Hepatoprotective effect of crude methanolic extract and fractions of Ring worm plant *Senna alata* (L. Roxb) leaves from Nigeria against carbon tetrachloride –induced hepatic damage in rats

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ABSTRACT

The methanolic extract and fractions (ethyl acetate and butanol) of *Senna alata*, a major medicinal plant used in traditional medicine in Nigeria were evaluated for possible hepatoprotective activity against carbon tetrachloride (*CCl*_4) – induced hepatotoxicity in wistar albino rats. Phytochemical screening indicates the presence of tannins, phlobatannins, alkaloids, anthraquinones, flavonoids, cardiac glycosides and saponins in the senna alata leaves. Administration of 2000mg/kg body weight of the crude methanolic extract did not produce any death in the rats within the observable period of 14 days. Treatment of rats with *CCl*_4-induced a significant decrease (p ≤ 0.05) in serum total protein and albumin and a significant increase (p ≤ 0.05) in total and direct bilirubin associated with a marked elevation in the activities of serum marker enzymes namely alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) when compared with the control group. Lipid peroxidation in *CCl*_4 – intoxicated rats was evidenced by a marked increase in the levels of thiobarbituric acid reactive substances (TBARS). However, simultaneous treatment of *CCl*_4 with methanolic extract and fractions (EtOAc and BuOH) significantly (p ≤ 0.05) restored total protein and albumin to near normal level while the activities of ALT, AST, ALP, total and direct bilirubin and liver TBARS were significantly decreased (p ≤ 0.05) as compared to *CCl*_4 – treated rats. The protective effects of methanolic extracts and fractions (EtOAc and BuOH) of leaves of *Senna alata* were further substantiated by histopathological examination of the liver hepatocytes. Our findings suggested that methanolic extract and fractions (EtOAc and BuOH) of leaves of *Senna alata* possess hepatoprotective effect against *CCl*_4 – induced liver damage in rats and the methanolic extract at 2000mg/kg body weight appeared to be safe and non toxic when administered orally.

Key words: *Senna alata*, extract and fractions, carbon tetrachloride, oxidative stress, hepatoprotective effect.
INTRODUCTION

The liver plays a crucial role in regulating various physiological processes in the body such as metabolism, secretion and storage. Liver diseases remain serious health problems and are caused among others by various chemicals, drugs, alcohol and toxins. Identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In the absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief (1). Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS).

Carbon tetrachloride (CCl₄) is a well known hepatotoxin and exposure to this chemical is known to induce oxidative stress and cause liver injury by the formation of free radicals (2). It has been found that metabolism of CCl₄ involves the production of free radicals through its activation by drug-metabolizing enzymes located in the endoplasmic reticulum (3).

_Senna alata_ (L.) Roxb) belongs to the Fabaceae family (subfamily Caesalpinioideae) and commonly known as candle bush, with reference to the shape of its inflorescences, or ringworm tree because of a traditional use (4). It is commonly referred to as “Asuwon oyinbo” by the Yoruba ethnic group in Southwestern Nigeria (5, 6). It is widely available in the tropics and has very important applications in folkloric medicine (7). In the northern part of Nigeria, particularly in Adamawa and Taraba States, the root, stem and leaves are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhea, gastrointestinal and upper respiratory tract infections (8). In Ghana and Ivory Coast, decoctions of the leaves and roots are used to treat diarrhea, dysentery and other gastrointestinal problems. The leaves are well known for their laxative property and due to the high content of chysophanic acid. The leaf extract is also used for skin diseases. In addition, leaves are also used for various diseases of the liver (9, 10). The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases (11). The decoction of the stem bark and roots are used to treat urinary tract infections, bronchitis and asthma (12).

In view of the numerous uses of the leaves, there is a need to conduct more studies on the plant. The primary objective of this investigation was to examine the phytochemical constituents, acute toxicity and hepatoprotective effect of the crude methanolic extract and fractions (EtOAc and BuOH) of leaves of _S. alata_ using carbon tetrachloride intoxicated rats as experimental model.

MATERIALS AND METHODS

Plant Materials:

Chemicals and reagents

All the chemicals and reagents used in the study were of analytical grade and they include : 2-thiobarbituric acid (TBA) (Sigma Chemical Co.), carbon tetrachloride (Fluka Chemica 87031), sodium lauryl sulphate (Sigma), Olive oil (Borges, Spain), Vitamin E (Sigma T3251), Ultra-Turrax T25 basic homogenizer, Spectra max plus -384 (Molecular devices, USA) spectrophotometer, Hitachi-902 -Roche- Japan auto analyzer.
Collection and Authentication of plant materials
The leaves of ringworm plant (S. alata) were collected from the Orchard near the Vice Chancellor’s Lodge, Delta Park, University of Port Harcourt, Port Harcourt, Rivers State Nigeria. The plant was identified by Mr. N.L Edwin-Wosu (a curator) in the Department of Plant Science and Biotechnology, University of Port Harcourt and Voucher specimens were deposited with the Herbarium in the Department.

Extraction of plant
The sun dried leaves of S. alata were pulverized into a fluffy mass. Five hundred grams of the powdered leaves were extracted with 8 litres of 80 % MeOH using Soxhlet extractor for 24 h. The extract was evaporated to dryness under reduced pressure (below 40 °C) using a rotary evaporator. The yield was 36 g (23.12% w/w) of powdered methanol extract, which was stored in the refrigerator for further use. This methanolic extract (MeE) was used for the toxicity test.

Phytochemical Screening
Freshly prepared crude methanolic extract of the leaves of S. alata was subjected to preliminary phytochemical screening for the detection of the presence of major chemical constituents (13).

Fractionation of the extract
The crude methanolic extract of S. alata was diluted with distilled water to 200 ml and further fractionated by successive solvent extraction with ethyl acetate (EtOAc) (2 x 100 ml) and n-butanol saturated with H2O (3 x 100 ml). Each extract was evaporated to dryness under reduced pressure to yield EtOAc fraction (4.28 g), butanol fraction (5.33 g) and remaining H2O fraction (5.86 g).

Experimental animals
Healthy male wistar albino rats weighing 120–180g from the Central Animal House of HEJ Research Institute of Chemistry, University of Karachi, Pakistan were used throughout the study. They were kept under standard environmental conditions at 25 °C with 12:12 h light–dark cycle in ventilated plastic cages. Animals were fed with a standard rat diet and water ad libitum. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and were approved by Institutional Animal Ethical Committee (IAEC) of University of Karachi.

Experimental Procedure:
Acute toxicity test
Healthy male wistar albino rats weighing 120-180g maintained under standard laboratory conditions were used for acute toxicity test according to Organisation for Economic Co-operation and Development (OECD) guidelines 425 (OECD guideline, 2000). A total of five animals were used which received a single oral-dose of 2000mg/kg body weight of crude methanolic extract (MeE) of S. alata. Animals were kept overnight fasting prior to drug administration of crude extracts by oral gavage. After administration of S. alata extract, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure),
autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted (14).

**Hepatoprotective Study**

Healthy and mature male Wister albino rats weighing 170-200g were equalized with respect to body weight and randomly divided into six groups of six animals each.

Group I (Control) received normal saline (5ml/kg, po) daily for 7 days. Group II received normal saline (5ml/kg, po) daily and CCl₄: olive oil (1:1 v/v) (1ml, ip) on alternate days for seven days. Group III was administered methanolic extract (400mg/kg) daily and CCl₄: olive oil (1:1 v/v) (1ml, ip) on alternate days for seven days. Group IV was administered EtOAc fraction (400mg/kg) daily and CCl₄: olive oil (1:1 v/v) (1ml, ip) on alternate days for seven days. Group V was administered BuOH fraction (400mg/kg po) daily and CCl₄: olive oil (1:1 v/v) (1ml, ip) on alternate days for seven days while Group VI was administered vitamin E (100mg/kg, po) daily and CCl₄: olive oil (1:1 v/v) (1ml, ip) on alternate days for 7 days.

The animals were sacrificed under light ether anaesthesia 24 h after the last treatment of CCl₄. On the 8th day, blood was collected by cardiac puncture, allowed to coagulate at 37°C for 30 min and serum separated by centrifugation at 2500 rpm for 10 min and stored at 4°C. The serum was used to estimate serum ALT, AST, ALP, total and direct bilirubin and total protein concentration using a Hitachi-902 -Roche- Japan Auto Analyzer. After collection of blood, the liver was immediately excised and rinsed in ice cold normal saline and divided into two parts. The first was kept at -20°C for TBARS analysis, while the second part was used for histopathological studies.

**Estimation of Thiobarbituric Acid Reactive Substances (TBARS):**

The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) were measured by the method of Ohkawa et al (15). The reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% (w/v) of liver homogenate. The mixture was brought to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged at 3000 rpm for 10 min. The organic layer was taken out and absorbance of the clear upper (n-butanol) layer was measured using Spectra Max Plus -384 (Molecular devices, USA) spectrophotometer at 532 nm. TBARS were quantified using an extinction coefficient of 1.56 x 10⁵ M⁻¹ cm⁻¹ and expressed as nmol of TBARS/mg tissue protein.

**Histopathological Study**

A portion of liver tissue of all animal groups were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and then processed for paraffin embedding. By using a microtome, sections of 5 µm thickness were taken, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin (16) and subjected to histopathological examination.
Statistical analysis
All the values are expressed as means ± standard error of mean (S.E.M). The results were analyzed statistically by one way Analysis of Variance (ANOVA) followed by the Turkey Multiple Comparison Test. Significance was accepted at a P-value of 0.05.

RESULTS

The results of the preliminary screening of *S. alata* showed the presence of secondary metabolites such as tannins, phlobatannins, alkaloids, anthraquinones, flavonoids, cardiac glycosides and saponins (Table 1). Observations from the results of acute toxicity studies showed no mortality or physical changes such as changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000mg/kg BW of crude methanolic extract (MeE) of *S. alata*.

Results in figure 1 show the effects of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* on the activities of liver marker enzymes ALT, AST and ALP. There was significant increase (p ≤ 0.05) in the activities of serum ALT, AST and ALP in the group treated with CCl₄ alone when compared with control. However, simultaneous administration of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* with CCl₄ significantly (p ≤ 0.05) reduced the activities of these enzymes when compared with the group treated with CCl₄ alone. Treatment of rats with CCl₄ alone significantly (p ≤ 0.05) increased serum total and direct bilirubin when compared with control. However, simultaneous administration of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* with CCl₄ significantly (p ≤ 0.05) reduced serum total and direct bilirubin in rats when compared with the group treated with CCl₄ alone (Table 2). Treatment of rats with CCl₄ alone significantly (p ≤ 0.05) reduced the total protein concentration in serum when compared with control (Table 2). However, group treated with CCl₄ with a simultaneous administration of 400mg/kg body weight of methanolic extract and fractions (EtOAc and BuOH) significantly (p ≤ 0.05) reversed the change. A significant decrease (p ≤ 0.05) in albumin concentration was observed in rats treated with CCl₄ alone when compared with control (Table 2). The change effected on albumin by the treatment with CCl₄ alone was significantly (p ≤ 0.05) reversed by the simultaneous administration of a fixed dose of 400mg/kg body weight methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* with CCl₄.

There was significant (p ≤ 0.05) increase in the levels of thiobarbituric acid reactive substances (TBARS) in rat group treated with CCl₄ alone when compared with control. However, the group treated with CCl₄ and simultaneously administered 400mg/kg body weight of methanolic extract and fractions (EtOAc and BuOH) significantly (p ≤ 0.05) reduced the TBARS level in the liver when compared with group treated with CCl₄ alone.

Histopathological examination of liver tissues of control group showed normal hepatic cells with well-preserved cytoplasm and prominent nucleus (Fig. 3a). Group treated with CCl₄ alone showed severe hepatic lesions characterized by necrosis of hepatocytes around the central vein, collapsed central vein with signs of necrosis such as karyolysis, karyorrhexis, pyknosis and
eosinophilia of the cytoplasm (Fig. 3b). Administration of a fixed dose of 400mg/kg BW of methanolic extract and fractions (EtOAc and BuOH) of leaves of S. alata followed by a simultaneous treatment with CCl₄ showed the appearance of normal hepatocytes and sinusoids with well defined central veins with no evidence of any granuloma or malignancy (Fig. 3c – e). Section of liver taken from group administered Vit. E showed a near normal architecture (Fig. 3f).

Table 1: Phytochemical screening

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Occurrence</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; ++ = Present in appreciable quantity

Figure 1: Effect of methanol extract and fractions of S. alata on various biochemical parameters in rats

GROUPS
Values are expressed as means ± S.E.M (n=6). Means with different superscripts (a-d) are significantly different at the 0.05 level

Me = methanolic extract, EtF = ethyl acetate fraction, BuF= butanol fraction, Vit.E = Vitamin E
Table 2: Effect of methanolic extract and fractions (EtOAc and BuOH) of S. alata on selected biochemical parameters in wistar albino rats

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Total bilirubin (mg/dL)</th>
<th>Direct bilirubin (mg/dL)</th>
<th>Serum Total Protein</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Albumin/Globulin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.38±0.05</td>
<td>0.12±0.01</td>
<td>6.39 ± 0.17</td>
<td>5.43 ± 0.12</td>
<td>3.11 ± 0.03</td>
<td>1.75 ± 0.03</td>
</tr>
<tr>
<td>CCl4 Only</td>
<td>1.09 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.11 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.61 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50 ± 0.05</td>
<td>0.75 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Me + CCl4</td>
<td>0.43 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.02 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.55 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EtF + CCl4</td>
<td>0.67 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.27 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.21 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.56 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.65 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.98 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BuF + CCl4</td>
<td>0.63 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.22 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.64 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.96 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.68 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.70 ± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vit. E + CCl4</td>
<td>0.32 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.28 ± 0.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.26 ± 0.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.02 ± 0.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.48 ± 0.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± S.E.M (n=6). Means in the same column with different superscript letters are significantly different at the 0.05 level

Me = methanolic extract, EtF = ethyl acetate fraction, BuF= butanol fraction, Vit.E = Vitamin E

Fig 2: Effect of Methanolic extract and fraction (EtOAc and BuOH) of leaves of S. alata on thiobarbituric acid reactive substances (TBARS)

Values are expressed as means ± S.E.M (n=6). Means with different superscripts (a-d) are significantly different at the 0.05 level

Me = methanolic extract, EtF = ethyl acetate fraction, BuF= butanol fraction, Vit.E = Vitamin E
Figure 3. Photomicrographs of Hematoxylin and Eosin-stained histological sections of rat liver. Control (3a); rats treated with CCl₄ alone (3b); rats administered 400mg/kg of MeOH fraction of S. alata and CCl₄ (3c); rats administered 400mg/kg of EtOAc fraction of S. alata and CCl₄ (3d); rats administered 400mg/kg BuOH fraction of S. alata and CCl₄ (3e); rats administered 100mg/kg of Vit E (3f).

DISCUSSION

Preliminary phytochemical study showed the presence of tannins, phlobatannins, alkaloids, anthraquinones, flavonoids, cardiac glycosides and saponins in the methanolic extract of leaves of S. alata. However, flavonoids (17, 18, 19), alkaloids (19), saponins (19, 20), glycosides (21) are reported to have hepatoprotective activity. Hence, the presence of secondary metabolites such as flavonoids, alkaloids, saponins and glycosides in the crude methanolic extract may be responsible for the observed antioxidant and hepatoprotective activity of leaves of S. alata.

The result of the acute toxicity test suggests that the crude extract of the plant was non-toxic to rats and hence one fifth of this dose i.e., 400 mg/kg, p.o. of crude Methanolic extract and fractions (EtOAc and BuOH) of S. alata was selected for in-vivo study.

In this study, hepatotoxicity was evidenced by a significant increase (p≤0.05) in the activities of serum marker enzymes namely serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum alkaline phosphatase (ALP), total and direct bilirubin in the group treated with CCl₄ only when compared with control. Increased levels of serum ALT, AST, ALP, total and direct bilirubin in plasma have been reported to be sensitive indicator in liver injury (22). This may be due to leakage from the cells through peroxidative damage of the membrane. However, the reduction in the levels of the marker enzymes in groups administered with methanolic extract and fractions (EtOAc and BuOH) is suggestive of regeneration process and repair of hepatic damage induced by CCl₄ (23, 24). Our findings corroborate the work of Thabrew et al. (25) who
reported that serum transaminases returned to normal activities with the healing of hepatic parenchyma and regeneration of hepatocytes.

The elevation in the levels of total and direct bilirubin observed in the group treated with CCl₄ alone may be attributed to hepatic dysfunction induced by CCl₄. Bilirubin is the main bile pigment that is formed from the breakdown of haeme in the red blood cells. It is transported to the liver from where it is secreted into the bile. The significant reduction (p≤0.05) in the level of serum total and direct bilirubin following treatment with CCl₄ and simultaneous administration of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* is suggestive of the hepatoprotective potential of the leaves in clearing bilirubin from the serum when it is elevated (26). The mechanism of action may be the activation of the constitutive androstane receptor (CAR), a key regulator in bilirubin clearance in the liver (27).

It is well established that CCl₄ administration causes a significant decrease in serum total protein and albumin levels (28). In the present investigation, treatment of rats with CCl₄ alone caused a significant decrease (p≤0.05) in the concentrations of serum total protein, albumin and albumin/globulin ratio. This is an indication that protein metabolism may have been adversely affected probably by inhibiting the synthesis of proteins such as albumin in the liver (29). However, the administration of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* at a fixed dose of 400mg/kg body weight significantly (p≤0.05) restored the serum protein levels close to control values. This observation may be due to increased protein synthesis. It has been reported that stimulation of protein synthesis has been advanced as a contributing hepatoprotective mechanism which accelerates regeneration of cells (30).

Estimation of thiobarbituric acid reactive substances (TBARS) is commonly used as index of lipid peroxidation. Elevated levels of TBARS observed in rats treated with CCl₄ only may indicate excessive formation of free radicals and activation of the lipid peroxidation system, resulting in hepatic damage. The significant decline in the concentration of these constituents in the liver homogenate of vitamin E and methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* administered rats indicates anti-lipid peroxidative effect of methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata*. Lipid peroxidation has been postulated as the mechanism of free radical- induced tissue injury and hence, free radical scavenging is established as the means by which antioxidants inhibit lipid peroxidation.

CCl₄-induced hepatic injuries are commonly used models for the screening of hepatic drugs and the extent of hepatic damage is assessed by the level of released cytoplasmic transaminases (ALT and AST) and alkaline phosphatase (ALP) in circulation (31, 32, 33). The hepatotoxic effects of CCl₄ depends largely on its reductive dehalogenation catalyzed by cytochrome P₄₅₀ in the hepatic endoplasmic reticulum, leading to the generation of an unstable complex of trichloromethyl peroxy radicals (•CCl₃). Trichloromethyl peroxy radical has been reported to be a highly reactive species (34, 35, 36). This free radical attacks membrane lipids, causing their peroxidation, and may also covalently bind to lipids and proteins, thereby initiating deleterious processes that ultimately lead to cell damage. Methanolic extract and fractions (EtOAc and BuOH) of leaves of *S. alata* may have protected the cell by impairing CCl₄-mediated lipid peroxidation, thus preventing the generation of free radical derivatives (37).
Our observation in the histological examination of hepatic tissues further validated the result of the biochemical studies. The near normal architecture of liver tissues of rats treated with CCl$_4$ and simultaneously administered 400mg/kg body weight of MeOH extract and fractions (EtOAc and BuOH) of $S$. alata is suggestive of the hepatoprotective nature of the leaves investigated. This result is comparable to the group administered with a known antioxidant Vit. E.

In conclusion, data obtained from this study suggest that methanolic extract and fractions (EtOAc and BuOH) of leaves of $S$. alata have significant antioxidant and hepatoprotective effects on CCl$_4$-induced hepatic damage in rats. These results however, indicate that the hepatoprotective effects of the leaves of $S$. alata on CCl$_4$-induced liver injury may be attributed to the antioxidant properties exhibited by their flavonoids, alkaloids, saponins and glycosides contents. A possible mechanism of hepatoprotective action of leaves of $S$. alata may be that the antioxidant effect impairs the activation of carbon tetrachloride into the reactive form. Further studies are underway to isolate and elucidate the active principle responsible for the hepatoprotective and antioxidant effects.

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