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Research Article
Toxicological Evaluation and Therapeutic Index of Ethanolic Leaf Extract of *Acanthus montanus* (Acanthaceae) in Mice

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Abstract

Background and Objectives: *Acanthus montanus* (Nees) T. Anderson (Acanthaceae) plant have been employed in folklore and traditional medicine in the treatment of various forms of disease conditions in Africa. Unquantifiable amounts have been consumed in the quest to obtain relief from these ailments. Thus, the work aimed to evaluate the effect of graded doses of Crude Ethanolic leaf Extract (CEE) of *A. montanus* in mice with regards to histopathological changes in vital organs such as the liver, heart, spleen, lungs, kidney and intestine and to determine its median Lethal Dose (LD_{50}). **Materials and Methods:** Mice were treated with (low, moderate, high and very high doses) of CEE or vehicle (distilled water) by oral gavage once, after a 12 hrs deprivation of water and observed for demeanour and/or signs of toxicity for 48 hrs and 21 days post-inoculation. A mouse from each group was humanely sacrificed and vital organs harvested. Tissues were trimmed, processed and haematoxylin-eosin stained. **Results:** There were no observable signs of toxicity and death of mice during the period of observation. Gross and histopathological findings were those of congestion, mononuclear cellular infiltration and cellular degeneration which were dose-dependent. The gross and histopathological changes improved within the period of observation. LD_{50} of the extract was greater than 10,000 mg kg^{-1}. **Conclusion:** An oral single dose of CEE of *A. montanus* leaf to mice at the rate of 10,000 mg kg^{-1} did not cause death in mice. This suggests that the plant might be practically non-toxic and have a high therapeutic index with regards to its use in folklore and ethnomedicine.

Key words: *Acanthus montanus*, ethanolic leaf extract, toxicological evaluation, therapeutic index, LD_{50}, histopathological changes, antiparasitic effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acanthus montanus (Nees) T. Anderson is a member of the Acanthaceae family[1,2]. The genus *Acanthus* has about 30 species of flowering plants. The generic name is derived from the Greek word Acanthus (*acanthos*), meaning "thorny"[3]. It is known and variously called by different names including "Ahon Ekon" (Yoruba), "Agameebu" or "Agamsoso" (Igbo), "Gautar Fadama" (Hausa), "Elele-nyijuo" (Igede), "Idumngbe" (Etulo) and "Shishaikyo", "Ityoukibua" or "Pevkyekye" (Tiv)[4-7] in Nigeria and across the world. The common name of this thorny herbaceous plant which grows in grasslands, woods, scrubs and rocky hills in different parts of the world include "Bear's breech", "Mountain thistle", "False thistle", "Alligator plant" and "Thorny pigweed"[2,8].

Burkill[2] asserts that different portions of the *A. montanus* plant are used in different parts of the world in the treatment of various ailments in humans including furunculosis, cough[6], pneumonia, fever, gastrointestinal upsets, heart troubles[9-13], urinary tract infections and other inflammatory processes[14,15], menstrual irregularities, 'morning sickness' in pregnant women, abortion[16-18], rheumatism, yaws, as well as in ceremonies of purification and exorcism. Recent research has demonstrated that leaf extracts of this plant have an antiparasitic effect against gastrointestinal nematodes[7,19,20].

Secondary metabolites such as cardiac glycosides, unsaturated steroids and sterols, saponins, tannins, anthracenes, triterpenes, flavonoids and alkaloids were previously detected in *A. montanus*[7] as well as a new phenylethanoid di-glycoside named 'Acanmontanoside' has been isolated[21]. Although, *A. montanus* seemed to have great potential in the treatment of myriads of infections and disease conditions, its toxicity studies may not have been conducted to ascertain its safety among the human population.

The aim of this study is, therefore, to evaluate the toxic effects and to determine the median lethal dose (LD_{50}) of ethanolic leaf extracts of *A. montanus* in mice.

MATERIALS AND METHODS

Study area: This study was carried out at Ahmadu Bello University, Zaria, Nigeria between July, 2011 to September, 2012.

Plant material collection and identification: Literature on ethnomedical uses and the folkloric claims warranted the choice of the plant materials. Fresh leaves of *Acanthus montanus* with stalks were collected/ harvested in March and April along a stream in the northern part of Katsina-Ala township of Katsina-Ala Local Government Area of Benue State, Nigeria. Katsina-Ala town is located on latitude 7°10'N and longitude 9°19'E in the middle belt (Guinea Savannah) of Nigeria. The plant name has been checked with The Plant List[22]. A sample of the plant was brought to Zaria and was identified/authenticated by a plant taxonomist at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen was deposited. It was assigned voucher number 7037.

Preparation and preservation of extracts: The method described by Oshadu *et al.*[7] was used to prepare the ethanolic leaf extract of *A. montanus* which was stored in an air-tight glass bottle until used.

Determination of maximum convenient concentrations (MCCs) and maximum convenient doses (MCDs) of extracts used in toxicity studies: The Maximum Convenient Concentrations (MCCs) of crude ethanolic leaf extract *A. montanus* (CEE) was prepared as described by Oghenesuvwe *et al.*[23]. One gram (1 g) of the extract was thoroughly mixed with a given volume (mL) of distilled water (0.05 mL at a time) until the solution could be delivered through an 18 gauge needle at room temperature. From the volume of the solvent used, the MCC of extract in g mL^{-1} was determined. Thus, the Maximum Convenient Doses (MCDs) for the extracts were prepared[23]. The Maximum Convenient Volume (MCV) that could be administered to mice by the oral route (gavage) is 5 mL kg^{-1} [23,24]. The MCDs (g kg^{-1}) was thus calculated by multiplying the MCCs (g mL^{-1}) by the MCV (mL kg^{-1}).

Laboratory animals and preliminary toxicity studies: This research was conducted following the internationally accepted principles for laboratory animal use and care and complies with ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines carried out with the UK Animal (Scientific Procedures) Act, 1987 with regards to EU Directive 2010/63/EU for animal experiments[25]. Twenty Albino mice, *Mus musculus* of 20-25 g were acquired and allowed to be acclimatized under standard laboratory conditions for 2 weeks. Fifteen of the mice of both sexes were randomly assigned to 5 groups of three mice each and the amount of CEE that would cause 0-100% death when administered orally was determined using Lorke's method[26] as modified by Dzenda *et al.*[27]. Group 1 received 10,000 mg kg^{-1} b.wt., Group 2 1,000 mg kg^{-1} b. wt., Group 3 and 4 received 100 and 10 mg kg^{-1} b. wt., respectively. Group 5 (the control group) were given distilled water at 5 mL kg^{-1} b.wt.

Stock solution: The stock solution was prepared in four well-labelled test tubes as previously described, by serial dilution. Briefly, 10,000 mg of CEE was thoroughly dissolved in 1 mL of distilled water (vehicle) to obtain 10,000 mg mL^{-1}. One milliliter of this was transferred into the second test tube containing 9 mL of distilled water and vigorously mixed to obtain 10 mL, giving 1,000 mg mL^{-1} of the solution. Again, 1 mL of this solution was transferred to the third test tube containing 9 mL of water and thoroughly mixed to obtain 10 mL of 100 mg mL^{-1} solution. Lastly, 1 mL of this solution was dissolved in the fourth test tube containing 9 mL of distilled water to obtain 10 mL of 10 mg mL^{-1}. The actual amount of this solution administered to each mouse was calculated based on the MCV (5 mg mL^{-1}) and the body weight of each mouse. Mice were deprived of water for about 12 hrs before extract administration and also about 3 hrs after. Treatments were administered by gavage (*per os*) directly into the oesophagus. Mice were observed closely for any change in behaviour and/or signs of toxicity for 48 hrs. Thereafter, one mouse from each group was sent to the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, for necropsy.

Postmortem examination of organs: The mice were euthanized in a chloroform chamber and necropsied. The liver, lungs, heart, kidneys, spleen and intestine were examined for gross pathologic changes by a Pathologist and recorded. Thereafter, tissue samples were taken from each of these organs and fixed in 10% buffered neutral formalin for Histopathology. At 21 days post-exposure, during which no death occurred in any group, one out of the two mice remaining in the group that took the highest dose was necropsied and the internal organs were examined for any gross lesions as described earlier. Tissues were trimmed and processed in tissue cassettes embedded in paraffin wax, sectioned at 5 µ. The processed tissues were stained with Haematoxylin and Eosin (H&E)[28].

RESULTS

Extractive yield, solubility, MCC and MCD of crude ethanolic leaf extract (CEE) of *A. montanus*: During extraction, 1,000 g of the pulverized leaf of *Acanthus montanus* yielded 25.58% (w/w) brown coloured Crude Ethanolic Extract (CEE) which was readily soluble in water with MCC and MCD of 0.8 g mL^{-1} and 4 g kg^{-1}, respectively, as shown in Table 1.

Observational effects of CEE on mice: Mice exposed to the varying doses of CEE of *A. montanus* did not die within 48 hrs and 21 days post-inoculation. None showed obvious signs of toxicity as shown in Table 2.

Postmortem findings: The gross lesions observed in a mouse exposed to crude ethanolic leaf extract of *A. montanus* during preliminary acute toxicity studies were those of congestion especially of the liver, lungs, heart and the spleen in the group exposed to 10,000 mg kg^{-1} of the extract. There was however, no significant gross pathologic changes in the groups exposed to 10-1,000 mg kg^{-1} and placebo (distilled water 5 mL kg^{-1}).

Histopathological results: Figure 1a shows a section of the liver of mouse exposed to distilled water at 5 mL kg^{-1} with no observable microscopic lesions, Fig. 1b shows a section of the liver of mouse after 48 hrs' exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1} showing periportal necrosis (D) around the congested central vein (C) and severe haemorrhages (H) and Fig. 1c shows a section of liver of mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1} with noticeable diffused hepatocellular necrosis (N), congested central vein (C) and vacuolar degeneration (V).

Figure 2a shows a section of the heart of a mouse exposed to distilled water at 5 mg kg^{-1}. No observable microscopic lesions, Fig. 2b shows a section of the heart of mouse 48 hrs after exposure to crude ethanolic leaf extract of

Table 1: Extractive yield, solubility, MCC and MCD of crude ethanolic leaf extract (CEE) of *A. montanus*

Crude ethanolic leaf extract							
Weight of powder (g)	Weight of extract (g)	Colour of extract	Yield (% w/w)	MCV (mL kg^{-1})	Solubility	MCC (g mL^{-1})	MCD (g kg^{-1})
1,000	255.84	Brown	25.58	5	Readily soluble	0.8	4

1.25 mL of solvent (distilled water) conveniently dissolved 1 g of CEE

Table 2: Results of preliminary acute toxicity studies of crude ethanolic leaf extract (CEE) of *A. montanus* in mice

Group	Dose (mg kg^{-1})	No. dead/No. alive	Clinical sign(s)
1	10,000	0/3	NOS
2	1,000	0/3	NOS
3	100	0/3	NOS
4	10	0/3	NOS
5	DW (5 mL kg^{-1})	0/3	NOS

5 mL kg^{-1} is the MCV, NOS: No observable sign

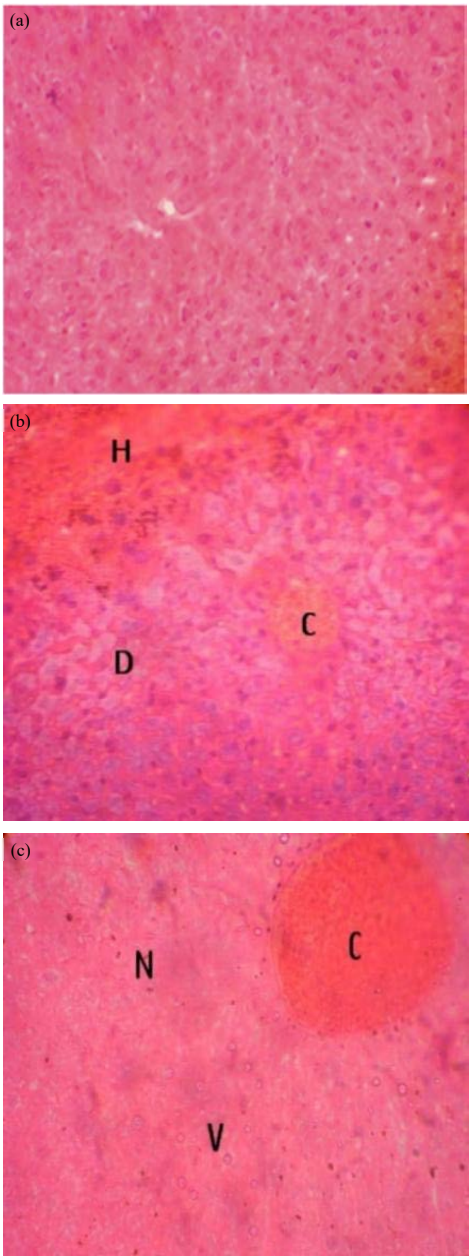

Fig. 1a-c: Photomicrograph of a section of liver of a mouse. (a) Mouse exposed to distilled water at 5 mL kg^{-1}, no observable microscopic lesions, (b) Mouse after 48 hrs exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}, showing, D: Periportal necrosis, C: Around the congested central vein, H: Severe haemorrhages and (c) Mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}, diffused N: Hepatocellular necrosis, C: Congested central vein and V: Vacuolar degeneration
(H&E, ×400)

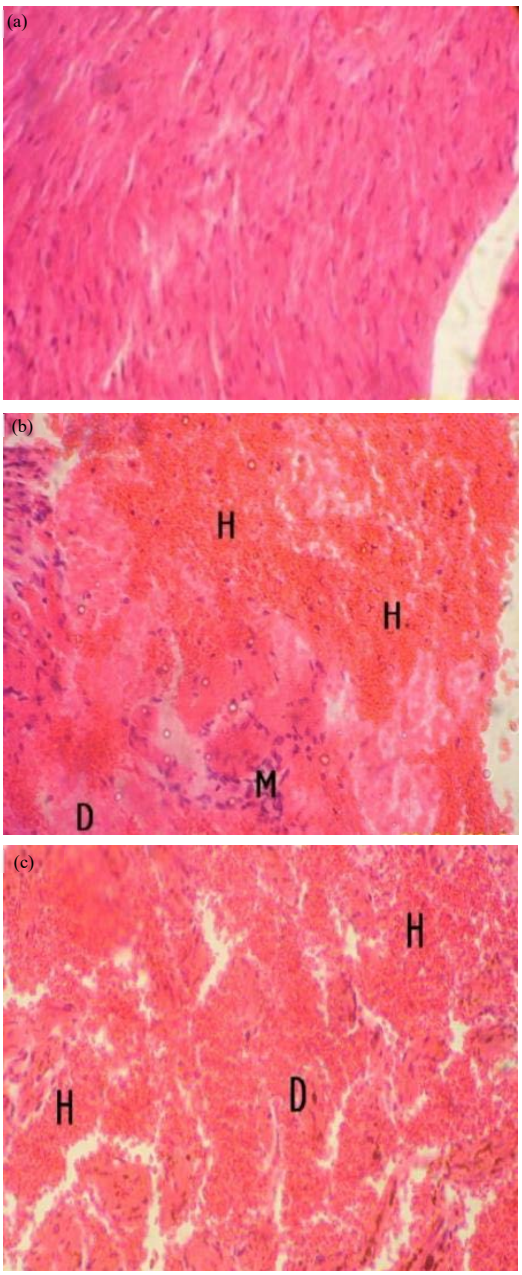

Fig. 2a-c: Photomicrograph of a section of the heart a mouse. (a) Mouse exposed to distilled water 5 mg kg^{-1}, no observable microscopic lesions, (b) Mouse 48 hrs after exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}, showing severe, H: Diffused haemorrhage, D: Myocardial degeneration, M: Mild focal mononuclear cellular infiltration and (c) Mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}, showing severe, H: Diffused haemorrhages, D: Myocardial degeneration/necrosis
(H&E, ×400)

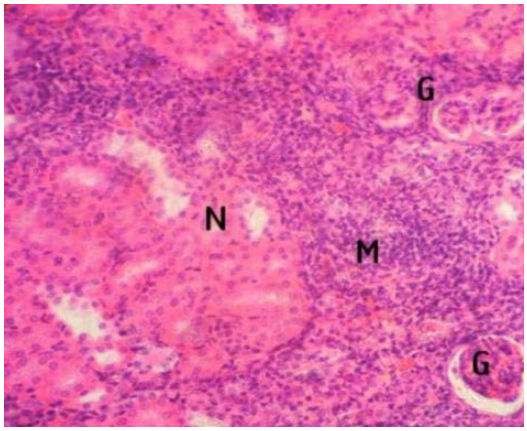

Fig. 3: Photomicrograph of a section of the kidney of a mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}

Necrosis and desquamation of renal tubular epithelial cells (N), glomerular degeneration/sclerosis (G) and marked mononuclear cellular infiltration (M) into the interstices. (H&E, ×400)

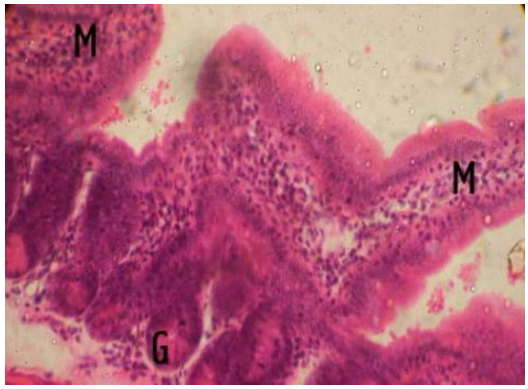

Fig. 4: Photomicrograph of a section of the intestine of a mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}

Regeneration of desquamated epithelium, mononuclear cellular infiltration (M) into the villi and hyperactive goblet cells (G). (H&E, ×400)

A. montanus at 10,000 mg kg^{-1}, showing severe diffused haemorrhage (H) and myocardial degeneration (D) and mild focal mononuclear cellular infiltration (M) and Fig. 2c is a photomicrograph of a section of the heart of mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}, showing severe, diffused haemorrhages (H) and myocardial degeneration/necrosis (D).

Figure 3 shows a photomicrograph of a section of the kidney of a mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1}. Necrosis and desquamation of renal tubular epithelial cells (N), glomerular degeneration/sclerosis (G) and marked mononuclear cellular infiltration (M) into the interstices were noticed. Figure 4, a photomicrograph of a section of the intestine of a mouse at day 21 post-exposure to crude ethanolic leaf extract of *A. montanus* at 10,000 mg kg^{-1} showing regeneration of desquamated epithelium, mononuclear cellular infiltration (M) into the villi and hyperactive goblet cells (G).

DISCUSSION

Subjecting 1,000 g pulverized leaf of *Acanthus montanus* to extractive process gave a yield of about 25% w/w which was readily soluble in water with MCC and MCD of 0.8 g mL^{-1} and 4 g kg^{-1}, respectively (Table 1). Secondary metabolites have been detected and some isolated from this plant[7,21] which holds great potential in ethnomedicne[9-13]. Oral administration of the highest dose (10,000 mg kg^{-1}) of the plant did not cause death (Table 2), however, there were some histopathological changes in vital organs of mice, some of which tend to resolve with time (Fig. 1-4).

Medicinal plants are vital sources of easily accessible remedies used in the countryside healthcare system[29]. Plants have sustained mankind not only as a source of food but also as medicine[30] and poisons utilized in various forms for varied purposes[31]. In a search to combat old and emerging diseases, plant medicines have given hope to man where many orthodox drugs have failed. Medicines of plant origin are said to be safer and better for human health than synthetic drugs since the ingredients in plants such as proteins, carbohydrates, lipids, vitamins and minerals are part of the body composition[29,30,32].

Ethnomedical uses of different parts (shoot, leaf, stem, stem bark and roots) of *Acanthus montanus* have been discussed[2,3,6,9-18]. The crude extraction of the pulverised leaf of *A. montanus* yielded about 25%. The crude ethanolic extract was readily soluble in water because of its hydrophilic nature i.e., they contain hydroxyl group (OH$^-$).

For the preliminary acute toxicity studies, all the treated mice were closely examined for signs of toxicity from the time of administration of extracts to 21 days post extract administration. None exhibited any obvious signs of toxicity or change of demeanour at doses ranging from 10-10,000 mg kg^{-1}. All the treated mice except those euthanized remained alive even long after the three weeks of observation. This suggests an oral median Lethal Dose (LD_{50}) greater than 10,000 mg kg^{-1}. The high LD_{50} value implies a remote risk of acute intoxication and a high degree of relative safety[24,27] when the extract is administered orally. It is, therefore, considered practically non-toxic and of high

therapeutic index. Earlier, Okoli *et al.*[15] established an LD_{50} greater than 5,000 mg kg^{-1} when the aqueous root extract of *A. montanus* was administered orally and intraperitoneally. In an earlier study, Paulin *et al.*[33] demonstrated LD_{50} of the same plant to be greater than 8,000 mg kg^{-1} in rats. This probably explains why an undetermined amount of aqueous leaf extract is taken locally in folk medicine without side effects. Attempts to grade dose-toxicity relationships of toxic substances under experimental conditions have been made. One of such grading, according to Diehl *et al.*[24], defined <1 mg kg^{-1} as extremely toxic, 1-50 mg kg^{-1} as highly toxic, 50-500 mg kg^{-1} as moderately toxic, 0.5-5 g kg^{-1} slightly toxic, 5-15 g kg^{-1} as practically non-toxic and >15 g kg^{-1} as relatively harmless. In this study, the extract tested in mice is therefore regarded as practically non-toxic, since the LD_{50} falls within the range of 5-15 g kg^{-1}.

Mice treated with a dose range of 10-1,000 mg kg^{-1} of crude ethanolic extract had no detectable pathology in the organs studied, but lesions, mainly congestion, were observed in the lungs, liver, heart and spleen of mice that were dosed with 10,000 mg kg^{-1}. It was discovered that the congestion grossly observed previously was resolved by 21 days post-exposure. This further confirmed the fact that the *A. montanus* plant might be practically non-toxic[16,24,26].

Histopathologic examination of internal organs of mice treated with varying doses of the crude ethanolic extract showed areas of haemorrhages, congestions, necrosis, desquamation of intestinal villi and in some cases, mononuclear cells infiltration into organs. For example, there was haemorrhages and hepatocellular degeneration around the sinusoids and central vein in the liver. While there was massive haemorrhage, myocardial degeneration and foci of mononuclear infiltration in the heart, the kidney had renal tubular necrosis and mononuclear cellular infiltration into the interstices (Fig. 1-4). Paulin *et al.*[33] found similar lesions in the kidney of Wistar rats when aqueous leaf extract of *A. montanus* was administered at a dose rate of 8,000 mg kg^{-1}. Theirs was glomerular degeneration in the renal parenchyma. These microscopic lesions were, however, marked in groups exposed to the highest dose (10,000 mg kg^{-1}). Most organs exposed to lower doses and distilled water were relatively normal. Three weeks post-treatment, some organs especially the intestine showed marked signs of villi regeneration as shown in the histopathological section of the intestine. It can be inferred from these results that the extract may be toxic to mice exposed to multiple doses of over 10,000 mg kg^{-1} for a prolonged period.

The implication of this study, therefore, is that oral consumption of a single dose of over 10,000 g kg^{-1} would not result in any health challenge, since it is practically non-toxic. This can be applied in pharmaceutics and its allied areas for potentials in the drug industry. The limitation of this study is the non-exposure to multiple doses of this plant extract to the subjects studied. It is, therefore, recommended that the active principle(s) of *A. montanus* be harnessed and detailed toxicological evaluation conducted to ascertain its use among human subjects.

CONCLUSION

In conclusion, the solubility and the Maximum Convenient Concentrations (MCCs) (g mL^{-1}) and Maximum Convenient Doses (MCDs) (g kg^{-1}) of crude ethanolic leaf extracts of *Acanthus montanus* were determined. Oral single-dose administration of the same extract to mice at a dose rate of 10,000 mg kg^{-1} did not cause death, although some gross and histopathological lesions were observed which tend to resolve with time. This suggests that the plant might be practically non-toxic and have a high therapeutic index as its use in folklore and ethnomedicine have no record of toxicity, thus confirming its widespread use by rural communities to treat several ailments and disease conditions. It is, therefore, recommended that chronic toxicity studies be conducted on subjects that frequently consume different parts of *A. montanus* to evaluate for its risk of remote toxicity.

SIGNIFICANCE STATEMENT

This study discovered the reason behind the consumption of an unquantified amount of extracts of *A. montanus* in ethnomedicine without reports of obvious signs of toxicity. This is due to its high mean Lethal Dose (LD_{50}) signifying a high degree of relative safety that can be beneficial in considering the plant as a pharmaceutical potential. This study will help researchers to uncover the critical areas of concern with regards to the high therapeutic index and a wide margin of safety in harnessing the bioactive compounds of this plant and investigating their possible mechanisms of action that many researchers are yet to explore.

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