British Journal of Pharmaceutical Research
4(4): 429-442, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effects of *Momordica charantia* on Serum Lipid Profile, Serum Protein Levels and Selected Markers of Cardiovascular Damage in Diabetic Rats

Omobola A. Komolafe[1*], David A. Ofusori[1], Olarinde S. Adewole[1] and Julius B. Fakunle[2]

[1]Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAK designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author DAO wrote the design of the research, literature search and statistical analysis. Author OSA coordinated all the aspects of experiments in this study. Author JBF coordinated the biochemical analysis in this study. All authors read and approved the final manuscript

Original Research Article

Received 14th June 2013
Accepted 5th October 2013
Published 21st December 2013

ABSTRACT

Aims: To investigate the effects of *M. charantia* on serum lipid profile, serum protein concentration and selected markers of cardiovascular damage in streptozotocin-induced diabetic Wistar rats.
Study design: Forty healthy adult Wistar rats of both sexes were randomly assigned into five groups A, B, C, D and E of eight rats each.
Place and Duration of Study: Department of Anatomy and Cell Biology, Obafemi Awolowo University, Nigeria, between January 2010 and March 2012.
Methodology: At the expiration of the research, the animals were sacrificed and blood samples were collected for biochemical analysis. Serum lipid profile, total protein and serum albumin, serum Creatine Kinase, Lactate Dehydrogenase and Glucose-6-Phosphate Dehydrogenase activities were determined using Randox assay kits. The

**Corresponding author: Email: bkomolaf@yahoo.com;*

levels of Serum globulin and albumin/globulin ratio were calculated**.** Serum nitric Oxide and Prostaglandin E_2 levels were determined using assay kits.
Results: The result showed a significant reduction ($p<0.05$) in the blood glucose levels in group D when compared with groups A, B, C and E. There was an increase in triglyceride ($p<0.05$), total cholesterol ($p>0.05$), low density lipoprotein ($p>0.05$) and very low density lipoprotein cholesterol ($p<0.05$) were increased in group B when compared with group D. The serum levels was presented a non significant reduction in total protein ($p>0.05$), albumin ($p>0.05$), globulin ($p>0.05$) and albumin/globulin ratio ($p>0.05$) when group B was compared with group D. Lactate dehydrogenase ($F=0.18$, $p>0.05$) and creatinine kinase ($F=1.96$, $p>0.05$) were increased ($p>0.05$) while the nitric oxide ($F=2.21$, $p<0.05$), PGE_2 ($F=1.25$, $p<0.05$) and G6PDH ($F=2.92$, $p<0.05$) were reduced ($p<0.05$) in group B when compared with A, C, D and E.
Conclusion: The presents study thus suggests that *M. charantia* could serve as a useful antidiabetic agent.

Keywords: Diabetes mellitus; Momordica charantia; serum lipid profile; protein concentration; cardiovascular complications.

1. INTRODUCTION

Diabetes mellitus is one of the most important world health problems, especially in developing countries where prevalence and incidence rates are highest. Diabetic patients are particularly prone to cardiovascular diseases including hypertension, atherosclerosis, diabetic cardiomyopathy, congestive heart failure and cardiac autonomic neuropathy [1]. Coronary atherosclerosis and cardiomyopathy occur as a result of the metabolic abnormalities associated with diabetes [2].

Individuals with diabetes have an increased prevalence of atherosclerosis, with increased rates of coronary artery disease, cerebrovascular disease and peripheral artery disease [3]; [4]. More than 50% of deaths in non-insulin-dependent diabetes mellitus are related to atherosclerosis, with coronary artery disease being the most frequent cause [1]. Although diabetes mellitus is frequently associated with coronary artery disease risk factors such as increased levels of total cholesterol triglycerides, and blood pressure, much of the increased risk for coronary artery disease is not explained by these and other standard cardiovascular risk factors [5,6]. Momordica charantia (Linn Family: Cucurbaceae) is one of the popular herbs that grow in different regions of Nigeria. It is commonly called Bitter melon, Bittergoud, Balsam pear. Various parts of M. charantia such as the seed, fruit and even the whole plant has been reported to have beneficial effects in prevention and treatment of many diseases in folkloric medicine, especially in the treatment of DM in individuals with non-insulin dependent diabetes [7,8]. It has hypoglycaemic properties as it significantly suppressed the rise in blood glucose concentrations in albino rats [7,9]. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. In addition, a protein found in bitter melon, momordin, has clinically demonstrated anticancerous activity against Hodgkin's lymphoma in animals. Other proteins in the plant, alpha- and beta-momorcharin and cucurbitacinB, have been tested for possible anticancerous effects. [10]. In numerous studies, at least three different groups of constituents found in all parts of bitter melon have clinically demonstrated hypoglycemic (blood sugar lowering) properties or other actions of potential benefit against diabetes mellitus [11]. These chemicals that lower blood sugar include a mixture of steroidal saponins known as charantins, insulin-like peptides, and

alkaloids. The hypoglycemic effect is more pronounced in the fruit of bitter melon where these chemicals are found in greater abundance.

To date, studies have demonstrated the blood sugar-lowering effect of this bitter fruit [12,13,14]. The fruit has also shown the ability to enhance cells' uptake of glucose, to promote insulin release, and to potentiate the effect of insulin [15,16]. In other in vivo studies, bitter melon fruit and/or seed has been shown to reduce total cholesterol. Some researchers reported that elevated cholesterol and triglyceride levels in diabetic rats were returned to almost normal after some weeks of treatment [17,18,19,20,21,22].

The present study investigated the effects of M. charantia on serum lipid profile, serum protein concentration and selected markers of cardiovascular damage (which includes serum levels of Lactate Dehydrogenase, Glucose-6-Phosphate Dehydrogenase, Nitric Oxide, Prostaglandin E2, and Creatine Kinase) in streptozotocin-induced diabetic Wistar rats and compared the effects with those of glimepiride, an oral blood-glucose-lowering drug of the sulfonylurea class [23].

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

2. MATERIALS AND METHODS

2.1 Animal Care

Forty healthy adult Wistar rats (*Rattus norvegicus* 8wks-12wks old) of both sexes, with average weight of 134.4g were used for the experiment. The rats were bred in the animal holding of College of Health Sciences, Obafemi Awolowo University, Ile-Ife. They were maintained on standard rat pellet (Capsfeed, Ibadan, Nigeria) and water was provided ad libitum.

The animals were randomly assigned into five groups A, B, C, D and E of eight rats each.

- Group A were the control (normal rats)
- Group B were the experimentally-induced diabetic rats administered with 10% tween 80
- Group C were the experimentally-induced diabetic rats treated with methanolic extracts of *Momordica charantia* dissolved in10% tween 80 for two weeks
- Group D were the experimentally-induced diabetic rats treated with methanolic extracts of *Momordica charantia* dissolved in 10% tween 80 for four weeks
- Group E were the diabetic rats treated with a standard diabetic drug (2mg/kg of glimepiride) dissolved in 10% tween 80 for four weeks

The animals received humane treatment as outlined in the “Care and Management of Laboratory Animals” published by the National Institute of Health (NIH, 1996).

2.2 Plant Material

Matured leaves of *Momordica charantia* (*Cucurbitaceae*) were collected during the raining season from suburban villages of Ile-Ife metropolis in Osun State of Nigeria. The leaves were taken to the Herbarium in the Department of Botany, Obafemi Awolowo University, Ile-

Ife to confirm identification and a voucher specimen number (UHI 16510) was placed in the Herbarium.

2.3 Preparation of Methanolic Extract of *M. charantia*

Leaves of *Mormodica charantia* (MC) were air dried and powdered in a warring blender. A 765 g of the powdered leaves were extracted in 1,950 mls of absolute methanol for 72 hours with intermittent shaking and filtered. The filtrate were concentrated in vacuo at 35^0C using a vacuum rotary evaporator (Büchi Rotavapor R110, Schweiz). The extract were partitioned between water and dichloromethane, the dichloromethane fraction (5.94%) was oven-dried at 37^oC and stored until it is ready to be used. The dichloromethane fraction of the extract were weighed and dissolved in 10% tween 80 for use on each day of the experiment.

2.4 Induction of Diabetes

Diabetes mellitus was experimentally-induced in groups B, C, D and E by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (Tocris Bioscience, UK) dissolved in 0.1M sodium citrate buffer (pH 6.3). Diabetes was confirmed in animals 48 hours after induction, by determining fasting blood glucose level using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips using blood samples obtained from the tail vein of the rats. Animals in group A were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally.

2.5 Administration of Extract and Anti-Diabetic Drug

Methanolic extracts of the leaves of *M. charantia* (100 mg/kg) was dissolved in10% tween 80 and administered daily (orally) by gastric intubation to the rats in groups C and D for 2 and 4 weeks respectively. The standard antidiabetic drug (glimepiride 2 mg/kg) was administered to group E rats for four weeks [24], while those in group B were left untreated.

2.6 Sacrifice of the Animals

At the end of the experimental period, all the animals were physically observed and anesthesized by chloroform inhalation. A midline incision was performed at the thoracic region. Blood was obtained by cardiac puncture using a 5 ml syringe with needle inserted into the left ventricle. The blood was centrifuged within 30 minutes of collection. The blood was centrifuged in a centurion scientific refrigirated centrifuge, R 8000 series (Oxford, UK) at 5000 R.P.M for 5 minutes to obtain the serum.

2.7 Biochemical Assays

Serum lipide profile, total protein and serum albumin were determined using Randox assay kits (Randox Laboratory, Northern Ireland). The lipid profile include; triglycerides (TGL), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) while low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated. The levels of Serum globulin and albumin/globulin ratio were calculated as described by Ettinger and Feldman, 2000 [25]. Lactase Dehydrogenase (LDH) and Glucose-6-Phosphate Dehydrogenase (G6PDH) activities were measured using Randox Kit (Randox laboratory, Northern Ireland). BioAssay Systems' QuantiChrom TM Nitric Oxide Assay Kit was used to measure Nitric Oxide (NO) levels in serum. Also serum Prostaglandin E_2 (PGE_2)

levels were determined using PGE_2 EIA Monoclonal assay kit (Arbor Assays, UK) and serum Creatine Kinase (CK-NAC) was determined using Randox Kit (Randox laboratory, Northern Ireland).

2.8 Statistical Analysis

All values were presented as means ±SEM. Data were analyzed using one way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) using Statistical Package for Social Science (SPSS 17).

3. RESULTS

3.1 Changes in Blood Glucose Level (BGL)

There was a progressive increase in the BGL in the STZ induced group such that at the expiration of the research, the BGL of the diabetic rats in group B remains hyperglycemic while the BGL of the rats in group C showed a significant ($p<0.05$) increase when the extract was withdrawn. Groups D and E rats were normoglycemic (233.20 ± 18.93 and 181.00 ± 60.67 respectively) on the 56^{th} day as shown in Table 1.

3.2 Changes in Serum Lipid Profiles

TG and VLDL-C in the diabetic group was significantly ($p<0.05$) increased, while TC and LDL-C was also increased non significantly ($p>0.05$) when compared with the control. HDL-C was however reduced significantly ($p<0.05$) in diabetic group when compared with the control. Administration of extract for two weeks only (group C), significantly increased TG and VLDL-C while TC, LDL-C and HDL-C was reduced when compared with group E. animals administered with extract for four weeks however reduced TG, LDL-C and VLDL-C significantly while HDL-C was significantly increased. The group administered with glimepiride (group E) compared favourably with the group administered with extract for four weeks (group D) as shown in Table 2.

3.3 Changes in Total Protein, Albumin, Globulin and Albumin/Globulin Ratio

There was a non significant (P=.05) decrease in the total protein concentration of the diabetic group when compare with the control. The rats treated with the *M. charantia* extract for four weeks (group D) increased the total protein concentration by 48.65% when compared with the group C rats. The animals treated with MC extract for two weeks (Group C) showed a non significant (P=.05) decrease in total protein concentration when compared with the group D rats. There was a slight difference in the total protein concentration of group D and group E animals (glimepiride) (Table 3).

There was a non significant ($p>0.05$) decrease in the albumin (ALB) increase in the concentration of the albumin 57.35% in the animals treated with MC extract for four weeks (Group D) when compared with group C. The rats (group E) treated with antidiadetic drug (glimepiride) (4.21±0.13) showed nearly the same albumin concentration when compared with the diabetic rats treated with *M. charantia* extract for four weeks (group D; 4.22± 0.20) (Table 3).

Table 1. The effect of *M. Charantia* on blood glucose level

	0	2	14	28	42	56
Group A	101.00±3.79^{a}	120.25±2.29^{a}	120.63±7.30^{a}	85.88±3.86^{a}	105.63±4.602^{a}	114.13±6.05^{a}
Group B	98.13±2.07^{a}	536.13±32.22^{b}	536.86±22.76^{c}	438.00±35.49^{b}	350.17±38.46^{c}	386.50±36.97^{c}
Group C	98.13±2.78^{a}	536.13±25.08^{b}	573.50±16.76^{c}	433.80±47.73^{b}	215.00±25.00^{b}	573.00±23.00^{d}
Group D	96.00±2.732^{a}	531.00±25.97^{b}	541.43±32.15^{c}	415.00±26.81^{b}	218.33±25.54^{b}	233.20±18.93^{b}
Group E	91.50±3.85^{a}	541.75±52.18^{b}	441.20±48.34^{b}	398.50±46.42^{b}	221.33±31.74^{b}	181.00±60.67^{ab}

Values are given as mean ± SEM for blood glucose level on days 0, 2, 14, 28, 42 and 56 in each group. a, b, c, d, ab within column signifies that means with different letters differs significantly at p < 0.05 while means with the same letters does not differ significantly at p < 0.05 (using one way ANOVA with Duncan multiple range test)

Table 2. The Effect of *M. Charantia* on the Lipid Profiles in STZ Induced Diabetic Rats

	TG(mg/dL)	TC(mg/dL)	HDL-C(mg/dL)	LDL-C(mg/dL)	VLDL-C(mg/dL)
Group A	78.40±18.65^{a}	47.59±9.96^{a}	52.40±12.21^{b}	17.44±1.36^{a}	15.68±3.73^{a}
Group B	107.86±29.08^{ab}	62.90±11.36^{a}	28.58±5.41^{a}	26.88±23.72^{a}	21.57±5.81^{ab}
Group C	170.54±7.63^{b}	45.93±11.62^{a}	29.01±8.12^{a}	16.92±3.50^{a}	34.11±1.53^{b}
Group D	85.27±13.82^{a}	62.64±13.93^{a}	37.08±2.37^{a}	20.37±8.13^{a}	17.05±2.76^{a}
Group E	89.72±27.36^{ab}	63.82±10.77^{a}	30.94±4.62^{a}	23.04±16.06^{a}	17.94±5.47^{ab}

Values are given as mean ± SEM for 5 biochemical parameters coded as TG, TC, HDL-C, LDL-C and VLDL-C in each group. a, b, ab, within column signifies that means with different letters differs significantly at p < 0.05 while means with the same letters does not differ significantly at p < 0.05 (using one way ANOVA with Duncan multiple range test).

The serum globulin (GLO) concentration in the diabetic group (1.42 ± 0.82) presented a significant ($p<0.05$) increase when compared with the control (3.20±0.12). Administration of MC extract for four weeks caused a significant ($p<0.05$) increase in the concentration of serum globulin 4.74±0.60. The animals in group C showed a non significant ($p>0.05$) decrease in serum globulin concentration by 41.77% when compared with the group D. There was a moderate increase in the serum globulin concentration in the group administered with glimepiride (group E) when compared with the animals treated with extract for four weeks (group D) after STZ induction (Table 3).

The ALB/GLO ratio in the diabetic group (Group B, 0.80 ± 0.46) was decreased ($p>0.05$) when compared with the control rats (group A, 1.04 ±0.08). The animals in group C showed a 64.52% decrease in the ALB/GLO ratio when compared with group D animals. However, the group administered with extract for four weeks showed a non significant ($p>0.05$) increase in ALB/GLO ratio by 13.98% when compared with the diabetic group. The group administered with glimepiride (group E) was increased by 5.00% when compared with group B animals (Table 3).

Table 3. The effect of *M. charantia* on Total Protein, Albumin, Globulin and Albumin/Globulin Ratio in STZ induced diabetic rats

	TP (g/dL)	ALB (g/dL)	GLO (g/dL)	ALB/GLO Ratio
Group A	6.53 ± 0.01^{a}	3.33 ± 0.13^{a}	3.20 ± 0.12^{a}	1.04 ± 0.08^{a}
Group B	8.01 ± 0.73^{a}	4.32 ± 0.22^{b}	4.39 ± 0.69^{a}	1.08 ± 0.21^{a}
Group C	9.61 ± 0.48^{a}	3.65 ± 0.05^{ab}	5.95 ± 0.42^{a}	0.61 ± 0.03^{a}
Group D	8.90 ± 0.69^{a}	4.22 ± 0.20^{b}	4.74 ± 0.60^{a}	0.93 ± 0.10^{a}
Group E	9.46 ± 1.14^{a}	4.21 ± 0.13^{b}	5.24 ± 1.01^{a}	0.84 ± 0.11^{a}

Values are given as mean ± SEM for 4 biochemical parameters coded as TP, ALB, GLO and ALB/GLO in each group. a, b, ab, within column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test).

3.4 Activities of LDH and G6PDH

The activity of LDH in diabetic group (2.43 ± 0.83) was moderately increased ($p>0.05$) when compared with the control group (2.33±0.62). Group C rats showed a significant increase ($p<0.05$) in the activity of LDH when compared with group D rats (2.12 ± 0.91) that was administered with MC extract for four weeks and control group (2.33±0.62). The group administered with glimepiride (group E) showed no significant ($p>0.05$) difference when compared with group D animals (Table 4).

There was no significant difference in the activity of G6PDH in the control (group A), diabetic group (Group B), and Group C. However, there was a significant increase ($p<0.05$) in the groups D (0.03±0.00) and E (0.03±0.00) when compared with groups A, B and C (Table 4).

Table 4. The effect of *M. charantia* on some enzymes (LDH and G6PDH) of Carbohydrate Metabolism in STZ induced diabetic rats

	LDH (U/L)	G6PDH (mU/mL)
Group A	2.33 ± 0.62[a]	0.02 ± 0.00[a]
Group B	2.43 ± 0.83[a]	0.02 ± 0.00[a]
Group C	3.67 ± 3.66[c]	0.02 ± 0.00[a]
Group D	2.12 ± 0.91[a]	0.03 ± 0.00[ab]
Group E	2.38 ± 0.13[a]	0.03 ± 0.00[b]

Values are given as mean ± SEM for 6 2 biochemical parameters coded as LDH and G6PD in each group. a, b, c, ab within column signifies that means with different letters differs significantly at p < 0.05 while means with the same letters does not differ significantly at p < 0.05 (using one way ANOVA with Duncan multiple range test).

3.5 Nitric Oxide (NO)

The result showed a significant (p<0.05) reduction in the NO of diabetic group (group B) as compared with the control (group A). Administration of extract for four weeks (group D), significantly (p<0.05) increased the NO level (0.52 ±0.04). Withdrawal of extract administration from animals in group C significantly reduced the NO concentration (0.13 ±0.12). The antidiabetic drug did not increase the NO level. (Table 5).

Table 5. The effect of *M. charantia* on Serum Level of Nitric oxide, Prostaglandin E_2 and Creatinine Kinase

	Nitric Oxide (mM)	Prostaglandin E_2x10^3 (pg/mL)	Creatinine kinase (U/L)
Group A	0.65±0.09[bc]	4.37±1.45[ab]	3.57±0.66[ab]
Group B	0.42±0.18[abc]	2.71±2.22[ab]	4.22±2.54[ab]
Group C	0.13±0.12[a]	2.56 ±2.49[ab]	1.75±1.70[a]
Group D	0.52±0.04[bc]	4.21±1.86[ab]	6.10±1.40[ab]
Group E	0.38±0.37[ab]	0.80±0.61[a]	2.27±2.20[a]

Values are given as mean ± SEM for 3 biochemical parameters named as Nitric Oxide, Prostaglandin E and Creatinine Kinase in each group. a, ab, bc, abc within column signifies that means with different letters differs significantly at p < 0.05 while means with the same letters does not differ significantly at p < 0.05 (using one way ANOVA with Duncan multiple range test)

3.6 Prostaglandin E_2 (PGE_2)

The result showed a non significant (p>0.05) reduction in the PGE_2 of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D) increased the PGE_2 level non significantly (p>0.05) (4.21 ±1.86). Withdrawal of extract administration from animals in group C reduced the PGE_2 concentration (2.56 ±2.49). The antidiabetic drug could not increase the PGE_2 concentration as compared with the diabetic group (Table 5).

3.7 Creatinine Kinase (CK-NAC)

The result showed a non significant (p>0.05) increase in the CK-NAC activity of diabetic group (group B) as compared with the control. Administration of extract for four weeks (group D), further increased the CK-NAC activity non significantly (6.10 ± 1.40). Withdrawal of extract administration from animals in group C reduced the CK-NAC activity (1.75 ± 1.70).

The antidiabetic drug could not increase the CK-NAC activity as compared with the diabetic group (Table 5).

4. DISCUSSION

Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes [26]. Low-density lipoprotein in diabetic patients leads to abnormal metabolism and is associated with increase in very low-density lipoprotein (VLDL) secretion and impaired VLDL catabolism. Ultimately this leads to atherosclerotic plaque [27]. A number of known factors for coronary artery disease such as hypertension, obesity and dyslipidemia are more common in diabetics than in the general population. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes, now as of 171 million, will touch 366 million or more by the year 2030 [28]. Patients with DM are more likely to develop microvascular and macrovascular complications than the non diabetic population [29]. Dyslipidemia is a frequent complication of DM and is characterized by low levels of high-density lipoprotein-cholesterol (HDL-C) and high levels of low density lipoprotein cholesterol (LDLC) and triglyceride (TG). Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease [30]. Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition [31].

The present study shows that withdrawal of *M. charantia* extract in group C rats produced a gradual increase in the lipids profile levels as shown by the parameters obtained while the rats in group D maintained a decrease in TG, TC, LDL-C levels and increases HDL-C level. Chait et al. [32] reported that high LDL-C levels in the blood of diabetic rats are due to low insulin levels. Insulin increases the number of LDL-C receptors at the cellular levels; therefore in an insulin-deficient state, a decrease in insulin receptors is expected which will consequently leads to delay in LDL-C clearance. Another reason could be the high level of glucose in the circulation. Witzum et al., [33] reported that two to three fold increases in glucose concentration causes glycosylation of lysine in apoprotein B. There is increasing evidence that hyperlipidemia plays an important role in the gradual development of atherosclerosis [34]. It has been established that diabetes is a risk factor for cardiovascular disease resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease which are the leading cause of death in the diabetic population [35].

The decrease observed in the serum concentrations of total protein, albumin, globulin and albumin/globulin ratio in the diabetic rats also supports other investigators [36,37,38]. Treatment of diabetic animals with extract was able to bring the serum total protein, albumin, globulin concentrations and albumin/globulin ratio close to normal. Withdrawal of *M. charantia* reduced the serum total protein, albumin, globulin concentrations and albumin/globulin ratio. Sundaram et al. [39] reported that deranged glucagons-mediated regulation of cyclic AMP formation in insulin deficiency is brought about by accelerated proteolysis of uncontrolled diabetes. In addition, secondary hypoalbumenia commonly observed in diabetic patients is generally attributed with the nephrotoxicity [39,40]. There was an improvement in albumin/globulin ratio in STZ diabetic rats treated with *M. charantia* and glibenclamide when compared with diabetic rats (group B).

Lactate dehydrogenase (LDH) in anaerobic glycolysis, catalyses the conversion of pyruvate to lactate which subsequently is converted to glucose in gluconeogenetic flux. The result of this study showed an increase in LDH concentration in the diabetic rats. Previous studies

also reported an increase in the concentration of lactate dehydrogenase [41,42]. When tissues are damaged by trauma or disease, higher amounts of the LDH enzyme are released into the bloodstream. While an LDH test can determine the presence of tissue damage, further tests often are necessary to assess the severity and location of the damage. LDH levels typically rise after people suffer damage to the heart muscle from a heart attack [43]. The increment noticed in the diabetic rats may be suggestive of cardiac damage in line with previous study [43]. Treatment with extract moderately restored the concentration of LDH.

Glucose-6-phosphate dehydrogenase (G6PDH) is an enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) [44]. A decrease in the concentration of G6PDH in the diabetic animals was noticed in this study. Investigations have shown that decrease in G6PDH in tissues may decrease NADPH generation required for glutathione reductase activity and glutathione production [44]. Treatment with extract moderately restored the concentration of G6PDH.

This study showed a significant decrease in the NO concentration in the diabetic rats. This decrement was normalized with the administration of *M. charantia* for four weeks. The decrease in NO may be due to its reaction with superoxide anion radical generated in diabetic state to form reactive peroxyl nitrite radicals. Mohan and Das (1998) [45] reported a decrease in NO in alloxan-diabetic rats, an effect which was abrogated by prio and simultaneous administration of L-arginine. NO is normally produced from L-arginine by endothelial nitric oxide synthase (eNOS) in the vasculature [46]. NO mediates endothelium-dependent vasorelaxation by its action on guanylate cyclase in vascular smooth muscle cells (VSMC), initiating a cascade that leads to vasorelaxation. NO also displays antiproliferative properties and inhibits platelet and leukocyte adhesion to vascular endothelium [46]. Therefore, NO is considered a vasculoprotective molecule. M. charantia showed a better protective potential when compared with glimepiride.

Prostaglandin E_2 has long been noted to have a role in regulating hematopoietic homeostasis. Prostaglandin signaling is active in mature erythrocytes, playing a role in cellular stress response by regulating K+ efflux and cell size [47,48]. This study showed a decrease in the PGE_2 concentration in the diabetic rats. The decrease was normalized with the administration of *M. charantia* for four weeks. Report has shown that PGE_2 is used in the management of pulmonary hypertension and also has vasodilatory effects [49]. This suggests that *M. charantia* has beneficial properties far beyond the antidiabetic drug which did not show any significant effect.

Creatinine kinase (CK-NAC) a powerful and sensitive predictor of increased cardiac complications [50]. In this study, CK-NAC level was increased in the diabetic rats indicative of cardiac damage. This increase was significantly lowered with the administration of *M. charantia* for two weeks. Continuation of the extract for four weeks showed a significant increase in the CK-NAC level. This observation is suggestive of the deletorius effect of *M. charantia* with prolonged treatment as seen in this study. This result also indicated that *M. charantia* treatment for two weeks showed a better protective potential against cardiac dysfunction when compared with glimepiride.

5. CONCLUSION

We thus conclude that the reason for non significant effect of *M. charantia* on some few parameters is yet to be understood and calls for further investigation. However, *M. charantia* to a reasonable extent has demonstrated its antidiabetic property and by extension useful in the management of associated cardiovascular diseases.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

We hereby acknowledge the contributions of Mr. Idowu Olawuni in the Department of Biochemistry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jarrett RJ. Cardiovvascular disease and hypertension in diabetes mellitus. Diabetes/Metabolism Reviews. 1989;5:547-558
2. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV et al, Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation. 1999;100:1134–1146.
3. Marks HH, Krall LP. Onset, course, prognosis, and mortality in diabetes mellitus In: Marble A, White P, Bradley RF, eds. Joslin's Diabetes Mellitus. Philadelphia: Lea & Febiger. 1971;227-228.
4. Pyoralla K, De Backer G, Graham I, Poole-Wilson P, Wood D. On behalf of the Task Force. Prevention of coronary heart disease in clinical practice. Recommendations of the Task force of the European society of Cardiology, European Atherosclerosis Society, and European Society of Hypertension. Eur Heart J 1994;110:121-61.
5. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: The Framingham study. Am J Cardiol. 1974;34:29-35.
6. UusitupaMIJ, Niskanen LK, Siitonen O. 5-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and non-diabetic subjects. Circulation. 1974;82:27-36.
7. Platel K, Srinivasan K. Plant foods in the management of diabetes mellitus: vegetables as potential hypoglycaemic agents. Nahrung. 1997;41(2):68-74.
8. Raman A and Lau C. Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (*Cucurbitaceae*).Phytomedicine. 1996;2;349-662.

9. Nicholas LB, Kolb Y, Prinssen EP. A combined marble burying ocomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. Eur J Pharmacol. 2006;547:106–115.
10. Nagasawa H, Watanabe K, Inatomi H. (2002): Effects of bitter melon *(Momordica charantia)* or ginger rhizome (*Zingiber offifinale* Rosc.) on spontaneous mammary tumorigenesis in SHN mice. Am. J. Clin. Me. 2002;30(2–3):195–205.
11. Tan FJ, Fire AZ, Hill RB. Regulation of apoptosis by C. elegans CED-9 in the absence of the C-terminal transmembrane domain. Cell Death Differ. 2007;14:1925-1935.
12. Han C, Hui Q, Wang Y. Hypoglycaemic activity of saponin fraction extracted from *Momordica charantia* in PEG/salt aqueous two-phase systems. Nat. Prod. Res. 2008;22(13):1112-9.
13. Omar S, Ojewole J. Hypoglycemic effect of the seeds of *Momordica charantia*. Fitoterapia. 2007;78(1):46-7.
14. Ojewole JA, Adewole, SO. and Olayiwola, G. Hypoglycaemic and hypotensive effects of *Momordica charantia* Linn (*Cucurbitaceae*) whole-plant aqueous extract in rats." Cardiovasc. J. S. Afr. 2006;17(5):227-32.
15. Shih CC, Lin CH, Lin WL. Effects of *Momordica charantia* on insulin resistance and visceral obesity in mice on high-fat diet. Diabetes Res. Clin. Pract. 2008;81(2):134-43.
16. Inayat-ur-Rahman., Malik SA., Bashir M, Khan R and Iqbal M. Serum sialic acid changes in non-insulin-dependent diabetes mellitus (NIDDM) patients following bitter melon *(Momordica charantia)* and rosiglitazone (Avandia) treatment. Phytomedicine. 2009;16(5):401-5.
17. Chaturvedi A, Bhawani G, Agarwal PK, Goel S, Singh A, Goel RK. Antidiabetic and antiulcer effects of extract of Eugenia Jambolana seed in mild diabetic rats. Study on gastric mucosal offensive acid – pepsin secretion. J Physiol pharmacol. 2009;53(2):137-146.
18. Chan LL, Chen Q, Go AG, Lam EK, Li ET. Reduced adiposity in bitter melon *(Momordica charantia*)-fed rats is associated with increased lipid oxidative enzyme activities and uncoupling protein expression. J. Nutr. 2005;135(11):2517-23.
19. Hsieh CL, Lin YC, Ko WS, Peng CH, Huang CN, Peng RY. Inhibitory effect of some selected nutraceutic herbs on LDL glycation induced by glucose and glyoxal. J. Ethnopharmacol. 2005;102(3):357-63.
20. Chaturvedi P. Role of *Momordica charantia* in maintaining the normal levels of lipids and glucose in diabetic rats fed a high-fat and low-carbohydrate diet, Br J Biomed Sci. 2005;62(3):124-6.
21. Senanayake GV, Maruyama M, Sakono M, Fukuda N, Morishita T, Yukizaki C et al. The effects of bitter melon *(Momordica charantia)* extracts on serum and liver lipid parameters in hamsters fed cholesterol-free and cholesterol-enriched diets, J. Nutr. Sci. Vitaminol. 2004;50(4):253-7.
22. Jayasooriya AP, Sakono M, Yukizaki C, Kawano M, Yamamoto K and Fukuda N. Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. J. Ethnopharmacol. 2000;72(1–2):331.
23. Hamaguchi T, Hirose T, Asakawa H. Efficacy of glimepiride in type 2diabetic patients treated with glibenclamide. Diabetes Res. Clin. Pract. 66 Suppl 1, 2004;129–132.
24. Mir SH, Darzi MM, Ahmad F, Chishti MZ, Mir MS. The Influence of Glimepiride on the Biochemical and Histomorphological Features of Streptozotocin-Induced Diabetic Rabbits. Pakistan Journal of Nutrition. 2008;7(3):404-407.
25. Ettinger SJ, Feldman EC. Textbook of Veterinary Internal Medicine. 5th ed. Philadelphia; W B Saunders & Co; 2000.

26. Gandhi HR. Diabetes and coronary arterydisease: Importance of risk factors; Cardiol. Today1. 2001;31-34.
27. Howard BV. Lipoprotein metabolism in diabetes mellitus; J. Lipid. Res. 1987;28:613–614.
28. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes; Estimates for the year 2000 and projections for 2030; Diabetes Care. 2004;27:1047–1053.
29. Baynes JW. Role of oxidative stress in development of complications in diabetes; Diabetes. 1991;40:405–412.
30. Betteridge J. Lipid disorders in diabetes mellitus; in Text book of diabetes (eds) J C ickup and G Williams. (London: Blackwell Science); 1997;2nd ed.
31. Segal P, Bachorik PS, Rifkind BM, Levy RI. Lipids and lipoproteinemia 1984; in Clinical diagnosis and management by laboratory methods (ed.) J B Henry (Philadelphia: W B Saunders). 1984;180–203.
32. Chait A, Bierman EL, Albers JJ. Regulatory role of insulin in the degradation of low density lipoprotein by cultured human skin fibroblasts. Biochem Biophys Acta. 1978;529:292-299
33. Witzum JL, Mahoney EM, Branks MJ, Fisher M, Elam R, Steinberg D. Nonenzymatic glucosylation of low density lipoprotein alters its biological activity; Diabetes. 1982;31283-291.
34. Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation. 2002;105(17):2107-2111.
35. The Diabetes Control and Complication Trial (DCCT). N. Engl.J. Med. 1993;329:977-986.
36. Sivajothi V, Dey A, Jayakar B, Rajkapoor B. Antihyperglycaemic property of *Tragia cannabina* in streptozotocin induced diabetic rats. J Med Food. 2007;10:361–65.
37. Porte DJ and Halter JB. The endocrine pancreas and diabetes. In: Williams RH, editor. Text Book of Endocrinology Philadelphia: WB Saunders Co. 1981;715.
38. Pepato MT, Migliorini RH, Goldberg AL, Kettelhut IC. Role of different proteolytic pathways in degradation of muscle protein from streptozotocin-diabetic rats. Am J Physio. 1996;271:240–7.
39. Sundaram EM, Uma Maheswara, Reddy P and Singh, K.P. Effect of Alcoholic Extracts of Indian Medicinal Plants on the Altered Enzymatic Activities of Diabetic Rats. Indian J Pharm Sci. 2009;71(5):594-598
40. Feling P, Marliss E, Ohman J and Cahill JR. Plasma amino acids level in diabetic ketoacidosis. Diabetes. 1970;19:727–30.
41. Pozzilli A, Signore A, Leslie RDG. Infection, immunity and diabetes In international textbook of diabetes mellitus. 1997;1231–1241.
42. Lemieux G, Aranda MR, Fournel P, Lemieux C. Renal enzymes during experimental diabetes mellitus in the rat: Role of Insulin, carbohydrate metabolism and ketoacidosis. Can. J. Physiol. Pharmacol. 1984;62:70–75.
43. e.how health (EHH); 2012
Available: http://www.ehow.com/list_7469479_causes- highldh.html. Retrieved on 21/01/12
44. Yadav P, Sarkar S, Bhatnagar D. Action of *Capparis decidua* against alloxan-induced oxidative stress and diabetes in rat tissues. Pharmacol. Res. 1997;36:221-228
45. Mohan IK, Das UN. Effect of L-arginine-nitric oxide system on chemical-induced diabetes mellitus. Free Radic Biol Med. 1998;25:757–765.

46. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. Am J Physiol Heart Circ Physiol. 2001;281(6):H2289-2294.
47. Young IS, Tate S, Lightbody JH, McMaster D, Trimble ER. The effects of esferrioxamine and ascorbate on oxidative stress in the streptozotocin diabetic rat. Free Radic Biol Med. 1995;18(5):833–840.
48. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: An overview. Meth Enzymol. 1990;186:1–85.
49. Komoto J, Yamada T, Watanabe K, Woodward D, Takusagawa F. Prostaglandin F2alpha formation from prostaglandin H2 by Prostaglandin-F synthase (PGFS): crystal structure of PGFS containing bimatoprost. Biochemistry. 2006;45(7):1987–96.
50. Howard-Alpe GM, Sear JW, Foex P. Methods of detecting atherosclerosis in non-cardiac surgical patients; the role of biochemical markers. Br J Anaesth. 2006;97(6):758-769.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=374&id=14&aid=2801