ACUTE AND SUB ACUTE TOXICITIES OF METHANOL / METHYLENE CHLORIDE (CH_3OH / CH_2CL_2) EXTRACT OF *LAPORTEA OVALIFOLIA* (URTICACEAE) IN RATS

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Summary

Acute toxicity studies of the aerial part of the plant of *Laportea ovalifolia* was carried out in Albinos wistar rats of both sexes. Single oral doses of 4, 8 and 12 g/kg of the mixture of methanol / methylene chloride of the plant were administered orally to the test group. These doses did not produce significant changes in the general behaviour of the animals after 7 days of treatment suggesting that the extract is not toxic after the study of acute toxicity

Sub acute toxicity of this extract was evaluated with the dose of 1000 mg/kg orally for 14 days. An extra group ($Test^+$ group) was given methanol / methylene chloride extract and kept for further 14 days after treatment. All animals did not show signs of toxicity during the experimental period. AST, ALT, creatinine and monocytes levels increased in animals that received the extract. The relative weights of the organs of the treated animals were not different from those of the control group which received corn oil. The minor lesions observed with this dose in the target organs (liver and kidney) were vascular congestion in the liver and degeneration of renal tubular cells. The product shows hepatic and renal toxicity after sub acute toxicity

Key words: *Laportea ovalifolia*, Acute toxicity, Sub acute toxicity; Biochemical, Haematological parameters.

Introduction

In recent times, focus on plant research has increased all over the world with increasing evidence showing immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using modern scientific approaches. The results from these plants have revealed the properties of medicinal plants in the area of pharmacology[1,2]
Laportea ovalifolia (L. ovalifolia) which belongs to the family of *Urticaceae* is a tropical plant commonly found in swampy areas in Cameroon, Morocco and others parts of the world in both dry and rainy seasons[3]. The leaves of *Laportea ovalifolia* are used in traditional medicine for bacterial infections, headache, urinary infections, pneumonia and epilepsy[4] .

Although, *L. ovalifolia* is widely used in Cameroonian folklore medicine, information on its toxicity is very scanty. This work was carried out to evaluate the acute and the sub acute toxicity of the methanol / methylene chloride extract of the aerial parts of *L. ovalifolia* on selected biochemical, hematological and histological parameters of matured rats.

Methods

Plant collection and identification

The aerial part (leaves and stem) of *Laportea ovalifolia* were collected in October and November 2002, in Yaounde, Cameroon and identified at the National Herbarium, Yaounde Cameroon, where a voucher sample had been deposited under the number 50623/HNC

Preparation of extracts

The plant material was air-dried for 15 days at room temperature and ground in to powder. This powder (1000g) was soaked in 5 l of a mixture of methanol/ methylene chloride (1:1) for 48h. This was filtered and concentrated to a small volume to remove the entire methanol and methylene chloride using rotatory evaporator. The small volume was later dried at room temperature.

The pulp extract was kept at 4°c for the experiments. The extract obtained (110g) corresponded to an 11 % yield.

Animals

Male and female albino rats of the wistar strain weighing 158g- 190g were obtained from the animal house of the Department of Biochemistry, University of Yaounde I, Cameroon. All animals were kept in a room maintained under environmentally controlled conditions of 12h light and 12h darkness with a temperature of 24 ±3°c. The animals had free access to water and a standard rat diet. Rats were deprived of food but not water 12-14h prior to administration of the test substance. The study was approved by institutional animal ethical committee.

Acute toxicity evaluation

The method of the World Health Organisation[5] was used for the acute toxicity test of *L. ovalifolia*. In order to study any possible toxic effect or change in normal behaviours, four groups of 12 rats (6 males and 6 females) were used in this experiment. The experimental groups were administred 4 ,8 and 12g/kg body weight (p.o) of the *L. ovalifolia* extract. The control group was given corn oil in the same volume (10ml/kg body weight). The animals were then observed closely for 5 hours for changes in activity, somatic functions, secretions and mortality. The number of surviving animals were noted after 48 h and were then maintained for a further 5days with once daily observation.

At the end of the experiment period, all surviving animals were sacrificed and the internal organs such (liver, heart, spleen, lungs and kidneys) examined macroscopically

Sub acute toxicity evaluation

Sub-acute toxicity study was carried out following OECD 407[6] guideline and the method of Witthawaskul [7] . According to this guideline, if an acute toxicity test at one dose level of at least 5 000 mg/kg body weight produce no observable toxic effects then a full study using three dose levels is considered not necessary and a dose of 1000 mg/kg given once daily for 14 days can be used to evaluate sub acute toxicity.

Two groups of 12 rats (6 males and 6 females) received the methanol / methylene chloride extract at dose of 1000 mg / kg / day or corn oil (control) for a period of 14 days. An extra group ($Test^+$ group) was given the extract orally at the dose of 1000 mg / kg / day for 14 days and kept for a further 14 days after treatment to observe the reversibility, persistence or delayed occurrence of toxic effects.

During the period of administration, the animals were weighed and observed daily to detect signs of toxicity. Daily visual observations were made and recorded systematically. Rats that died during the test period were examined pathologically.

At the end of the experiment, all surviving animals were fasted overnight before being anaesthetized by ether. Blood samples were collected from a common carotid into EDTA and dry centrifuge tubes. The EDTA blood was used for haematological studies immediately after collection. The serum was separated by centrifugation at 3000 (rpm for 10 min) from the blood collected in to the dried tubes and stored at -20°C for biochemical parameters.

Blood analysis

White Blood Cells (WBC) and differential leukocyte count as well as hematocrit, Red Blood Cell (RBC) and haemoglobin were estimated [8]. The biochemical parameters including Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) [9], alkaline Phosphatase (ALP) [10], creatinine [11], serum urea[12] , total protein[13], Glucose[14] and triglycerides[15] were determined.

Tissue preparation

After blood collection, animals were sacrificed for tissue studies. The heart, lungs, kidney, liver and spleen were removed, blotted free of blood and weighed immediately using an electronic balance for subsequent analysis. Portions of the liver and kidney of both control and test groups were removed and fixed with 10 % formalin for subsequent histological work. Sections were cut, stained with hematoxylin (H) and eosin (E) and mounted on the neutral mounting medium (DPX).

Statistical Analysis

Results are expressed as mean ± S.E.M. Statistical significance was determined by ANOVA and post hoc Dunnett Test. P – values less than 0.05 were considered significant.

Results

Acute toxicity

All rats treated (males and females) with different doses of the methanol / methylene chloride extract of *Laportea ovalifolia* remained alive for the 7 days duration of the experiment. The test

material administered at different doses did not have an effect on the weight of animals (table 1) nor did treated animals show any signs of toxicity or change in general behaviour or other physiological activities.

The pathological examination of the internal organs: heart, lungs, kidney, liver and spleen revealed that there were no abnormal signs.

The body weight of the control and treated groups were not different in both sexes. Moreover, no lethality was recorded and no signs of toxicity observed during the experimental period.

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Table 1 : Body weight of rats after oral acute toxicity of methanol /methylene chloride extract of *Laportea ovalifolia*.

	Body weight (g)	
Groups	**Day 0**	**Day 7**
Male		
Group I	162.77 ± 2.92	177.00 ±2.96
Group II	169.50 ± 5.64	183.00 ± 4.14
Group III	160.67 ± 8.47	177.83 ± 7.22
Group IV	159.83 ± 5.27	173.33 ± 6.68
Female		
Group I	166.17 ± 1.94	175.83± 3.40
Group II	177.33 ± 2.66	185.50 ± 2.43
Group III	163.00 ± 4.00	177.51 ± 5.50
Group IV	166.17 ± 6.33	173.50 ± 4.84

The methanol / methylene chloride extract of whole plant was given by oral gavage to groups of wistar rats (n = 6) at doses : I(0 g/kg, control), II (4 g/kg), III (8 g/kg) and IV (12 g/kg). Values are expressed as mean ± S.E.M.;

Sub acute toxicity

The present study indicates that this extract did not cause any change in animal behaviour, and the body weight gains (table 2) were not significantly different in the treated rats compared to the control.

Table 2: Body weight of rats in sub acute toxicity of methanol /methylene chloride extract of *Laportea ovalifolia*

	Body weight (g)		
Groups	**Day 0**	**Day 14**	**Day 28**
Male			
Control	162.50 ± 5.82	189.83 ± 9.81	-
Test	159.50 ± 6.12	195.50 ± 6.25	-
Test $^{+}$	173.33 ± 6.18	200.80 ± 6.90	222.33 ± 6.37
Female			
Control	156.10± 5.11	170.33 ± 3.00	-
Test	163.66 ± 5.88	178.83 ± 6.52	-
Test $^{+}$	165.16 ± 3.75	185.16 ± 3.70	202.16 ± 8.70

Values are expressed as mean ± S.E.M.; n = 6. Test Group was given 1000 mg/kg daily for 14 day.

Test^{+} Group was given 1000 mg/kg daily for 14 days followed by no treatment for 14 days.

Effects of sub acute oral administration of *L. ovalifolia* extract on hematological and biochemical parameters of rats

The effect of sub acute oral administration of methanol / methylene chloride of *Laportea ovalifolia* on the haematological parameters shown that for the various parameters measured, the values for the treated group rats were not significantly different from those of the control group (table 3). In both male and female rats, treatment with the extract of *Laportea ovalifolia* brought about an increase in the number of monocytes as well as a decrease in the number of neutrophils (table 4) with no changes in the other blood parameters (haemoglobin, RBC, MCV, MCH, MCHC and WBC). Different parameters measured in the blood are shown in table 5 for male and female rats respectively. AST, ALT and creatinine values of the Test^{+} treated group and AST of the Test treated male group were significantly higher compared to that of the control group.

Table 3 : Haematological values of rats in sub acute toxicity of methanol /methylene chloride extract of *Laportea ovalifolia*

	Haemoglobin (g/dl)	RBC (10^6/µl)	Hematocrit (%)	MCV (ft)	MCH (pg)	MCHC (g/%)
Male						
Control	14.94 ± 0.85	7.93 ± 0.64	44.03 ± 2.58	54.43 ± 4.70	18.50 ± 0.54	34.08 ± 3.24
Test	14.57 ± 1.07	7.91 ± 0.66	45.24 ± 3.20	55.34 ± 3.20	17.73 ± 0.74	32.12 ± 2.09
Test$^+$	14.76 ± 0.70	8.25 ± 0.24	44.95 ± 2.39	54.77 ± 2.80	17.88 ± 0.58	33.40± 1.92
Female						
Control	13.45 ± 0.55	7.03 ± 0.22	40.56 ± 1.75	57.82 ± 2.56	19.16 ± 0.59	33.80 ± 2.20
Test	13.73 ± 0.69	7.35 ± 0.31	40.3 ± 1.78	54.27 ± 2.16	18.67 ± 0.57	34.40± 4.70
Test$^+$	13.58 ± 0.56	7.22 ± 0.28	40.75 ± 2.52	57.19 ± 6.17	17.67 ± 0.62	33.40 ± 0.92

Values are expressed as mean ± S.E.M.; n = 6. Test Group was given 1000 mg/kg daily for 14 day. Test$^+$ Group was given 1000 mg/kg daily for 14 days followed by no treatment for 14 days.

Table 4 : Differential White Blood Cell count of rats in sub acute toxicity of methanol / methylene chloride extract of *Laportea ovalifolia*

Groups	WBC (10^3/µl)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophiles (%)
Male					
Control	7.19 ± 0.50	61.5 ± 2.17	37.67 ± 2.32	0.00	1.33 ± 0.50
Test	7.52 ± 0.57	63.00 ± 3.63	34.33 ± 3.76*	1.50 ± 0.54*	1.50 ± 1.20
Test+	7.71 ± 0.39	65.17 ± 3.00	32.67 ± 3.32*	1.17 ± 0.40*	1 ± 0.60
Female					
Control	5.07 ± 0.89	63.83 ± 3.48	35.17 ± 3.25	0.00	1.30 ± 0.50
Test	4.75 ± 0.17	70.67 ± 3.07*	26.67 ± 2.94*	0.83 ± 0.40*	1.50 ± 0.54
Test+	4.75 ± 0.70	66.60 ± 4.56*	29.83 ± 4.02*	1.33 ± 0.51*	2.17 ± 0.98

Values are expressed as mean ± S.E.M.; n = 6. Test Group was given 1000 mg/kg daily for 14 day. Test+ Group was given 1000 mg/kg daily for 14 days followed by no treatment for 14 days. Significantly different from control: $P < 0.05$.

Table 5 : Blood chemistry values of male and female rats in sub acute toxicity of methanol/methylene chloride extract of *Laportea ovalifolia*

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Total Protein (g/dl)	Glucose (mg/dl)	Triglycerides (mg/dl)
Male								
Control	108.58 ± 7.06	27.88 ± 2.72	84.94 ± 9.00	0.55 ± 0.05	22.5 ± 1.08	10.08 ± 0.43	91.25 ± 5.24	64.80 ± 4.49
Test	119.33 ± 10.40*	29.70 ± 2.99	72.77 ± 3.129	0.54 ± 0.06	24.02 ± 2.32	9.46 ± 0.77	87.83 ± 6.36	59.35 ± 2.63
Test+	122.42 ± 12.08*	40.63 ± 4.93*	90.50 ± 12.30	0.72 ± 0.06*	25.05 ± 2.75	10.24 ± 0.37	93.5 ± 8.10	62.12 ± 5.11
Female								
Control	76.50 ± 6.56	27.03 ± 0.90	71.24 ± 4.45	0.54 ± 0.07	20.11 ± 0.81	10.19 ± 0.29	83.45 ± 4.50	60.83 ± 6.55
Test	81.09 ± 8.12	26.13 ± 2.55	74.11 ± 4.84	0.50 ± 0.05	21.1 ± 0.87	9.92 ± 0.40	83.65 ± 5.81	56.00 ± 3.34
Test+	109.83 ± 8.28*	37.6 ± 6.50	80.72 ± 4.80	0.60 ± 0.06*	23.05 ± 1.08	10.27 ± 0.37	88.55 ± 7.76	58.00 ± 3.34

Values are expressed as mean ± S.E.M.; n = 6. Test Group was given 1000 mg/kg daily for 14 day.
Test+ Group was given 1000 mg/kg daily for 14 days followed by no treatment for 14 days.
* Significantly different from control: *P < 0.05.

The administration of this extract did not affect the levels of urea, glucose, triglyceride, ALP and total protein. No significant changes in relative weights of various organs were observed between the experimental group and the control group (table 6).

Table 6: Relative organ weights (g/100g body weight) of male rats in sub acute toxicity of methanol/methylene chloride of *Laportea ovalifolia*

Groups	Liver	Kidney	Heart	Spleen	Lungs
Male					
Control	3.01 ± 0.43	0.46 ± 0.06	0.36 ± 0.02	0.26 ± 0.05	0.53 ± 0.05
Test	2.91 ± 0.23	0.51 ± 0.06	0.39 ± 0.03	0.28 ± 0.06	0.47 ± 0.05
Test$^+$	3.22 ± 0.46	0.48 ± 0.06	0.37 ± 0.02	0.27 ± 0.02	0.54 ± 0.05
Female					
Control	3.20 ± 0.22	0.45 ± 0.04	0.36 ± 0.01	0.24 ± 0.03	0.6 ± 0.07
Test	3.01 ± 0.15	0.41 ± 0.04	0.39 ± 0.04	0.24 ± 0.02	0.65 ± 0.77
Test$^+$	3.10 ± 0.10	0.41 ± 0.03	0.36 ± 0.03	0.25 ± 0.01	0.66 ± 0.09

Values are expressed as mean ± S.E.M.; n = 6. Test Group was given 1000 mg/kg daily for 14 day.
Test$^+$ Group was given 1000 mg /kg daily for 14 days followed by no treatment for 14 days.

Pathology

The histological examination of section of the livers and kidneys from male and female rats did not reveal any difference between the Test groups and the control. However tissue samples of liver of the Test$^+$ group showed moderate vascular congestion while hepatocytes and hepatic parenchyma were normal (Figure 1 and 2).

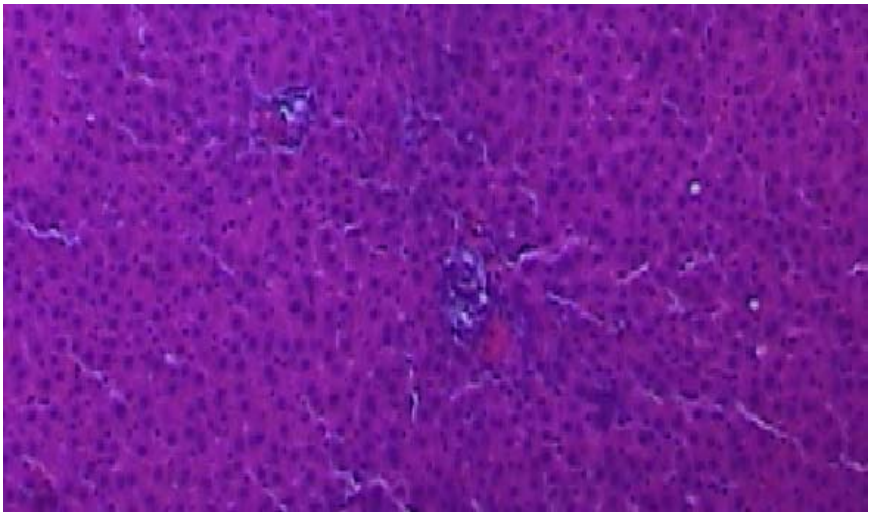

Figure 1.Section of a liver from a rat of control group that receive corn oil. The section shows normal portal space and liver cells (H & E x100).

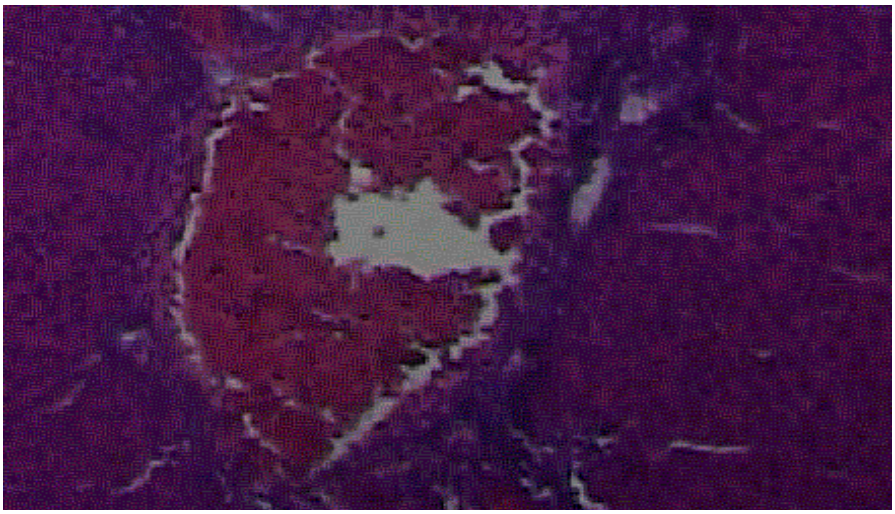

Figure 2. Section of a liver from rat from Test^{+} group that received 1000 mg/kg of methanol / methylene chloride of *Laportea ovalifolia* daily for 14 days. The section shows vascular congestion (H & E x 100)

Kidney samples of the Test^{+} group showed degeneration of renal tubular cells and aggregates of polynuclear, lymphocytes and plasmocyte while glomeruli remained normal (Figure 3 and 4).

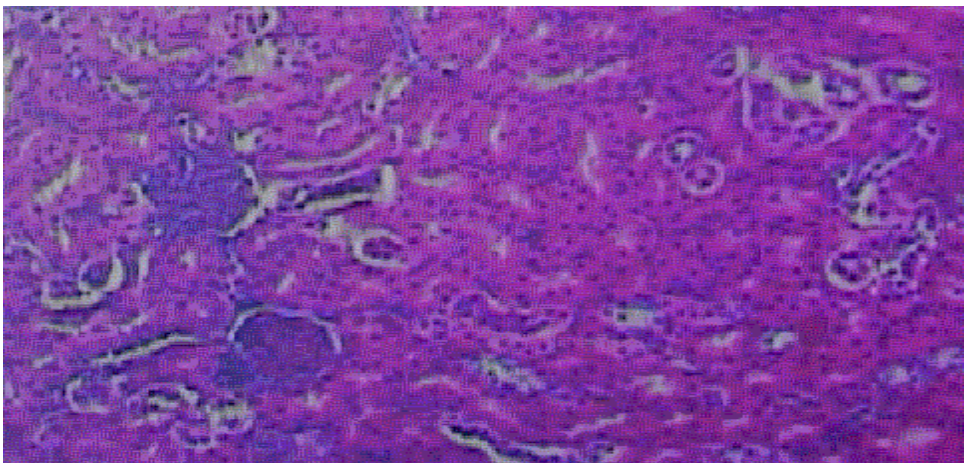

Figure 3.Section of a kidney from a rat of control group that receive corn oil. The section shows normal renal cells and normal glomerulus (H & E x100).

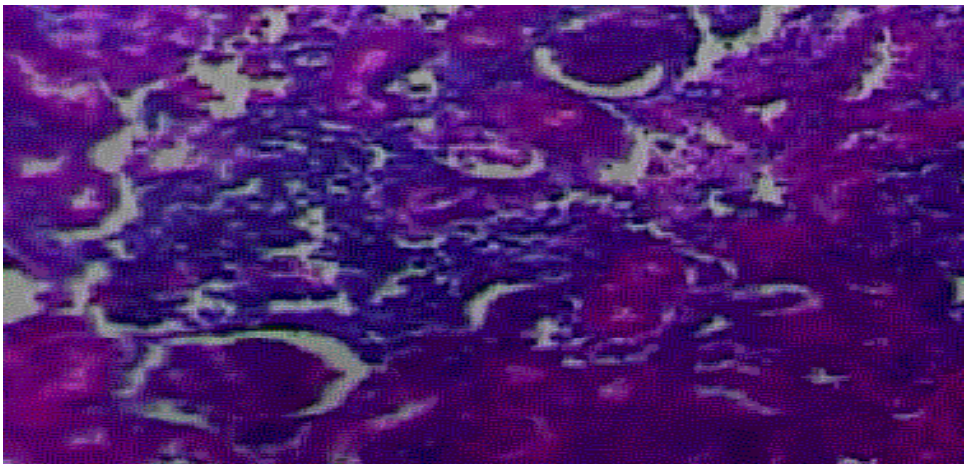

Figure 4. Section of a kidney from a rat from Test^{+} group that received 1000 mg/kg of methanol / methylene chloride of *Laportea ovalifolia* daily for 14 days. The section shows degeneration of renal tubular cells and aggregates of polynuclear, lymphocytes and plasmocyte (H & E x 100)

Discussion

The results obtain after the single oral administration of *L. ovalifolia* suggesting that the LD_{50} of the extract was higher than 10 g/kg.

The results obtained in this acute toxicity study indicate that a high dose of *Laportea ovalifolia* (12 g/kg body weight) did not produce any symptoms of toxicity, nor did any of the rats died after 7 days of observation suggesting that the LD_{50} of the extract was higher than 12 g/kg. Going by the OMS guidelines for testing of chemicals, the results of acute toxicity test indicate that the methanol / methylene chloride extract of *Laportea ovalifolia* is non-toxic.

The rational for sub acute toxicity determination is the use of *Laportea ovalifolia* by Cameroonian traditional medical for the treatment of bacterial infections and diabetes [1,3]. The results obtain suggest that this extract did not affect animal behaviour nor the food intake.

The oral administration of methanol / methylene chloride of *Laportea ovalifolia* on the haematological parameters in sub acute toxicity may indicate that the extract had a selective action on circulating differential white blood cells. The difference in value in the lymphocyte count of the treated female group compared to the control group falls within the reference values [16] suggesting the absence of alterations of clinical importance.

Damage to liver cells with necrosis causes the release of intracellular constituents into the blood stream, and the transaminases are sensitive indications of such damage. Other enzymes such as ALP are also increased [17] . In liver cells, AST is found both in the mitochondria and cytoplasm, while ALT is found in the cytoplasm. In the present study, both AST and ALT levels increased slightly in $Test^{+}$ group while only AST levels increased in the Test male group. On the other hand, all the values of ASAT and ALT are above the reference values (96 – 200 U/L) and (21-52 U/L) respectively [18]. Consequently hepatic function alteration may be suggested [19] since the ALT in blood increases when the hepatic cellular permeability is changed.

No significant changes in relative weights of various organs were observed between the experimental group and the control group. The lack of effect observed in relative weights of differents internal organs indicates little or no macroscopical changes brought about by the extract.

Lesions observed in this study indicate that the extract can cause hepatic and renal toxicity when administered orally for longer periods. The mechanism where by this extract constituent injures body tissues is not stated in the present study, but damage to these organs probably contributes to the increased activities of AST and ALT as well as the creatinine concentration in the serum.

Conclusion

Considering the importance and widespread use of this plant in the treatment and management of various illnesses in Cameroon, its safety needs to be ascertained. Additional clinical and toxicological evaluations are will therefore needed.

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