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Hypoglycemic, antidyslipidemic, and antioxidant activities of methanol extract of *Struchium sparganophora* leaves in alloxaninduced oxidative stress-mediated diabetes in rats



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Abstract

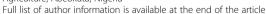
Background: Diabetes mellitus is clinically underlined by hyperglycemia and dyslipidemia. In view of this, the current study assessed the glycemic and lipidemic control potentials of methanol extract of *Sruchium sparganophora* leaves (SPA) in the alloxan-induced diabetic model using male *Wistar* rats. Experimental diabetes was induced through a single intraperitoneal injection of 120 mg/kg freshly prepared alloxan. Thirty-six rats were randomly assigned into six groups of normoglycemic control, untreated diabetic group, and diabetic treated with (i) metformin (12 mg), (ii) metformin 12 mg + SPA 300 mg/kg, (iii) SPA 300 mg/kg, and (iv) SPA 600 mg/kg *per os* twice at 9.00 and 18.00 h daily for 10 days. Fasting blood glucose (FBG) level and markers of dyslipidemia, and oxidative stress markers were determined.

Results: SPA at selected doses decreased fasting blood glucose which was significantly (p < 0.05) raised by alloxan. Increase in plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) concentrations and decrease in HDL-cholesterol (HDL-C) concentration (p < 0.05) caused by alloxan were significantly moderated by SPA at selected doses. Glutathione-s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activities reduced by alloxan (p < 0.05) in both the liver and pancreas were reversed by SPA 300 and 600, and its combination with metformin. Decreased reduced glutathione (GSH) concentration in alloxan diabetic rats was also reversed by the extract, while the level of malondialdehyde (MDA) exacerbated by alloxan (p < 0.05) in the tissues was decreased by the extracts.

Conclusion: *Struchium sparganophora* possesses considerable antihyperglycemic, antidyslipidemic, and antioxidant potentials without compromising organ functionality.

Keywords: Alloxan, Diabetes, Dyslipidemia, Oxidative stress

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Background

Diabetes mellitus (DM) is a disease associated with fat, carbohydrate, and protein metabolic disorders due to defects in insulin secretion, insulin action (sensitivity), or both [1]. Patients with diabetes have a high incidence of cardiovascular disease especially hypertension, atherosclerosis, cardiomyopathy, and microvascular damage. In fact, it is the most comorbid condition reported in patients with hypertension [2]. Besides hyperglycemia, several other comorbidities such as dyslipidemia (imbalance in lipid homeostasis) and free radical generation are involved in the development of diabetes-related cardiovascular complications which are the major cause of morbidity and death.

Struchium sparganophora (Linn.) Ktze. belongs to the Asteraceae family. It is a fibrous culinary shrub that grows in the tropical Africa. The leaf is commonly called water bitter leaf and locally called "ewuro odo" among the Yoruba tribe of South Western Nigeria. It is a water plant consumed as a leafy vegetable in soup preparations among the rural occupants in Nigeria. It is used ethnomedicinally for the management of diabetes mellitus, malaria, measles, headache, and gonorrhea [3-6]. The nutritional, antioxidant, antimicrobial, and antimalarial activities of leaves of S. sparganophora have been documented [6-8]. Sesquiterpene lactone has been isolated from the plant [5]. In vitro study on α -amylase and α glucosidase inhibitory activities suggested the leaves could possess hypoglycemic activity [9]. Although there has been increasing information on the nutritional and medicinal significance of the plant in question, there is little available information on the possible pharmacological mechanisms of actions of the plant to substantiate the antidiabetic property ascribed to it from ethnomedicinal and in vitro studies. This study, therefore, sought to determine the hypoglycemic potential and possible pharmacology mechanisms of actions of methanol extract of S. sparganophora leaves in alloxaninduced diabetic rats

Methods

Chemical and drugs

Alloxan was obtained from Sigma-Aldrich (Germany) and metformin HCl from Shuangwei Pharm. Co. Ltd (China). Other reagents used for this study were of analytical grade.

Plant sample preparation

Struchium sparganophora was obtained from Badeku farm in Ibadan and was authenticated and deposited in herbarium with voucher number UIH 22655 by Mr. Donatus, a botanist at the University of Ibadan, Ibadan, Nigeria. The leaves were separated from the stems, dried, and pulverized at room temperature. Three

hundred grams of the powdered leaves was weighed and extracted with 2.4 L of absolute methanol, centrifuged at 2000 rpm for 5 min; the supernatant was collected; and the solvent was evaporated at 40 °C on a rotary evaporator to obtain the crude methanol extract of the leaves.

Assay for drug active content

Standard metformin HCl active content was assayed using the standard procedure before use [10].

Experimental design and animal care

Thirty-six male rats weighing 145–153 g were obtained from the Department of Veterinary Medicine, the University of Ibadan, transported to the animal house, Department of Biochemistry, Lead City University, Ibadan. The rats were handled humanely and managed according to the protocol of the Animal Research Ethics Committee of Lead City Ethical Review Board (LCU/ERB). The rats were kept in plastic cages in 12-h light and dark conditions. They were acclimatized for 2 weeks prior to the study and served vital feed and water throughout the study.

Using stratified randomization based on bodyweight and blood glucose level, the rats were divided into six groups (n = 6/group). Group A was the control (no treatment), and diabetes was induced in groups B-F by intraperitoneal injection of alloxan (120 mg/kg). Rats that showed fasting blood glucose levels of above 220 mg/dL were taken to be diabetic. The animal groups were assigned treatment as follows: B-no treatment (ALO); C-metformin as standard, 12 mg/kg (MET); and D—metformin 12 mg/kg + SPA 300 mg/kg (MET + SPA 300), SPA 300 mg/kg (SPA 300), and SPA 600 mg/kg extract (SPA 600). The rats were treated twice daily at 9.00 h and 18.00 h for 10 days. ACCU-CHECK glucometer with strips was used to check blood glucose concentration. Bodyweight of the animals was determined before administration of alloxan (day 0) and after treatment (day 10). Fasting blood glucose concentration was checked before administration of alloxan, 48 h after administration of alloxan (before treatment), and on the last day of the treatment. On the 10th day of treatment, the rats were fasted overnight (8 h). Blood was collected by ocular puncture into plain and lithium heparinized bottle; the blood samples were centrifuged at 3000 rpm for 15 min to obtain the serum and plasma, respectively. The rats were euthanized with ether, and their liver, kidney, and pancreas were excised, washed (in 1.15% KCl), and homogenized in 10 mM phosphate buffer pH 7.4 (1: 4), and centrifuged at 12,500rpm to obtain the postmitochondria fractions

Serum and plasma biochemical analyses

In the serum, alanine aminotransferase and aspartate aminotransferase were assayed using the method

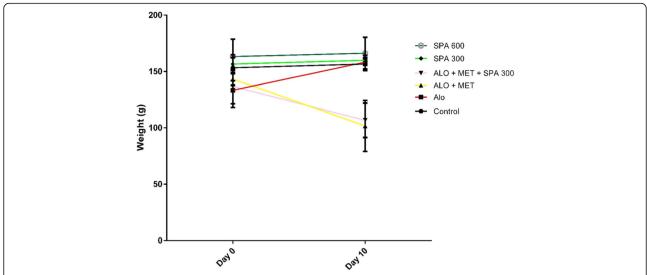


Fig. 1 Effect of methanol extract of *S. sparganophora* leaves on the bodyweight of alloxan-induced hyperglycemic rats. Results were expressed as mean + standard deviation

described by Reitman and Frankel [11], and alkaline phosphatase was assayed with the method described by Englehardt et al. [12]. In the plasma, triglyceride was determined using the method of Fossati and Prencipe [13], HDL-C was determined with the method of Lopez-Virella et al. [14], and total cholesterol was determined using the method of Zollner and Kirsch [15]. LDL-C and VLDL-C were calculated from the obtained lipid parameters using the formula by Friedewald et al. [16]. Cholesterol ratio (CR) was calculated with the formula:

$$Cholesterol\ ratio = \frac{TC}{HDL - C}$$

Atherogenic index (AI) was calculated using the following formula:

$$AI = \frac{TC - HDL - C}{HDL - C}$$

Tissue oxidative stress markers

The following were determined in the post-mitochondria fractions of the liver, kidney, and pancreas of the rats: total protein (TP) [17], lipid peroxidation (MDA) [18], reduced glutathione (GSH) [19], glutathione-s-transferase (GST) [20], superoxide dismutase activity (SOD) [21], and catalase activity (CAT) [22].

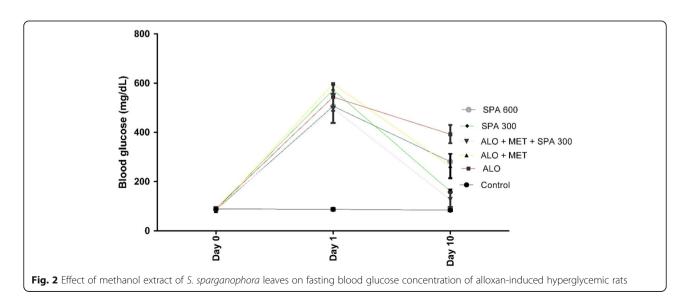


Table 1 Effect of methanol extract of *S. sparganophora* leaves on liver enzyme activities in alloxan-induced hyperglycemic rats

Groups	ALT (IU)	AST (IU)	ALP (IU)
Control	4.68 ± 0.36	17.67 ± 0.21	50.03 ± 6.00
ALO	7.52 ± 0.67^{a}	20.29 ± 0.11 ^a	68.93 ± 1.47^{a}
MET	10.96 ± 0.11 ^{a,b}	18.66 ± 0.52	55.20 ± 0.08^{b}
MET + SPA 300	$5.42 \pm 0.37^{b,c}$	$14.07 \pm 1.08^{a,b,c}$	49.98 ± 2.29 ^b
SPA 300	$5.23 \pm 0.32^{b,c}$	15.71 ± 0.05 ^{b,c}	53.36 ± 4.25 ^b
SPA 600	$5.56 \pm 0.47^{b,c}$	$13.42 + 2.30^{a,b,c}$	49.45 ± 1.22 ^b

The results were expressed in mean \pm standard deviation

Data analyses

Analyses were performed using GraphPad Prism 6.05 (GraphPad Inc., USA). Parameters were presented as mean ± standard deviation of animals in each group. Values in all groups were tested for differences with one-way analysis of variance coupled with Tukey's multiple comparisons test.

Results

Prior to treatment, the average weight of alloxan diabetic rats used was 147.78 ± 11.63 , and 18% increase in bodyweight was observed in rats that were administered alloxan alone, while metformin and metformin supplemented with 300 mg/kg BW caused 29.4% and 22.1% decrease in bodyweight of the rats. The trend in the effect of SPA on the bodyweight of alloxan-induced diabetic rats can be found in Fig. 1.

Alloxan at 120 mg/kg BW produced the hyperglycemic conditions in the rats. This was effectively reversed by a combination of metformin with SPA 300. Considerable reductions of blood glucose were seen in rats treated with metformin alone, and extract at selected doses (Fig. 2).

Alloxan caused an increase in serum ALT and AST activities. ALT was further in the rats that were treated with metformin alone. The extract was able to reverse the effect of alloxan on serum ALT and AST activities (Table 1). Alloxan also increased the serum activity of ALP, and treatment with the extract (SPA) or when the

low dose is combined with metformin reversed the effect of alloxan on serum ALP (see Table 1).

Intraperitoneal administration of $120\,\mathrm{mg/kg}$ BW alloxan causes a marked increase in total cholesterol, and this effect was reversed (p < 0.05) by treatment with the plant extract alone or in combination with metformin. Serum HDL-cholesterol reduced in rats administered alloxan alone. The combination of low-dose extract and metformin markedly increases the HDL-cholesterol level. More information on the effect of S. spargano-phora on the lipid profile of alloxan-induced diabetic rats can be found in Table 2. Increased cardiac risk and atherogenic risk index were noted in rats administered alloxan alone when compared to the control and treatment groups (see Table 3).

Treatment with metformin markedly increased total protein concentration in the liver of the rats. More detail on total protein concentration in the liver, kidney, and pancreas can be found in Table 4. SPA effectively reversed (p < 0.05) increased the level of malondialdehyde caused by alloxan in the liver, kidney, and pancreas of the rats (Table 4). This extract markedly increased (p < 0.05) reduced glutathione concentration in the liver and pancreas of hyperglycemic rats better than metformin, though only SPA 600 increased level of glutathione in the kidney (Table 4).

SPA markedly increased catalase activity in the liver, kidney, and pancreas of alloxan diabetic rats. The extract also increased activities of SOD in the liver and pancreas, though kidney SOD activities were found to decrease in rats treated with the extract. In this finding, GST activity was markedly decreased by alloxan in the liver and kidney of the rats; meanwhile, SPA reverses the effect by increasing the activity in a manner comparable to metformin, though treatment with SPA and metformin does not normalize GST activity in alloxan diabetic rats. Further detail on antioxidant enzymes can be found in Table 5.

Discussion

Green leafy vegetables are plants of medicinal values that have been used in the management of different diseases.

Table 2 Effect of methanol extract of S. sparganophora leaves on lipid profile of alloxan-induced hyperglycemic rats

Groups	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)	VLDL-C (mg/dL)
Control	19.13 ± 0.57	7.14 ± 0.41	1.39 ± 0.46	49.25 ± 7.40	9.855 ± 1.48
ALO	21.72 ± 0.69^{a}	3.27 ± 0.42^{a}	3.29 ± 0.45^{a}	75.99 ± 4.30^{a}	15.40 ± 0.56^{a}
MET	19.29 ± 0.19 ^b	6.94 ± 0.73^{b}	2.57 ± 0.43	46.34 ± 3.32^{b}	9.265 ± 0.66^{b}
MET + SPA 300	18.75 ± 0.69 ^b	6.52 ± 0.39^{b}	1.69 ± 0.81^{b}	53.95 ± 4.40^{b}	10.29 ± 0.37^{b}
SPA 300	18.23 ± 1.63^{b}	6.38 ± 0.33^{b}	1.96 ± 0.53^{b}	41.18 ± 1.65 ^b	8.235 ± 0.33^{b}
SPA 600	17.29 ± 1.17 ^b	6.22 ± 0.49^{b}	2.13 ± 0.63	38.26 ± 6.57^{b}	$7.653 \pm 1.31^{a,b}$

The results were expressed in mean \pm standard deviation

^aSignificant when compared to control at p < 0.05

^bSignificant when compared to ALO at p < 0.05

^cSignificant when compared to ALO + MET at p < 0.05

^aSignificant when compared to control at p < 0.05

^bSignificant when compared to ALO at p < 0.05

cSignificant when compared to ALO + MET at p < 0.05

Table 3 Effect of methanol extract of *S. sparganophora* leaves on cardiovascular disease index in alloxan-induced diabetic rats

Groups	Cardiac risk index	Atherogenic risk index
Control	2.67	1.68
ALO	6.64 ^a	5.64 ^a
MET	2.78 ^b	1.78 ^b
MET + SPA 300	2.88 ^b	1.87 ^b
SPA 300	2.86 ^b	1.85 ^b
SPA 600	2.78 ^b	1.78 ^b

^aSignificant when compared to control at p < 0.05

This study evaluated the antihyperglycemic, antidyslipidemic, and antioxidant potentials of methanol extract of Struchium sparganophora leaves in the alloxan diabetic model. The ardent antihyperglycemic and antihyperlipidemic efficacies produced by this plant extract, especially when combined with metformin, revealed that the plant possesses phytoconstituent(s) with hypoglycemic efficacy or capable of being synergistic or potentiating the effect of metformin. The hypoglycemic efficacies of this plant involve bodyweight reduction. Increased bodyweight and dyslipidemia are comorbid and complications of diabetes mellitus. Consumption of vegetable-rich diets has been reported to serve a beneficial role in preventing obesity and diabetes mellitus and promoting health as they are rich sources of dietary fibers and phenolic antioxidants with low calories [23, 24]. Major features of dyslipidemia in diabetes mellitus are elevated plasma level of TC, LDL-C, and triglycerides, with decreased level of HDL-C. There is limited glucose utilization by body cells, which call for the need for another source of energy; hence, there is increased secretion of fatty acids in adipose tissues which are converted in the hepatocytes to triglycerides and cholesterol then released into circulation. There is also increased VLDL-C, which accumulatively caused decreased HDL-C concentration.

In the current study, the irregular lipid homeostasis was observed in rats administered alloxan alone, and this finding corroborates with several other studies [25, 26]. This effect has resulted in increased cholesterol ration and atherogenic risk index. SPA at selected dosages seems to sufficiently mitigate dyslipidemia via reducing storage form of fat (triglycerides) and increasing HDL-C to an extent comparable to metformin. Treatment with SPA moderated LDL-C, VLDL-C, and total cholesterol level in the plasma of alloxan rats. Several other alcohol extracts of medicinal plants have been documented with antidyslipidemic effects in alloxan and another model of diabetes rats. Akinloye and Solanke [25] reported a marked reduction in serum triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol in alloxan-induced hyperglycemic rats treated with Pigeon pea methanol extract. Sapium ellipticum leaf ethanol extract has been reported to maintain lipid homeostasis in streptozocin-induced diabetic rats [27].

In addition to the hypoglycemic effect and moderation of dyslipidemia, the leaf extract exhibited medicinal efficacies by reducing some markers of tissue injuries. SPA decreases plasma activities of ALT, AST, and ALP activities which were increased in alloxan diabetic rats in this study. Administration of alloxan could have resulted in leakage of these enzymes from the liver and some other tissues into circulation, indicating the injury of these tissues. The effect of SPA on liver enzyme markers is in agreement with several other studies on medicinal plant effects in diabetic rats [27, 28].

Administration of methanol extract of *Struchium sparganophora* leaves to alloxan diabetic rats moderated tissue oxidative stress markers in the selected tissues. Oxidative stress in the selected tissues, as seen in alloxan diabetes rats, occurs when superoxide dismutase, catalase, and glutathione systems (the endogenous antioxidant system) which effectively break down hydrogen peroxide and hydroperoxides to harmless molecules are

Table 4 Effect of methanol extract of *S. sparganophora* leaves on total protein, malondialdehyde, and reduced glutathione in alloxan-induced hyperglycemic rats

Group	TP (mg/g tissue)			MDA (nM MDA/mg protein tissue)			GSH (nM GSH/mg protein tissue)		
	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas
Control	33.06 ± 2.11	35.41 ± 1.48	39.74 ± 0.89	32.97 ± 4.09	45.25 ± 3.73	35.04 ± 2.12	32.26 ± 2.14	42.26 ± 2.14	3.01 ± 0.23
ALO	48.60 ± 9.39^{a}	36.59 ± 2.08	34.18 ± 0.56^{a}	64.75 ± 3.11	75.68 ± 6.11^{a}	40.14 ± 6.01	20.09 ± 4.12^{a}	37.09 ± 4.12	2.05 ± 0.07^{a}
MET	$58.39 \pm 0.27^{a,b}$	35.44 ± 0.50	$36.84 \pm 0.79^{a,b}$	$23.53 \pm 0.40^{a,b}$	$61.09 \pm 0.08^{a,b}$	49.30 ± 0.25^{a}	26.95 ± 1.75^{b}	40.00 ± 2.75	2.77 ± 0.04^{b}
MET + SPA 300	39.91 ± 2.68 ^c	$28.18 \pm 0.67^{a,b,c}$	39.41 ± 0.83b	$45.42 \pm 5.12^{a,b,c}$	$49.53 \pm 4.44^{b,c}$	24.67 ± 0.18^{b}	$26.23 \pm 3.04^{a,b}$	41.23 ± 3.04	3.20 ± 0.35^{b}
SPA 300	$31.08 \pm 1.15^{b,c}$	$28.99 \pm 2.41^{a,b,c}$	$41.21 \pm 1.07^{b,c}$	$51.44 \pm 5.61^{a,b,c}$	$44.15 \pm 9.25^{b,c}$	31.71 ± 0.18^{c}	29.30 ± 3.70^{b}	39.30 ± 3.70	2.85 ± 0.22^{b}
SPA 600	$36.46 \pm 0.47^{b,c}$	$31.19 \pm 1.89^{a,b,c}$	$39.70 \pm 2.19^{b,c}$	$52.93 \pm 2.49^{a,b,c}$	$43.21 \pm 1.11^{b,c}$	39.27 ± 0.94 ^c	$33.04 \pm 1.08^{\circ}$	43.04 ± 1.08^{b}	3.25 ± 0.29^{b}

Results were expressed in mean \pm standard deviation

^bSignificant when compared to ALO at p < 0.05

 $^{^{}c}$ Significant when compared to ALO + MET at p < 0.05

^aSignificant when compared to control at p < 0.05

 $^{^{\}mathrm{b}}$ Significant when compared to ALO at p < 0.05

^cSignificant when compared to MET at p < 0.05

Table 5 Effect of methanol extract of *S. sparganophora* leaves on antioxidant enzymes in alloxan-induced hyperglycemic rats

Group	CAT (µmol H ₂ O ₂ consumed/min/mg protein)			SOD (nmol/min/mg protein)			GST (nmol CDNB-GSH/min/mg protein)		
	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas
Control	23.46 ± 1.30	18.09 ± 1.30	7.64 ± 0.65	43.95 ± 2.06	33.95 ± 2.06	20.47 ± 0.98	43.66 ± 3.30	34.82 ± 3.30	30.82 ± 3.30
ALO	$17.31 + 0.97^{a}$	15.31 ± 0.97^{a}	5.90 ± 0.70^{a}	27.87 ± 3.22^{a}	30.87 ± 3.22	13.67 ± 1.2^{a}	27.44 ± 2.10^{a}	31.44 ± 4.10	17.44 ± 2.10^{a}
MET	21.95 ± 1.76^{b}	15.95 ± 1.76	6.00 ± 0.65^{a}	33.59 ± 2.11^{a}	30.59 ± 2.11	18.67 ± 2.57^{b}	26.97 ± 3.40^{a}	35.07 ± 3.40	$25.07 \pm 3.40^{a,b}$
MET + SPA 300	22.32 ± 0.55^{b}	18.11 ± 0.55^{b}	6.41 ± 0.21^{a}	33.21 ± 3.41^{a}	31.21 ± 3.41	17.98 ± 1.01 ^b	$32.05 \pm 0.95^{a,b}$	37.00 ± 0.95^{b}	27.00 ± 0.95^{b}
SPA 300	$21.04 \pm 0.40^{a,b}$	17.60 ± 1.40	6.35 ± 0.22^{a}	27.10 ± 3.90^{a}	29.10 ± 3.90	18.03 ± 0.22^{b}	29.03 ± 0.40^{a}	34.03 ± 0.40	$20.03 \pm 0.40^{a,c}$
SPA 600	22.09 ± 1.09^{b}	18.77 ± 1.18 ^c	6.21 ± 0.85^{a}	$35.39 \pm 2.88^{a,b}$	29.39 ± 2.88	17.95 ± 0.58 ^b	$37.90 \pm 1.74^{a,b}$	32.90 ± 1.74	$22.90 \pm 1.74^{a,b}$

Results were expressed in mean ± standard deviation

compromised as a result of overproduction of reactive species [29]. It has been reported in several studies that alloxan (a glucose analog used for diabetic models in animals) caused an increase in markers of oxidative stress in the liver, kidney, and pancreas of rats [30-32]. Also, diabetes has been reported to have a detrimental effect on the activities of endogenous antioxidant enzymes, and concentrations of antioxidant substances [33]; these effects are often attributed to uprising reactive oxygen species that further lead to organ impairment. In fact, one of the mechanisms through which alloxan pathophysiology occurs in the pancreatic β-cell is selective inhibition of thiol groups which serve as a glucose sensor for triggering glucose-induced insulin secretion [34]. Our study revealed that the methanol extract of Struchium sparganophora leaves may be a good source of exogenous antioxidants for rat tissues. The extract increased the level of reduced glutathione (a thiol antioxidant) and decreased the tissue concentration malondialdehyde (a product of oxidized lipid). In addition, this plant may help in the process required in the reduction and/or scavenging of free radicals. The extract, either administered in combination or alone, increased the SOD and GST activities in the liver and pancreas, while it also increases catalase activities in the liver and kidney. Some therapeutically promising antidiabetic medicinal plants and plant products have been documented with similar antioxidant efficacy [35–38].

Conclusion

The methanol extract of *Struchium sparganophora* leaves (SPA) exhibited worthy antihyperglycemic, antidyslipidemic, and antioxidant efficacies when administered alone or in combination with metformin in alloxan diabetic rats. These effects are achieved by the extract through its ability to moderate dyslipidemic events, and reduce markers of oxidative stress in alloxan-induced diabetic rats.

Abbreviations

ALO: Alloxan; Al: Atherogenic index; CAT: Catalase; Cl: Cardiac index; FBG: Fasting blood glucose; GSH: Reduced glutathione; GST: Glutathione-s-transferase; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; MDA: Malondialdehyde; MET: Metformin; SOD: Superoxide dismutase; SPA: Methanol extract of *Struchium sparganophora*TCTotal cholesterol; TP: Total protein; VLDL-C: Very-low-density lipoprotein cholesterol

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Authors' contributions

All authors have read and approved the submission of this manuscript. AMA designed and organized the project, FOA monitored the laboratory works and was involved in the internal review of the manuscript, OMO was involved in the writing and internal review of the manuscript, BAO was involved in the bench work and funding of the research, and OAA monitored the progression and supervised the project.

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Availability of data and materials

All data is available upon request.

Ethics approval and consent to participate

Ethical approval (reference number: LCUERB205) was obtained from Lead City University Ethical Review Board (LCU/ERB) to perform this animal research.

Consent for publication

Not applicable

Competing interests

No competing interest to declare

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^aSignificant when compared to control at p < 0.05

^bSignificant when compared to ALO at p < 0.05

^cSignificant when compared to MET at p < 0.05

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