Original Research Article (Experimental)

Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of Synsepalum dulcificum in type 2 diabetic rats

T.O. Obafemi [a, b, *], A.C. Akinmoladun [a], M.T. Olaleye [a], Stephen O. Agboade [a], Amos A. Onasanya [b]

[a] Phytomedicine, Drug Metabolism and Toxicology Unit, Department of Biochemistry, The Federal University of Technology, PMB 704, Akure, Nigeria
[b] Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, PMB 5454 Ado — Ekiti, Nigeria

ARTICLE INFO

ABSTRACT

Background: Synsepalum dulcificum is a plant indigenous to West Africa. The fruit is used to modify taste of foods to sweetness.
Objectives: This study aims to investigate the antidiabetic potentials of both methanolic and flavonoid-rich leaf extracts of S. dulcificum (MSD and FSD respectively) in type 2 diabetic Wistar albino rats.
Materials and methods: Sixty three rats were randomly distributed into nine groups of seven animals each with group 1 serving as the normal control. Groups 2 to 7 were given 10% fructose in their drinking water for 14 days, after which 40 mg/kg of streptozotocin was administered. Group 2 animals served as the diabetic control, while groups 3, 4, 5, 6 and 7 were treated with 30 mg/kg MSD, 60 mg/kg MSD, 30 mg/kg FSD, 60 mg/kg FSD and 5 mg/kg glibenclamide respectively. Groups 8 and 9, contained healthy animals, and were treated with only 60 MSD, and 60 mg/kg FSD respectively. Biochemical parameters such as liver and kidney function tests, lipid profile, as well as lipid peroxidation and antioxidant enzymes were assessed in addition to histopathology.
Results: It was observed that daily oral administration of MSD and FSD for 21 days significantly ($p < 0.05$) improved the observed pathological changes as a result of type 2 diabetes.
Conclusion: It could be deduced from results obtained in this study that methanolic and flavonoid-rich leaf extracts of S.dulcificum have antidiabetic potential in type 2 diabetic rats.
© 2017 Transdisciplinary University, Bangalore and World Ayurveda Foundation. Publishing Services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia and alteration of carbohydrate, proteins and lipids metabolism as a result of abnormal secretion and/or activity of insulin [1]. Over 346 million people have diabetes, of which, type 2 diabetes mellitus (T2DM) makes up 90% of these cases [2]. Type 1 diabetes is caused by lack of insulin due to the destruction of β-cells in the pancreas usually as a result of autoimmune destruction. Some of the causes of type 1 diabetes include genetic susceptibility, environmental factors, viruses and infections [3]. Type 2 diabetes is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance; and environmental factors such as obesity,

overeating, lack of exercise, stress as well as aging [4]. T2DM patients live in a chronic state of hyperglycemia due to progression of pancreatic beta cell dysfunction and insulin resistance [5].

About 40% of prescription drugs are derived from herbs and about half of the world's best-selling drugs are derived from plants. Moreover, several studies have shown that flavonoids are known to exhibit strong antidiabetic and antioxidant activities [6,7]. Consumption of flavonoids or flavonoid-rich compounds protects the body against free radicals and other pro-oxidative compounds, thereby reducing the risk of diabetes [8,9]. Therefore, search for new antidiabetic drugs from natural plants is still attractive because they contain substances which have alternative and safe effect on diabetes mellitus. Concurrently, phytochemicals identified from traditional medicinal plants are presenting exciting opportunities for development of new drug therapies for diabetes [10].

Synsepalum dulcificum is also known as miracle fruit, magic fruit, miraculous or flavor fruit [11]. Compounds such as β-sitosterol, stigmasterol, pheophytin-a, pheophytin-b, lupeol, lupenone, lupeol

* Corresponding author. Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, PMB 5454, Ado — Ekiti, Nigeria.
E-mail address: oobafemi@abuad.edu.ng

acetate, and α-tocopheryl quinone were isolated from the leaves of *S. dulcificum* [12]. It was reported that miracle fruit may be used as an adjuvant for treating diabetic patients with insulin resistance because this fruit has been shown to have the ability to improve insulin sensitivity [13]. We have identified active principles in the methanolic extract of *S. dulcificum* leaves using high performance liquid chromatography (HPLC). Rutin, quercetin, isoquercitrin, quercitrin, kaempferol, ellagic acid, caffeic acid, chlorogenic acid, catechin, gallic acid, epicatechin, tocopherol, β-carotene and lycopene have all been identified in the extract [14]. This study reports the antidiabetic potentials of methanolic and flavonoid-rich extracts of *S. dulcificum* leaves in type 2 diabetic Wistar albino rats.

2. Materials and methods

2.1. Chemicals and reagents

Glibenclamide and streptozotocin were purchased from Sigma-Alrich (St-Louis, MO, USA). Reduced glutathione and epinephrine were purchased from Santa Cruz Biotechnology, Heidelberg, Germany. All other reagents used were of analytical grade.

2.2. Plant material and preparation

Fresh *S. dulcificum* leaves were obtained from Olode village, Osun State, Nigeria, and authenticated at the Botany Department, University of Ibadan, Ibadan, Nigeria. A voucher number UIH-22457 was obtained for the leaf. The leaves were air-dried for three weeks and pulverized. A portion of the pulverized sample (700 g) was extracted in 80% methanol by maceration for 72 h. The methanolic extract was concentrated in a rotary evaporator, lyophilized and preserved for further use.

2.3. Extraction of flavonoids

A known gram of the methanol extract was dissolved in 20 ml of 10% H_2SO_4 and hydrolysed by heating in the water bath for 30 min at 100 °C. The mixture was placed on ice for 15 min for precipitation of the flavonoid aglycones. The flavonoid aglycones were then dissolved in 50 ml of warm 95% ethanol, filtered and concentrated by rotary evaporation [15].

2.4. Induction of diabetes and experimental design

Type 2 diabetes was induced according to the method of Rachel and Shahidul [16]. After giving water containing 10% fructose to rats for 14 days, streptozotocin (40 mg/kg body weight) in ice-cold 0.1 M citrate buffer (pH 4.5) was administered to the animals after an overnight fast. After 72 h of streptozotocin administration, blood glucose level was checked using Accu-check® glucometer and animals with glucose levels ≥ 250 mg/dl were considered diabetic. The animals were then divided into nine groups with seven animals apiece. Group 1 served as the normal control, group 2 was the diabetic control while groups 3, 4, 5, 6 and 7 were diabetic animals treated with 30 mg/kg methanol extract, 60 mg/kg methanol extract, 30 mg/kg flavonoid-rich extract, 60 mg/kg flavonoid-rich extract and 5 mg/kg glibenclamide respectively. Groups 8 and 9 were administered 60 mg/kg methanol extract and 60 mg/kg flavonoid-rich extract respectively. All animals were administered the extract for 21 days, and sacrificed 24 h after the last dose of the extract. Animal studies adhered to the Principles of Laboratory Animal Care (NIH publication #85−23, revised in 1985). All animal experiments were approved by the animal care committee of the Afe Babalola University Research Center, Ado-Ekiti, Ekiti State, Nigeria with ethical number ABUAD-SCI04/03/15/013.

2.5. Oral glucose tolerance test (OGGT)

Oral glucose tolerance test was conducted to assess glucose utilization in experimental animals. On day 21, animals in groups 1−7 were fasted overnight prior to the administration of extracts and glibenclamide at 0 h. Glucose (2 mg/kg) was administered to all the groups and blood glucose was checked at 0 h (before any treatment), 60 min and 120 min.

2.6. Biochemical assays

Glucose, urea, ALT, AST, ALP, HDL, total cholesterol, triglyceride and total protein levels were estimated according to the protocol provided by the kit manufacturer (Randox Laboratories Crumlin, United Kingdom). Catalase activity was estimated using the method of Sinha [17] and assessment of lipid peroxidation was done using the method of Varshney and Kale [18]. Glutathione S-transferase activity was measured using the method of Habig et al.[19], superoxide dismutase activity was measured using the method of Misra and Fridovich [20] while glutathione peroxidase activity was measured using the method of Rotruck et al. [21].

2.7. Histopathological study

Liver, pancreas and kidney tissues of animals were used for histopathological study. Tissues were fixed in 10% buffered formalin, routinely processed and embedded in paraffin wax. Sections were cut on glass slides at a thickness of 4 μm and stained with hematoxylin and eosin (H&E) (Culling, 1974). The slides were examined under a light microscope and the magnified images of the tissue structures were captured [22].

2.8. Statistical analysis

Results were expressed as mean value ± standard error of mean (SEM). Data analysis was done using GraphPad Prism 5 software by one-way analysis of variance (ANOVA) followed by Tukey-test. In all instances *p* values <0.05 were considered statistically significant.

3. Results

3.1. Effect of treatment on body weight

Treatment of diabetic animals with MSD, FSD and glibenclamide led to increase in body weight in most of the animals. The diabetic control group showed a significant ($p < 0.05$) reduction in body

Table 1
Effect of crude methanol and flavonoid-rich fractions of *S. dulcificum* on body weight in both diabetic and normal rats.

Groups	Initial weight (g)	Final weight (g)	% change in weight
Normal control	220.89 ± 2.52*	236.03 ± 6.18*	6.93 ± 3.02*
Diabetic control	198.74 ± 4.30	176.28 ± 11.98	−11.56 ± 4.95
Diabetic + 30 mg/kg MSD	204.94 ± 4.23	220.94 ± 13.40*	8.075 ± 6.87*
Diabetic + 60 mg/kg MSD	207.24 ± 4.46	219.38 ± 5.16*	6.14 ± 3.24*
Diabetic + 30 mg/kg FSD	204.20 ± 4.03	202.89 ± 6.55**	−0.66 ± 2.43*
Diabetic + 60 mg/kg FSD	205.71 ± 3.51	207.54 ± 4.35*	0.92 ± 1.60*
Diabetic + 5 mg/kg glib.	210.07 ± 1.36	205.63 ± 6.24	−2.19 ± 2.51*
Normal + 60 mg/kg MSD	212.94 ± 5.38*	229.04 ± 4.35*	7.72 ± 1.55*
Normal + 60 mg/kg FSD	208.41 ± 5.01	234.04 ± 6.02*	12.31 ± 1.35*

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p < 0.05$) different from the diabetic control while values with ** in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of *S. dulcificum* leaves, FSD = Flavonoid-rich extract of *S. dulcificum* leaves.

weight when compared with the normal control, extracts and glibenclamide treated groups (Table 1).

3.2. Serum glucose level

The extract and glibenclamide treated groups showed a significantly lower serum glucose levels when compared with the diabetic control group and their glucose levels were closer to that of the normal control (Table 2). At the end of the study, the glucose

levels of animals treated with extracts only were not significantly ($p < 0.05$) different from that of control.

3.3. Oral glucose tolerance test

The extract and glibenclamide treated diabetic animals showed better utilization of glucose when compared with the diabetic control group (Table 3).

3.4. Serum level of ALP, AST, ALT, total protein, urea and creatinine

There were significantly ($p < 0.05$) lower serum levels of ALP, AST, ALT, urea and creatinine in the MSD, FSD and glibenclamide treated diabetic animals when compared with the diabetic control (Table 4). AST and ALT levels of the extract and glibenclamide treated diabetic animals were significantly ($p < 0.05$) different from the normal control. Serum total protein level of the diabetic control group was however significantly ($p < 0.05$) lower than the normal control, extract and glibenclamide treated groups.

3.5. Lipid profile

Total-cholesterol, triglyceride and LDL-cholesterol levels of the diabetic control group were significantly ($p < 0.05$) higher when compared with the normal control, extract and glibenclamide treated groups (Table 5). The HDL-cholesterol was however significantly ($p < 0.05$) lower in the diabetic control group than in the normal control group, extract and glibenclamide treated diabetic groups; and normal animals treated with extract only.

3.6. Liver total protein and MDA levels, and antioxidant enzyme activities

A significantly ($p < 0.05$) lower total protein level, SOD, GST and GPx and catalase activities were observed in the liver of diabetic control group when compared with the normal control and MSD treated groups (Table 6). Catalase activity in FSD and glibenclamide treated groups were not significantly different from the diabetic control group. However, a significantly ($p < 0.05$) higher hepatic MDA level was observed in the diabetic control group when compared with the other groups in the study.

3.7. Pancreatic total protein and MDA levels, and antioxidant enzyme activities

A significantly ($p < 0.05$) lower pancreatic total protein, SOD, GST, GPx and catalase levels were observed in the diabetic control group when compared with the normal control, FSD and

Table 2
Effect of methanol and flavonoid rich extract of S. dulcificum on serum glucose levels in type 2 diabetic rats after confirmation of diabetes.

Groups	1st day (mg/dl)	3rd day (mg/dl)	21st day (mg/dl)
Normal control	79.71 ± 3.05	103.00 ± 2.35*	67.85 ± 0.71*
Diabetic control	71.00 ± 2.20	301.71 ± 20.39	254.27 ± 3.47
Diabetic + 30 mg/kg MSD	78.43 ± 3.61	347.14 ± 28.88**	78.14 ± 0.70**
Diabetic + 60 mg/kg MSD	73.43 ± 2.97	319.14 ± 17.39	73.91 ± 0.72**
Diabetic + 30 mg/kg FSD	73.86 ± 3.44	274.14 ± 6.97**	77.03 ± 1.49**
Diabetic + 60 mg/kg FSD	79.00 ± 3.20	272.43 ± 9.57**	72.89 ± 0.95**
Diabetic + 5 mg/kg glib.	79.14 ± 4.18	290.43 ± 8.60**	72.89 ± 0.66**
Normal + 60 mg/kg MSD	101.43 ± 3.83**	100.57 ± 1.86*	65.05 ± 1.07*
Normal + 60 mg/kg FSD	102.29 ± 4.13**	104.00 ± 1.60*	66.90 ± 1.13*

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p < 0.05$) different from the diabetic control while values with ** in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves, FSD = Flavonoid-rich extract of S. dulcificum leaves.

Table 3
Effect of oral administration of MSD and FSD on blood-glucose level in glucose loaded rats.

Groups	0 min mg/dl	60 min mg/dl	120 min mg/dl
Normal control	74.86 ± 6.96*	89.00 ± 9.75*	82.57 ± 7.25*
Diabetic control	387.71 ± 64.22	409.29 ± 65.67	404.14 ± 63.27
Diabetic + 30 mg/kg MSD	74.14 ± 17.17*	96.57 ± 22.13*	78.43 ± 19.42*
Diabetic + 60 mg/kg MSD	81.86 ± 20.92*	101.14 ± 17.66*	90.00 ± 15.79*
Diabetic + 30 mg/kg FSD	66.71 ± 11.68*	77.14 ± 15.03*	73.85 ± 15.29*
Diabetic + 60 mg/kg FSD	83.57 ± 15.47*	102.71 ± 16.47*	90.00 ± 13.38*
Diabetic + 5 mg/kg glib.	143.14 ± 57.14*	153.14 ± 50.09*	142.43 ± 51.42*

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p < 0.05$) different from the diabetic control while values with ** in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves, FSD = Flavonoid-rich extract of S. dulcificum leaves.

Table 4
Effect of MSD and FSD on ALP, AST, ALT, total protein, urea and creatinine in serum of type 2 diabetic rats.

Groups	ALP U/l	AST U/l	ALT U/l	Total protein g/dl	Urea g/dl	Creatinine µmol/l
Normal control	86.74 ± 3.94*	105.71 ± 5.55*	38.57 ± 6.49*	3.60 ± 0.11*	47.08 ± 2.05*	7.40 ± 0.13*
Diabetic control	118.29 ± 5.09	215.00 ± 2.89	126.86 ± 5.25	2.59 ± 0.04	64.92 ± 1.40	16.64 ± 0.40
Diabetic + 30 mg/kg MSD	74.91 ± 5.09*	200.00 ± 7.16**	79.71 ± 2.16**	3.35 ± 0.07*	48.00 ± 2.09*	10.21 ± 0.74**
Diabetic + 60 mg/kg MSD	82.80 ± 6.02*	178.57 ± 6.33**	65.14 ± 3.72**	2.88 ± 0.11**	39.84 ± 2.06**	8.58 ± 0.69*
Diabetic + 30 mg/kg FSD	78.86 ± 3.94*	165.00 ± 5.00**	72.21 ± 3.88**	2.95 ± 0.13**	47.69 ± 1.79*	7.46 ± 0.62*
Diabetic + 60 mg/kg FSD	82.80 ± 0.00*	185.71 ± 1.70**	71.57 ± 2.69**	3.26 ± 0.15**	58.92 ± 0.80**	8.15 ± 0.77*
Diabetic + 5 mg/kg glib.	78.86 ± 2.86*	173.57 ± 3.22**	72.21 ± 3.21**	3.47 ± 0.11*	62.61 ± 0.61	8.76 ± 1.10*
Normal + 60 mg/kg MSD	86.74 ± 2.96*	93.57 ± 7.05*	88.07 ± 5.09**	2.94 ± 0.07**	46.31 ± 0.47*	7.87 ± 0.77*
Normal + 60 mg/kg FSD	82.20 ± 6.02*	168.57 ± 5.64**	66.00 ± 2.04**	2.93 ± 0.02**	53.07 ± 0.77**	6.02 ± 0.36*

Each value is a mean of seven determinations ± SEM. Values with * in the same column are significantly ($p < 0.05$) different from the diabetic control while values with ** in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves; FSD = Flavonoid-rich extract of S. dulcificum leaves; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 5
Effect of MSD and FSD on lipid profile of type 2 diabetic rats.

Groups	Total-chol mg/dl	HDL-chol mg/dl	Triglyceride mg/dl	LDL-chol mg/dl
Normal control	68.95 ± 8.70[*]	59.81 ± 7.04[*]	105.33 ± 3.69[*]	57.02 ± 1.83[*]
Diabetic control	206.84 ± 3.68	33.04 ± 1.18	184.12 ± 6.12	343.81 ± 7.91
Diabetic + 30 mg/kg MSD	86.41 ± 5.07[*]	78.22 ± 3.17[**]	137.90 ± 3.09[**]	67.01 ± 4.85[*]
Diabetic + 60 mg/kg MSD	73.87 ± 5.92[*]	66.07 ± 6.78[*]	112.58 ± 4.39[*]	59.15 ± 3.67[*]
Diabetic + 30 mg/kg FSD	116.40 ± 9.32[**]	38.35 ± 1.02	56.90 ± 4.31[**]	183.07 ± 8.99[**]
Diabetic + 60 mg/kg FSD	47.46 ± 1.44[*]	52.21 ± 3.94[*]	127.64 ± 2.57[**]	17.17 ± 3.67[*]
Diabetic + 5 mg/kg glib.	124.01 ± 3.27[**]	62.65 ± 2.85[*]	92.43 ± 1.87[**]	166.88 ± 6.71[**]
Normal + 60 mg/kg MSD	67.60 ± 4.04[*]	53.35 ± 5.51[*]	121.19 ± 1.80[**]	57.62 ± 3.99[*]
Normal + 60 mg/kg FSD	105.66 ± 4.97[**]	47.47 ± 2.47[*]	87.76 ± 2.48[**]	146.30 ± 9.14[**]

Each value is a mean of seven determinations ± SEM. Values with [*] in the same column are significantly ($p < 0.05$) different from the diabetic control while values with [**] in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves, FSD = Flavonoid-rich extract of S. dulcificum leaves.

Table 6
Effect of methanol and flavonoid rich extracts of S. dulcificum on liver levels of total protein, MDA, SOD, GST, GPx, and catalase activities of type 2 diabetic rats.

Group	Total Protein g/dl	MDA $\times10^6$ (nmol/ml)	SOD % inhibition/mg protein	GST µmole/min/mg protein	Gpx µmole/min/mg protein	Catalase µg/mgH$_2$O$_2$/consumed/mg protein
Normal control	1.72 ± 0.18[*]	8.27 ± 2.02[*]	77.38 ± 2.38[*]	0.94 ± 0.11[*]	134.26 ± 4.42[*]	20.29 ± 2.10[*]
Diabetic control	0.28 ± 0.03	69.62 ± 7.66	34.52 ± 2.17	0.10 ± 0.01	57.15 ± 8.07	9.39 ± 2.39
Diabetic + 30 mg/kg MSD	1.82 ± 0.10[*]	4.00 ± 0.42[*]	82.14 ± 3.37[*]	0.80 ± 0.05[*]	136.19 ± 9.71[*]	23.40 ± 0.65[*]
Diabetic + 60 mg/kg MSD	0.77 ± 0.01[**]	12.20 ± 1.09[*]	61.91 ± 4.40[**]	0.36 ± 0.01[**]	118.13 ± 3.60[**]	14.73 ± 0.27[**]
Diabetic + 30 mg/kg FSD	0.58 ± 0.03[**]	18.34 ± 1.71[**]	67.86 ± 1.19[**]	0.30 ± 0.02[**]	107.31 ± 8.96[**]	7.96 ± 1.67
Diabetic + 60 mg/kg FSD	0.70 ± 0.02[**]	15.55 ± 1.17[*]	55.95 ± 1.54[**]	0.38 ± 0.01[**]	114.60 ± 7.50[**]	9.97 ± 0.62
Diabetic + 5 mg/kg glib.	0.63 ± 0.02[**]	18.35 ± 0.72[**]	63.10 ± 1.68[**]	0.34 ± 0.01[**]	110.92 ± 10.97[**]	8.91 ± 0.84
Normal + 60 mg/kg MSD	0.96 ± 0.09[**]	8.25 ± 1.23[*]	47.62 ± 1.54[**]	0.50 ± 0.05[**]	122.96 ± 4.09[**]	16.71 ± 1.81[*]
Normal + 60 mg/kg FSD	1.01 ± 0.03[**]	8.58 ± 0.93[*]	51.19 ± 1.19[**]	0.55 ± 0.02[**]	125.49 ± 8.78[*]	18.22 ± 0.63[*]

Each value is a mean of seven determinations ± SEM. Values with [*] in the same column are significantly ($p < 0.05$) different from the diabetic control while values with [**] in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves, FSD = Flavonoid-rich extract of S. dulcificum leaves MDA = malondialdehyde, SOD = superoxide dismutase, GST = glutathione S-transferase, GPx = glutathione peroxidase.

Table 7
Effect of methanol and flavonoid rich extracts of S. dulcificum on pancreatic levels of total protein, MDA, SOD, GST, GPx, and catalase of type 2 diabetic rats.

Group	Total Protein g/dl	MDA $\times10^6$ (nmol/ml)	SOD % inhibition/mg protein	GST µmole/min/mg protein	Gpx µmole/min/mg protein	Catalase µg/mgH$_2$O$_2$/consumed/mg protein
Normal control	3.13 ± 0.21[*]	16.06 ± 0.67[*]	42.29 ± 2.29[*]	0.79 ± 0.04[*]	99.64 ± 5.06[*]	3.32 ± 0.44[*]
Diabetic control	1.86 ± 0.09	55.63 ± 2.96	24.00 ± 2.47	0.28 ± 0.01	46.36 ± 6.30	1.04 ± 0.30
Diabetic + 30 mg/kg MSD	3.20 ± 0.22[*]	24.10 ± 2.23[**]	41.14 ± 2.72[*]	0.62 ± 0.05[**]	101.21 ± 5.07[*]	1.75 ± 0.10
Diabetic + 60 mg/kg MSD	3.20 ± 0.16[*]	24.10 ± 2.23[**]	37.71 ± 1.48[*]	0.68 ± 0.04[**]	101.75 ± 4.86[*]	1.822 ± 0.14
Diabetic + 30 mg/kg FSD	3.67 ± 0.29[**]	13.45 ± 1.57[*]	37.71 ± 2.88[*]	0.83 ± 0.06[*]	109.48 ± 8.11[*]	3.18 ± 0.45[*]
Diabetic + 60 mg/kg FSD	2.56 ± 0.07[**]	17.77 ± 1.63[*]	38.86 ± 2.72[*]	0.60 ± 0.01[**]	83.62 ± 2.65[**]	3.25 ± 0.35[*]
Diabetic + 5 mg/kg glib.	3.06 ± 0.17[*]	17.97 ± 0.57[*]	38.86 ± 1.72[*]	0.74 ± 0.05[*]	98.23 ± 4.87[*]	2.85 ± 0.34[*]
Normal + 60 mg/kg MSD	2.51 ± 0.06[**]	18.38 ± 0.43[*]	41.14 ± 2.72[*]	0.60 ± 0.02[**]	82.01 ± 2.20[**]	3.18 ± 0.11[*]
Normal + 60 mg/kg FSD	2.94 ± 0.09[*]	18.10 ± 1.09[*]	48.00 ± 3.02[*]	0.74 ± 0.02[*]	96.25 ± 2.54[*]	3.15 ± 0.16[*]

Each value is a mean of seven determinations ± SEM. Values with [*] in the same column are significantly ($p < 0.05$) different from the diabetic control while values with [**] in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves, FSD = Flavonoid-rich extract of S. dulcificum leaves MDA = malondialdehyde, SOD = superoxide dismutase, GST = glutathione S-transferase, GPx = glutathione peroxidase, CAT = catalase.

Table 8
Effect of methanol and flavonoid rich extracts of S. dulcificum on total protein level and antioxidant enzyme activities in kidney of type 2 diabetic rats.

Group	Total Protein g/dl	SOD % inhibition/mg protein	GST µmole/min/mg protein	Gpx µmole/min/mg protein	Catalase µg/mgH$_2$O$_2$/consumed/mg protein
Normal control	2.61 ± 0.05[*]	83.67 ± 3.73[*]	0.61 ± 0.02[*]	355.81 ± 2.01[*]	5.88 ± 0.15[*]
Diabetic control	0.78 ± 0.06	57.14 ± 0.00	0.11 ± 0.01	121.03 ± 7.68	2.75 ± 0.47
Diabetic + 30 mg/kg MSD	1.89 ± 0.07[**]	83.67 ± 2.04[*]	0.35 ± 0.02[**]	318.65 ± 5.60[**]	6.08 ± 0.10[*]
Diabetic + 60 mg/kg MSD	2.20 ± 0.08[**]	71.43 ± 0.00[**]	0.42 ± 0.02[**]	337.74 ± 3.87[*]	6.60 ± 0.13[**]
Diabetic + 30 mg/kg FSD	2.77 ± 0.13[*]	73.47 ± 3.73[**]	0.57 ± 0.03[*]	360.38 ± 3.74[*]	7.00 ± 0.13[**]
Diabetic + 60 mg/kg FSD	2.06 ± 0.04[**]	75.51 ± 2.64[*]	0.43 ± 0.01[**]	330.78 ± 2.05[*]	6.03 ± 0.90[*]
Diabetic + 5 mg/kg glib.	1.64 ± 0.10[**]	83.67 ± 2.04[*]	0.35 ± 0.03[**]	297.47 ± 9.00[**]	4.68 ± 0.24[*]
Normal + 60 mg/kg MSD	2.28 ± 0.07[**]	78.57 ± 2.20[*]	0.48 ± 0.02[**]	341.75 ± 3.35[*]	5.95 ± 0.21[*]
Normal + 60 mg/kg FSD	3.01 ± 0.04[*]	83.67 ± 3.72[*]	0.64 ± 0.01[*]	368.51 ± 1.05[*]	6.83 ± 0.16[**]

Each value is a mean of seven determinations ± SEM. Values with [*] in the same column are significantly ($p < 0.05$) different from the diabetic control while values with [**] in the same column are significantly ($p < 0.05$) different from both positive and normal control.
MSD = Methanolic extract of S. dulcificum leaves; FSD = Flavonoid-rich extract of S. dulcificum leaves; SOD = superoxide dismutase; GST = glutathione-S-transferase; GPx = glutathione peroxidase.

glibenclamide treated groups (Table 7). Catalase activity in diabetic animals treated with MSD was not significantly different from diabetic control group. However, a significantly ($p < 0.05$) higher MDA level was observed in the diabetic control group when compared with the other groups in the study.

3.8. Kidney total protein level and antioxidant enzyme activities

Total protein, SOD, GST, GPx and catalase activities in the kidney of diabetic control group was significantly ($p < 0.05$) lower when compared with the normal control and both extract and glibenclamide treated groups (Table 8).

4. Discussion

Reduction in body weight of diabetic animals has been linked to degradation of structural proteins and muscle wasting [23]. Our result as presented in Table 1 shows that there was a significant reduction in the body weight of diabetic control animals. Treatment with MSD and FSD however improved body weight to a reasonable extent as observed from the percentage change in body weights of experimental animals in the course of the study.

Research has shown that sustained reduction in hyperglycemia associated with diabetes will decrease the risk of developing microvascular diseases and reduce diabetes complications [24]. Furthermore, the antihyperglycemic property of several medicinal plants has been well documented [25,26]. Our study as shown in Table 2 shows that the diabetic control group showed a persistently higher serum glucose levels throughout the experimental period while administration of MSD, FSD and glibenclamide significantly ($p < 0.05$) reduced the serum glucose levels in the treated diabetic animals. It has been severally stated that treatment of diabetic animals with medicinal plants could activate β-cells and normalize their granulation [27]. This could result in potentiation of insulin secretion from the remaining pancreatic β-cells, or increased utilization of glucose by tissues. We propose that our extracts may exert their antihyperglycemic effect through the aforementioned mechanisms.

The results of the OGTT presented in Table 3 show that the glucose levels of the animals reached a peak 60 min after glucose load. It is however instructive to note that 120 min after glucose load, blood glucose level in the diabetic control group showed a wider difference (16.43 mg/dl) from the glucose level at 0 min when compared with other groups. The glucose levels of both the normal control, MSD, FSD and glibenclamide treated diabetic groups reverted to levels that were closer to the levels at 0 min. It was also observed that glucose level at 120 min in the glibenclamide treated group was actually lower than the value at glucose load. OGGT indicates the relative roles of insulin secretion and insulin resistance in the progression of glucose intolerance. Moreover, a prolonged elevation of plasma glucose level (after glucose load) is an indication of impaired glucose tolerance and insulin resistance [28]. These observations indicate that the diabetic control group had a slower glucose utilization rate than other groups in this study, a hallmark of diabetes. We therefore suggest that both FSD and MSD could be useful in improving glucose intolerance and insulin resistance associated with type 2 diabetes.

Experimentally induced diabetes indicates several alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis which is an energy dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing glucogenic amino acids [29]. Our results as presented in Table 4 showed a marked reduction in the total serum protein in the animals in diabetic control group as compared with the normal control group. However a significant improvement in

the serum protein levels was observed in the animals treated with extracts and the standard drug. This suggests that the extract has the potential to ameliorate the alterations in amino acid metabolism associated with diabetes mellitus.

Renal damage consequent upon persistent hyperglycemia associated with diabetes is linked with increased levels of urea and creatinine. The World Health Organization states that diabetes is a leading cause of kidney failure which is responsible for 10%–20% of deaths in diabetic people [30]. Moreover, creatinine was recently discovered to be a new risk factor for T2D and insulin resistance (IR) [31]. In our results presented in Table 4 it was observed that the diabetic control group had a significantly ($p < 0.05$) higher serum level of urea and creatinine than both the normal control and extract treated groups. This might be due to the hypoglycemic effect of both MSD and FSD on the diabetic rats which by extension prevented the kidney damage that could be caused by prolonged hyperglycemia.

The fact that increase in the serum levels of biomarker enzymes, such as AST, ALT, and ALP (as observed in diabetic rats) indicates organ damage has been well documented [32,33]. This is usually as a result of leakage of the enzymes from organs where they are located into the blood stream. Our results as presented in Table 4 corroborates this fact as a significantly higher level of the enzymes were observed in the serum of animals in the diabetic control group when compared with the other groups.

Diabetes is a metabolism-associated disease, particularly closely related to lipid metabolism, affecting the serum lipid and lipoprotein profile [34]. Type 2 diabetes-associated cardiovascular complications are due to lowered HDL and elevated triglyceride, low-density lipoprotein (LDL) and cholesterol levels [35]. Antidiabetic agents have been reported to reduce the cardiovascular risk by controlling the lipid profile levels in diabetic patients [36,37]. In the present study, Table 5 shows that lipid metabolism in diabetic control rats was markedly deranged. This was evident in significantly ($p < 0.05$) lower level of HDL-cholesterol with a corresponding increase in the levels of triglyceride, total-cholesterol and
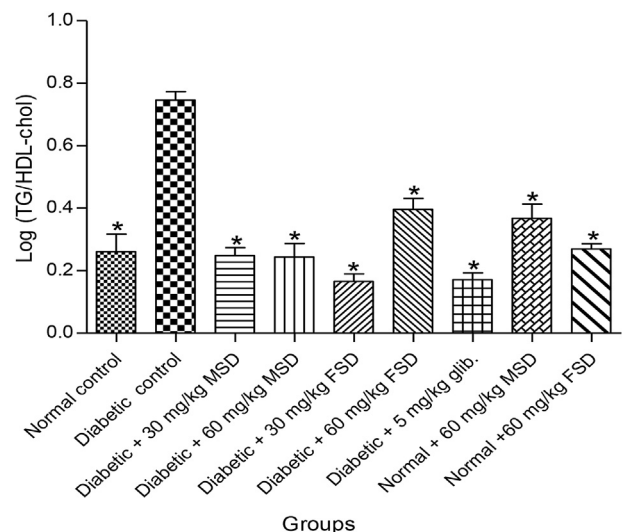

Fig. 1. Effect of methanolic and flavonoid-rich extracts on atherogenic plasma index-log (triglyceride/HDL-cholesterol) in type 2 diabetic rats. Each result is a mean of seven determinations ± SEM. Bars with * are significantly ($p < 0.05$) different from the diabetic control. The diabetic groups treated with MSD, FSD and glibenclamide have a significantly lower atherogenic plasma index when compared with the diabetic control group. Furthermore, the atherogenic plasma index of the diabetic animals treated with extracts and glibenclamide were comparable with that of normal control. MSD = Methanolic extract of *S. dulcificum* leaves, FSD = Flavonoid-rich extract of *S. dulcificum* leaves.
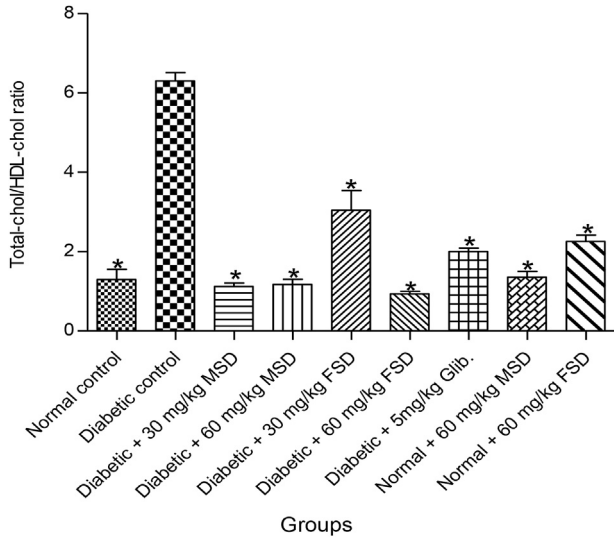
Fig. 2. Effect of methanolic and flavonoid-rich extracts on atherogenic index in type 2 diabetic rats. Each result is a mean of seven determinations ± SEM. Bars with * are significantly ($p < 0.05$) different from the diabetic control. The diabetic control group has a significantly higher atherogenic index when compared with both the normal control group and the treated groups. The atherogenic index of the MSD, FSD and glibenclamide treated groups were also comparable with that of normal control. MSD = Methanolic extract of *S. dulcificum* leaves, FSD = Flavonoid-rich extract of *S. dulcificum* leaves.

LDL-cholesterol. However, this aberration was significantly ameliorated in the MSD, FSD and glibenclamide treated groups. In an attempt to optimize the predictive capacity of the lipid profile, several lipoprotein ratios or atherogenic indices have been defined [38]. Several observational studies reported that the total/HDL cholesterol ratio is a more powerful coronary risk predictor than independently used total cholesterol, LDL cholesterol and HDL cholesterol [38]. Furthermore, an atherogenic plasma index [log (triglycerides/HDL cholesterol)] over 0.5 has been proposed as the cut-off point indicating atherogenic risk [39]. Likewise individuals with a high total-cholesterol/HDL cholesterol ratio have greater cardiovascular risk owing to the imbalance between the cholesterol carried by atherogenic and protective lipoproteins [40]. In our results as presented in Figs. 1 and 2 it was observed that the diabetic control group had a high atherogenic and cardiovascular risks respectively when compared with the normal control, extracts and glibenclamide treated groups. Circulating FFAs derived from adipocytes are elevated in insulin-resistant states. The link between increased circulating FFAs and both type 2 diabetes or insulin resistance might involve accumulation of triglycerides and other fatty acid-derived metabolites and a low HDL-cholesterol levels. Insulin resistance further exacerbates this atherogenic dyslipidemia by increasing the hepatic secretion of VLDL and other apolipoprotein (apo) B-containing lipoprotein particles such as LDL [41]. Moreover, it has been observed that increased cholesterol synthesis is observed in obese subjects, patients with metabolic syndrome
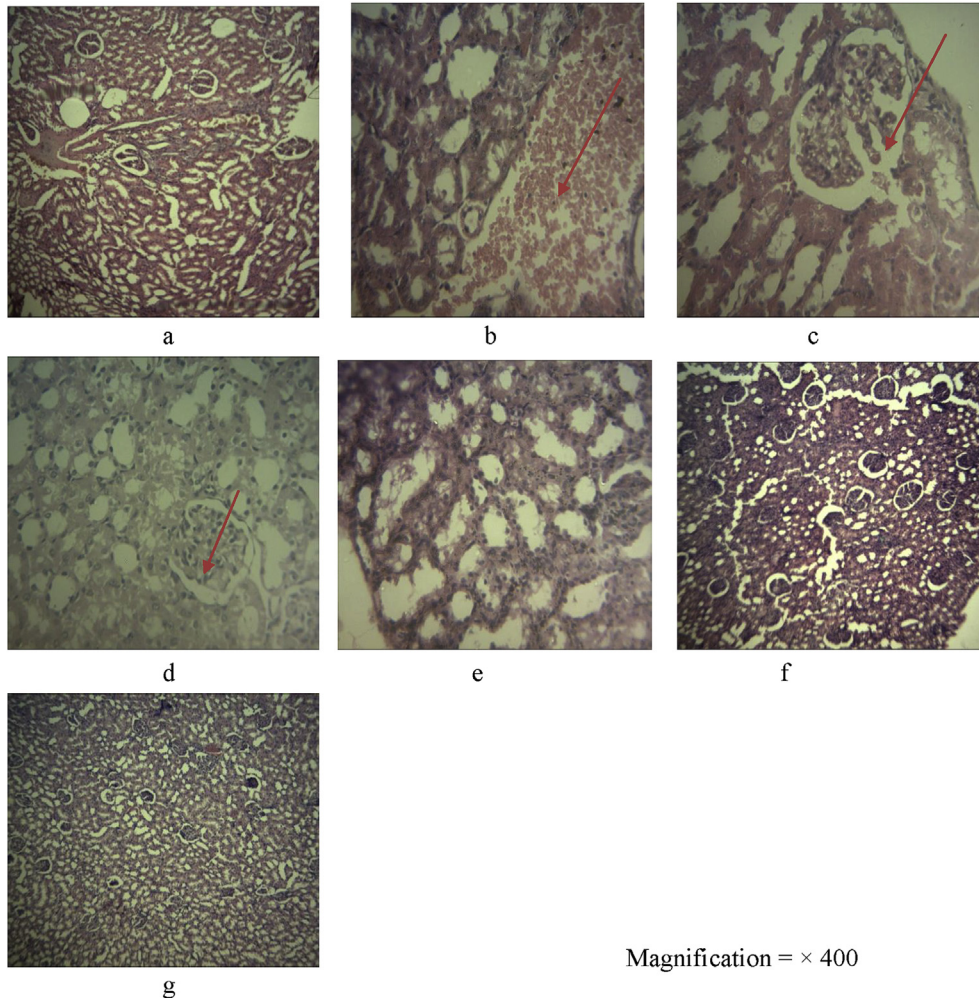

Magnification = × 400

Fig. 3. Kidney sections of normal and diabetic rats. Groups a, e, f and g showed no visible lesion. Group b showed severe congestion and hemorrhage into renal interstitium. Group c showed a mild congestion of renal interstitium. Group d showed diffuse tubular degeneration. a = normal control, b = diabetic control, c = diabetic + 30 mg/kg MSD, d = diabetic + 60 mg/kg MSD, e = diabetic + 30 mg/kg FSD, f = diabetic + 60 mg/kg FSD, g = diabetic + 5 mg/kg glibenclamide.

[42], and type 2 diabetes patients [43], conditions characterized by insulin resistance. We propose that MSD and FSD could thus be relevant in preventing derangement of lipid metabolism and cardiovascular complications associated with type 2 diabetes by improving insulin resistance condition.

It has been reported that hyperglycemia generates abnormally high levels of free radicals by a mechanism involving autoxidation of glucose, followed by oxidative degeneration and protein glaciation [44]. Lipid peroxide mediated tissue damage has also been observed in both type I and II diabetes mellitus [45—47]. In the present study, Tables 6 and 7 showed that the malondialdehyde level in the liver and pancreas of animals, a measure of the extent of lipid peroxidation in the organs was significantly ($p < 0.05$) higher in the diabetic control group than both the normal control; and the extract and glibenclamide treated groups. This could be as a result of the depletion of natural antioxidant mechanisms of the animals, a condition that was ameliorated by the administration of the standard drug and extracts. Research has shown that streptozotocin treatment of animals decreases their antioxidant systems when compared with controls. SOD, CAT, GST and GPx are all antioxidant enzymes which play important roles in scavenging the toxic intermediates of incomplete oxidation. The activities of these enzymes were found to be significantly lower in the liver, pancreas and kidney of diabetic control animals which is an indication of oxidative stress in the organs [48]. However as shown in our results in Tables 6—8, administration of glibenclamide, MSD and FSD significantly ($p < 0.05$) increased the antioxidant enzymes activities in the liver, pancreas and kidney respectively to those comparable with that of the normal control group. Catalase activity in the liver of diabetic animals treated with FSD and glibenclamide, and pancreas of diabetic animals treated with MSD were not significantly ($p < 0.05$) different from diabetic controls.

Sections of the kidney, pancreas and liver are shown in Figs. 3—5 respectively. Treatment for 21 days with both extract and glibenclamide was observed to protect/prevent the organ damage that was still evident in the diabetic control group at the end of the study. This further corroborates the protective effect of the extracts on the organs of diabetic animals as observed from biochemical analyses.
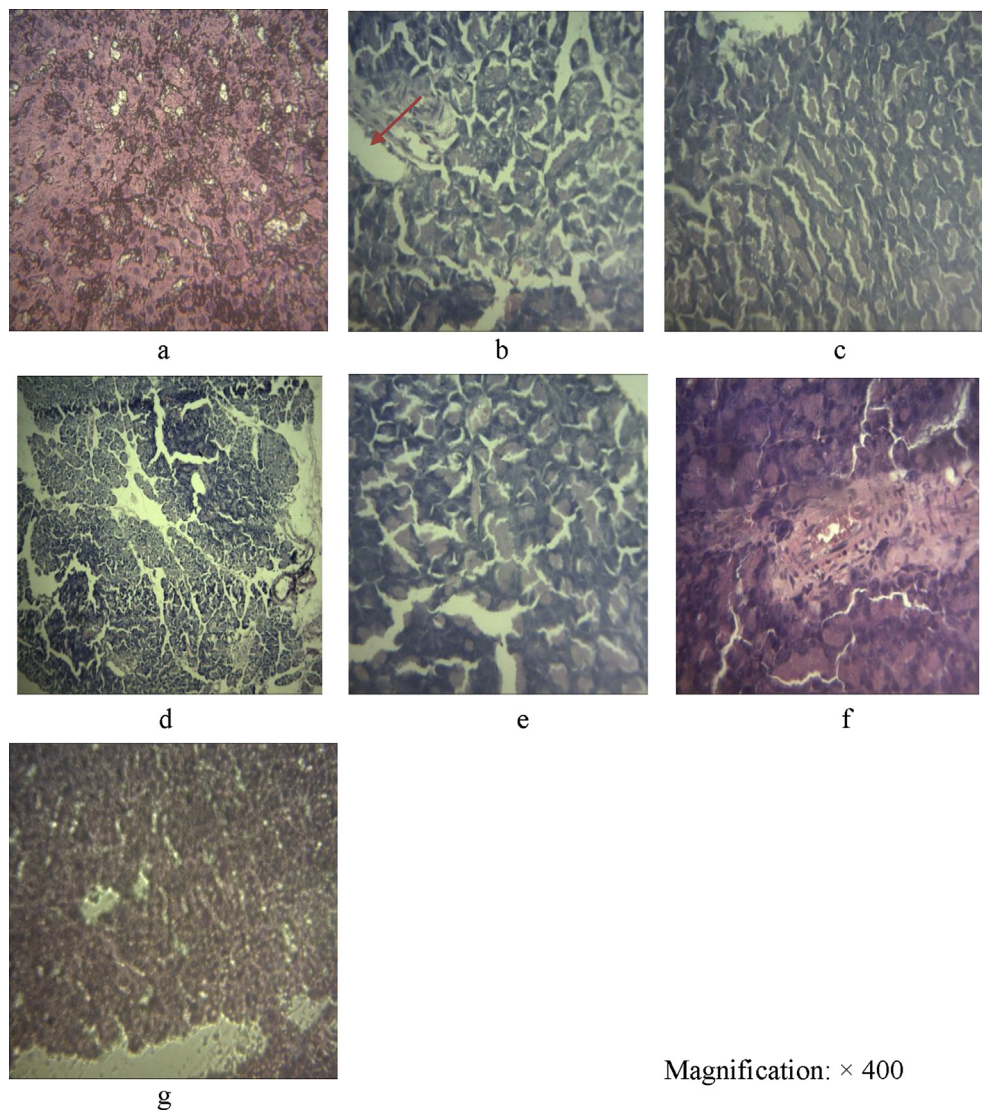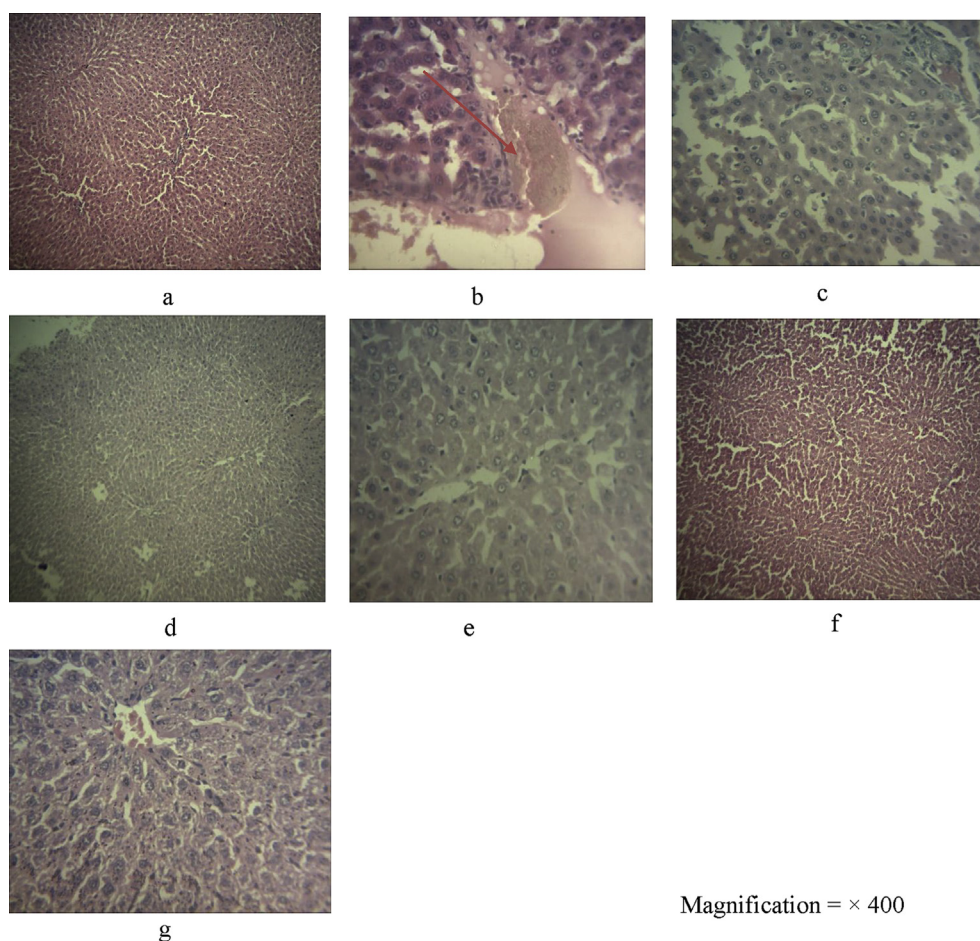

Magnification: × 400

Fig. 4. Pancreas sections of normal and diabetic rats. Groups a, c, e, f and g showed no visible lesion. Groups b and d showed an area of extensive necrosis and moderate necrosis of the acini cells respectively. a = normal control, b = diabetic control, c = diabetic + 30 mg/kg MSD, d = diabetic + 60 mg/kg MSD, e = diabetic + 30 mg/kg FSD, f = diabetic + 60 mg/kg FSD, g = diabetic + 5 mg/kg glibenclamide.

Magnification = × 400

Fig. 5. Liver sections of normal and diabetic rats. Groups a, c, d, e, and f showed no visible lesion. Group b showed a portal congestion with mild periportal cellular infiltration by mononuclear cells while and g has a diffuse hydropic degeneration of hepatocytes. a = normal control, b = diabetic control, c = diabetic + 30 mg/kg MSD, d = diabetic + 60 mg/kg MSD, e = diabetic + 30 mg/kg FSD, f = diabetic + 60 mg/kg FSD, g = diabetic + 5 mg/kg glibenclamide.

5. Conclusion

The methanolic and flavonoid-rich extracts of *S. dulcificum* leaves have definite antidiabetic activities, and compared favorably with glibenclamide. We suggest that the antidiabetic potential of both MSD and FSD is due to the presence of the identified polyphenols in the extract. However, further studies should focus on the characterization of these active principles. This will enhance studies on the mechanism of action of the principles which may be acting singly or in synergy to bring about the observed activity.

Sources of funding

None

Conflict of interest

None

References

[1] Ahmad F, Khalid P, Khan MM, Chaubey M, Rastogi AK, Kidwai JR. Hypoglycemic activity of *Pterocarpus marsupium* wood. J Ethnopharmacol 1991;35: 71–5.
[2] World Health Organization. Diabetes fact sheet. 2011. no. 312.
[3] National Institute of diabetes and digestive and kidney diseases publication. 2014. p. 14–5164.
[4] Kaku K. Pathophysiology of type 2 diabetes and its treatment policy. JMAJ 2010;53:41–6.
[5] Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9: 193–205.
[6] Shukla R, Barve V, Padhye S, Bhonde R. Synthesis, structural properties and insulin- enhancing potential of bis (quercetinato) oxovanadium (IV) conjugate. Bioorg Med Chem Lett 2004;14:4961–5.
[7] Singab ANB, El-Beshbishy HA, Yonekawa M, Nomura T, Fukai T. Hypoglycemic effect of Egyptian *Morus alba* root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. J Ethnopharmacol 2005;100:333–8.
[8] Kandaswami C, Middleton JE. Free radical scavenging and antioxidant activity of plant flavonoids. Adv Exp Med Biol 1994;366:351–76.
[9] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933–56.
[10] Kalpana R, Vijay KS, Parag JS, Prakash R, Zabeer A, Veena DS. In-vitro and in-vivo antiadipogenic, hypolipidemic and antidiabetic activity of *Diospyros melanoxylon* (Roxb). J Ethnopharmacol 2014;155:1171–6.
[11] Duke JA, Ducellier JL. Handbook of alternative cash crops. CRC Press; 1993. p. 433–43.
[12] Chen CY, Wang YD, Wang HM. Chemical constituents from the leaf of *Synsepalum dulcificum*. Chem J Nat Compds 2010;46:495.
[13] Chen CC, Liu IM, Cheng JT. Improvement of insulin resistance by miracle fruit (*Synsepalum dulcificum*) in fructose-rich chow-fed rats. Phytother Res 2006;20:987–92.
[14] Obafemi TO, Akinmoladun AC, Olaleye MT, Onasanya A. High Performance Liquid Chromatography (HPLC) fingerprinting, mineral composition and in vitro antioxidant activity of methanol leaf extract of *Synsepalum dulcificum* (Sapotaceae). J App Pharm Sci 2017 (in press).
[15] Chu YF, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activity of common vegetables. J Agric Food Chem 2002;50:6910–6.
[16] Rachel DW, Shahidul I. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. Pharmacol Rep 2012;64:129–39.
[17] Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389–94.

[18] Varshney R, Kale RK. Effects of calmodulin antagonist. Int J Rad Biol 1990;58: 733—43.

[19] Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130—9.

[20] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170—9.

[21] Rotruck JT, Pope AL, Ganther HE, Swason AB. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973;179:588—90.

[22] Culling CFA. Handbook of histopathological and histochemical techniques. 3rd ed. London: Butterworth and Co; 1974. p. 96.

[23] Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian pac J Trop Biomed 2011;1(5):353—8.

[24] Gaster B, Hirsch IB. The effects of improved glycemic control on complications in type 2 diabetes. Arch Int Med 1998;158:134—40.

[25] Neelesh M, Sanjay J, Sapna M. Antidiabetic potential of medicinal plants. Acta Pol Pharm 2010;67(2):113—8.

[26] Adeyi AO, Nneji Lotanna M, Idowu BA. Ameliorative potentials of medicinal plants on the pathophysiological complications of diabetes mellitus: a review. J Med Plants Res 2015;9(8):262—88.

[27] Gray AM, Flatt RP. Insulin secreting activity of the traditional antidiabetic plant *Viscum album*. J Endocrinol 1999;160:409—14.

[28] Koletsky RJ, Ernsberger P. The glucose tolerance test as a laboratory tool with clinical implications. Glucose Tolerance. InTech; 2012. p. 3—14. http://dx.doi.org/10.5772/54785.

[29] Amos FJL, Jolin T, Salinas M, Dominguez F, Herrera E. The effects of streptozotocin diabetes on brain protein synthesis in the rat. Diabetes Metab 1985;11:92—7.

[30] World Health Organization Media centre. Diabetes. 2013.

[31] Harita N, Hayashi T, Sato KK, Nakamura Y, Yoneda T, Endo G, et al. Lower serum creatinine is a new risk factor of type 2 diabetes: the Kansai healthcare study. Diabetes Care 2009;32:424—6.

[32] Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Toxicol 2003;17:24—38.

[33] Jung CH, Zhou S, Ding GX, Kim JH, Hong MH, Shin YC, et al. Antihyperglycemic activity of herb extracts on streptozotocin-induced diabetic rats. Biotechnol Biochem 2006;70:2556—9.

[34] Picton SF, Flatt PR. Differential acute and long term actions of succinic acid monomethyl ester. Int J Exp Diabetes Res 2001;2:19—27.

[35] Carmena R. Type 2 diabetes, dyslipidemia, and vascular risk: rationale and evidence for correcting the lipid imbalance. Am Heart J 2005;150:859—70.

[36] Papanas N, Maltezos E. Oral antidiabetic agents: antiatherosclerotic properties beyond glucose lowering? Curr Pharm Des 2009;15:3179—92.

[37] Molavi B, Rassouli N, Bagwe S, Rasouli N. A review of thiazolidinediones and metformin in the treatment of type 2 diabetes with focus on cardiovascular complications. Vasc Health Risk Manag 2007;3:967—73.

[38] Jesús M, Xavier P, Anna M, Manuel Z, Joan R, Luis F, et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manag 2009;5:757—65.

[39] Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein depleted plasma (FERHDL). Clin Biochem 2001;34:583—8.

[40] Criqui MH, Golom BA. Epidemiologic aspects of lipid abnormalities. Am J Med 1998;105:48S—57S.

[41] Pihlajamäki J, Gylling H, Miettinen TA, Laakso M. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res 2004;45:507—12.

[42] Miettinen TA, Gylling H. Cholesterol absorption efficiency and sterol metabolism in obesity. Atherosclerosis 2000;153:241—8.

[43] Simonen PP, H. K, Gylling HK, Miettinen TA. Diabetes contributes to cholesterol metabolism regardless of obesity. Diabetes Care 2002;25:1511—5.

[44] Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and antioxidative glycosylation;Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and aging. Biochem J 1998;256:205—12.

[45] Halliwell B, Gutteridge MC. Free radical and other reactive species and disease. Free radicals in biology and medicine. 3rd ed. Oxford University Press; 1999. p. 639—46.

[46] Esterbauer A. Aldehydic of lipid peroxidation. Free radicals, lipid peroxidation and cancer. Academic Press; 1990. p. 101.

[47] Chattopadhyay SM, Ramanothan J, Das Bhattacharva SK. Animal models in experimental diabetes mellitus. Exp Bio 1997;35:1141—5.

[48] Karuppusamy A, Thangaraj P. Antidiabetic and enzymatic antioxidant properties from methanol extract of *Ficus talboti* bark on diabetic rats induced by streptozotocin. Asian Pac J Reprod 2014;3(2):97—105.