African Journal of Biotechnology Vol. 5 (3) pp. 283-289 2 February, 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae)

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Accepted 18 January, 2006

We investigated the acute and subacute toxicities of hydro-ethanolic extract of leaves of *Senna alata* (L.) Roxb. in Swiss mice and Wistar albino rats. The mice were divided into 6 groups of 10 animals and each group received once by intra-gastric gavages 0, 4, 8, 12, 16, 20 times 1000 mg/kg dose of extract. Distilled water served as the control. For the subacute toxicity, three groups of 10 rats (5 males and 5 females) were treated *per os* with distilled water (control), 500 or 1000 mg/kg of extract every 48 h for 26 days. At the end of treatment blood sample and 20% liver homogenates were collected for biochemical analyses. The results indicated that the medium lethal dose (LD_{50}) was about 18.50 g/kg of body weight. Significant variation ($P<0.05$) of the body weight was observed after 26 days of treatment, in some biochemicals index of serum and 20% liver homogenates (glutathion , alkaline phosphatase (APL), aspartate aminotransferase (AST)), haematological parameters (platelet) also in the female relative weight of heart of rat. Some of parameter investigated in this study showed dose responsive. The histopatological study of the liver did not show any features after the treatment but, the extract seems to ameliorate the liver architecture.

Keys words: Acute toxicity, alkaline phosphatase, hematological and histopatological study.

INTRODUCTION

The traditional use of plant in the treatment of different infections is widely practised in Cameroon and other developing countries. The World Health Organization has recommended that this should be encouraged especially in countries where access to the conventional treatment is not adequate (WHO, 1980). For this reason, various medicinal plants have been studied using modern scientific approaches. The studies have shown that due to their various biological components, many medicinal plants have a variety of properties and can be used to treat various diseases.

Senna alata (L) Roxb. belongs to Ceasalpiniaceae family. This plant grows in several regions of Cameroon and can also be found in other countries (Palanichamy et al., 1990; Ibrahim et al., 1995; Awal et al., 2004). In Cameroon, the leaves and stem bark of *S. alata* are used to treat hepatitis, skin diseases, jaundice, gastroenteritis, intestinal helminthiasis, eczema, tryphoenteritis and ringworm. The young leaves are used in rural areas to treat constispation and food poisoning**.** In Bangladesh various parts of the plants are often used for boils carbuncles, cut wound, foul ulcer, dysentery, ringworm, itches eczema, helminthiasis and various intestinal

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troubles (Awal et al., 2004; Adjanohou et al., 1996 Ghani, 1998). Some authors have reported the antifungal activity of the leaves of this plant. Awal et al. (2004) showed that the leaves of *S. alata* have antibacterial activity on Gram positive and negative bacteria (*Sarcina lutea, Bacillus megaterium, Streptococcus hemlyticus, Staphylococus aureus, Salmonella thyphi, Esherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae*), while its seeds exhibited little activity on *K. pneumoniae and S. Lutea.* So far, other workers have reported diverse biological properties of *S. alata* (Thamlikitkul et al., 1990; Moriyama et al., 2003). Despite the popular use of this plant in the area of pharmacology in Cameroon, no information has been given about its toxicity. This work is carried out to evaluate the acute and subacute toxicity of the hydro-ethanolic extract of the plant *S. alata* in mice and albino rats. In the subacute toxicity study, the effect of this extract on biochemical, hematological and histopathological parameters were investigated.

MATERIALS AND METHODS

Plant material

Fresh leaves of *S. alata* were collected near the Eloundem mountain in Yaoundé, the capital city of Cameroon. The sample was identified at the National Herbarium and a voucher specimen was deposited there under the number 1871YA.

Preparation of the extract

The collected plant material was dried at room temperature (30±3°C), pulverized and finely sieved. The powder obtained (200 g) was macerated in 1000 ml of a mixture of ethanol/water (4:1, v/v) for 72 h. The extract was filtered using Whatman filter paper N° 1 and concentrated in an air circulating oven at 54°C until total dryness. The experiment was repeated twice and 25 g of hydro-ethanolic extract obtained was stored at 5°C.

Experimental animals

Sexually mature male and female Wistar albino rats (102-134 g) and sexually mature Swiss albino mice (15-27 g) were obtained from the animal laboratory of the Biochemistry Department of the Yaoundé I University, Cameroon. All the animals were kept under standard environmental condition (27±2°C). The animals had free access to water and standard diet. Rats and mice were deprived of food but not water (16-18 h) prior to administration of the extract. The principles of laboratory animal care were followed and the Department’s ethical comittee approved the use of the animals and the study design.

Acute toxicity

The bioassays were conducted according to the World Health Organisation guideline for the evaluation of the safety and efficiency of herbal medicines (WHO, 1992). For the study, Swiss albino mice were divided into six groups of 10 animals (5 males and 5 females). The control group received distilled water *per os* throughout the experiment. The remaining groups (2-6) received once 4, 8, 12, 16, 20 doses of 1000 mg/kg body weight of the extract, respectively. Observations were made and recorded systematically 1, 2, 4 and 24 h after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. The number of survivors was noted after 24 h and the futher 8 days where their weights were recorded . The LD_{50} was then determined at the end of the experiment based on Lorke (1983) method.

Subacute toxicity

Three groups of 10 rats (5 males and 5 females) received by intra-gastric gavages the plant extract at the dose of 500 mg/kg, 1000 mg/kg body weight or distilled water (control) every 48 h for 26 days. During the period of administration, the animals were weighed, and food and water intake were monitored. After 26 days, all surviving animals were fasted overnight. Animals were sacrificed by decapitation and blood samples were collected into heparinized tube for haematological parameters and non-heparinized centrifuge tubes. The liver, heart, kidney and lung were collected and weighed. After instantaneous washing, a part of the liver tissue was kept in frozen containers (-20°C) for further analysis of biochemical parameters and another part was used for histopathological studies.

Biochemical estimations

Blood collected into non heparinized tubes were then centrifuged at 3000 rpm for 10 min. The serum separated was analysed to evaluate the liver enzymes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using the method of Reitman and Frankel (1957); alkaline phosphatase (ALP) was analysed by the method of Bessey et al. (1946) Lowry et al, (1954); creatinine by Barterls (1972); protein was evaluated by the method of Gornall et al. (1949); and cholesterol by the method of Zlatkis et al. (1952) modified by Taga et al. (1998). The 20% liver homogenates were also subjected to the same biochemical estimation except that instead of cholesterol, glutathione was assayed using the method of Ellman (1959).

Haematological assay

Blood samples collected in the heparinized tubes were used to investigate white blood cells (WBC), red blood cells (RBC) and platelets using the visual method (Dacie, 1991).

Histopathological study

Histopathological investigation of the liver were done according to the method described by Lamb (1981).The organ pieces (3-5 μm thick) were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis

Values were expressed as mean ± SD. The statistical analyses of variance was done by ANOVA followed by the student-Newman

Table 1. Body weights of rats in sub acute toxicity after 26 days of oral administration of hydro-ethanolic extract of *S. alata*.

Body weight (g)			
Female	**Day 0**	**Day 26**	**Weight gained in 26days**
Control	143.59 ± 7.37 (135.02-154.26)	169.25 ± 8.00 (164.02-183.55)	26.63 ± 5.30[a] (19.60-30.33)
500 mg/kg	124.12 ± 18.65 (119.34-132.24)	156.36 ± 14.85 (136.98-172.82)	32.27 ± 16.15[b] (33.65-47.26)
1000 mg/kg	132.81± 11.36 (116.84-142.89)	157.36 ± 15.75 (142.19 – 184.17)	24.55 ± 6.51[a] (20.09 – 35.37)
Male			
Control	126.34 ±20.53 (102.22- 158.87)	176.26 ± 29.26 (169.07 –189.53)	49.72 ± 18.55[b] (16.88 – 45.11)
500 mgKg	147.45± 6.82 (142.01 –158.87)	176.90 ± 8.46 (169.02 – 189.53)	29.45 ± 12.87[a] (12.21 –45.11)
1000 mg/kg	161.42 ± 15.93 (113.29 – 148.05)	162.38 ± 6.35 (153.32 –171.06)	33.93 ± 16.65[a] (16.67 –46.68)

Values are expressed as mean±SD. Parentheses are expressed as range values, n=5. Means with the same letter superscript within a column in each group (sex) are not significantly different (P<0.05).

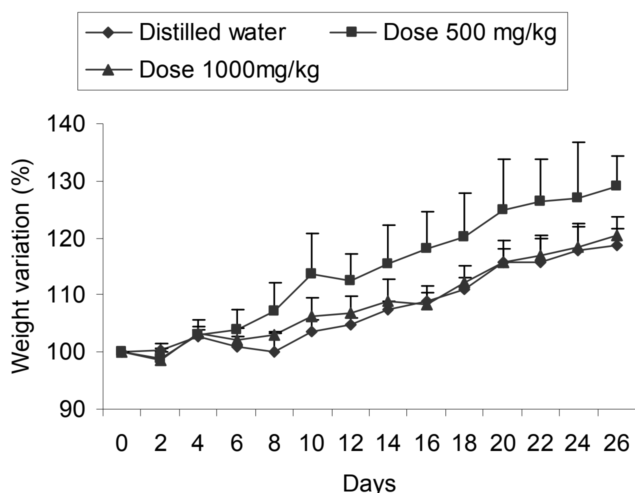

Figure 1. Effect of hydro-ethanolic extract of *S. alata* on the body weight of female rats during the subacute toxicity.

Kerls test. $P < 0.05$ was considered as the level statistical significance.

RESULTS AND DISCUSSION

The extraction of 200 g of sample gave a yield of 12.5%. Although the hydro-ethanol extract was used for the study, ethanol was included in the extraction to enhance different bioactive components, tannins, polyphenols and saponins. Moreover, the Cameroonian population only takes the aqueous preparations for their diseases.In the acute toxicity, mice treated with the dose of 20 g/kg body weight showed some behavioral changes 120 min after oral administration. These changes included slow response to external stimuli, reduction of mobility and aggression, slight excitability sketching and sluggishness. However, after 24 h all the changes observed before disappeared. On the contrary, no adverse changes were noted in mice treated with less than 12 g/kg body weight. Death of mice was noted at 16 and 20 g/kg. Weight gain recorded was increased in both sexes 8 days after oral administration of *S. alata*. The DL_{50} value obtained was 18.5 g/kg body weight. According to Schorderet (1992), subtances with DL_{50} values greater than 5 g/kg of body weight are considered to show low toxicity. Thus the aqueous ethanol extract of *S. alata* can be classified in the category of substances with low toxicity.

In the subacute toxicity, the hydro-ethanol extract of *S. alata* at doses of 5000 mg/kg and 1000 mg/kg given *per os* every 48 h for 26 days did not result in death of the animals. No sign of observable toxicity was detected during the experimental period. According to the OECD guideline, if an acute toxicity test at one dose level of at least 500 mg/kg body weight produced no observable toxic effects (Witthawaskyl et al., 2003)**,** the full study at a dose of 1000 mg/kg given once daily for 14 days can be used to evaluate subacute toxicity. Rats treated with various doses of hydro-ethanolic extract of *S.alata* had a progressive weight gained. This increase in weight is significantly different (P< 0.05) from that of the control (Table 1). The progressive increase in body weight at doses of 500 and 1000 mg/kg of female and male rats during 26 days (Figure 1) of administration of aqueous ethanol extract of *S. alata* may indicate the improvement

Table 2. Haematological values of rats in sub acute toxicity after 26 days of administration of hydro-ethanolic extract of *S. alata*.

Treatment (mg/kg)	**WBC (10^3/mm^3)**	**RBC (10^7/mm^3)**	**Platelet (10^7/mm^3)**
Female			
Control	2.02 ± 1.11 (1.20 – 2.31)	3.29 ± 1.40 (2.36 – 5.80)	2.03 ± 0.29^a (1.52 – 2.32)
500 mg/kg	2.21 ± 0.59 (1.62 –5.27)	3.35 ± 1.46 (2.02 - 5.76)	2.87 ± 0.62^a (2.01 – 3.50)
1000 mg/kg	2.33 ± 0.6 (1.60 – 2.82)	4.50 ± 1.05 (3.36 – 5.28)	3.05 ± 0.15^b (2.94 – 3.28)
Male			
Control	2.60 ± 1.74 (1.62 – 2.27)	3.38 ± 0.38 (2.60 – 3.23)	1.18 ± 0.14^a (0.76 – 1.32)
500 mg/kg	2.73 ± 1.11 (1.21 – 4.41)	3.44 ± 1.74 (3.08 – 3.88)	1.38 ± 0.25^a (0.81 – 1.6)
1000 mg/kg	2.96 ± 1.51 (2.20 – 3.60)	3.50 ± 0.88 (3.04 – 4.72)	2.52 ± 0.09^b (0.76 – 3.12)

Values are expressed as mean±SD, n=5. Parentheses are expressed as range values. Means with the same letter superscript within a column in each group (sex) are not significantly different (P<0.05).

Table 3. Weights of organs of rats (g/100g of body weights) in sub acute toxicity of hydro ethanolic extract *S. alata*.

Treatment (mg/kg)	**Heart**	**Lung**	**Kidney**	**Liver**
Female				
Control	0.40 ± 0.01^b (0.38 – 0.42)	0.58 ± 0.02 (0.58 – 0.62)	0.61 ± 0.08 (0.57 – 0.74)	3.55 ± 0.34 (3.71 – 3.98)
500 mg/kg	0.37 ± 0.05 ^{b} (.28 – 0.41)	0.61 ± 0.01 (0.59 – 0.3)	0.71 ± 0.07 (0.62 – 0.79)	3.50 ± 0.17 (3.38 –3.84)
1000 mg/kg	0.31 ± 0.06^a (0.26 – 0.47)	0.64 ± 0.06 (0.58 – 0.74)	0.71 ± 0.14 (0.55 – 0.94)	3.67 ± 0.27 (3.33 – 3.84)
Male				
Control	0.37 ± 0.08 (0.31 – 0.52)	0.58 ± 0.11 (0.44 – 0.71)	0.66 ± 0.13 (0.54 – 0.87)	3.31± 0.11 (3.17- 3.47)
500 mg/kg	0.30 ± 0.02 (0.29 – 0.39)	0.62 ± 0.06 (0.53 – 0.71)	0.68 ± 0.11 (0.55 – 0.71)	3.33 ± 0.18 (3.12 – 3.61)
1000 mg/kg	0.37 ± 0.04 (0.30 – 0.39)	0.63 ± 0.02 (0.53 – 0.66)	0.74 ± 0.10 (0.73 – 0.82)	3.43 ± 0.27 (3.24 – 3.91)

Values are expressed as mean±SD. Parentheses are expressed as range values, n=5. Means with the same letter superscript within a column in each group (sex) are not significantly different (P<0.05).

of the nutritional state of the animal. The growth response effect could be as the result of increased food and water intake.

As shown in Table 3, the calculated relative weights of the control and treated animals groups varied from one organ to another. For the hearts and livers from the control group, the relative weights were quite similar to those of the treated groups (female and male rats). A decrease of the values of hearts were observed in the female control group with significant difference (P<0.05) in both organs of female group with significant difference (P<0.05) in the heart values. No significant differences (P<0.05) were noted in the relative weights of the other organs (liver, lung, kidney). However, there is no correlation between the relative weights of the organ and the doses of the extract of *S. alata* administered.

The haematological status (Table 2) after 26 days of oral administration of hydroalcoholic extract of *S. alata* was also assessed. No significant variation (P<0.05) for RBC and WBC were observed. However, the variation was significantly different (P<0.05) for platelets. In general the results showed that the values for the RBC and WBC were slightly increased in female and male groups compared to the control. The small transient of

Table 4. Blood biochemical indices of rats in sub acute toxicity of hydro-ethanolic extract of *S. alata*.

Biochemical indices	Treatment (mg/kg)		
	Control	500 mg/kg	1000 mg/kg
Female			
ALT (UI/ml)	40.01 ± 0.12 (39.48 – 40.26)	40.06 ± 0.25 (39.88 – 40.39)	39.77 ± 0.18 (39.96 – 40.24)
AST (UI/ml)	83.63 ± 0.06 (83.56 – 83.68)	83.66 ± 0.12 (83.54 – 83.86)	83.69 ± 0.05 (83.69 – 83.76)
APL (UI/ml)	46.34 ± 2.51 (39.31 – 48.55)	46.72 ± 2.40 (46.02 – 48.45)	47.49 ± 1.00 (43.52 – 49.50)
Creatinine (mg/ml)	16.61 ± 1.27 (12.50 – 16.66)	15.85 ± 0.41 (15.85 – 0.41)	16.34 ± 0.47 (16.34 – 0.47)
Protein (mg/ml)	0.17 ± 0.01 0.17 ± 0.01	0.17 ± 0.02 0.17 ± 0.02	0.16 ± 0.02 0.16 ± 0.02
Cholesterol (g/L)	2.04 ± 0.39 (1.64 – 2.84)	2.05 ± 0.40 (1.52 – 2.39)	1.85 ± 0.14 (1.71 – 1.93)
Male			
ALT (UI/ml)	40.01 ± 0.12 (39.91 – 40.23)	40.06 ± 0.25 (0.00 – 40.14)	39.77 ± 0.18 (39.77 – 40.31)
AST (UI/ml)	83.80 ± 0.15 (83.68 – 84.06)	83.88 ± 0.28 (83.72 – 84.39)	83.73 ± 0.07 (83.62 – 83.82)
APL (UI/ml)	48.56 ± 0.75 (47.04 – 49.05)	48.59 ± 0.45 (47.97 – 48.98)	48.70 ± 0.59 (47.97 – 49.05)
Creatinine (mg/ml)	16.20 ± 0.42[a] (13.33 – 16.66)	15.69 ± 0.72 [a] (14.95 – 16.60)	17.46 ± 1.07[b] (15.50 – 20.00)
Protein (mg/ml)	0.16 ± 0.03 (0.14 – 0.20)	0.19 ± 0.04 (0.17 – 0.21)	0.17 ± 0.00 (1.71 – 1.93)
Cholesterol (g/ml)	2.23 ± 0.49 (1.64 – 2.84)	1.94 ± 0.32 (1.52 – 2.39)	1.83 ± 0.07 (1.71 – 1.93)

Values are expressed as mean±SD. Parentheses are expressed as range values, n=5. Means with the same letter superscript within the same line are not significantly different (P>0.05).

values observed in blood haematology did not show any dose responsiveness. Thus the hydroalcoholic extract of *S. alata* affect slightly some haematological parameters.

Results of biochemical analyses in both female and male rats are shown in the Tables 4 and 5 for the serum and the 20% liver homogenate, respectively. After 26 days dosage of hydroalcoholic extract of *S. alata,* there were no significant changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (APL) activities in the serum of both sexes and the 20% homogenate liver samples (female group). A small but not significantly different (p<0.05) increase was noted on alkaline phophatase (APL) activities of the serum in both sexes and 20% liver homogenate in the female group only. This variation of APL activity was statistically different (P<0.05) in the 20% homogenate from male liver. In the same group, a slight reduction of the level of ALT and APL were noted with statistical differences (P<0.05). ALT and AST are two liver enzymes that are associated to the hepatocellular damage. Although both AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, only ALT is remarkably specific for liver function since AST is mostly present in the myocardium, skeletal muscle, brain and kidneys (McIntyre et al., 1987; Witthawaskul et al., 2003). In general with liver disease, serum levels of AST and ALT rise and fall at the same time (Sacher et al., 1991). A mild elevation of AST level has been shown to be associated with liver injury or myocardial infarctions (Stroev, 1989). The higher the activity of AST, the larger the infarctions size (Roberts et al., 1975; Haweroft, 1987). This result indicates that the hydroalcoholic extract of *S. alata* when taken for long periods of time may not cause liver disease. A typical myocardial infarction gives an AST/ALT ratio greater than 1 while an AST/ALT ratio less than 1 is as a result

Table 5. Biochemical indices of 20% homogenate liver of rat in sub acute toxicity of hydro- ethanolic extract of *S. alata*.

Biochemical indices	Control	500	1000
Female			
ALT (UI/ml)	68.42 ± 3.43 (64.03 – 73.37)	67.03 ± 3.00 (63.44 – 71.66)	65.42 ± 4.37 (59.83 – 69.69)
AST (UI/ml)	84.06 ± 0.22 (83.83 – 84.29)	83.73 ± 0.27 (83.73 – 83.93)	84 .00± 0.29 (83.60 – 84.10)
APL (UI/ml)	21.74 ± 0.25 (21.49 – 21.49)	22.85 ± 1.12 (21.21 – 23.53)	23.78 ± 2.44 (20.12 – 26.62)
Protein (mg/ml)	0.07 ± 0.01 (0.06 – 0.09)	0.09 ± 0.01 (0.07 – 0.11)	0.09 ± 0.01 (0.07 – 0.12)
Glutathione (mM/g of liver)	13.79 ± 2.50[a] (11.61 – 17.35)	17.51 ± 1.61[b] (15.66 – 19.11)	19.27 ± 2.02[b] (17.02 – 22.05)
Male			
ALT (UI/ml)	68.05 ± 2.32[b] (65.81 – 70.94)	65.38 ± 2.38[ab] (62.13 – 68.57)	61.48 ± 5.07[a] (55.49 – 61.47)
AST (UI/ml)	83.79 ± 0.08 (83.79 – 83.91)	83.78 ± 0.27 (83.51 – 84.12)	84.12 ± 0.40 (83.68 – 84.12)
APL (UI/ml)	31.76 ± 2.54[b] (28.13 – 34.77)	29.56 ± 1.83[ab] (29.18 – 31.75)	28.51 ± 1.05[a] (28.20 – 30.10)
Protein (mg/ml)	0.08 ± 0.01 (0.07– 0.10)	0.09 ± 0.00 (0.08 – 0.10)	0.11 ± 0.03 (0.07 – 0.15)
Glutathione (mM/g of liver)	19.48 ± 4.40 (17.95 – 21.58)	20.71 ± 1.63 (19.19 – 23.16)	22.46 ± 4.99 (18.27 – 24.93)

Values are expressed as mean±SD. Parentheses are expressed as range values, n=5. Means with the same letter superscript within the same line are not significantly different (P>0.05).

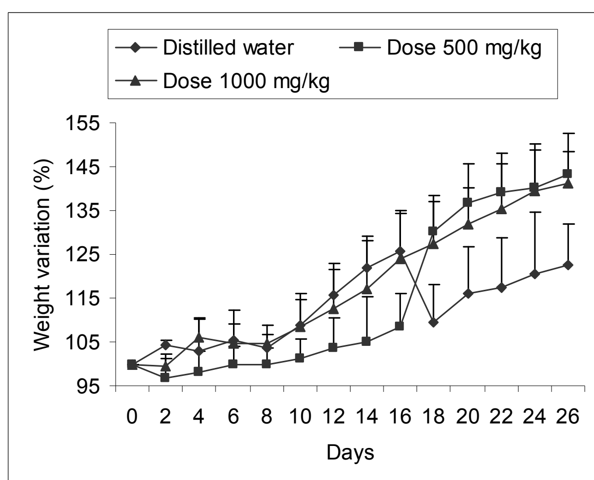

Figure 2: Effect of hydro-ethanolic extract of *S. alata* on the body weight of male rats during the subacute toxicity. Each data point represent the mean ± SD (n = 5).

the release of ALT from the affected liver (Haweroft, 1987). AST/ALT ratio of more than 2 indicates alcoholic hepatitis or cirrchosis (McIntyre et al., 1987). However, this result did not show any dose responsive effect from the results of ALT, AST, and APL. The liver glutathione level increases gradually at doses of 500 and 1000mg/kg body weight compared to the control after 26 days of administration. This increased level of glutathion is not statistically significant (P<0.05) for the male but this variation was significantly different (P<0.05) for the female.

The histopatological study of the liver of differents groups of rats showed a normal architecture. Rats treated orally with the extract of *S. alata* for 26 days, Figure 2 showed little abnomalities such as steatosis, clarification and balloning of hepatocytes. These signs not found in the control groups are mostly seen in the rats which received 1000 mg/kg body weight. The presence of steatosis also in the control groups suggested that this may be caused by diet of the animals. However no necrosis, infiltration, oedema and conjunction, which are the signs of hepatotoxicity were found. The effect of *S. alata* seems to have a protective effect on hepatocytes and improves liver architecture.

In conclusion, this study presents strong evidence of the nontoxic effect of the hydroethanolic extract of *S.*

alata. These results showed that the use of the extract of *S. alata* is safe and explained the extensive utilisation of the plant in traditional medicine.

ACKNOWLEDGMENTS

The authors acknowledge the technical support of the Laboratory of Nutrition and Toxicology of the Department of Biochemistry through Pr. Moundipa F. Paul, The Chinese cooperation and the Cameroon National Herbarium.

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