Protein Isolates from Fermented Melon Seeds Promoted Differential Expression in Liver and Pancreas of Streptozotocin Induced Diabetic Male Wistar Rats

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Authors' contributions

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ABSTRACT

Diabetes mellitus (DM) is the most common endocrine disorder of human. However, the anti-diabetic activity of protein isolates from fermented plants seed for DM remains enigmatic. The prevalence of diabetes in Africa is exponentially increasing with more deaths occurring directly from diabetes mellitus or from its associated complications. The current study investigated the effect of Protein isolate from fermented melon seeds (Ogiri; OPI) of *Cucumeropsis manni* on blood glucose, hepatic and pancreatic protein profile, histopathological parameters, identification and characterisation of expressed proteins in streptozotocin (STZ)-induced diabetic rats. Thirty Male wistar rats were divided into nondiabetic control, STZ-diabetic control, STZ-Ogiri protein isolate supplemented group (STZ-OPI; 200 mg/kg diet), STZ-Ogiri protein isolate supplemented group (STZ-OPI; 600 g/kg diet) and STZ-glibenclamide treated group (STZ-GBN; 0.5 mg/kg diet). Diabetes was induced by a single injection of STZ (60 mg/kg BW) freshly dissolved in 0.1 mol/L citrate buffer (pH 4.5) into the intraperitonium. Diabetes was confirmed by measuring the fasting

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blood glucose concentration 48-h post-injection. The rats with blood glucose level above 290 mg/dL were considered to be diabetic. Ogiri protein isolates was supplemented in the diet for 6 weeks. The supplementation OPI reduced (P< 0.05) the blood glucose concentration of the STZ-induced diabetic rats. OPI supplemented groups had significantly higher percentage body weight gain. The high dose OPI supplemented group had a lowest liver protein concentration (19.39 mg/dl) but a significantly (P< 0.05) higher pancreas protein concentration when compared to all the diabetic control. Histological sections of examined tissue revealed accumulation of fat in the liver of diabetic rats and necrosis of the islet of Langerhans were observed in the pancreas. 1DE SDS-PAGE of hepatic and pancreatic tissue homogenates revealed differential expression of 150 kDa proteins in rats treated with 200 mg/kg body weight of OPI only and 20-25 kDa proteins in rats treated with 600 mg/kg body weight of OPI respectively. This result show that OPI supplementation may impose a direct or indirect inhibitory or post translational modification on specific proteins implicated in hyperglycemia and diabetes and as such be a potential antihyperglycemic agent in the management of diabetes.

1. INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Being an endocrine metabolic disorder, it is characterized by persistent blood glucose accumulation and often followed by extreme fatigue, excessive urination, polyuria, weight loss and stupor [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3,4].

Diabetes being a polygenic disease with expected alterations in many proteins; proteomics is a powerful experimental approach to study the pathophysiological mechanisms of alteration in these proteins as well as the mechanism of action of plant-derived remedies in Streptozotocin (STZ)-induced diabetic rats [5,6,7].

Available literature reports show that there are more than 400 plant species showing antidiabetic activity [8,9] and the demand to use natural products in the treatment of diabetes is increasing worldwide [10,11]. The effects of these plants have been shown to delay the development of diabetic complications and correct some metabolic abnormalities. In the past few years some of the new bioactive drugs isolated from hypoglycemic plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy [12]. 'Ogiri' an oily paste condiment is produced mainly from melon seeds (*Cucumeropsis mannii*), sesame seeds (*Sesamum indicum*) [13,14] through the process of fermentation.

Currently, the main treatment for *Diabetes mellitus* is using Insulin and hypoglycemic drugs. Although there are many proven side effects for these compounds. Hence, there is growing interest in the use of natural health products; as an alternative approach to current medications. Plant sources has become a target to explore new drugs and in searching for biologically active compounds.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used in this study were of analytical grade. They are products of Sigma Chemical Co St Louis USA, BDH Laboratory Supplies, Poole Bh 15 1 TD, England; Glibenclamide was obtained at a drug store in Lagos, Nigeria. Glucometer Kits (Accucheck® sensor, Roche Diagnostics GmbH, Mannheim).

2.2 Plant Material and Preparation

Ogiri was purchased from the new local market in ijebu -ode, Ogun State, Nigeria. The seeds were identified and authenticated by the Department of Botany, Lagos State University (Ojo). *Cucumeropsis mannii* fermented seeds were oven-dried (500°C) and pulverized to obtain a coarse powder using an electric blender. Two kilograms of uniform powdered seeds was defatted with hexane to obtain the defatted extract. Briefly, Ogiri samples were suspended in hexane in a screw cap container; the suspension was shaken vigorously and allowed to settle overnight. And the clear residue was later re-suspended in the same fresh solvent. This

process was repeated twice to effect complete extraction.

The residue was recovered and excess extract solvent was removed by squeezing in muslin cloth. Total defatting was confirmed by pressing one gram of defatted sample on a white sheet of paper, non-soiling of the paper with oil, indicated complete defatting of sample.

The defatted extract was air-dried and then extracted (1:10 w/v) with butanol to remove possible anti-nutrients. Protein isolate was obtained from the defatted extract using the method described by Nkosi and co-workers [15]. Briefly, the dry defatted extract was suspended in 1.0M NaCl solution (ratio 1:5), stirred for 30 mins at ambient temperature and adjusted to pH 10 using 1.0M NaOH. Thereafter, the resultant suspension was filtered (using a Whatman filter paper) to remove debris and the filtrate adjusted to pH 5 with 1.0N HCl, followed by centrifugation at 15,000 x g for 20 min at 4°C. Supernatant collected and re-adjusted to pH 4.0 and further centrifuging at 15,000x g for 20 mins at 4°C. The supernatant was discarded and the pellet containing the protein isolate retained and freeze-dried to yield a brown extract. The lyophilized extract was kept dry until needed.

2.3 Experimental Animals

Thirty (30) adult healthy male wistar rats were purchased from the animal house of the Department of Veterinary Medicine, University of Ibadan, Nigeria and housed at the animal house of the Department of Biochemistry, faculty of Science, Lagos State University (Ojo), Lagos, Nigeria. The animals were acclimatized for a period of one week under standard environmental conditions, with an appropriately 12 hours light / dark cycle in a controlled temperature (24±2°C) and relative humidity (40-60%) room. The animals were fed with standard laboratory diet and water provided *ad libitum*. After one week of acclimatization, the animals were subjected to various treatments for a period of six weeks. All animal experiments were carried out in accordance with the recommendations of the Lagos State University Ethics Committee.

2.3.1 Induction of diabetes

Following an overnight fasting, diabetes was induced in the selected rats by a single intraperitoneal injection of freshly prepared STZ (Sigma-Aldrich Co., St. Louis, MO, USA) at a dose of 60 mg/kg body weight; dissolved in 0.1 M ice-cold citrate buffer, pH 4.5 [16]. Diabetes was confirmed in the rats 72 h after STZ administration.

2.4 Experimental Design

Thirty (30) healthy male albino rats of the average body weight of about 120–170 g was used in this study. The Animals were grouped into five groups, each containing six animals based on their body weight range.

The blood samples was collected (from tail vein using capillary tubes) to measure their fasting blood glucose (FBG) levels using glucometer. Rats with FBG level above 290 mg/dL were considered diabetic and selected for the study [17].

Treatment commence on the fourth day [18], Ogiri Protein isolate (OPI) was suspended in distilled water and administered orally through orogastric tube at dose of 200 mg/kg and 600 mg/kg body weight. The administration of the protein isolate and standard drug was carried out every day for a period of forty two days [18]. The animals were treated daily 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 600 mg/kg body weight.
Group 5 (STZ) diabetic rats treated with glibenclamide.

2.5 Recording of Body Weight

Body weight was measured before and after inducing with streptozotocin and continued every day during treatment in all groups for the period of six weeks [19].

2.6 Blood Glucose Monitoring

Blood Glucose concentration determination was done using glucometer kits; blood from the animal tails was used to determine the glucose concentration in mg/dl (Accucheck® sensor, Roche Diagnostics GmbH, Mannheim) and strips [20,21]. Blood samples for estimation of blood glucose was collected from each animal from the tip of the tail under mild ether as an anesthesia

on day 0 (before treatment) and subsequently every week (during treatment) and the last day of treatment [22,23].

2.7 Organ Collection

At the end of 42 days of treatment with OPI, the rats were fasted overnight prior to sacrifice. Animals were anesthetized with ketamine and the blood samples were collected through venipuncture with 5 ml syringes. The blood samples for hematological parameters were collected from each animal by venipuncture into EDTA sample tubes and then transport immediately for analysis.

The liver and Pancreas were excised, trimmed of connective tissues, weighed, rinsed with normal saline to eliminate blood contamination and then stored in formal saline and then sent for histopathological investigation. Samples for proteomics analysis were rinsed in ice - cold sterile phosphate buffer saline (PBS) and stored in RIPA buffer at -20°C until further analysis.

2.8 Preparation of Liver and Pancreas Homogenate

Liver and Pancreas samples for molecular analyses were rinsed separately in 500 µl ice cold 1x PBS (Phosphate buffer saline) and homogenized in 500 µl ice cold RIPA buffer using mortar and pestle. The homogenate is then centrifuge at 10000 rpm for 10 min at 4°C. Supernatant is discarded intro a fresh tube and 500 µl of 10% TCA added and then incubated on ice for 30 min. Centrifuged again at 10000 rpm for 10 min at 4°C. Supernatant is discarded, Pellet is washed with 500 µl ice cold acetone to get rid of TCA, centrifuge at 10000 rpm for 10 mins at 4°C; supernatant discarded and pellet is dried at 40°C until Acetone is gotten rid of.

2.9 Histopathological Analysis

After tissues have been harvested and fixed in formal-saline solution, they were dehydrated in concentrated ethanol. The dehydrated organ was soaked in xylene to remove alcohol and embedded in melted paraffin wax, the resulting block is mounted on a microtome and a knife mounted in a microtome is used to cut tissue sections 10 micrometers thick which are mounted on a glass microscope slide. The slices are affixed to microscope slides at which point the wax is removed with a solvent and the tissue slices attached to the slides are rehydrated and are ready for staining.

2.9.1 Hematoxylin and eosin staining

Sections of liver and pancreatic slides were deparaffinized by dipping in 2 changes of xylene for 10 minutes each. They were re-hydrated in 2 changes of absolute alcohol for 5 minutes each followed by soaking in 95% alcohol for 2 minutes and 70% alcohol for 2 minutes then washed briefly in distilled water. The slides were stained in Harris hematoxylin solution for 8 minutes then washed in running tap water for 5 minutes. They were differentiated in 1% acid alcohol for 30 seconds then washed under running tap water for 1 minute. Bluing was done in 0.2% ammonia water or for 30 seconds to 1 minute followed by washing under running tap water for 5 minutes then rinsed by dipping 10 times in 95% alcohol. Counterstaining in eosin-phloxine solution was done for 30 seconds. The slides were dehydrated via 2 changes of 95% alcohol, for 5 minutes each. Clearing was done in 2 changes of xylene for 5 minutes each. The slides were mounted with a xylene based mounting medium [24].

2.9.2 Pancreas Gomori's aldehyde fuchsin stained sections

This was carried out according to [25]. The slides were first deparaffinized and hydrated in water. They were placed in 70% ethanol for 1 min, followed by Aldehyde Fuchsin Solution for 15 minutes. They were then dipped in 3 changes of 95% ethanol- 3 min., 2 min., 1 min respectively. The slides were dipped in 70% ethanol, and then washed in tap water for approximately 15 minutes. They were stained in Mayer's Hematoxylin for 10 seconds then washed in running tap water for at least 10 minutes to blue. The slides were rinsed in distilled water followed by 2-3 dips in 50% ethanol, then 3 dips in Eosin. Slides were quickly dehydrated in 2 changes of 95% and Absolute ethanol. Clearing was done in several changes of Xylene, and then slides were mounted with a synthetic resin.

2.10 Molecular Analysis

2.10.1 Protein quantification

Proteins were quantified using a NanoDrop 2000/2000c (Thermo Scientific) following the manufacturer's instructions.

2.10.2 One dimensional (1-DE) Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)

Ten microgram of liver - and pancreas protein from rat were resuspended in 100 µl of 7M urea buffer and separated respectively on 1-DE SDS-PAGE as described by Laemmli (1970). Two types of gels are used in this system *viz.* the stacking and the separating gel. For 1-DE gels, the Mini-PROTEAN® Electrophoresis Cell gel casting system (BIO-RAD) was used. Separating gels of 12.5% (v/v) were prepared from a 40% Acrylamide/Bis stock solution 37.5: 1 (2.6% C) using 1.5 M Tris-HCl separating buffer (pH 8.8). The separating gel was poured first and overlaid with 1 ml of 100% isopropanol and allowed to polymerise. Then isopropanol was poured off and the separating gel was rinse thoroughly with distilled water and gel surfaces were dried off using filter paper. The stacking gels 5% (v/v) were prepared from a 40% Acrylamide/Bis stock solution 37.5: 1 (2.6% C) using 0.5 M Tris-HCl gel buffer, pH 6.8. The separating gel was overlaid with the stacking gel and the 1 mm (10-well comb) (BIO-RAD) was put in place. Protein samples were mixed with an equal volume of 2 DE SDS sample loading buffer, boiled for 5 min at 95°C on a heating block (Dry Block Heater, FMH Instruments).

Samples were centrifuged for a few seconds before loading on the gel. Total protein quantities of 10 µg per well were loaded onto the 10-welled gels and then electrophoresed in 1DE SDS electrophoresis running buffer using a PowerPacTM Universal Power supply (BIO-RAD). The run was started with 100 V for the first 30 minutes and thereafter increased to 140 V. Electrophoresis was stopped when the dye front reached the bottom of the separating gel. The gels were removed from the plates, stained with Coomassie Brilliant blue.

2.10.3 Coomassie brilliant blue staining of SDS-PAGE gels

Total protein samples loaded on 1-DE SDS-PAGE were detected by using CBB R-250 staining protocol. Following electrophoresis, the gels are placed in CBB staining solution, heated for 1 min in a microwave oven and incubated for 30 minutes with shaking at room temperature. The staining solution was discarded. Following the staining process, the gels were destained by immersing in destaining solution with shaking at room temperature until the protein bands were clearly visible against a clear background. The gels were imaged using a Molecular Imager PharosFX Plus System (BIO-RAD).

2.11 Statistical Analysis

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 (200 mg/kg body weight); ## P<0.05: Statistically significant from high dose OPI (600 mg/kg body weight); Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One-way ANOVA was used, 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, 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 after every two weeks. On the first day, a significant (P< 0.05) weight difference when comparing rats treated with high dose OPI (130 g) and standard drug (GBN) (110 g) to the control group (120 g). A comparison of the weights of the diabetic control (120 g) and the low dose OPI treated diabetic rats (150 g) also revealed a significant (P< 0.05) weight difference. The rats had 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 increase in weight while the diabetic control had a 10 g reduction in weight. There was significant difference in the weight of the OPI 600 mg/kg treated group when compared to the diabetic control group.

Fig. 2 shows no significant difference in the protein concentration in liver homogenate of the diabetic control compared to the control. The diabetic control group had a lower protein concentration (27.12 mg/dl) compared to the control (30.38 mg/dl). Low dose OPI treated group showed a higher protein concentration (31.12 mg/dl) compared to the diabetic control.

The high dose treated group had a lower protein concentration (19.39 mg/dl) compared to both the diabetic control group and the low dose treatment group. GBN treated group had a higher protein concentration (27.62 mg/dl) compared to the diabetic control group.
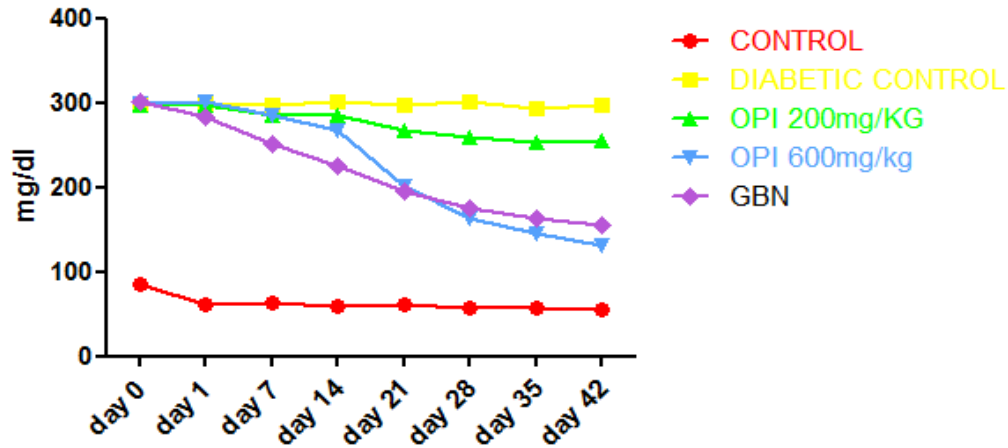

Fig. 1. Effect of Ogiri Protein Isolate (OPI) on Fasting Blood Glucose (FBG) level of control and STZ diabetic rats

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 (200 mg/kg body weight); d $P<0.05$: Statistically significant from high dose OPI (600 mg/kg body weight)
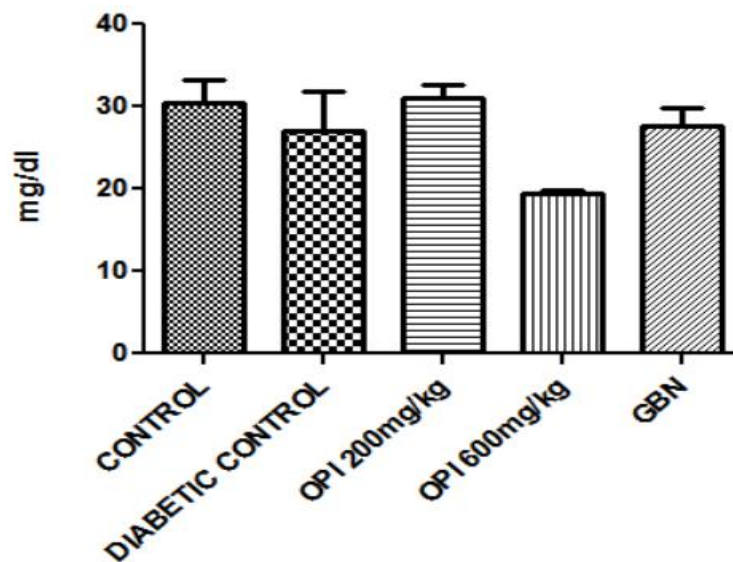

Fig. 2. Effect of Ogiri protein isolate on liver protein concentration

Fig. 3 shows a significant increase in the pancreas protein concentration of the OPI 600 mg/kg (68.91 mg/dl) compared to the diabetic control (42.26 mg/dl). The diabetic control group had a lower protein concentration (42.26 mg/dl) compared to the control (46.59 mg/dl). Low dose OPI treated group showed a lower protein concentration (46.26 mg/dl) compared to the control. The high dose treated group had the highest protein concentration (68.91 mg/dl) compared to both the diabetic control group and the low dose treatment group. GBN treated group had a higher protein concentration (49.94 mg/dl) compared to the diabetic control group.
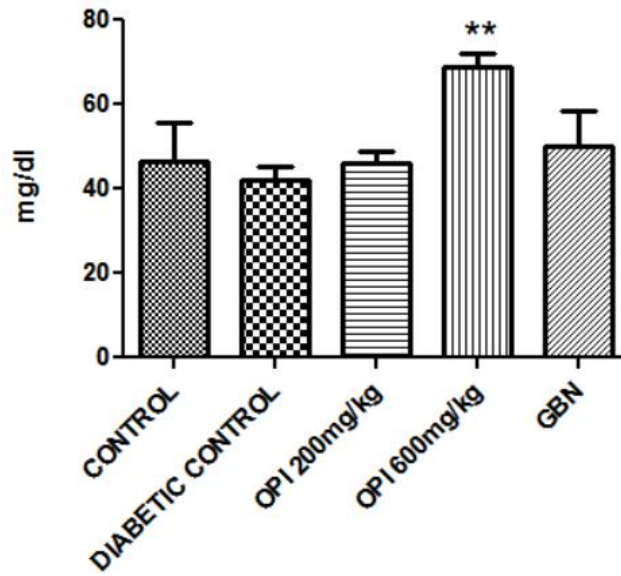

Fig. 3. Effect of Ogiri protein isolate on pancreas protein concentration
*Results are expressed as mean ±SEM; n=6 animals in each group; ** P<0.05: Statistically significant from diabetic control*
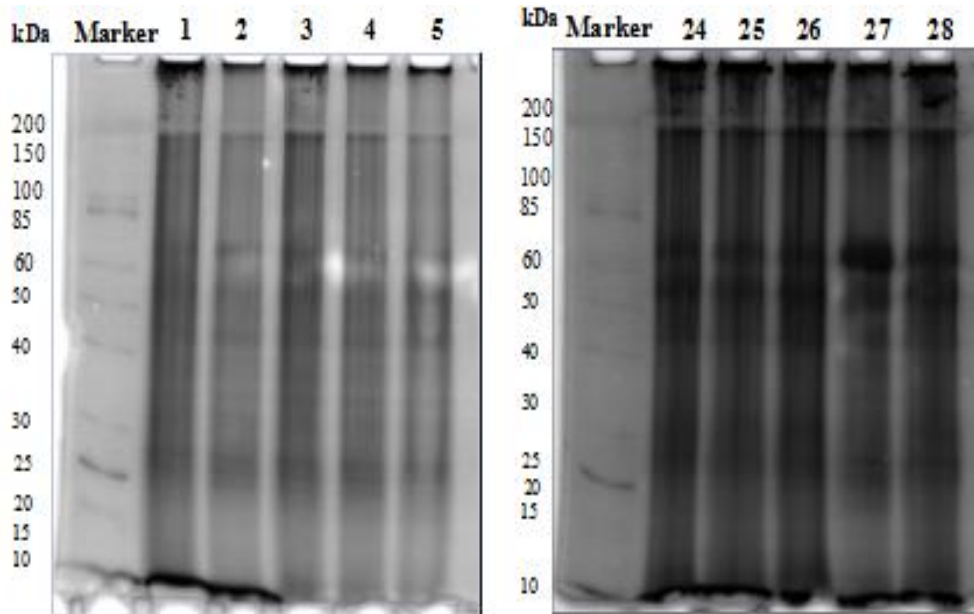

Plate 1. 1-DE PAGE profile of total protein extracted from liver and pancreas of rats in control group. Lane 1 shows the protein ladder, Lanes 1-5 pancreas and lanes 22-28 (second gel) liver. Each lane has 10 μg of total protein
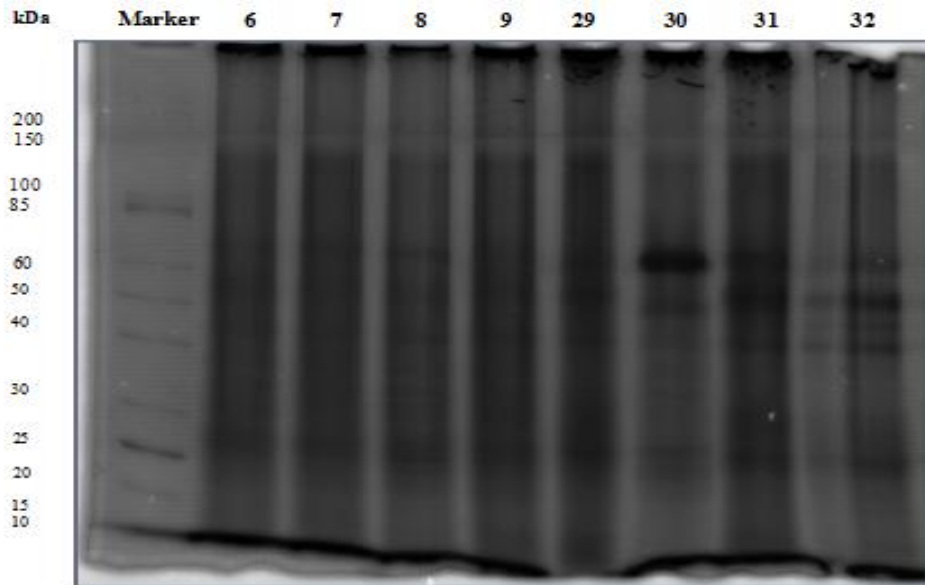
Plate 2. 1-DE PAGE profile of total protein extracted from liver and pancreas of OPI 600 mg/kg treated diabetic group. The first Lane shows the protein ladder, Lanes 15-18 pancreas and lanes 38-41 (second gel) liver. Each lane has 10 µg of total protein
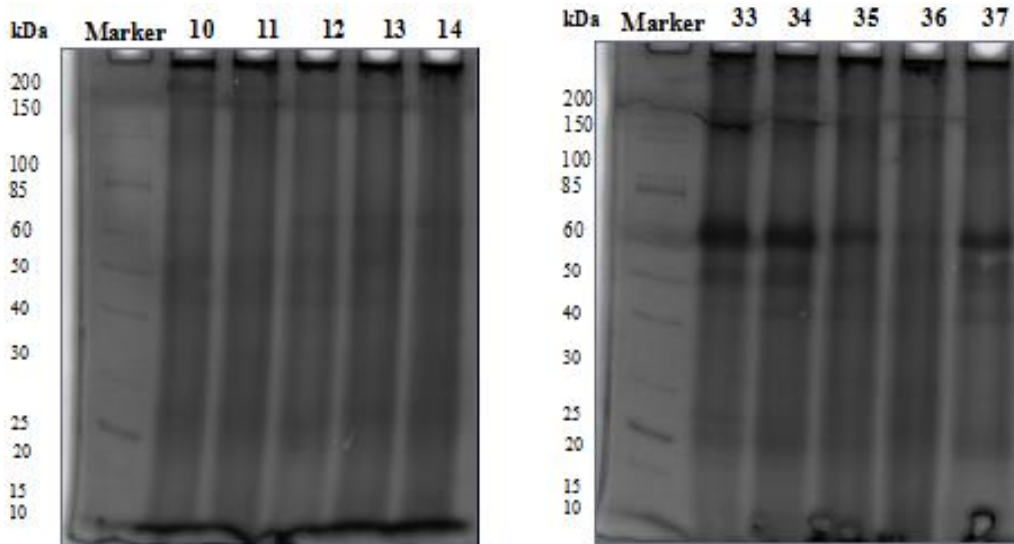

Plate 3. 1-DE PAGE profile of total protein extracted from liver and pancreas of OPI 600 mg/kg treated diabetic group. The first Lane shows the protein ladder, Lanes 15-18 pancreas and lanes 38-41 (second gel) liver. Each lane has 10 µg of total protein

Histological section of normal control show hepatocytes arranged in thin plates separated by fine vascular sinusoids. The hepatocytes are large polyhedral cells with round nuclei, peripherally dispersed chromatin and prominent nucleoli. Occasional portal tracts are observed. The latter is composed of collagenous connective tissue within which are terminal branches of portal veins, bile ducts and hepatic veins.

Histological sections of Normal Pancreas show epithelial elements disposed in lobules. These epithelial elements consist of acini, ducts and islets of Langerhans. The acini and tubules, which are closely packed, are lined by regular

cuboidal epithelial cells with abundant granular eosinophilic cytoplasm. There is minimal intralobular and interlobular connective tissue within which are traversing neurovascular bundles. Lobules of mature adipocytes are seen at the pheriphery.
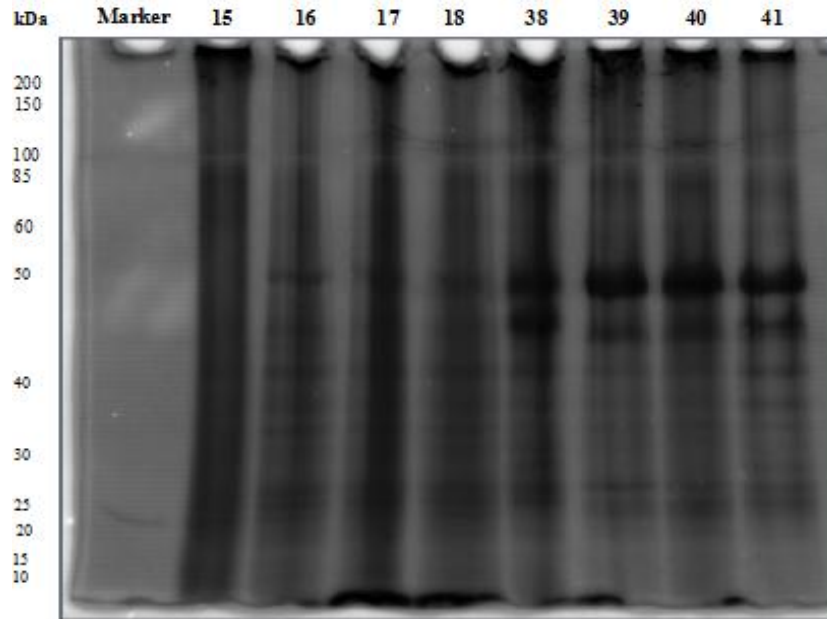

Plate 4. 1-DE PAGE profile of total protein extracted from liver and pancreas of Glibenclamide treated diabetic group. The first Lanes shows the protein ladder, Lanes 15-18 pancreas and lanes 38-41 (second gel) liver. Each lane has 10 μg of total protein
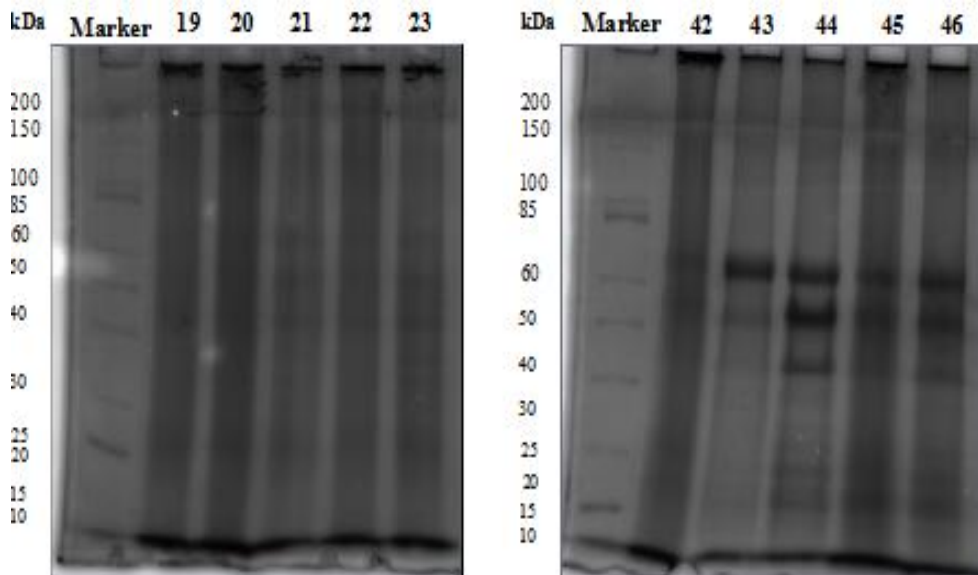

Plate 5. 1-DE PAGE profile of total protein extracted from liver and pancreas of Glibenclamide treated diabetic group. The first Lanes shows the protein ladder, Lanes 19-23 pancreas and lanes 42-46 (second gel) liver. Each lane has 10 μg of total protein
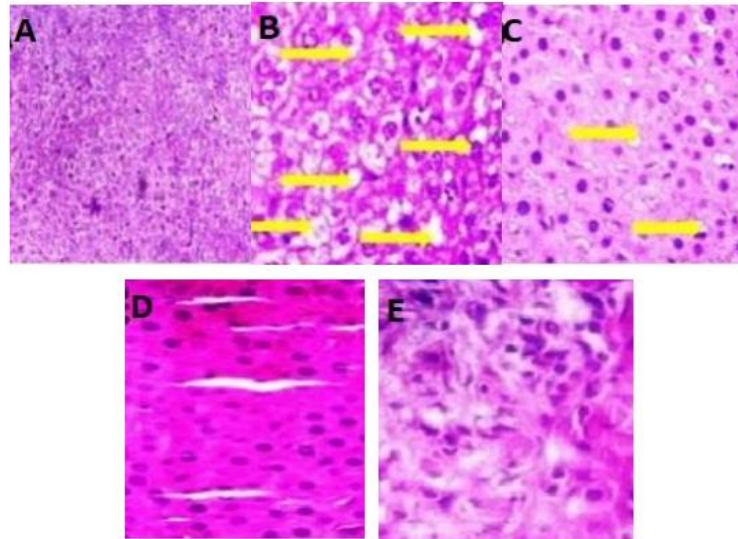
Plate 6. Effect of OPI on liver fat accumulation in different groups of rats (A) Normal control: (B) Diabetic control: Diabetic control histopathology of rat liver shown the micro fat droplet deposition (yellow arrow) (C) OPI (200 mg/kg): Histopathology there was only few micro fat droplets was present (yellow arrow). (D) OPI (600 mg/kg): histopathology similar to the glibenclamide treated group. (E) Glibenclamide (0.5 mg/kg): Standard drug treated group shown histopathology similar to the normal control groups. The samples were obtained from the same liver anatomical regions (X40)
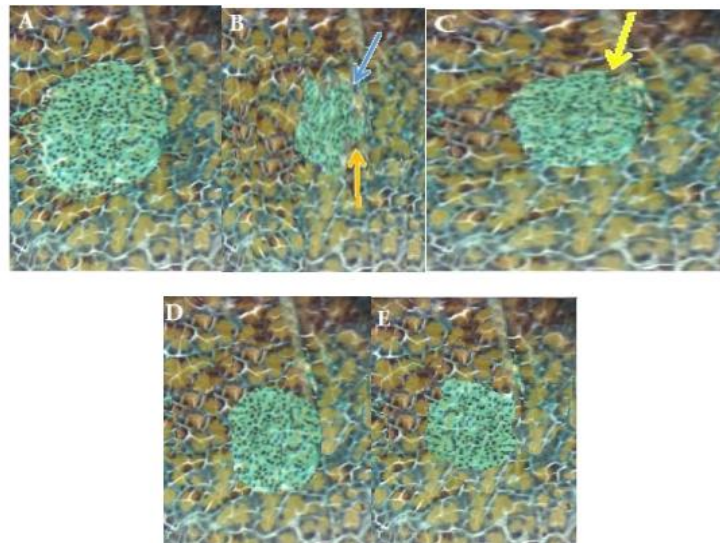

Plate 7. Photomicrographs of histological changes of rat pancreas of islets of Langerhans; (A) Normal control: Normal histological structure of rat pancreas showing normal islet (B) Diabetic control: Diabetic control rat showing dilated acini (blue arrow) and focal necrosis (yellow arrow) (C) OPI (200 mg/kg): Showing focal necrosis (D) OPI (600 mg/kg): Showing nearly restored islet of Langerhans (E) Glibenclamide (0.5 mg/kg)

4. DISCUSSION

Diabetes is the largest growing metabolic disorder in the world [26] and as knowledge about this disorder's heterogeneity is advanced, the need for more appropriate therapy is increasing [27,28].

According to American Diabetes ADA (2016), chronic diabetes-related hyperglycemia causes

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the presence of lesions in organs such as the kidneys, skin, nerves and blood vessels and glycemic control in order to prevent or postpone the development of these complications [29].

In this study, the continuous treatment of 200 mg/kg body weight and 600 mg/kg body weight Ogiri Protein Isolate (OPI) for a period of forty two days, showed a slight increase in blood glucose concentration without indication of hypoglycemic effect compared to synthetic glibenclamide drug which is similar to the report of [30]. The OPI could also possibly regulate blood glucose concentration by enhancing the sensitivity of insulin and by the regeneration of β-cells in the pancreas [31,32,33]. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus and unexpected weight loss, etc [34]. The results indicate that administration of OPI leads to weight gain in all the groups except in diabetic untreated animals. This agrees with the result of [35]. The OPI supplement exhibited no wasting or catabolism of muscle tissues and that the dietary protein was well utilized by the rats [36].

This study data shows that OPI significantly reduced the elevated fasting blood glucose level when compared to the diabetic control animals. 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 other extra-pancreatic mechanisms such as enhanced blood glucose transport to peripheral tissue and increased utilization of glucose via several enzymatic pathways or the inhibition of intestinal absorption of glucose, similar to earlier reported studies [37]. The diabetogenic effect of STZ and hypoglycemic properties of OPI were supported by the comparative histopathological studies of liver and pancreas tissues of diabetic control animals as well as OPI treated and standard drug treated animals. Diabetic rats showed reduced number of islet cells which were restored to near normal upon treatment with OPI as was found in the histology of the tissues of treated group animals and that substantiates the cytoprotective action of the OPI in diabetic condition. Similar protective effects were observed in the case of liver and pancreas tissues of the treated groups as well.

In the liver tissue of diabetic rats, OPI increased the total protein content to a similar extent as glibenclamide. Total protein loss in experimentally induced diabetic rats may be attributable to microproteinuria, a major systemic predictor of diabetic nephropathy, or increased protein catabolism [38]. In diabetic patients with vascular complications, significant changes in the metabolism of carbohydrates, lipids, and proteins such as increased lipid peroxidation, dyslipidemia have been recorded. Hyperglycemia is also associated with autoxidation of glucose, protein glycation, and the consequent oxidative degradation of glycated proteins leading to higher reactive oxygen species (ROS) output [39]. Upon supplementation with OPI, the increase in total protein content suggests that this protein isolate has the potential to correct hyperglycaemia related complications. This finding is confirmed by rat liver gel electrophoresis. The electrophoresis plates revealed low-dose OPI treated group liver with bands at 150kDa (Plate 4) that are not expressed in other study groups, supporting the outcome of total protein determination (Fig. 2) indicating that the administration of OPI at 200 mg / kg body weight may have triggered the expression of protein(s) that may have been inhibited at a higher dose. A differential expression of proteins was observed in the pancreas. 20-25 kDa proteins were expressed in rats administered with OPI 600 mg/kg body weight in contrast to other groups expressing bands between 25-30 kDa. This could be an expression of different proteins entirely or an expression of similar but modified proteins. Similar results have been observed [40] where Immunoblot analysis of cells expressing myc-tagged wild-type human eNOS confirmed the reciprocal increase in *O*-linked N-acetylglucosamine and decrease in *O*-linked serine 1177 phosphorylation in response to hyperglycemia. In contrast, when myc-tagged human eNOS carried a mutation at the Akt phosphorylation site (Ser1177), *O*-linked N-acetylglucosamine modification was unchanged by hyperglycemia and phospho-eNOS was undetectable [40].

5. CONCLUSION

The data of this research work showed that Ogiri Protein Isolate (OPI) of fermented *Cucumeropsis mannii* seeds have anti- diabetic effect in streptozotocin - induced diabetes in rats. Thus, OPI has a good potential for clinical applications, but further investigations are still on going on OPI mechanism of action. From this present investigation, it can be concluded that oral administration of Ogiri Protein isolate extracts possesses significant hypoglycemic effect in controlling the blood glucose level and OPI could

be a valuable candidate for developing new phytotherapeutics agent with antidiabetic activity.

ETHICAL APPROVAL

All the experimental procedure and protocols follow the approved protocols by the Institutional Animal Ethical Committee (IAEC) and monitored by the University ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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