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Antioxidant and antidiabetic effects of aqueous extract of *Senna alata* on alloxan-induced diabetic rats

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Abstract

This study aimed to evaluate the antioxidant effect and the state of the liver following injury by alloxan monohydrate and treatment with an aqueous extract of *Senna alata* (SA) leaf. A total of 24 male Albino Wistar rats assigned into 4 groups (A-D) of 6 rats per group were used in this study. Forty-eight hours following administration of alloxan monohydrate and confirmation of diabetes mellitus in groups B-D rats, group C were treated with SA while group D rats were treated with glibenclamide. Rats in groups A and B received distilled water. The treatments were through oral administration, once daily for 21 consecutive days. On day 21 post-treatment, serum samples were collected for *in vivo* antioxidants and liver enzyme assays. The liver tissues were also collected for macroscopic examination and histomorphology. The *in vitro* antioxidant activity of SA was also determined using DPPH photometric model. The SA-treated rats recorded significantly reduced activities of ALT, ALP and total bilirubin values when compared to rats in the diabetic untreated group. There was also a significant increase in the activities of catalase, superoxide dismutase and glutathione in SA-treated rats compared to those of the untreated diabetic group. The study also recorded 67.33% *in vitro* antioxidant activity of SA at 400 µg/ml. Liver photomicrographs of rats treated with the extract were comparable to those of the normal control while diabetic untreated rats showed congestion of the central vein, degeneration and necrosis of hepatocytes. SA has both *in vitro* and *in vivo* antioxidant activity and protected the liver against damage by alloxan monohydrate.

Keywords: Alloxan monohydrate, Antidiabetic, Antioxidants activity, Histopathology, Oxidative stress markers

Introduction

The liver is the largest and very important organ in the body. Liver cells (hepatocytes) are involved in the biotransformation of endogenously and exogenously administered substances (Ramasamy, 2009). They are also responsible for detoxification of noxious substances, storage of some chemical substances and

immunological roles (Aba & Asuzu, 2016). These vital functions of the liver expose it to generation of various highly reactive oxygen species (ROS) which are injurious to it.

Studies have shown that diabetes mellitus (DM) which is an endogenous and metabolic disease, affects the liver (diabetic hepatopathy) (Messeri *et al*., 2012). Alloxan monohydrate has been the foremost substance used to induce experimental DM due to its selective destruction of insulin-producing pancreatic beta islets (Lenzen, 2008). Alloxan monohydrate induced hyperglycemia in experimental rats after 48 hours of its injection (Siddique *et al*., 1989). Insulin is critical in transporting glucose via the blood circulation to cells and tissues of various organs (Akuador *et al.,* 2014). Therefore, low production of insulin occasions hyperglycemia which leads to DM (Lenzen, 2008). Alloxan monohydrate is also known to generate ROS which affects certain organs including liver (Szkudelski, 2001; Cichoz-Lach & Michalak, 2014).

Antioxidants are substances which have the tendency to delay or inhibit unwarranted peroxidation and prevent oxidative damage to cells and molecules (Banafsheh & Sirous, 2016). Elevated levels of free radicals in the body have the ability to damage major biochemical components of cells and tissues. Several plant species and food stuffs have been shown to possess such antioxidants in form of bioactive compounds (Findik *et al*., 2011). Therefore, screening of plants to identify new potent antioxidants is necessary.

Senna alata (L.) Roxburgh is an ornamental tropical shrub belonging to the Leguminosae family. The plant is widely distributed in Africa, Australia and Asia and has many common names including Candle bush, Ringworm plant, Acapuco and Craw craw plant (Kumar *et al*., 2008). In Nigeria, it is commonly used in the treatment of several infections including skin diseases, waist and abdominal pains, constipation, convulsion and heart failure (Benjamin & Lamikanra, 2008; Igoli *et al*., 2013; Oladeji *et al*., 2020). Previous studies showed that *S. alata* possesses phytochemicals such as flavonoids, phenols, tannins, saponins, alkaloids, steroids, terpenoids and glycosides (Moriyama *et al*., 2003; Fatmawati & Abubakar, 2020). These phytochemicals exhibit various pharmacological and biochemical actions when ingested by man and animals (Usunobun & Okolie, 2016). For instance, flavonoids have been reported to possess hepatoprotective properties as a result of the well-known antioxidant activity, scavenging free radicals and rendering them inactive (Aiyegoro & Okoh, 2009; Omoregie *et al*., 2011; Sarker & Oba, 2020). Safety of *Senna alata* has been investigated and reports showed that oral administration of leaf extract of the plant produced no toxicity even at high doses in rats and mice, indicating the safety of the plant and support its use as an alternative system of medicine (Roy *et al*., 2016; Da Filkpiere *et al*., 2020). Previous studies have also shown that *S. alata* possess antibacterial and analgesic activities (Palanichamy & Nagarajan, 1990; Sugumar *et al*., 2016; Uwazie *et al*., 2020). However, reports on the antioxidant and hepatoprotective effects of the plant are limited. This study was therefore undertaken to examine the possible antioxidant and antidiabetic effects of *S. alata* on alloxan-induced diabetic rats.

Materials and Methods

Ethical approval

The study was carried out after obtaining Institutional Animal Ethical Committee's clearance dated 12-12-2021 (FVM-UNN-IACUC-2021-0334).

Experimental animals

Male Wistar rats (120-140g) used for this study were acclimatized for two weeks during which they were housed in a stainless wire mesh cage in a controlled environment (temperature 25 ± 2 ^{o}C; 12 h dark/light cycles) and fed with standard laboratory diet (Vital feed®, Vital feed LTD, Jos, Nigeria) *ad libitum*.

Reagents and chemicals

Chemicals and reagents used in this study were procured as follows: Alloxan monohydrate (Sigma Aldrich, UK); Glibenclamide (Hovid Hong Kong); Alanine Aminotransferase (ALT) activity assay kit (ab105135) (Biovison, Abcam company, Cambridge UK); Alkaline phosphatase (ALP) kit (ab83369) (Biovison, Abcam company, Cambridge UK); Bilirubin assay kit (Sigma Aldrich, UK); Superoxide dismutase (SOD) kit (Merk KGaA Darmstadt, Germany); Catalase (CAT) kit (Hardy diagnostics, Santa Maria, California); Malondialdehyde (MDA) kit (Eagle Biosciences, Armherst, New Harmshire, Germany), Glutathione (GSH) kit (Randox Laboratories, Crumlin, County Austrim, UK). All the chemicals used are of analytical grades.

Plant material and extract preparation

Fresh leaves of *S. alata* were collected during rainy season (September, 2021) from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka and were authenticated by a botanist from the same Department. The voucher specimen (*S. alata*:

INTERCEED/2852) was retained in the herbarium. The leaves of the plant were washed under tap water and shade-dried for 10 days. Thereafter, the leaves were pulverized into powder using an electric grinder (Ridgeyard, Ridgeyard Co, LTD, London, UK). One hundred grams of the powdered plant leaves were taken and soaked in 200 ml distilled water for 2 days with intermittent shaking after every two hours. Then the aqueous extract was obtained after filtration using No. 1 Whatman filter paper. The aqueous extract was lyophilized and stored in airtight container in a refrigerator at 4^{o}C until needed for study.

Percentage yield of the plant material was calculated using the formula;

% Yield = [(weight of dried extract) / (weight of dried plant material)] X 100

Experimental induction of diabetes mellitus

Diabetes mellitus was induced in overnight fasted Wistar rats by a single intraperitoneal injection of 160 mg/kg of alloxan monohydrate. Confirmation of DM was by determining the fasting blood glucose concentration using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek glucometer, Roche Diabetes care Inc. Swiss City, Switzerland) 48 hours after administration of alloxan monohydrate. Animals with fasting blood glucose levels above 126 mg/dl were considered diabetic and were selected for the study (ADA 2021).

Experimental protocol

Twenty-four rats were used for this study. After 48 hours of alloxan monohydrate administration and confirmation of fasting blood glucose level ≥126 mg/dl, the animals were sorted and treated as shown below;

Group A: Un-induced normoglycemic rats treated with 10 ml/kg distilled water (Normal control)

Group B: Alloxan-induced diabetic rats treated with 10 ml/kg distilled water (Negative control)

Group C: Alloxan-induced diabetic rats treated with 400 mg/kg aqueous extract of *S. alata*

Group D: Alloxan-induced diabetic rats treated with 2 mg/kg glibenclamide (Standard control)

The animals were treated daily for 21 days through the oral route. At the end of the study, 3 mls of blood samples were collected from 3 animals in each group, into lithium heparin coated bottles for serum biochemical determinations. The liver tissues were also freshly collected from 3 different animals per group and were fixed in 10% buffered neutral formalin for histopathology. The dosage of *S. alata* was chosen based on effects observed with its use in previous reports (Ramasamy *et al.,* 2009, Sugumar *et al.,* 2016, Uwazie *et al*., 2020).

Sample collections

Blood samples for sera used for the determination of serum biochemical markers of liver damages (ALT, ALP, total and direct bilirubin) and *in vivo* antioxidants (SOD, CAT, GSH and MDA) assays were collected through the medial canthus of the eye into lithium heparin coated sample bottles. The blood samples were centrifuged at 10,000 rpm for 10 min.

Determination of serum biochemical parameters

Determination of serum ALT activity was done according to Reitman-Frankel spectrophotometric method (Reitman & Frankel, 1957; Colville, 2002; Aba & Okorie-Kanu, 2017). The ALP activity was determined by phenolphthalein monophosphate method (Klein *et al*., 1960; Coville, 2002)**.** Conjugated and total bilirubins were analysed using the method of Doumas *et al.* (1971).

Determination of oxidative stress markers

Oxidative stress markers such as SOD, CAT, GSH and MDA were detected by the specified standard methods and statistically compared (Sinha, 1972; Rotruck *et al.,* 1973; Marklund & Marklund, 1974; Habig & Jakoby, 1978).

Gross examination and histopathology of liver tissues

After humane euthanasia of the rats under chloroform anaesthesia, the liver tissues were freshly harvested and washed with normal saline, examined grossly and the ratio of wet liver to body weights of each rats were calculated using the formula:

Wr = 100 g X [**W**organ g / **W**animal g]

Thereafter, the liver was excised for histopathological studies which were done according to the method of Drury *et al*. (1967).

Data analysis

Results were expressed as mean ± standard error of the mean (SEM). Data generated were analysed using SPSS version 23 One-way Analysis of Variance (ANOVA), followed by Duncan's multiple range post hoc test used to separate the variant means and $p < 0.05$ was accepted as significant.

Results

A yield of 10.5 g (5.25% w/w) was obtained from the aqueous leave extract of SA. There were significantly

Table 1: Effects of the aqueous leaf extract of *Senna alata* on liver function of alloxan-induced diabetic rats

Group	ALT (Iμ/L)	ALP (Iμ/L)	D.BIL (mg/dl)	T.BIL (mg/dl)
A	28.35 ± 0.83^{a}	58.63 ± 0.71^{a}	1.36 ± 0.02^{c}	1.64 ± 0.03^{b}
B	47.70 ± 1.58^{c}	72.29 ± 0.62^{c}	0.86 ± 0.03^{a}	2.45 ± 0.08^{c}
C	36.43 ± 0.42^{b}	63.43 ± 0.54^{b}	1.06 ± 0.03^{b}	1.25 ± 0.07^{a}
D	31.33 ± 0.54^{ab}	61.57 ± 0.89^{b}	1.10 ± 0.04^{b}	1.61 ± 0.05^{b}

Superscripts a, b and c indicate significant difference at $p < 0.05$ down the columns (across the groups).
ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; D. BIL: Direct Bilirubin; T. BIL: Total Bilirubin

Table 2: Effects of aqueous leaf extract of *Senna alata* on average live weight of treated animals

Groups	Liver weight / 100 g body
A	2.02 ± 0.03^{a}
B	3.94 ± 0.04^{b}
C	2.12 ± 0.02^{a}
D	2.15 ± 0.01^{a}

Superscripts a and b indicate significant differences at $p < 0.05$ down the column (across the groups)

($p < 0.05$) higher activities of ALT and ALP in rats of diabetic untreated group (Group B) compared with that of the normal control rats (Group A) and the rats of Group C treated with the extract. The direct bilirubin values were significantly ($p < 0.05$) higher in the treated groups compared to the negative control group (Table 1).

The result of the effects of aqueous leaf extract of *Senna alata* on wet liver weight indicated significantly ($p < 0.05$) higher wet liver weight in rats of diabetic untreated group compared to rats of groups treated with glibenclamide and the aqueous extract of *S. alata* which compared favourably with rats of the control group (Table 2).

Result of *in vitro* antioxidant activities using DPPH model showed that the antioxidant activities of aqueous extract of *S. alata* was linear in increasing concentration-dependent manner. The antioxidant activity at 400 µg/ml recorded was 67.33% while ascorbic acid (the standard reference) recorded an antioxidant activity of 93.30% (Figure 1).

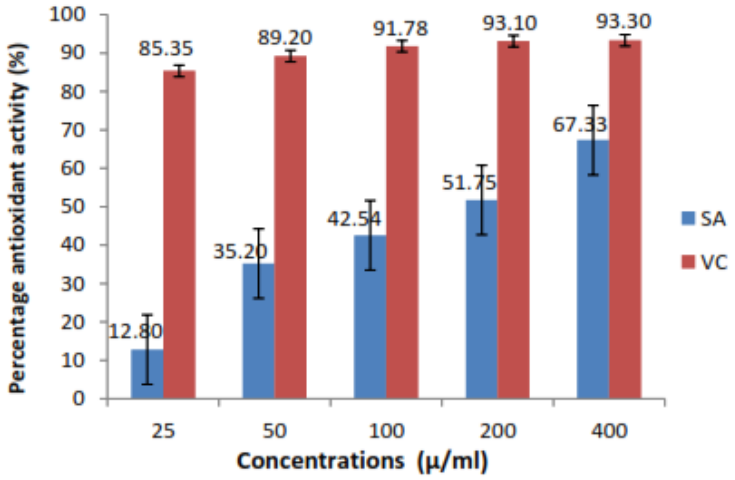

Figure 1: The *in vitro* antioxidant activity of *Senna alata* using DPPH model
SA: *Senna alata*
AA: Ascorbic acid

The results of the effects of aqueous leaf extract of *S. alata* on oxidative stress markers indicated significant ($p < 0.05$) increase in the activities of SOD, catalase and GSH in rats treated with glibenclamide and the extract, compared with rats of diabetic untreated group. The result also recorded significant ($p < 0.05$) reductions in MDA values in the rats treated with glibenclamide and the extract compared to rats of diabetic untreated group (Table 3). Macroscopic observation of the liver showed that the liver was enlarged in diabetic untreated rats but appeared normal in the normal control and treated groups. There was also a significant higher wet liver to body weight ratio in diabetic untreated group compared to treated groups.

The liver photomicrograph of Group B rats showed congestion of the central vein. The hepatocytes of the glibenclamide-treated rats (Group D) and those treated with aqueous extract of *S. alata* (Group C) appeared normal and comparable to that of the normal control rats (Plate I).

Table 3: Effects of the aqueous leaf extract of *Senna alata* on oxidative stress markers of alloxan-induced diabetic rats

Group	Catalase (μ/m)	SOD (μ/m)	MDA (mg/dl)	GSH (mg/dl)
A	7.58 ± 0.24^{c}	0.09 ± 0.01^{b}	0.25 ± 0.01^{a}	0.19 ± 0.01^{c}
B	4.00 ± 0.12^{a}	0.04 ± 0.01^{a}	1.44 ± 0.03^{d}	0.05 ± 0.01^{a}
C	6.15 ± 0.19^{b}	0.08 ± 0.01^{b}	0.95 ± 0.01^{c}	0.13 ± 0.01^{b}
D	7.40 ± 0.14^{c}	0.08 ± 0.01^{b}	0.50 ± 0.01^{b}	0.12 ± 0.01^{ab}

Superscripts a, b, c and d indicate significant difference at $p < 0.05$ down the columns (across the groups).
SOD: Superoxide dismutase; MDA: Malondialdehyde; GSH: Reduced glutathione

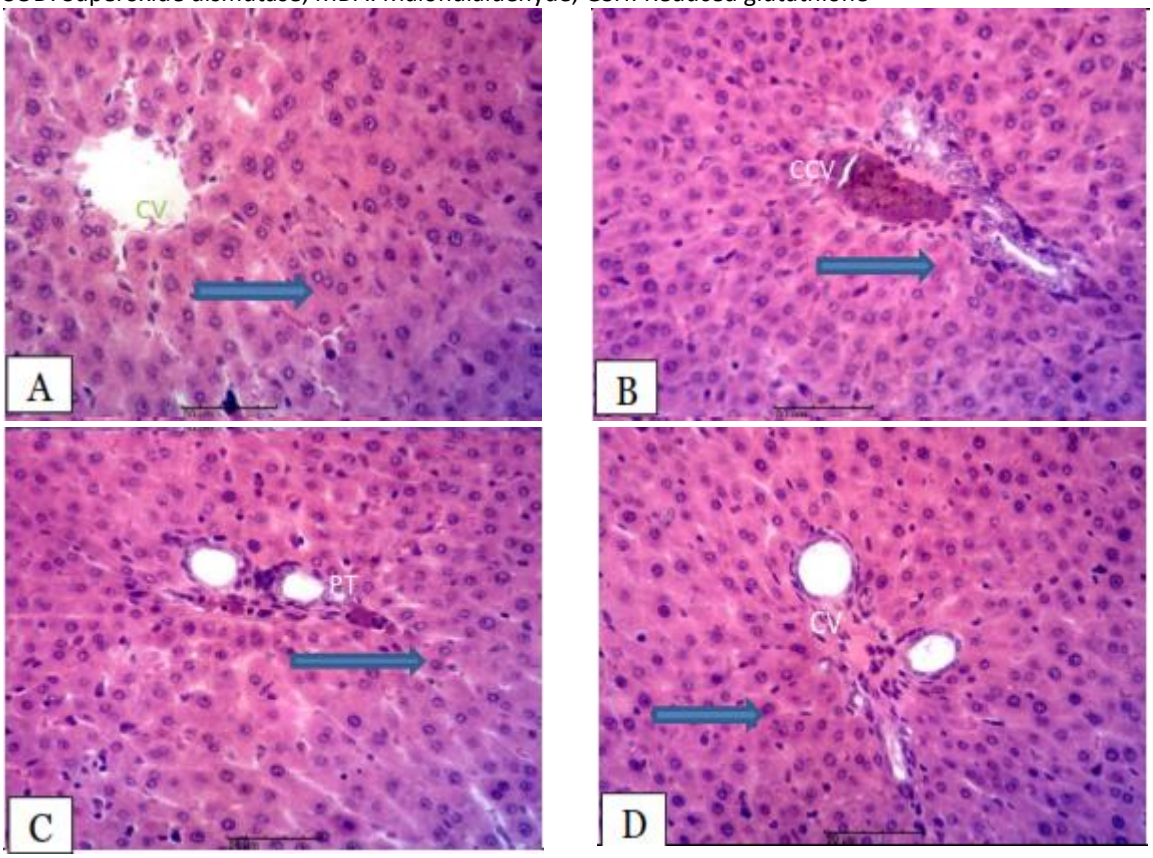

Plate I: A –Photomicrograph of the liver of group A rats (Un-induced + 10 ml/kg distilled water) showing central vein (CV) and liver cells in cords (arrows); B –Photomicrograph of the liver of group B rats (Diabetic + 10 ml/kg distilled water) showing congested central vein (CCV) (arrows); C -Photomicrograph of the liver of group C rats (Diabetic + 400 mg/kg of *Senna alata* extract) showing the portal triad (PT) and liver cells in cords (arrows); D –Photomicrograph of the liver of group D rats (Diabetic + 2 mg/kg Glibenclamide) showing the central vein and liver cells in cords (arrows). H&E X400

Discussion

The serum biochemical parameters showed that ALT and ALP activities were significantly higher in the diabetic untreated rats when compared to treated groups, indicating impaired liver function and the ameliorative effect of *S. alata* extract, respectively. This observation is consistent with the work of other researchers (Akah *et al.*, 2009; Soliman, 2016), who reported that elevated activities of serum ALT are common signs of liver diseases which are observed more often in diabetic condition. The increase in serum activities of these enzymes indicates that alloxan-induced DM produced alterations in hepatic function, which consequently results in leakage of these hepatic enzymes from the liver cytosol into the bloodstream. Therefore, restoration of these biomarker enzymes towards normal levels in treated groups signifies decreased diabetic complications.

This finding also supports the work of Sugumar *et al*. (2016) who reported that *S. alata* has hepatoprotective activity.

Rats in treated groups recorded significantly lower values of total bilirubin when compared to rats of the negative control group. Breakdown of heme in red blood cells leads to formation of bilirubin (Nelson and Cox, 2000). Heme is transported to the liver where it is conjugated before its secretion into the bile (Nelson & Cox, 2000). Impairment of the liver function was justified by the significant increase in the level of total bilirubin in the serum of the diabetic untreated group when compared with control and extract-treated groups. Defects in the liver often lead to its inability to conjugate bilirubin which results in an increased level of total bilirubin in the blood (Fevery, 2008). Therefore, the extract is able to significantly reduce the level of total bilirubin compared with the diabetic untreated group indicating the hepatoprotective effect of *S. alata*.

Macroscopic observation of the liver showed that the liver was enlarged in alloxan-induced diabetic untreated rats but was normal in the normal control and treated groups. This finding was corroborated by a significantly higher wet weight of the liver in the alloxan-induced diabetic untreated group compared to treated groups. Thus, this also indicates the hepatoprotective ability of *S. alata* extract. This finding is in agreement with the work of Ramasamy *et al*. (2009) who reported the hepatoprotective effect of the dried leaf infusion of the plant in paracetamol-induced hepatotoxic rats.

The present study recorded the antioxidant activity of *S. alata* at 400 µg/ml on DPPH to be 67.33% while ascorbic acid that served as the reference showed 93.30% scavenging activity at the same concentration. The scavenging ability of DPPH free radical is widely used to analyse the antioxidant potential of naturally derived plants, as plants act as electron donors because of their content of phenolic compounds (Duthie & Dubson, 1999). Antioxidants are known to mop up free radicals or ROS (Devasagayam *et al*., 2004). This may justify the DPPH radical scavenging power noted in the extract tested. The antioxidant activities of the aqueous extract and ascorbic acid were linear in increasing concentration-dependent manner with highest activities at 400 µg/ml concentration. This result corroborates with previous study which demonstrated that DPPH scavenging properties of plant extracts increase with concentrations of extract (Gul *et al.,* 2013; Kumar *et al*., 2014).

The results of the *in vivo* antioxidant activities recorded significant increases in the SOD, CAT and GSH levels in rats of the treated groups compared to those of the alloxan-induced diabetic untreated group. This is a pointer to *in vivo* antioxidant potentials of *S. alata* extract. Catalase is an *in vivo* antioxidant frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide to less reactive gaseous oxygen and water molecules (Gaetani *et al*., 1996). Catalase deficiency has been reported to increase the likelihood of diabetic conditions (Aba & Asuzu, 2018). Superoxide dismutase is also an *in vivo* antioxidant that catalyses the reduction of superoxide anions to hydrogen peroxide and thereby plays a critical role in cell defence against the damaging effects of free radicals (McCord *et al*., 1976). Therefore, SOD is an important antioxidant defence in nearly all cells exposed to oxygen (Borgstahl *et al*., 1996). Reduced glutathione is an antioxidant capable of preventing damage to important cellular components caused by ROS. Reduced glutathione is found in high concentrations in cellular systems and plays a major role in the detoxification of various electrophilic compounds. Glutathione also regenerates important antioxidants such as vitamins C and E (Jeanne & Drisko, 2018). Therefore, a deficiency of GSH puts the cell at risk of oxidative damage. Malondialdehyde on the other hand, is the major reactive aldehyde resulting from the peroxidation of polyunsaturated fatty acids constituent of biological membranes and is a by-product of prostaglandin synthesis (Vaca *et al*., 1998). Free radicals generate the lipid peroxidation process in an organism. An increase in free radicals causes the overproduction of MDA, therefore, it serves as a marker of oxidative stress (Del Rio *et al*., 2005). Rats in the alloxan-induced diabetic untreated group had the highest level of MDA compared to those of treated and normal control groups which further validate the antioxidant ability of the plant extract.

The photomicrographs of the liver of the alloxan-induced untreated rats present lesions of congestion of the central vein, degeneration and necrosis of the hepatocytes. This implies that alloxan monohydrate is capable of causing degenerative and necrotic injuries to the liver. The finding is in tandem with the submissions of earlier researchers (El –Demardash *et al*., 2005; Onyema *et al*., 2006). The observations of reduced and or absence of lesions in the liver photomicrographs of the alloxan-induced and treated groups also showed that the aqueous extract of *S. alata* mitigated hepatic injuries occasioned by alloxan monohydrate administration.

In conclusion, the aqueous extract of *S. alata* has both *in vitro* and *in vivo* antioxidant activity and ameliorated hepatic injuries occasioned by the administration of alloxan monohydrate in Wistar rats.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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