ACUTE AND SUBACUTE TOXICITY STUDIES OF SANSEVIERIA LIBERICA AQUEOUS LEAF EXTRACTS

Achi, Ngozi K* and Ohaeri, O. C.

Department of Biochemistry, Michael Okpara University of Agriculture, Umudike PMB 7267 Abia State, Nigeria.

ABSTRACT

The toxicological effects of various concentrations of an aqueous extract of Sansevieria liberica on some hepatospecific markers were investigated in Wister strain rats. The extract was prepared by macerations in sterile distilled water and soaking for 48h. A yield of 11.3% w/w dry extract was obtained for the aqueous extractions. Oral administration of aqueous extract of S. liberica at the doses ranging from 50-1000 mg/kg body weight did not produce mortality or any significant change in treated animals over a 14-day period when compared to the control. Acute toxicity test in rats gave an LD50 of 4570 mg/kg. There were no significant difference (P<0.05) in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities. Total, conjugated and unconjugated bilirubin levels, albumin, globulin and total protein levels were not altered by the extract in the experimental groups when compared with the control group. The concentrations of plasma creatinine and urea were also significantly not affected. The aqueous extracts of S. liberica showed no effect in rats and therefore no evidence of hepatotoxicity of the extract was established.

Keywords: Sansevieria liberica, acute toxicity, LD50, aqueous leaf extract

INTRODUCTION

Herbal medicines are an inherent part of African traditional medical practices[1-3]. They are much cheaper than conventional drugs and therefore easily available, readily accepted and widely used [4-5]. However, many of these plant extracts have been reported to possess a toxic effect on some key enzymes of liver metabolism. The literature abounds with reports of hepatotoxicity and other toxic effects of herbal remedies to both human and animals [6].

Sansevieria liberica (Gerome and Labroy)(Agavaceae) is an ornamental perennial plant with thick woody rhizomes. In Nigeria, the leaves and roots of S. liberica are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, monorrhagia, piles, sexual weakness,
snake bites and wounds of the foot. The root part is used in ethnomedicine in the treatment of fever, headache and cold, as well as analgesic, antibiotic, a sedative, an anticonvulsant and an anti-inflammatory agent. Adeyemi et al. reports that the aqueous root extract of S. liberica was found to possess antidiarrhoeal property perhaps due to inhibition of gastrointestinal propulsion and fluid secretion, which is possibly mediated through inhibition of the nitric oxide pathway. The leaves are also used for the treatment of several ailments, which include acute malaria, convulsion and stomachache in young children in the eastern part of Nigeria. Notwithstanding the widespread use of infusions, decoctions and poultices of S. liberica in the Nigerian folk medicine, the toxicology of the plant has not been intensively studied. According to Amida et al., administration of the plant extract orally up to 20 g/kg, similar to those used in folk medicine, did not produce any visible toxicity or mortality within 14 days of single treatment, but i.p. administration caused mortalities with LD₅₀ of 668.3±47.6 mg/kg. Also, in the chronic tests, neither mortality nor visible signs of lethality was seen in rats. The benefits of the traditional use of Sanseviera liberica have been supported by the isolation and identification of several possible active chemical constituents, including flavonoids, saponins, tannins, and anthraquinones. The same observations were previously reported by Adeyemi et al., including the presence of carbohydrates, alkaloids, saponins, reducing sugars, and oils in the aqueous root extract. Similarly, the phytochemical constituents of the related species S. trifasciata, have shown the presence of carbohydrates, saponins, glycosides, steroids in the leaves. Presence of these compounds could help to account for both anti-diarrheal activity and embryotoxicity. The expression of toxicity of xenobiotics as reported by Owu et al. is usually determined biochemically by the monitoring of some plasma enzymes and lipids. Determination of its phytochemical constituents, as well as toxicological profile will provide supportive scientific evidence in favour of its continuous usage. The present investigation was therefore, undertaken to evaluate the toxicity of an aqueous extract of Sanseviera liberica leaves on biochemical parameters and body weight in rats and establish the safety of the plant in traditional medicine.

MATERIALS AND METHODS

Plant material and authentication
The leaves of *Sansevieria liberica* were collected in March, 2008 from a single population of the plant from Amaekpu-Ohafia, Abia State. The species was authenticated by Mr. Ibe Ndukwe of the Department of Forestry and Environmental Management, Michael Okpara University. A voucher specimen was prepared and deposited in the Department of Biological Sciences Herbarium of the University.

**Phytochemical screening**

The dried extracts were first reconstituted in water used for extraction and then tested by standard phytochemical methods \[^{16-17}\] for presence of alkaloids, saponins, flavonoids, tannins, glycosides, proteins, oils, steroidal glycons, and mucilages.

**Experimental animals**

Twenty-five (25) male Wistar rats weighing between 75-80g were obtained from the Animal House of the Department of Zoology University of Calabar, Nigeria. The rats were housed in wire cages placed in well ventilated house conditions. Animals were maintained under a constant 12-h light and dark cycle and an environmental temperature of 25 ± 1°C. A standard pellet diet and water were given ad libitum throughout the period of the experiment. The study was conducted with strict adherence to the ethical procedure of the Michael Okpara University of Agriculture, Umudike, on the use of animals for experiment.

**Preparation of extract**

The fresh leaves of *S. liberica* were carefully rinsed under running tap water, sun-dried then pounded and homogenized in a Waring blender. The powdered leaves (800g) were extracted thrice in water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The combined extracts were filtered through Whatman No.1 filter paper and concentrated using a rotary vacuum evaporator under reduced pressure at 40°C. The leaf water extract was later reconstituted in distilled water to give the required doses of 50, 200 and 600 and 1000mg/kg respectively.

**Animal grouping and extract administration**

The animals were grouped into five consisting of five rats each. Group A (control), received orally, 0.5 ml of distilled water for 14 days while Groups B, C, D and E were treated like the control except that they received 50, 200, 600 and 1000mg/kg respectively.
body weight of the plant extract. The extract and distilled water were administered daily using metal oropharyngeal cannula.

Preparation of serum samples

The method described by Yakubu et al., \cite{18}, was adopted for the preparation of the serum. Briefly, after cervical dislocation, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were cut with sterile scalpel blade and an aliquot of the blood was collected into BD vacutainer sample bottles for the haematological analysis. The remainder was allowed to clot for 10 min at room temperature, and then centrifuged at 1282 g × 5 min using Hermle Bench Top Centrifuge (Model Hermle Z300, Hamburg, Germany). The sera were used within 12 h of preparation for the various biochemical assays. The liver and the kidney were thereafter removed from the animals and weighed for the determination of the organ-body weight ratio.

Acute toxicity test

This assay was carried out to evaluate any possible toxic effect or changes in normal behavior of Wistar rats. Thirty (30) Wister male rats were randomly distributed into three (3) groups of ten (10) rats each. The first group (A) served as control and received 0.9 ml of normal saline (solvent system). The second (B), third (C), fourth (D) and (E) groups received 50, 200,600 and 1000 mg/kg body weight of \textit{S. liberica} extract respectively. All rats in this evaluation received their respective doses daily and had free access to food and water at all times. The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1 h after treatment, then intermittently for 4 h, and thereafter over a period of 24 h. The mice were further observed for up to 14 days following treatment for behavioral changes and signs of toxicity and/or death, and the latency of death. The LD$_{50}$ values were determined according to the Lorke's method \cite{19} by administering extracts in normal saline via oral and intraperitoneal administration. The number of dead animals was expressed in percentage. At the end of 14 days, the rats were sacrificed by cervical dislocation.

Determination of biochemical parameters

Adopting the method of Tietz et al., \cite{20}, the levels of creatinine, uric acid, calcium, chloride, sodium and potassium ions, phosphorus and urea were determined.
Cholesterol, albumin, bilirubin (total and conjugated), total protein, alkaline phosphatase, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Randox Assay kit based on the standard methods as described by Reitman and Frankel [21].

Organ weight studies.

The rats were sacrificed after cervical dislocation and examined for gross and microscopic changes. The following vital organs from each rat such as liver, lungs, kidney and heart were excised, blotted and weighed.

Statistical analysis

The values expressed as mean ± SD. Statistical significance was determined by one-way ANOVA and means were separated by the Duncan Multiple Range Test. A value of p < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Herbal preparations and their use in the treatment of disease are very common in the rural communities of Nigeria. *S. liberica* roots have been used in the traditional treatment of many ailments including convulsion, children’s cough and malaria [11]. Evidence of the antimicrobial activity of this extract was also demonstrated (Adeyemi et al., [10]. The importance of this plant in folk medicine makes it is necessary to characterize its effects on bio-systems, including its toxicological activities and safety following repeated exposure.

The constituent compounds detected by qualitative phytochemical analysis of the crude extract of the plant material are shown in Table 1. The aqueous extracts of *S. liberica* contained some bioactive substances such as alkaloids, flavonoid, saponins and steroids and were present at the relative concentrations indicated. Cardiac glycosides, tannins and phenolic compounds were also present. However, protein, cyanogenic glycosides were present in low concentrations. The samples were devoid of gums and mucilages, reducing sugar, and volatile oil. Other chemical compounds such as chlorogenic acid, anthraquinone glycosides, amino acids were not determined. In comparison, chemical analysis of the root of *S. liberica* has in addition, proven the existence also of anthraquinones, and Adeyemi et al., [8]. Tannins have been indicated as the cause of death produced by the pods of *Acacia nicotica* in goats in South Africa,
giving rise to tachycardia, anorexia, ruminal stasis, anaemia, dyspnoea and recumbency \[22\]. The hydrolysable tannins are astringents and apparently bind the proteins in plasma and organs causing coagulation and necrosis\[23\]. Saponins are also known to cause poisoning in animals.

**TABLE 1: PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACTS OF S. LIBERICA**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Metabolite</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fehling</td>
<td>Reducing carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Mucilages</td>
<td>Mucilages</td>
<td>-</td>
</tr>
<tr>
<td>Molish</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>Phenols/tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanidines</td>
<td>Anthocyanidins</td>
<td>+</td>
</tr>
<tr>
<td>Dragendorff</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Cyanogenic glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Mayer</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
</tbody>
</table>

It was not possible to calculate the LD$_{50}$ orally, for even a dose of 8000 mg/kg did not produce deaths up to seven days following administering of the extract. Through i.p. (3000 to 8000 mg/kg), the extract induced death in the animals, which allowed the 50% lethal dose to be calculated at 4570 mg/kg suggesting a very low toxicity of this product.

Acute toxicity studies through oral administration of the aqueous leaf extracts of *S. liberica* in low and high doses (that is from 100 to 1000mg/kg body weight ) did not produce significant changes in the animals’ behaviour, such as in breathing, cutaneous effect, sensory and nervous system responses or, on gastro-intestinal effects. No adverse effects were observed during the experimental period and no death occurred, which may
indicate that the administration of the herb in the doses indicated presented little toxicity in the animals studied. Furthermore, the absence of mortality after the administration of 1000mg/kg of extract by a 28 days treatment, allowed us to conclude that the extracts obtained from the plant species, possess very low risk of toxicity within a reasonable margin of therapeutic safety.

Rats orally administered various doses of the leaf extracts of *S. liberica* did not develop any clinical signs of toxicity to show significant changes in behaviours, breathing, and nervous and gastrointestinal effects either immediately or during the post-treatment period. In the 24 h and 14 days single dose study, oral administration of 50, 100, 200 and 1000 g/kg of *S. liberica* produced neither mortality nor changes in behavior or any other physiological activity in mice. Independent of the extract utilized, the pathological analyses of the different organs did not show alterations or differences when compared with the control groups.

The results of the body weight changes after administration of the aqueous extracts are shown in Table 2. The body weight of each animal was evaluated weekly until the 14th day observation, showing a normal increment without differences between groups. Further, the relative organ weight of various organs was significantly different compared to controls. Table 2 shows the result of the changes in weight of the heart and kidney of rats administered with different doses of the extracts from *S. liberica*. In general, although the values obtained for heart and kidney differed, they were not significantly different (p<0.05) from the values obtained from the controls for these organs.
TABLE 2: EFFECT OF ADMINISTRATION OF AQUEOUS LEAF EXTRACTS OF S. LIBERICA ON BODY AND RELATIVE ORGAN WEIGHTS OF RATS

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Body (gm)</th>
<th>Liver (gm)</th>
<th>Heart (gm)</th>
<th>Left kidney (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.30±</td>
<td>0.084±</td>
<td>0.002±</td>
<td>0.002±</td>
</tr>
<tr>
<td>9.48 x 10^-2</td>
<td>74.60±</td>
<td>6.67±</td>
<td>0.16±</td>
<td>0.16±</td>
</tr>
<tr>
<td>50</td>
<td>0.63</td>
<td>2.3x10^-2</td>
<td>6.32x10^-3</td>
<td>5.83x10^-3</td>
</tr>
<tr>
<td>200</td>
<td>75.01±</td>
<td>0.087±</td>
<td>0.002±</td>
<td>0.0024±</td>
</tr>
<tr>
<td>7.74x10^-3</td>
<td>7.07x10^-3</td>
<td>3.10x10^-3</td>
<td>9.69x10^-3</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>75.02±</td>
<td>0.089±</td>
<td>0.0021±</td>
<td>0.0024±</td>
</tr>
<tr>
<td>1.96x10^-2</td>
<td>5.02x10^-2</td>
<td>3.02x10^-3</td>
<td>5.09x10^-3</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>72.08±</td>
<td>0.099±</td>
<td>0.0024±</td>
<td>0.18±</td>
</tr>
<tr>
<td>3.74x10^-3</td>
<td>0.19</td>
<td>3.17x10^-3</td>
<td>2.4x10^-3</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M

The effects of oral administration of various doses of S. liberica extracts on the activity of serum enzymes are shown in Table 3. ALT activities ranged between 67 and 73 U/L. The control rats had lower ALT activities (68.0 U/L) than those of 1000mg/kg-administered rats (p<0.05).

The ALP activities of all test groups were higher than in the control groups. Increasing the weight of the leaf extract administered produced dose-related changes in ALP values as shown in Table 3. The serum AST activity was 83.04 U/L in the control group. The value was lower than that of the 1000mg/kg administered group, but higher than that of the 1000mg/kg administered groups. In addition, changes in several parameters such as P, Na, Ca and were observed in all the treatment groups, although the degree of variation were small in extent and lacked obvious dose dependence as shown in Table 4.

During tissue damage, it is noted that some biomolecules may find their way into the serum, probably by leakage through disrupted cell membranes. Results of the in vivo screening of serum enzyme activity can provide an indication of general
hepatotoxicity at concentrations possessing potentially useful bioactivities\textsuperscript{[25]}. Serum enzyme measurement therefore, provides a valuable tool in clinical diagnosis as well as toxicity studies. Estimation of the serum activity alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury\textsuperscript{[26]}. The changes in serum enzyme activities observed in this study showed dose-dependent relationships with the groups administered 1000mg/kg body weight having lower values than other groups. The differences between the alkaline phosphatase (ALP) activities of all the test animals and those of the control were not significant (p< 0.05). Serum ALP is a sensitive detector for early intrahepatic and extrahepatic bile obstruction or the presence of infiltrative diseases of the liver\textsuperscript{[15]}. ALP levels observed in these studies were within the normal physiological range of 20 to 90 IU/L\textsuperscript{[27-28]}. Thus, it is likely that the levels of the leaf extracts used in this study did not adversely interfere with the metabolic activities mediated by ALP.

**TABLE 3 EFFECT OF AQUEOUS LEAF EXTRACTS OF S. LIBERICA ON SERUM ENZYMES IN RATS.**

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153.12 ± 0.27</td>
<td>83.04 ± 0.67</td>
<td>72.62 ± 0.42</td>
</tr>
<tr>
<td>50</td>
<td>153.02 ± 1.45</td>
<td>82.44 ± 4.68</td>
<td>74.05 ± 3.50</td>
</tr>
<tr>
<td>200</td>
<td>153.97 ± 0.43</td>
<td>80.71 ± 0.0152</td>
<td>70.20 ± 0.02</td>
</tr>
<tr>
<td>600</td>
<td>153.37 ± 0.042</td>
<td>80.89 ± 0.0164</td>
<td>74.156 ± 0.20</td>
</tr>
<tr>
<td>1000</td>
<td>158.98 ± 0.15</td>
<td>89.11 ± 0.15</td>
<td>74.14 ± 0.14</td>
</tr>
</tbody>
</table>

Values are the means ± SEM (n=5)
ALP = Alkaline Phosphatase
AST = Aspartate transaminase
AT = Alanine transaminase
TABLE 4 EFFECT OF AQUEOUS LEAF EXTRACTS OF *S. LIBERICA* ON BIOCHEMICAL INDICES IN RATS

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>urea (mg/dl)</th>
<th>creatinine (mg/dl)</th>
<th>protein (g/dl)</th>
<th>total bilirubin (mg/dl)</th>
<th>conjugate bilirubin (mg/dl)</th>
<th>albumin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.210 ± 3.16x10^-3</td>
<td>34.082 ± 3.63x10^-2</td>
<td>6.562 ± 2.53x10^-2</td>
<td>1.220 ± 6.32x10^-3</td>
<td>0.314 ± 2.44x10^-3</td>
<td>5.806 ± 4.0x10^-3</td>
</tr>
<tr>
<td>50</td>
<td>0.312 ± 4.89x10^-3</td>
<td>34.128 ± 4.83x10^-2</td>
<td>6.826 ± 8.71x10^-3</td>
<td>0.324 ± 1.32x10^-2</td>
<td>0.518 ± 8.00x10^-3</td>
<td>5.206 ± 6.78x10^-3</td>
</tr>
<tr>
<td>200</td>
<td>0.450 ± 1.54x10^-2</td>
<td>28.01 ± 3.74x10^-3</td>
<td>6.752 ± 9.69x10^-3</td>
<td>1.604 ± 5.09x10^-3</td>
<td>0.748 ± 1.07x10^-2</td>
<td>4.912 ± 5.82x10^-3</td>
</tr>
<tr>
<td>600</td>
<td>0.404 ± 5.09x10^-3</td>
<td>36.500 ± 3.01x10^-2</td>
<td>6.612 ± 9.69x10^-3</td>
<td>1.783 ± 1.39x10^-2</td>
<td>0.900 ± 1.37x10^-2</td>
<td>5.680 ± 3.0x10^-2</td>
</tr>
<tr>
<td>1000</td>
<td>0.452 ± 6.35x10^-3</td>
<td>36.706 ± 8.78x10^-3</td>
<td>6.710 ± 1.04x10^-2</td>
<td>1.746 ± 1.50x10^-3</td>
<td>1.606 ± 2.40x10^-3</td>
<td>5.800 ± 1.30x10^-2</td>
</tr>
</tbody>
</table>

Values are the means ± SEM (n=5), P<0.05 compared to the control.

The differences observed between the aspartate transaminase (AST) activities in rats administered 1000mg/kg body weight leaf extracts and the control was not significant (p< 0.05). One of the causes in transaminase activities is the dietary intake of pyridoxine, which is an essential cofactor for transamination reactions. Decreased transaminase activities follow a decreased pyridoxine intake [29-30]. According to Moore and Dalley [31], an increase in organ-body weight ratio is an indication of inflammation while a reduction in the same parameter can be adduced to cellular constriction. Therefore, the non-effect of the extract on the liver-body weight ratio in this study has suggested that the extract did not cause inflammation or constriction of the hepatocyte.
This may also explain the non-effect of the 50 and 100 mg/kg body weight of the extract on the kidney-body weight ratio. However, the increase in the parameter observed with the 200 mg/kg body weight may be explained by the inflammation of the nephrons. Thus the absence of significant effects relating to acute or chronic toxicity might be a result of adaptation to the drug indicating that S. liberica is safe in oral administration in rats.

The main serum proteins are albumin and globulin. Albumin is synthesized entirely in the liver and is present in greater concentration than globulin in the plasma and serum. The liver is a common site of damage following exposure to toxic xenobiotics. The concentrations of total proteins, bilirubin and albumin in the serum may indicate the state of the liver and the type of damage [32]. Increase in serum total protein is usually associated with infections and liver disease [33]. In the sub-acute toxicity studies, some of the indices of kidney and liver function were not significantly altered when compared with the controls. An increase in bilirubin concentration in the serum is called jaundice which occurs in toxic or infectious liver diseases [4]. The nonsignificant change in the levels of total and direct bilirubin when compared with the control suggests that the aqueous extract of S. liberica has no obvious effect on the liver at all doses. The fact that the total bilirubin, albumin, globulin and total protein levels were not altered by the extract suggest that the secretory function of the liver may not have been affected. However, the non-definite pattern shown by the extract on the conjugate bilirubin level may be explained as adaptation by the animals to the effect of the extract. Similarly, no damage or structural modification was observed during the macroscopic evaluation of important organs such as the liver, kidney and heart of the treated animals when compared with the control group.

REFERENCES


   Analysis. Chapman and Hill, New York. 1973:
17. Trease GE, and Evans WC: A textbook of Pharmacognosy 13\textsuperscript{th} Edn. Bucilliere
   Tinalia Ltd. London. 1989:
18. Yakubu MT, Akanji MA and Oladiji AT: Haematological evaluation in male
   albino rats following chronic administration of aqueous extract of \textit{Fadogia agrestis} stem. Pharmacol. Manag. 2007; 3: 34-38
   54: 275-287.
21. Reitman S and Frankel S: \textit{In vitro} determination of transaminase activity in
22. Bothaa CJ Penrith M-L: Poisonous plants of veterinary and human importance in
   Hodson E and Levi PE (eds) 2\textsuperscript{nd} edn. McGraw-Hill Companies: Singapore, 2000:
   pp 1–25.
   assessment of \textit{Byrsocarpus cocineus} Schum. and Thonn. (Connaraceae) aqueous
   3(2): 1-11,
26. Adewunmi CO and Ojewole JAO: Safety of traditional medicines, complementary and
   alternative medicines in Africa. African Journal of Traditional, Complementary and
   Publications, Carleifornia1985: pp 88-125

For Correspondence:
Achi, Ngozi K Email: omekachi@yahoo.com