

**AN EXPERIMENTAL STUDY OF THE TOXICITY EFFECTS OF
CALOTROPIS PROCERA (AITON) ASCLEPIADACEAE IN
SPRAGUE-DAWLEY RATS**

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Article Received on
26 July 2017,

Revised on 16 August 2017,
Accepted on 05 Sept. 2017

DOI: 10.20959/wjpr201711-9500

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ABSTRACT

Calotropis procera Aiton (*Calotropis*) is a wild-growing plant used for a variety of disease conditions such as leprosy, ulcers, tumor and healing of wounds. The present study was conceived to evaluate the toxicological effect of the latex following systemic use in Sprague-Dawley rats with a view to understanding its potential safety in treating wounds and preventing keloid formation in human. Haematological evaluation revealed a significant and progressive reduction of the packed cell volume (PCV) and red blood cell count at high doses of the latex. There was no significant effect on the differential count except for lymphocytes which showed significant reduction following increasing concentrations of the latex. Similarly, the latex exhibited no significant effect on the renal indices of the rats: uric acid, urea and

creatinine levels. A significant ($P<0.05$) decrease in blood glucose was

observed in the group that received 0.85mg/kg of the latex as compared with the control. The latex produced a significant ($p < 0.05$) decrease in the levels of aspartate transaminases at a dose of 0.35mg/kg IP and also the level of total cholesterol had a significant ($p < 0.05$) decrease at the same dose, but there was no significant change in the levels of the albumin, alanine transaminases and alkaline phosphatases in all the treated groups. Histopathological studies showed no remarkable effect on the tested organs except for the testes where there was destruction of the seminiferous tubules. In conclusion, these results suggest that *Calotropis* latex is relatively safe when used acutely especially through the oral route and in lower doses when administered through the intra-peritoneal route in rats. However, there is need for caution when higher doses are administered because of the toxic effects on the genitourinary systems particularly in males.

KEYWORDS: *Calotropis procera* (Calotropis) latex; toxicity; Sprague-Dawley rats.

INTRODUCTION

Calotropis procera Aiton (subsequently referred to as Calotropis) is a wild-growing plant that belongs to the family Asclepiadaceae. It is known by various names like swallow wort, Dead Sea apple, Sodom apple and milkweed. Latex from the plant has been reported to be useful in wound healing^[1,2] and has potential anti-keleoidal activity.^[2] Singhi and co-workers^[3] proposed the possibility of utilizing the latex as a larvicidal agent against *Aedes aegypti* and *Aedes albopictus* while Sehgal et al.^[4] studied the inhibitory effect of extracts of the latex against *Candida albicans*. Calotropis latex has been investigated for antibacterial, analgesic and *in-vitro* schizonticidal activities.^[5,8] The aqueous extract of the dried latex has been reported to induce histamine-mediated inflammation^[9] which can be reversed by anti-inflammatory drugs.^[4] Topical application of Calotropis latex produces ocular and dermal toxicities.^[10] Due to their easy accessibility, availability and relatively low cost, medicinal plants are relied upon for the primary health care needs of some 80% of the population in developing countries.^[11] This use is on the rise as a result of the efficacy of some of these medicinal plant materials and misconceptions that since they are natural, they should be safer than synthetic drugs. Few studies have been carried out to assess the toxicity of traditional remedies and those that do have revealed their toxicity potentials.^[12,13] As a follow up to our previous publication on wound-healing activity and antikeleoidal potential of Calotropis latex,^[2] the present study was undertaken to evaluate its possible toxicological effect using Sprague-Dawley rats.

MATERIALS AND METHODS

Collection and preparation of plant material

Calotropis latex was collected from various sites at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. It was identified and authenticated by Mr. Gabriel Ighanesebhor of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria and a voucher specimen (number UHI-16332) has been deposited at the Ife Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. Fresh latex was collected from healthy plants by making small incisions on the stems and on the leaf stalks closest to the leaves, allowing the latex to flow into a clean Mac Cartney bottle. The latex was gently handled to maintain its integrity during transport to the laboratory.^[6,7] The latex was triturated with glass mortar and pestle so as to finely disperse the colloidal portion of the latex and to make it more soluble when dispersed in water. Doses were prepared by diluting the raw latex with distilled water.

Animals

Male Sprague-Dawley rats (100-250g) obtained from the Animal Facility Centre, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used in the study. The rats were fed standard laboratory diet and water, and maintained under good laboratory conditions throughout the experimental period. They were kept at the facility and were allowed to acclimatize for 14 days. All experiments were performed according to the Principles of Laboratory Animal Care (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals).

TOXICOLOGICAL STUDIES

Acute toxicity study

An acute toxicity study was carried out using the method of Lorke^[14] which was carried out in two phases. In the first phase: 9 rats were randomly divided into 3 groups of 3 rats per group and were given doses of 0.01 mg/kg, 0.1 mg/kg and 1 mg/kg of the latex intraperitoneally (IP), respectively. The rats were kept under the same conditions and observed for signs of toxicity which included but not limited to grooming, rearing, respiratory distress and mortality for the first critical 4 h and 24 h.

In the second phase of the study, 1 mg/kg, 2 mg/kg and 4mg/kg IP of the latex, respectively, were administered to another 3 groups of 3 rats per group; each of these rats were observed for signs of toxicity and mortality for the first critical 4 h and 24 h, thereafter. The intraperitoneal median lethal dose was calculated as the geometric mean of doses that caused

zero and 100% mortality, respectively. The first phase of the procedure was repeated using oral route of latex administration. The second phase included oral doses of 2 mg/kg, 4 mg/kg, 5 mg/kg of the latex and were given to another fresh sets of 3 groups of 3 rats each. These rats were also observed for signs of toxicity and mortality for the first critical 4 hours and 24 hours thereafter. The oral median dose was calculated as the geometric mean of doses that cause 0 and 100% mortality, respectively. (The acute toxicity study was carried out both in the oral and IP routes using same doses in the two phases).

Sub-acute toxicity study

Twenty five rats were divided into 5 groups of 5 rats each. Group 1, which served as the control, received distilled water while rats in groups 2, 3 and 4 were given intraperitoneal doses of 0.35 mg/kg, 0.65 mg/kg and 0.85mg/kg of the latex, respectively, daily for 28 days. Group 5 were dosed orally at 2 mg/kg daily. All the rats had free access to food and water throughout the study and were observed daily for general symptoms of toxicity and mortality.

Feed and water intake

The weight of feed and volume of water consumed by rats in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 h.

Body weight change

Rats in all the groups were weighed twice weekly during the period of treatment and on the last day of study. Doses of the latex administered were adjusted based on the observed weight.

Sample collection for haematological and biochemical analyses

On the 29th day of the experiment, all the rats were euthenized after Diethyl ether inhalation and blood collected by cardiac puncture. One portion was collected into K⁺ EDTA bottles for estimation of packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelets, neutrophil, lymphocyte, white blood cell counts (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), using an automated blood analyser (H18 Light [SFRi Medical Diagnostics, France] previously standardized for routine laboratory procedure). Another portion of the collected blood sample was dispensed into non-heparinized bottles, allowed to clot and centrifuged at 3500 rpm for 10 min. The serum was separated, stored at -

4°C and used for evaluation of biochemical parameters which included: alanine transaminase (ALT), aspartate transaminase (AST) levels, alkaline phosphatase (ALP), total cholesterol, urea, triglycerides, and glucose, albumin and creatinine contents.

Histopathological evaluation

The organs (liver, spleen, lungs, testes, brain and heart) were removed immediately the animals were sacrificed and fixed in formalin. The fixed tissues were dehydrated using graded concentrations of ethanol and defatted (or cleared) in xylene. After clearance, tissues were infiltrated with soft paraffin wax at 58⁰ C in three successions at an interval of 1 h. Subsequently, the tissues were embedded in molten paraffin wax and tissues embedded in blocks were then produced ready for sectioning. The tissue blocks were sectioned at 5-7 µm thickness using a rotary microtome (Leica, Germany), fixed on clean slides and stained with Haematoxylin-and-Eosin stains (H & E). The slides were reviewed under a light microscope (Leitz Orthholux II, Germany) and photomicrographs were taken using 100X and 400X objectives.

Statistical analysis

All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using a Student t-test followed by one way analysis of variance (ANOVA). Difference between means was considered significant at $p < 0.05$.

RESULTS

General/gross observation of the animals

There was no change in the character of the stool, urine and eye colour of all the animals used in the test and control groups for both the acute and sub-acute studies.

Acute toxicity study of Calotropis latex on rats

The results of the acute toxicity of Calotropis latex in rats showed no mortality after 24 h of oral administration at doses of 0.01 mg/kg, 0.10 mg/kg and 1 mg/kg. Even at a high dose of 4mg/kg of the latex, the rats showed no remarkable behavioural changes or mortality. However, single intraperitoneal injection of the latex at similar doses produced a dose-dependent increase in adverse effects and mortality rate. Hence, the intraperitoneal LD50 of the latex was 0.7 mg/kg whereas oral LD50 was greater than 5 mg/kg since there was no death at this dose.

Sub-acute toxicological assessment of *Calotropis latex*

Effect of *Calotropis latex* on feed intake of rats

In the sub-acute toxicity study, there was a significant ($P < 0.05$), dose-dependent reduction in the intake of the rats in all the treatment groups when compared to the control (Fig. 1). While the reduction in feed intake was significantly reduced in a time-dependent manner across the dose-groups up to 3 weeks, the amount of food consumed slightly increased on week 4.

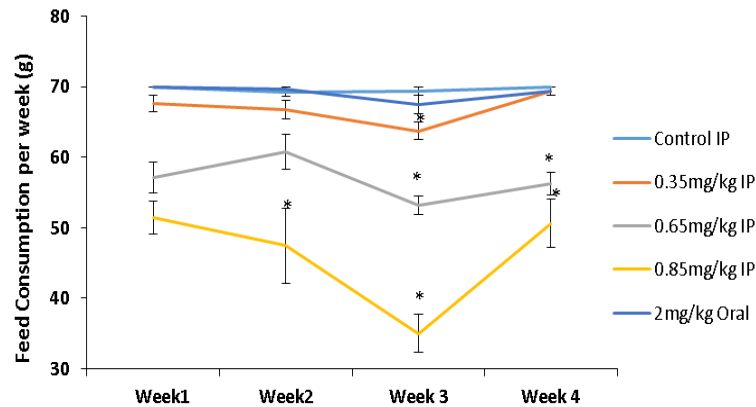


Figure 1: Feed intake by rat in g/100g body weight during the 4 weeks of sub-acute toxicology treatment with *Calotropis latex*

Effect of *Calotropis latex* on water intake in rats

There was a significant ($p < 0.05$), dose-dependent reduction in the volume of water consumed among the rats dosed intraperitoneally with *Calotropis latex* (Fig.2). The amount of water supplied was 250 ml/24 h. However, orally-dosed rats did not experience any significant change in their water consumption.

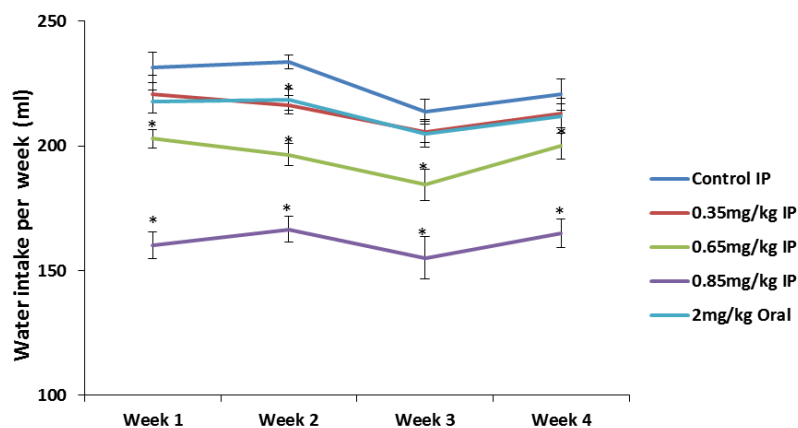


Figure 2: Water intake by rats (ml) during the 4 weeks of sub-acute toxicology treatment with *Calotropis latex*.

Effect of *Calotropis latex* on average body weight (g) of rats

In the sub-acute toxicity study, there was no significant difference in the weight of the rats in all the treatment groups when compared to the control (Fig. 3).

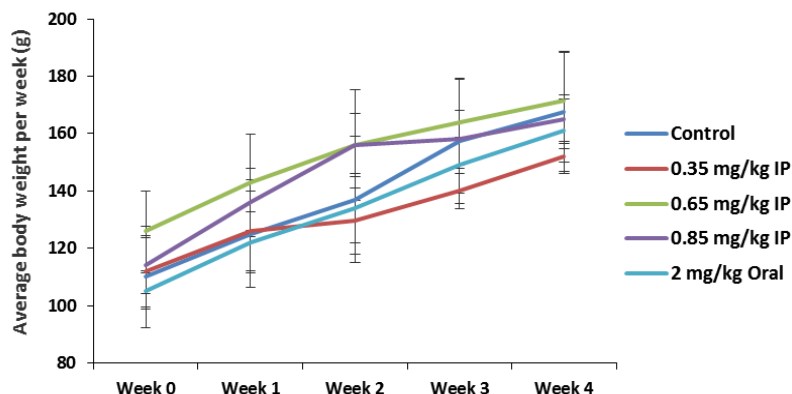


Figure 3: Average body weight (g) of rats during the 4 weeks of sub-acute toxicology treatment with *Calotropis latex*.

Haematological analysis

Haematological analysis revealed that there was a progressive decrease in the packed cell volume (PCV) and the haemoglobin concentration (Hb) with increasing concentrations of *Calotropis latex*, it was significant ($p < 0.05$) for groups treated with dose of 0.85 mg/kg IP of the latex compared to the control treated with distilled water (Table 1). There were no statistical significance ($p > 0.05$) for all the groups with respect to hemoglobin and mean corpuscular hemoglobin concentrations (Table 1). We also observed a progressive decreasing value for the white blood cells and platelets across the groups to a limit of 0.85mg/kg IP.

Table 1: Effect of *Calotropis latex* on hematological indices

Parameters	Control IP	0.35mg/kg IP	0.65mg/kg IP	0.85mg/kg IP	2mg/kg Oral
PCV (%)	0.44 ± 0.02	0.45 ± 0.02	0.41 ± 0.01	0.31 ± 0.05*	0.41 ± 0.02
HB (g/dl)	14.68 ± 0.76	14.80 ± 0.71	13.74 ± 0.34	12.20 ± 1.42	13.94 ± 0.69
RBC	9.50 ± 0.48	4.42 ± 0.92 *	5.79 ± 1.20*	7.36 ± 1.39	8.12 ± 0.43
MCV	0.04 ± 0.00	0.12 ± 0.02 *	0.11 ± 0.02 *	0.06 ± 0.01	0.05 ± 0.00
MCH	1.57 ± 0.13	3.91 ± 0.71*	3.52 ± 0.76*	1.86 ± 0.42	1.72 ± 0.08
MCHC (g/l)	33.36 ± 0.02	32.86 ± 0.49	33.34 ± 0.02	33.34 ± 0.04	33.34 ± 0.02
WBC (X10 ³)	14.89 ± 3.06	17.66 ± 3.85*	11.01 ± 1.75*	5.46 ± 2.43 *	5.46 ± 2.25*
Platelet (X10 ³)	125.00 ± 7.91	22.40 ± 4.70*	18.40 ± 2.42*	22.20 ± 2.91*	17.60 ± 2.99*
NEUT (%) (X10 ³)	2.22 ± 0.64	3.56 ± 0.78	3.78 ± 0.94	1.53 ± 0.77	0.63 ± 0.44
LYMPH (%) (X10 ³)	12.67 ± 2.44	17.06 ± 4.31*	7.30 ± 1.39	4.11 ± 1.86*	4.83 ± 2.03*

*significantly different from the control at $p < 0.05$, $n = 5$. Mean ± SEM; IP – intraperitoneal route

BIOCHEMICAL ANALYSIS

Effects of Calotropis on glucose level in rats.

The result in Table 2 shows a significant ($P < 0.05$) dose-dependent decrease in glucose level in the intraperitoneally-dosed mice as compared with the control.

Effects of Calotropis latex on liver and renal indices in rats

The latex produced no significant change in the levels of the hepatic and renal indices in all the treated groups regardless of route of exposure. The exception was in aspartate aminotransaminase and total cholesterol where there was a significant ($p < 0.05$) reduction at 0.35 mg/kg IP and the 2 mg/kg orally dosed rats (Table 2).

Table 2: Effect of Calotropis latex on liver and renal function indices.

Parameters	Control IP	0.35mg/kg IP	0.65mg/kg IP	0.85mg/kg IP	2mg/kg Oral
Glucose (mmol/L)	8.10±1.97	4.14±1.29	4.18±1.52	2.62±0.67*	8.50±0.95
Urea (mmol/L)	9.52±2.36	8.52±1.36	12.62±2.71	12.04±0.97	8.78±1.82
Creatinine (µmol/L)	82.00±8.53	77.60±11.78	71.80±11.73	64.00±8.35	71.80±12.72
Uric acid (µmol/L)	1156.60±574.97	429.40±62.74	788.80±125.19	611.60±95.95	398.60±60.08
ALP (U/L)	257.34±78.90	246.46±74.90	350.52±105.39	473.62±63.57	436.64±58.39
ALT (U/L)	30.20±12.00	12.90±4.78	24.60±2.49	25.56±3.49	14.20±3.32
AST (U/L)	145.20±10.92	50.90±6.76*	123.20±16.48	142.80±14.40	80.00±23.13*
Albumin (g/L)	29.20±1.93	25.80±3.32	24.20±0.80	24.20±0.80	27.60±1.21
TC (mmol/L)	3.52±0.62	2.06±0.20*	2.55±0.29	2.55±0.29	2.18±0.31*
Triglyceride (mmol/L)	0.87±0.20	1.07±0.50	1.23±0.19	2.07±0.52	2.30±1.13

*significantly different from the control at $p < 0.05$, $n = 5$.

ALT: Alanine-aminotransaminase, AST: Aspartate-aminotransaminase, ALP: Alkaline phosphatase, TC: Total Cholesterol, IP: Intraperitoneal route.

HISTOPATHOLOGY

The histological findings of the testes of the rats which received 0.85mg/kg IP of the latex showed marked destruction of the seminiferous tubules with significant interstitial fibrosis (Fig. 4, Plate C) when compared with rats which received 0.35mg/kg (Fig. 4, Plate B) intraperitoneally and the control group (Fig. 4, Plate A).

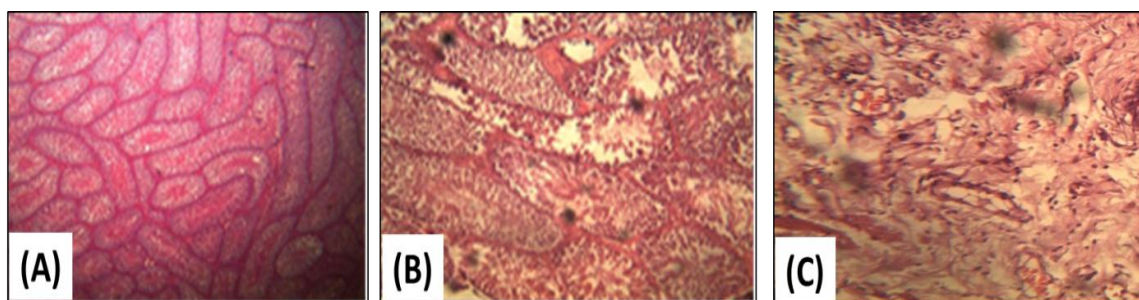


Figure 4: *Plate A:* Photomicrograph of the testis showing seminiferous tubules of different sizes and shapes in the control group. *Plate B:* Photomicrograph of the testis showing progressive destruction of the seminiferous tubules in rats that received 0.35mg/kg of intraperitoneal treatment. *Plate C:* Photomicrograph of the testis of the group that received 0.85mg/kg of intraperitoneal treatment showing tissue not reminiscent of the testis but showing severe destruction of the seminiferous tubules with loss of spermatogenic cell series and increased interstitial fibrovascular stroma and conspicuous presence of Leydig cells singly in area. (H&E staining (400X)).

The histological sections from the lungs of the groups that received both the intraperitoneally and orally administered doses of the latex showed pulmonary oedema and congestion (Fig. 5 Plates *A and B*).

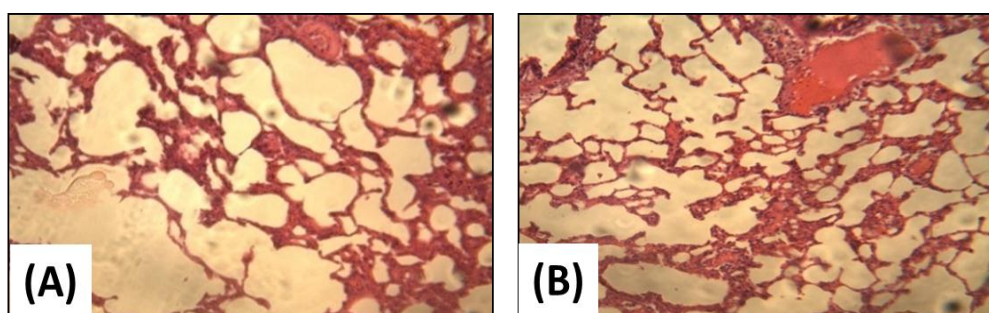


Figure 5: *Plate A:* Photomicrograph of the lung showing dilated alveolar spaces with thickened alveolar septae in the groups which received intraperitoneal treatment. *Plate B:* Photomicrograph of the lung showing pulmonary congestion in the group that received oral treatment of the latex. (H&E staining (400X)).

The histology of the sections from the brain, heart, liver, kidney and spleen were essentially normal.

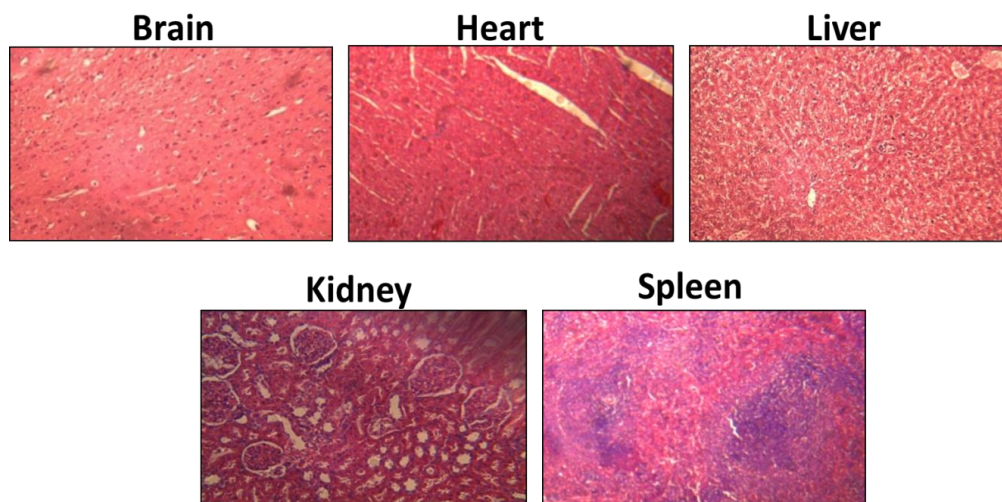


Figure 6: Photomicrographs of the brain showing viable brain tissue, the heart showing bundles of cardiac muscles separated by thin myocardial fibres, the liver showing a well preserved portal tract, parenchyma and central vein of the liver. Photomicrograph of the kidney showing numerous glomeruli with preserved Bowman's space, unremarkable interstitium, tubules and blood vessels. Photomicrograph of the section of the spleen showing the red pulp consisting of thin walled vascular channel and white pulp consisting of aggregates of lymphocytes. (H&E staining (400x).

DISCUSSION

As a result of the ethnomedicinal use of the latex of *Calotropis procera*, it has become necessary to evaluate its toxicity. The assessment of haematological, biochemical and histopathological parameters of animals following the administration of chemical compounds including plant extracts plays significant roles in the evaluation of toxicity risk or safety of such compounds, especially in humans.

Basak et al.^[15] reported that *Calotropis* latex contained such alkaloids as calotropin, catotoxin, calcilin and gigantol which have toxicity potentials. These might be responsible for the few adverse effects observed with the acute toxicity tests. The lack of adverse effects after oral administration of the latex in the rats may be suggestive of low bioavailability of the toxic component(s) due to poor absorption from the gastrointestinal tract unlike the intraperitoneal route that gives a better and faster absorption into the circulatory system. This finding is similar to that of Mohammed et al.^[16] who reported lower toxicity following oral exposure to the latex of *C. procera*. Caution is, however, needed in the use of the latex in wound-healing since absorption from the open wounds could potentially induce some systemic effects.^[1,2]

It has been documented that changes in body weight and feed intake can be used as indicators of adverse effects of drugs and chemicals.^[17] This present work indicated that the average body weight of rats in all the groups treated with *Calotropis latex*, both intra-peritoneally and orally, were not significantly ($P < 0.05$) affected in terms of reduction in weight as compared with the control groups.

The toxic effects of *Calotropis latex* following oral administration in ruminant animals and rats have been reported.^[18,19] This study evaluated the haematologic toxic effect of the latex following intra-peritoneal administration. There was a significant but non-progressive reduction of the PCV and red blood cell count following increasing doses of the latex to a limit of 0.85mg/kg IP. This is similar to what was observed by Mahmoud *et al.*^[20] and El-Badwi *et al.*^[21] following oral administration and potentiation by phenobarbital in sheep. The mechanism of this effect could not be explained by the present study design but may be related to direct cytolytic effect (red cell lysis which is more profound at higher doses of the latex). Similarly, there was a progressive and significant reduction ($P < 0.05$) in platelet count following increasing doses of the latex. There was no significant effect on the differential count except for the lymphocytes which showed significant reduction following increasing concentrations of the latex. The total WBC showed an initial rise in groups 1 and 2 similar to the observation of El-Badwi *et al.*^[21], Udoh *et al.*^[22] but, subsequently, in groups 3, 4 and 5 showed a marked and significant reduction in the total white cell counts. This observation may be related to the immunosuppressive effect on cell components of the leucocytes.^[23]

A significant ($P < 0.05$) decrease in glucose level was also observed in the treated groups especially the group administered with 0.85mg/kg of the latex as compared with the control. This indicates that *Calotropis latex* may have hypoglycaemic property as previously reported by Basak *et al.*^[15] who reported a decrease in blood glucose of rats. There was no significant change in the levels of albumin, alanine transaminases and alkaline phosphatase in all the treated groups. However, there were decreases in the levels of aspartate transaminases and total cholesterol. The reduction in the level of the total cholesterol may be a potential hypolipidaemic property, which can reduce the risk of the development of coronary heart disease, a known cause of sudden cardiac death. No significant changes were observed in uric acid, urea and creatinine levels for all the treated groups in this study indicating that the latex may not have adverse effects on the kidney.

In the histological study, the testes in the male rats showed appearance consistent with severe testicular atrophy. The severity of the testicular atrophy observed in the male rats increased in a dose-dependent manner regardless on the route of administration. This indicates that the latex exerted significant toxic effects on the physiological function of the male reproductive system. This observation is further confirmed by the report of an earlier study^[24] which showed that the *Calotropis* latex had significant influence on the spermatogenesis of male rats.

The lungs showed significant histopathological features at all the doses except in the control groups. There were varying degrees of dilatation of the alveolar ducts with the dilated alveoli filled with eosinophilic materials. This appearance is suggestive of pulmonary oedema. The toxic effect of the latex appears to be both dose and route dependent as the pulmonary oedema was more prominent at higher doses and in those treated with the latex through the intraperitoneal route. Histopathologic examination of some other organs like the brain, heart, liver, kidney, and spleen of both the treated and control animals showed normal architecture, suggesting no detrimental changes and morphological disturbances.

CONCLUSION

This study suggests that *Calotropis* latex is relatively safe when used acutely especially through the oral route and in low doses than when administered through the intraperitoneal route in rats. Its use in wound-healing and prevention of keloid formation, however, involves its direct contact with the circulatory system which may produce toxic effects similar to those observed for the intraperitoneal route. The marked progressive destruction of the seminiferous tubules in the male rats indicates that the latex may induce infertility and, therefore, great caution is required when extrapolating its use in humans. It would be informative to carry out this study in female rats in order to see its effect on the female reproductive organs. Nonetheless, clinical assays are recommended to confirm the low toxicity of the latex in humans. It is recommended that more studies be carried out in establishing the potential of *Calotropis* latex in reducing blood glucose and cholesterol for clinical purposes.

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