Hepatoprotective Effect of an Aqueous Extract of the Leaves of *Sansevieria liberica* Gerome and Labroy Against Carbon Tetrachloride Induced Liver Injury in Wistar Rats.

Catherine Chidinma Ikewuchi

Department of Biochemistry, Faculty of Science, University of Port Harcourt,
PMB 5323, Port Harcourt, Nigeria

E-mail: okaraonye@yahoo.com

ABSTRACT

In this study, the ability of an aqueous extract of the leaves of *Sansevieria liberica*, to protect against carbon tetrachloride induced liver damage was investigated in Wistar albino rats. The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight. The extract was administered to both normal and carbon tetrachloride treated rats at 100, 200, and 300 mg/kg. Compared to test control, the treatment dose dependently produced significantly lower (P<0.05) plasma alkaline phosphatase, aspartate, and alanine transaminase activities, and total bilirubin level. Histopathological studies showed that carbon tetrachloride caused fatty degeneration of hepatocytes; while pre-treatment with the extract exhibited protection, which confirmed the results of the biochemical studies. These results indicated that treatment with the plant extract protects the liver against carbon tetrachloride induced hepatotoxicity. This supports the use of *Sansevieria liberica* in African traditional health care for the treatment of liver problems.

(Keywords: *Sansevieria liberica* Baker; carbon tetrachloride; histopathology; hepatospecific markers; plasma total bilirubin)

INTRODUCTION

Herbs play a major role in the management of various liver disorders. *Sansevieria liberica* is one of such plants used in African traditional health care for the treatment of liver diseases. *Sansevieria liberica* belongs to the family Agavaceae (Ruscaceae or Dracaenaceae). It is one of the bowstring hemp species (Evans, 2005), with concave, short petioled leaves that are in part transversely banded with light and dark green, also linearly striated with whitish to light green and dark green striations (Reed, 1978). They are grown as ornamental plants (United States Department of Agriculture, 2008), and are widely distributed throughout the tropics. Their leaves are very rich in fibers (Osabohien and Egboh, 2008; Ikewuchi *et al*., 2010a), protein (Ikewuchi *et al*., 2010a), potassium, calcium, magnesium, vitamin C, biotin, and riboflavin (Ikewuchi and Ikewuchi, 2009a). The leaves also contain alkaloids, allicins, carotenoids, flavonoids, glycosides, saponins and tannins (Ikewuchi *et al*., 2010a, 2011a). Fibers for local use are obtained from the leaves in various countries for making string, nets, coarse fabrics and bows. Osabohien (2009) reported that bowstring hemp fiber produced appreciable reinforcement of natural rubber, though inferior to CB(N330) filler, but gave harder vulcanizates.

In traditional health care practice, the leaves are used as pain killers, and in the treatment of small pox, chicken pox, measles and most venereal diseases. The pressed juice of the leaves is dropped in the eyes and ears for the treatment of infections and inflammations. The fumes from the burning leaves are inhaled to relieve feverish headaches and cold. A decoction of the roots is drunk as a remedy for convulsions and as a vermifuge. In Nigeria, the leaves and roots of *Sansevieria liberica* are used in traditional health care practice for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, diabetes mellitus, monorrhagia, piles, sexual weakness, wounds of the foot, and alleviating the effects of snake bites (Gill, 1992; Amida *et al*., 2007; Osabohien and Egboh, 2008; Osabohien, 2009; Adeyemi *et al*., 2009).

The hypotensive effect of aqueous extract of the leaves (Ikewuchi *et al*., 2011b), and their ability to

moderate hematological indices and plasma chemistry (Ikewuchi *et al*., 2010b) have been reported. The present study investigated the hepatoprotective effect of an aqueous extract of the leaves of *Sansevieria liberica* Gerome and Labroy against carbon tetrachloride induced liver injury in Wistar rats.

MATERIALS AND METHODS

Preparation of Plant Extract

Samples of fresh *Sansevieria liberica* plants were procured from a horticultural garden by Air Force Gate, Aba Road, Port Harcourt, and another at the University of Port Harcourt's Abuja campus, and from behind the Ofrima complex, University of Port Harcourt, in Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, the identity was confirmed/authenticated by Dr. Michael C. Dike of Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; and Mr. John Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria.

They were cleaned of dirt and the leaves were removed, oven dried at 55 °C and ground into powder. The resultant powder was soaked in hot, boiled distilled water for 12 h, after which the resultant mixture was filtered and the filtrate was stored in the refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract.

Experimental Design for the Hepatoprotective Study

Wistar albino rats (160-190 g) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Studies were conducted in compliance with the applicable laws and regulations for handling experimental animals. The rats were weighed and sorted into eighth groups (Table 1) of five animals each, so that their average weights were approximately equal. The animals were housed in plastic cages at the animal house of the Department of Biochemistry, University of Port Harcourt. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced. The extracts were administered orally on daily basis for eight days. The dosages of administration of the extracts were adopted and modified from Ikewuchi *et al*. (2010b, 2011b). The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight of carbon tetrachloride, on days 4 and 8.

The dosage and method of administration of carbon tetrachloride was adopted from Obi and Uneh (2003), with modification. Twenty four hours after the last administration of carbon tetrachloride, the rats were weighed and anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed and blood was collected from each rat into heparin sample bottles, after which their livers were collected and preserved in 10% formalin, for histological studies. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which their plasma was collected and stored for subsequent analysis.

Table 1: Experimental Design for the Hepatoprotective Screening.

S/N	ID	Treatment
1	Normal	Olive oil (1 mL/kg) and Normal saline and Water
2	Test control	Carbon tetrachloride (1 mL/kg) and water
3	Treatment control I (LLC1)	Olive oil (1 mL/kg) and extract (100 mg/Kg)
4	Treatment control II (LLC2)	Olive oil (1 mL/kg) and extract (200 mg/Kg)
5	Treatment control III (LLC3)	Olive oil (1 mL/kg) and extract (300 mg/Kg)
6	Treatment I (LL1)	Carbon tetrachloride (1 mL/kg) and extract (100 mg/Kg)
7	Treatment II (LL2)	Carbon tetrachloride (1 mL/kg) and extract (200 mg/Kg)
8	Treatment III (LL3)	Carbon tetrachloride (1 mL/kg) and extract (300 mg/Kg)

Assay of Plasma Hepatospecific Markers

The plasma activities of alanine and aspartate transaminases, and alkaline phosphatase, were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The activities of alanine and aspartate transaminases were respectively measured by monitoring at 546 nm the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of alkaline phosphatase was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm.

Plasma total bilirubin and protein concentrations were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The wavelength for the determination of total bilirubin was 578 nm, while that of total protein was 560 nm.

Determination of Percentage Protection

The percentage protection provided by the extract against carbon tetrachloride induced liver damage was calculated using the following formula adapted from Al-Qarawi *et al.* (2004).

$$\% \text{ Protection} = \frac{(A - B) \times 100}{A - C} \qquad [Eqn\ 1]$$

Where A = $Parameter_{Test\ control}$; B = $Parameter_{Treatment}$; C = $Parameter_{Control}$

Histopathological Study

The histopathology study was carried out by Professor S.O. Nwosu, of the Department of Anatomical Pathology, University of Port Harcourt Teaching Hospital. Small pieces of liver tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 µm in thickness were cut, mounted on slide and stained with hematoxylin and eosin. The sections were then examined via light microscopy (Opticphot-2; Nikon, Tokyo, Japan) at 100× magnification.

Statistical Analysis of Data

All values are reported as the mean ± s.d. (standard deviation). The values of the various parameters were analyzed for statistical significant differences between the groups, using the Student's *t*-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). $P<0.05$ was assumed to be significant. Graphs were drawn using Microsoft Office Excel, 2010 software.

RESULTS AND DISCUSSION

Table 2 shows the effects of an aqueous extract of the leaves of *Sansevieria liberica* on the plasma hepatospecific markers of normal and carbon tetrachloride treated rats. The test control had significantly ($P<0.05$) higher plasma alkaline phosphatase, alanine and aspartate transaminase activities than those of the other groups. The plasma total bilirubin content of the test control group was significantly higher ($P<0.05$) than those of control, LLC1, LLC2, LLC3, LL1, and LL3, but not significantly higher than LL2. The plasma total protein content of the test control group was significantly higher ($P<0.05$) than those of control, LLC1, LLC2 and LLC3, but not significantly higher than those of LL1, LL2 and LL3.

The hepatoprotective activity of an aqueous extract of the leaves of *Sansevieria liberica* on carbon tetrachloride-induced hepatotoxicity in Wistar rats is shown in Figure 1. The protection seemed to be dose dependent, with the 200 and 300 mg/kg doses being more effective. The frequency distribution of the effects of an aqueous extract of the leaves of *Sansevieria liberica* on the liver histology of normal and carbon tetrachloride treated rats is shown in Figure 2.

Sections of the liver samples are shown in Figure 3. This histopathological result showed that carbon tetrachloride caused fatty degeneration and necrosis of hepatocytes, which was prevented by pre-treatment with aqueous extract of the leaves of *Sansevieria liberica*; thus confirming the results of the biochemical studies.

Carbon tetrachloride induced liver cirrhosis is probably the best-studied model of liver cirrhosis (Cornelius, 1993).

Table 2: Effects of an Aqueous Extract of the Leaves of *Sansevieria liberica* on the Plasma Hepatospecific Markers of Normal and Carbon Tetrachloride Treated Rats.

Treatment group	Magnitude				
	Alkaline phosphatase activity (U/L)	Alanine transaminase activity (U/L)	Aspartate transaminase activity (U/L)	Total bilirubin content (µmol/L)	Total protein content (mg/dL)
Normal	327.53±9.29[a]	23.19±2.74[a]	17.85±1.08[a]	7.52±0.17[a]	58.45±0.88[a]
Test control	476.97±8.98[b]	62.52±3.15[c]	31.44±2.82[c]	9.18±0.18[c]	61.91±0.66[c]
LLC1	253.00±17.15[e]	35.87±3.99[d]	17.48±2.65[a]	3.15±0.33[d]	55.01±1.42[b]
LLC2	310.50±16.21[a]	22.12±2.50[a]	16.97±2.40[a]	2.71±0.27[e]	55.17±1.18[b]
LLC3	382.41±13.78[c]	26.62±6.46[a]	20.83±2.67[a,b]	3.09±0.17[d,f]	53.60±1.53[b]
LL1	431.83±10.63[d]	52.90±3.51[b]	26.61±1.98[d]	8.90±0.28[b]	59.86±1.49[a,c]
LL2	388.24±16.40[c]	53.23±3.09[b]	27.28±1.70[d]	8.77±0.20[b,c]	60.72±1.42[c]
LL3	441.60±7.81[d]	39.91±2.90[d]	22.05±1.89[b]	7.97±0.45[h]	61.25±1.18[c]

Values are mean ± s.d., n=5, per group.
[a,b,c]Values in the same column with different superscripts are significantly different at $P<0.05$.

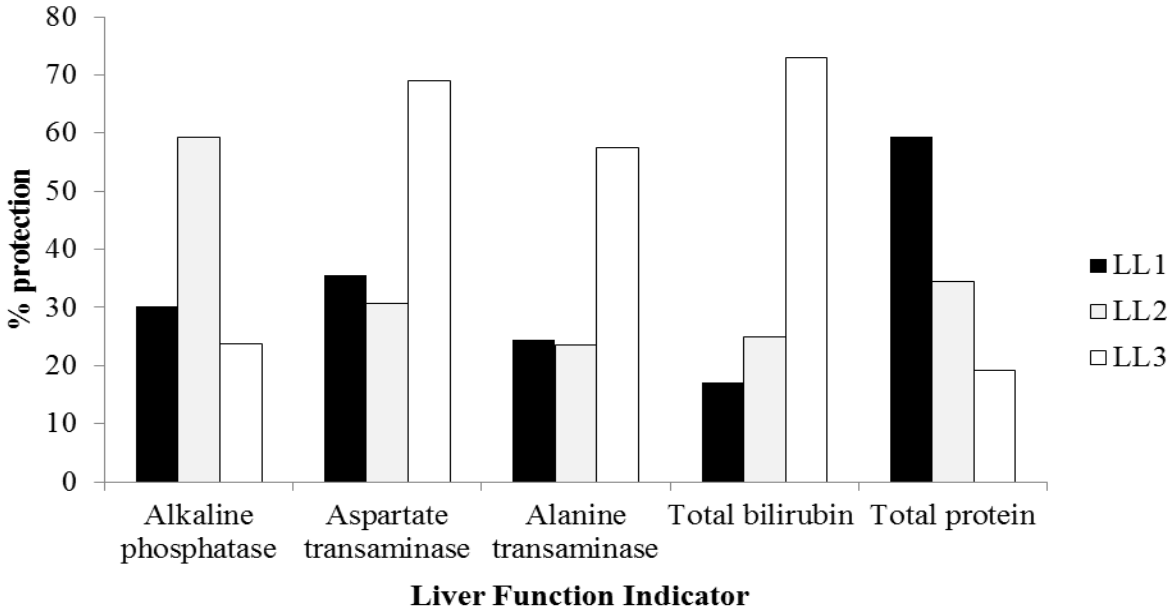

Figure 1: Hepatoprotective Activity of an Aqueous extract of the Leaves of *Sansevieria liberica* on Carbon Tetrachloride-Induced Hepatotoxicity in Wistar Rats.

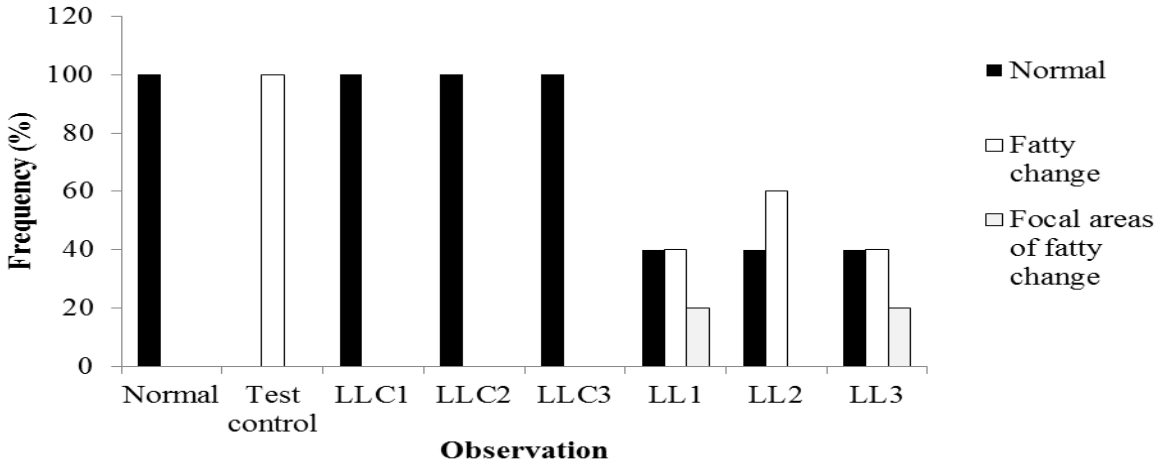

Figure 2: Frequency Distribution of the Effects of an Aqueous Extract of the Leaves of *Sansevieria liberica* on the Liver Histology of Normal and Carbon Tetrachloride Treated Rats.

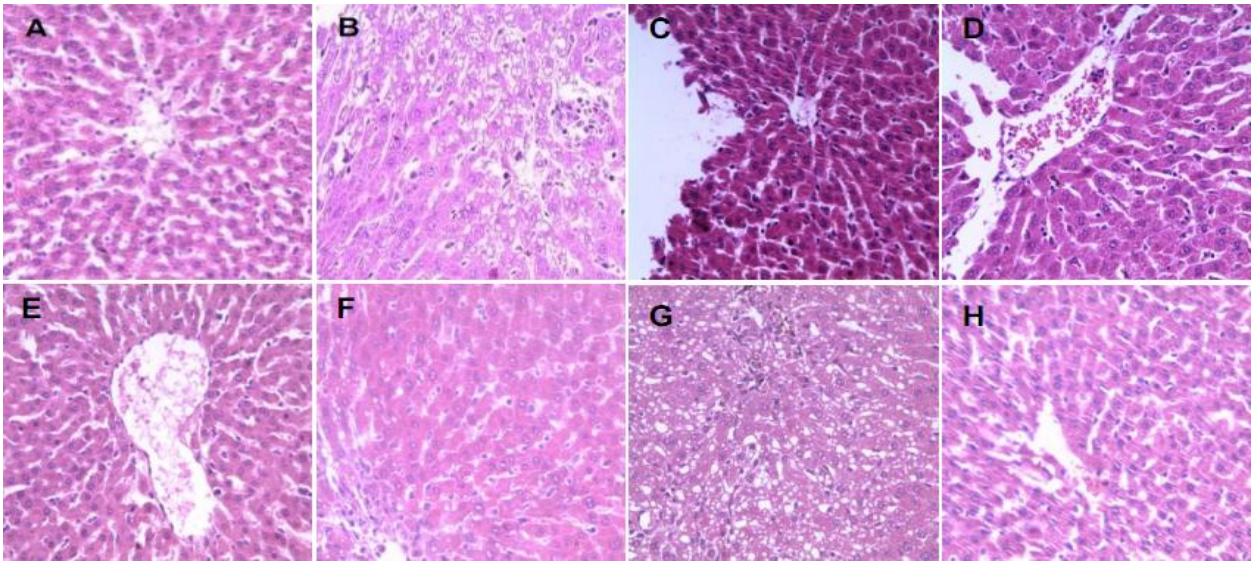

Figure 3: Sections (×20) of the Liver Samples Showing Effect of an Aqueous Extract of the Leaves of *Sansevieria liberica* on the Liver Histology of Normal and Carbon Tetrachloride Treated Rats.

A: Section of the liver of rats administered olive oil (1 mL/kg) and treated with water, showing normal cells. B: Section of the liver tissue of rats administered carbon tetrachloride (1 mL/kg) and treated with water, showing fatty change. C: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 100 mg/kg extract, showing normal cells. D: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 200 mg/kg extract, showing normal cells. E: Section of the liver of rats administered olive oil (1 mL/kg) and treated with 300 mg/kg extract, showing normal cells. F: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 100 mg/kg extract, showing normal cells. G: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 200 mg/kg extract, showing fatty change. H: Section of the liver of rats administered carbon tetrachloride (1 mL/kg) and treated with 300 mg/kg extract, showing focal areas of normal cells.

It is often characterized by elevated plasma activities of aspartate and alanine transaminases and alkaline phosphatase, as well as high levels of total bilirubin and protein. Therefore, the reduction of the carbon tetrachloride-induced elevation of these parameters, in animals pretreated with the aqueous extract of the leaves of *Sansevieria liberica* shows the ability of the leaves to protect normal functional integrity of the poisoned liver, and also to protect against subsequent carbon tetrachloride hepatotoxicity. This hepatoprotective activity may have been produced through a number of mechanisms.

Initiation of lipid peroxidation and the resultant toxicity of carbon tetrachloride can be suppressed by lowering the metabolic activation of carbon tetrachloride to trichloromethyl free radical by cytochrome P450 (Middleton *et al*., 2000). Therefore, any hepatoprotective agent should be able to lower the metabolic activation of carbon tetrachloride, thereby favoring liver regeneration. So, it can be suggested that flavonoids in *Sansevieria liberica* leaves (Ikewuchi *et al*., 2010a), could be responsible for its hepatoprotective ability. This family of compounds has been reported to inhibit lipid peroxidation by inhibiting cytochrome P450 aromatase (Kowalska *et al*., 1990; Middleton *et al*., 2000), and/or exerting a membrane-stabilizing action (Middleton *et al*., 2000).

Another component of *Sansevieria liberica* leaves that may also have contributed to its hepatoprotective activity is vitamin C, a compound that has been reported to be abundant in the leaves of *Sansevieria liberica* (Ikewuchi and Ikewuchi, 2009). Studies have shown that hepatic cytochrome P450 (Rikans *et al*., 1978), and hepatic microsomal drug metabolism (Sato and Zannoni, 1976; Burtis and Ashwood, 2001), are significantly reduced in ascorbic acid-deficiency, and are augmented when high supplements of the vitamin are given to guinea pigs.

CONCLUSION

This study clearly demonstrated that aqueous extract of the leaves of *Sansevieria liberica* is an effective agent in the treatment and prevention of carbon tetrachloride-induced hepatic cytotoxicity. The data suggests that the daily oral consumption of the extract was prophylactic to carbon tetrachloride poisoning. This supports the use of *Sansevieria liberica* in traditional health care for the treatment of liver problems.

REFERENCES

1. Adeyemi, O.O., Akindele, A.J., and Ogunleye, E.A. 2009. “Evaluation of the Antidiarrhoeal Effect of *Sanseviera liberica* Gerome and Labroy (Agavaceae) Root Extract”. *Journal of Ethnopharmacology*. 123(3): 459-463.

2. Al-Qarawi, A.A., Mousa, H.M., Ali, B.E.-D.H., Abdel-Rahman, H., and El-Mougy, S.A. 2004. “Protective Effect of Extracts from Dates (*Phoenix dactylifera* L.) on Carbon Tetrachloride–Induced Hepatotoxicity in Rats”. *International Journal of Applied Research in Veterinary Medicine*. 2(3): 176 – 180.

3. Amida, M.B., Yemitan, O.K., and Adeyemi, O.O. 2007. “Toxicological Assessment of the Aqueous Root Extract of *Sanseviera liberica* Gerome and Labroy (Agavaceae)”. *Journal of Ethnopharmacology*. 113(1):171-175.

4. Burtis, C.A. and Ashwood, E.R. 2001. *Tietz’s Fundamentals of Clinical Chemistry*. Volume 2. WB Saunders: Philadelphia.

5. Cornelius, C.E. 1993. *Animal Models in Liver Research.* Academic Press: San Diego.

6. Evans, W.C. 2005. “A Taxonomic Approach to the Study of Medicinal Plants and Animal-Derived Drugs. In: Evans, WC (ed.): *Trease and Evans Pharmacognosy*, 15th edition. 15-40. Elsevier: India.

7. Gill, L.S. 1992. *Ethnomedical Uses of Plants in Nigeria*. Uniben Press: Benin City, Nigeria.

8. Ikewuchi, C.C. and Ikewuchi, J.C. 2009. “Amino Acid, Mineral, and Vitamin Composition of *Sansevieria liberica* Gérôme and Labroy”. *Pacific Journal of Science and Technology*. 10(1):477-482.

9. Ikewuchi, C.C., Ayalogu, E.O., Onyeike, E.N., and Ikewuchi, J.C. 2011a. “Study on the Alkaloid, Allicin, Glycoside and Saponin Composition of the Leaves of *Sansevieria liberica* Gérôme and Labroy by Gas Chromatography”. *Pacific Journal of Science and Technology*. 12(1):367-373.

10. Ikewuchi, C.C., Ayalogu, E.O., Onyeike, E.N., and Ikewuchi, J.C. 2011b. “Effect of Aqueous Extract of the Leaves of *Sansevieria liberica* Gérôme and Labroy on Blood Pressure Indices and Pulse Rates of Sub-Chronic Salt-Loaded Rats”. *Journal of Natural Remedies* (in press).

11. Ikewuchi, C.C., Ikewuchi, C.J., Ayalogu, O.E. and Onyeike, N.E. 2010a. “Proximate and Phytochemical Profile of *Sansevieria liberica* Gérôme and Labroy”. *Journal of Applied Science and Environmental Management*. 14(2):103–106.

12. Ikewuchi, C.C., Ikewuchi, J.C., Onyeike, E.N., and Ayalogu, E.O. 2010b. “Effect of *Sansevieria liberica* Gérôme and Labroy on Plasma Chemistry and Hematological Indices of Salt-Loaded Rats”. *Research Journal of Science and Technology*. 2(5): 110-114.

13. Kowalska, M.T., Brandt, M.E., and Puett, D. 1990. “Inhibition of Cytochrome P-450 Aromatase Activity by Plant Extracts”. *Planta Medica*. 56: 675–677.

14. Middleton, E., Jr., Kandaswami, C., and Theoharides, T.C. 2000. “The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer”. *Pharmacology Reviews*. 52: 673-751.

15. Obi, F.O. and Uneh, E. 2003. “pH Dependent Prevention of Carbon Tetrachloride–Induced Lipoperoxidation in Rats by Ethanolic Extract of *Hibiscus rosasinensis* Petal”. *Biokemistri*. 13: 42-50.

16. Osabohien, E. 2009. “Effects of Epoxidation on the Thermal Conductivity and Equilibrium Swelling Properties of Bowstring Hemp Fibre Natural Rubber Composites”. *Advances in Natural and Applied Science Research*. 7:169 – 176.

17. Osabohien, E. and Egboh, S.H.O. 2008. “Utilization of Bowstring Hemp Fiber as a Filler in Natural Rubber Compounds”. *Journal of Applied Polymer Science*. 107:210–214.

18. Reed, D.W. 1978. “Sansevieria - Identification and Culture”. *Louisiana Society for Horticultural Research*. 14(2):30-77.

19. Rikans, L.E., Smith, C.R., and Zannoni, V.G. 1978. “Ascorbic Acid and Cytochrome P-450”. *Journal of Pharmacology and Experimental Therapy*. 204: 702–705.

20. Sato, P.H. and Zannoni, V.G. 1976. “Ascorbic Acid and Hepatic Drug Metabolism”. *Journal of Pharmacology and Experimental Therapy*. 198:295–307.

21. United States Department of Agriculture, Agricultural Research Service, Germplasm Resources Information Network (GRIN). GRIN taxonomy for plants [database on the Internet]. c2008 [cited 2008 July 23]. Available from: www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?33057

SUGGESTED CITATION

Ikewuchi, C.C. 2012. “Hepatoprotective Effect of an Aqueous Extract of the Leaves of *Sansevieria liberica* Gerome and Labroy Against Carbon Tetrachloride Induced Liver Injury in Wistar Rats”. *Pacific Journal of Science and Technology*. 13(1):512-518.

Pacific Journal of Science and Technology