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Solanum anomalum Leaf Extract and Fractions Attenuate Oxidative Stress and Liver Injuries in Alloxan-Induced Diabetic Rats

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Abstract

The leaf of *Solanum anomalum* used in ethnomedicine for the treatment of various ailments such as diabetes was evaluated for antioxidative stress and hepatoprotective potentials against hepatic injuries in alloxan-induced diabetic rats. Antioxidative stress and hepatoprotective activities of leaf extract and fractions (70-210 mg/kg) were assessed by determining oxidative stress markers levels, liver function indices and histopathological study of livers of treated rats. The leaf extract and fractions caused significant ($p<0.05 – 0.001$) increases in the levels of oxidative stress markers (SOD, CAT, GPx, GSH) in the livers of the treated diabetic rats. The extract/fractions treatment caused reduction in liver enzymes (ALT, AST and ALP), total and direct bilirubin. Histology of the livers revealed absence or significant reductions in pathological features in the treated diabetic rats compared to untreated diabetic rats. The results show that the leaf extract and fractions of *S. anomalum* has antioxidative stress and hepatoprotective potentials which may be due to the antioxidant activities of their phytochemical constituents.

Keywords: *Solanum anomalum*; Medicinal plant; liver protective; antioxidant; antioxidative stress.

Abbreviations: Gas chromatography mass spectrometry (GCMS)

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder which is associated with increased generation of free radicals especially Reactive Oxygen Species (ROS) (Okutana *et al*., 2005). Alloxan biotransformation to dialuric acid in the body is accompanied by generation of H_2O_2, •OH and superoxide radicals via iron catalyst which attack organs like kidney, liver and pancreas etc (Mathews and Leiter, 1999) and cause oxidative stress which is implicated in the pathogenesis of diabetes complications in animals and humans (Baynes and Thorpe,1999). ROS produce cellular and tissue injury through covalent binding, DNA strand breaking, lipid peroxidation and augment fibrosis which is also implicated in other disease conditions (Paradies *et al.*, 2011). Medicinal plants which are used traditionally in the management of diabetes, therefore serve as a great repository for anti-diabetic remedies having the advantage of being safer and providing many therapeutic effects.

Solanum anomalum Thonn. ex Schumach, a plant whose fruits and leaves are used medicinally and nutritionally, is commonly found growing in West and East Africa sub-regions. Its parts are utilised locally to treat diabetes, gastrointestinal disorders, infections, inflammation and pains (Burkill, 2000; Bukenya and Hall, 1988; Offor and Ubengama, 2015). Hypoglycemic and antidiabetic activities of the fruits and leaves have been reported (Offor and Ubengama ,2015; Okokon *et al.*, 2022). More so, *in vivo* and *in vitro* antiplasmodial (Okokon *et al.*, 2016; Okokon *et al.*, 2017a), anti-oedema (Okokon *et al*., 2017b), antioxidant and antiulcer (Okokon *et al.*, 2019a), anticonvulsant and depressant (Okokon *et al*., 2019b), analgesic (Okokon *et al.*, 2020) and antidiarrhoeal (Udobang *et al.*, 2022) properties of the leaf extract have also been reported. Phytochemical constituents such as alkaloids, flavonoids, saponins, tanins, diosgenin, a diosgenin glycoside (25(R)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been reported on the leaves of the plant (Okokon *et al.*, 2016; Okokon *et al.*, 2022). We report in this study the effect of the leaf extract and fractions *S. anomalum* on oxidative stress markers, liver function indices and histology of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plants collection

Fresh leaves of *Solanum anomalum* were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2020. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

Extraction

Fresh leaves of *S. anomalum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using electric grinder. The powdered leaves material was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ±2 °C). While the other part, (1.5 kg) was successively and gradiently macerated for 72 hours in each of these solvents (2 x 5L), *n*-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding fractions of these solvents. These were thereafter filtered and the liquid filtrates were concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract and fractions were stored in a refrigerator at -4°C, until used for the proposed experiments.

Animals

Wistar rats (138-150 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the Faculty of Pharmacy Animal Ethics committee, University of Uyo.

Induction of Experimental Diabetes using Alloxan Monohydrate

Sixty (60) healthy albino Wistar rats (male and female) of known weights were fasted for 24 hours, they were reweighed before the induction by a single intra peritoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl solution). According to the method of Pari and Saravanan, (2002), the animals were given 2 mL of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycaemia. A rest period of 72 hours was allowed during which the rats were allowed access to food and water and the diabetes to be fully developed during these 72 hours. After the rest period, rats with moderate diabetes, having persistent glycosuria, and hyperglycaemia (i.e with blood glucose levels 200 mg/dL and above), (Lenzen, 2008) were considered diabetic and selected for the experiments.

The diabetic animals were randomised and divided into 9 (nine) treatment groups of 6 rats each. Based on the value of previously determined median lethal dose (LD_{50}) (Okokon *et al.*, 2022), suitable dose regimens were selected and the rats were treated as follows. Group 1 was given 10 mL/kg/day of normal saline orally for 14 days, Group 2 was administered with 5 mg/kg/day of Glibenclamide orally for 14 days, Group 3 was given 70 mg/kg/day of *S. anomalum* leaf extract orally for 14 days and group 4 rats were given 140 mg/kg/day of *S. anomalum* leaf extract orally for 14 days, Group 5 was administered with 210 mg/kg/day of *S. anomalum* leaf extract orally for 14 days. Groups 6 -9 were respectively administered with 140 mg/kg/day of *n*-hexane, dichloromethane, ethyl acetate and methanol fractions of *S. anomalum* leaves orally for 14 days.

Effect of Administration of Leaf Extract and Fractions of *S. anomlum* on Fasting Blood Glucose of Alloxan-induced Diabetic Rats.

The fasting blood glucose (FBG) of all the rats was measured after 14 days of administration of the leaf extract and fractions. The method that was employed was "the tail-tipping method". The blood obtained from the tail vein of the rats was dropped on the dextrostix reagent pad and the pad inserted into a microprocessor digital blood glucometer and the readings were recorded (WHO, 1980).

All the treatments were administered between 7.00-8.00 am daily throughout the experimental period and food was withdrawn from the experimental animals 12 hours before measurement of FBG to create the necessary fasting period for measurement of the fasting blood glucose concentrations.

Determination of the Body Weights Changes of the Treated Diabetic Rats

Throughout the experimental period the body weights of the experimental animals were monitored and recorded at the following points; just before the fasting in preparation for induction of the diabetes, after induction, on stabilization of diabetes and after the prolonged study

Collection of Blood Samples and Organs

After 14 days of treatment (24 hours after the last administration) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used immediately. Blood were collected into plain centrifuge tubes and EDTA bottles. The blood in the centrifuge tubes were centrifuged immediately at 1500 rpm for 15 min to separate of serum at room temperature to avoid haemolysis and used for biochemical assays. Blood that was collected into EDTA bottles were taken for haematological analysis. The livers and kidneys of the diabetic rats were surgically removed, weighed and fixed in 10% formaldehyde for histological process.

Hematological Study

After the animals were sacrificed under diethyl ether anesthesia, blood samples were collected from each rat by cardiac puncture using 21 gauge (21 G) needles mounted on a 5 ml syringe into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles for analyzed. Hematological parameters such as Red blood cell count (RBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and total and differential white blood cell count (WBC). These parameters were analyzed using automatic hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

Liver Function Test

The following parameters were determined; Aspartate transaminase (AST), alanine aminotransferase (ALT), total cholesterol, alkaline phosphatase (ALP), total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols (Tietz, 1976) at the Chemical Pathology Department of University of Uyo Teaching Hospital.

Evaluation of the Protective Effect of the Leaf Extract and Fractions on Biochemical Parameters and Histology of Livers of Alloxan-induced Diabetic Rats

Serum was separated from the blood of each animal sacrificed and the sera were stored at -20^{o}C until used for biochemical determinations such as total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphotase (ALP), direct and total bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols (Reitman and Frankel, 1957; Tietz, 1976)).

The livers of the animals were surgically removed, weighed and a part of each fixed in 10% formaldehyde for histological processes, while the other part was washed with ice cold 0.9% NaCl and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4˚C and the supernatants were used for the assays of superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha,1972), glutathione peroxidase (GPx) (Lawrence and Burk,1976), and reduced glutathione (GSH) (Ellman,1959). Malondialdehyde (MDA) and gutathione-S-transferase (GST) were measured using commercial diagnostic kits (Sigma-Aldrich, USA) according to standard manufacturer's protocols.

Gas Chromatography-Mass spectrometry (GCMS) Analysis

GC-MS analyses of hexane and dichloromethane fractions were performed using an Agilent 7890A gas chromatograph connected with an 5975C MSD detector (Agilent Technologies, USA). 1-2 µL of each fraction was injected to a HP5-MS column (5 % phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 µm) and eluted with helium gas under a pressure of 10 psi. The oven temperature gradient was: keeping at 150 °C for 3 min, increasing to 300 °C at 10 °C/min, and keeping at 300 °C for 5 min. Electron ionization mode of GC-MS at 70 eV was applied (Okokon *et al*., 2022). The compounds in the hexane and Dichloromethane fractions were determined by comparison of spectral data in the NIST 2011 database.

Statistical Analysis

Data obtained from this work were analysed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software, (San Diego, USA). Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of Leaf Extract and Fractions on Body Weights of Rats

The body weights of the treated and untreated alloxan-induced diabetic rats were observed to have changed considerably within the period of study (Table 1). Treatment of the diabetic rats with the leaf extract and fractions produced a non – dose dependent increases in the body weight of the diabetic rats with the middle dose (140 mg/kg) having the highest weight increase. These increases which were significant ($p<0.05$-0.001) when compared to control were highest in the middle dose treated group (10.02%) followed by methanol fraction-treated group (8.77 %). (Table 1).

Effect of Extract and Fractions on Weights of Organs

Treatment of alloxan–induced diabetic rats with leaf extract and fractions of *S. anomalum* caused prominent decreases in the liver weights of the treated rats though non dose-dependently (Table 1). These decreases which were pronounced in groups treated with higher doses of the extract (140 and 210 mg/kg) and n-hexane fraction were only significant ($p<0.001$) in the group treated with methanol fraction (Table 1).

Antidiabetic Activity of the Leaf Extract and Fractions during Prolonged Treatment

The leaf extract produced significant ($p<0.05$-0.001) reductions in FBG levels of the diabetic rats following repeated treatment. These reductions which were non-

dose dependent were sustained throughout the duration of the study. These effects were comparable to that the standard drug, glibenclamide. On day 14, the effects were 64.08%, 63.43%, 65.43% and 64.54% for 70, 140, 210 mg/kg and glibenclamide respectively (Table 1). The various fractions produced sustained significant ($p<0.05$-0.001) reductions in FBG of the diabetic rats. The effects of the fractions on day 14 were 67.99, 54.66, 66.08 and 66.77% respectively for n-hexane, dichloromethane, ethyl acetate and methanol fractions. The effects exerted by *n*-hexane, ethyl acetate and methanol were better than that of standard drug, glibenclamide (Table 1).

Effect of Leaf extract and Fractions on Liver Function Test Parameters of Diabetic Rats.

Treatment of the diabetic rats with leaf extract and fractions of *S. anomalum* caused significant ($p<0.05$-0.001) reductions in the levels of total bilirubin, ALT, ALP and AST when compared to control. Direct bilirubin level was not affected by treatment with the extract and fractions. Although reductions were observed in AST levels of the treated diabetic rats, it was only significant ($p< 0.01$-0.001) at the highest dose (210 mg/kg) and methanol fraction treated group (Table 2).

Effect of Leaf Extract and Fraction on Liver Antioxidant Enzymes.

Treatment of alloxan-induced diabetic rats with leaf extract and fractions *of S. anomalum* caused significant ($p<0.05$-0.001) dose- dependent elevation in the levels of the antioxidant enzymes (SOD, CAT, GPX) when compared to control. Similarly, GSH level was significantly ($p<0.001$) elevated following treatment with the extract and fractions when compared to control. *n*-hexane fraction followed by methanol fraction exerted the highest activity. Similarly, there were significant ($p<0.05$-0.01) reductions in the level of MDA of the treated rats with ethyl acetate and DCM fractions having the most significant effects. Also, significant ($p<0.05$-0.01) increases in enzymes and GSH levels as well as significant ($p<0.01$) reductions in MDA level were observed with the standard drug, glibenclamide (Table 3).

Effect of Extract and Fractions on the Histology of Livers of Diabetic Rats

Histologic sections of livers of untreated diabetic rats revealed numerous hepatocytes with pyknotic nucleus, cellular and vascular degeneration, vascular congestion, hepatocytes hyperplasia and periportal inflammation. Livers of diabetic rats treated with glibenclamide (10 mg/kg) revealed reduced pathological signs with normochromic pyknotic nucleus. (Figure 1). Livers of rats treated with leaf extract (70 – 210 mg/ kg) revealed hyperchromic cellular profile with vascular congestion and numerous pyknotic nucleus. Livers of diabetic rats treated with the middle dose of the extract (140 mg/kg) showed no visible cellular abnormality (Figure 1). Livers of diabetic rats treated with n-hexane, dichloromethane, ethyl acetate and n-butanol fractions (140 mg/kg) revealed normochromic cellular profile containing periportal inflammation and pyknotic nuclei. There was no obvious cellular abnormality in the livers of diabetic rats treated with ethyl acetate fraction of the leaf. It could be considered slightly affected. (Figure 1).

Effect of Leaf extract and Fractions on Haematological Indices of Diabetic Rats

Administration of the leaf extract and fractions of *S. anomalum* caused significant ($p< 0.05$-0.001) increases in the levels of white blood cells, lymphocytes, and eosinophils, which was significant at the middle dose (140 mg/kg) when compared to control. Neutrophils, monocytes, basophils and platelets were reduced, while RBC, Hb concentration, platelets and pack cell volume were not affected significantly ($p>0.05$) by the administration of extract and fraction (Table 4).

Gas Chromatography-Mass Spectroscopy (GCMS) analysis

The phytochemical analysis of the most active fractions (n-hexane and dichloromethane) of *S. anomalum* revealed the presence of major compounds. The compounds in *n*-hexane fraction were bicyclo[3.1.1]heptanes-2,5,6-trimethyl-(1.alpha 2-beta, 5alpha-, squalene and beta.-sitosterol trimethylsilyl ether (Table 5). While 3,7,11,15-tetramethyl-2-hexadecen-1-ol, squalene and heptacosane were revealed to be present in DCM fraction (Table 6).

Table 1. Effect of leaf extract and fractions of *Solanum anomalum* on body and Liver weights of alloxan- induced diabetic rats.

Treatment	Dose mg/kg	Body Weight (g)			Weights of Liver (g)	Fasting Blood Glucose (mg/dl)	
		Day 0	Day 15	% Increase		0 HR	14th Day
Control normal saline	-	132.6 ± 18.34	129.3± 20.43	-2.48	6.72±0.98	266.0±17.16	249.0±14.01
Glibenclamide	10	132.0 ± 9.45	141.3± 12.33	7.04	6.10±0.15	233.0±12.00	82.6±8.19[b]
Extract	70	152.8 ± 15.56	163.0± 9.29	6.67	6.28±0.23	279.3±14.74	100.3±16.69[b]
	140	143.6 ± 12.55	158.0 ± 3.15	10.02	5.80±0.32	260.6±8.32	95.3±11.29 [a]
	210	140.8 ± 13.29	152.6 ± 7.22	8.38	5.82±0.67	257.0±3.71	88.3±8.83[b]
n- hexane fraction	140	142.9 ± 8.54	153.6 ± 6.48	7.48	5.82±0.65	264.3±14.50	84.6±40.05[c]
Dichloromethane fraction	140	138.4 ± 6.26	144.6± 13.71	4.68	6.14±0.35	236.0±37.60	107.0±14.52[b]
Ethyl acetate fraction	140	144.31 ± 8.50	156.6± 9.88	8.51	7.07±0.22	271.3±13.04	92.0±14.29[a]
Methanol fraction	140	145.8 ± 7.45	158.6± 10.55	8.77	0.98±0.07[c]	245.6±12.33	81.6±18.49[b]

Data are expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, when compared to control. (n=6).

Table 2. Effect of *S. anomalum* leaf extract and fractions on the liver function parameters of alloxan-induced diabetic rats.

Treatment	Dose (mg/ kg)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	Total Bilirubin (µmol/L)	Direct Bilirubin (µmol/L)
Control	10 mg/ml	97.00± 4.35	109.53±8.95	172.63±10.68	2.16±0.14	1.60±0.10
Crude extract	70	77.90±9.49	92.11± 2.67	162.42± 1.45	2.06± 0.29	1.16±0.03
	140	75.10±12.61	70.73±8.67	149.69±15.94	1.80± 0.05	1.10±0.15
	210	53.23±2.25[c]	61.92±8.67[b]	128.07±18.57[a]	1.50± 0.05	1.10±0.00
n- hexane Fraction	140	63.73±3.91[b]	100.23±9.68	130.48±8.54	1.60±0.00	1.10±0.10
Dichloromethane fraction	140	68.86±3.33[a]	62.92±6.76[b]	155.42±18.27	1.43±0.20[a]	1.10±0.05
Ethyl acetate fraction	140	70.40±4.19	67.43±5.44[b]	145.13±15.21	1.86 ± 0.31	1.10±0.11
Methanol fraction	140	58.2±5.51[c]	53.33±6.09[c]	106.98±0.75[c]	1.36± 0.08[a]	1.10±0.10
Glibenclamide	10	61.12±10.94[b]	60.40±6.54[b]	122.00±4.00[b]	1.66± 0.12	1.30±0.10

Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, when compared to control. (n=6).

Table 3. Effect of *S. anomalum* leaf extract on liver antioxidative stress markers in alloxan-induced diabetic in rats.

Parameters/ Treatment	Dose mg/kg	SOD (µg/mL)	CAT (IU/L)	GST (µg/mL)	GSH (µg/mL)	GPx (µg/mL)	MDA (µMol/mL)
Diabetic Control	10 ml/kg	0.18±0.03	3.68± 0.28	0.16±0.01	0.71±0.02	0.039±0.001	0.54±0.02
Crude extract	70	0.24±0.04[a]	4.11±0.23	0.21±0.02	0.72±0.03	0.055±0.002	0.52±0.02
	140	0.20±0.02	2.95±0.28	0.23±0.01[a]	0.81±0.02 [a]	0.056±0.002[c]	0.48±0.03
	210	0.30± 0.02[c]	3.53±0.17	0.31±0.01[c]	1.01±0.03[c]	0.059±0.002[c]	0.41±0.02[c]
n-hexane fraction	140	0.30±0.02[c]	5.86± 0.48[c]	0.23±0.01[a]	0.98±0.02[c]	0.057±0.002[c]	0.44±0.0[b]
Dichloromethane fraction	140	0.20 ±0.04	2.87±0.18	0.31±0.01[c]	0.82±0.01[a]	0.058±0.002[c]	0.38±0.01[c]
Ethyl acetate fraction	140	0.33± 0.02[c]	4.64±0.26	0.26±0.01[c]	0.92± 0.02[c]	0.048± 0.002[a]	0.31± 0.02[c]
Methanol fraction	140	0.37± 0.03[c]	4.33±0.56	0.32±0.01 [c]	0.87± 0.02[c]	0.049± 0.002[a]	0.43± 0.02[b]
Glibenclamide	10	0.29±0.02[c]	5.69±0.18 [b]	0.25±0.02 [b]	0.85±0.01[c]	0.051±0.003[b]	0.40±0.02[c]

Data were expressed as mean ± SEM. Significant at [a]p< 0.05, [b]p< 0.01, [c]p< 0.001 when compared to diabetic control. (n = 6).

Table 4. Effect of *Solanum anomalum* leaf extract and fractions on hematological parameters of alloxan-induced diabetic rats.

Treatment	Dose mg/kg	WBC (L)	NEUT. (%)	LYM (%)	MONO (%)	EOSINO (%)	BASO (%)	RBC (L)	HGB (g/dL)	PCV (%)	PLATELETS. (L)
Control normal saline	10	9.27± 1.95	59.00±1.60	35.66±1.65	4.66± 0.28	0.26± 0.21	1.00± 0.10	6.00± 0.28	12.13± 0.78	43.76±3.22	940.0± 35.76
Crude extract	70	11.60±1.56	51.86± 3.16	45.86±2.06 [a]	2.96± 0.24 [a]	0.16 ±0.06[c]	0.90± 0.05	6.72 ± 0.53	12.63± 0.56	43.20±2.36	709.3± 54.14[a]
	140	15.15±3.34 [a]	50.40±0.64	45.30±0.88 [a]	2.63± 0.29 [a]	0.76 ± 0.12[c]	1.10± 0.20	7.83± 0.78	14.10± 1.25[a]	53.76±7.61[a]	684.6± 92.13[a]
	210	9.86±1.66	57.93 ± 0.60	36.53±4.27	3.76± 0.38	0.66± 0.12[c]	0.50± 0.00	7.17± 0.18	11.10± 0.68	40.80±1.55	925.6± 61.50
n- hexane Fraction	140	9.90±1.51	59.50 ± 0.60	34.90±1.44	4.96± 0.03	0.43 ± 0.17[b]	0.60± 0.26	6.08± 0.69	12.46± 0.08	44.50±0.40	768.0± 82.92
Dichloromethane fraction	140	9.32±2.31	48.10±14.13	65.30±5.71[c]	3.40± 0.58	0.36 ± 0.51	0.90 ± 0.05	6.65 ± 0.90	12.56± 1.07	45.90±7.50	723.0± 35.80
Ethyl acetate Fraction	140	10.31±1.64	50.66±0.82	43.46±0.73	3.60 ± 1.01	0.30± 0.51	0.50± 0.17	7.28± 0.17	12.13± 1.21	43.90±4.43	775.0±59.53
MethanolFraction	140	14.55 ± 0.98[a]	47.60±6.45[a]	46.93±6.59 [a]	4.16± 0.72	0.40± 0.15[c]	0.53 ± 0.03	6.70 ± 0.63	13.33 ± 0.74	48.90±2.82	867.3±74.71
Glibenclamide	10	12.03±3.76	33.16±0.79[b]	61.56±1.66 [c]	2.23± 0.28 [a]	0.06± 0.03[c]	0.55± 0.03	7.56 ± 0.31	12.66±1.18	47.90±2.82	888.0±47.05

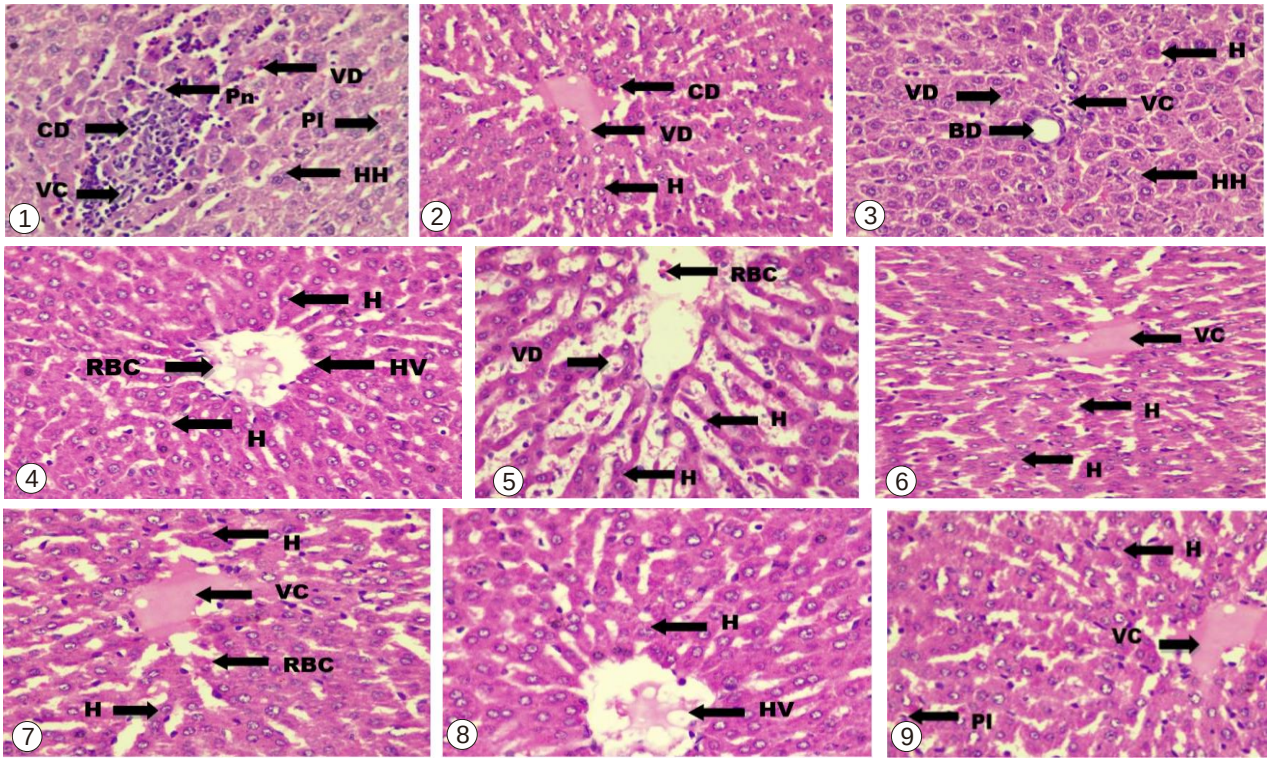
Data is expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$, when compared to control. (n=6).

Table 5. GCMS analysis of column hexane fraction of *S. anomalum*.

PEAK	RT	COMPOUND NAME	FORMULA	MOL. MASS
1.	10.044	Bicyclo[3.1.1]heptanes-2,5,6-trimethyl-(1.alpha 2-beta, 5alpha-	$C_{19}H_{38}O_5Si_4$	458.18
2.	18.315	Squalene	$C_{30}H_{50}$	410.39
5.	18.326	.beta.-Sitosterol trimethylsilyl ether	$C_{32}H_{58}OSi$	486.43

Table 6. GCMS analysis of column DCM fraction of *S. anomalum*.

PEAK	RT (min)	COMPOUND NAME	FORMULA	MOL. MASS
1.	10.044	cis-Pinane	$C_{10}H_{18}$	138.25
2.	10.524	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.1
3.	10.547	1,4-Eicosadiene	$C_{20}H_{38}$	278.5
4	18.315	Squalene	$C_{30}H_{50}$	410.39
5.	22.836	Heptacosane	$C_{27}H_{56}$	380.44



Keys: Vascular congestion (**VC**), Vascular degeneration (**VD**), Cellular degeneration (**CD**), Periportal inflammation (**PI**), Pyknotic nucleus (**Pn**), Red blood cell (**RBC**), Hepatocytes (**H**), Hepatocytes hyperplasia(**H**). Hepatic vein (**HV**), Bile duct (**BD**), and Pyknotic nucleus (**Pn**)

Figure 1: Histological sections of Livers of alloxan-induced diabetic rats treated with Normal saline 10 mL/kg (1), Glibenclamide 10 mg/kg bw (2), leaf extract 70 mg/kg bw (3), leaf extract 140 mg/kg bw (4), leaf extract 210 mg/kg bw (5), n-hexane fraction 140 mg/kg (6) dichloromethane fraction 140 mg/kg (7), ethyl acetate 140 mg/kg (8), Methanol fraction 140 m/kg (9), at Magnification A(x400), stained with H&E Method.

Discussion

The leaf of *S. anomalum* is use in ethnomedicine for the treatment of various ailments such as malaria, gastrointestinal disorders and diabetes (Okokon *et al.*, 2016). This work was designed to evaluate antioxidative and hepatoprotective potentials of leaf extract and fractions of *S. anomalum* in alloxan-induced diabetic rats.

The body weights of diabetic rats were found to increase significantly following treatment with the leaf extract and fractions. Diabetes is associated with a severe loss in body weight due to loss or degradation of structural proteins (Rajkumar and Govindarajulu, 1991). Treatment with the leaf extract and fractions remedied this situation perhaps due to the alleviation of hyperglycemic state and stimulation of protein synthesis.

The leaf extract and fractions treatments were observed in this study to cause significant increases in WBC, lymphocytes and eosinophils, while neutrophils, monocytes and basophils were significantly reduced. Other parameters were not affected. The increases maybe due to immunological responses by the body defence mechanism to heal or repair the injuries caused by alloxan (Soetan *et al.,* 2013) which can be attributed to the immunostimulatory effect of diosgenin (Nimbalkar *et al.,* 2018) and squalene.

Metabolism of alloxan to dialuric acid causes the production of H_2O_2, •OH and superoxide radical (Mathews and Leiter, 1999)., which destroys β-cell necrosis and induces diabetes by partial destruction of pancreatic β-cells of islet of Langerhans (Cakici *et al.,* 1994; Abdel-Barry *et al.,* 1997). This reduces insulin levels, raise the blood glucose level thereby leading to type 2 diabetes mellitus, but with residual pancreatic β-cells with potentials to secrete insulin (Sheela and Augusti, 1992; Subramoniam *et al*., 1996). In this study, *S. anomalum* leaf extract/fractions were observed to demonstrate sustained significant antidiabetic activities during prolonged study with the methanol, n-hexane and ethyl acetate fractions exerting prominent activities. The fasting blood glucose (FBG) levels of the treated diabetic rats were significantly reduced when compared to those of untreated diabetic rats (control). The antidiabetic results observed in this study corroborate that of other species of *Solanum* such as *S. nigrum* (Sengottaiyan *et al.,* 2012; Umamageswari *et al*.,2017), *S. trilobatum* (Doss *et al.,* 2009), *S. xanthocarpum* (Selvi and Yogananth, 2016) and *Solanum villosum* (Nyaga *et al.,* 2019) Thus, confirming strongly the antidiabetic potentials of this plant in ethnomedicine.

The leaf extract and fractions were observed in this study to cause significant decrease in weight of liver. Generally, internal organs weights are considered as important indicator to injury and toxicities (Farah *et al.,* 2013). Hypertrophy of organs often indicates toxicity and damaged to organ (Ping *et al*., 2013). This often results from oedema due to inflammation of the organs. Free radicals generated during alloxan metabolism cause destruction of hepatic, pancreatic and kidney cells and tissues (Mathews and Leiter, 1999). The decrease in weights of liver by the extract/fractions especially DCM and methanol fractions-treated group, is as a result of protective effect of the fractions against the effect of free radicals generated by alloxan and diabetic condition perhaps due to its hypoglycemic and antioxidant activities of the phytoconstituents (Okokon *et al.,* 2019a) such as diosgenin (Kanchan *et al.,* 2016), 1-octacosanol and octacosane (Leng *et al.,* 2020; Sengupta *et al.,* 2018; Rhetso *et al.,* 2018), squalene (Gunes, 2013; Micera *et al.,* 2020), β-sitosterol (Gupta *et al.,* 2011; Baskar *et al.,* 2012) and phenolic compounds in the leaf extract.

Metabolism of alloxan leads to the generation of free radicals which attack organs and cause oxidative stress which contributes to diabetes complications in animals or humans (Baynes and Thorpe, 1999). Reactive oxygen species (ROS) produce cellular and tissue injury through covalent binding, DNA strand breaking, lipid peroxidation (LPO) and augment fibrosis which is also implicated in other disease conditions (Sirnivasan *et al.,* 2007; Paradies *et al.,* 2011).

The results of this study show that oxidative stress was duly induced by alloxan in the diabetic rats as reflected in the marked reductions in the levels of SOD, CAT, GSH and GPx in hepatic tissues as well as significant increase in MDA levels in the liver of the diabetic rats. This finding agrees with earlier findings that the activities of these antioxidants are known to reduce during diabetes (Parmar and Kar,2008; Kostolanska *et al.,* 2009; Dixit and Kar, 2010). The oxidative stress status of the diabetic rats is further supported by marked elevations in the serum levels of AST, ALT, ALP, and total bilirubin levels of the diabetic rats. Reduction in liver levels of these oxidative markers following treatments with the leaf extract and fractions strongly suggest the great potentials of leaf extract and fractions in attenuating oxidative stress associated with type II diabetes mellitus which was probably mediated via free radical scavenging activities and improving glutathione status in the tissues by its phytochemical constituents such as diosgenin, 1-octacosanol, octacosane and β-sitosterol (Gupta *et al.,* 2011; Baskar *et al.,* 2012;Kanchan *et al*., 2016;Leng *et al*., 2020; Sengupta *et al*., 2018; Rhetso *et al.,* 2018).

Disease conditions such as diabetes affect the liver and changes the hepatic function as shown by increases in the levels of AST, ALT, ALP, total and direct bilirubin indicates liver damage which are also detected in human diabetes (Takaike *et al.,* 2004). Damage to liver cells often results in leakage of enzymes such as aspartate aminotransferase (AST), and alanine aminotransferase (ALT) into the blood, while blood levels of alkaline phosphatase (ALP) rise when the bile flow is slow or blocked (Prat and Kaplan, 2000).

In this study, treatment of the diabetic rats with the leaf extract and fractions was found to cause significant reductions in the levels of ALT, ALP and total bilirubin. The integrity of hepatocytes is assessed by levels of the serum liver enzyme markers such as AST, ALT and ALP (Moss and Henderson, 1999). Serum aminotransferases levels (ALT and AST) are two of the most useful measures of liver parenchymal cell injury. AST is raised in acute liver damage, but is also present in RBCs, kidney, testis, cardiac and skeletal muscles, so it is not specific to the liver, while ALT is almost exclusively found in the liver (Nyeblom *et al.,* 2006). Elevated levels of AST and ALT indicate liver damage but are not good measures of liver function since they do not reliably reflect the synthetic ability of the liver and may come from tissues other than the liver such as muscles. The leaf extract and fractions produced

significant reduced the elevated levels of these enzymes. This further corroborates histologic findings. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of diabetics and are also responsible for the increased gluconeogenesis and ketogenesis (Udayakumar *et al.,* 2009). Diabetes and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic rats (Udayakumar *et al.,* 2009). This suggests that alloxan-induced diabetes caused lipid peroxide mediated tissue damage in the liver. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and then migrating into the blood stream. The decrease in AST, ALT and ALP levels in leaf extract and fractions treated groups indicates the protective effect on the liver.

Total bilirubin level which was increased in the untreated diabetic rats was observed in this study to be significantly reduced in groups treated with the husk extract and fractions. Bilirubin, is a metabolic product of hemoglobin which undergoes conjucation with glucuronic acid in hepatocytes to increase its water solubility. Determination of bilirubin represent an index for the assessment of hepatic function, severity of necrosis, conjugation and excretory capacity of hepatocytes and any abnormal increase in the level of bilirubin in the serum indicate hepatobililiary disease and severe disturbance of hepatocellular function (Martin and Friedman, 1992). Decrease in serum bilirubin level of the diabetic rats after treatment with the leaf extract and fractions indicated extract/fractions effectiveness to restore normal functional status of the liver.

The above activities of the extract/fraction show that the leaves extract and fractions posses hepatoprotective potential against alloxan-induced hepatic injury. These results corroborate histologic findings which show significant hepatoprotective potentials of the extract/fractions. This effect could be due to the antioxidant /free radical scavenging activities of the extract and fractions (Okokon *et al.,* 2019a), squalene, β-sitosterol (Ivorra *et al.,* 1988; Gupta *et al.,* 2011; Baskar *et al.,* 2012; Micera *et al.,* 2020) as well as the isolated compounds; diosgenin, 1-octacosanol and octacosane (Kanchan *et al*., 2016; Leng *et al*., 2020; Sengupta *et al*., 2018; Rhetso *et al.,* 2018).

Reports have been indicated that persistent hyperglycemia causes increased production of oxidative stress in alloxan-induced diabetes (Bonnefont *et al.,* 2000). Hence, excessive ROS produced leads to oxidative damage and increased Lipid Peroxidation (LPO). The results of this study showed a significant increase in LPO levels resulting in high level of MDA in alloxan-induced diabetic rats liver with accompanying reduction in the levels of oxidative stress markers (SOD, CAT, GPx and GSH) in the liver. This reduction in the levels of antioxidant enzymes in this study corroborates those of earlier reports (Parmar and Kar, 2008; Kostolanska *et al.,* 2009; Dixit and Kar, 2010).

In *in vivo* experimental models, tissue oxidative stress markers such as SOD, CAT and GSH are useful and reliable markers of antioxidant status while MDA is a sensitive and reliable marker for lipid peroxidation (Kumar *et al*., 2010; Feillet-Coudray *et al.,* 1999). SOD plays an important role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide, which in mammals is readily reduced to water principally by CAT and GPx. GPx plays a pivotal role in minimizing the oxidative stress. GPx and GST work together with GSH and decompose H_2O_2 and other organic hydroperoxides to non-toxic products. Glutathione, reduced form GSH is ubiquitous tripeptide thiol, is a vital intra/extra-cellular protective antioxidant against oxidative/nitrosative stress. Glutathione reductase is an enzyme responsible for its conversion back to the reduced state (Kayali and Tarhan, 2006; Okutana *et al.,* 2005). The antioxidant enzymes levels were found to be reduced in untreated alloxan-induced diabetic rats. Hence, the changes of these biomarkers is in accordance with the decrease in antioxidant state in the body as other reports (Ei-Missiry and Ei-Gindy, 2000; Meral *et al.,* 2001). In the present study, administration of the extract and fractions significantly counteract the changes of oxidative stress biomarkers in alloxan-induced rats thus preventing the accumulation of excessive oxidative stress with corresponding increases in the levels of antioxidant enzymes/oxidative stress markers. This activity is due to the antioxidant activities of the phytochemicals such as squalene, β-sitosterol, diosgenin, 1-octacosanol, octacosane and other phenolic compounds found to be present in the leaf extract. Similarly, β-sitosterol and diosgenin present in the extract/fractions have been reported to cause reduction in hepatic lipid peroxidation and elevation in the activities of catalase, superoxide dismutase and glutathione (Gupta *et al.,* 2011b; Baskar *et al.,* 2012; Kanchan *et al.,* 2016).

The liver of the extract/fractions treated diabetic rats were found to be significantly protected from the effects of free radicals generated by alloxan. This protection was visible as the livers of the treated rats lacked or had reduced pathological signs such as vascular degeneration and congestion as well as periportal inflammation observed in the untreated diabetic rats. These findings corroborated that of chemical pathology and therefore suggest hepatoprotective activity against oxidative stress induced by alloxan. The protection is due to the free radical scavenging potentials of the phytoconstituents of the extract and fractions such as squalene, β-sitosterol, diosgenin, 1-octacosanol, octacosane as well as the antioxidant activity of other phenolic compounds present in the extract (Okokon *et al.,* 2019a). The hepatoprotective effect of *Solanum anomalum* in this study corroborate that previously

reported on other species of *Solanum* such as *S. nigrum, S. macrocapon and S. xanthocarpum* (Lin *et al.*, 2008; Gupta *et al.*, 2011a; Adewale *et al.*, 2015; Liu *et al.*, 2016).

CONCLUSION

The results of this study show that the leaf fractions of *Solanum anomalum* possess liver protective and antioxidant potentials which may be attributed to the activities of its phytochemical constituents.

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