Original Research Article

Toxicity study of aqueous leaf extracts of *Sarcocephalus latifolius* (Rubiaceae) in rats

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Abstract

This study was designed to evaluate the acute and sub acute toxicity of the aqueous leaf extract of *Sarcocephalus latifolius*. The albino rats were orally administered with doses ranging from 500 to 2000 mg/kg body weight and observed continuously for the first 0h, then hourly for the next 12h and finally for 24h. Control animals received normal saline. The clinical signs of toxicity manifested in rubbing of nose and mouth on the floor of the cage, weaknesses and dizziness, loss of appetite and restlessness. To determine the toxicity characteristics of the medicinal plants such parameters as the lethal dose (LD50) as well as effects on the functions of vital body organs such as the liver and kidney were evaluated in the albino rats. For subacute toxicity, 4 groups of 4 rats of both sex each received normal saline (control), 250, 450, and 583 mg/kg of the extract respectively, once daily for 28 days. There was significant increase (p<0.05) in serum enzymes such as alanine transaminase, aspartate transaminase and alkaline phosphatase level against the control group. However, histological examination of tissue sections of liver and kidney revealed histopathological evidence of pathological lesions. The results of this study showed the toxicity characteristics of the aqueous leaf extract of *S. latifolius* for long time treatment at the doses used.

Keywords: *Sarcocephalus latifolius*, alanine transaminase, aspartate transaminase, alkaline phosphatase Acute/subacute toxicity, Histopathology, Rat.

INTRODUCTION

*Sarcocephalus latifolius* (*Nuclea latifolia*) belongs to the family Rubiaceae. It is locally known as Egbesi (Yoruba), *Tafashiya* or *tuwon bari* (Hausa), *Ubuluinu* in (Igbo), *mawyann* (Fali) language, in Nigeria and African Peach, in English. *Sarcocephalus latifolius* is a savannah tree or shrub up to 12m high, with a twisted bole up to 30 cm in diameter, a spreading open crown with a flexible entangled branches erect then dropping. The stem is cracked dark grey brown with fibrous reddish slash. It is multi-stemmed and has an open canopy flowers with terminal spherical head like cymes of small whitish flowers. The fruit is a syncarp, the individual fruits being fused together into a fleshy mass with characteristic pitted surface. The seeds are minute and embedded in a pinkish flesh with straw-berry scent (Michel, 2004) It is commonly found in Senegal, Cameroon, Nigeria and as far as Sudan, tropical and Southern Africa. It is scattered, common and locally abundant in lowlands of Sudan-Guinea and Guinea savannahs, on moist more or less well drained soil (Michel, 2004). Three other closely related species *Sarcocephalus pobeguini*, *Sarcocephalus diderichi* and *Sarcocephalus vandergushtii* are forest trees (Yesufu and Hussaini, 2014; Burkill, 1997).

Traditional healers throughout Africa use these species of Rubiaceae for many medicinal purposes.
These include treatment of tooth decay, jaundice, indigestion, hernia, Leaf, wounds, swellings, leprosy, syphilis, diabetes fever, malaria, constipation and kidney failure and diabetes. Studies have shown that the fruit is eaten as a cough remedy and the leaf is used by traditional healers to treat diabetes and as a cure for malaria fevers (Orwa et al., 2009). Akubue and Mittal, (1982) and Oye (1990) also reported that the medicinal applications of Sarcoccephalus latifolius are varied and numerous, for example the bark and the root extracts are said to be useful in malaria treatment. It is also used as a tonic and remedy for treating fever, toothaches, dental cures, septic mouth, and diarrhea and in dysentery treatment and also used as chewing stick (Etkin et al., 1990, Lamidi et al., 1995). The bark is said to be useful in the treatment of wounds, cough and gonorrhea in Nigeria (Madubunyi, 1995, Isah et al, 2012). The leaves are claimed to be useful in the treatment of fever while the roots and bark are claimed to be useful in the treatment of venereal disease and wounds (Pedro and Antonio, 1998). Medicinal plants though apparently believed to be non-toxic by most traditional healers plant products requires scientific tests, on different vital organs, because these natural products may contain some harmful ingredients in them as secondary metabolites (Nakamura and Yamamoto, 1982) which may have perilous side effects including mutagenic potentials, it is therefore, important to carry out toxicity studies of crude plants extracts to determine their safety use (Singh and Singh, 2012). Despite its long-time and varied uses, there has been little or no report on its toxicity. Therefore, this study was undertaken to determine the acute and sub acute toxicity of the aqueous leaf extract of Sarcoccephalus latifolius given orally to albino rats, with the hope of encouraging the use of this medicinal plant in primary health care. Sarcoccephalus latifolius is reported to have a wide range of medicinal properties and its medicinal uses vary from one traditional setting to another; common traditional uses include fever, pain, dental caries, septic mouth, malaria, hypertension, dysentery, diarrhea, and diseases of the central nervous system such as epilepsy (Amos et al., 2005; Ngo Bum et al., 2009; Abbah et al., 2010). The aqueous extract of leaves of the plant has been used as a remedy for diabetes in northern Nigeria (Gidado et al., 2005). The anticonvulsant, anxiolytic and sedative properties of S. latifolius roots decoction (Ngo Bum et al., 2009) have already been reported as well as the antihypertensive and laxative activities (Akpanabantu et al., 2005). Almost all the parts of Sarcoccephalus latifolius have been found medicinally useful with different parts linked to particular pharmacological activities. Products of herbal medicine are consumed as dietary supplements, being sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Owing to the diverse nature of plant components, some may complicate health problems or be the reason for a health challenge. There may also be a problem of interaction with other drugs leading to alteration of activities. These uncertainties culminate in toxicity associated with certain products. To the best of our knowledge no study was carried out in our areas that investigated the toxicity characteristics of this medicinal plant as it is used in herbal medicines for the management of the reported ailment. Therefore, this study was carried out in order to determine the toxicity characteristics of the aqueous leaf extract of this plant.

MATERIALS AND METHODS
Sample collection and preparation
Plant material
The leaf of Sarcoccephalus latifolius; family Rubiaceae was collected locally from Vimtim, in Mubi North local government area, Adamawa State, Nigeria in November, 2011. The plant was identified by Mr. Jarafu Ulam Mamza of the Department of Biological Sciences Adamawa State University, Mubi. A voucher specimen was deposited. The sample was shade dried in the laboratory and pulverized using wooden pestle and mortar. Finally, this was stored in an air-tight polythene bag and kept away from moisture until needed for analysis.

Preparation of aqueous extract
About 100 g of the powdered leaf was dissolved in 1000 ml of distilled water for 24 h on a hot plate. The mixture was sequentially filtered through a cheese cloth, cotton wool and Whatman filter paper no.1, respectively. The filtrate obtained was concentrated on water bath with a consistent heating. Concentration of the sample was done by evaporation in a water bath. The concentrate (extract) was stored in the fume hood and later used for administration to the experimental animals.

Experimental animals
Wister albino rats of either sex were obtained from the animal house of the National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria. Rats of both sexes (200g-250g) aged 5-6 months were used for the experiment. The animals were housed in iron cages kept in an adequate animal house environment and were acclimatized for two weeks before the commencement of the study. The animals were adequately fed with appropriate standard animal diet (standard chew) and had free access to water.
Acute toxicity studies (LD50)

The LD50 of the extract was determined as described by Yeo et al., (2012) with modifications. The number of deaths in each group within 24 h was recorded and the final LD50 values were calculated using the arithmetical method of Karber as modified by Aliu and Nwode (1982). A toxicity test on experimental animal was carried out with an initial test dose to determine the approximate lethal and non-lethal doses of the extract according to method of (Turner, 1965). Twelve (12) rats of either sex were divided into four groups of three (3) rats per group. The animals were divided into a control group and three treatment groups each group consisting of three rats. The control group (Group 1) received orally, normal saline while Groups 2 to 4 received a single dose of 500, 1500 and 2000 mg/kg body weight, respectively. The animals were observed for 24 h for signs of toxicity including death and the number of dead rats were recorded and used in the calculation of the acute toxicity value (LD50).

Subacute toxicity test

The animals were divided into four groups of four animals each. The treatments were given by oral intubation. Group (1) served as control and received normal saline, while Groups 2 to 4 received 250, 450 and 583mg/kg body weight, respectively. The animals were observed every daily for 28 days. The animals were observed every daily for behavioral changes, toxicity symptoms, signs of poisoning and mortality for a period of 28 days. All the animals were sacrificed according to their groups about 24 hours after the last administration (Enemor et al., 2013). The blood was drained from each animal by cardiac puncture with sterile syringes and needles and emptied into labeled centrifuge tubes for serum separation. They were left standing at room temperature for thirty minutes before centrifuging at 4000 rpm for five minutes in an 800 electric centrifuge (B – Bram Scientific and Instrument Co. England). The liver and kidney from both the treated and control groups was fixed in 10% formaldehyde for histopathological examinations.

Enzyme Assay of subacute toxicity

The serum separated was analysed spectrophotometrically by URINT-810 an automatic Chemistry Analyzer and Reflectron Plus Roche -5069865, the Chemical Kits (Randox Diagnostic kits) to evaluate the liver and kidney enzymes [Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP)], using the method of Pieme et al. (2006).

Histopathological examination of subacute toxicity

The method described by Biswas et al. (2010) was adopted with some modifications. The kidney and the liver from both the treated and control groups was processed with automatic tissue processor (STP 120) by tissue processing method as described by Galen and Gambino (1975). To perform histology of tissues 4 µm sections were prepared with the help of Microtome (Leica, RM 2145). These sections then deparaffinated in xylene, dehydrated through a degraded ethanol series, and stained with haematoxylin and cleared in xylene I and xylene II and these organs were preserved for microscopic examination.

The slides prepared by this process were observed under microscope (Model Nikon Labophot. 223425 Japan) and photographed through Nikon labophot Advanced Research Microscope, Model 223425 Japan, with Sony Digital 12.1 MEGA PIXELS.

Statistical analysis

The obtained results are presented as mean ± SD (standard deviation). All differences are considered significant at 5% level, therefore P-values less than 0.05 (P<0.05) were considered statistically significant at p<0.05 using Analyse-it version 2.3 statistical software for Microsoft Excel. Significant elemental concentration differences in plants samples were determined by analysis of variance (ANOVA).

RESULTS

The results obtained for the enzyme activities are shown in Table 1 and figures 1-3. The effects of the leaf extracts on the liver and kidney are also shown on figures 4-11.

Serum alkaline phosphates (ALP) ranged between 255.70 and 953.90 U/L but significant difference was observed between control group and any other dose group (Figure 1). Serum Alanine transaminase (ALT) levels in the S. latifoliuus treatment groups and the control ranged between 4.45 U/L and 9.15 U/L. In the plants extract treatment groups there was significant difference against control groups (Figure 2). Serum aspartate aminotransferase (AST) levels in the groups treated with S. latifoliuus ranged between 33.40 U/L and 57.00 U/L. In the plants extract treatment groups there was significant difference between control groups and any other treatment groups p<0.05 (Figure 3).

On administration of the extract, immediate behavioral changes were noted, which manifested in rubbing of nose and mouth on the floor of the cage, weaknesses and dizziness, loss of appetite, restlessness and deaths occurred as was observed in the two groups that received higher doses of
Table 1. Effects of the leaf extracts of *S. latifolius* of rats Serum enzyme activities (U/L).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Plant</th>
<th>Dose mg/kg</th>
<th>Mean ±SD</th>
<th>SE</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td><em>Sacrocephalus</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>latifolius</em></td>
<td>Control</td>
<td>637.93 ±332.610</td>
<td>192.033</td>
<td>255.70</td>
<td>861.50</td>
<td>796.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>798.12 ±69.086</td>
<td>39.887</td>
<td>741.40</td>
<td>875.06</td>
<td>777.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450</td>
<td>808.70 ±70.503</td>
<td>40.705</td>
<td>727.30</td>
<td>850.50</td>
<td>848.30</td>
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<td></td>
<td></td>
<td>583</td>
<td>864.00 ±143.505</td>
<td>82.853</td>
<td>698.50</td>
<td>953.90</td>
<td>939.60</td>
</tr>
<tr>
<td>ALT</td>
<td><em>Sacrocephalus</em></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td><em>latifolius</em></td>
<td>Control</td>
<td>5.46 ±1.031</td>
<td>0.595</td>
<td>4.45</td>
<td>6.51</td>
<td>5.41</td>
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<td></td>
<td></td>
<td>250</td>
<td>7.24 ±1.438</td>
<td>0.830</td>
<td>5.58</td>
<td>8.08</td>
<td>8.06</td>
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<tr>
<td></td>
<td></td>
<td>450</td>
<td>5.90 ±0.188</td>
<td>0.108</td>
<td>5.79</td>
<td>6.12</td>
<td>5.80</td>
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<tr>
<td></td>
<td></td>
<td>583</td>
<td>7.52 ±1.922</td>
<td>1.110</td>
<td>5.40</td>
<td>9.15</td>
<td>8.01</td>
</tr>
<tr>
<td>AST</td>
<td><em>Sacrocephalus</em></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td><em>latifolius</em></td>
<td>Control</td>
<td>36.50 ±2.961</td>
<td>1.710</td>
<td>33.40</td>
<td>39.30</td>
<td>36.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>43.47 ±7.207</td>
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<td>38.30</td>
<td>51.70</td>
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<td>450</td>
<td>43.20 ±8.412</td>
<td>4.857</td>
<td>35.90</td>
<td>52.40</td>
<td>41.30</td>
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<tr>
<td></td>
<td></td>
<td>583</td>
<td>52.57 ±4.008</td>
<td>2.314</td>
<td>49.20</td>
<td>57.00</td>
<td>51.50</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD (n=3); p<0.05. Significantly different from controls.

![Figure 1](image)

Figure 1. Effects of the extract of *S. latifolius* on liver enzymes level.

*Note: values represent the mean ± SD (n=3);* x p<0.05. Significantly different from controls.
Figure 2. Effects of the extract of *S. latifolius* on liver enzymes level. 
*Note: values represent the mean ± SD (n=3); x p<0.05. Significantly different from controls.*

Figure 3. Effects of the extract of *S. latifolius* on liver enzymes level. 
*Note: values represent the mean ± SD (n=3); x p<0.05. Significantly different from controls.*

the extracts 1000 mg/kg and 2000 mg/kg compared to the control group.

**Histopathology Examination**

Histopathological data on the liver and kidney of the rats are shown in Figures 4-11, respectively. No changes were observed in the liver and kidney of the rats in the control groups. However, structural changes were observed in the livers and kidneys following extract administration showing pathological lesion.

**DISCUSSION**

The four weeks oral administration of the extracts of *S. latifolius* had some significant effects on the rats’ vital
Figure 4. (40X) Section of liver of control rats sinusoids appeared normal.

Figure 5. (40X) Section of rat treated with 250mg/kg of water extract of S.latifolia showing, severe congestion of central vein with multiple hemorrhagic foci.

Figure 6. (40X) Section of rat treated with 450mg/kg of water extract of S.latifolia effects on Liver of rats after subacute toxicity showing, congestion and destruction of the sinusoids.

Figure 7. (40X) Section of rat treated with 583 mg/kg of water extract of S.latifolia effects on Liver of rats after subacute toxicity showing, severe congestion of the central vein and destruction of the sinusoids.

Figure 8. (40X) Section of control rat showing normal histological appearance of kidney.

Figure 9. (40X) Section of rat treated with 250mg/kg of water extract of S.latifolia effects on kidney of rats after subacute toxicity showing, degeneration of few glomeruli leading to congestion and lost of architecture.
Figure 10. (40X) Section of rat treated with 450mg/kg of water extract of S.latifolia effects on kidney of rats after subacute toxicity showing lost of glomeruli. Hemorrhages within corpuscles of glomeruli and congestion of vessels.

Figure 11. (40X) Section of rat treated with 583mg/kg of water extract of S.latifolia, effects on kidney of rats after subacute toxicity showing, congested vessels of glomeruli and few foci of hemorrhage.

organ as seen in the results obtained from this study. It was observed that the extract of S.latifolia is toxic as revealed by the activities of the marker enzymes and histopathological evidence. Though S.latifolia has been used by traditional medical practitioners without report of any mortality due to toxicity, but this studies has shown that oral treatment of over 500 mg/kg body weight of the extract causes death of the animals. This finding suggests that the extract is toxic considering the parameters under study. Rats treated with various doses of the extract (250, 450 and 583mg/kg) respectively showed significant decrease in body weights in relation to the control animals, indicating that S.latifolia have adverse effects on the body weight, which is used to assess the response to therapy of drugs and to indicate adverse effects of drugs (Schorderet, 1992; Teo et al., 2002). The changes in the liver in groups treated with 583 mg/kg of water extract is in agreement with the research conducted by Fafioye et al. (2004), who observed histopathological changes in the liver of fish treated with barks of p. biglobosa. The impact of the aqueous extracts S.latifolia on vital organ such as the liver and kidney was assessed through blood enzyme activity of Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Alkaline phosphates level is raised in any condition of biliary obstruction (Hashemi, et al., 2008). ALP is an enzyme in the cells lining of the biliary duct of the liver, osteoblasts of bone, cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. (Hashemi et al., 2008). ALT and AST are sensitive indicators of hepatocellular injury but they lack specificity as they are also present in cardiac and skeletal muscles, kidney and red blood cells (Thapa and Walia, 2007). Liver cell damage is characterized by a rise in plasma enzymes (Clementine and Tar, 2010); therefore S.latifolia induced hepatocellular changes. A rise in plasma alkaline phosphates (ALP) level is usually a characteristic finding in cholestatic liver disease (Kaneko, 1989; Builders et al., 2012)). The significant increase in ALP levels by the aqueous extracts of S.latifolia shows that possible cholestasis occurred at the dose levels tested (Orisakwe et al., 2003). ALP levels in serum will rise with large bile duct obstruction (Orisakwe et al., 2003). Serum AIP is related to the functions of hepatic cells. Increase in serum level of ALP is due to the increase in the synthesis of the enzymes in the presence of increasing biliary pressure (Ojo et al., 2006; Onyeylli et al., 1998). In the treatment groups there was an increase in AIP levels as compared to the control (Figure 1), which indicated some adverse effect on the liver and kidney of the animals. Choudhari and Deshmukh (2007) reported that alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic substance. In this study marked alteration were observed in the blood serum enzymes levels after treatment with aqueous leaf extracts Table 1. This could be indicator signs of toxicity (Ghasi et al., 2011). The extracts of S.latifolia alter the ALP, AST and ALT levels of the treated rats compared to the control. Under normal circumstances, these enzymes mostly reside within the cells of the liver and kidney. But when the liver or kidney is injured for any reason, these enzymes are spilled into the blood stream (Ghasi et al., 2011). The leaf extracts of the medicinal plant on oral administration showed significant increase (P<.05) in serum enzyme activity of AST, ALT and ALP against the control group (figures 1-3). Therefore, a significant increase in the levels of AST,
A desktop computer was observed comparable to the control groups (P<.05). These enzymes are useful biomarkers of liver and kidney injury (Ojo et al., 2006; Ghasi et al., 2011). AST and ALT elevation indicate liver cell necrosis. In liver necrosis membrane damage releases the enzyme into circulation, which can be measured in serum. Higher levels of AST and ALT indicate liver and kidney damage. The rise in the AST is usually accompanied by elevation in the levels of ALT, (Hoffbrand and Pettit, 1997, Dash et al., 2007). The levels of ALT and AST can be used to monitor possible adverse effect of drugs on the liver and kidney functions (Vijayalakshmi et al., 2000). The ALT was a reliable indicator of liver necrosis in small animals (Choudhari and Deshmukh, 2007). AST is an enzyme of the biliary tract, indicative of injury to the hepatobiliary system resulting in cholestasis, cholecystitis and hepatic necrosis (Dash et al., 2007). In this study marked increment of AST and ALT was observed after four weeks oral administration of the S.latifolius leaf extracts. The rise in the levels of AST and ALT was in a dose dependent manner of the extracts as revealed by the activities of the enzymes (figures 1-3). The increase in the levels of the enzymes were found to be dose dependent in the sense that the severity of damage are likely to increase as the dose administered increases. The levels ALT after administration of S. latifolius extracts, 250 (mg/kg) were 7.24±1.438 (U/L), 450 (mg/kg), 5.90±0.188 (U/L) and 583 mg/kg 7.52±1.922 (U/L). It was observed that there was significant increase (P<.05) in serum ALT against the control group. The probable reason for the increase of these enzymes in the liver serum and kidney is inhibition of these enzyme molecules, or damaged to the liver and kidney by the components of the plants extracts. Alanine aminotransferase is present in high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart (Crook, 2006). The serum or plasma levels of ALT become raised whenever there is liver cell damage (Vijayalakshmi et al., 2000).

The levels of AST after administration of S. latifolius at 250 (mg/kg) 43.47±7.207 (U/L) and 583 (mg/kg) 52.57±4.008 (U/L) increased significantly (P<.05) compared to the control group. AST is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes (Bumah et al., 2005). Damage to any of these tissues may increase plasma AST levels. Besides numerous causes such as pathological, various drugs can cause increases in AST levels. Generally, increase of these enzyme parameters by administered substances could cause liver and kidney damage (Vijayalakshmi et al., 2000).Related studies have reported changes in ALT, AST, ALP, and GGT (gamma glutamyl transferase) activities in animals treated with plant extracts (Nada et al., 1997; Udosen and Ojong, 1998; Bumah et al., 2005; Akpanabiantu et al., 2005; Lienou et al., 2007), for instance, they reported insignificant effect of Aspilia africana leaves extract however reported significant increases in ALP, ALT and AST. Therefore, normal values for these parameters after administration of extracts would mean absence of damage to the liver and kidney (Vijayalakshmi et al., 2000). The effect of the extracts on the liver and kidney has been confirmed further by the histopathological examination of the tissue sections of liver and kidney (figures 4-11).However, the confirmatory histopathological studies carried out in this study remain to elucidate further implications of the extracts administered. The histopathological evidence revealed in the pathological lesions showed destruction, lost of architecture and damages to the organs of Liver and Kidney tissues (Himri et al., 2005). The toxic irritant substances brought to the kidney by circulatory blood cause degenerative changes in the kidney tissues according to Varely (1987).

The histopathological lesion was similar to manifestations due to enzyme activities markers (Samuel et al., 2012). This was observed on Liver and Kidney tissue sections which showed spotty necrosis and destruction of sinusoids.

CONCLUSION

The results of this study showed structural changes in the kidney and liver of rats following oral administrations of S. latifolius leave extract. Furthermore, mortality was observed following oral administration. The increase in concentrations of enzymes in the serum is an indication of its toxicity potentials which was confirmed by pathological lesions. These extracts induced necrosis of cells in the portal and central vein of the liver and kidney showed lost of architecture, degeneration of glomeruli, destruction of sinusoids and hemorrhages, phenomenon that could compromise the medicinal use of this plant in traditional medicine. However, this study provides the basis for further study on the hematological, biochemical parameters assay and detailed toxicological and pharmacological studies on the leaf extract of S.latifolia and its active components to confirm this evidence. The study showed the aqueous extracts of S.latifolia is toxic.

REFERENCES


