Nephrotoxic and Hepatotoxic effects of Orally-administered Aqueous root bark extract of *Securidaca longepedunculata* (Polygalaceae) in female Wistar Rats.

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ABSTRACT

Background: *Securidaca longepedunculata* (Polygalaceae) is a savannah shrub commonly used by traditional medical practitioners in Nigeria. The plant is reported to have over one hundred medical indications.
Objective: This study was designed to investigate the hepatotoxic and nephrotoxic effect of oral administration of the aqueous extract of the root bark of *S. longepedunculata (SLE)* in female Wistar rats for 14 days.
Methods: Thirty (30) acclimatized, healthy, female rats weighing 110g - 126g were randomly divided into five groups. Animals in group 1 served as control and received only distilled water. Animals in group 2, 3, 4, 5 received 50, 75, 100, 125 mg/kg body weight of aqueous extract of the root bark of *S. longepedunculata* orally for 14 days respectively. Animals were anaesthetized on the 15th day after an overnight fast. Venous blood samples were analyzed for liver and kidney function parameters using standard protocols. Excised organs were used for the histological examination of the liver and kidneys.
Results: Phytochemical analysis of the plant extract revealed the presence of saponins, flavonoids, tannins, alkaloids and carbohydrates. The aqueous extract of the root bark of *S. longepedunculata* caused a significant ($P < 0.05$) increase in alkaline phosphatase activity, cholesterol and urea levels. Aqueous extract of the root bark of *S. longepedunculata* did not induce any marked pathological lesions in the liver and kidneys.
Conclusion: Oral administration of Aqueous root bark extract of *S. longepedunculata* did not have any marked adverse effect on kidney and liver functions in female rats.

Keywords: *S. Longepedunculata*, root extract, nephrotoxicity, hepatotoxicity, rats

INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs[1]. Estimates show about 80% of world population utilize plant as their primary source of medicinal agents[2]. Few plant species that provide medicinal herbs have been scientifically evaluated for their possible medical application. Both the general consumer and health-care professionals need up-to-date, authoritative information on the safety and efficacy of medicinal plants[1].
Securidaca longipedunculata is a semi-deciduous shrub or small tree that grows to 12 m tall, with an often flattened or slightly fluted bole. It is spiny and much branched, with an open, rather straggly looking crown[3]. It has different local names in different parts of Africa: Afrikaans (krinkhout); Amharic (es a manahi); Arabic (saggat, alali); Bemba (mupapi); English (violet tree, fibre tree, Rhodesian violet); Hausa (uwar magunguna, sanya); Nyanja (mwinda, mpuluka); Shona (mufufu); Swahili (muteya, mzigi); Tigrigna (shotora); Tongan (njefu, bwazi, mufufuma); Wol of (fouf); Yoruba (ipeta).
The plant parts especially the root is used in treating various medical ailments in Western and Southern Africa. They are used for eye complaints such as conjunctivitis, malaria, venereal diseases, urethral discharges, stomach problems, dysentery, rheumatism, fibrositis, toothache, headache, sleeping sickness, cough, chest complaints, snakebite, and wound dressing, and as an aphrodisiac, vermifuge and expectorant[3].
Medicinal plants contain several phytochemicals which have curative properties but could also be detrimental to the health. Such toxic effects manifest as changes in the structure and functions of organs as manifested by changes in biochemical parameters and tissue architecture.
The present study aims to give comprehensive information on its potential toxicity on the liver and kidney of female Wistar rats.

MATERIALS AND METHODS

Collection of plant material

Fresh *Securidaca longepedunculata* root bark was collected from Osogbo, Nigeria, identified and authenticated in the Department of Botany, University of Lagos by a taxonomist. A voucher specimen was deposited at the University of Lagos Herbarium with no LUH 3593.

Preparation of extract

The freshly collected plant material was rinsed under tap water and air dried under shade for 14 days and grinded. one kg of the powder material was soaked in distilled water for 48 hours at room temperature. The mixture was then filtered using a clean muslin cloth and then, Whatman No1. filter paper. The filtrate was then evaporated to dryness using a rotary evaporator attached to a vacuum pump and concentrated in vacuo using a lyophilizer. Freeze dried extract was stored at 2 to 8°C till further use.

Preparation of different concentrations
Different concentrations of crude extract (50, 75, 100 and 125 mg/kg b.wt) were prepared by dissolving the extract in sterile distilled water.

Phytochemical Analysis
The methods of Sofowora[4] were used for the qualitative determination of phytochemical constituents.

Acclimatization and Treatment of Animals
Thirty healthy, acclimatized, female Wistar rats weighing between 110 and 130 g were used for this study. Rats were divided into five groups prior to treatment. Group 1 served as a control and received only distilled water while groups 2, 3, 4 and 5 were orally administered 50, 75, 100 and 125 mg/kg bodyweight of extract respectively for 14 days. All animals were given access to rat feed and water *ad libitum* throughout the duration of the experiment.
Animals were fasted overnight on the 14th day. On the 15th day, animals were sacrificed under either anaesthesia and blood was collected into plain sample bottles via left ventricular cardiac puncture. The blood was centrifuged at 2,500 g for 10 minutes and supernatant carefully separated from blood cells using Pasteur pipette.

Biochemical Assay
The blood sera were used for the analyses of Biochemical parameters using the following methods: triglycerides[5,6], cholesterol[7], HDL-cholesterol[8], albumin[9], creatinine[10], Total Protein[11], Urea[12], aspartate aminotransferase[13], alanine aminotransferase[13] and alkaline phosphatase[14].

Histopathological Analysis
Histological tissue sections of the vital organs from each animal were fixed in 10% formaldehyde and processed for haematoxylin-eosin staining. Photomicrographs of the prepared slides haematoxylin-Eosin stained tissue sections were taken with a camera attached to the compound light microscope in the Department of Morbid Anatomy, College of Medicine, University of Lagos.

Statistical Analysis
SPSS version17.0 was used for data entry analysis. Results were expressed as Mean $\pm$ Standard error of mean. The difference between the test groups and control were compared using Student's t-test. P values $\leq$0.05 were considered significantly different

RESULTS

The result of the phytochemical screening of *S. longepedunculata* aqueous root bark extract revealed the presence of alkaloid, saponins, tannins, steroid and flavanoids. Reducing sugar, cyanogenic glycosides and phlobatannins were found to be absent.
The biochemical studies showed significant ($p < 0.05$) increases in serum urea levels only in animals receiving 75 mg/kg and 100 mg/kg bodyweight of SLE. There was no significant difference in creatinine levels in animals receiving varied doses of SLE compared to control. Cholesterol level at low dose of 50mg/kg significantly increased. There was no significant difference recorded in serum triacylglycerols levels of rats receiving varied doses of extract compared to control. Similarly, there was no significant difference in total protein levels of rats in all groups compared to the control. There was no significant difference in glucose levels in animals at all treatment levels compared to the control (Table 1).
There was a significant ($P<0.05$) increase in the activity of ALP in experimental rats receiving 125 mg/kg doses of extract. No significant difference was observed in the activities of AST and ALT in animals receiving varied doses of extract (Table 2). In addition, there was no significant difference in the level of albumin.
The histopathological features of the kidneys of the animals in the control and those treated with the extract showed normal histological characteristics (Figures 3 and 4). Results of histopathological studies do not reveal any gross damage to the liver in the experimental animals receiving varied dose of the aqueous extract of *Securidaca longepedunculata.*

Table 1
Effect of 14 days Oral Administration of Aqueous root bark extract of *Securidaca longepedunculata* on Kidney function parameters in female Wistar Rats.

Groups	Treatment (mmol/l)	TG (mmol/l)	Chol (mmol/l)	Glucose (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)
1	Distilled Water	1.36 + 0.22	1.58 + 0.12	13.60 + 2.83	7.03 +0.17	56.29 + 3.69
2	50 mg/kg b.wt SLE	1.59 + 0.39	2.29 + 0.12*	9.89 + 1.86	10.83 +1.70	44.35 + 7.00
3	75 mg/kg b.wt SLE	0.93 + 0.19	1.49 +0.12	7.37 + 0.07	10.56 + 0.81*	53.79 + 4.41
4	100 mg/kg b.wt SLE	1.11 + 0.16	1.28 + 0.12	7.88 +0.98	8.56 + 0.46*	50.69 + 5.90
5	125 mg/kg b.wt SLE	1.13 + 0.14	1.41+ 0.15	12.99+ 3.51	8.00 + 0.81	54.61+ 8.12

Values represent Mean $\pm$ SEM Total 5 rats and triplicate determinations - IG - triacylglycerols, chol - cholesterol
*$p<0.05$ significant difference between control and test group

Table 2
Effect of 14 days Oral Administration of Aqueous root bark extract of *Securidaca longepedunculata* on Liver function parameters in female Wistar Rats.

Group	Treatment	Total Prot (g/l)	Albumin (g/l)	ALT (U/L)	AST (U/L)	ALP (U/L)
1	Distilled water	75.66 + 1.76	50.00 + 1.52	18.66 + 3.52	228.00 + 64.00	6.66 + 1.20
2	50 mg/kg b.wt SLE	74.66 + 2.18	48.33 + 4.40	14.00 + 1.15	186.33 + 39.19	9.00 + 1.73
3	75 mg/kg b.wt SLE	82.66 + 5.36	49.00 + 2.64	13.33 + 3.52	166. 00 + 21.00	7.66 + 1.20
4	100 mg/kg b.wt SLE	71.66 + 3.48	38.66 + 7.35	20.00 + 2.30	352.66 + 14.52	6.66 + 1.20
5	125 mg/kg b.wt SLE	86.00 + 5.29	44.66 + 2.84	16.00 + 2.30	287.33 + 39.87	11.66 + 0.66*

Values represent Mean + SEM of 5 rats and triplicate determinations -

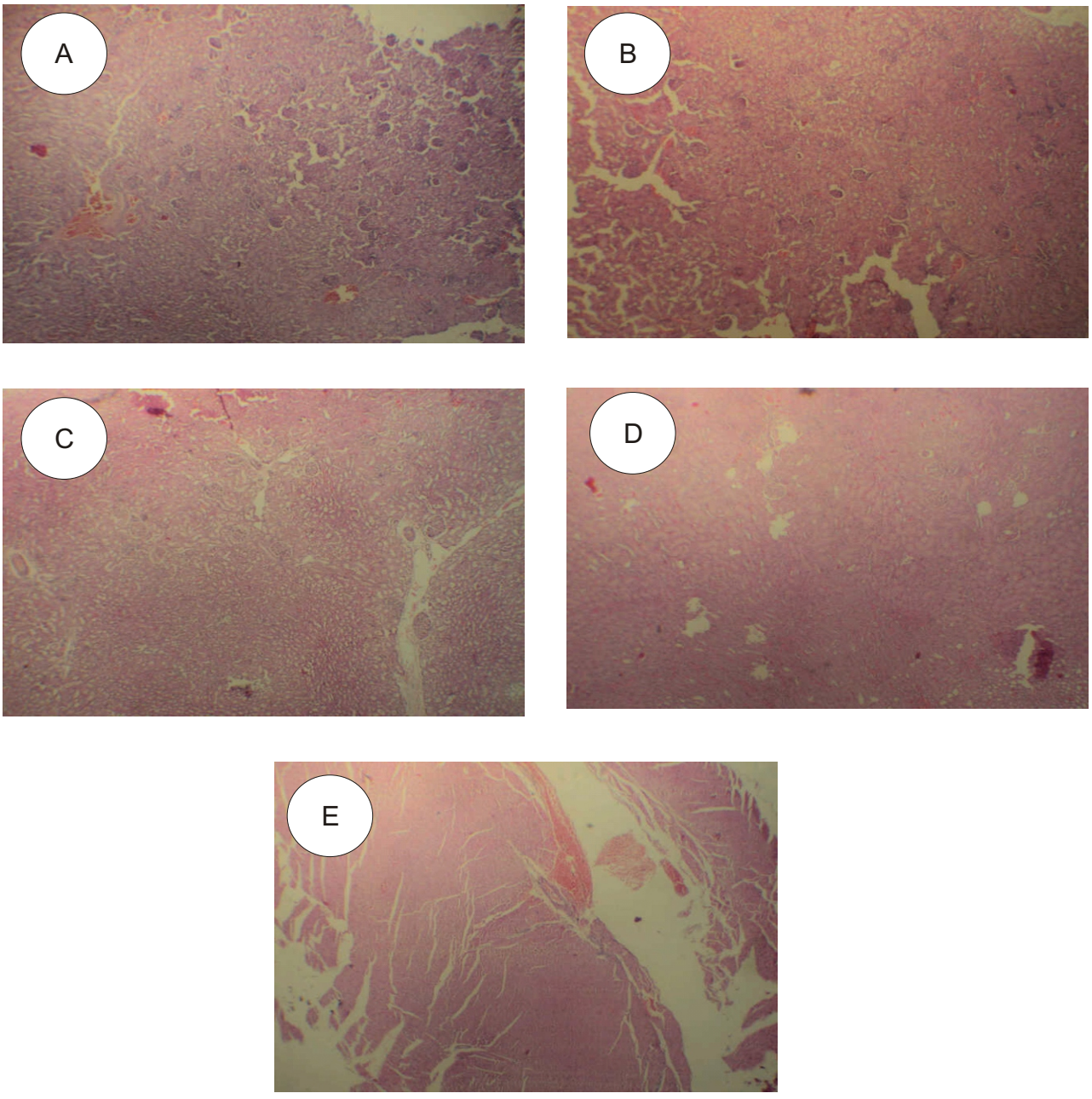

Figure 1. Photomicrographs of the kidneys of female Wistar rats administered doses of (A) Control (B) 50 (C) 75 (D) 100 and (E) 125mg/kg (X40)

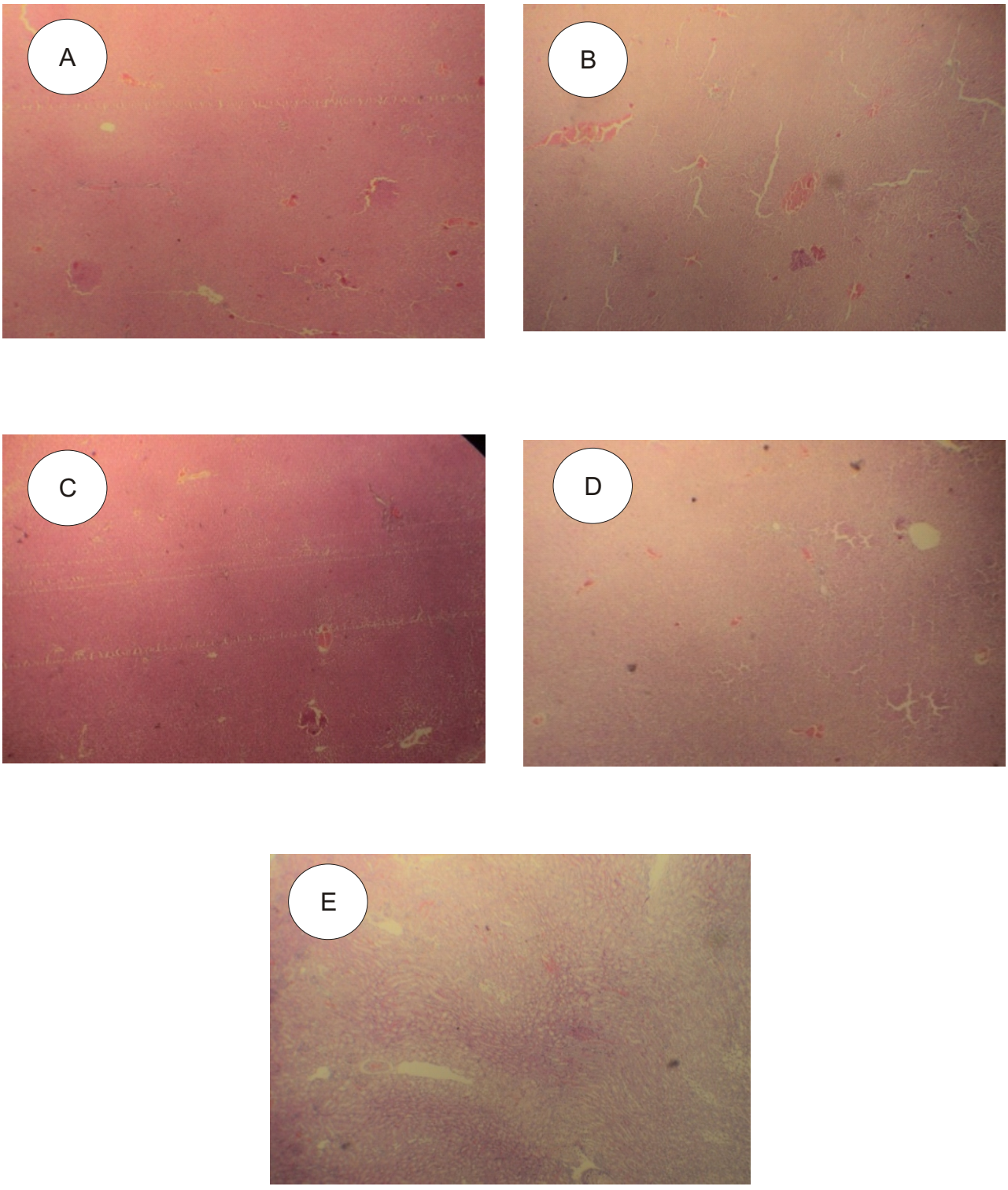

Fig. 2. Photomicrographs of the livers of female Wistar rats administered doses of (A) Control (B) 50 (C) 75 (D) 100 and (E) 125mg/kg (X40).

DISCUSSION

Phytochemical analysis showed the presence of steroids, alkaloids, flavonoids, saponins and tannins. The presence of these secondary metabolites confirms plant use for pharmaceutical manufacturing and drug discovery.

Plasma urea is a major end product of protein and amino acid catabolism. It is water soluble and easily excreted in the urine. An increased level of urea is an indication of azotaemia[15]. End products of protein metabolism are amino acids which are deaminated in the liver to produce ammonia. Ammonia does not enter the blood stream directly but is converted into urea by a series of reaction in the liver. Ammonia is toxic to the brain. Therefore the formation of urea and its excretion in the urine is the principal means by which the body is able to free its self of excess ammonia. The measurement of plasma urea is an essential analysis in the investigation of renal function. Causes of increased serum urea include pathological conditions such as acute renal failure. In patients with known renal dysfunction, plasma urea parallels plasma creatinine[16]. The increase in blood urea observed in animals administered 75 mg/kg and 100 mg/kg body weight of extract may be associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea[17].

Creatinine is a major catabolic product of the muscle and it is excreted in the kidney. Creatinine levels are used as indicator of renal failure[18]. As long as there is no increase in muscle mass, an increase in serum creatinine almost always reflects decrease in glomerular filtration rate. Adinarayana *et al*. [19] had reported that any condition that impairs the function of the kidney will probably raise the creatinine level in the blood. The reduction observed in the serum creatinine levels at all doses compared to the control (though not statistically significant) may indicate that glomerular filtration of the kidney is maintained. Serum creatinine alone is not a sensitive indicator of early renal failure because serum creatinine rises very little until fifty percent of nephrons are gone[16].

Cholesterol has been severally described as the major sterol in animals[16]. Cholesterol is synthesized by all cells of the body except the red blood cells. Large amounts of cholesterol are synthesized in the liver. Cholesterol is carried in the plasma or bloodstream as mainly LDL-cholesterol[20]. The cholesterol level in animals receiving 50 mg/kg body weight of the extract of *Securidaca longepedunculata* increased compared to the control. This increased blood levels of cholesterol are commonly associated with genetic disorders of lipid metabolism as well as secondary causes such as nephritic syndrome or chronic hepatitis as a result of drug use. It has been noted that high concentration of all lipid except HDL-cholesterol is associated with an increased risk of atherosclerosis.

Primary increased plasma triglyceride (i.e. primary hyper triglyceridaemia) may be seen in the adult male and female. Such on increase is associated with hypertension[15]. Secondary increased blood triglyceride (i.e. secondary hyper- triglyceridaemia) may be caused by renal disease and protenuria amongst several other factors[15]. However, there was no significant difference in triglyceride levels ($P>0.05$). Normal healthy adults are said to excrete 30 mg of protein per 24 hours. The proteins include albumin, immunoglobulins and globulins similar to those found in plasma or serum[15]. There was no statistical difference in the level of serum protein of rats administered 50, 75, 100 and 125 mg/kg body weight of the extract of *Securidaca longepedunculata* when compared to control. Increased protein level in the urine, (proteinuria) becomes significant when urinary excretion exceeds 150 mg in 24 hours[15]. The principal causes of proteinuria include mild glomerular disease and nephrosclerosis which are indications of gross kidney toxicity. There was no significant difference in the level of serum glucose in rats administered 50, 75, 100 and 125 mg/kg body weight of the extract.

Histological sections revealed kidney architecture were preserved.

The liver is the largest solid organ in the body; it is the center of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated or biodegraded in the liver. Enzymes synthesized in the liver cells include the Aspartate-aminotransferase, Alanine aminotransferase, alkaline phosphatase. Other proteins include albumin which is the most significant of the liver protein. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage[21, 22, 23, 24, 25, 26].

Drugs and toxins could cause hepatic cell damage. The damage to hepatocyte will lead to release of intracellular constituent into circulation. Serum enzyme measurement therefore, provides a valuable tool for clinical diagnosis of the liver damage as well as toxicity studies[27]. Result indicates that in experimental animals receiving 50-125mg/kg of aqueous extract of *Securidaca longepedunculata* root bark, the serum levels of AST and ALT were not significantly different from control.

Mounnissamy *et al*.[28] had reported that elevated activities of AST, ALT and ALP are indicative of hepatotoxicity. Dhanasekaran and Ganapathy[29] pointed out that AST and ALT may be elevated in other conditions such as the dysfunction of heart and skeletal muscle which also posses these marker enzymes.

The significant increase ($P < 0.05$) of Alkaline phophatase (ALP) in experimental animals receiving doses of aqueous extract of *Securidaca longepedunculata* at 125 mg/kg could imply cellular damage of the liver[27].

The level of albumin and total protein in the experimental animals receiving aqueous extract of *Securidaca longepedunculata* at all doses was not significantly different from control. These indicated that the secretory function of the liver was not impaired.

The presence of bio-active agents identified during phytochemical screening could also play a role in the selective toxicity observed[30]. The high amount of saponins in the extract may cause lysis of red blood cell by destroying the erythrocyte membrane[31, 32]. Saponin therefore could serve as an agent contributing to hepatotoxicity. Flavonoids are also found to have antioxidant properties[33].

CONCLUSION

Results of histopathology studies do not reveal any gross damage to both the liver and kidney in the experimental animals receiving varied doses of the aqueous extract of *Securidaca longepedunculata.* However, liver function indices indicate that the extract may not be completely safe for usage.

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