mg, 2000 mg/Kg BW of EtCP and control group at 400 × . Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. Spleen, brain, heart and lungs showed normal architecture.

raised serum urea and creatinine due to kideny toxicity (Bertrand et al., 2011), but in our study, EtCP flowers did not increase serum urea and creatinine at higher doses of EtCP flower (2000 mg/Kg). Changes in the hematopoietic parameters would reflect physiological and pathological status as a result of toxicity of test drug (Li et al., 2010). Hematological data (WBC, PCV, and platelets) and differential leucocyte counts were found statistically insignificant ($P > 0.05$). These data suggested the normal physiological process during the growth. Effect of EtCP flower on the weight was not observed in acute and subacute toxicity study whereas weight loss was found in rabbit by *Calotropis procera* leaves in an earlier study (Dada et al., 2002).

Histological studies of the liver, kidney, lungs, liver, brain, and spleen revealed no significant changes, which is consistent with biochemical and hematological parameters, whereas previous research on aqueous extract of leaves revealed toxicity of kidney congestion, necrosis, and blood infiltration in the alveoli of the lungs. (Jato et al., 2016). In the acute and subacute toxicity studies, no mortality was found at the dose of 2000 mg/Kg of EtCP flower, whereas in an earlier toxicity study of aqueous extract of *Calotropis procera* on rabbits, 33.3% and 100% were found at doses of 800 mg/Kg and 1600 mg/Kg respectively (Jato et al., 2016).

## 5. Conclusion

Acute and subacute toxicity of EtCP was performed on mice orally. Oral administration of single dose of EtCP 2000 mg/Kg did not cause mortality. 28 day treatment did not show mortality at the dose of 300, 1000, 2000 mg/Kg. Biochemical and hematological parameters were within the normal range and there was no significant difference was recorded. Histopathology of liver, kidney, brain, spleen, lungs and heart revealed that no major toxicity was found. Therefore, it is concluded that acute and subacute treatment by EtCP flowers did not cause significant toxicity. These findings indicate that the no-observed-adverse-effect-level (NOAEL) of *Calotropis procera* flower ethanolic extract was greater than 2000 mg/kg/day.

## Author agreement

We, the undersigning authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons, who satisfied the criteria for authorship, but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100224.

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