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Hypoglycemic Potential of Dietary Supplementation of Protein Isolate from Fermented *Cucumeropsis manii* in Streptozotocin Induced Hyperglycemic in Male Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AOA and AOL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AOL managed the analyses of the study. Authors AOA and AOL managed the literature searches. Authors PAA and ASO managed all correspondence involving the research. All authors read and approved the final manuscript.

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ABSTRACT

The effect of Protein isolate from fermented melon seeds (Ogiri Protei Isolates; OPI) of *Cucumeropsis manii* on blood glucose, lipid profile, and antioxidant enzyme activities in streptozotocin (STZ)-induced diabetic rats was investigated. Thirty Male Wistar rats were divided into five equal groups. GThe first control group with no exposure. The second group of rats with Streptozotocin-induced non-treated diabetes. The 3rd and 4th groups of rats with Streptozotocin-induced diabetes supplemented with Ogiri protein isolates (200, 600 mg/kg in diet). And the 5th group of rats with Streptozotocin-induced diabetes administered glibenclamide in a dose 500 ug/kg

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in diet [17]. The OPI was administered for 6 weeks. The administration of OPI reduced the blood glucose concentration of the STZ-induced diabetic rats. Sera and hepatic superoxide dismutase, activities of the STZ-induced diabetic rats were significantly (P< 0.05) increased in comparison with the diabetic control rats. Lipid peroxidation of the supplemented OPI diabetic rats was significantly (P< 0.05) decreased in comparison with the diabetic control rats as the administration of OPI to the STZ-induced diabetic rats significantly increased the enzymes' activities. The concentration of low-density lipoproteins in the OPI supplemented rats was significantly elevated. These data demonstrate that OPI supplements might be beneficial for correcting hyperglycemia but the consumption of OPI can modulate some tissue lipids in a direction not beneficial for CVD risk in patients with diabetes.

Keywords: Ogiri; Ogiri protein isolate; streptozotocin; glibenclamide; antidiabetic activity; hypolipidemic activity; antioxidant activity.

1. INTRODUCTION

Diabetes mellitus is a major health issue affecting people of all social conditions. It is an endocrine metabolic disorder characterized by persistent blood glucose accumulation and often followed by extreme fatigue, excessive urination, polyuria,and weight loss [1]. Type 1 and 2 diabetes prevalence is complicated by the fact that it is a multi-factor disorder, frequently linked with a group of pathologies involving hypertension, hypertriglyceridemia, reduced glucose tolerance, and sensitivity to insulin [2]. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues [3,4]. Type 1 diabetes accounts for 5% to 10% of all cases of diabetes. Its risk factors include autoimmune, genetic, and environmental factors [5,6] while type 2 diabetes accounts for 90% to 95% of all diagnosed diabetes cases [2].

A protein isolate is a dietary supplement and food product that is produced by extracting components from food sources including vegetables, seed plants, animal sources, etc [7]. Vegetable proteins are highly bioavailable, absorbed very quickly into the body, and have the high concentration of branched-chain amino acids (BCAAs) that are highly concentrated in muscle tissue, used to fuel working muscles and promote protein synthesis [8,9].

Cucumeropsis manii is a species of melon native to tropical Africa where it is grown for food and as a source of oil [10]. Its common names include egusi in Yoruba and agushi in Hausa. The seeds are commonly processed into soups and oil products and are also eaten individually as a snack. The seed is an excellent vegetable protein and is ideal for battling nutritional debilitations [11].

Ogiri refers to the fermented oily paste produced from *Cucmeropsis manii* in South-western Nigeria [12,13] that is used as soup condiments for its strong smell. It is prepared by the traditional method of uncontrolled solid-state fermentation of melon seeds involving the use of natural inoculation or chance fermentation [11]. Studies have shown that protein isolates may carry potentials in the management and/or eventual eradication of the diabetes pandemic [14].

The present work investigated the hypoglycemic, hypolipidemic, as well as the antioxidant effects of oral administration protein, isolate from *Cucumeropsis manii* of streptozotocin-induced diabetes in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Fermented lemon seeds were purchased from New-market in Ijebu-ode, Ogun State, Nigeria. The seeds from the production batch were identified and authenticated by the Department of Botany, Lagos State University (Ojo).

2.2 Extraction of Ogiri Protein Isolate

The procedure for crude protein isolation was done according to Nkosi et al. [15].

2.3 Collection and Acclimatization of Animals

Healthy male Wistar rats weighing 120-170g were used for this study. Before starting the experiment, the animals were acclimatized to the laboratory conditions for 2 weeks at ambient temperature (24±2 ºC) and relative humidity (40-60%). The animals were fed with standard

laboratory diet and water ad libitum and were fasted overnight before the study but had a free approach to water. All animal experiments were carried out in accordance with the recommendations of the Lagos State University Ethics Committee.

Diabetes was induced by a single administration of streptozotocin (60 mg/kg) dissolved in 0.1M citrate buffer, pH 4.5 was intraperitoneally injected to overnight fasted rats. The blood samples were collected from the tail vein using capillary tubes. Blood glucose level was measured and the rats having a blood glucose level of more than 290 mg/dl were considered as fit for the study [16].

2.4 Experimental Design

Evaluation of the antidiabetic effect of protein isolate was done on five groups of rats by randomly selecting six rats for each group. The first group served as control while the others were induced with diabetes. The groups are as follows.

Group 1 (control – not induced) was given distilled water only.
Group 2 (STZ), diabetic control, was given citrate buffer only.
Group 3 (STZ OPI 200) diabetic, was treated with protein isolate 200 mg/kg body weight.
Group 4 (STZ OPI 600) diabetic, was treated with protein isolate 600mg/kg body weight.
Group 5 (STZ) diabetic rats treated with glibenclamide (500 µg/kg B.wt) [17].

Determination of the dose to be administered to each animal in the group was done according to the bodyweight before administration. All animals in the groups were repeatedly exposed by oral gavage to the respective dose of their treatment for six weeks after which they were sacrificed.

2.5 Recording of Body Weight

Body weight was measured before and after the streptozotocin administration every day during the treatment in all groups [17].

2.6 Blood Sampling

By the end of the experiment, after six weeks treatment with OPI and glibenclamide, blood samples were collected from all animal groups after overnight fasting from the tail vein [17]. FBG was done immediately and serum was separated by centrifugation at 3000 rpm for 15 min. The clean and clear serum has proceeded for lipid profile and antioxidant activity determination [18,19].

2.7 Blood Glucose Level

Blood sugar estimation was done by using a glucometer (Accu-check® sensor, Roche Diagnostics GmbH, Mannheim) and strips [20,21].

2.8 Preparation of Tissue Homogenate

The rats were euthanized on the last day with ketamine. The organs were harvested, weighed and organs designated for homogenizing were rinsed in ice-cold phosphate buffer saline, cut into sections and homogenized in phosphate buffer at ratio 1:4 (w/v). Organs to be used for histopathological studies were rinsed in phosphate buffer and stored in Formal Saline.

2.9 Estimation of Lipid Profile

Lipid profile (Total cholesterol, Triglyceride, LDL, HDL, and VLDL) was estimated by using Star 21 bio auto-analyzer (E114947) at 505 nm by standard kits (Span Diagnostics Ltd. India) [20].

2.10 Determination of selected antioxidant markers

2.10.1 Determination of Superoxide dismutase (SOD) activity

The levels of SOD activity were determined by the method of Misra and Fridovich [22].

2.10.2 Determination of Catalase activity

Catalase activity was determined according to the method of Clairborne [23].

2.11 Assessment of Lipid Peroxidation

Lipid peroxidation was determined by measuring the Thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation according to Al-Jassabi et al. [24]

2.12 Statistical Analysi

The results were reported as mean ±SEM; n=6 animals in each group; * $P<0.05$: Statistically significant from control; ** $P<0.05$: Statistically significant from diabetic control; # $P<0.05$:

Statistically significant from low dose OPI (200mg/kg body weight); ## P<0.05: Statistically significant from high dose OPI (600mg/kg body weight); Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). Two-way ANOVA followed by Bonferroni's post hoc test was used to analyze the fasting blood glucose and Bodyweight change of the rats. One-way ANOVA was used to analyze other data, followed by Tukey's multiple comparison tests.

3. RESULTS

Fig. 1 shows a significant (P<0.05) increase in fasting blood glucose concentration in all STZ treated groups (301 mg/dl, 297.25 mg/dl, 300 mg/dl, 300 mg/dl) 72 hours after induction with STZ. At the end of the treatment, the high dose treatment group had the least blood glucose levels (132 mg/dl) although not significantly different from the GBN treated group (156 mg/dl), it was significantly lower to the low dose treated group.

Table 1 shows the different body weights of the rats every two weeks. The rats had an increase in weight, with the groups treated with GBN having significant (P<0.05) (130 g) increase in weight compared to the OPI treated groups (160 g and 170 g respectively). On the final day, the control group and all treated groups were recorded to have an increase in weight while the diabetic control had a 10 g reduction in weight. There was a significant difference (P<0.05) in the weight of the OPI 600 mg/kg treated group when compared to the diabetic control group.

GBN treated group had the highest (2.05 ± 0.17 mmol/L) serum total cholesterol levels compared to the diabetic control group (1.74 ± 0.08 mmol/L) while the Low dose OPI treated group showed the lowest of serum total cholesterol (1.60 ± 0.06 mmol/L) compared to the diabetic control group (1.74 ± 0.08 mmol/L). Serum Total cholesterol levels in control group was 1.83 ± 0.07 mmol/L while for the high dose treated group was 1.89 ± 0.11 mmol/L.

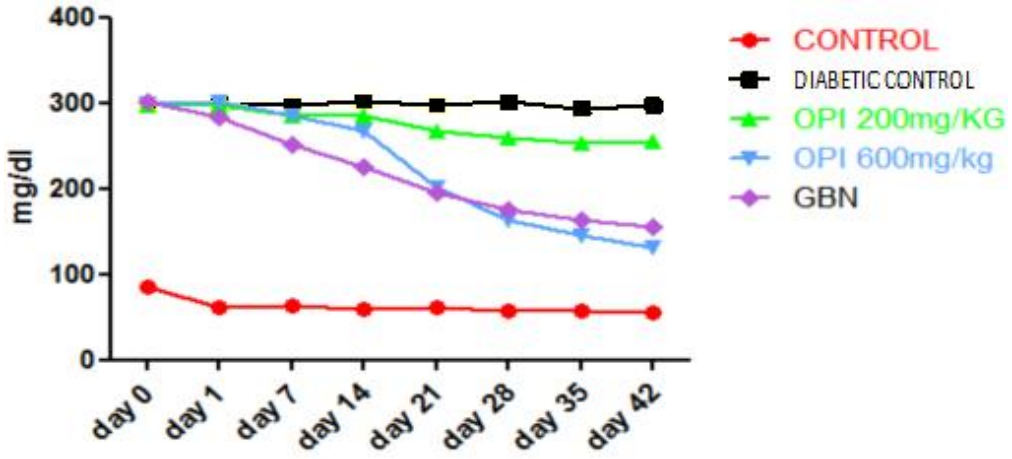

Fig. 1. Effect of Ogiri protein Isolate (OPI) on fasting blood glucose (FBG) level of control and STZ diabetic rats

Table 1. Effect of Ogiri protein Isolate (OPI) on weight gain level of control and STZ diabetic rats

Groups	Day 1	Day 14	Day 28	Day 42
Control	120 ± 2.1	150 ± 2.4	160 ± 9.0	190 ± 11
Diabetic control	120 ± 2.6c	140 ± 7.0	160 ± 7.6	150 ± 4.8
OPI 200mg/kg	150 ± 4.6b	160 ± 8.2	180 ± 9.7	190 ± 14
OPI 600mg/kg	130 ± 6.4a	170 ± 6.6	190 ± 14	230 ± 18b
GBN	110 ± 12abc	130 ± 11cd	160 ± 10	190 ± 9.9

Results are expressed as mean ±SEM; n=6 animals in each group; a P<0.05: Statistically significant from control; b P<0.05: Statistically significant from diabetic control; c P<0.05: Statistically significant from low dose OPI (200mg/kg body weight); d P<0.05: Statistically significant from high dose OPI (600mg/kg body weight)

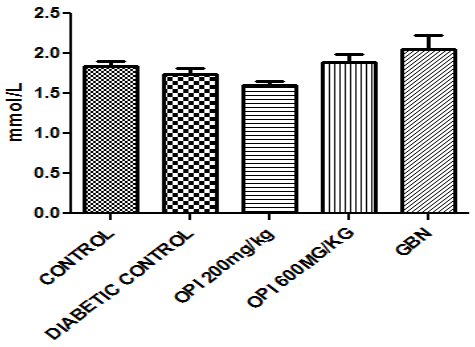

Fig. 2. Effect of Ogiri protein isolate on serum total cholesterol levels in control and STZ diabetic rats

Results are expressed as mean ±SEM; n=6 animals in each group

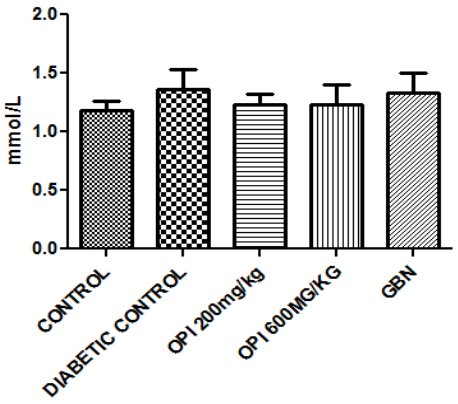

Fig. 3. Effect of Ogiri protein isolate on serum triglycerides levels in control and STZ diabetic rats

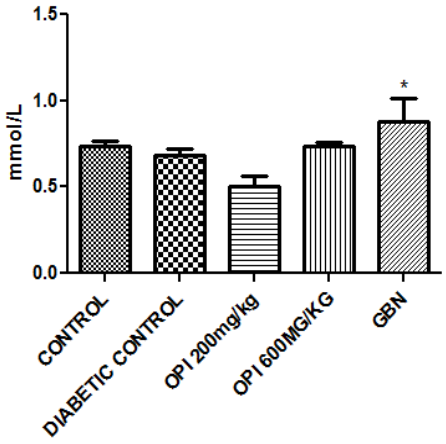

Fig. 4. Effect of Ogiri protein isolate on serum HDL-cholesterol levels in control and STZ diabetic rats

** P<0.05: Statistically significant from control*

The diabetic control group had a higher serum triglycerides levels (1.37 ± 0.16 mmol/L) compared to the control (1.18 ± 0.08 mmol/L). The high dose OPI treated group also showed a lower serum triglyceride (1.23 ± 0.09 mmol/L) level compared to the diabetic control (1.37 ± 0.16 mmol/L) but both OPI treated groups had higher levels when compared to the control group (1.18 ± 0.08 mmol/L). GBN treated group had a higher level (1.33 ± 0.17 mml/L) compared to the diabetic control group (1.37 ± 0.16 mmol/L).

This result shows a significant (P<0.05) increase in the serum HDL cholesterol level of the GBN treated group (0.876 ±0.14 mmol/L) compared to the control (0.736 ± 0.03mmol/L). The low dose OPI treated group showed a lower serum HDL cholesterol (0.5 ± 0.08 mmol/L) compared to the diabetic control (0.68 ±0.04 mmol/L). The high dose OPI treated group also showed a lower serum HDL cholesterol (0.735 ±0.03 mmol/L) compared to the diabetic control (0.68 ±0.04 mmol/L). A comparison of the serum HDL cholesterol levels of the OPI treated groups showed that the high dose OPI treated group had a higher level (0.735 ±0.03 mmol/L).

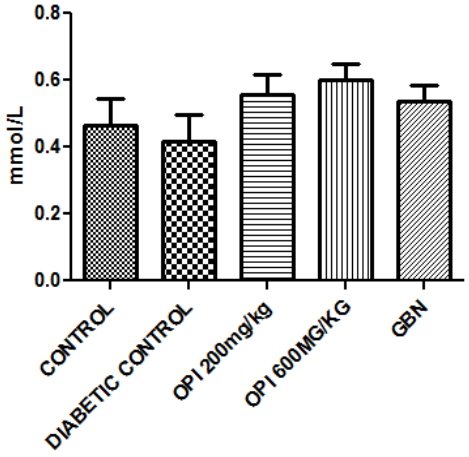

Fig. 5. Effect of Ogiri protein isolate on serum LDL-cholesterol levels in control and STZ diabetic rats

Rats treated with the high dose of the OPI had an elevated level (0.60 ± 0.05 mmol/L) of serum LDL-cholesterol when compared to the diabetic control group (0.42 ± 0.08 mmpl/L) and a reduction when compared to the control group (0.46 ±0.08 mmol/L). Rats treated with the low dose of OPI had an elevated (0.56 ± 0.06 mmol/L) when compared with the control and diabetic control groups. It also showed a reduced

level of serum LDL-cholesterol levels in rats treated with the low dose of OPI (0.56 ± 0.06 mmol/L) when compared with those treated with high dose OPI (0.60 ± 0.05 mmol/L).

A reduction in the Liver total cholesterol levels in diabetic control rats (0.76 ± 0.24 mmol/L) when compared with the control group (0.96 ± 0.20 mmol/L) was observed. Rats treated with the high dose of the OPI had an elevated level (0.92 ± 0.26 mmol/L) of liver total cholesterol when compared to the diabetic control group and a non-significant reduction when compared to the control group. Rats treated with the low dose of OPI had a reduced (0.61± 0.26 mmol/L) when compared with the control (0.96 ± 0.20 mmol/L) and diabetic control groups (0.76 ± 0.24 mmol/L). It also showed a reduced level of liver total cholesterol in rats treated with the low dose of OPI (0.61 ± 0.16 mmol/L) when compared with those treated with high dose OPI (0.92 ± 0.26 mmol/L).

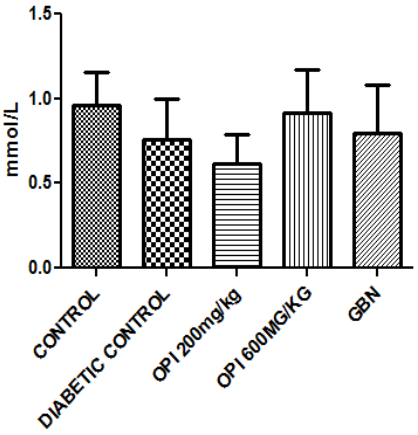

Fig. 6. Effect of Ogiri protein isolate on liver total cholesterol levels in control and STZ diabetic rats

Fig. 7. revealed a reduction in the Liver triglyceride levels in diabetic control rats (1.62 ± 0.22 mmol/L) when compared with the control group (1.75 ± 0.18 mmol/L). Rats treated with the low dose of the OPI had a reduced level (1.04 ± 0.35 mmol/L) of liver triglycerides when compared to the diabetic control group (1.62 ± 0.22 mmol/L) and the control group (1.75 ± 0.18 mmol/L). Rats treated with the high dose of OPI had an elevated (2.46 ± 0.57 mmol/L) when compared with the control (1.75 ± 0.18 mmol/L) and diabetic control groups (1.62 ± 0.22 mmol/L). It also showed an increased level of liver triglycerides when compared with rats treated with the low dose of OPI (1.04 ± 0.35 mmol/L).

Fig. 8 shows an increase in the liver HDL cholesterol level of the diabetic control group (1.6 ± 0.67 mmol/L) compared to the control (1.0 ± 0.002 mmol/L). The low dose OPI treated group showed a lower liver HDL cholesterol (1.4 x ± 0.24 mmol/L) compared to the diabetic control (1.6 ± 0.67 mmol/L). The high dose OPI treated group also showed a lower liver HDL cholesterol (1.3 ± 0.24 mmol/L) compared to the diabetic control (1.6 ± 0.67 mmol/L). Comparison of the liver HDL cholesterol levels of the OPI treated groups showed that the low dose OPI treated group had a higher level (1.4 ± 0.25 mmol/L) compared to the low dose OPI treatment group (1.3 ± 0.24 mmol/L).

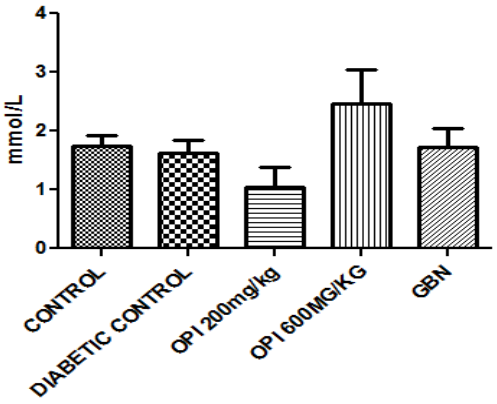

Fig. 7. Effect of Ogiri protein isolate on liver triglycerides levels in control and STZ diabetic rats

Fig. 9 shows no significant difference among compared means. The GBN treated group had a lower (1.2 ± 0.08 mmol/L) compared to the diabetic control group and OPI treated groups while a reduction in the liver LDL-cholesterol levels in rats induced with dibetic treated group (0.19 ± 0.09 mmol/L) when compared with those treated with the control group (0.27 ± 0.08 mmol/L). Rats treated with a low dose of the OPI had an reduced level (0.8 ± 0.05 mmol/L) of liver LDL-cholesterol when compared to the diabetic control group and a reduction when compared to the control group. Rats treated with the high dose of OPI had an elevated (0.9 ± 0.05 mmol/L) when compared with the control and diabetic control groups. It also showed a reduced level of liver LDL-cholesterol levels in rats treated with the low dose of OPI (0.8 ± 0.05 mmol/L) when compared with those treated with high dose OPI (0.9 ± 0.05 mmol/L). Rats treated with the standard drug (GBN) had a higher (0.14 mmol/L) when compared with those treated with high-dose OPI (0.9 ± 0.05 mmol/L).

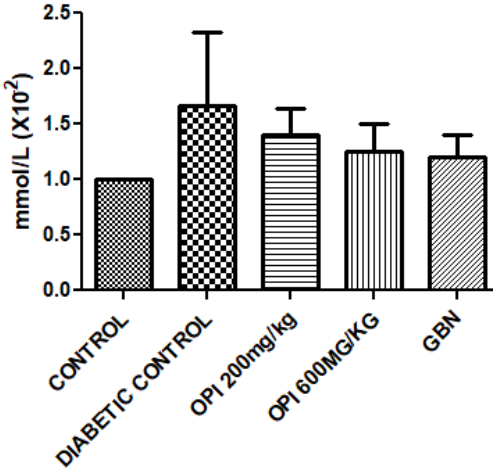

Fig. 8. Effect of Ogiri protein isolate on liver HDL levels in control and STZ diabetic rats

Table 2 shows that rats treated with high dose OPI had a significantly higher activity when compared with the diabetic control group. There was however no significant difference in the remaining comparisons made in this result; although, rats treated with low dose had a higher activity when compared with those treated with control and diabetic control groups. A significant increase in the serum Catalase activity of rats induced with STZ only when compared with the control group. Rats treated with the high dose of the OPI had a low-level units/mg protein) of serum Catalase activity when compared to the diabetic control group. Rats treated with the standard drug (GBN) had a significantly higher activity when compared with the diabetic control group. GBN treated group also had a higher activity when compared with the low dose OPI group and high dose OPI group. The table also reveals a significant increase in the lipid peroxidation of male rats induced With STZ only when compared with those treated with the control group. OPI treatment groups did not have any significant difference when compared to the control and diabetic control groups. Rats treated with the low dose of OPI had a higher when compared with those treated with high dose OPI. Rats treated with the standard drug (GBN) had a higher when compared with those treated with high-dose OPI. GBN treated group also had a higher when compared with the control group.

Table 3 shows that treatment with high dose OPI had a significantly higher activity when compared with the diabetic control group. there was also significantly increased activity when low dose OPI was compared to the high dose. There was however no significant difference in the comparison of the control and diabetic control. Low dose OPI treatment had higher inhibitory when compared to the diabetic control group but had a lower inhibitory when compared to the control GBN treated group had lower inhibitory compared to low dose OPI.

There was an increase in the liver catalase activity of diabetic control rats when compared with the control group. Rats treated with the high dose of the OPI had significantly increased activity of liver catalase when compared to the diabetic control group. Rats treated with the low dose of OPI had significantly reduced activity when compared with high dose OPI. Rats treated with the standard drug (GBN) had the higher activity when compared with the diabetic control group and low dose OPI group but were lower when compared to the high dose OPI group. The table also shows a significant increase in the lipid peroxidation in the liver of rats induced with STZ only when compared with those treated with the control group. Rats treated with the high dose of the OPI had a significantly low level of liver lipid peroxidation when compared to the diabetic control group and a non-significant reduction when compared to the control group. Rats treated with the low dose of OPI had the higher when compared with those treated with high dose OPI. Rats treated with the standard drug (GBN) had the higher when compared with those treated with high-dose OPI. GBN treated group also had the higher when compared with the control group but a reduced level when compared to the diabetic control group.

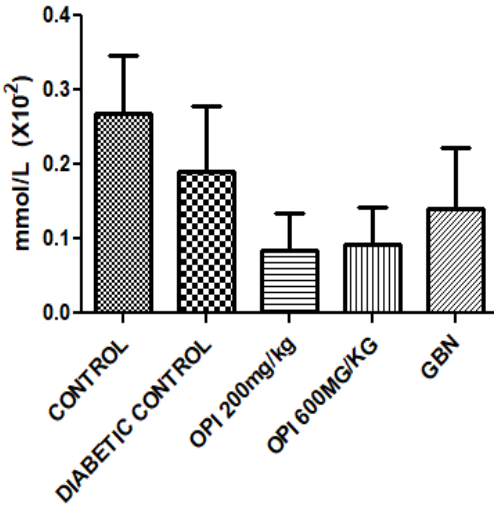

Fig. 9. Effect of Ogiri protein isolate on liver LDL levels in control and STZ diabetic rats

Table 4 shows that rats treated with high dose OPI had a significantly higher activity when compared with the diabetic control group. There was a significantly low activity observed in GBN treated groups when compared to the high dose and there was significantly increased activity when low dose OPI was compared to the high dose. There was a decrease in the Pancreas catalase activity of diabetic control rats when compared with the control group. Rats treated with the high dose of the OPI had lower activity when compared to the diabetic control group. Rats treated with the low dose of OPI had increased activity when compared with high dose OPI. Rats treated with the standard drug (GBN) had a higher activity when compared with the diabetic control group. GBN treated group also had a higher activity when compared with the high dose OPI group. There were no significant differences between all compared groups. The table also shows an increase in the pancreas lipid peroxidation of rats induced with STZ only when compared with those treated with the control group. Rats treated with the high dose of the OPI had a low level of pancreas lipid peroxidation when compared to the diabetic control group and a non-significant decrease when compared to the control group. Rats treated with the low dose of OPI had low when compared with diabetic control and control. Rats treated with the standard drug (GBN) had a higher when compared with those treated with high-dose OPI. GBN treated group also had a lower when compared with the control group, the diabetic control group, and low dose OPI. There were no significant differences between all compared groups.

4. DISCUSSION

Diabetes is the largest growing metabolic disorder in the world [25], associated with elevated blood sugar levels, dyslipidemia and several other complications like obesity, hypertension and several other atherogenic diseases [26]. The data inform that OPI significantly reduced the elevated fasting blood glucose level when compared to the diabetic control animals [27]. This hypoglycemic effect may be ascribed to the natural action of pancreatic secretion of insulin from the existing and regenerated cells of the islets as well as

Table 2. Effect of Ogiri protein isolate on selected antioxidant activity in the serum of control and STZ-induced diabetic rats

	SOD (UI)	CAT (H_2O_2/g hb)	LPO (MDA units/mg hb)
Control	24.1 ± 4.46	102.6 ± 4.52	5.66 ± 0.76
Diabetic Control	14.8 ± 2.34	132.8 ± 4.44[a]	9.30 ± 1.13
OPI 200 mg/kg	29.6 ± 5.49[d]	115.9 ± 6.17	8.46 ± 0.95
OPI 600 mg/kg	38.9 ± 3.21[b]	122.0 ± 3.17	4.85 ± 0.83[b]
GBN	22.2 ± 3.51[d]	125.8 ± 5.9[a]	6.69 ± 0.77

Results are expressed as mean ±SEM; n=6 animals in each group; a P<0.05: Statistically significant from control; b P<0.05: Statistically significant from diabetic control; c P<0.05: Statistically significant from low dose OPI (200mg/kg body weight); d P<0.05: Statistically significant from high dose OPI (600mg/kg body weight).SOD= Superoxide dismutase; CAT= Catalase; LPO; Lipid Peroxide; STZ= Streptozotocin; GBN = Glibenclamide; OPI = Ogiri protein Isolate

Table 3. Effect of Ogiri protein isolate on selected antioxidant activity in the Liver of control and STZ-induced diabetic rats

	SOD (UI)	CAT (H_2O_2/g protein)	LPO (µmol/mg prot/mg protein)
Control	25.0 ± 4.56	270 ± 19	22.21 ± 3.59
Diabetic Control	12.5 ± 3.42	330 ± 56	46.96 ± 8.65[a]
OPI 200 mg/kg	19.8 ± 3.39[d]	260 ± 14[d]	35.91 ± 3.00
OPI 600 mg/kg	35.9 ± 3.93[b]	410 ± 11[a]	19.15 ± 2.58[b]
GBN	17.5 ± 1.25[d]	290 ± 25	31.42 ± 1.00

Results are expressed as mean ±SEM; n=6 animals in each group; a P<0.05: Statistically significant from control; b P<0.05: Statistically significant from diabetic control; c P<0.05: Statistically significant from low dose OPI (200mg/kg body weight); d P<0.05: Statistically significant from high dose OPI (600mg/kg body weight). SOD= Superoxide dismutase; CAT= Catalase; LPO; Lipid Peroxide; STZ= Streptozotocin; GBN = Glibenclamide; OPI = Ogiri protein Isolate

Table 4. Effect of Ogiri protein isolate on selected antioxidant activity in the Pancreas of control and STZ-induced diabetic rats

	SOD (UI)	CAT (H_2O_2/g protein)	LPO ($X10^5$ (µmol/mg prot)
Control	31.0 ± 5.6	172.9 ± 20.67	11.23 ± 3.9
Diabetic Control	12.0 ± 4.2a	169.1 ± 13.06	11.47 ± 0.97
OPI 200 mg/kg	24.0 ± 5.8[d]	159.1 ± 11.56	10.09 ± 1.28
OPI 600 mg/kg	44.0 ± 4.8[b]	105.1 ± 4.54	4.80 ± 0.19
GBN	22.0 ± 1.5[d]	153.9 ± 18.25	9.67 ± 0.6

Results are expressed as mean ±SEM; n=6 animals in each group; a P<0.05: Statistically significant from control; b P<0.05: Statistically significant from diabetic control; c P<0.05: Statistically significant from low dose OPI (200mg/kg body weight); d P<0.05: Statistically significant from high dose OPI (600mg/kg body weight). SOD= Superoxide dismutase; CAT= Catalase; LPO; Lipid Peroxide; STZ= Streptozotocin; GBN = Glibenclamide; OPI = Ogiri protein Isolate

other extra-pancreatic mechanisms such as enhanced blood glucose transport to peripheral tissue and increased utilization of glucose via several enzymatic pathways or the activity of intestinal absorption of glucose, similar to earlier reported studies [28,29].

Lipids play a vital role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor coronary heart disease [30]. High levels of total cholesterol and more importantly LDL-cholesterol in the blood are major coronary risk factors [31]. The abnormal high concentration of serum lipids in the diabetic subjects is due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase [32]. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [16]. A significant lowering of total cholesterol and the rise of HDL-cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and ischemic conditions [33].

The exact mechanism by which vegetable proteins show their hypolipidemic effect is still obscure. it has been reported by several researchers that the amino acids or the non-protein components such as isoflavones could be responsible for the hypolipidemic effects observed [34]. The hypolipidemic effect of protein isolates from vegetables is well documented. Isolates from Soybean and sesame have been studied and the cholesterol-lowering effects of plant protein compared to animal proteins have been acknowledged in both experimental models and farm animals for several years [14]. In this study, two different doses of OPI were administered to the experimental animals and a non-distinctive difference in their effects would suggest that a higher dose may be produce desired effects or incorporated into pre-existing therapies. OPI decreased serum total cholesterol and triglyceride in both OPI treated groups when compared to the control and diabetic control group and an increase in the HDL-cholesterol in the serum and liver of treated groups compared to the diabetic control group at the high dose. Studies revealed that whey protein fed to animals increases weight gain and affects blood lipid levels [32] as well as the hypocholesterolemic lowering ability of crude dietary protein isolates from soy producing a significant effect than milk protein [35].

Hyperglycemia is reported to increase oxidative stress through free radical formation [36]. Endogenous oxygen-free radicals scavenging enzymes can respond to such conditions of oxidative stress in diabetes with a compensatory mechanism. The concentration of lipid peroxides was increased in the serum, liver, and pancreas of diabetic rats, indicating an increased free radical generation and/or exhaustion of the endogenous antioxidant system. The present finding showed significant attenuation of the lipid peroxide level by the treatment of OPI as well as Glibenclamide. Catalase is involved in the elimination of H_2O_2 and plays a vital role in cellular stress reduction [37]. The induction of SOD activity by OPI may be attributed to the activity of the generation of active oxygen species from autoxidation of glucose generation from the action of STZ. The increased activity of SOD accelerates the dismutation of superoxide radicals to H_2O_2, which is removed by CAT. This indicates that the OPI treatment has

altered the SOD, CAT, Treatment of OPI increased the enzyme level in the serum and tissues of heart, liver, kidney, and pancreas and thus may help to minimize the free radical generation and hence oxidative stress in diabetes [38,39] Therefore the recovery of the antioxidant status of diabetic rats by treatment with OPI reveals the antioxidant property of OPI by which it can significantly reduce the imbalance between the free radical generation and endogenous antioxidant system. This may be beneficial for minimizing the complications of the disease [40].

5. CONCLUSION

From the present study, it can be concluded that oral administration of Ogiri protein isolates produces a significant hypoglycemic effect in the control of blood glucose levels. Additionally, it possesses a potent antioxidant effect by the significant reversal of hyperglycemia-induced oxidative stress. However, from this study, ogiri protein isolates cannot be referred to posses a reliable hypolipidemic activity. Hence, protein isolates from fermented *Cucumeropsis manii* can be considered a potent source of hypoglycemic agent but not cardioprotective agents. Further studies to elucidate the mechanism of action involved should be done.

ETHICAL APPROVAL

All animal experiments were carried out in accordance with the recommendations of the Lagos State University Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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