HET

Human and Experimental Toxicology
29(8) 679–688

DOI: 10.1177/0960327109357218
het.sagepub.com
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Aqueous extract of *Securidaca longepedunculata* root induce redox imbalance in male rat liver and kidney

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Abstract

The effect of aqueous extract of *Securidaca longepedunculata* root on redox homeostasis in male rat liver and kidney was investigated. Rats were grouped into four: A, B, C and D, where A (the control) received orally 1 mL of distilled water; B, C and D (test groups) received orally 200, 400 and 800 mg/kg body weight of the extract, respectively, for 28 days. Extract administration significantly reduced ($p < .05$) alkaline phosphatase activity in the liver and kidney with corresponding increases in the serum. Acid phosphatase activity increased significantly ($p < .05$) in the liver and kidney, while there was no significant change ($p > .05$) in the serum acid phosphatase activity. There was also significant decrease ($p < .05$) in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the liver and kidney. Liver and kidney levels of GSH, vitamins C and E were also significantly reduced ($p < .05$). Serum malonidialdehyde and lipid hydroperoxide increased significantly ($p < .05$) in all the extract-treated groups. The available data from this study revealed that aqueous extract of *S. longepedunculata* root exerted its toxicity in the animals by depleting the antioxidant systems. This may consequently expose the cells and cellular macromolecules to oxidative damage by reactive oxygen species generated either from the metabolism of the extract or other *in vivo* means.

Keywords

Antioxidant system, lipid peroxidation, malondialdehyde, redox homeostasis, *Securidaca longepedunculata*

Introduction

Phytomedicine research is gaining more grounds than ever as majority of people are relying heavily on herbal medicine, which occasionally has been shown to be less toxic and cheaper compared to the synthetic orthodox medicines.[1] The general acceptability of traditional medicines is however limited by lack of dose regimen and adequate toxicity data to evaluate their safety.[2] Toxicological evaluation of phytomedicines to ascertain their safety for consumption and their possible mechanisms of action is thus needed to increase their acceptabiltiy.

Securidaca longepedunculata, known as violet tree, Rhodes's violet, wild vesteria (English) and *Uwar magunguna* (Hausa), is a shrub or small (2–10 m high) flowering savannah plant. The flowers are sweet scented, bright purple or violet in racemes, and the fruit is winged; the plant is widespread throughout tropical Africa. In many parts of Africa, the plant is employed in traditional medicine principally for its psychotropic properties; aqueous extracts of this root are used as psychopharmacological agents.[3] The roots and bark are taken orally either powdered or as infusions for treating chest pain, headache, inflammation, abortion, ritual suicide, rheumatism, tuberculosis, infertility, venereal diseases and constipation.[4,5]

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The analgesic, antinflammatory and hypoglyceamic acitvity of *S. longepedunculata* root bark has also been reported.[6] Its use in bacterial and malarial chemotherapy have also been documented.[7,8] Wannag *et al*.[9] also reported the anti-snake venom activity of the aqueous root extract of *S. longepedunculata*.

Phytochemical investigations of the root bark revealed the presence of flavonoids, alkaloids, saponins, triterpenoids and volatile oils.[10] Other secondary metabolites like sapogenins; presenegenin, indole alkaloid; securinine and some ergot alkaloids have also been reported to be present in the root of this plant.[11]

S. longepedunculata root has also been reported to possess some level of toxicity on mice liver and kidney.[12] Dapar *et al*. showed that intraperitoneal administration of 100 mg/kg body weight resulted in signs of toxicity and death.[12] Also, the cellular enzymes (alanine and aspartate aminotransferases) were elevated in *S. longepedunculata*-treated rats.[12] In contrast, Etuk *et al*.[13] observed no toxicity following a 28-days oral administration of *S. longepedunculata* root in mice with the LD_{50} more than 2700 mg/kg body weight.[13] Although, the effects of plant extract on tissue marker enzymes and histopathological examination have provided an insight into the toxic effects of extracts, more studies such as effect on redox hoemoestasis and drug metabolizing enzymes are needed to further ascertain the mechanism of toxicity. This study thus provides information on the effect of aqueous extract of *S. longepedunculata* root on redox homoestasis of rat liver and kidney.

Materials and methods

Materials

Plant material. *S. longepedunculata* roots collected at Idu Industrial Layout, Idu-Abuja, Nigeria, by Mallam Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria was identified by Mrs. Grace Ugbabe of the herbarium unit of the Department.

Animals. A total of sixty, apparently healthy, 3 months old, male albino rats (*Rattus norvegicus*) of Wistar strain, weighing 195.08 $\pm$ 3.58 g were obtained from the Animal Holding Unit of the Department of Bio chemistry, University of Ilorin, Ilorin, Nigeria. They were kept in clean aluminum cages of dimensions $33.0 \times 20.5 \times 19.0$ contained in well-ventilated house conditions (temperature: 28-31°C; photoperiod: 12 hour natural light and 12 hour dark; humidity: 50%–55%) with free access to rat pellets (BendelFeeds and Flour Mills Ltd., Ewu, Nigeria) and tap water.

Assay kits. Alkaline and acid phosphatase (ALP & ACP) assay kits were products of Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany. Glutathione peroxidase (GPx) was a product of Randox Laboratories Co-Antrim, United Kingdom. All other reagents used were products of Sigma-Aldrich Inc., St Louis, USA.

Methods

Extraction. The *S. longepedunculata* roots werewashed, sliced into pieces and then air-dried under shade for 2 weeks to constant weight. The dried pieces were then pulverized with an electric blender (Blender/Miller III, model MS–223, Tapei, Taiwan) and the resulting powder stocked in a plastic container. The powder (300 g) was extracted in 1 L of distilled water for 48 hours. This was later filtered with Whatman No. 1 filter paper and the resulting filtrate concentrated on a steam bath to give 107.40 g of the residue, which is equivalent to a yield of 35.80%. The reconstituted extract which was completely homogenous was stored at 4°C each time after administration.

Animal treatment. Male rats were completely randomized into four groups (A, B, C and D) of 15 animals each. Rats in groups B, C and D were orally administered once daily with 1 ml containing 200, 400 and 800 mg/kg body weight of the extract at 24 hours interval for 28 days, respectively. Group A, which served as the control was treated like the test groups except that the animals equal volume of distilled water (vehicle).

The animals were allowed free access to rat pellets and tap water before and after administration. Five animals each were sacrificed from all the groups, 24 hours after 1, 14 and 28 days of their daily doses. This study was carried out following approval from the Ethical Committee on the use and care of animals of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were also handled according to the NIH Guide for the Care and Use of Laboratory Animals[14] as well as in accordance with the principles of Good Laboratory Procedure (GLP).[15]

Tissue and serum preparation. The rats were sacrificed by placing them in a jar containing cotton wool

soaked in ether. To collect blood, their jugular vein was sharply cut with clean sterile scalpel blade after they were unconscious. The blood samples collected were allowed to clot for 15 min and centrifuged at 300g for 5 min for serum preparation. The clear supernatant was used for the determination of biochemical parameters. Liver and kidney excised from the rats were cleaned of blood and immersed in ice-cold 0.25 M sucrose solution (after decapsulation of kidney) to maintain the integrity of the organs. The organs were thereafter blotted with tissue paper, cut thinly with sterile scalpel blade and then homogenized separately in ice-cold 0.25 M sucrose solution (1:5 w/v).[16] The homogenates were centrifuged at 800 g for 10 min at 4°C; the resulting supernatant was frozen at –20°C to ensure the maximum release of the enzymes located in the cell before being used for the enzyme assay.

Enzyme assay

Acid phosphatase assay was done according to the method described by Hillmann.[17] The 'optimized standard method' according to the recommendation of the German Clinical Chemistry Association (Deutsche Gesellschaft für Klinische Chemie)[18] was used for assaying ALP activity. Superoxide dismutase (SOD) and catalase (CAT) were assayed as reported by Misra and Fridovich[19] and Beers and Sizer,[20] respectively. Glutathione peroxidase (GPx) and glutathione reductase (GR) assays were also done according to the methods described by Rotruck et al.[21] and Mavis and Stellwagen,[22] respectively. Reduced Glutathione (GSH) concentration was determined according to the method of Ellman[23] while vitamins C and E were assayed according to the methods described by Omaye et al.[24] and Desai,[25] respectively. Biuret method was used for the determination of protein concentration in the tissues.[26] Malondialdehyde (MDA) and lipid hydroperoxide (LPO) were determined using the procedures described by Buege and Aust[27].

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test were used to detect significant differences among the means as well as the interactions between the variables using SPSS 15.0 Version. Differences were considered statistically significant at $p < .05$.

Results

While there was no significant ($p < .05$) effect on the liver and kidney alkaline phosphatase activity following the single administration of 200 mg/kg body weight of the extract, the enzyme activity was decreased by the 400 and 800 mg/kg body weight (Table 1). Further administration produced dose-dependent decrease in the enzyme activity of the organs. Serum ALP activity was also elevated right from day 1 of administration and was sustained throughout the experimental period (Table 1).

There was significant ($p < .05$) increase in liver and kidney acid phosphatase activities (Table 2) following the administration of the extract at all the doses. This increase in the activity of the enzyme was sustained for all the doses throughout the period of the experiment. In contrast, there was no significant ($p < .05$) change in the serum enzyme activity (Table 2).

Antioxidant enzymes were significantly ($p < .05$) reduced following administration of aqueous extract of *S. longepedunculata* root in a dose-dependent manner (Tables 3-6). The highest dose produced 68.43%, 77.09%, 70.77% and 45.27% decrease in the liver; SOD, CAT, GPx and GR, respectively, by the end of the experimental period, whereas the kidney SOD, CAT, GPx and GR activities were decreased by 50.78%, 59.16%, 51.54% and 79.75%, respectively, following the administration of 800 mg/kg body weight of aqueous extract of *S. longepedunculata* root by the end of the exposure period.

The levels of GSH and vitamins C and E were also significantly reduced (Tables 7 and 8). While the highest dose of aqueous extract of *S. longepedunculata* root produced a 68.44%, 59.11% and 66.67% decrease in the liver GSH, vitamins C and E levels, respectively, a 72.93%, 56.30% and 76.49% decrease, respectively, were observed in the kidney at the end of the experimental period (day 28).

In contrast, the levels of malonidialdehyde and lipid hydroperoxides (LPO), in the liver and kidney of rats (Tables 9 and 10) were significantly ($p < .05$) elevated following the administration of aqueous extract of *S. longepedunculata* root, except the 200 mg/kg body weight of the extract on day 1. The highest dose of the extract by the end of the experimental period produced a 101.03 and 94.91% increase in liver malonidialdehyde and LPO levels, respectively, while a 98.17% and 101.29% increase, respectively, were observed in the kidney.

Table 1. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver, kidney and serum alkaline phosphatase activity

	Liver			Kidney			Serum		
Doses/days	1	14	28	1	14	28	1	14	28
Control	1.64 ± 0.04[a]	1.58 ± 0.02[a]	1.61 ± 0.06[a]	19.25 ± 0.02[a]	18.98 ± 0.06[a]	19.05 ± 0.04[a]	0.52 ± 0.01[a]	0.55 ± 0.02[a]	0.52 ± 0.02[a]
200 mg/kg body weight	1.45 ± 0.02[a]	1.25 ± 0.08[b]	1.12 ± 0.03[b]	17.35 ± 0.09[a]	13.95 ± 0.05[c]	9.43 ± 0.01[c]	0.72 ± 0.09[b]	0.98 ± 0.09[c]	1.35 ± 0.09[c]
400 mg/kg body weight	1.11 ± 0.01[b]	0.98 ± 0.03[c]	0.82 ± 0.07[c]	15.18 ± 0.01[b]	10.10 ± 0.09[c]	7.49 ± 0.02[d]	1.01 ± 0.07[c]	1.39 ± 0.03[c]	1.52 ± 0.01[d]
800 mg/kg body weight	1.02 ± 0.03[c]	0.85 ± 0.06[c]	0.59 ± 0.01[d]	14.27 ± 0.03[b]	8.12 ± 0.07[d]	6.02 ± 0.08[d]	1.44 ± 0.02[d]	1.61 ± 0.04[d]	1.84 ± 0.03[d]

NOTE: The results are mean ± SD for five rats. Enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. Values carrying superscripts different from the control and for each organ are significantly different ($p < .05$).

Table 2. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver, kidney and serum acid phosphatase activity

	Liver			Kidney			Serum		
Doses/Days	1	14	28	1	14	28	1	14	28
Control	0.63 ± 0.02[a]	0.69 ± 0.02[a]	0.68 ± 0.02[a]	0.82 ± 0.02[a]	0.77 ± 0.02[a]	0.79 ± 0.02[a]	0.39 ± 0.02[a]	0.42 ± 0.07[a]	0.41 ± 0.02[a]
200 mg/kg body weight	0.85 ± 0.09[b]	1.47 ± 0.03[c]	1.91 ± 0.06[d]	1.42 ± 0.04[b]	1.73 ± 0.05[c]	2.25 ± 0.06[d]	0.45 ± 0.03[a]	0.47 ± 0.09[a]	0.38 ± 0.03[a]
400 mg/kg body weight	1.03 ± 0.01[b]	1.65 ± 0.05[c]	2.49 ± 0.08[d]	1.63 ± 0.03[b]	2.05 ± 0.07[d]	2.72 ± 0.02[e]	0.42 ± 0.01[a]	0.38 ± 0.02[a]	0.41 ± 0.08[a]
800 mg/kg body weight	1.45 ± 0.09[c]	1.89 ± 0.09[d]	2.58 ± 0.09[d]	1.81 ± 0.02[c]	2.43 ± 0.08[d]	3.04 ± 0.06[e]	0.44 ± 0.03[a]	0.46 ± 0.01[a]	0.46 ± 0.05[a]

NOTE: The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. Values carrying superscripts different from the control and for each organ are significantly different ($P < 0.05$).

Table 3. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver and kidney superoxide dismutase activity

	Liver			Kidney		
Doses/Days	1	14	28	1	14	28
Control	53.4 ± 1.2[a]	54.2 ± 2.3[a]	52.9 ± 1.5[a]	50.2 ± 0.7[a]	49.4 ± 1.3[a]	51.0 ± 0.2[a]
200 mg/kg body weight	44.2 ± 2.3[b]	36.0 ± 1.5[c]	27.9 ± 2.4[d]	48.1 ± 1.3[a]	42.6 ± 0.6[c]	37.4 ± 0.9[d]
400 mg/kg body weight	41.4 ± 1.7[b]	30.2 ± 1.7[c]	22.4 ± 1.8[d]	46.9 ± 2.1[b]	38.5 ± 0.4[d]	30.6 ± 0.3[e]
800 mg/kg body weight	38.6 ± 1.1[c]	24.2 ± 1.5[d]	16.7 ± 1.5[e]	42.4 ± 1.6[c]	34.3 ± 1.1[d]	25.1 ± 1.5[e]

NOTE: The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. Values carrying superscripts different from the control and for each organ are significantly different ($p < .05$).

Table 4. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver and kidney catalase activity

	Liver			Kidney		
Doses/Days	1	14	28	1	14	28
Control	35.2 ± 0.8[a]	37.1 ± 1.3[a]	35.8 ± 1.6[a]	32.8 ± 1.2[a]	33.1 ± 0.9[a]	33.3 ± 1.6[a]
200 mg/kg body weight	28.6 ± 2.4[b]	21.7 ± 1.8[c]	13.2 ± 2.1[e]	28.2 ± 2.5[b]	24.6 ± 1.7[c]	19.8 ± 1.4[d]
400 mg/kg body weight	25.6 ± 1.6[b]	18.2 ± 1.4[d]	11.6 ± 1.1[e]	26.2 ± 1.3[b]	21.6 ± 1.0[c]	17.3 ± 1.9[d]
800 mg/kg body weight	22.6 ± 2.5[c]	14.6 ± 1.3[e]	8.2 ± 1.7[e]	24.3 ± 1.8[c]	18.7 ± 1.3[b]	13.6 ± 1.2[d]

NOTE: The results are mean ± SD for five rats. Enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. Values carrying superscripts different from the control and for each organ are significantly different ($p < .05$).

Table 5. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver and kidney glutathione peroxidase activity

	Liver			Kidney		
Doses/Days	1	14	28	1	14	28
Control	56.3 ± 1.5[a]	55.8 ± 2.0[a]	56.1 ± 1.7[a]	52.7 ± 0.1[a]	52.0 ± 0.5[a]	51.8 ± 1.9[a]
200 mg/kg body weight	46.4 ± 3.2[b]	32.6 ± 1.7[c]	23.2 ± 1.5[d]	48.4 ± 3.2[b]	43.2 ± 1.8[b]	36.7 ± 0.6[d]
400 mg/kg body weight	40.1 ± 1.8[b]	29.9 ± 2.3[d]	20.1 ± 1.9[e]	45.2 ± 0.3[b]	40.3 ± 1.2[c]	32.4 ± 0.5[d]
800 mg/kg body weight	36.2 ± 2.5[c]	28.1 ± 1.6[d]	16.4 ± 1.1[e]	40.3 ± 1.1[c]	31.3 ± 0.7[d]	25.1 ± 1.9[e]

NOTE: The results are mean ± SD for five rats. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$ protein. Values carrying superscripts different from the control and for each organ are significantly different ($p < .05$).

Table 6. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver and kidney glutathione reductase activity

	Liver			Kidney		
Doses/Days	1	14	28	1	14	28
Control	2.21 ± 0.3[a]	2.15 ± 0.1[a]	2.43 ± 0.5[a]	1.50 ± 0.8[a]	1.73 ± 0.3[a]	1.58 ± 0.6[a]
200 mg/kg body weight	1.93 ± 0.5[b]	1.75 ± 0.2[c]	1.49 ± 0.6[d]	1.32 ± 0.8[a]	0.95 ± 0.5[b]	0.73 ± 0.2[c]
400 mg/kg body weight	1.86 ± 0.2[b]	1.63 ± 0.5[c]	1.43 ± 0.5[d]	1.09 ± 0.6[b]	0.82 ± 0.3[c]	0.56 ± 0.4[d]
800 mg/kg body weight	1.72 ± 0.1[c]	1.51 ± 0.3[d]	1.33 ± 0.3[e]	0.91 ± 0.2[b]	0.66 ± 0.1[d]	0.32 ± 0.1[d]

NOTE: The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol min^{-1} mg^{-1} protein. Values carrying superscripts different from the control and for each organ are significantly different ($p < .05$).

Discussion

The consumption of drugs (either synthetic or phytomedicine) may bring about significant changes in the structure, function and metabolic transformation of all classes of biomolecules, enzymes and metabolic pathways. These alterations that may be rapid or slow, may lead to different biochemical mechanisms producing similar pathological, clinical and laboratory findings. This study, thus, investigates the effect of aqueous extract of *S. longepedunculata* root on the redox homoestasis of rat liver and kidney with a view to providing an insight into the cellular mechanism of toxicity.

The measurement of enzyme activities in body fluid provides a significant and well-known aid in investigation and diagnosis[28] of assault on the organ/tissue, and to a reasonable extent on the toxicity of chemical compounds or drugs. Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by conventional histological techniques.[29] Enzymes from a damaged tissue or diseased tissue find their way into the serum by leakage,[30] this leakage depends on the proximity of the enzyme to the plasma membrane. Serum enzyme measurements are therefore valuable tools in clinical diagnosis, providing important

Table 7. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver non-enzymic antioxidant system

	Glutathione (nmol mg^{-1} protein)			Vitamin C (mg dL^{-1})			Vitamin E (mg dL^{-1})		
Doses/Days	1	14	28	1	14	28	1	14	28
Control	29.6 ± 0.4^{a}	29.1 ± 0.6^{a}	28.2 ± 0.1^{a}	55.2 ± 1.3^{a}	56.8 ± 0.5^{a}	56.0 ± 0.9^{a}	23.1 ± 1.2^{a}	23.9 ± 0.3^{a}	22.8 ± 2.2^{a}
200 mg/kg body weight	23.4 ± 1.2^{b}	19.2 ± 0.7^{b}	12.1 ± 1.7^{d}	49.1 ± 0.2^{b}	40.8 ± 0.3^{c}	35.9 ± 1.6^{d}	20.2 ± 1.8^{b}	15.6 ± 0.1^{c}	12.8 ± 0.6^{d}
400 mg/kg body weight	20.8 ± 0.8^{b}	17.9 ± 0.2^{c}	10.3 ± 1.2^{d}	43.1 ± 0.5^{c}	33.2 ± 1.1^{d}	27.0 ± 1.5^{e}	18.6 ± 2.1^{b}	12.9 ± 0.3^{d}	9.2 ± 0.3^{d}
800 mg/kg body weight	18.2 ± 2.1^{c}	14.8 ± 1.3^{d}	8.9 ± 0.5^{d}	38.1 ± 1.6^{c}	30.7 ± 0.2^{d}	22.9 ± 1.4^{e}	16.7 ± 0.3^{c}	10.1 ± 0.9^{d}	7.6 ± 1.3^{e}

NOTE: The results are mean $\pm$ SD for 5 rats. Values carrying superscripts different from the control and for each day are significantly different ($p < .05$).

Table 8. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the kidney non-enzymic antioxidant system

	Glutathione (nmol mg^{-1} protein)			Vitamin C (mg dL^{-1})			Vitamin E (mg dL^{-1})		
Doses/Days	1	14	28	1	14	28	1	14	28
Control	22.4 ± 0.2^{a}	21.8 ± 1.3^{a}	22.9 ± 0.6^{a}	46.7 ± 2.1^{a}	45.9 ± 0.2^{a}	46.0 ± 1.1^{a}	26.2 ± 2.3^{a}	27.3 ± 1.2^{a}	26.8 ± 0.3^{a}
200 mg/kg body weight	18.3 ± 2.3^{b}	15.6 ± 1.2^{b}	11.1 ± 0.3^{d}	41.3 ± 0.5^{b}	34.2 ± 0.6^{c}	26.7 ± 0.3^{d}	22.8 ± 0.1^{b}	17.2 ± 0.8^{c}	14.1 ± 1.2^{d}
400 mg/kg body weight	16.7 ± 0.1^{b}	13.8 ± 0.5^{c}	8.9 ± 0.3^{d}	40.2 ± 1.5^{c}	31.2 ± 0.7^{d}	22.3 ± 0.8^{e}	19.9 ± 0.6^{b}	15.1 ± 0.5^{d}	11.1 ± 0.7^{d}
800 mg/kg body weight	14.1 ± 0.3^{c}	10.2 ± 0.3^{d}	6.2 ± 0.2^{d}	37.6 ± 0.1^{c}	29.2 ± 0.7^{d}	20.1 ± 0.5^{e}	16.2 ± 0.2^{c}	12.4 ± 0.3^{d}	6.3 ± 0.9^{e}

NOTE: The results are mean $\pm$ SD for 5 rats. Values carrying superscripts different from the control and for each day are significantly different ($P < 0.05$).

Table 9. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the liver lipid peroxidised product

	Malonialdehyde			Lipid hydroperoxide		
Doses/Days	1	14	28	1	14	28
Control	389.3 ± 1.2^{a}	382.1 ± 1.9^{a}	379.2 ± 0.7^{a}	405.8 ± 1.7^{a}	411.7 ± 0.3^{a}	406.5 ± 2.2^{a}
200 mg/kg body weight	426.7 ± 0.3^{a}	593.2 ± 1.1^{c}	682.7 ± 1.1^{d}	481.3 ± 4.3^{b}	589.1 ± 2.9^{c}	723.4 ± 1.1^{e}
400 mg/kg body weight	478.2 ± 0.6^{b}	612.5 ± 0.7^{c}	716.5 ± 1.4^{d}	514.2 ± 0.9^{b}	608.3 ± 1.7^{c}	761.7 ± 3.9^{e}
800 mg/kg body weight	501.8 ± 1.2^{b}	667.9 ± 0.5^{d}	762.3 ± 0.9^{e}	564.2 ± 5.1^{c}	631.7 ± 1.8^{d}	792.3 ± 2.0^{e}

NOTE: The results are mean $\pm$ SD for 5 rats. MDA and lipid hydroperoxide concentrations are expressed as nmol mg^{-1} protein. Values carrying superscripts different from the control and for each day are significantly different ($p < .05$).

Table 10. Effect of administration of aqueous extract of *Securidaca longepedunculata* root on the kidney lipid peroxidised product

	Malonialdehyde			Lipid hydroperoxide		
Doses/Days	1	14	28	1	14	28
Control	372.1 ± 0.5[a]	377.6 ± 3.8[a]	377.9 ± 0.7[a]	383.6 ± 3.6[a]	380.7 ± 1.3[a]	386.2 ± 1.9[a]
200 mg/kg body weight	401.3 ± 1.1[a]	579.3 ± 3.5[c]	658.7 ± 0.1[d]	466.4 ± 0.5[b]	549.8 ± 1.6[c]	701.1 ± 2.5[d]
400 mg/kg body weight	457.9 ± 0.7[b]	595.6 ± 1.3[c]	696.3 ± 0.2[d]	491.3 ± 1.1[b]	572.7 ± 0.3[c]	742.5 ± 1.2[d]
800 mg/kg body weight	489.2 ± 0.9[b]	644.7 ± 2.2[d]	748.9 ± 1.3[e]	544.6 ± 2.3[c]	619.3 ± 0.8[c]	777.4 ± 1.7[d]

NOTE: The results are mean ± SD for 5 rats. MDA and lipid hydroperoxide concentrations are expressed as nmol mg^{-1} protein. Values carrying superscripts different from the control and for each day are significantly different ($p < .05$).

information on the extent and nature of pathological damage to any tissue.

ALP, an ectoenzyme of the plasma membrane,[31] is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum.[30] It is often used to assess the integrity of the plasma membrane,[32] such that any alteration in the activity of the enzyme in the tissue and serum would indicate likely damage to the external boundary of cells (plasma membrane). The reduction in both the liver and kidney ALP activities (Table 1) may be attributed to either loss of membrane components (including ALP) into the extracellular fluid, the serum,[33] inactivation of the enzyme molecule *in situ*[34] or inhibition of the enzyme activity at the cellular/molecular level.[16] It may also be due to a reduction in concentration or total absence of specific phospholipids required by this membrane-bound enzyme to express its full activity.[35] ALP has been reported to be associated with brush borders of the renal convoluted tubule; the loss in activity in the kidney may adversely affect the transfer of metabolites or required ions across the cell membrane, which may result in insufficient ions and metabolites for the renal cells.[32] A corresponding increase in the activity of the serum enzyme (Table 1), confirms that the integrity of the plasma membrane has been compromised. The loss of the enzyme from the tissues (liver and kidney) into the serum could be attributed to disruption of the ordered lipid-bilayer of the membrane structure, probably by peroxidation of the membrane polyunsaturated fatty acids.

Lysosomes are essential for controlled intracellular digestion of cellular components by autophagy, heterophagy and endocytosis.[36] A significant increase in ACP, a 'marker' enzyme of the lysosomal membrane, was observed in both the liver and kidney (Table 2). This could lead to indiscriminate hydrolysis of phosphate esters, which are potential energy source for the cell.[37] This implies a possible threat to the well-being of the organs as it may result in autolysis and consequently cell death.[38]

The mitochondria are the power house of the cell, and a proton gradient across their inner membrane drives the turbines that produce adenosine triphosphate (ATP), the fuel used by the machinery of the cell. There is evidence that if the proton gradient gets too high, superoxide is generated, which dismutates into hydrogen peroxide, and eventually to nontoxic molecules (H_2O and O_2) by catalase. It may also undergo Fenton reaction, producing hydroxyl radicals that lead to oxidative damage. Maintenance of normal cellular functions in the presence of oxygen largely depends on the efficiency of the defence mechanisms against free radical-mediated oxidative stress.[39] The enzymic antioxidant systems, which includes SOD, CAT, GPx, GR, as well as G6PD, plays a coordinated role in the prevention of oxidative damage by reactive oxygen species (ROS; O_2^- and H_2O_2). However, natural defences of the organism (enzymatic, nonenzymatic or dietary origin) are overwhelmed by excessive generation of ROS.

The administration of aqueous extract of *S. longepedunculata* root at all doses produced a significant ($p < .05$) decrease in the activity of SOD (Table 3) in the liver and kidney, throughout the experimental period. The reductions are indications of hepatocellular and nephrocellular toxicities by aqueous extract of *S. longepedunculata* root. This could result in gross accumulation of O_2^-, which can either initiate lipid peroxidation directly[40] or indirectly, through the product of its metabolism (singlet oxygen [$O^{\cdot}$] and hydroxy radical [$OH\cdot$]). O_2^- accumulation could also inhibit catalase,[41] exposing the cells and the cellular macromolecules to H_2O_2-mediated oxidative attack. The least reactive of the ROS, H_2O_2 diffuses throughout mitochondria and crosses cell membranes enabling it to inflict many types of cellular injury.[42] Upon entry into cytosol, it interacts with metal ions, such as iron or

copper, to form highly toxic OH·, which often cause DNA alteration.

It directly cause damage to the cell membrane by releasing arachidonic acid, which may be responsible for the prolonged damage in H_2O_2-treated cells even after H_2O_2 has been scavenged.[43] The reduction in the activity of catalase (Table 4) could have resulted from either accumulation of O_2^- or exhaustion of the enzyme. The consequential effect of this reduction include: lipid peroxidation of cellular macromolecules, DNA alteration and redox homeostasis perturbation.

H_2O_2 and hydroperoxides are reduced by GPx at the expense of GSH. Oxidized glutathione (GSSG) is reduced by GR in a nicotinamide adenine dinucleotide phosphate-oxidase (NADPH)-dependent reaction. The normal functioning of this closed system (redox cycle) maintains a high intracellular GSH:GSSG ratio and protects cells against oxidative damage.[44] Thus, a reduction in the activities of GPx and GR. (Tables 5 and 6) following treatment with aqueous extract of *S. longepedunculata* root could alter the normal redox cycle and expose the cellular macromolecules to oxidative attack by ROS.

Non-enzymic antioxidant system is available to complement the activity of the enzymic antioxidant system in excessive oxidative stress by acting as free radical (O·, O_2^-, OH· and H_2O_2) scavenger and modulating enzymes *in vivo*. Vitamins C and E play a significant role in the prevention of oxidative stress of cells,[45] as well as cellular macromolecules by acting as free radical scavengers. GSH functions by scavenging O·, O_2^- and OH·. It protects lipids, proteins, and nucleic acids from the attack of electrophilic compounds by blocking the electrophilic centre through its thiol group (–SH).[46] The levels of these molecules (GSH, vitamins C and E) in the liver and kidney of rats (Tables 7 and 8) decreased significantly following treatment with aqueous extract of *S. longepedunculata* root. Therefore, the reduction in GSH level could affect the GSH:GSSG ratio, and enhance oxidative attack of cellular macromolecules.

Oxidative stress is a critical event in the opening of mitochondrial membrane permeability transition pores and the breakdown of the mitochondrial membrane potential,[47,48] which leads to ATP depletion and cell death by oncotic necrosis.[49] The dose-dependent increase in MDA and LPO concentration of the liver and kidney of extract-treated animals (Tables 9 and 10) may be the consequence of enhanced lipid peroxidation possibly by the component of the extract. Peroxidation of lipids on the cell membranes of these organs may account for the depletion of antioxidant system (enzymic and non-enzymic) of the animals by aqueous extract of *S. longepedunculata* root.

Conclusion

The available data from this study revealed that aqueous extract of *S. longepedunculata* root is toxic to the animals; the mechanism may be through depletion of the antioxidant systems (enzymic and non-enzymic) and thus exposing the cells and cellular macromolecules to oxidative attack by ROS generated either from the metabolite of its constituents or other *in vivo* means.

Declaration of conflicting interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Yakubu MT, Akanji MA, Oladiji AT. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *Pharmacog Rev* 2007; 1: 39–46.
2. Pousset JL. Medicinal plants used as a source of raw material for the pharmaceutical industry, problems and prospects. In: *Drug production from natural products*. Proceedings of the Seventh International Symposium, DRPU, OAU, Ife, Nigeria, 1988, pp.37–41.
3. Winkleman M, Dobkin de Rios M. Psychoactive properties of kung bushmen medicinal plants. *J. Psychoactive Drugs* 1989; 21: 51–59.
4. Neuwinger HD. African ethnobotany, poisons and drugschemistry, pharmocology, toxicology London, UK: Chapman and Hall, 1996, pp.743–753.
5. Van Wyk BE, Gericke N. *A guide to useful plants of southern Africa*. Pretoria, South Africa: Briza Publications, 2000.
6. Ojewole JAO. Analgesic, anti-inflammatory and hypoglycaemic effects of *Securidaca longepedunculata* (Fresen.) [Polygalaceae] root-bark aqueous extract. *Inflammopharmacol* 2008; 15: 174–181.
7. Msonthi JD. The status of medicinal plant research and traditional medicine in malaria. In: A Sofowora (ed.) *The state of medicinal plants research in Nigeria*. Ibadan: University Press, 1986, pp. 335–349.
8. Akinniyi JA, Manawadu D, Sultanbawa M. Ethnobotany and ethnopharmacology of Nigerian medicinal plants research and traditional medicine in malaria.

In: A Sofowora (ed.) *State of medicinal plants research in Nigeria*, Ibadan: University Press, 1996, pp.35–349.
9. Wannang NN, Wudil AM, Dapar LMP, Bichi LA. Evaluation of anti-snake venom activity of the aqueous root extract of *Securidaca longepedunculata* in rats. *J Pharm Bio Res* 2005; 2: 80–83.
10. Ekpendu TO, Anyago P, Itywgh D, Akpan F. Niegrian ethnomedicine and medicinal plant flora; The Benue experience – Part 3. *Nig J Nat Prod Med* 2000; 4: 13–22.
11. Van Wyk BE, Van Quadshoom B, Gericke N. *Medicinal Plants of South Africa*. 1st ed. Pretoria, South Africa: Briza Publication, 1997, p.236.
12. Dapar LPM, Aguiyi CJ, Wannang NN, Gyang SS, Tanko MN. The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. *Afr J Biotech* 2007; 6: 591–595.
13. Etuk EU, Adebiyi RA, Elsa AT, Agaie BM. Acute and subchronic (28) oral toxicity studies of the aqueous root extract of *Securidaca longepedunculata* Fresen (Polygyalaceace) in mice. *Intl J Pharmacol* 2006; 2: 421–425.
14. NIH. Guide for the care and use of laboratory animals. *NIH Publication* 1985; 83: 127–138.
15. WHO. Basic OECD Principles of GLP. Geneva, Switzerland: World Health Organization. Available at: www.who.int/tdrold/publications/pdf/pr15/info.pdf (access August 2009).
16. Akanji MA, Ngaha EO. Effect of repeated administration of berenil on urinary excretion with corresponding tissue pattern in rats. *Pharmacol Toxicol* 1989; 64: 272–275.
17. Hillmann G. Acid phosphatase. *Z Klin Chem Klin Biochem* 1971; 9: 273.
18. German Society for Clinical Chemistry. Recommendations of the German society for clinical chemistry. *Z Kim Chem KIm Biochem* 1970; 8: 659.
19. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; 247: 3170–3175.
20. Beers RF Jr., Sizer IW. A spectrophotmetric method for measuring breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; 195: 133–140.
21. Rotruck JT, Pope AL, Ganther HF, Swason AB. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973; 179: 588–590.
22. Mavis RD, Stellwagen E. Purification and subunit structure of glutathione reductase from bakers' yeast. *J Biol Chem* 1968; 243: 809–814.
23. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70–77.
24. Omaye ST, Turbull TP, Sauberchich HC. Selected methods for determination of ascorbic acid in cells, tissues and fluids. *Methods Enzymol* 1979; 6: 3–11.
25. Desai ID. Vitamin E analysis method for animal tissues. *Methods Enzymol* 1984; 105: 138–143.
26. Gornall AC, Bardawill CJ, David MM. Determination of serum protein by means of biuret reaction. *J Biol Chem* 1949; 177: 751–756.
27. Buege JA, Aust SD. Microsomal lipid peroxidation. *Meth Enzymol* 1978; 52: 302–310.
28. Malomo SO. Toxicological implication of ciftriaxone administration in rats. *NJBMB* 2000; 15: 33–38.
29. Akanji MA. A comparative biochemical study of the interaction of some trypanosides with rat tissue cellular system. PhD Thesis, University of Ife, Ile-Ife, Nigeria, 1986.
30. Wills DE. Evaluation of lipid peroxidation of lipids and biological membranes. In K Snell, B Mullak (eds.) Biochemical toxicology: a pratical approach. Oxford: IRL. Press, 1985.
31. Shahjahan M, Sabitha KE, Mallika J, Shyamala-Devi CS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. *Indian J Med Res* 2004; 120: 194–198.
32. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of rat cellular system. *Toxicol* 1993; 81: 173–179.
33. Malbica JO, Hart LG. Effect of adenosine triphosphate (ATP) and some anti-inflammatory agents on purified lysosomal fraction having high acid phosphatase and labile glucuronidase activity. *Biochem Pharmacol* 1971; 20: 2017–2026.
34. Umezawa H, Hooper IR. *Amino-glycoside antibiotic*., Hadelberg, NY: Springer-Verlag Berlin, 1982.
35. Yakubu MT, Olatunji IK, Akanji MA. Comparative effects of administration of halofantrine hydrochloride and dihydroartemisinin on some enzymes of selected rat tissues. *NISEB J* 2002; 2: 175–180.
36. Anandakumar P, Kamaraj S, Jagan S, Ramakrishnan G, Devaki T. Lysosomal abnormalities during benzo(a)pyrene-induced experimental lung carcinogenesis – defensive role of capsaicin. *Fund Clin Pharmacol* 2009; 23: 97–103.
37. Butterwort PJ, Moss DW. The effect of urea on human kidney alkaline phosphatase. *Biochem J* 1966; 99: 9–10.
38. Akanji MA, Nafiu MO, Yakubu MT. Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. *Afr J Biomed Res*. 2008; 11: 87–95.

39. Sinha M, Manna P, Sil PC. Protective effect of arjunolic acid against arsenic-induced oxidative stress in mouse brain. *J Biochem Mol Toxicol* 2008; 22: 15–26.
40. Yen G, Duh P. Scavenging effect of methanolic extract of peanut hulls on free radical and active oxygen species. *J Agric Food Chem* 1994; 42: 629–632.
41. Kono Y, Fridovich I. Superoxide radicals inhibit catalase. *J Biol Chem* 1982; 257: 5751–5754.
42. Ray G, Husain SA. Oxidants, antioxidants and carcinogenesis. *Indian J Exp Biol* 2002; 40: 1213–1232.
43. Banmeyer I, Marchand C, Clippe A, Knoops B. Human mitochondrial peroxiredoxin 5 protects from mitochondrial DNA damages induced by hydrogen peroxide. *FEBS Lett* 2005; 579: 2327–2333.
44. Kozer E, Evans S, Bar J, et al. Glutathione, glutathione-dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol. *Br J Clin Pharmacol* 2003; 55: 234–240.
45. Pesh-Imam M, Recknagel RO. Lipid peroxidation and the concept of anti-oxygenic potential; Vitamin E change in acute experimental CCl4, and ethanol induced liver injury. *Toxicol Appl Pharmacol* 1977; 42: 463–475.
46. Ahluwalia P, Tewari K, Choudhary P. Studies on the effect of monosodium glutamate (MSG) on oxidative stress in erythrocytes of adult male mice. *Toxicol Lett* 1996; 84: 161–165.
47. Kon K, Kim JS, Jaeschke H, Lemasters JJ. Mitochondrial permeability transition in acetaminophen-induced necrotic and apoptotic cell death to cultured mouse hepatocytes. *Hepatol* 2004; 40: 1170–1179.
48. Reid AB, Kurten RC, McCullough SS, Brock RW, Hinson JA. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. *J Pharmacol Exp Ther* 2005; 312: 509–516.
49. Gujral JS, Knight TR, Farhood A, Bajt ML, Jaeschke H. Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? *Toxicol Sci* 2002; 67: 322–328.