Journal of Diabetes & Metabolic Disorders (2022) 21:669–680
https://doi.org/10.1007/s40200-022-01029-9

RESEARCH ARTICLE

Synclisia scabrida protects against oxidative stress, hepatotoxicity and hyperglycaemia in alloxan-induced diabetic rats

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Received: 15 September 2021 / Accepted: 12 March 2022 / Published online: 18 March 2022

Abstract

Background *Synclisia scabrida* is commonly used in traditional medical practices for the management of diseases like diabetes and its complications. This study seeks to establish a scientific rationale for this practice.

Methods Thirty Wistar rats were randomly and equally grouped into six. Alloxan was used to induce diabetes in the rats in groups 2 to 6. The diabetic rats in group 2 were treated with glibenclamide, while those in group 3 were not treated. Also, the diabetic rats in groups 4, 5 and 6 were, respectively, treated with the ethanol extracts of the stem, root and leaf of *S. scabrida*. After 28 days of treatment, blood and organ samples were collected for biochemical studies.

Results *S. scabrida* possesses high amounts of useful phytochemicals. It also exhibits high total reducing capacity, FRAP activity, DPPH and ABTS scavenging ability. The inhibition of the α-glucosidase and α-amylase activities by the methanol extracts of *S. scabrida* stem, leaf and root were significantly ($p < 0.05$) higher than that of glibenclamide. Administration of *S. scabrida* extracts to the alloxan-induced diabetic rats caused significant ($p < 0.05$) decreases in the blood glucose, total bilirubin, AST, ALT, and ALP of the treated groups as compared to that of the untreated group. Treatment with the extracts also resulted in significantly ($p < 0.05$) lower LPO and significantly ($p < 0.05$) higher levels of GSH, SOD and CAT.

Conclusion *S. scabrida* extracts exhibited antioxidative, hepatoprotective and hypoglycaemic properties which are similar to that of the standard drug, glibenclamide.

Keywords *Synclisia scabrida* · Glibenclamide · Antioxidants · Antidiabetic · Hepatoprotective

Abbreviations

FRAP	Ferric Reducing Antioxidant Power
DPPH	1,1-Diphenyl-2-picrylhydraxil
TAN	Tannin
QUE	Quinine Equivalent
GAE	Garlic Acid Equivalent
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase

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ALP	Alkaline Phosphatase
GSH	Reduced Glutathione
SOD	Superoxide Dismutase
CAT	Catalase
LPO	Lipid Peroxidation
DMSO	Dimethyl Sulphoxide
TPTZ	Tripyridyltriazine
TEAC	Trolox Equivalent Antioxidant Capacity
DNSA	Dinitrosalicyclic Acid
GBM	Glibenclamide
BW	Body Weight
TBARS	Thiobabituric Acid Reactive Substances

Introduction

Management of blood glucose level is an essential approach in controlling diabetes and its complications. α-amylase and α-glucosidase are key enzymes involved in carbohydrate metabolism and intestinal absorption. Inhibitions of these enzymes prevent an increase in blood glucose level, which is possibly an important strategy in managing diabetes mellitus [1]. However, commonly used synthetic inhibitory drugs often present with negative effects.

In traditional medicine practice, various medicinal herbs or plants have been used directly as medication in the management of different diseases like diabetes and the complications associated with them [2–5]. Clinically effective substances are now being obtained from medicinal herbs due to their easy accessibility and acclaimed lesser side effects. Several classes of α-amylase and α-glucosidase inhibitory substances such as hydrolysable tannins, flavonoids, xanthones, fatty acids, terpenoids, peptides, procyanidins, and caffeoylquinic acid derivatives, have been derived from medicinal herbs [1, 6]. *Synclisia scabrida,* which belongs to the family Menispermaceae, is a common medicinal shrub in tropical Africa and in southern Nigeria, Cameroon, Gabon, Democratic Republic of Congo and Angola [7]. It is commonly used as fodder for domestic animals but has also been reputed in folklore as herbal remedy for lower abdominal pains, restlessness, mental strain, certain sexually transmitted diseases and diabetes mellitus [8, 9]. However, there are limited reports on the health benefits of *S. scabrida* in the treatment of diabetes. This study was carried out to establish a scientific rationale for the ethno-medicinal use of *S. scabrida* stems, roots and leaves in the treatment of diabetes. In this study, we evaluated the hypoglycaemic, hepatoprotective and antioxidative properties of the methanol extracts of *S. scabrida* stems, roots and leaf. The standard antidiabetic drug, glibenclamide, was used as a positive control.

Materials and methods

Chemicals and Reagents

Commercially available reagent kits for glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were procured from Randox laboratory, United Kingdom. Alkaline phosphatase (ALP) assay kit (from TECO diagnostics, U.S.A), Bilirubin (from Crest Coral Clinical Systems, Goa, India), Methanol solution, Distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Dimethyl Sulfoxide (DMSO), Potassium ferricyanide ($K_3Fe(CN)_6$), Phosphate buffer, Trichloroacetic Acid, Folin-Ciocalteu reagent, Sodium trioxocarbonate ($NaCO_3$), Aluminium trichloride ($AlCl_3$), Gallic Acid Equivalent (GAE), Tripyridyl Triazine (TPTZ), Dinitrosalicylic Acid (DNSA), Thiobarbituric Acid Reactive Substances (TBARS), Trolox (6-Hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid). All chemicals used were of analytical grade.

Plant sample collection and preparation

Fresh samples of *S. scabrida* plant were harvested in local gardens within the university environment. The samples were authenticated by a Botanist in the Department of Botany, Faculty of Life science, Ambrose Alli University. Edo State Nigeria. The leaves, stem and roots were washed with distilled water, air-dried and ground into fine powder. Methanol extracts of the different parts were prepared by soaking 450 g each of the powdered samples in 2100 ml of methanol for 72 h with intermittent stirring. The mixtures were then filtered using a muslin cloth and the filtrates (extracts) concentrated using a rotary vacuum evaporator (Re-52A, lab science, England) at 37 °C. The concentrated extracts (leave, stem and root) were kept in sterilized sample bottles and stored at 4 °C in a refrigerator pending further biochemical investigations. The percentage yield was calculated as 12% using the equation below;

$$\%Yield = (Weight\ of\ dry\ extract \div Weight\ of\ sample) \times 100$$

Phytochemical analysis

Phytochemical analysis of the leave, stem and root extracts of *S.scabrida* was carried out to identify some chemical constituents (flavonoid, saponin, tannin, phenols) using standard methods.

Determination of total flavonoid content

The total flavonoid content was determined using the Dow method [10]. About 2 ml of 2% aluminium trichloride ($AlCl_3$) was mixed with the same volume of extract solution. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 415 nm using a spectrophotometer (Jenway 6705 UV/VIS). The total flavonoid content was determined as a microgram of rutin equivalent using a standard curve.

Determination of total saponin content

Determination of total saponin content was done by mixing appropriate dilutions of the extracts with 250 µl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol), then 2.5 ml of 72% sulphuric acid was added with proper mixing of the reaction mixture. The reacting solution was kept in the water bath at 60 °C for 10 min, after which it was cooled in ice cold water and absorbance was read at 544 nm. The values were expressed as Quinine equivalent (mg QUE/g extract) using standard curve [11].

Determination of total tannin content

The total tannin content of the extracts was determined according to the method of Polshettiwar et al., [12]. About 100 µl each of the extracts and standard solution of tannic acid was taken and made up to 1 ml with distilled water. Afterwards, 0.5 ml of follin-denis reagent and 1 ml of Na_2CO_3 solution were added to the mixture and the volume was also made up to 10 ml with distilled water. The absorbances were measured at 700 nm. The total tannic acid content was expressed as tannic acid equivalent (mg TAN/g extract).

Determination of total phenolic contents

Total soluble phenolic compounds of the extracts were determined using Folin-Ciocalteu reagent according to the method of Slinkard [13]. Pyrocatechol was used as a standard phenolic compound. About 1 ml each of the extracts were diluted with distilled water (46 ml) and 1 ml of Folin-Ciocauteu reagent was added to the content of the flask and thoroughly mixed. After 3 min, 3 ml of Na_2CO_3 (2%) was added and the mixture was then allowed to stand for 2 h with intermittent shaking. The absorbances were measured at 760 nm in a spectrophotometer (Jenway GBR) against a blank consisting of all the reagents and distilled water in place of the extract. The total concentration of phenolic compounds in the extracts was determined as microgram of pyrocatechol equivalent using an equation that was obtained from standard pyrochatecol graph.

Determination of total reducing capacity

The total reducing power of the extracts was determined according to the method of Oyaizu [14]. About 1 ml each of the extracts were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6 and Potassium ferricyanide, K_3 $[Fe(CN)_6]$ (2.5 ml, 1%). The mixtures were incubated at 50^0C for 20 min. Then trichloroacetic acid (10%, 2.5 ml) was added to the mixture and centrifuged. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml: 0.1%). The absorbance of the solution was measured at 700 nm in a spectrophotometer (Jenway GBR). Blank was prepared with all the reaction agents without the extracts. The higher absorbance of the reaction indicated that the reducing power is increased. Ascorbic acid, butylated hydroxyanisole (BHA) and Alpha-tocopherol were used as standards.

Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM Tripyridyltriazine (TPTZ) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples of the extracts at different concentrations were added to 3 ml of FRAP reagent and the reaction mixtures were allowed to incubate for 30 min at 37 °C. Absorbance was read at 593 nm and fresh working solution of Fe_2SO_4 was used for calibration [15].

Determination antioxidant (DPPH) free radicals scavenging activity

The free radical scavenging activity of the extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The method used is similar to the method previously used by some authors [16, 17], but with slight modifications. About 2 ml of methanol solution of DPPH radical (0.05 mg/ml) and 1 ml each of the plant extracts were placed in cuvettes. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm against methanol as blank in spectrophotometer (Jenway GBR). The DPPH radical concentration was calculated using the following equation;

$$DPPH\ Scavenging\ Effect\ (\%) = [(Ao - A1) \div Ao] \times 100$$

Determination of ABTS assay

This was done by using the ABTS free radical decolorization assay developed by Re et al., [18] with some modifications. Briefly, the preformed radical modification of ABTS was

generated by reacting ABTS solution (7 nM) with 2.45 nM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stay for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 + 0.2 units at 750 nm. The plant extracts were separately dissolved in ethanol to obtain a concentration of 1 mg/ml. Aliquots of 20 ul of ethanol test solutions of each sample were added to 180 ul of ABTS free radical solution. The absorbance was measured spectrophotometrically at 750 nm. All measurements were done in triplicate. The free radical scavenging activity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC), which was obtained by comparing the antioxidant change at 750 nm in a reaction mixture containing a sample of plant extract with that containing Trolox solution. This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract [19]. The ABTS value was calculated as follows;

$$\%ABTS\ of\ Extract = (ABTS\ for\ Extract \div ABTS\ for\ Vit\ C) \times 100$$

Enzyme inhibition assays

Alpha-amylase assay

α-Amylase assay was done according to the method reported by Worthington [20]. Appropriate dilutions of each plant extract were mixed with 500 µl of 0.02 mol/L sodium phosphate buffer containing hog pancreatic α-amylase (0.5 mg/ml). The mixtures were incubated at 25 °C for 10 min and the reaction stopped with 1.0 ml of dinitro-salicylic acid (DNSA) colour reagent. The reacting mixtures were then incubated in a boiling water bath for 5 min and cooled to room temperature. They were then diluted by adding 10 ml of distil water and absorbances were measured at 540 nm. Glibenclamide was used as the positive control, the α-amylase inhibitory activity was expressed as the percentage inhibition as follows;

$$\%Inhibition = \left[(Abs\ control - Abs\ sample) \div Abscontrol\right] \times 100$$

where

Abs control	Absorbance of control
Abs sample	Absorbance of sample extracts

Alpha-glucosidase assay

α-Glucosidase assay was performed in accordance with the method reported by Apostolidis et al., [21]. Appropriate dilutions of the sample extracts were mixed with 100 µl of the alpha-glucosidase solution and incubated at 25 °C for 10 min after which 50 µl of *P*-nitrophenyl-α-D-glucopyranoside solution was added. The reacting mixtures were then incubated at 25 °C for 5 min and absorbances were read at 450 nm. Glibenclamide was used as the positive control. Inhibitory activity of α-glucosidase was expressed as percentage inhibition as indicated below;

$$\%Inhibition = \left[(Abs\ control - Abs\ sample) \div Abs\ control\right] \times 100$$

where

Abs control	Absorbance of control
Abs sample	Absorbance of sample extracts

Animals

Healthy Wistar albino rats of both sexes weighing between 180 and 220 g, used for the experiment, were obtained from the animal house of the Department of Biochemistry, Federal University of Technology Akure, Nigeria. They were kept in clean, disinfected cages in the animal house of the department of biochemistry, Ambrose Alli University, Nigeria. They were subjected to natural photoperiod of 12 h light and 12 h dark circles per day, with free access to water and feed (grower's mash from Top feeds, premier feed mills limited, Ibadan, Nigeria). The rats were allowed to acclimatize to the new environment for two weeks before the commencement of the experiment.

Experimental design and procedure

An acute toxicity study was carried out to determine the median lethal dose (LD_{50}) of the extracts (stem, root and leaf) using the modified Lorke's method [22]. The study was carried out in two phases. In the first phase, three groups of four (4) rats each were administered with different doses of the extracts (1000, 2000 and 3000 mg/kg) and the rats were monitored for a period of 24 h for signs of toxicity and possible death. In the second phase, three groups of four rats each were administered with increasing doses of the extracts (4000, 5000 and 6000 mg/kg) and the rats were monitored for 24 h for signs of toxicity and death. After 24 h, no death was recorded. However, signs like depression, weakness and loss of appetite were observed at the highest dose (6000 mg/kg) administered. The second phase was used in the calculation of the median lethal dose (LD_{50}) and 250 mg/kg body weight was determined as the dose for oral administration to the experimental animals.

Afterward, thirty rats were randomly assigned to 6 groups of 5 rats each. Diabetes was induced via intraperitoneal administration of 150 mg/kg alloxan monohydrate dissolved in normal cold saline. After 48 h, the experimental rats with blood glucose level higher than 200 mg/dl were classified as diabetic and used for the experiment. The various groups

were treated as follows; group **I** (normal control) rats were neither induced with diabetes nor treated, group **II** (positive control) rats were induced with diabetes and treated with glibenclamide (GBM, 10 mg/kg B.W), group **III** (negative control) rats were induced with diabetes but not treated, group **IV** rats were induced with diabetes and treated with methanol extract of *S. scabrida* stem (500 mg/kg B.W), group **V** rats were induced with diabetes and treated with methanol extract of *S. scabrida* root (500 mg/kg B.W), group **VI** rats were induced with diabetes and treated with methanol extract of *S. scabrida* leaf (500 mg/kg B.W). Glibenclamide and the extracts were administered orally for a period of 28 days.

After 28 days of treatment, the rats were allowed to fast over night before been anaesthetised with chloroform. Blood samples were collected by cardiac puncture technique, using sterilized needles and syringes, into clean plain sample bottles. The serum in each tube was separated by centrifugation at 4000 rpm for 10 min. The samples where then stored in the freezer for further analysis. The Committee approved the experimental design and procedures for the Care and Use of Laboratory Animals in the Department of Biochemistry, Ambrose Alli University Ekpoma. According to the NIH and University Guidelines for the Care and Use of Laboratory Animals, it was carried out.

Liver function assay

Total bilirubin was determined by caffeine, which releases albumin-bound bilirubin by the reaction with diazotized sulfanilic acid [23]. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine while ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine [24]. ALP acts upon AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which is measured photometrically [25].

Preparation of tissue homogenates

About 2 g each of the organs (liver, kidney and heart) were homogenized in ice cold phosphate buffer using mortar and pestle. The smooth homogenates obtained were centrifuged at 4000 rmp for 15 min and the supernatants were collected and stored at -4 °C till use.

Antioxidants determination

Lipid peroxidation (LPO) activity was determined by measuring the thiobarbituric acid reactive substance (TBARS) produced during lipid peroxidation as described by Rice-Evans et al., [26]. The method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde, an end product of lipid peroxidation. On heating in acidic pH, a pink coloured product (chromophore) was formed which maximally absorbs at 532 nm. Catalase (CAT) activity was determined by Cohen et al. method [27], where it converts hydrogen peroxide to water and oxygen. Reduced glutathione (GSH) activity was determined by the method of Beutler et al., [28]. The method is based on the development of a relatively stable coloured (yellow) product formed when 5′5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman's reagent reacts with the reduced glutathione which absorbs maximally at 412 nm. Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich [29]. In an alkaline medium, adrenaline undergo autoxidation rapidly to adrenochrome in the presence of superoxide anions. SOD inhibits the autoxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 480 nm.

Table 1 Phytochemical analysis of *S. scabrida* plant extract

Phytochemicals	S.S.Leaf	S.S.Root	S.S.Stem
Flavonoids	360.2 ± 1.46^{a}	392.2 ± 0.48^{b}	347.2 ± 0.30^{c}
Saponin	157.2 ± 0.72^{a}	279.2 ± 1.03^{b}	112.2 ± 0.22^{c}
Total Reducing Capacity	800.3 ± 31.69^{a}	285.2 ± 1.22^{b}	197.2 ± 86.28^{b}
FRAP	522.4 ± 2.70^{a}	361.4 ± 2.16^{b}	201.5 ± 4.90^{c}
Tannin	439.8 ± 0.17^{a}	592.9 ± 0.51^{b}	497.6 ± 0.10^{c}
Total Phenol	208.2 ± 0.32^{a}	193.2 ± 2.20^{b}	236.2 ± 0.53^{c}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c}$) are significantly different ($p < 0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values ± standard error of the mean. Data analyses were performed using an instant statistical package (IBM-SPSS statistics version 23) followed by the analysis of variance (ANOVA) and Duncan's comparison test for significance. The difference was considered statistically significant at $p < 0.05$ and insignificant at $p > 0.05$.

Results

S. scabrida leaf, root and stem extracts possess tangible amounts of useful phytochemicals

In Table 1, the phytochemical screening of *S. scabrida* root revealed a significantly ($p<0.05$) higher amount of flavonoid (392.2 ± 0.48 mg QUE/ g extract) compared to that of the leaf (360.2 ± 1.46 mg/QUE/g) and stem (347.2 ± 0.30 mg/QUE/g). The saponin content of *S. scabrida* root was significantly ($p<0.05$) higher (279.2 ± 1.03 mg/g) than that of the stem (112.2 ± 0.22 mg/g) and leaf (157.2 ± 0.72 mg/g). For tannins, it was observed that *S. scabrida* root had a significantly ($p<0.05$) higher amount (592.9 ± 0.51 mg/TAN/g) when compared to that of the stem (439.8 ± 0.17 mg/TAN/g) and leaf (497.6 ± 0.10 mg/TAN/g). The stem extract of *S. scabrida* was shown to possess a significantly ($p<0.05$) higher amount of phenol (236.2 ± 0.53 mg/GAE/g) compared to that of the root (208.2 ± 0.32 mg/GAE/g) and leave (193.2 ± 2.20 mg/GAE/g). The screening further revealed that the total reducing capacity of *S. scabrida* leaf extract was significantly ($p<0.05$) higher (800.3 ± 31.69 mg/g) as compared to that of the root (285.2 ± 1.222 mg/g) and stem (197.2 ± 86.28). While the FRAP activity was significantly ($p<0.05$) higher in *S. scabrida* leaf (522.4 ± 2.704 mg/g) as compared to that of the root (361.4 ± 2.164 mg/g) and stem (201.5 ± 4.904 mg/g).

S. scabrida leaf, root and stem extracts possess DPPH free radical scavenging and ABTS activities, as well as inhibit DPPH activity invitro.

Table 2 shows the DPPH free radical scavenging and ABTS activities of the extracts of *S. scabrida* leaf, root and stem. From the results, *S. scabrida* leaf extract had a significantly ($p<0.05$) higher DPPH scavenging ability (4.39 ± 1.22 Ug/ml) compared to the root and stem which are (3.090 ± 0.66 Ug/ml) and (2.73 ± 0.75 Ug/ml) respectively. Also, the leaf extract showed a significantly higher ABTS activity (4.43 ± 0.01 Ug/ml) compared to the root (4.06 ± 0.620 Ug/ml) and stem (1.78 ± 0.03 Ug/ml). In Table 3, the IC50 values for the inhibitory effects of the extracts of *S. scabrida* on ABTS and DPPH activities are significantly ($p<0.05$) higher than that of the standard, ascorbic acids. While in Table 4, at different concentrations, the inhibitory activity of the different extracts of *S. scabrida* on the invitro activity of DPPH were significantly ($p<0.05$) higher than that of the standard, ascorbic acids.

Table 2 DPPH and ABTS analysis of *S.scabrida*

Parameters	S.S.Leave	S.S.Root	S.S.Stem
DPPH	4.39 ± 1.22^{a}	3.09 ± 0.66^{b}	2.73 ± 0.75^{b}
ABTS	4.43 ± 0.01^{a}	4.06 ± 0.62^{b}	1.78 ± 0.03^{c}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c}$) are significantly different ($p<0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

Table 3 IC50 value for the inhibitory effect of *S. scabrida* extracts on ABTS and DPPH activities

	IC50 (µg/ml)			
	Ascorbic acid	S.S.Stem	S.S.Root	S.S.Leaf
DPPH	0.51 ± 0.001^{a}	4.43 ± 0.68^{b}	4.06 ± 0.62^{b}	6.54 ± 1.34^{c}
ABTS	0.41 ± 0.001^{a}	1.68 ± 0.03^{b}	0.97 ± 0.11^{c}	1.78 ± 0.03^{b}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c}$) are significantly different ($p<0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

Antidiabetic study

S. scabrida extracts (stem, root and leaf) inhibit the activities of α-Glucosidase and α-Amylase

As indicated in Table 5, the inhibition of α-glucosidase activity by the methanol extracts of *S. scabrida* stem (68.76 ± 7.23), leaf (73.87 ± 6.62) and root (78.25 ± 4.61) were significantly ($p<0.05$) higher as compared with that of the positive control (62.79 ± 2.29). Also, the inhibition of α-amylase activity by the methanol extracts of *S. scabrida* root (70.88 ± 6.09), leaf (78.93 ± 3.60) and stem (71.77 ± 4.80) were significantly ($p<0.05$) higher as compared with that of the positive control (glibenclamide, 42.03 ± 1.11). While in Table 6, the IC50 values of the stem, root and leaf extracts of *S. scabrida* on the activities of α-amylase and α-glucosidase were significantly ($p<0.05$) lower than that of the standard drug, glibenclamide.

S. scabrida extracts (stem, root and leaf) reduced the blood glucose levels in alloxan-induced diabetic rats

Table 7 shows the blood glucose values of the experimental diabetic rats, before and after alloxan induction, followed by treatment with the stem, root and leaf extracts of *S. scabrida.* After 48 h, alloxan induction resulted in significantly ($p<0.05$) higher glucose values in all the test groups as compared to that of the control group (group 1). From week 1 to week 4 of treatments with the extracts and the standard drug (group II), there were steady significant ($p<0.05$) decreases in the blood glucose values of the treated groups as compared to that of the untreated group (group III). The blood glucose lowering effects of the extracts are similar

Table 4 Inhibitory activity of *S. Scabrida* plant extracts on DPPH invitro activity

Concentration (µg/ml)	% Inhibition			
	Ascorbic acid	S.S.Stem	S.S.Root	S.S.Leaf
40	32.78 ± 0.35^{a}	0.64 ± 0.13^{b}	1.21 ± 0.70^{c}	1.95 ± 0.44^{d}
80	48.17 ± 0.24^{a}	3.56 ± 1.32^{b}	2.63 ± 0.83^{c}	2.39 ± 0.73^{c}
120	60.46 ± 0.01^{a}	4.39 ± 1.22^{b}	3.09 ± 0.66^{c}	2.73 ± 0.75^{c}
160	78.04 ± 0.05^{a}	8.90 ± 0.82^{b}	7.77 ± 0.65^{b}	4.94 ± 1.32^{c}
200	90.42 ± 0.12^{a}	14.58 ± 1.96^{b}	19.34 ± 4.41^{c}	11.28 ± 2.19^{d}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c, d}$) are significantly different ($p < 0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

Table 5 Alpha-Glucosidase and Alpha-Amylase inhibitory activities of alloxan-induced diabetic rats treated with *S. scabrida* extracts

Enzymes	Positive Control	S.S.Leaf	S.S.Root	S.S.Stem
α-Glucosidase	62.79 ± 2.29^{a}	73.87 ± 6.62^{b}	78.25 ± 4.61^{b}	68.76 ± 7.23^{c}
α-Amylase	42.03 ± 1.11^{a}	78.93 ± 3.60^{b}	70.88 ± 6.09^{c}	71.77 ± 4.80^{c}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c}$) are significantly different ($p < 0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

Table 6 IC50 value of *S. scabrida* plant extracts on Alpha Amylase and Alpha Glucosidase activity

Assays	IC50 (mg/ml)			
	Glibenclamide	S.S.Stem	S.S.Root	S.S.Leaf
Alpha Amylase	1.45 ± 0.10^{a}	0.69 ± 0.03^{b}	0.68 ± 0.02^{b}	0.74 ± 0.01^{b}
Alpha Glucosidase	0.97 ± 0.03^{a}	0.81 ± 0.06^{b}	0.90 ± 0.06^{a}	0.73 ± 0.08^{b}

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ($^{a, b, c}$) are significantly different ($p < 0.05$). S.S.Leaf—*S. scabrida* leaf extract; S.S.Root—*S. scabrida* root extract; S.S.Stem—*S. scabrida* stem extract

to that of the standard drug, glibenclamide (group II) as observed.

Liver function study

S. scabrida stem, root and leaf extracts protect against hepatotoxicity in alloxan-induced diabetic rats

Table 8 shows the effects of the stem, root and leaf extracts of *S. scabrida* on serum parameters that are determinants of the functional status of the liver. Alloxan induced diabetic rats treated with the different extracts showed significantly ($p < 0.05$) lower total bilirubin concentration as compared with the untreated group (group III). The effects are similar to that of the standard drug, glibenclamide (group II). Alloxan-induced diabetic rats treated with stem (22.60 ± 1.63 IU/L) and leaf (25.00 ± 1.30 IU/L) extracts showed significantly ($p < 0.05$) lower AST concentration when compared with that of the root (39.80 ± 1.62) and untreated group (57.27 ± 3.44 IU/L). Treatment with the stem and leaf extracts resulted in significantly ($p < 0.05$) lower ALT concentration as compared with that of the Root (20.80 ± 0.58 IU/L), glibenclamide ((17.40 ± 1.21 IU/L) and untreated group (41.32 ± 2.49 IU/L). For ALP, alloxan induced diabetic rats treated with the leaf extracts showed a significantly ($p < 0.05$) lower (20.68 ± 1.76 IU/L) concentration when compared with that of the stem (36.58 ± 4.55 IU/L) and root (57.06 ± 1.80) extracts. The reducing effects on the liver function parameters protect against alloxan's hepatic toxicity.

Antioxidant study

S. scabrida stem, root and leaf extracts protect against LPO in selected tissues of alloxan-induced diabetic rats

Table 9 shows the results for the LPO level in the blood, liver, heart and kidney tissue homogenates of the alloxan induced diabetic rats. LPO level was significantly ($p < 0.05$) lower in all the extract treated groups (groups IV, V and VI) compared to that of the untreated group (group III). The effects of the extracts were similar to that of the standard

Table 7 The blood glucose levels of alloxan-induced diabetic rats treated with *S. scabrida* extracts

Glucose Level	CONTROL			TEST		
	Group I	Group II	Group III	Group IV	Group V	Group VI
Before Induction	96.00 ± 4.76[a]	98.33 ± 2.60[a]	98.33 ± 1.76[a]	94.33 ± 1.01[a]	97.00 ± 2.1[a]	92.66 ± 0.6[a]
After Inducing	96.97 ± 3.76[a]	596.33 ± 1.36[b]	594.00 ± 2.08[b]	593.00 ± 0.90[b]	596.33 ± 1.36[b]	593.66 ± 1.90[b]
Week 1	97.20 ± 2.62[a]	519.00 ± 6.25[b]	559.33 ± 6.69[c]	517.00 ± 6.00[b]	543.66 ± 6.00[c]	543.00 ± 6.00[c]
Week 2	98.00 ± 3.46[a]	426.70 ± 22.04[b]	489.33 ± 7.69[c]	377.00 ± 14.40[d]	451.33 ± 5.30[b]	400.33 ± 6.00[e]
Week 3	95.33 ± 2.91[a]	201.00 ± 20.31[b]	379.40 ± 7.69[c]	192.00 ± 0.70[b]	211.00 ± 7.40[b]	210.33 ± 6.60[b]
Week 4	95.60 ± 1.96[a]	99.00 ± 2.08[a]	278.00 ± 15.70[b]	178.00 ± 15.17[c]	104.00 ± 2.80[a]	102.66 ± 1.00[a]

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ([a, b, c, d, e]) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract.

Table 8 The liver function test of alloxan-induced diabetic rats treated with *S. scabrida* extracts

Markers	CONTROL			TEST		
	Group I	Group II	Group III	Group IV	Group V	Group VI
Tot.Bil. (mg/dl)	1.36 ± 0.12[a]	0.29 ± 0.03[b]	2.25 ± 0.13[c]	0.36 ± 0.04[b]	0.30 ± 0.03[b]	0.28 ± 0.05[b]
AST (U/L)	11.60 ± 0.93[a]	27.00 ± 1.58[b]	57.27 ± 3.44[c]	22.60 ± 1.63[b]	39.80 ± 1.62[d]	25.00 ± 1.30[b]
ALT (U/L)	5.60 ± 0.81[a]	17.40 ± 1.21[b]	41.32 ± 2.49[c]	8.00 ± 0.71[a]	20.80 ± 0.58[b]	9.20 ± 0.66[a]
ALP (U/L)	6.59 ± 0.59[a]	12.51 ± 1.49[b]	63.94 ± 1.93[c]	36.58 ± 4.55[d]	57.06 ± 1.80[c]	20.68 ± 1.76[e]

Data are expressed as mean ± SEM (n = 3). Values in same row with different alphabet ([a, b, c, d, e]) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract

Table 9 Effect of *S. scabrida* extracts on LPO level (u/g protein) of Alloxan Induced Diabetic Rats

Group	Blood	Liver	Heart	Kidney
Group I	10.14 ± 0.66[a]	23.36 ± 2.93[a]	31.58 ± 6.45[a]	91.43 ± 8.13[a]
Group II	10.12 ± 1.12[a]	43.84 ± 4.72[b]	44.70 ± 5.55[b]	268.52 ± 14.63[b]
Group III	39.94 ± 1.97[b]	95.7 ± 2.05[c]	81.50 ± 3.48[c]	269.64 ± 15.75[b]
Group IV	10.29 ± 0.56[a]	26.84 ± 5.69[d]	64.70 ± 4.42[d]	98.64 ± 15.75[a]
Group V	9.12 ± 0.65[a]	40.76 ± 7.55[b]	45.13 ± 4.35[b]	252.0 ± 19.05[b]
Group VI	26.62 ± 1.23[c]	28.18 ± 7.25[d]	46.77 ± 5.81[b]	257.3 ± 18.75[b]

Data are expressed as mean ± SEM (n = 3). Values in same column with different alphabet ([a, b, c, d]) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract

drug, glibenclamide (group II). However, non – significant ($p > 0.05$) decreases were observed in the kidney of rats treated with *S. scabrida* root and leave extracts.

S. scabrida stem, root and leaf extracts increase the activities of GSH, SOD and CAT, in selected tissues of alloxan-induced diabetic rats

Table 10 Effect of *Syncliasia scabrida* Extracts on GSH activity (mg/gprotein) of Alloxan Induced Diabetic Rats

Group	Blood	Liver	Heart	Kidney
Group I	12.59 ± 0.99^{a}	10.55 ± 1.17^{a}	4.56 ± 41^{a}	5.98 ± 0.65^{a}
Group II	20.36 ± 1.41^{b}	9.73 ± 1.57^{a}	4.62 ± 0.37^{a}	5.56 ± 1.01^{a}
Group III	8.49 ± 0.37^{c}	7.42 ± 0.29^{b}	2.86 ± 0.25^{b}	4.66 ± 0.33^{b}
Group IV	12.84 ± 0.99^{a}	9.33 ± 1.00^{a}	4.09 ± 0.47^{a}	7.85 ± 0.69^{c}
Group V	10.15 ± 1.64^{d}	10.25 ± 1.05^{a}	4.70 ± 0.46^{a}	5.02 ± 0.69^{a}
Group VI	10.49 ± 1.12^{d}	11.62 ± 1.39^{c}	4.43 ± 0.37^{a}	8.26 ± 0.62^{c}

Data are expressed as mean ± SEM (n = 3). Values in same column with different alphabet ($^{a, b, c, d}$) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract

Table 11 Effect of *Syncliasia scabrida* Extracts on the SOD activity (U/g protein) of Alloxan Induced Diabetic Rats

Group	Blood	Liver	Heart	Kidney
Group I	1.29 ± 0.10^{a}	1.56 ± 0.05^{a}	0.92 ± 0.15^{a}	1.68 ± 0.02^{a}
Group II	1.33 ± 0.14^{a}	1.59 ± 0.03^{a}	1.16 ± 0.04^{b}	1.82 ± 0.04^{b}
Group III	1.38 ± 0.06^{b}	1.58 ± 0.02^{a}	1.18 ± 0.03^{b}	1.79 ± 0.02^{b}
Group IV	1.55 ± 0.03^{c}	1.53 ± 0.03^{a}	1.29 ± 0.07^{c}	1.88 ± 0.01^{c}
Group V	1.56 ± 0.03^{c}	1.56 ± 0.02^{a}	1.31 ± 0.06^{c}	1.85 ± 0.01^{c}
Group VI	1.56 ± 0.01^{c}	1.75 ± 0.02^{b}	1.26 ± 0.04^{c}	1.87 ± 0.03^{c}

Data are expressed as mean ± SEM (n = 3). Values in same column with different alphabet ($^{a, b, c}$) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract

Table 10 shows the results for the GSH levels of the blood, liver, heart and kidney homogenates. It was observed that treatment with *S. scabrida* stem, root and leaf extracts resulted in significant ($p < 0.05$) increases in the levels of GSH as compared to that of the negative control group (rats induced but not treated). The effects of the extracts were similar to that of the positive control group (diabetic rats treated with glibenclamide).

Table 11 shows the results for the SOD activity in the blood, liver, heart and kidney tissue homogenates. The SOD activities in the blood, heart and kidney significantly ($p < 0.05$) increased in the extracts (stem, root and leaf) treated groups as compared to that of the negative control group (untreated group). However, SOD activity was significantly ($p < 0.05$) higher only in the rats treated with *S. scabrida* leaf extract as compared to other groups.

Table 12 shows the results for the CAT activity in the blood, liver, heart and kidney tissue homogenates. CAT activity significantly ($p < 0.05$) increased in the blood, liver, heart and kidney of the rats treated *S. scabrida* stem, root and leaf extracts as compared to that of the untreated group (group III). However, there was a non-significant ($p > 0.05$) increase in the CAT activity in the liver of rats treated with *S. scabrida* leaf extract. The effects observed due to the treatments with the extracts were similar to that observed due to the treatment with glibenclamide (group II).

Discussion

The quantitative phytochemical screening of the extracts of *S. scabrida* stem, root and leaf revealed significant amounts of flavonoids, saponin, tannins and phenols. The screening further revealed high reducing capacity and FRAP activity of the extracts. Flavonoids and saponins are known to possess hypoglycaemic and antioxidative properties. Flavonoids tend to improve derangement in glucose and oxidative metabolism in the diabetic state as well as stimulate the secretion of insulin by altering the concentration of Ca^{2+}. They have been reported as active constituents of herbal medicines and are known to be powerful antioxidants that could protect organs against the toxicity and potential damage caused by agents such as alloxan [30]. As a measure of the DPPH radical scavenging and ABTS activities of *S.*

Table 12 Effect of *S. scabrida* extracts on the CAT activity (nmol/min/ml) of Alloxan Induced Diabetic Rats

Group	Blood	Liver	Heart	Kidney
Group I	0.042 ± 0.001^{a}	0.054 ± 0.001^{a}	0.054 ± 0.001^{a}	0.054 ± 0.001^{a}
Group II	0.046 ± 0.002^{b}	0.056 ± 0.001^{a}	0.055 ± 0.003^{a}	0.0052 ± 0.001^{a}
Group III	0.036 ± 0.001^{c}	0.044 ± 0.001^{b}	0.051 ± 0.001^{b}	0.052 ± 0.001^{a}
Group IV	0.044 ± 0.001^{b}	0.066 ± 0.003^{c}	0.055 ± 0.004^{a}	0.059 ± 0.001^{b}
Group V	0.050 ± 0.001^{d}	0.052 ± 0.001^{a}	0.053 ± 0.004^{a}	0.057 ± 0.001^{b}
Group VI	0.045 ± 0.001^{b}	0.046 ± 0.002^{b}	0.055 ± 0.004^{a}	0.060 ± 0.001^{b}

Data are expressed as mean ± SEM (n = 3). Values in same column with different alphabet ($^{a, b, c, d}$) are significantly different ($p < 0.05$). Group I – Normal control; Group II – Positive control (Diabetes + Glibenclamide); Group III – Negative control (Diabetes + Untreated); Group IV – Diabetes + *S. scabrida* stem extract; Group V – Diabetes + *S. scabrida* root extract; Group VI – Diabetes + *S. scabrida* leaf extract

scabrida, the different extracts (stem, root and leaf) were observed to possess DPPH free radical scavenging activities, high amount of ABTS activities as well as inhibit DPPH activity invitro. These activities were comparatively higher than that of ascorbic acids and thus indicated the strong potential of the plant as a useful antioxidant in the biological system, especially in cases of metabolic derangements.

Diabetes is a form of metabolic derangement that affects blood glucose utilisation in the biological system. Therefore, blood glucose management is necessary for the control or prevention of diabetic complications. A useful approach is the inhibition of α-amylase and α-glucosidase, which are key enzymes involved in the metabolism of carbohydrates. Inhibition of these enzymes have been reported to prevent increase in blood glucose level after a carbohydrate diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus [1]. On analysis of the inhibitory effects of the methanol extracts of *S. scabrida* stem, leaf and root on the activities of α-amylase and α-glucosidase, it was observed that the extracts showed high inhibitory effects which are concentration dependant and comparable to that of glibenclamide (standard drug). These inhibitory effects, possibly due to the presence of tannins, flavonoids and phenols in *S. scabrida,* makes it a potential good antidiabetic agent. Further antidiabetic study revealed that treatment with *S. scabrida* extracts resulted in reductions in the blood glucose levels of the alloxan-induced diabetic rats. The extracts of *S. scabrida* caused steady significant decreases in the blood glucose values of the treated diabetic rats throughout the duration of treatment. These steady decreases were similar to those caused by the treatment with the standard drug (glibenclamide). The similarity between the hypoglycaemic effects of *S. scabrida* and glibenclamide is indicative of the possible use of *S. scabrida* in ethnomedicine for the management of diabetic conditions.

Alloxan-induced diabetic complications include injuries to the hepatocytes which results in increases in membrane permeability [31]. Consequently, hepatic enzymes are aberrantly released into the blood resulting in increases in the levels of these enzymes. As observed, alloxan induction resulted in increases in the levels of ALT, AST, ALP and total bilirubin in the serum. Increased levels of ALP and bilirubin are indicative of biliary tract obstruction while elevated levels of ALT and AST are indicative markers of liver injury. Treatment with the stem, root and leaf extracts of *S. scabrida* resulted in decreases in the levels of ALT, AST, ALP and total bilirubin. The effects were observed to be similar to that of the standard antidiabetic drug, glibenclamide. The reducing effects of *S. scabrida* on the liver function parameters indicates its protective role against the hepatic toxicity due to alloxan and alloxan-induced diabetic complications. These may also be linked with the antioxidant potential of *S. scabrida* which probably protects against membrane lipid peroxidation, thereby maintaining the integrity of the biological membranes of the hepatocytes.

LPO is a hallmark of oxidative stress in which reactive oxygen species interact with polyunsaturated fatty acids leading to the formation of lipid products such as 4-hydroxynonenal, which then causes damage to the membrane components of the cell, cell necrosis and inflammation [32, 33]. It has been demonstrated that increased lipid peroxidation plays an important role in the progression of diabetes by altering the trans-bilayer fluidity gradient, which could affect the activities of membrane-bound enzymes and receptors [34]. We observed that alloxan-induced diabetes resulted in the increases in LPO in selected tissues (blood, liver, heart and kidney) of the experimental rats, which indicates possible increases in damage to membrane components of cells of these tissues. These increases were significantly reduced on treatment with the stem, root and leaf extracts of *S. scabrida* in a similar manner to treatment with glibenclamide. Thus, *S. scabrida* protects against the LPO and its associated damage in selected tissues in the alloxan-induced experimental diabetic rats. Our study also revealed that treatment with *S. scabrida* stem, root and leaf extracts resulted in increases in the activities of GSH, SOD and CAT in the diabetic experimental rats. This is against the decreases in these antioxidant enzymes occasioned by induction with alloxan. It has been reported that the activity of antioxidant enzymes such as SOD and CAT are often reduced in the tissues of diabetic rats which may result in a number of deleterious effects due to the accumulation of reactive oxygen species. A possible mechanism for this reduction may be inactivation caused by excess free radicals and/or by non-enzymatic glycation due to the persistent hyperglycaemia common in diabetes [35]. The reduction in GSH due to alloxan induction, as observed in the untreated group, can increase the susceptibility to oxidative damage in hepatic and renal cells and may contribute to the pathogenesis of complications associated with the chronic diabetic state [36]. Antioxidant enzymes are the first line of defence against reactive oxygen species which play an important role in scavenging the toxic intermediates of incomplete oxidation. CAT and SOD play major roles in the removal of reactive oxygen species in vivo. SOD catalyzes the dismutation of superoxide anion (O_2) into hydrogen peroxide (H_2O_2), which is then degraded to H_2O by CAT or by GSH. A decrease in the activity of these antioxidants may lead to an excess amount of O_2 and H_2O_2, which in turn generates hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation [37]. The increases in the activities of GSH, SOD and CAT in the rats treated with the extracts of *S. scabrida* point at the strong antioxidant potential of *S. scabrida* and its ability to offset oxidative stress, thereby protecting against the complications associated with diabetes.

Conclusion

S. scabrida leaf, root and stem extracts possess tangible amounts of useful phytochemicals, DPPH free radical scavenging and ABTS activities, as well as inhibited the activities of α-glucosidase and α-amylase. The extracts exhibited hypoglycaemic, hepatoprotective and antioxidative properties which are comparable to that of the standard drug, glibenclamide. This may justify the use of *S. scabrida* in ethnomedicine in managing diabetes and its complications.

Authors' contributions Godwin Ezemwenghian Orumwense, Marshall Arebojie Azeke and Aishat Mary Osagie, conceived, designed and performed the experiments; Godwin Ezemwenghian Orumwense, Marshall Arebojie Azeke, Aishat Mary Osagie, Sylvia Oghogho Omage and Kingsley Omage performed the analysis and interpretation of the data; while Sylvia Oghogho Omage and Kingsley Omage prepared the draft of the manuscript. All authors have reviewed and approved the final draft of the manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability Not Applicable.

Code availability Not Applicable.

Declarations

Ethics approval The ethical approval for this research was obtained from the Research Ethics Committee of the Department of Biochemistry, Ambrose Alli University, Ekpoma Nigeria. The use of rats for the study was also according to the Ethical Guidelines Involving Whole Animal Testing of the Research Ethics Committee of the Department of Biochemistry, Ambrose Alli University, Ekpoma Nigeria.

Consent to participate Not Applicable.

Consent for publication Not Applicable.

Conflicts of interest We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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