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In-Vivo Antimicrobial Activities of *Calotropis Procera* (Linn) Using Experimental Animals

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ABSTRACT

Indigenous medicine comprises of knowledge systems that developed over subsequent years within different societies before the period of modern medicine. This work investigated on the *in-vivo* antimicrobial activities of the extracts (solvent used in this study are acetone, ethanol, aqueous and methanol) of stem and leaf of *Calotropis procera* using experimental animal (albino rats), whereby haematological and biochemical analyses were determined using standard methods. Based on the result in this study, there was no death recorded but there was a mild toxicity effects on the liver, kidney, stomach and heart of the animals at 1000-3000 mg/kg of the rats in this research.

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Introduction

Calotropis procera (Linn): The yoruba name (Bomubomu) is a shrub, which appears to be a serious weed in pastures and overgrazed rangelands. It is common in West Africa such as Angola, North and East African, Madagascar, the Arabian Peninsula, Southern Asia, India and China to Malaysia.

Calotropis was placed in the family of *Asclepiadaceous* (the milkweed family), which is now considered a subfamily of the Apocynaceae [1]. The common names of this plant are rubber bush and king's crown, it is a green fruits that contains a milky sap that is toxic and extremely bitter which turns into a gluey coating which is resistant to soap. It is a plant that is found along degraded roadsides, lagoon edges and in overgrazed native pastures. It is often found in areas of abandoned cultivation, especially in sandy soils in areas with little amount of rainfall. This is a large family of plants including 415 genera and about 4555 species distributed largely throughout the tropics. It is often dominant in areas of abandoned cultivation, especially on sandy soils in areas of low rainfall. It can be used as an indicator of over-cultivation [2]. *Calotropis procera* holds a reasonable position as a medicinal plant in different systems of medicine in India. All the parts of this plant possess a valuable medicinal properties.

Root bark is diaphoretic and it cures different diseases like asthma and syphilis. Flowers are analgesic, astringent and cure inflammations and tumors. Basu and Chaudhuri found anti-inflammatory activity in rats of a chloroform-soluble fraction from the roots [3]. Jain et al. investigated antimicrobial activity of *C. procera*, the maximum inhibitory activity was observed in ethanol extracts of root bark against *Enterobacter cloaceae* and of stem against *Fusarium moniliforme* [4].

The latex of this plant is used in traditional medicine for a lot of qualities such as a purgative, antisyphylitic and antiodontalgic agent and as a cure for verrucas. Extracts from latex, leaves and flowers in Morocco had more effect on yeasts than on fungi. According to the Unani system of Medicine, this plant is useful against leprosy, scabies, and ringworm of the scalp, piles, asthma, liver and spleen enlargement and dropsy. *Calotropis procera* extracts can be used as a coagulant in cheese-making [5].

The leaves of the plant are used for the treatment of asthma, the sap which is milky is used as a rubefacient and is also strongly caustic. The latex is used for treating infections like ringworm, quinea worm, blisters, scorpion stings, venereal sores and ophthalmic disorders and it is also used as a laxative, it is used in India in the treatment of skin diseases has caused severe bullous dermatitis leading sometimes to hypertonic scars.

Acute Toxicity

Acute toxicity describes the adverse effects of a product which result either from a single exposure. To be described as acute toxicity, the adverse effects should occur within 14days of the administration of the substance [6].

Materials and Method

Collection of Plant Samples

The plant (*Calotropis procera*) was collected from Elekute Street in Ado-Ekiti, Ekiti State Nigeria. The plant parts (stems and leaves) were always collected and packed inside a cleaned sac.

Preparation of Plant Extracts

The plant parts (leaves and stem) were dried at room temperature (25 + 2 °C) and then the plant parts were ground to powder with a mechanical grinder (Thomas Wiley machine, model 5 USA) and were soaked for 48 hours with sterile water, ethanol, methanol and acetone in a glass jar separately. The extractant were filtered using membrane filter of size 0.45 µm and after which the rotary evaporator (RE- 300DB England) was used in order to reduce the solvents from the extracts, the supernatant were collected and concentrate to dryness and were then labelled respectively.

Investigation of the In-Vivo Effects of the Plant's Extracts

Acute Toxicity Study

The bioassay was conducted according to the method in World Health Organization (WHO) guideline for the evaluation of the safety and efficiency of herbal medicine and the Organization of Economic Co-operation and Development (OECD) (OECD, 2010). Eighty-four mice comprising both male and female were divided into six groups after six hours of fasting period. The albino rats in group six received normal saline (10 ml/kg oral) while the albino rats in groups one to five (1-5) received oral doses of the extracts (500, 1000, 2000, 3000 mg/kg) of the five plant extracts that were having good antimicrobial activities. The animals were not observed for obvious toxic symptoms within 14 days based on the method of Lorke [7]. The result for the median lethal dose of the extract (LD50) was estimated using probit analysis [8]. The plant extracts used for each group are:

Group 1: acetone leaf extract,
Group 2: methanol stem extract,
Group 3: methanol leaf extract,
Group 4: aqueous leaf extract,
Group 5: aqueous stem extract and
Control: normal saline.

Haematological Studies of the Albino Rats: The haematological parameters used are white blood cells count (WBC), total red blood cells count (RBC), differential count of leucocytes which are neutrophil, lymphocyte, monocyte, haemoglobin, and packed cell volume (PCV)

Packed Cell Volume (PCV) (%)

Centrifugation method was employed in this experiment. Parallel sided tubes (capillary tubes) were filled with blood until three quarters full. One end was then sealed with plastic in. The capillary tubes were spun in a micro haematocrit centrifuge (Hawksley and sons, London) for 10 minutes at 12,000 rpm. The PCV values were read using the Hawksley Haematocrit Reader. The value was read on the haematocrit reader as a percentage of the total blood volume using the formula: [9].

$$\text{PCV (\%)} = \frac{\text{Height of packed cell column}}{\text{Height of whole blood column}}$$

Red blood Cell Count

The method of Rodak was used. The RBC was determined using the Neubeauer counting chamber, dilution pipette, dilution [10]. The Neubeauer counter was mounted under compound microscope and cells were counted with a hand tally. The formula below was used to calculate total RBC.

$$\text{Red cell count} = \frac{N \times DF}{A \times D} \times 10^6$$

Where A = the area of chamber counted, N = number of cell, DF = Dilution factor, convert to cell per litre and D = depth of chamber.

White blood Cell Count

The method of Rodak was used [10]. Ethylene diamine tetra acetic acid (EDTA)-treated whole blood at 1:20 ratio with blood cell diluting fluid (made up of 3.8 gramme sodium citrate, 0.21 gramme neutral formalin and 0.5 gramme brilliant cresol blue and 100 ml distilled water). The diluted blood sample was then mixed and loaded into counting chamber. The WBC in the chamber was counted leaving out the edges of the chamber. Total WBC was determined using the formula below.

$$\text{WBC} = \frac{N \times DF}{A \times D} \times 10^6$$

Where A = the area counted, N = number of cells. DF = Dilution factor, D = depth of chamber.

Leucocytes Differential Counts

Differential count of monocyte, neutrophil, lymphocyte and eosinophil were carried out using the criteria of Berend, Siekmeier et al. [11-12]

Determination of Haemoglobin Concentration

Blood sample (0.02 ml) were mixed for sixty seconds and drawn using a 0.02 ml micro-pipette and expelled into a tube containing 4 ml of Drakins solution. The tube was stopped, mixed and left for 5 minutes to allow full colour development. A standard was prepared using a blood sample of known hemoglobin concentration. Using plain Drakin's solution both the sample and standard blood dilution were then read on the colorimeter (Corning colorimeter, 253) at 550 nm [13]. The haemoglobin concentration in the blood samples were calculated using the formula:

$$\text{Sample Hb concentration (\%)} = \frac{\text{Absorbance of test blood sample}}{\text{Absorbance of standard blood sample}} \times \text{Standard Hb concentration}$$

Liver Biochemistry of the Albino Rats: Assay for aspartate aminotransferase activity, (AST), Assay for alanine aminotransferase activity (ALT) and Assay for alkaline phosphate (ALP) were examined using the method of Bergmeyer et al. [14].

Statistical Analysis

All experiments were carried out in triplicates, the data are mean ± SD error and the mean values were analysed with their control using the one-way analysis of variance (ANOVA) by the statistical package for social science (SPSS 20).

Results

The weight of Experimental Albino Rats

Acetone, aqueous and methanol extract of the leaf and stem were highly effective after carrying out the *in-vitro* activity.

It was noticed that there was no variation in the weight of the rats but there was reduction at the first three days but after some days of experiment the weights were regained but varied from each other. The weight is between 140-235 grams (Tables 1 and 2)

Table 1: Weight of experimental albino rats (grams)

Extracts	AL	ML	MS	AQL	AQS
Day 1 (A)	155±2.31^{c}	178±1.89^{c}	185±2.56^{d}	131±0.45^{a}	140±0.23^{a}
(B)	213±5.67^{f}	144±0.22^{c}	235±0.32^{g}	175±1.32^{f}	179±1.23^{a}
Day 3 (A)	153±3.12^{b}	186±0.67^{d}	189±1.78^{e}	132±0.55^{a}	141±2.34^{a}
(B)	214±5.34^{f}	147±0.55^{d}	233±1.76^{g}	172±0.67^{e}	184±2.22^{b}
Day 5 (A)	152±1.27^{a}	177±2.43^{b}	188±0.98^{e}	141±1.08^{b}	143±0.22^{b}
(B)	197±2.60^{e}	142±1.90^{b}	225±0.34^{f}	142±0.44^{b}	209±1.90^{c}
Day 7 (A)	151±1.00^{a}	172±0.43^{a}	184±0.67^{d}	140±0.33^{b}	144±0.55^{b}
(B)	180±1.40^{b}	135±3.45^{a}	222±3.44^{e}	139±2.22^{a}	209±1.01^{c}
Day 9 (A)	162±2.44^{d}	172±1.20^{a}	180±1.32^{c}	145±0.56^{c}	142±0.66^{b}
(B)	173±0.45^{a}	145±2.34^{c}	220±0.47^{d}	158±1.55^{c}	210±1.56^{c}
Day 11 (A)	162±1.01^{d}	176±1.45^{b}	180±2.67^{c}	145±0.67^{c}	145±1.23^{b}
(B)	182±0.22^{c}	146±0.67^{d}	213±1.46^{c}	159±2.32^{c}	214±0.89^{d}
Day 13 (A)	163±0.67^{d}	179±2.34^{c}	174±1.50^{a}	147±0.34^{d}	148±2.22^{d}
(B)	182±1.02^{c}	147±0.43^{d}	209±0.88^{a}	162±1.23^{d}	217±0.55^{e}
Day 14 (A)	165±0.11^{d}	179±1.56^{c}	179±0.55^{b}	147±0.21^{d}	147±0.88^{c}
(B)	185±0.78^{d}	147±1.22^{d}	210±1.50^{b}	162±0.36^{d}	217±0.77^{e}

Values with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4)

KEY: AL- Acetone Leaf, ML-Methanol Leaf, MS-Methanol Stem, AQL-Aqueous Leaf, AQS-Aqueous Stem. A- 500 mg/kg, B-1000 mg/kg

Table 2: Weight of experimental albino rats (grams) cont

Extracts	AL	ML	MS	AQL	AQS
Day 1 (C)	190±3.31^{e}	174±0.89^{a}	221±0.56^{d}	176±0.45^{b}	160±0.33^{a}
(D)	223±1.67^{e}	161±1.22^{c}	140±0.32^{c}	140±1.0^{2}	158±1.03^{d}
Day 3 (C)	200±3.12f	184±0.47c	213±0.78c	162±0.35a	161±0.34a
(D)	226±4.34f	160±0.55c	139±0.76c	120±0.47	156±1.22c
Day 5 (C)	186±2.27d	183±2.41b	212±0.88c	182±1.08d	176±0.02d
(D)	208±1.60d	158±0.90b	136±0.34b	140±0.14	154±1.80b
Day 7 (C)	169±0.70c	182±0.43b	208±0.57b	181±1.33d	161±0.25a
(D)	190±1.40c	157±0.45a	132±1.44a	140±0.22	149±0.01a
Day 9 (C)	162±2.44a	182±1.20b	206±0.32a	176±0.36b	162±0.66ab
(D)	181±1.45a	171±2.24d	145±0.97d	141±0.55	159±1.56
Day 11 (C)	162±1.01a	184±1.45c	206±2.07a	178±2.67c	172±1.13c
(D)	181±0.92a	172±2.67d	146±1.46d	143±1.32	159±0.69d
Day 13 (C)	165±0.67b	184±2.34c	206±0.50a	182±0.14d	174±2.52d
(D)	182±1.67b	171±1.43d	147±0.18de	145±0.23	160±0.15de
Day 14 (C)	165±0.11b	184±1.16c	212±0.15c	182±0.21d	176±0.28d
(D)	181±0.78a	172±1.22d	147±0.50de	145±0.16	160±0.77de

Value with different alphabets along colomn are significantly different from each other at P≤0.05. (mean±SEM; n=4) .

KEY: AL- Acetone Leaf, ML-Methanol Leaf, MS-Methanol Stem, AQL-Aqueous Leaf, AQS-Aqueous Stem. C-2000 mg/kg, D-3000mg/kg

Effect of *C. procera* Extracts on Haematological Parameters of Albino Rats

The haematology parameters are the haemoglobin, red blood cell, packed cell volume, white blood cell, neutrophils, monocytes, lymphocytes and eosinophil (PCV, WBC, HB, RBC, N, L, E and M) of the rats which were analyzed for group 1-5 with the concentration of the extracts that varied from 500-3000 mg/kg. The packed cell volume varied from (38.50-52.67%), white blood cell varied from (2.80-8.69 mm3), haemoglobin (10.65-18.00 g/d), red blood cell (3.48-5.85 ×10 g/l), neutrophil (40.50-50.75%), lymphocytes (38.25-54.75%), monocytes (2.25-7.25%) and eosinophil (2.25-4.25%) respectively (Tables 3-7). The haematology parameters fall within the normal range.

Table 3: Effect of Acetone Leaf Extract of *c. Procera* on Haematological Parameters of Albino Rat

GROUPS (mg/kg)	PCV (%)	WBC (thousand/mm^3)	HB(g/d)	RBC(*10g/l)	N (%)	L (%)	M (%)	E (%)
CONTROL	47.00±1.8^b	3.01±0.53^b	13.77±0.55^b	4.50±0.07^b	45.66±0.98^b	43.07±0.6^b	2.78±0.5^a	3.66±0.3^b
500	40.75±1.7^a	2.80±0.28^a	17.15±0.08^d	4.38±0.2^b	43.75±0.48^b	38.25±0.75^a	2.50±0.29^a	2.75±0.48^a
1000	44.75±1.10^a	3.03±0.55^b	15.75±0.48^c	4.05±0.03^b	40.50±0.96^a	43.25±0.63^b	3.25±0.48^b	2.50±0.29^a
2000	47.75±1.65^b	3.73±1.01^c	12.83±0.44^a	3.85±0.06^a	48.25±0.75^c	45.00±0.71^c	4.50±0.29^c	3.50±0.29^b
3000	49.25±4.53^c	4.01±1.20^c	11.15±1.46^a	3.55±0.35^a	49.65±2.31^c	46.00±0.41^c	4.25±0.63^c	3.25±0.25^b

Values with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: PCV-Packed Cell Volume, HB- Haemoglobin, RBC- Red Blood Cell, WBC-White Blood Cell, N-Neutrophil, L-Lymphocyte, M-Monocytes, E-Eosinophil

Table 4: Effect of Methanol-Leaf Extract of *C. procera* on Haematological Parameters of Albino Rat

GROUPS (mg/kg)	PCV (%)	WBC (mm^3)	HB(g/d)	RBC(*10g/l)	N (%)	L (%)	M (%)	E (%)
CONTROL	47.00±1.80^b	3.01±0.53^a	13.77±0.55^a	4.50±0.07^a	45.66±0.9^a	43.07±0.67^a	2.78±0.45^a	3.66±0.34^b
500	43.25±2.21^a	3.00±1.82^a	14.33±0.71^b	4.00±0.11^a	47.50±1.3^b	45.25±0.25^a	2.25±0.25^a	2.75±0.25^a
1000	43.00±1.47^a	3.67±0.07^a	14.58±0.34^b	4.25±0.15^a	47.00±1.0^b	50.00±0.41^b	3.00±0.41^b	2.25±0.25^a
2000	49.25±2.78^c	4.76±1.87^b	14.15±0.15^b	4.03±0.02^a	49.50±0.6^c	50.00±0.41^b	3.75±0.48^b	3.75±0.25^b
3000	49.75±1.54^c	4.08±0.43^b	16.30±0.60^c	4.85±0.09^a	49.75±1.4^c	54.75±1.70^c	3.25±0.48^b	2.25±0.25^a

Values with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: PCV-Packed Cell Volume, HB- Haemoglobin, RBC- Red Blood Cell, WBC-White Blood Cell, N-Neutrophil, L-Lymphocyte, M-Monocytes, E-Eosinophil

Table 5: Effect of Methanol-Leaf Extract of *C. procera* on Haematological Parameters of Albino Rat

GROUPS	PCV (%)	WBC (mm^3)	HB(g/d)	RBC(*10g/l)	N (%)	L (%)	M (%)	E (%)
CONTROL	47.00±1.80^b	3.01±0.53^a	13.77±0.55^a	4.50±0.07^a	45.66±0.9^a	43.07±0.67^a	2.78±0.45^a	3.66±0.34^b
500	43.25±2.21^a	3.00±1.82^a	14.33±0.71^b	4.00±0.11^a	47.50±1.3^b	45.25±0.25^a	2.25±0.25^a	2.75±0.25^a
1000	43.00±1.47^a	3.67±0.07^a	14.58±0.34^b	4.25±0.15^a	47.00±1.0^b	50.00±0.41^b	3.00±0.41^b	2.25±0.25^a
2000	49.25±2.78^c	4.76±1.87^b	14.15±0.15^b	4.03±0.02^a	49.50±0.6^c	50.00±0.41^b	3.75±0.48^b	3.75±0.25^b
3000	49.75±1.54^c	4.08±0.43^b	16.30±0.60^c	4.85±0.09^a	49.75±1.4^c	54.75±1.70^c	3.25±0.48^b	2.25±0.25^a

Value with different alphabets along colomn are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: PCV-Packed Cell Volume, HB- Haemoglobin, RBC- Red blood cell, WBC-White blood cell, N-Neutrophil , L-Lymphocyte M-Monocytes, E-Eosinophil

Table 6: Effect of aqueous leaf extract of *C. procera* on haematological parameters of albino rat

GROUPS (mg/kg)	PCV (%)	WBC(mm^3)	HB(g/d)	RBC(*10g/l)	N(%)	L(%)	M(%)	E(%)
CONTROL	47.00±1.80^b	3.01±0.53^a	13.77±0.55^b	4.50±0.07^b	45.66±0.98^a	43.07±0.67^a	2.78±0.45^a	3.66±0.34^b
500	43.25±1.03^a	6.56±0.54^b	18.00±0.41^d	3.63±0.22^a	47.25±0.48^b	42.25±0.85^a	4.75±0.48^b	3.75±0.25^b
1000	43.00±1.08^a	5.78±1.88^b	14.57±0.25^c	3.70±0.24^a	48.75±0.63^c	43.75±0.48^a	6.75±0.48^c	2.25±0.25^a
2000	48.25±0.95^b	7.68±0.54^c	12.40±0.57^a	3.45±0.21^a	48.00±1.08^c	44.25±0.75^b	6.25±0.48^c	2.25±0.25^a
3000	48.50±2.10^b	8.69±0.78^c	12.48±0.40^a	3.50±0.21^a	49.89±0.75^d	46.50±0.65^c	7.25±0.48^d	3.25±0.25^b

Value with different alphabets along colomn are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: PCV-Packed Cell Volume, HB- Haemoglobin, RBC- Red Blood Cell, WBC-White Blood Cell, N-Neutrophil, L-Lymphocyte, M-Monocytes, E-Eosinophil

Table 7: Effect of Aqueous Stem Extract of *C. procera* on Haematological Parameters of Albino Rat

GROUPS (mg/kg)	PCV (%)	WBC (mm^3)	HB(g/d)	RBC(*10g/l)	N (%)	L(%)	M(%)	E (%)
CONTROL	47.00±1.80^b	3.01±0.53^a	13.77±0.55^b	4.50±0.07^b	45.66±0.98^a	43.07±0.67^b	2.78±0.45^a	3.66±0.34^b
500	38.50±1.44^a	4.56±0.78^b	10.65±0.70^a	3.48±0.17^a	47.25±1.18^b	42.50±0.87^b	4.75±0.48^b	2.75±0.25^b
1000	45.25±0.85^b	4.05±1.73^b	13.33±0.52^b	4.15±0.12^b	48.75±1.38^b	40.75±0.48^a	5.75±0.25^c	3.25±0.25^c
2000	48.50±1.19^c	5.00±0.54^c	17.83±0.28^d	4.23±0.06^b	50.00±0.71^c	46.25±1.11^c	5.06±0.48^c	3.25±0.25^c
3000	52.67±5.17^d	6.78±1.74^d	15.65±1.15^c	4.13±0.21^b	50.75±2.46^c	46.50±1.32^c	6.25±0.75^d	2.50±0.29^b

Values with different alphabets along colomn are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: PCV-Packed Cell Volume, HB- Haemoglobin, RBC- Red Blood Cell, WBC-White Blood Cell, N-Neutrophil, L-Lymphocyte, M-Monocytes, E-Eosinophil

Effect of *C. procera* Extracts on Biochemical Parameters (Liver function test) of the Albino Rats

The rats were tested on the biochemical parameters of their liver function; The enzyme markers are: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) ; ALT, AST and ALP ranges from values of 32-51 IU/L, 160-204 IU/L and 67- 160 IU/L respectively. ALT of the rats fed with acetone-leaf extract at 2000 mg/kg was not significantly different from 3000 mg/kg, AST of all the varied concentrations were significantly different from each other and ALP of the control was not significantly different from 2000 mg/kg (Table 8).

The ALT of the rats at 1000 mg/kg was not significantly different from 2000 mg/kg, the AST of the control was not significantly different from the rats fed with 2000 mg/kg extract and the ALP of the rats fed with 2000 mg/kg was not significantly different from the rats fed with 3000 mg/kg (Table 9).

The ALT of the rats fed with methanol leaf extract at 2000 mg/kg was not significantly different from 3000 mg/kg, AST of the rats at 1000 mg/kg was not significantly different from 2000 mg/kg and ALP of the rats were significantly different from each other except the control and the rat fed with 2000 mg/kg (Table 10)

The ALT of the rats were not significantly different from each other but the ALP of the varied concentrations are significantly different from each other (Table 11).The ALT of the rats in these groups were not significantly different from each other except the rat fed with 3000 mg/kg while the ALP of the rats are significantly different from each other (Table 12).

Table 8: Effect of Acetone-Leaf Extract of *C. procera* on Biochemical Parameters of Albino Rats

GROUPS (mg/kg)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
CONTROL	32.8±0.64^a	194.15±1.08^e	125.36±0.85^b
500	35.50±2.09^b	160.48±10.33^a	112.70±2.39^a
1000	37.85±2.92^c	172.53±0.65^b	160.72±2.67^d
2000	42.45±0.45^d	175.50±11.38^c	121.31±7.88^b
3000	40.55±2.32^d	190.33±1.26^d	135.25±5.40^c

Value with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4)

KEYS: ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alanine phosphate.

Table 9: Effect of Methanol-Stem Extract of *C. procera* on Biochemical Parameters of Albino Rats

GROUPS (mg/kg)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
CONTROL	32.8±0.64^a	194.15±1.08^d	125.36±0.85^d
500	48.85±2.92^c	190.35±11.97^c	123.23±0.48^c
1000	45.18±4.09^b	187.15±6.87^b	115.16±0.12^a
2000	43.70±6.59^b	195.93±9.23^d	120.72±4.72^b
3000	51.38±0.71^d	175.23±0.95^a	120.81±0.03^b

Values with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4)

KEYS: ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alanine phosphate

Table 10: Effect of Methanol-Leaf Extract of *C. procera* on Biochemical Parameters of Albino Rats

GROUPS (mg/kg)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
CONTROL	32.8±0.64^{a}	194.15±1.08^{c}	125.36±0.85^{d}
500	39.35±0.94^{b}	185.68±1.63^{a}	116.28±1.40^{b}
1000	41.20±0.66^{c}	189.38±0.43^{b}	105.55±2.15^{a}
2000	43.50±0.26^{d}	189.55±1.81^{b}	128.31±0.31^{d}
3000	43.00±0.59^{d}	185.66±0.67^{a}	125.57±1.37^{c}

Value with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4).

KEYS: ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alanine phosphate

Table 11: Effect of Aqueous-Leaf Extract of *C. procera* on Biochemical Parameters of Albino Rats

GROUPS (mg/kg)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
CONTROL	32.8±0.64^{a}	194.15±1.08^{d}	125.36±0.85^{e}
500	37.15±0.46^{b}	184.50±1.94^{a}	100.44±0.21^{a}
1000	36.85±0.83^{b}	191.03±2.03^{b}	110.88±1.16^{b}
2000	40.25±0.97^{c}	201.53±0.63^{c}	121.11±0.42^{c}
3000	42.93±0.82^{c}	204.83±1.33^{c}	129.81±1.06^{d}

Values with different alphabets along column are significantly different from each other at P≤0.05. (mean±SEM; n=4)

KEYS: ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alanine phosphate

Table 12: Effect of Aqueous-Stem Extract of *C. procera* on Biochemical Parameters of Albino Rats

GROUPS (mg/kg)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
CONTROL	32.8±0.64^{a}	194.15±1.08^{d}	125.36±0.85^{e}
500	40.30±1.64^{a}	158.00±1.08^{b}	67.25±0.85^{a}
1000	41.15±0.30^{b}	155.28±1.64^{a}	79.83±0.44^{c}
2000	41.45±1.58^{b}	175.38±14.04^{c}	105.09±0.15^{d}
3000	45.95±1.57^{c}	176.13±2.68^{c}	71.63±3.18^{b}

Value with different alphabets along column are significantly different from each other at P≤0.05 (mean± SEM; n=4)

KEYS: ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alanine phosphate

Discussion and Conclusion

During the in-vivo study, the LD50 of the plant extracts were prepared and it revealed that there was no lethal dose even at 5000 mg/kg, these findings correlates with Bezerra et al. [15]. Different concentration of plant extracts of 500 - 3000 mg/kg were administered, it was observed that the extracts were mildly toxic at higher concentration (1000-3000mg/kg) but was not posing any toxic effect on the organs.

The effect of the extracts on the haematological parameters studied was observed to be of varied reactions on plant parts and extract concentrations. Hence, the plant extracts were found not to have interference with the physiological properties of the albino rats. The low haemoglobin recorded which fell within value (11-19%) showed that the blood cells are intact. Neutrophil values count is essential aspect in different counts because it is the most common type of white blood cell in vertebrates responsible for protecting the body against infection. Excessive increase above standard in the neutrophil count denotes severe haematology defect in the white blood cell of the albino rats leading to Neutropenia. The observed result in leukocytes count is in agreement with the results obtained by (Patchree et al., [16]. Therefore, the membrane stabilization of the plant extracts compounds such as flavonoids and phenol may be responsible for the enhanced essential improvement observed in the cellular integrity of the neutrophil, thereby inhibiting radicals from damaging the cells.

ALT activity is the most frequently biomarker of hepatoxicity because it plays a vital role in amino acid metabolism and gluconeogenesis [17]. AST (Aspartate aminotransferases) is another liver enzyme found in the liver and other organs including heart, muscle, brain and kidney. ALP (alkaline phosphate) is an enzyme maker for plasma membrane and endoplasmic reticulum damage which is frequently used to assess the integrity of the plasma membrane and is required in certain amounts for proper functioning of organs. These enzyme markers gave a predictive level of hepatocellular damages [18]. The liver functioning test carried out showed that the livers were not damaged and these results obtained is in accordance with Gowda et al. whose report stated that biochemical markers helps in accurate diagnosis and also for assessing of problems and adopting therapy which improves clinical outcomes and also with Mohamed et al. who stated that popularity of herbal remedies is increasing globally and at least one quarter of patience with liver disease use ethno botanicals [19]. However, it can be said with this research study that *Calotropis procera* is safe for human consumption at low

concentration (maximum of 500 mg/kg) and there is need for good hygiene practices during the preparation.

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