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Myrothamnus flabellifolius attenuates streptozotocin-high energy diet-induced type 2 diabetes in male sprague dawley rats

Tebogo Elvis Kwape[1,2*], Padmaja Chaturvedi[2], Goabaone Gaobotse[1], Kabo Masisi[1], Keagile Bati[1] and Runner Majinda[3]

[1]Department of Biological Sciences and Biotechnology, Faculty of Sciences, Botswana International University of Science and Technology, Palapye, Botswana.
[2]Department of Biological Sciences, Faculty of Sciences, University of Botswana, Gaborone, Botswana.
[3]Department of Chemistry, Faculty of Sciences, University of Botswana, Gaborone, Botswana.

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Several medicinal plants have anti-diabetic properties and can be considered as an alternative therapy because of their actions which are like those of conventional antidiabetic drugs. In this study, we investigated *Myrothamnus flabellifolius* (MF) ethanol: water (70:30) extract for its possible anti-diabetic potential in streptozotocin-high energy diet (STZ-HED) induced type 2 diabetes mellitus (T2DM) rats and its possible mechanisms of action. Diabetic rats were divided into MF-50, MF-100, MF200, MF-300, MF-400, and Metformin (MET)-500 groups, where the numbers represent doses in mg.kg.bw that were administered to the groups. Normal (NC) and diabetic (DC) controls were administered distilled water. The animals had their fasting blood glucose levels and body weights determined weekly for 21 days. Blood samples, liver, pancreas, and muscle were collected and used for biochemical and histological examination. Our study showed that MF extract lowered glucose levels, body weight, glycated hemoglobin, thiobarbituric acid reactive substances, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, Homeostatic model assessment of insulin resistance, and alkaline phosphatase ($p<0.05$; $p<0.01$) in T2DM. While adiponectin, catalase, leptin, lipid profile, insulin Homeostatic model of assessment of β cell function, and superoxide dismutase levels were elevated ($p<0.05$; $p<0.01$). Furthermore, MF extract preserved both the liver and the islets structure of T2DM rats and enhanced muscle glucose transporter 4 (GLUT 4) expression. *M. flabellifolius* extract normalized blood glucose and body weight in T2DM. Further investigations are needed to explore other possible mechanisms of action and clinical potential of MF extracts.

Key words: *Myrothamnus flabellifolius,* T2DM, Lipid profile, blood glucose, oxidative stress, streptozotocin-high energy diet, Sprague dawley rats.

INTRODUCTION

Diabetes is a social problem affecting more than 463 million people worldwide of which 87-91% suffer from

*Corresponding author. E-mail: kwapet@biust.ac.bw. Tel: +267-493-1887.

type 2 diabetes mellitus. It has been predicted that the number of diabetic patients will increase to 578 million and 700 million in 2030 and 2045 respectively (International Diabetes Federation, 2019). Type 2 diabetes mellitus arises due to the combined effects of insulin resistance and insufficient insulin secretion by the pancreatic β-cells (Kahn et al., 2014; Axelsson et al., 2017). Uncontrolled hyperglycemia in type 2 diabetes can result in vascular complications affecting the kidney, heart, nervous system, and eyes (Faselis et al., 2020). Some of the risk factors of type 2 diabetes mellitus are obesity and physical inactivity. High fat and high sugar diets are also responsible factors (Guo et al., 2018; Abu-Saad et al., 2019). Accumulating evidence indicates that several medicinal plants have anti-diabetic properties and can be used as alternative therapy due to their actions that are like conventional antidiabetic drugs (Gupta et al., 2017; Bahmani et al., 2014).

According to the World Health Organization (WHO), 65-80% of the populations in developing countries currently use medicinal plants as remedies for preventing diseases such as type 2 diabetes (Singh et al., 2018). It is estimated that of the 300, 000 plant species in the world, only 15% have been evaluated to determine their pharmacological potential (De Luca et al., 2012). *Myrothamnus flabellifolius* (MF) is a wild African plant found in Southern Africa that is known for its many traditional uses. The plant is widely sold by the local street vendors due to its appealing medicinal value. *M. flabellifolius* is reported to treat asthma, backaches, kidney problems, microbial infections (Molefe-Khamanga, 2012), stroke, and shingles (Setshogo and Mbereki, 2011). It is reported to possess flavonoids, alkaloids, gums, steroids, tannins, glycosides, and phenolics which may be responsible for its diverse medicinal use (Molefe-Khamanga, 2012; Cheikyoussef et al., 2015). Some studies have reported the anticancer, antioxidant, antimicrobial, antiviral effects of the different extracts of *M. flabellifolius* as reviewed by Erhabor et al. (2020). However, the effects of *M. flabellifolius* on metabolic risk factors are not well documented. Therefore, in this study, we hypothesize that *M. flabellifolius* reduces metabolic risk factors in diabetic animal models. To test our hypothesis, we evaluated the impact of *M. flabellifolius* treatment on metabolic risk factors in diabetic Sprague Dawley (SD) rats induced with Streptozotocin-High Energy Diet.

MATERIALS AND METHODS

Chemicals and materials

Streptozotocin and all other chemicals of analytical grade were purchased from Sigma-Aldrich (St Louis, MO, USA). Primary and secondary antibodies for GLUT 4 were purchased from Cell Signaling Technology (Danvers, MA, USA). The ELISA and all other kits were purchased from Aggape Diagnostics (Agappe Hills, Pattimattom PO, Kochi, Kerala 683562, India).

Preparation of 70% ethanol/water *M. flabellifolius* extracts (EW70)

M. flabellifolius shoots (leaves and twigs) were collected from Ranaka hills in the Southern part of Botswana. Plant specimen authentication was performed by Dr. M. Muzila of the University of Botswana Herbarium. The plant shoots were then washed with distilled water; sun-dried and thereafter ground using a laboratory grinder purchased from Fisher Scientific (Bartlesville, OK 74003, USA) to obtain a powder. The powder was soaked and extracted in 70% ethanol/water at room temperature. The solvent was evaporated with a rotary evaporator purchased from Fisher Scientific (Bartlesville, OK 74003, USA) while the extract was freeze-dried before storage at 4°C until use.

Animal husbandry and care

Protocols and methods used to maintain rats in this study were approved by the University of Botswana's Animal Use and Care Committee (Ref no: UBR/RES/ACUC/001). Hundred and twenty male Sprague Dawley rats (120 to 170 g) were obtained from the University of Botswana breeding unit. These rats were initially purchased from South African Vaccine Producers. Five rats were housed per galvanized cage with access to food (Epol Rodent Chow), water *ad libitum*, and under optimal vivarium conditions (12 h/12 h light-dark cycle, 24±1°C and 40-50% relative humidity). After the acclimation period, rats were randomly divided into eight groups (n=5/group) and given similar high energy diets and different concentrations of ethanol/water *M. flabellifolius* extract, water, or metformin through oral gavage. Average food consumption, body weight, and fasting blood glucose levels were determined weekly. Oral glucose tolerance tests (OGTTs) were conducted at the beginning and towards the end of the experiments. The glucose level of rats that had been fasting for 16 h was monitored at 1 h intervals for 3 h after the animals had been fed a 2 g/kg body weight glucose solution.

Diet and experimental design

Seven-week-old rats were randomly divided into eight groups (n=5/group) and fed with high energy diets (HED) consisting of powdered commercial food pellets (with minerals and vitamins) (365 g/kg), animal fat (310 g/kg), casein 75 (g/kg) and sugar (250 g/kg). The HEDs were used to induce type 2 diabetes. They were followed by intraperitoneal injection of streptozotocin at 35 mg/kg body weight. Normal controls were un-injected animals. The animals received oral gavage with distilled water for the normal control group or MF dosages (50, 100, 200, 300, and 400 mg.kg body weight) or 500 mg metformin for the experimental period of 21 days. Blood and tissue samples were collected in anesthetized and euthanized rats at the end of the experimental period after rats had been fasting for 12 h.

Final body and organ weight

At the end of the study, final body weight was measured. Furthermore, organs and tissues were excised. The organs were rinsed with physiological saline solution and weighed. Portions of the liver and pancreas were fixed with 10% formalin for histological analysis. Fresh muscle tissue was excised for the estimation of glucose transporter 4 (GLUT 4). All tissues and organs were kept at -80°C until analysis.

Quantification of blood and plasma biochemical parameters

Blood samples from tails of rats were collected throughout the experiments and analyzed for cholesterol, triglycerides, glycemia, and glucose using reactive strips (Accu-Check Plus; Roche Diagnostics, Mannheim, Germany). Plasma from blood collected by cardiac puncture at the end of the study was obtained by centrifugation at 1000×g for 15 min and stored at - 80°C until further analysis. Plasma lipid profiles, glycated hemoglobin (HbA1C), Thiobarbituric acid reactive substances (TBARS), glutamate oxaloacetate (GOT), Alkaline phosphatase (ALP), and glutamate pyruvate transaminase (GPT) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Aggape diagnostics, Switzerland). Plasma insulin, adiponectin, leptin reduced glutathione (GSH), SOD, and catalase were used in the estimation by ELISA kits (Elabscience, China).

Western blot

Western blot was performed as described by Benhaddou-Andaloussi et al. (2011), with some modification. Samples of the skeletal muscle tissues were ground in liquid nitrogen and subsequently lysed. Sucrose lysis buffer (20 mM, Tris-HCL pH 7.4, 255 mM sucrose, 1 mM EDTA) was used for GLUT 4. A protease inhibitor cocktail was added (Roche, Mannheim, Germany) as well as 1 M phenylmethanesulfonyl fluoride and phosphatase inhibitors (1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 10 mM sodium fluoride). Cells were lysed for 30 min on ice and then centrifuged at 12000xg for 10 min. Supernatants were then stored at -80°C until analysis. Protein content was assayed by the bicinchoninic acid method standardized to bovine serum albumin (Roche, Laval, QC, Canada). Lysates were diluted to a concentration of 1.25 mg/mL total protein and boiled for 5 min in reducing sample buffer (62.5 mM Tris-HCL pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.01% bromophenol blue). Twenty microlitre of each sample was separated on 10% polyacrylamide mini-gel and transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA). The membrane was blocked for 2-h at room temperature with Tween-20 and 5% skim milk in TBS (20 mM Tris-HCL, pH 7.6, and 137 mM NaCl). The membrane was then incubated overnight at 4°C in blocking buffer with appropriate phospho-specific or pan-specific antibodies against GLUT at 1:1000. The membrane was washed 5 times and incubated for 1.5-h at room temperature in TBS plus tween-20 with anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies at 1:100000 to 1:50000. Band visualization was performed using the enhanced chemiluminescence method and luminescence captured to blue-light sensitive film (Amersham Bioscience, Buckinghamshire, England).

Histological analysis

Formalin-fixed unprocessed liver and pancreas tissues were dehydrated in a graded ethanol series (70%-100) before hematoxylin and eosin (H&E) staining to determine histomorphological features.

Statistical analysis

The results were presented as means ± SEM. Statistical analysis was performed with univariate analysis, one-way analysis of variance (ANOVA), and Tukey Kramer multiple comparison procedure. Data analysis was performed using the statistics software Sigmastat 3 purchase from Systat Software Inc, USA. Statistical significance of the difference among means was estimated at $p < 0.05$.

RESULTS

EW70 improves glucose tolerance of the normal rats

Almost all dosages of EW70 tested showed a significant ($p<0.01$) and dose-dependent improvement in the glucose tolerance of the experimental rats when compared to the control rats which were fed glucose only. Only the 50 mg.kg.bw extract showed poor enhancement of glucose tolerance when compared to the control group which was fed glucose only. The improvement in glucose tolerance shown by lowering glucose level over 3-h increased with an increase in the extract dosage (Figure 1).

Chronic administration of EW70 reduces weight gain

STZ-HED induced type 2 diabetic rats showed a significant weight gain when compared to the normal control group. EW70 administration led to a significant reduction in weight gain when compared to STZ –HFD induced type 2 diabetic control (Table 1). Values are expressed as mean ± SEM; column with *****($p<0.05$) or **($p<0.01$) indicate statistical differences when compared to normal control as determined by the Tukey Kramer's multiple range test; column with ^{a}($p<0.05$) or ^{aa}($p<0.01$) indicate statistical differences when compared to diabetic control as determined by the Tukey Kramer's multiple range test;. E1 (50 mg.kg.bw extract), E2 (100 mg.kg.bw), E3 (200 mg.kg.bw extract), E4 (300 mg.kg.bw), E5 (400 mg.kg.bw).

EW70 administration leads to short term hourly anti-diabetic effect

An hourly effect of EW70 was performed by administering graded doses of EW70 followed by an hourly observation of the glucose level for 3-h. The EW70 resulted in significantly ($p<0.01$) lowered glucose level on the 3rd when compared to diabetic control. The glucose-lowering effect increased with an increase in extract dosage. Metformin offered the most significant ($p<0.05$) glucose-lowering effect when compared to both diabetic and the experimental groups (Figure 2).

Chronic oral administration of EW70 lowers glucose levels

A daily oral gavage of graded doses of EW70 prevented a rise in glucose level compared to in HFD-STZ induced type 2 diabetic rats. It resulted in a significant ($p<0.05$) lowering effect in the glucose level when compared to the diabetic control. The glucose-lowering effect was dose-dependent. The highest dosage showed the most significant lowering effect on day 21 when compared to

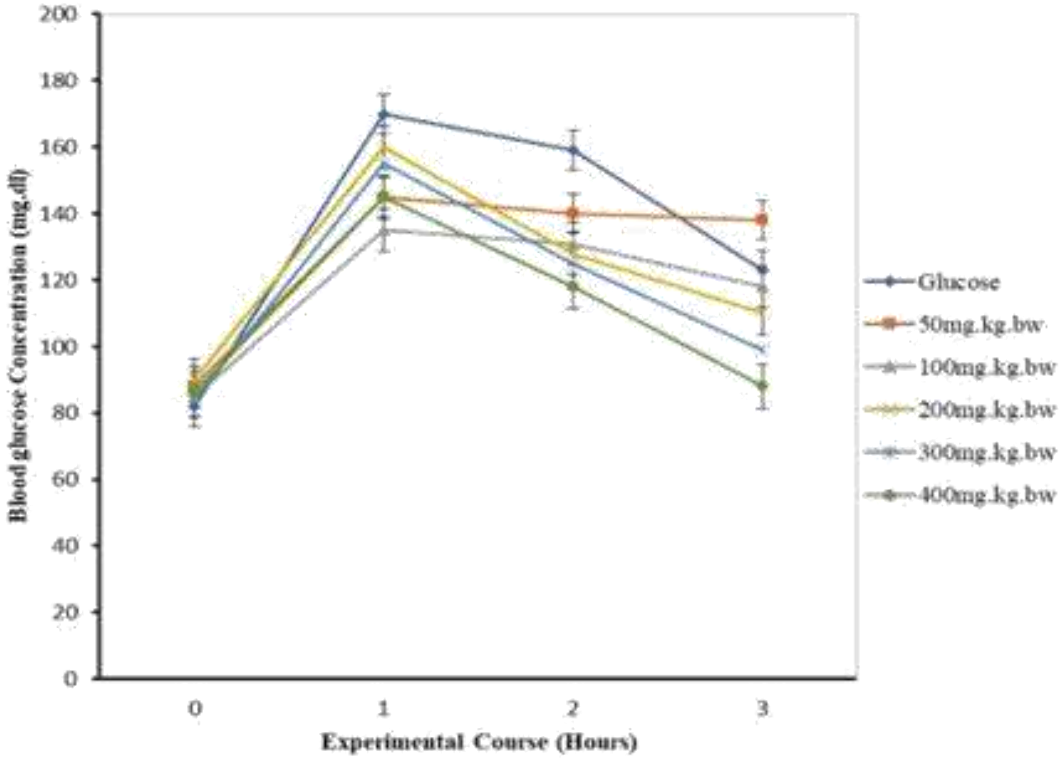

Figure 1. Effect of EW70 on oral glucose tolerance test in normal rats. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

Table 1. Effect of the chronic administration of EW70 extraction the bodyweight of STZ-HED (DC) induced type 2 diabetic rats

Treatment	Body weight (grams) in days				
Groups	**Before**	**Initial**	**7**	**14**	**21**
NC					
DC	182.75±1.11*	183.73±2.54*	189.62±1.82*	205.75±1.44*	225.48±2.31*
E1	184.41±2.34*	185.92±2.33*	188.51±1.99*	204.19±2.54*	223.32±2.33*
E2	180.31±2.55*	182.55±1.79*	186.74±3.24*/[a]	202.04±2.00*/ [a]	221.74±3.59*/
E3	181.42±1.97*	181.45±3.50*	184.55±2.88*/ [a]	200.37±2.74*/[a]	199.50±1.78*/
E4	180.77±2.88*	180.51±2.59*	181.39±1.64*/ [a]	190.98±2.33*/ [a]	185.11±1.88*/
E5	181.64±3.22*	180.72±1.58*	181.89±3.33*/ [a]	182.53±4.15*/ [a]	184.93±3.00*/

diabetic control. Metformin, a standard diabetic drug showed the most significant lowering effect when compared to diabetic control and the experimental groups (50 -300 mg.kg.bw) (Figure 3).

Chronic oral administration of EW70 lowers glycated hemoglobin (HbA1c)

Glycated hemoglobin of diabetic control was significantly ($p<0.05$) elevated when compared to normal control. An administration of EW70 graded doses to diabetic rats resulted in a significant decline in the level of HbA1c. The decrease in HbA1c followed an increase in the dosage of the extract. The highest dosage of 400 mg.kg.bw resulted in the most significant ($p<0.01$) decrease in HbA1c when compared to the diabetic control and the normal control (Figure 4).

Chronic administration of EW70 elevates adiponectin levels

The level of adiponectin was significantly ($p<0.05$) lower

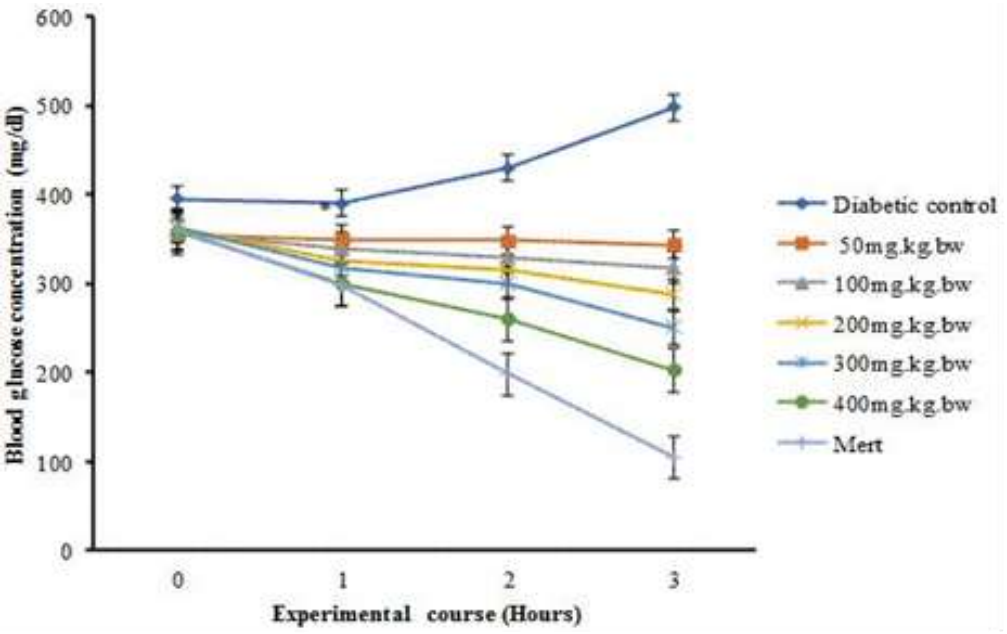

Figure 2. Hourly effect of different doses of EW70 and Met on glucose level during three hours in STZ-HED diabetic rats. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

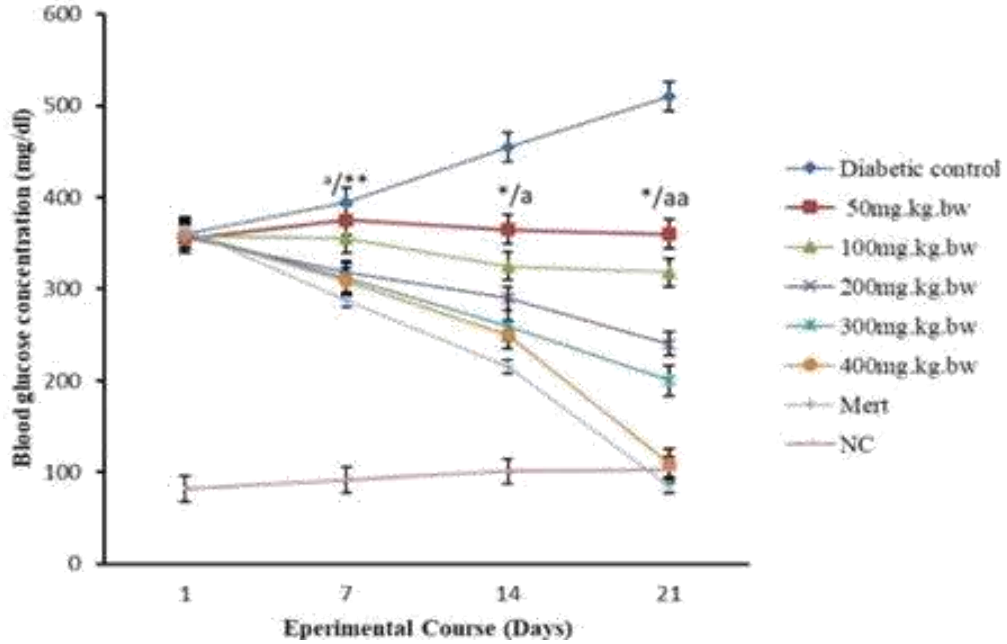

Figure 3. Effect of different dosages of EW70 and Met on glucose level in STZ-HED induced diabetic rats over 21 days. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

in the diabetic control when compared to the normal control. Graded doses of EW70 administered to experimental groups resulted in significantly ($p<0.05$) elevated adiponectin levels when compared to the diabetic control. The increase in the level of adiponectin was dependent on an increase in the dosage of the

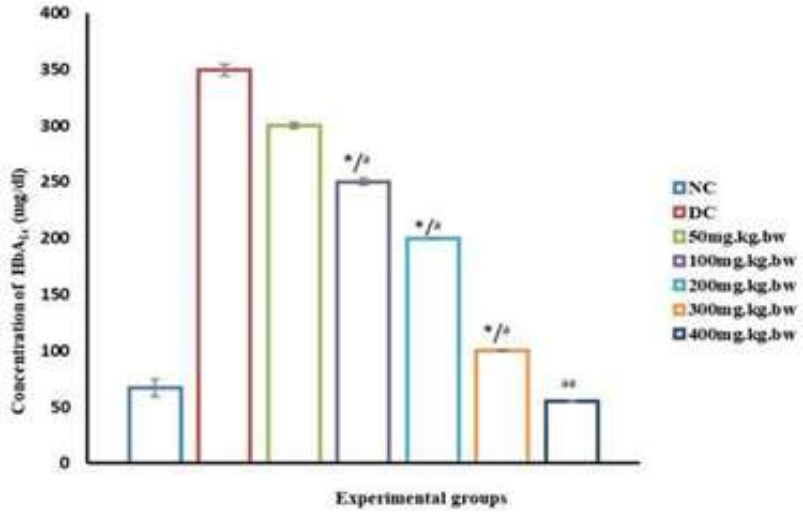

Figure 4. Effect of EW70 on glycated hemoglobin of STZ-HED induced diabetic rats compared with normal control. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

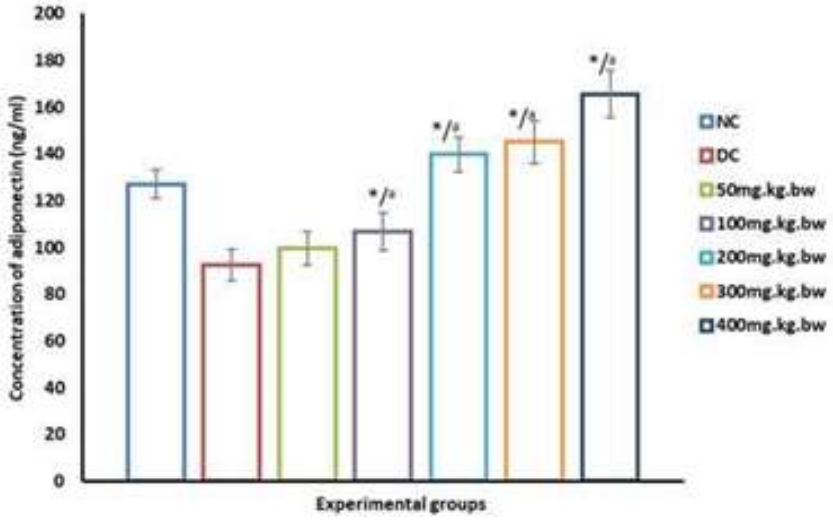

Figure 5. Effect of different concentrations of EW70 on plasma adiponectin levels compared with normal and diabetic controls. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

extract (Figure 5).

Chronic administration of EW70 increases leptin levels

Leptin level was significantly ($p<0.05$) decreased in the diabetic control when compared to the normal control. A significant increase in leptin levels occurred in response to EW70 administration when compared to the diabetic control. A more pronounced significant ($p<0.05$) increase was marked in the 300 and 400 mg.kg.bw dosages. This marked increase was significantly ($p<0.05$) higher than both the normal control leptin levels (Figure 6).

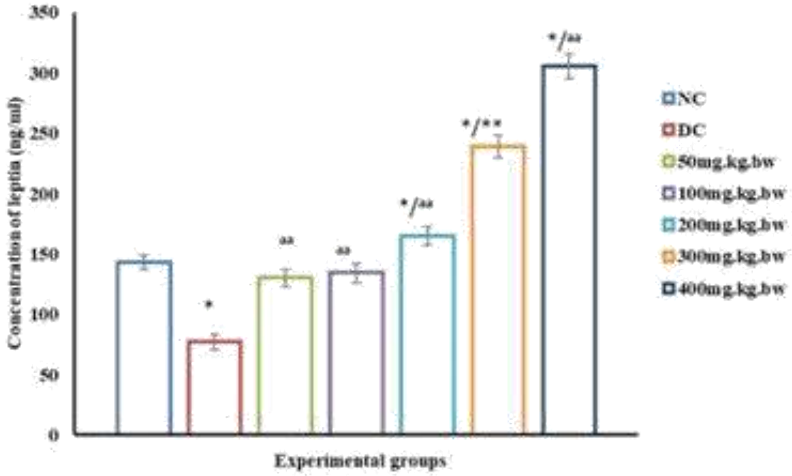

Figure 6. Effect of varying concentrations of EW70 on plasma leptin levels compared with normal and diabetic controls. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively (p<0.05; P<0.01 Tukey-Kramer's multiple range test).

Table 2. Effect of EW70 extract on lipid and lipoprotein profiles in plasma from HFD-STZ induced type 2 diabetic rats

Groups	HDL (mg/dL)	LDL (mg/dL)	Cholesterol (mg/dL)	TP (mg/dL)	Triglycerides (mg/dL)
NC	61.01± 0.13	12.03± 1.12	13.82± 1.21	10.00 ± 0.05	65.21± 5.33
DC	12.40± 0.55**	131.01 ± 2.12*	55.76± 1.22*	1.98± 0.01*	230.18 ±5.44*
E1	14.08± 0.44 ^{a} /**	101.05 ± 5.33*/ ^{a}	45.61± 1.13*/ ^{aa}	2.11± 0.04*/ ^{aa}	188.29 ± 4.21*/ ^{a}
E2	22.61± 0.25 ^{a} /**	75.65± 3.15*/ ^{aa}	38.39± 1.04*/ ^{aa}	3.52± 0.44*/ ^{aa}	98.67± 4.19*/ ^{aa}
E3	35.11± 0.25 ^{a} /**	47.08± 3.47*/ ^{aa}	30.54± 1.01*/ ^{aa}	5.58± 0.66*/ ^{aa}	71.77± 4.88*/ ^{aa}
E4	51.18± 0.33 ^{aa}/*	35.06± 2.35*/ ^{aa}	28.11± 0.88*/ ^{aa}	7.33± 0.07*/ ^{aa}	68.81± 3.22*/ ^{aa}
E5	58.10± 0.18 ^{aa}	15.19± 1.68 ^{aa}	12.45± 1.78 ^{aa}	8.01± 0.05 ^{aa}	62.23± 6.15 ^{aa}

Chronic administration of EW70 promotes a healthy balance in plasma lipid profiles

The HDL and total protein (TP) levels in the STZ-Diabetic group were lowered significantly (p<0.05) when compared to the normal control. EW70 was observed to significantly increase HDL and TP levels when compared to the diabetic control. LDL, Cho, and Triglycerides levels increased significantly (p<0.01) in the diabetic control (DC) group when compared to levels in the normal control. The levels of LDL (p<0.01), Cho (p<0.01), and Triglycerides (p<0.01) decreased in EW70 fed rats when compared to the diabetic control group (Table 2).

Values are expressed as mean ± SEM; column with *(p<0.05) or **(p<0.01) indicate statistical differences when compared to normal control as determined by the Tukey Kramer's multiple range test; column with a (p<0.05) or aa (p<0.01) indicate statistical differences when compared to diabetic control as determined by the Tukey Kramer's multiple range test; E2 (100 mg.kg.bw), E3 (200 mg.kg.bw), E4 (300 mg.kg.bw), E5 (400 mg.kg.bw).

Chronic administration of EW70 elevates insulin levels

Significantly lowered insulin levels were demonstrated in HED-STZ induced type 2 diabetic rats when compared to the normal control. EW70 fed experimental groups expressed significant (p<0.05) but gradual increases in insulin levels when compared to diabetic controls. The increases in the insulin level for all the experimental groups were significantly (p<0.05) lower than the normal control group (Figure 7).

Chronic administration of EW70 increases HOMA-β and reduces HOMA-IR

Significantly (p<0.05) elevated Homa-IR was observed in the DC when compared to the NC. Significantly (p<0.05) reduced Homa-IR was observed in EW70 fed rats when compared to both the diabetic and normal control. The Homa-β function was significantly (p<0.05) lowered in DC rats when compared to the NC and the EW70 group. A

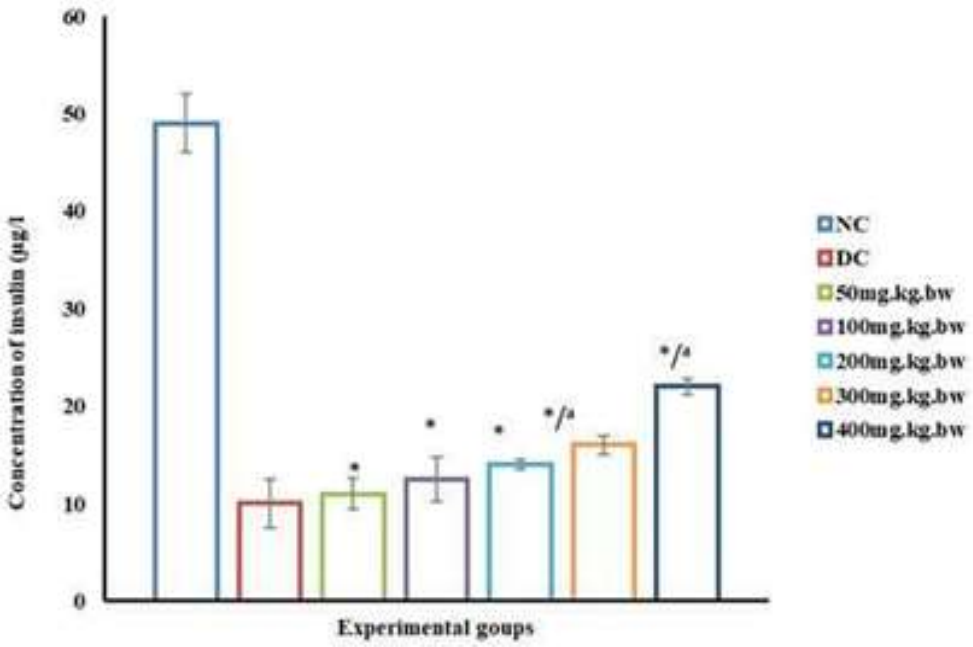

Figure 7. Effect of varying concentrations of EW70 on plasma insulin levels compared with normal and diabetic controls. Data presented are mean ± SEM (n=5). Letters and asterisk indicate significant differences compared to normal control and diabetic control respectively ($p<0.05$; $p<0.01$ Tukey-Kramer's multiple range test).

Table 3. Effect of EW70 on HOMA-IR and HOMA-β levels in plasma from HFD-STZ induced type 2 diabetic rats.

Group	IR	Homa-β (%)
NC	1.34±0.21	106.2±0.14
DC	5.85±0.88*/ [a]	5.3±0.41*
EW70	0.63±0.19*/ [a]	115.81±0.25*/ [a]

significant ($p<0.05$) increase of Homo-β function was observed in EW70 administered rats when compared to the DC and NC (Table 3). Values are expressed as mean ± SEM; column with ***** ($p<0.05$) or ** ($p<0.05$) indicate statistical differences when compared to normal control as determined by the Tukey Kramer's multiple range test; column with [a]($p<0.05$) or [aa]($p<0.01$) indicate statistical differences when compared to diabetic control as determined by the Tukey Kramer's multiple range test.

Chronic administration of EW70 increases GLUT 4 expression

A marked increase in Glut 4 expression was observed in the EW70 administered group which was shown by the more visible band on the EW70 group when compared to fainter and low-intensity bands for the DC and the NC groups (Figure 8).

Chronic administration of EW70 lowers the levels of TBARS, SGPT, SGOT and ALP

The diabetic control showed elevated ($p<0.05$) levels of TBARS compared to the normal control. Graded doses of EW70 were observed to lower the level of TBARS when compared to the diabetic control. An increase in EW70 dosage demonstrated a reduced level of TBARS. Levels of plasma marker enzymes SGOT ($p<0.01$), SGPT ($p<0.001$), and ALP ($p<0.01$) increased in STZ-diabetic rats when compared to the normal control. The EW70 fed rats were found to have significant decreases in the SGOT ($p<0.05$), SGPT ($p<0.05$), and ALP ($p<0.05$) levels (Table 4). Values are expressed as mean ± SEM; column with ***** ($p<0.05$) or ** ($p<0.01$) indicate statistical differences when compared to normal control as determined by the Tukey Kramer's multiple range test; column with [a]($p<0.05$) or [aa] ($p<0.01$) indicate statistical differences when compared to diabetic control as

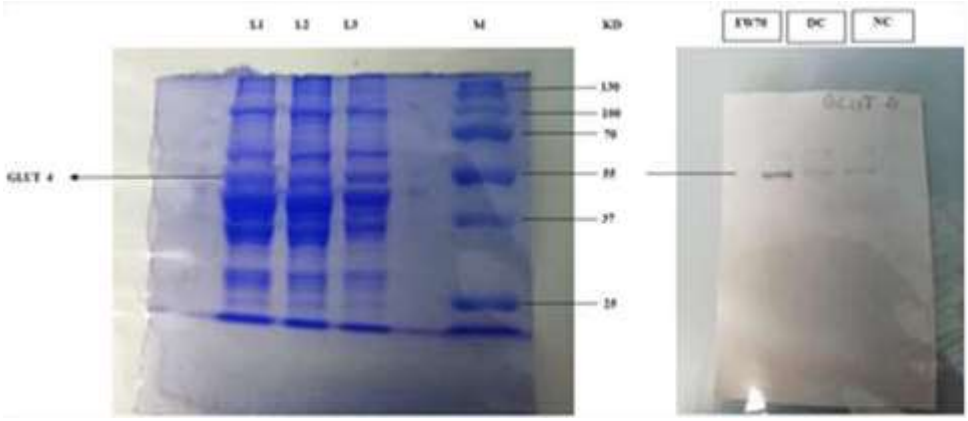

Figure 8. Expression of GLUT 4 in the skeletal muscles of rats fed EW70 compared with DC and the NC, after 21 days of treatment. L1 = EW70, L2 = DC, L3 = NC, M = protein ladder.

Table 4. Effects of EW70 on plasma marker enzymes from STZ-HED induced type 2 diabetes

Groups	TBARS(nmol/ml)	SGOT(U/L)	SGPT(U/L)	ALP(U/L)
NC	0.21± 0.16	46.12± 0.21	48.05± 0.51	69.41± 0.61
DC	5.11± 0.11*	350.07±0.66*	340.42±0.17*	561.32±0.18*
E1	2.84± 0.19*/ ^{aa}	240.15±0.41*/ ^{aa}	287.53±0.21*/ ^{aa}	516.44±0.14*/ ^{aa}
E2	2.11± 0.22*/ ^{aa}	176.28±0.10*/ ^{aa}	246.18±0.19*/ ^{aa}	238.71±0.21*/ ^{aa}
E3	1.92± 0.13*/ ^{aa}	70.31± 0.78*/ ^{aa}	144.31±0.43*/ ^{aa}	111.38±0.15*/ ^{aa}
E4	1.68± 0.11*/ ^{aa}	60.21± 0.34*/ ^{aa}	90.42± 0.13*/ ^{aa}	87.21± 0.49*/ ^{aa}
E5	0.80± 0.12*/ ^{aa}	50.33± 0.21*/ ^{aa}	47.21± 0.10 ^{aa}	75.65± 0.61 ^{aa}
EO	0.25± 0.12^{aa}	38.54± 0.11*/ ^{aa}	37.09± 0.81 ^{aa}	60.58± 0.29 ^{aa}

Table 5. Effects of EW70 on SOD and catalase in plasma from STZ-HED induced type 2 diabetes.

Groups	SOD (U/dL)	Catalase (U/dL)
NC	20.88± 0.33	487.99± 0.55
DC	7.99± 1.66*	251.11± 2.88*
E1	8.11± 0.33*/ ^{aa}	260.31± 3.29*/ ^{aa}
E2	10.44± 1.31*/ ^{aa}	271.22± 0.33*/ ^{aa}
E3	11.44± 0.14*/ ^{aa}	280.84± 2.99*/ ^{aa}
E4	13.71± 0.39*/ ^{aa}	301.99± 2.11*/ ^{aa}
E5	15.89± 0.89 ^{aa}	354.14± 0.38*/ ^{aa}

determined by the Tukey Kramer's multiple range test; E2 (100 mg.kg.bw), E3 (200 mg.kg.bw extract), E4 (300 mg.kg.bw), E5 (400 mg.kg.bw), EO (400 mg.kg.bw).

Chronic administration of EW70 increases SOD and catalase activities

The STZ-HED diabetic group showed reduced activities of SOD and catalase in comparison to the normal control ($p<0.05$). A slight increase ($p<0.01$) was observed in SOD and catalase activities for EW70 administered group when compared to the diabetic control (DC). An increase in the activities of the enzymes occurred when the EW70 extract dosage was increased (Table 5). Values are expressed as mean ± SEM; column with * ($p<0.05$) or ** ($p<0.01$) indicate statistical differences when compared to normal control as determined by the Tukey Kramer's multiple range test; column with ^{a} ($P<0.05$) or ^{aa} ($P<0.01$) indicate statistical differences when compared to diabetic

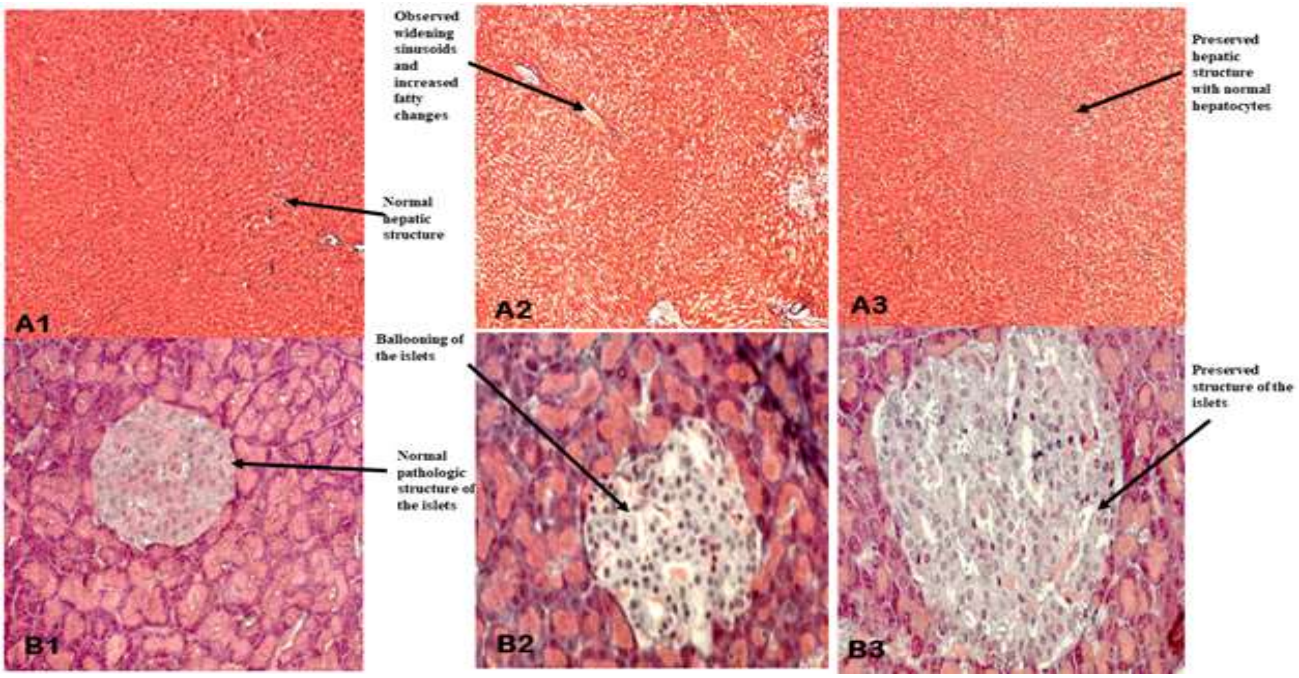

Figure 9. Photomicrographs of hematoxylin and eosin-stained and formalin-fixed liver and pancreatic islets cells. (A1) Normal control showing normal hepatocytes (X100), (A2) Diabetic control with a lot of fatty changes and evidence of widening sinusoids (X100), (A3) EW70 fed group showing preserved hepatocytes (X100). (B1) normal control showing the normal pathological structure of the islets (X4000). (B2) HED-STZ induced T2DM group showing evidence of islets ballooning (X400). (B3) EW70 fed T2DM model with evidence of islets preservation.

control as determined by the Tukey Kramer's multiple range test; E1 (STZ + 50 mg/kg.bw extract), E2 (STZ + 100 mgkg.bw), E3 (200 mg/kg.bw extract), E4 (300 mg kg.bw), E5 (400 mg/kg.bw).

Chronic administration of EW70 preserves liver and pancreatic beta cells

The preventive effects of EW70 ware revealed when compared to the diabetic control. The diabetic control was associated with changes in the pathological structure shown by fatty changes and membrane loss of the liver. The pancreatic islets show partial destruction of beta cells with no adverse pathological changes. The normal group showed normal hepatic and pancreatic islets pathologies with no pathological changes. The rats administered with only EW70 showed no signs of hepatic damage (Figure 9 A1, A2, A3, B1, B2 and B3).

DISCUSSION

M. flabellifolius (MF) has multiple health benefits such as the management of diabetes mellites (Motlhanka and Matlhapa, 2011). However, this plant has not been scientifically tested for its antidiabetic potential, its effects on related parameters, and its possible mechanisms of action. Our study thus investigated MF for its possible anti-diabetic potential in T2DM and its possible mechanisms of action. The HED-STZ T2DM rats were subjected to hourly 21-day long chronic administration of EW70. In both hourly effect and chronic administration studies, EW70 led to a significant reduction in blood glucose levels. The hypoglycaemic effect of the EW70 extracts may be attributed to the presence of compounds like flavonoids, terpenoids, triterpenoids, and many other compounds as reported by Kwape et al. (2016). Enhanced glucose uptake could be due to increased insulin sensitivity and/or increased insulin synthesis and release, as well as increases in glucose transporters. This increased insulin sensitivity is indicated by the Homa-IR and Homa-β scores. Homa-IR is significantly reduced with EW70 administration. The lowered Homa-IR could be due to the lowered glucose and insulin levels which signal improved insulin sensitivity (Kwape et al., 2016). The chronic administration of EW70 resulted in significantly increased Homa-β score when compared to the diabetic control with dysfunctional β cells. The increase is an indication of the functional improvement of β cells (Wilson and Islam, 2012). Histological examination of the pancreatic islets relative to the diabetic control attests to this functional improvement (Figure 9, B1, B2).

GLUT 4, an insulin-regulated glucose transporter plays a pivotal role in ascertaining glucose transfer to the target cells for metabolic functions. Translocation of GLUT 4 to

the cell membrane from the cytoplasm occurs during a normal physiological process of glucose homeostasis which is mainly triggered by insulin. GLUT 4 then acts as a glucose gateway to enter the cell through the membrane surface (Wang et al., 2018). The translocation of GLUT 4 to the cell membrane inevitably increases glucose uptake by the muscles or adipose tissues (Kuo et al, 2016). In this study, immunoblotting was employed to estimate the level of GLUT 4 expression in the skeletal muscle tissues of diabetic rats, EW70 extract fed rats, and normal control rats. The presence of GLUT 4 at low levels in muscle cells was observed in diabetic and normal rats while a marked increase in GLUT 4 expression in muscles of EW70 fed rats. Enhancement of GLUT 4 translocation by EW70 seems to be one of the mechanisms used to lower glucose levels. This is possibly a result of increased glucose uptake by muscles. In this way, GLUT 4 is used as a gateway to enter the muscle tissues, hence a reduction in blood glucose levels. Increased GLUT 4 expression may be a result of increasing insulin sensitivity which is depicted by significant decreases in the Homa-IR scores. Increased insulin sensitivity or reduced insulin resistance is due to an uninterrupted insulin signaling pathway during the stimulation of GLUT 4 translocation to the membrane (Takaguri et al., 2016). Increased β cell function may be a contributing factor to increased GLUT 4 expression. This is so because an increase in β cell function leads to adequate insulin supply and secretion for GLUT 4 stimulation. In the diabetic control rats, GLUT 4 expression was low, possibly because of the reduced Homa-β scores, which indicate β cell dysfunction. During β cell dysfunction, inadequate insulin is secreted to trigger or stimulate the translocation of GLUT 4 from the cytoplasm vesicles to the cell membrane.

Pancreatic beta cells are known to secrete insulin to control blood glucose levels. STZ is well known for its selective cytotoxicity on pancreatic islet beta cells which induce diabetes (King, 2012). The absence of or substantial reduction in beta cells leads to equally reduced insulin production and action. In the present study, the DC insulin levels were significantly lowered after 21-days when compared to the normal control. A significant rise in the insulin level was observed after the administration of EW70. The rise in insulin levels was dosage increase dependent and significantly lower than the NC insulin levels. The increase in Homa-β cell function because of the EW70 administration coincided with significant elevation in insulin levels of the treatment groups when compared to the diabetic control. Improvements in beta-cell function and significant increases in insulin levels in the treatment groups in comparison to the diabetic control are supported by findings of the histological examination of the pancreatic islets (Figure 9).

Adiponectin is a blood plasma adipokine that is specifically expressed in adipose tissues and has been found to directly sensitize the body to insulin through its receptors AdipoR1 and AdipoR2 (Wang and Scherer, 2016). Plasma insulin has been seen to increase in mice administered with high-fat diets. Replenishing of adiponectin significantly ameliorated high-fat diet insulin resistance, hence proposing adiponectin as an insulin-sensitizing adipokine (Kim et al., 2017). Adiponectin is also said to improve plasma clearance of free fatty acids, glucose, triglycerides, and to also suppress hepatic glucose production, as well as to stimulate adipogenesis (Tao et al., 2014). These effects of adiponectin are associated with increased cellular fat oxidation and appear to involve activation of AMP-activated protein kinase (AMP-Kinase) in the liver and skeletal muscles (Ding et al., 2016). In the present study adiponectin levels were significantly decreased in HFD-STZ induced type 2 diabetes rats when compared to NC rats. A significant increase in adiponectin levels is observed after EW70 administration (Figure 7). Increases in adiponectin levels can be linked to increased insulin sensitivity as shown by significantly lowered Homa-IR scores in HED-STZ T2DM rats administered with EW70 when compared to increased Homa-IR scores in DC rats. The significant increases in adiponectin levels after the administration of MF extract may also possibly be involved in the triglycerides, cholesterol, and LDL clearances. EW70 might have achieved these functions through the activation of AMP-activated protein kinase. Our results are thus in agreement with previous reports that outlined adiponectin's role in the stimulation of glucose utilization and lipid oxidation by activating AMP-protein kinase (Kim et al., 2017).

Leptin is an important hormone that helps prevent excessive eating by inhibiting hunger (Gruzdeva and Borodkina, 2019). In the present study, leptin levels were significantly lowered in DC rats compared to NC rats while a significant dose-dependent increase in leptin level was observed in EW70 fed rats (Figure 6). The mechanism employed by the extract to attain normal hormonal balance may make it an important supplement to use to curb excessive eating which inevitably causes obesity. A reduction in weight gain rather than prevention in weight gain is observed. A significant reduction in weight gain was observed in the highest dosage of EW70. This coincides well with increased levels of leptin. A controlled eating habit because of upregulated leptin corresponds with a moderate weight gain in diabetic rats.

In normal physiological function, insulin induces the action of lipolytic hormone on the peripheral fat which hydrolyses triglycerides and prevents mobilization of free fatty acids. However, insulin deficiency during diabetes inactivates lipoprotein lipase and promotes the liver conversion of free fatty acids into phospholipids and cholesterol which gets discharged into the bloodstream to elevate lipoprotein levels (Mohan et al., 2013). In the present study, Cho, LDL, and triglycerides were observed to rise significantly in DC rats when compared to NC rats.

The level of HDL declined significantly in DC rats when compared to NC rats. The administration of EW70 (Table 2) resulted in a significant elevation of HDL to almost normal levels. It also caused a significant decline in the levels of LDL, cholesterol, and triglycerides. This is a clear indication that the MF extracts might play an important role in preventing bad lipid accumulation in STZ- induced type 1 and type 2 diabetes. LDL is known to carry cholesterol to the peripheral tissues where it can be deposited. This increases the risk of atherosclerosis, cardiovascular attack, and peripheral vascular disease among other diabetic complications (Storey et al., 2018). LDL accumulation is thus very bad for the body.

Orally administered MF extracts seem to offer benefits by increasing the level of the HDL in rat plasma. Unlike LDL, HDL hastens the removal of cholesterol from peripheral tissues to the liver for catabolism and excretion (Besler and Lu, 2012). High levels of HDL may compete with LDL receptors or arterial smooth muscle cells thus partially inhibiting the uptake and degradation of LDL against oxidation *in vivo*. This is because lipids in HDL are partially oxidized before those in LDL (Bartelt et al., 2017).

Decreases in plasma protein content have been associated with STZ induced diabetes (Gandhi and Sasikumar, 2012). A decline in protein level was observed in diabetic rats and restoration of protein level occurred after EW70 extract administration due to controlled glucose levels. The ability of the plant extract to attain desirable lipoprotein levels suggests the ability of the plant to protect against diabetes linked-cardiovascular complications. Our results on the lowering of LDL and the increase in HDL by MF extracts show possible arterial prevention in arterial atheroma accumulation. Our results are thus in agreement with Setshogo and Mbereki (2011)'s report on the traditional use of MF in the treatment of stroke.

The levels of glycated hemoglobin (HbA1C) are elevated during diabetes due to prolonged hyperglycemia which results in the glycation of hemoglobin and the ultimate potentiating formation of glycation products that contribute to oxidative stress (Cavero-Redondo et al., 2017). The concentration of HbA1C has been related to diabetes complications such as retinopathy, nephropathy, and neuropathy. HbA1C is usually used as an important measure for the diagnosis and prognosis of diabetes-associated complications (Cavero-Redondo et al., 2017). In the present study, an elevation in HbA1C was observed in the diabetic control group and administration of the highest dose of EW70 to HFD-STZ induced T2D rats (Figure 4.) significantly suppressed HbA1C level to normal. This lowering effect on HbA1C is dose dependent. This glucose-lowering effect is possibly brought about by normal plasma glucose levels.

Apart from the glycation products mentioned above, other reasons for oxidative stress in diabetes are; increased production of free radicals by glucose autoxidation, and dismutation of oxidized glucose to hydrogen peroxide, leading to the formation of reactive hydroxyl radicals, which in turn leads to lipid peroxidation (Asmat et al., 2016). In the present study, alterations in enzymatic antioxidants were observed. Catalase, SOD, and GSH were significantly lowered in the DC group when compared to the NC group. These endogenous antioxidants possibly reduced because they had been overwhelmed by increases in diabetes linked oxidative stress. Administration of EW70 extract resulted in significant increases in enzymatic and non- enzymatic antioxidants SOD, catalase, and GSH. Lipid peroxidation was evidenced by significant increases in the levels of TBARS and hepato-specific enzymes SGOT, SGPT, and ALP in DC rats when compared to NC rats. EW70 extract was found to reduce lipid peroxidation as depicted by a significant decline in the TBARS, SGPT, SGOT, and ALP levels across all treatment groups when compared to the diabetic control group. This is an important protective mechanism since lipid peroxidation is the most potent oxidative defect that affects cells during diabetes linked oxidative stress (Rehman and Akash, 2017). SGPT, SGOT, and ALP are liver enzymes that leak from the liver into the blood as a result of lipid peroxidation damage to the liver which in this case may be caused by STZ-induced type 2 diabetes (Safhi et al., 2019). The ability of EW70 extracts to prevent lipid peroxidation could possibly occur through the boosting of the endogenous antioxidant system by the quenching of free radicals, providing room for the recovery of endogenous antioxidants. Histology of the liver provides supportive evidence of the ability of EW70 to prevent liver damage as evidenced by the preservation of hepatocytes from treatment group rats when compared to the DC control rats (Figure 9). These preventative effects may be attributed to compounds mentioned earlier.

Conclusion

In conclusion, MF improves lipid profile, blood glucose, and oxidative stress in Sprague Dawley rats induced with diabetes by acting through several mechanisms. Most importantly, MF increased circulating insulin and enhanced the sensitivity of peripheral tissues to the hormone. The latter effect can be attributed in part to the activation of AMPK pathways in skeletal muscle to an increased content of GLUT4 in skeletal muscle. Such pleiotropic actions provide strong evidence in support of the medicinal use of MF for treating diabetes. These warrant further high-quality clinical studies are necessary to determine the optimal conditions for complementary or alternative treatment in diabetic patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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