ACUTE TOXICOLGICAL STUDIES OF *ACANTHUS MONTANUS* (NEES) T. ANDERSON (ACANTHACEAE) IN WISTAR RATS

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Summary

Acanthus montanus is a plant widely used in Cameroon to treat various diseases specifically pain, inflammation and epilepsy. No toxicological investigations have been carried out on this plant. The present study was to evaluate oral acute toxicity of *A. montanus* aqueous leaves extract on Wistar rats. Using single doses of 0, 500, 1000, 2000, 4000, 8000 mg/kg, the rats were observed for behavioural changes and mortality for 7 consecutive days and then sacrificed. Blood was collected for hematological and biochemical examinations. Organs were removed, weighed and kept in 10% formalin solution for pathohistological studies. The results showed that a single oral dose of aqueous extract did not produce significant changes in the general behaviour or mortality at the doses up to 4000 mg/kg. However there was a reduced reaction to pinch in animal

treated with the dose of 8000 mg/kg. At 4000 and 8000 mg doses, there were significant reductions in water and food intakes. Body weight gain and relative organs weights did not significantly vary. Cholesterol and creatinine increased significantly in animals treated with 8000 mg/kg compared to the control whereas serum total proteins and transaminases activities remained unchange compared to the control. Histological analysis did not reveal any pathology on liver and lungs but caused degenerative changes on the kidneys of 8000 mg/kg treated rats which was corroborated by creatinine increase. The results suggested that the plant was devoid of toxic effects in rats at the doses often used by the population.

Key words: *Acanthus montanus*, acute toxicity, mortality, histopathology, rat.

Introduction

The use of plants for healing purposes is getting increasingly popular as they are believed to be beneficial and free from adverse effects. However, most of the information available to the consumer about the medicinal herbs is not backed by credible scientific data. Medicinal herbs have their use as drugs, based simply on perpetuated traditional folk use. The plant *Acanthus montanus* (Nees) T. Anderson of the Acanthaceae family is a quick-growing evergreen herb distributed mainly in tropical region. In Traditional Medicine and Pharmacopoeia of Cameroon, *Acanthus montanus* is cited as being beneficial for the treatment of pain, cough, epilepsy, dysmenorrhoea, miscarriages and false labour [1,2]. In Nigeria, the leaves are used for cough, rheumatism, hypertension, skin infections, and witches [3]. The same plant is used in Gabon to treat cough, heart troubles, rheumatic pain and syphilis. Few of these claims have been investigated such as antiinflammatory, analgesic and antipyretic activities [4] and intestinal smooth muscle relaxant effects [5]. Due to widespread use of this plant by rural communities to treat several

diseases, the objectives of the present study were to obtain preliminary safety data of the extract. The acute oral toxicity of the aqueous extract from leaves of this plant was assessed on selected biochemical, hematological and histological parameters of matured rats.

Methods

Plant collection and identification

A. montanus (Nees) T. Anderson (Acanthaceae) plants were collected in Nsimeyong area of Yaounde, Cameroon in October 2004 and identified in the National Herbarium, Yaounde. The voucher number was 1652SRFCAM.

Preparation of extracts

The aqueous extract was prepared by maceration of 3.8 kg in 10 L of boiled distilled water for 24h. The extract was later filtered, and the solvent eliminated by concentration in a rotor evaporator to give a yield of 400 g (10.5%) of aqueous extract.

Animals

The experimental animals used in this study were Wistar male and female rats of sixty days old. They were raised in the Animal House of the Faculty of Science. Their weights were 120±10 g. They were acclimatized in the laboratory conditions for 10 days with water and standard rat chow given throughout the experimental period. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. No. FWA-IRB00001954). Following clearance from the Institutional Ethical Committee, the rats were sacrificed with ether.

Acute toxicity evaluation

The animals were divided into control and five treated groups, each consisting of ten animals (five males and five females). All the animals were subjected to 15 hours fasting prior to treatment. The control group received distilled water and each treated group received any of these doses; 500, 1000, 2000, 4000 and 8000 mg/kg aqueous extract by gavage. These doses were 10-160 times higher than effective doses in other studies. The animals were observed continuously for 4 hours each day for 7 consecutive days after administering the extract to check for any changes in comportment.. On day 8, the animals were weighed, sacrificed by ether anaesthesia and dissected. Blood was collected through cardiac puncture into EDTA and dry tubes. The anticoagulated blood (EDTA) was

analysed immediately for hematological parameters. The second tube was centrifuged at 6000 rpm at 4°C for 15 min to obtain the serum which was stored at -20°C for biochemical parameters. Selected organs (liver, heart, spleen, kidneys and lungs) were removed for macroscopic analysis, weighed and kept in 10% formalin buffer solution for histology.

Blood analysis

The hematological parameters (total red blood cell (RBC), leucocyte (WBC), and platelets counts, hematocrit and hemoglobin) were determined using an autoanalyzer (System H1, Bayer Diagnostics). The biochemical parameters, serum creatinine, total protein, cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercial kits.

Tisssue preparation

Microscopic tissue slides were processed using standard procedures [6]. Paraffin sections of 4-5µm were stained with hematoxilin and eosin.

Statistical Analysis

The results are presented as mean ± s.d. and the statistical significance differences between the groups were analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test as post test. P values less than 0.05 were considered as significant.

Results

Clinical signs

Oral administration of *Acanthus montanus* aqueous extract at doses ranging from 500 to 8000 mg/kg did not produce significant changes in behavior, breathing and cutaneous effects but decreased sensation to pain (pinch). All treated rats remained alive during the 7 days of observation. As shown in Table 1, insignificant differences were observed in body weight gain and organs relative weights. However, the quantity of food and fluid intakes decreased appreciably ($p<0.05$) for two highest doses (4000 and 8000 mg/kg).

Table 1: Effects of acute treatment with aqueous extract from leaves of *Acanthus montanus* on rats.

Parameters	Aqueous extract (mg/kg)					
	0	**500**	**1000**	**2000**	**4000**	**8000**
Number of death	0	0	0	0	0	0
Body weight gain (g)	6.48±0.50	6.04±0.60	5.62±0.39	5.68±0.66	4.86±0.62	4.42±0.49
Food consumption (g/kg/day)	86.30±4.99	83.72±4.80	77.78±3.72	74.34±5.56	65.48±6.38*	63.98±4.78*
Water consumption (ml/kg/day)	77.32±3.89	74.88±3.62	76.66±2.63	70,76±3.45	71.38±4.79	61.36±4.82*
Relative organs weight (%)						
Liver	3.97±0.17	3.90±0.07	3.76±0.19	3.79±0.07	3.96±0.24	4.05±0.13
Spleen	0.36±0.03	0.38±0.03	0.40±0.01	0.37±0.02	0.41±0.04	0.40±0.03
Lungs	0.72±0.03	0.71±0.02	0.73±0.03	0.69±0.02	0.74±0.06	0.85±0.07
Kidneys	0.60±0.05	0.58±0.03	0.51±0.03	0.53±0.02	0.58±0.02	0.53±0.06
Heart	0.32±0.02	0.34±0.01	0.32±0.02	0.34±0.01	0.35±0.01	0.33±0.03

Values are expressed as means ± s.d. of 10 animals. * $p<0.05$ Vs. Control group (ANOVA).

Effects of acute oral administration of *A. montanus* extract on hematological and biochemical parameters of rats

The effects of acute oral administration of *A. montanus* aqueous extract on hematological parameters are presented in Table 2. Hematological parameters of rats treated were not significantly different from control groups ($p>0.05$).

A study of the biochemical parameters showed that the aqueous extract induced hypercholesterolemia ($p<0.01$) and hypercreatininemia ($p<0.05$) compared with the control (Table 3). However, no changes were recorded in transaminase activities and proteins serum levels.

Pathology

The extract did not produce significant pathology at dosages of 500 to 4000 mg/kg after the acute treatment. Degenerative changes were, however, observed in the kidneys of rats treated with 8000 mg/kg (Figure 1). These changes were glomerulosclerosis characterised by glomerula retraction and necrosis of some cells.

Table 2: **Hematological parameters 7 days after acute treatment with aqueous extract from *Acanthus montanus* leaves on rats.**

Parameters	Aqueous extract (mg/kg)					
	0	500	1000	2000	4000	8000
Red blood cells (10^3/mm^3)	8.38±1.05	8.22±0.31	8.75±1.01	8.65±0.36	8.76±0.23	8.43±1.01
White blood cells (10^3/mm^3)	6.33±1.12	6.98±0.77	6.60±0.33	6.57±0.91	7.14±0.58	6.23±0.47
Platelets (10^9/L)	803.84±84.53	791.63±89.76	789.23±69.85	782.62±46.54	784.49±84.88	786.23±92.25
Haemoglobin (g/dL)	13.56±2.35	14.71±3.35	14.18±1.81	13.42±2.21	13.03±3.49	13.90±2.04
Heamatocrit (%)	45.75±7.55	44.43±7.27	45.34±3.42	43.71±4.03	42.17±4.45	46.32±5.62

Values are expressed as means ± s.d. of 10 animals (ANOVA). No significant difference was observed in any parameter.

Table 3: Effect of acute treatment with *A. montanus* aqueous extract on some biochemical parameters in rats

Parameters	**Extract (mg/kg)**					
	0	**500**	**1000**	**2000**	**4000**	**8000**
Creatinine (mg/dL)	0.78±0.23	1.18±0.08	1.21±0.14	1.31±0.16	1.39±0.27	2.07±0.45**
Cholesterol (mmol/L)	1.29±0.11	1.33±0.12	1.60±0.22	1.63±0.18	2.31±0.43	2.63±0.53*
Proteine (mg/mL)	68.95±4.14	57.19±3.48	59.79±3.48	57.06±4.35	56.60±3.31	55.42±8.06
ALT (U/L)	44.67±5.91	43.18±5.87	41.40±3.42	43.84±6.22	49.78±5.24	48.71±4.72
AST (U/L)	98.56±12.13	93.41±24.96	91.80±20.68	96.80±17.45	108.71±27.57	104.51±14.38

Values are expressed means ± s.d., n = 10, *$p < 0.05$, **$p < 0.01$ Vs. Control group (ANOVA).
ALT = alanine aminotranferase
AST = aspartate aminotransferase

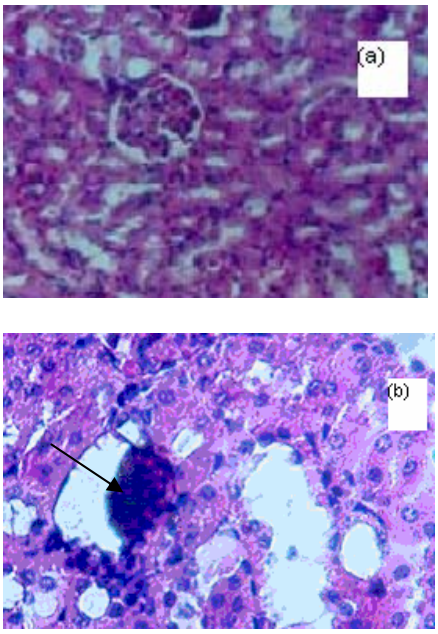

Figure 1 : Kidney lesions observed on 8000 mg/kg treated rats following a single daily oral acute treatment with *Acanthus montanus* aqueous leaves extract. (a) kidney of control rat, (b) kidney of treated rat. Note the glomerular degeneration (⟶) in the renal parenchyma.(200X)

Discussion

The importance of this plant in folk medicine as well as its promising pharmacological properties verified in our laboratory make this toxicity studies very essential. Oral administration of aqueous extract of *A. montanus* did not cause significant clinical signs at doses up to 4000 mg/kg body weight. No death occurred in any group. These results showed that single dose of *A. montanus* had no acute toxic effects, indicating that the medium lethal dose (LD 50) is higher than 8000 mg/kg for rats. Therefore, oral administration of *A. montanus* aqueous leaves extract is safe in rats. The doses used in this study were 10 -160 times higher than those used in other pharmacological studies, such as inhibition of pain by orally administered extract (50 mg/kg) to reverse writhing reflex in mice [4].

The administration of this extract to rats caused reduced reaction time to pinch. This decreased sensitivity to pain may be due to prostaglandins inhibition [4], since prostaglandins have been reported to function in regulating the perception of pain [7]. Various mediators that prevent the perception of pain inhibit the conversion

of arachidonic acid by inhibiting cyclooxygenase or the release of prostaglandin synthetase [8].

The slight reduction in body weight gain observed in this study correlated to the significant reductions in food and water intakes which could be attributed secondarily to the feeling of fullness or loss of appetite [9].

Changes in the status of bone marrow activity and intravascular effects of *A. montanus* were monitored by hematological examination. No treatment related outcomes on the hematological parameters were noticed. The plant extract did not induce significant changes in transaminases activities which are good indicators of liver function [10]. These results could indicate that liver function was preserved and was corroborated by the lack of pathological changes in the liver. It is important to mention that an *in vivo* studies on another species of the genus Acanthus, showed its hepatoprotective activity [11].

Unlike other doses which slightly and insignicantly increased the cholesterol, the 8000 mg/kg dose caused serum hypercholesterolemia. Familial hypercholesterolemia, which is genetically linked is much less common, however, diseases such as renal failure, diabetes mellitus, gout, hypothyroidism, obstructive jaundice and cirrhosis of the liver can cause secondary hypercholeslesterolemia [12]. The creatinine serum level was also significantly increased at the dose of 8000 mg/kg. This could be due to toxic effects of aqueous extract on some glomeruli. The reduction in the glomerular flow of filtration was accompanied by a parallel increase in the serum creatinine level. These metabolic disturbances were corroborated by the histological deteriorations observed in the kidneys.

Conclusion

In summary, our study demonstrated that aqueous extract of *Acanthus montanus* is non toxic at therapeutic doses (< 200 mg/kg) when given orally and acutely. It affects the kidneys at 8000 mg/kg. However, subacute and chronic toxicity tests are mandatory for the assessment of adverse reactions following multiple dosing.

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