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Potential hepatic dysfunction associated with the use of the leaf and latex extracts of *Calotropis procera* W. T. Aiton (Asclepiadaceae) in Sprague-Dawley rats: an acute toxicity study

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ABSTRACT

Calotropis procera is an evergreen shrub of the family Asclepiadaceae. It is widely used traditionally in the treatment of various ailments, including dysentery and fever. Experimental data also report that *C. procera* has anticancer activity, among others. This study seeks to investigate the toxicity profile of *C. procera* in Sprague-Dawley rats. The aqueous, and ethanolic extracts of the dried leaves and the milky fluid (latex) of *C. procera* were prepared and analysed for phyto-constituents. Male and female Sprague-Dawley rats were administered with different doses of the aqueous and ethanolic leaf extracts, as well as latex extract of *C. procera via* the oral route. The animals were observed for signs of toxicity and euthanized on the 14th day (and blood samples were collected *via* cardiac puncture for hematological and biochemical analysis). Internal organs were also excised from the rats. The results showed no toxic effect based on the hematological and biochemical indices. Histopathological examinations did not reveal any significant effect on the internal organs except for mild changes in the liver architecture for which care should be taken upon chronic consumption.

KEYWORDS: *Calotropis procera*, extract, latex, toxicity, histopathology.

1. INTRODUCTION

Calotropis procera is a woody perennial shrub belonging to the family *Asclepiadaceae*. It is native to the tropics, especially West Africa.

Excavation at Helwan, Egypt, showed that *C. procera* was used for medicinal indications in the Neolithic period [1]. Ancient medical papyri indicate that the plant was recommended for the treatment of nodular leprosy [2, 3]. A decoction of the plant is used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism and as an expectorant [3-6]. Reports suggest that the capsulated root bark powder of *C. procera* is effective in the treatment of cholera, dysentery, indigestion, snake bite, syphilis [3] diarrhea [7] and management of asthma [3]. The latex is applied externally for treatment of boils, black scar on the face, ascites,

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leprosy, migraine, haemorrhoids, ring worm, scabies and tooth ache [3]. Additionally, extract of this plant has been reported to possess anticancer [8], antimicrobial [3, 9], anthelmintic [10, 11] and anti-inflammatory activities [12]. Although *C. procera* is used in Ghana and various parts of the world as medicinal plant, there is paucity of data on the toxicity profile of extracts of its leaves and latex. Furthermore, there are conflicting reports on the safety of extracts of the root bark and aerial parts of the plant [3, 13, 14]. With increasing interest in the use of natural products for therapeutic indications, and the already existing use of extracts of the leaves and latex of *C. procera* among indigenes of Ghana, it has become imperative that *in vivo* toxicological studies on *C. procera* is conducted.

Thus, the aim of this study was to assess the acute toxicity of extracts of the leaves and latex of *C. procera* in Sprague-Dawley rats and also establish their "no observed effect level" (NOEL)/ "no observed adverse effect level" (NOAEL).

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of *C. procera* were collected from the University of Ghana botanical garden and authenticated at the Ghana Herbarium. A voucher specimen (CPOOISP) was prepared and deposited at the herbarium. The leaves were then air-dried for 7 days and pulverized into coarse powder. The latex (milky fluid) was collected fresh from the plant on the day of dosing and used immediately.

2.2. Preparation of leaf extracts

Aqueous extract

The aqueous extract of *C. procera* was prepared by subjecting one hundred and fifty (150) g of the powdered leaves to cold maceration with agitation in distilled water. This was done 3 times successively and decanted after every 24 hours until exhaustion. The collected extract was then filtered and freeze-dried and stored in sterile bottles.

Ethanolic extract

The ethanolic extract was prepared by cold maceration. One hundred and twenty (120) g of the powdered leaves was placed in 80% ethanol and allowed to stand with agitation for 24 hours and this was then filtered. The filtrate was then concentrated under vacuum in a rotary evaporator at 50-60 ºC. The concentrate was then lyophilized, weighed and stored in sterile bottles.

2.3. Phytochemical screening

The preliminary phytochemical evaluation was done on all the extracts of *C. procera* using standard methods as described [15].

Animals

Twelve (12) adult male Sprague-Dawley rats and twelve (12) adult female Sprague-Dawley rats weighing between 100 g and 130 g were used for this study. The female rats were nulliparous and non-pregnant. The animals were kept in the animal house of the University of Ghana Medical School, Korle-Bu, Ghana. They were kept in metal cages and maintained under standard experimental conditions of room temperature (25 ºC) and 12 hour light/darkness cycle in cages with bedding material (wood shavings), fed with normal diet, given clean water and maintained under laboratory conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle). Spontaneous behaviors of all rats were observed in cages before experimental procedures were carried out. Animals were put into 4 groups of 6 (3 males and 3 females). The groups were randomly created and were made up of rats administered: aqueous extract, ethanolic extract, latex and distilled water. All animal procedures and techniques used were in accordance with the National Institute of Health Guidelines for the care and use of laboratory animals.

Acute toxicity

The latex, vehicle and extracts were administered orally (by gavage) on day 1 to rats in respective groups to mimic the traditional folkloric route of administration. After administration, the rats in each group were observed every 7 hours within a period of 48 hours for clinical signs of toxidromes, such as changes in movement, salivation, mydriasis, respiration, piloerection, frequency and consistency of stool and mortality. In each case a high dose of 4500 mg/kg was administered to the animals.

The rats were then monitored daily for 12 consecutive days for any clinically observed toxidromes and mortality. On day 14, rats were euthanized and blood samples were collected from each animal *via* cardiac puncture into EDTA tubes (BD microtainer) and gel separator tubes (BD vacutainer SST – II Advance) for hematological and biochemical analysis, respectively. Hematological analysis conducted included full blood count, while biochemical assays included total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase enzyme assays.

The rats were immediately autopsied and the heart, kidney, liver, lungs, small intestines, spleen, testis and uterus harvested and stored in formalin. Gross necropsy was later performed and post-mortem examinations conducted.

2.4. Ethical approval

The experiment was approved by the research ethics committee of the School of Pharmacy, College of Heath Sciences, University of Ghana, Legon (ETH-IAG003).

3. RESULTS

Percentage Yield of *Calotropis procera* leaves

The freeze-dried aqueous extract obtained was 22.5 g, a yield of 15%, and that of the ethanolic extract was 25.0 g, a yield of 20.8%.

Phytochemical screening of the *Calotropis procera* extracts

The phytochemical analysis revealed that the latex, aqueous and ethanolic extracts contained alkaloids, flavonoids, saponins, phenolic compounds, glycosides, triterpenes, sterols and tannins. Results are shown in Table 1.

3.1. Acute toxicity studies

Clinical observations

There were no observable clinical signs of toxidromes such as mydriasis, twitching, salivation, morbidity and piloerection. The absence of these

Table 1. Phytochemical screening of the extracts[a] of *Calotropis procera*.

Test	Observation	Inference
1. Extract + Dragendorff's Reagent 2. Extract + Mayer's Reagent 3. Extract + Wagner's Reagent	Reddish brown colouration Cream precipitate formed Yellow precipitate formed	Alkaloids are present in extract of *Calotropis procera*
Extract + 80% aqueous methanol + iron (III) chloride	Greenish blue colouration	Tannins are present in extract of *Calotropis procera*
Extract + ferric chloride	Blue colouration	Phenols are present in extract of *Calotropis procera*
Extract + water + shake	Foam formation	Saponins are present in extract of *Calotropis procera*
Extract + Chloroform + conc. H_2SO_4	No colour was observed at interface	Cardiac glycosides are absent in extract of *Calotropis procera*
Extract + 80% ethanol + magnesium turnings + conc. HCl	Light pink colouration	Flavonoids are present in extract of *Calotropis procera*
Extract + chloroform + heat + conc. H_2SO_4	Reddish brown ring	Triterpenoids are present in extracts of *Calotropis procera*
Extract + chloroform + acetic anhydride + conc. H_2SO_4.	Reddish violet colouration	Phytosterols are present in extracts of *Calotropis procera*

[a]extracts refer to the latex, aqueous and ethanolic extracts of *C. procera*.

signs might possibly indicate that the extracts, at these dose levels, have no visible effects on the central nervous system.

The histopathological results revealed no signs of toxic effects in the heart, kidneys, lungs, small intestines, spleen, testes and uterus (Figures 1a-g). Observation parameters such as degeneration, congestion, oedema and cellular infiltration were absent for all the extracts. However, the liver showed mild to moderate signs of congestion, cellular infiltration, coagulation and oedema formation (Figure 1h). The hematological results also revealed no effects of the extracts on the blood. There was normal red blood cell coloration, normal red blood cell size, normal red blood cell shape, normal red blood cell distribution, normal white blood cell distribution and a normal platelet distribution in the blood smears of all the test rats (Figure 2).

3.2. Heart

In both gross and histopathology, the hearts of all the test rats were similar to that of the controls. There was no myofibril degeneration; nucleus was not affected, no cellular infiltration, edema and no congestion in the hearts of all the test rats as shown in Figure 1a.

3.3. Kidneys

The kidneys of all the test rats were similar to that of the controls. There was no degeneration of the glomerulus, no tubular cast, no cellular infiltration or edema and no congestion in the kidneys of any of the test rats as shown in Figure 1b.

3.4. Lungs

The lungs of all the test rats were similar to that of the controls. There was no degeneration of the lining of the bronchus, no cell proliferation, no cellular infiltration, no edema and no congestion in the lungs of any of the test rats as shown in Figure 1c.

3.5. Small intestines

The small intestines of all the test rats were similar to that of the controls. There was

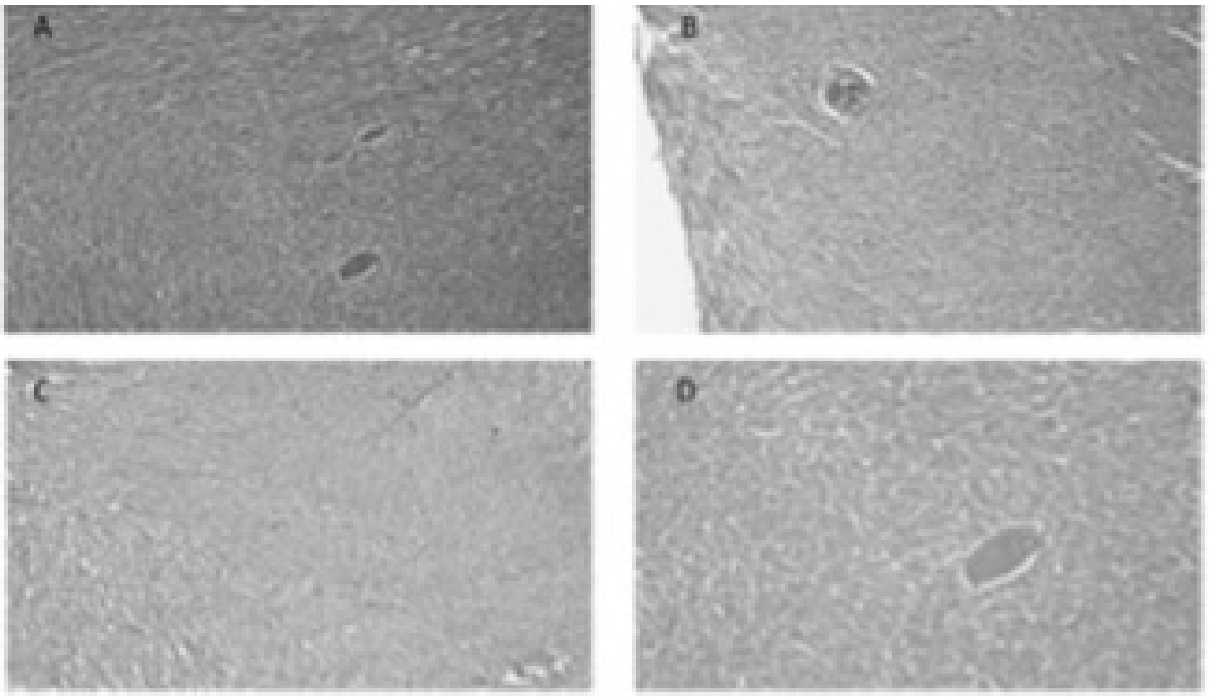

Figure 1a. Photomicrographs of paraffin sections of the hearts of the rats showing no difference between the control rats and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

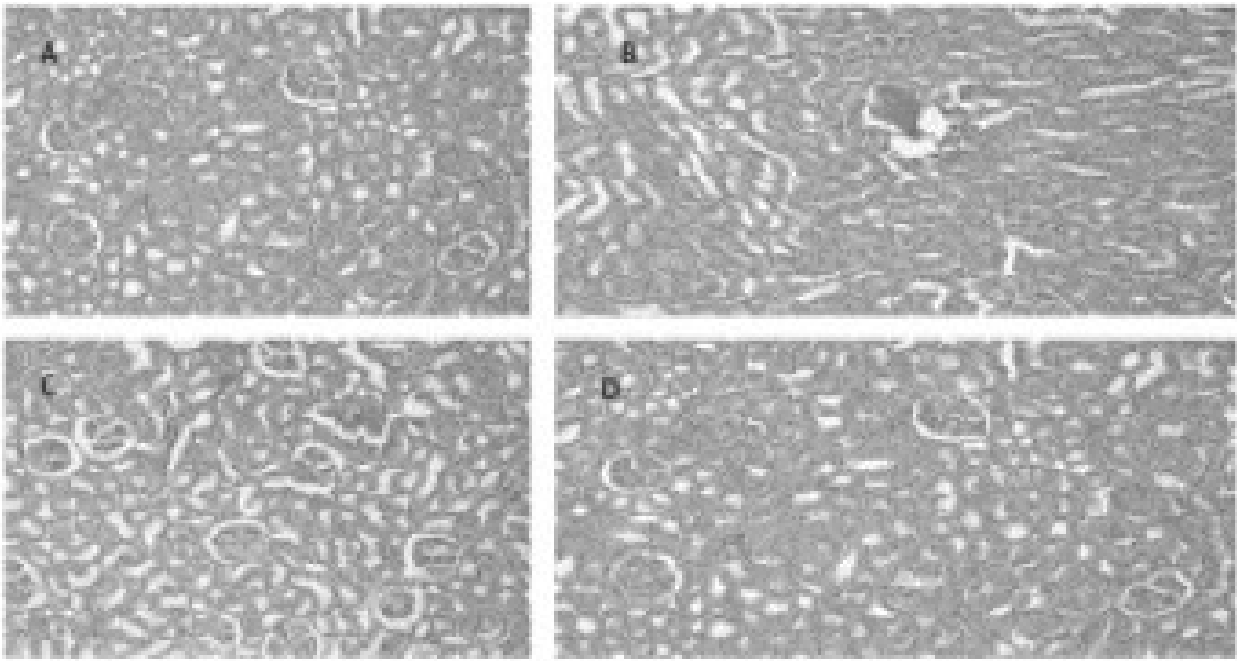

Figure 1b. Photomicrographs of paraffin sections of the kidneys of the rats showing no difference between the control rat and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

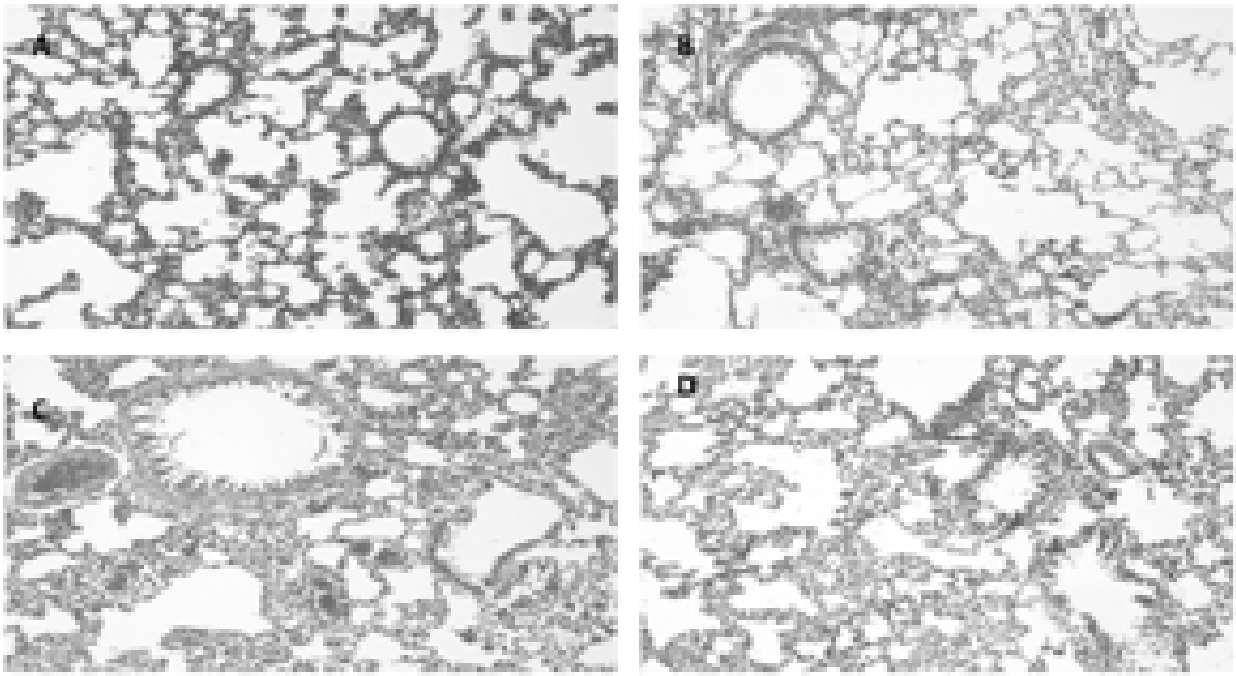

Figure 1c. Photomicrographs of paraffin sections of the lungs of the rats showing no difference between the control rat and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

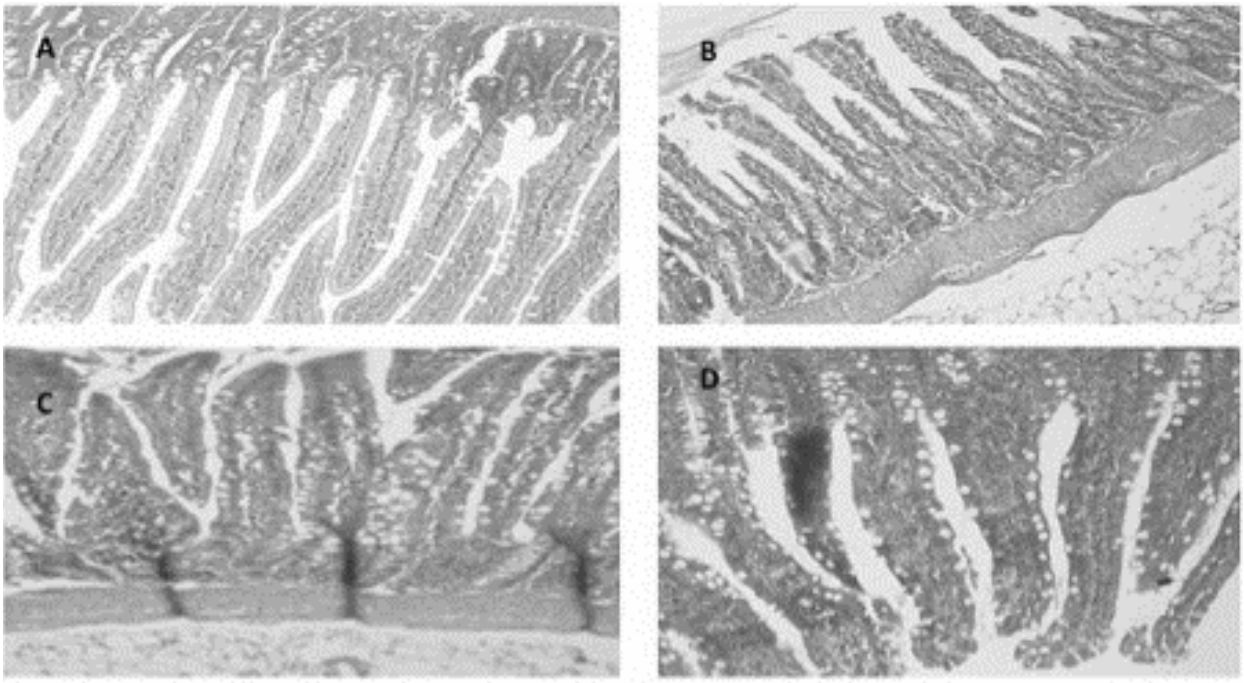

Figure 1d. Photomicrographs of paraffin sections of the small intestines of the control rats showing no difference from those of the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

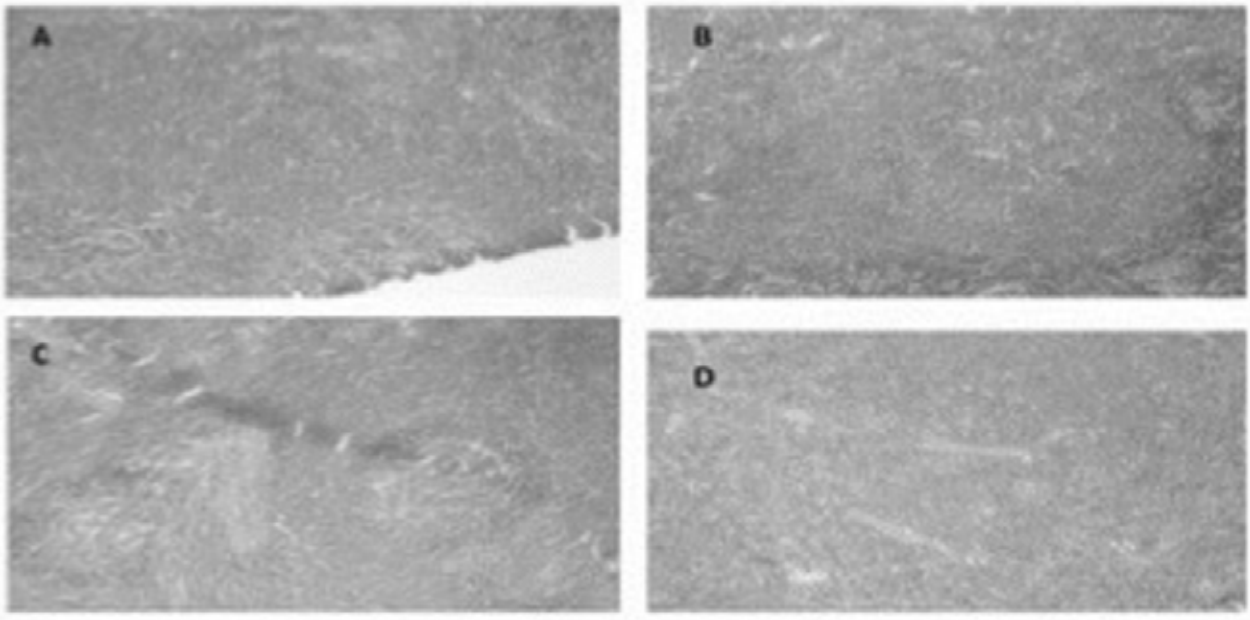

Figure 1e. Photomicrographs of paraffin sections of the spleens of the rats showing no difference between the control rat and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

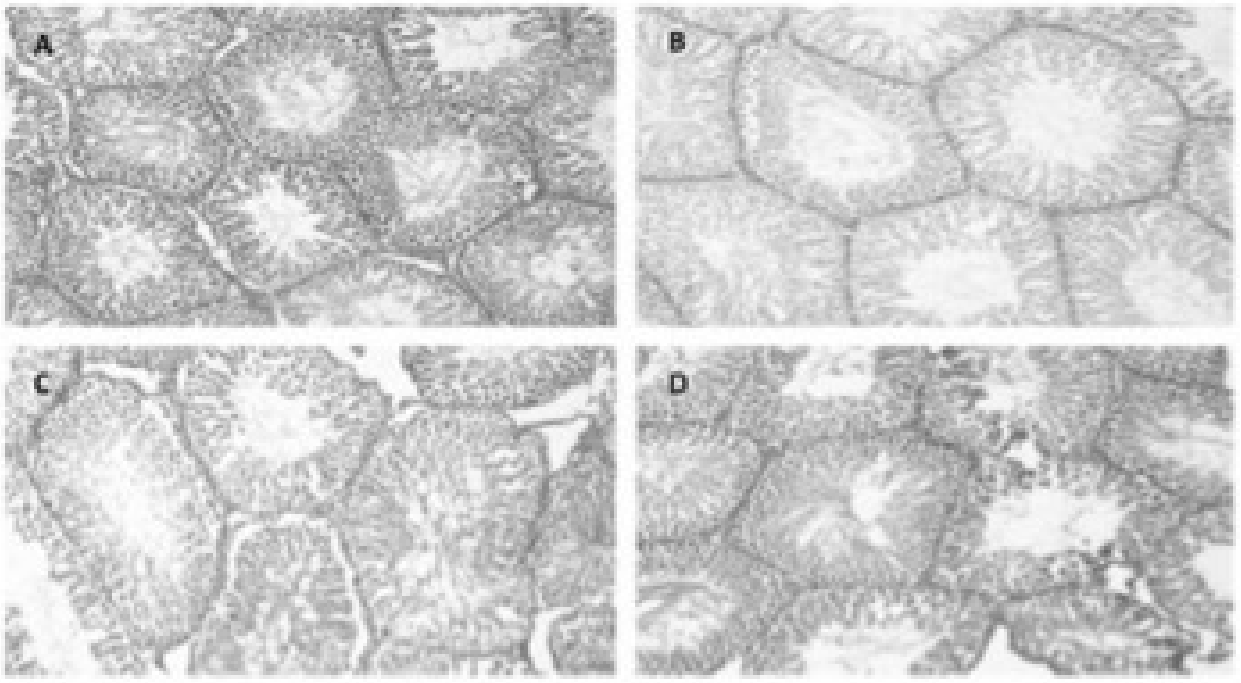

Figure 1f. Photomicrographs of paraffin sections of the testes of male rats showing no difference between the male control rats and the male test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

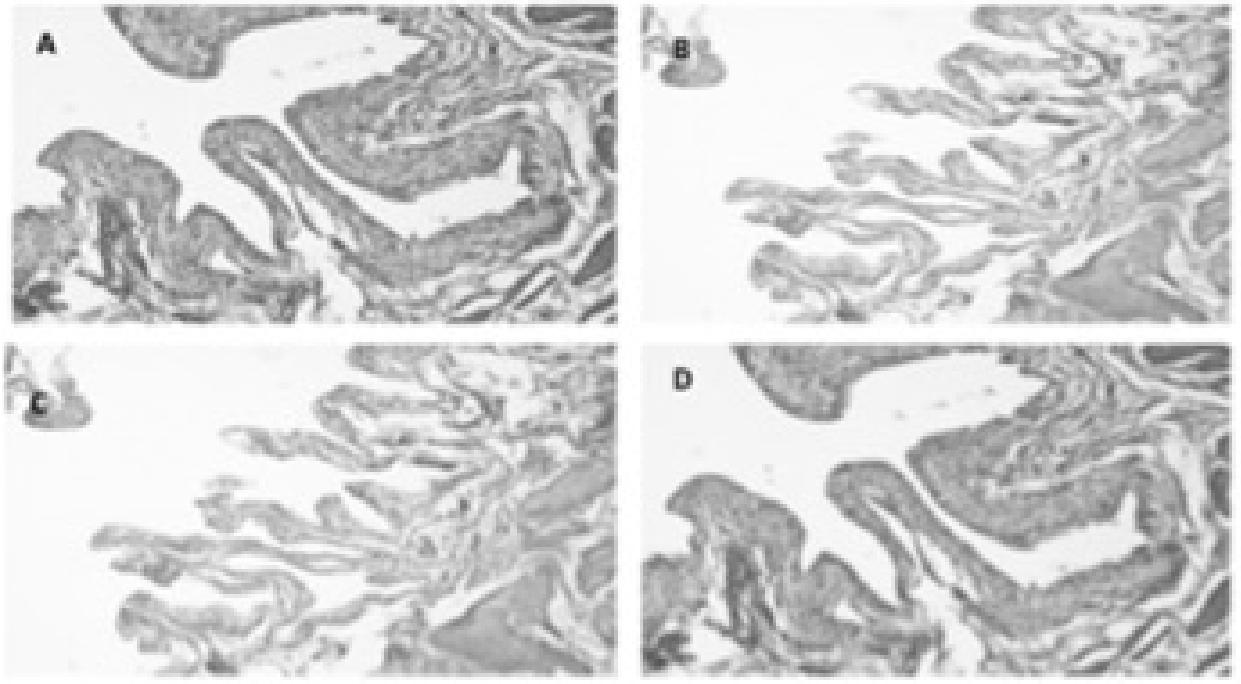

Figure 1g. Photomicrographs of paraffin sections of the uteri of the rats showing no difference between the control rat and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (H & E X20).

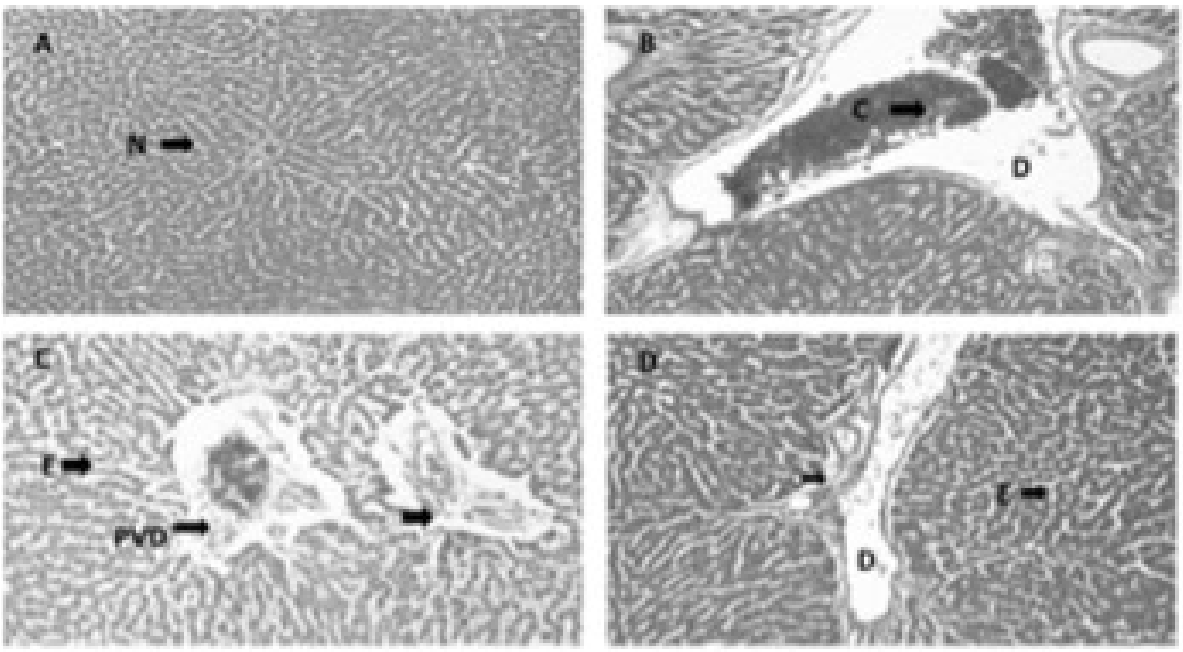

Figure 1h. Photomicrographs of paraffin sections of the livers of the rats showing marked differences between the liver of the control and those of the test rats. A: control showing normal (N) liver architecture. B: 2 mL of 1/100 dil. latex showing, dilated (D) and congested (C) central vein. C: 2500 mg/kg aqueous ext. showing edema (E) and perivascular degeneration (PVD) of liver parenchyma. D: 2500 mg/kg ethanolic extract showing dilated (D) central vein and mild edema (E). (H & E x20).

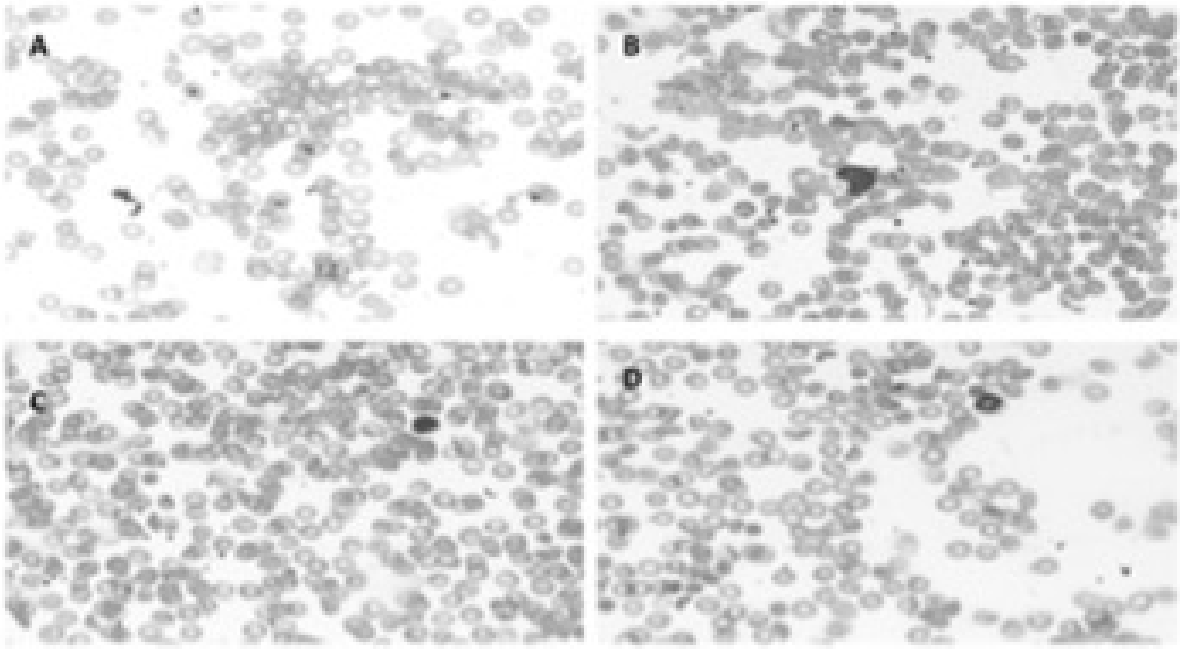

Figure 2. Photomicrographs of blood smears from control and test rats showing no difference between the control rat and the test rats: – A: control, B: 2 mL of pure latex, C: 4500 mg/kg aqueous extract and D: 4500 mg/kg ethanolic extract. (Leishman', stain x20).

no degeneration of the villi, no lining ulceration, no cellular infiltration, no edema and no congestion in the small intestines of any of the test rats as shown in Figure 1d.

3.6. Spleens

The spleens of all the test rats were similar to that of the controls. There was no degeneration, no edema or lymphoid hyperplasia in the spleens of any of the test rats as shown in Figure 1e.

3.7. Testes

The testes of all the test rats were histologically similar to that of the controls. There was no degeneration, no impaired spermatogenesis, no cellular infiltration or edema in the testes of any of the test rats as shown in Figure 1f.

3.8. Uteri

The uteri of female test rats were similar to that of the female controls. There was no degeneration of the endometrial lining, no cellular infiltration, no edema and no hypertrophy of the uteri walls of any of the test rats as shown in Figure 1g.

3.9. Livers

The livers of all the test rats were different from that of the controls. There was cellular spacing (edema), cellular infiltration, congestion and perivascular degeneration of liver tissues of the test rats (Figure 1h).

3.10. Blood smears

The morphology of the blood cells in the blood smear of all the test rats was no different from the controls. The red cells were normocytic and normochromic, with normal red blood cell distribution, normal white blood cell distribution and a normal platelet distribution in the blood smears of all the test rats, Figure 2.

4. DISCUSSION

A number of studies have reported the pharmacological potentials of *C. procera*, Mascolo's group and Mueen Ahmed's group [12, 16, 17]. Toxicity studies have also been conducted on different parts of the plant including the aerial parts and root bark [13, 14], however, there is a paucity of data on the toxicity of the latex and leaves of this plant.

In this acute toxicity study, a dose level of 4500 mg/kg body weight in a single oral administration of the aqueous and ethanolic extracts showed that both extracts have LD_{50} higher than 4500 mg/kg. This suggests that these extracts have low oral toxicity according to classifications by the World Health Organisation [18]. This corroborates the study that showed that ethanolic extract of the aerial parts of *C. procera* does not cause oral toxicity in mice at doses up to 3000 mg/kg [19]. Ouedraogo's group also proved that extracts of the plants have LD_{50} greater than 2000 mg/kg [14].

Administration of 2 mL pure latex of *C. procera* did not cause any mortality or signs of toxicity during the period of observation in rats of both sexes. A similar study found that the daily oral administration of latex of *C. procera* to rats for 7 and 14 days, respectively, did not cause any visible toxic effects [20]. On the contrary, a study has shown 20% - 40% mortality in Wistar rats administered both aqueous decoction and ethanolic extract of *C. procera* roots (obtained from Mali) at doses of 1500 and 2000 mg/kg [21]. The difference could be partly due to process of extraction, the type of animals used, and also soil factors that can influence the final chemical composition of extracts [22].

In this present acute toxicity study there were no significant changes in the hematological parameters (full blood count, blood cells morphology and distribution) between the control and the experimental groups (Figure 2). The hematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of toxicity in animals and humans. Thus, there is a low likelihood that ingestion of *C. procera* would have adverse effects on cellular components of blood in humans. These results are comparable to others where a daily oral administration of the latex of *C. procera* in rats during a 7 to 14-day administration had no significant effects on blood parameters [20]. Ouedraogo's group [14] and other studies [3, 5, 6, 18] also showed the non-toxic potential of *C. procera* on the hematological system [14].

Histopathological examinations of the liver of treated animals portrayed cellular infiltration, congestion, edema and coagulation (Figure 1h).

This was observed in all the different extracts administered: latex, aqueous and ethanolic extracts. This showed that the tested extracts of *C. procera* had some effect on liver cells and could potentially affect liver function. These results are similar to one reported by Ouedraogo's group where changes in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed (Table 2) [14]. Congestion and dilatation of central veins of liver seen in histological examination was the reason given behind the slight elevation of transaminases ALT and AST [14]. Generally, the level of hepatic enzyme present in circulation is directly proportional to the number of damaged liver cells [23]. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferases (AST) are usually elevated in conditions associated with injuries or diseases affecting the liver which leads to the release of these hepatocellular enzymes into the bloodstream [24]. In this present study there were significant differences in the ALT, AST, GGT, ALB, direct bilirubin and total bilirubin between the test rats and controls (Table 2). Further, there was some level of reduction in albumin and total proteins as decreased levels of proteins are indicative of liver damage or malnutrition. It is important to note that although the term "liver function tests" is used commonly, it is imprecise since many of the tests reflecting the health of the liver are not direct measures of its function and also some of the tests may also be abnormal even in patients with a healthy liver. These results however show some level of injury caused to the liver cells by the extracts (Figure 1h).

Histopathology of the heart, kidneys, lungs, small intestines, spleen, testes and uterus did not vary significantly compared to the controls (Figures 1a-g). The same was observed by Ouedraogo's group where no significant changes in the kidneys, lungs and small intestines were recorded [14]. In the kidney, there were no thickening of glomerular basement membrane and proliferation of the endothelial cells surrounding the outer surface of the glomerular capillary tuft. This study has provided some clarity on the relatively non-toxic potential of *Calotropis procera* when

Table 2. Biochemical analysis.

Parameters	Units	Control	Pure latex 2 ml	Aqueous extract 4500 mg/kg	Ethanolic extract 4500 mg/kg	P value
Total bilirubin	umol/l	0.4000 ± 0.2309	1.200 ± 0.0	0.8500 ± 0.08660	1.100 ± 0.05774	0.0035
Direct bilirubin	umol/l	0.6650 ± 0.04907	1.515 ± 0.1357	0.8850 ± 0.1010	0.8450 ± 0.1415	0.0010
GGT	U/l	0.5000 ± 0.2887	3.500 ± 0.8660	8.500 ± 1.443	4.000 ± 1.732	0.0047
AST	U/l	1 ± 0.5774	33.00 ± 10.39	36.50 ± 0.0	31.00 ± 9.238	0.0032
ALB	g/l	40.08 ± 0.05196	41.14 ± 1.319	30.36 ± 4.633	86.88 ± 26.63	0.0497
ALT	U/l	126.5 ± 1.443	90.00 ± 3.464	86.50 ± 4.907	116.0 ± 9.815	0.0008
Total protein	g/l	71.05 ± 1.819	64.40 ± 0.9815	50.35 ±8.227	55.76 ± 9.206	0.1479
ALP	U/l	591.0 ± 62.93	602.5 ± 126.2	553.0 ±113.2	631.0 ± 326.8	0.9927

administered orally. The non-toxic aspect of these extracts is likely to be linked to their components and the phytochemical constituents [3, 5, 6, 18]. It is however advisable that liver function tests be conducted regularly during administration of these extracts during prolonged use. Further, drugs that have known adverse effects on the liver should be avoided during concomitant administration with these extracts.

5. CONCLUSION

Acute toxicity assessment showed that the latex, aqueous and ethanolic extracts of the leaves of *Calotropis procera* had no observable adverse effect on the heart, kidney, lungs, spleen, small intestines, testes, uterus and blood cells of rats. However, the extracts showed appreciable toxic effects on the liver. These results suggest that the latex, aqueous and ethanolic extracts of *C. procera* leaves are relatively safe, aside from possible hepatotoxic effects, when administered orally.

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CONFLICT OF INTEREST STATEMENT

The authors hereby declare there is no conflict of interest regarding this study.

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