Semi-modified okara whey diet increased insulin secretion in diabetic rats fed a basal or high fat diet

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Abstract Lifestyle and diet preferences are primarily responsible for developing type 2 diabetes. In this study, okara was manufactured into okara whey crackers (OWC) to investigate its dietary role in controlling diabetes in streptozotocin-diabetic rats with and without a high-fat diet. Forty-eight rats were divided into eight groups. G1–G4 were nondiabetic and fed a basal diet, a basal diet with 30% crackers, high fat diet, and a high-fat diet with 30% crackers, respectively. G5–G8 were diabetic groups that received similar diets as previous groups. Blood glucose, liver function, lipid pattern, pancreas and liver histopathology, and insulin immunohistochemistry were performed. OWC improved measured parameters and histopathology of the liver and pancreas in diabetic rats. The area % of positive insulin cells was increased in G6

(5.20%) and G8 rats (2.83%) fed OWC compared to diabetic rats (1.17%). In conclusion, the use of 30% OWC in a semi-modified diet has controlled the hyperglycemia and hyperlipidemia associated with diabetes.

Keywords Okara · Diabetes · Histopathology · Insulin · Fat diet · Fiber diet

Introduction

Type 2 diabetes affects many individuals worldwide. Individuals with uncontrolled diabetes and chronic hyperglycemia may develop complications like macrovascular disease, nephropathy, neuropathy, and retinopathy, in addition to being more vulnerable to infections (Wu et al., 2014). Diabetes damage is caused by the production of reactive oxygen species which causes oxidative damage to vital cellular structures as well as membranes (Giacco and Brownlee, 2010). A healthy diet and exercise can delay diabetic complications by keeping blood glucose levels and cholesterol within normal ranges (Hosokawa et al., 2016).

Dietary fiber is a term that is used for plant-based carbohydrates. Cereals, fruits, and vegetables are a rich source of dietary fiber (Ercisli et al., 2008; Senica et al., 2019; Zia-UI-Haq et al., 2014). New sources of dietary fibers (DF) are considered in designing ‘new food systems’. Agronomic by-products are now being evaluated as a potential source of dietary fiber and further investigations are still required to elucidate the functionality of DF (Rodríguez et al., 2006).

Okara, a by-product produced during the soymilk production process, represents a suitable dietary additive due to its high dietary fiber and protein. It has about 50% dietary fiber, 25% protein, 10% lipid, and other nutrients. It

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can be used in biscuits and snacks as a dietary supplement to prevent diabetes, hyperlipidemia, and obesity (Li et al., 2012).

Whey is produced from milk after casein precipitation and has a high biological value as it contains several groups of proteins such as lactoferrin, lactoperoxidase, cytokines, B-lactoglobulin, and lactoalbumin as well as a protein like insulin (Sukkar and Bounous, 2004). Each one has a specific structure and properties which act together to produce one or more specific effect as antioxidant proteins. They contain several amino acids such as valine, leucine, and isoleucine which are required for repair and growth of tissue. They are rich in cysteine with an antioxidant thiol group, glutamic acid, and glycine which form the glutathione reductase one of the major antioxidants in the cell (Bland et al., 2004). Whey also contains some antioxidant vitamins, high-quality protein fraction of good water holding and emulsifying qualities, and a peptide with anti-hypertension effects (O'Tool, 1999). Therefore the use of okara and whey can be considered as a therapeutic approach for oxidative stress-associated diseases such as diabetes.

The objective of this work was to investigate the role of using okara whey crackers as a semi-modified diet in the management of diabetes in experimental rats.

Materials and methods

Animals

Forty-eight adult male Wistar rats weighing between 150 and 180 g were raised in the animal house of Agricultural Research Center (ARC, Giza, Egypt). The animals were kept under normal laboratory conditions at a temperature of 25 ± 2 °C and humidity 50–60%. The animals were fed on a normal basal diet which consisted of casein 15%, corn oil 10%, cellulose 5%, salt mixture 4%, vitamin mixture 1%, and starch 65% or fatty diet which consisted of casein 15%, animal fat 30%, cellulose 5%, salt mixture 4%, vitamin mixture 1%, and starch 45%. Rats had free access to water. This study was performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC), Cairo University, and all institutional and national guidelines for the care and use of laboratory animals were followed.

Preparation of okara whey crackers

Okara was obtained from Soybean Factory, Food Technology Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Okara was dried in an oven at 40 ± 1 °C for 24 h and powdered using a lab

grinder. Then stored at -4 °C. Whey was obtained from Dairy Sciences Department, Faculty of Agriculture, Cairo University and was used freshly.

Chemical composition of dried okara and whey such as moisture, ash content, crude protein, crude fiber, total lipid, and total carbohydrate (by difference) were determined according to the methods of A.O.A.C. (2005). Casein, salts, and vitamins were purchased from Technogen Company (Giza, Egypt).

Our study showed that replacing 50% wheat flour with okara powder to make cracker biscuits (using whey instead of water) was appropriate and these okara and whey-containing foods had almost similar taste and quality to normal foods.

The procedure for the preparation of okara whey cracker was carried out according to Bose and Shams-Ud-Din (1970). The wheat flour, okara flour, and other ingredients were mixed. Whey was added to form a dough. The dough was rolled to a thickness of 3 mm. The crackers were cut with a round cutter of 5.5 cm diameter and baked at 200 °C for 10–15 min. Then they were cooled at ambient temperature and packed in high-density polyethylene bags. The basic formulations of cracker whey biscuits were 100 g flour, 6 g fat, 2 g salt, 4 g sugar, 2 g baking powder, and 30 g whey.

Experimental design

Forty-eight male rats were divided into eight groups (six rats/group). The 1st group (G1) rats were fed a basal diet. The 2nd group (G2) rats were fed on a 70% basal diet and 30% okara whey crackers. The 3rd group (G3) rats were fed on a fatty diet. The 4th group (G4) rats were fed on a 70% fatty diet and 30% okara whey crackers. The rats in diabetic groups (groups 5–8) were injected intraperitoneally with a single dose of Streptozotocin (STZ) (40 mg/kg body weight), dissolved in 0.01 M citrate buffer immediately before use (Coskun et al., 2005). The 5th group (G5) rats were fed on the basal diet and injected with STZ. The 6th group (G6) rats were fed on a 70% basal diet and 30% okara whey crackers and injected with STZ. The 7th group (G7) rats were fed on a fatty diet and injected with STZ. The 8th group (G8) rats were fed on a 70% fatty diet and 30% okara whey crackers and injected with STZ.

At the end of the experiment (60 days), rats were anesthetized by diethyl ether, and blood samples were withdrawn from the eye veins by fine capillary glass tubes. Serum was collected after centrifugation at 1000 $\times$ g and kept at -20 °C for further investigations.

Biochemical analysis

The blood glucose, liver function, and lipid pattern [total cholesterol, triacylglycerols, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)] were determined using commercial kits according to manufacturer protocol (Biodiagnostic Co., Egypt). The determination of HDL-cholesterol (HDL-c) was carried out according to the method of Lopez-Virella et al. (1977). Serum LDL-c-cholesterol (LDL-c) was calculated by difference according to Wallach (2007) using the following equation:

$$\text{LDL-c (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-cholesterol}$$

The serum total protein, albumin, alkaline phosphatase (AP), alanine transaminase (ALT), and aspartate transaminase (AST) activities were measured using commercial kits according to manufacturer protocol (Biodiagnostic Co.).

Histopathology and immunohistochemistry

Specimens of pancreas and liver were fixed in 10% neutral formalin buffer. The samples were processed by paraffin embedding technique, sectioned (3–4 μm thick), stained using routine hematoxylin and eosin stain, examined using a light microscope, and photographed using an Olympus XC30 camera (Tokyo, Japan).

Immunohistochemistry of insulin in paraffin-embedded tissue sections of the pancreas of all groups was performed using anti-insulin antibodies (Invitrogen, Thermo-Fisher Scientific, USA) and the avidin–biotin–peroxidase complex according to kit manufacturer protocol (Dako, North America, Inc., MI, USA). Color development was performed using 3,3'-Diaminobenzidine. The area % of beta cells in the pancreatic islet was measured using Image J in three photos/rat in each group at a 400 $\times$ magnification power.

Statistical analysis

Statistical analysis was carried out using statistical package SPSS, version 8.0 (SPSS Inc., Chicago, IL, USA). The data were analyzed using a one-way analysis of variance followed by the Duncan and Tamhane test. Results were expressed as a mean $\pm$ standard error. P values less than 0.05 were considered significant.

Results and discussion

Chemical composition of okara (% based on dry weight)

The proximate analysis of okara showed that the moisture content was found to be $4.37 \pm 0.31\%$. This result is nearly similar to those of Grizotto et al. (2010) who found it 6.51% in okara. The protein content of okara was $33.64 \pm 0.38\%$ which is slightly lower than that of Elref-faei et al. (2014) who found 40.0% protein and was nearly similar to that found by Préstamo et al. (2006) who found it 33.4%.

Okara contained crude fiber ($18.58 \pm 0.08\%$), fat (21.08%), ash ($4.67 \pm 0.09\%$), and carbohydrate ($22.03 \pm 0.72\%$). Total dietary fiber (TDF) of okara comprised $45.03 \pm 0.19\%$ of which $38.92 \pm 0.22\%$ is insoluble dietary fiber (IDF). Grizotto et al. (2010) reported comparable results to ours. On the other hand, whey contained protein ($0.82 \pm 0.03\%$), ash ($0.48 \pm 0.02\%$), fat ($0.5 \pm 0.02\%$), and carbohydrate as lactose ($4.79 \pm 0.11\%$) without any fiber fraction.

Okara was made into crackers because they are generally low in calories but still a good source of energy as well. They are also an ideal snack when being hungry. The percentage of okara whey crackers incorporation into the diet was selected based on the average DF consumption by humans. Healthy adults should consume about 20–35 g of DF each day in which cereals can constitute up to 50% of the daily fiber consumed (Li et al., 2012).

Hypoglycemic effect of okara whey crackers

The average values of the blood glucose level of rats in all eight groups at the beginning of the experiment were the same. After STZ had been injected in rats (Groups 5, 6, 7, and 8) the blood glucose level was raised to a maximum value of 407.67 mg/dl after 8 weeks in Group 7 diabetic rats receiving a high-fat diet. STZ-induced diabetes is one of the widely used animal models that mimic human diabetes mellitus. The selective destruction of insulin-producing β -cells of the pancreas by STZ is mediated by induction of high levels of DNA strand breaks in these cells, causing activation of poly (ADP-ribose) polymerase (PARP), resulting in a reduction of cellular NAD⁺, and cell death (Bolzan and Bianchi, 2002). The metabolism of glucose, proteins, and lipids is abnormal in diabetes due to insulin secretion defects, which leads to various metabolic disorders and complications (Wu et al., 2014).

Data in Table 1 show that the serum glucose in non-diabetic groups G1, G2, G3, and G4 are normal. But the glucose level of diabetic G6 rats receiving okara whey

Table 1 Serum glucose level (mg/dl) in male albino rats during the experimental period

Groups	Zero time (mg/dl)	60 day (mg/dl)
G1	92 ^c ± 3	99 ^b ± 11
G2	108 ^c ± 2	122 ^b ± 4.36
G3	114.33 ^c ± 1.53	125.67 ^b ± 10.69
G4	101 ^c ± 2	111.67 ^b ± 13.32
G5	380.33 ^a ± 70.22	379.33 ^a ± 92.79
G6	251.67 ^b ± 36.17	195.33 ^b ± 34.02
G7	396.67 ^a ± 94.52	407.67 ^a ± 110.59
G8	370.33 ^a ± 66.03	340 ^a ± 13.89

Means of each raw followed by the same letter are not significantly different at the 5% level

crackers was significantly lower compared with diabetic G5 rats. Also, the glucose level of diabetic G8 rats receiving a high-fat diet and okara whey crackers was significantly lower compared with the diabetic G7 rats receiving a high-fat diet. Therefore, okara whey crackers have decreased the serum glucose level in diabetic rats. These findings were comparable to those of Hosokawa et al. (2016). Likewise, diabetic rats fed diets supplemented with 10% okara caused a significant reduction in fasting serum glucose level from (136.75 mg/dl) compared with (260.75 mg/dl) positive control group (Ahmed et al., 2010). DF, isoflavones, and β -conglycinin found in okara are the main bioactive components of the okara. Okara retains about 12–30% soybean isoflavones with antioxidant capabilities after soy milk processing which could counteract the oxidative stress present with diabetes. Also, the DF in okara foods probably increases the indigestible carbohydrate portion in small and large intestines with the subsequent reduction in the rate of dietary carbohydrate absorption (Lu et al., 2013). Recently, Hosokawa et al. (2016) showed that okara can improve glucose tolerance. The mRNA expression levels of PPAR γ , adiponectin, and

GLUT4, which up-regulate the effects of insulin, were increased in epididymal adipose tissue by the okara diet.

Lipid profile

G1 and G2 rats had almost similar levels of cholesterol, HDL-c, and LDL-c. The cholesterol, triglycerides, and LDL-c significantly increased and HDL-c was decreased in G3 more than G4 rats (Table 2). The nature of the diet had a great effect on the lipid profile. It was found that increased intake of total saturated fatty acids would result in increased serum levels of larger LDL-c particles (Dreon et al., 1998).

The G5 and G7 rats had elevated cholesterol, triglycerides, and LDL-c and a decreased HDL-c. Type 2 diabetes mellitus is usually associated with changes in the circulating lipids, including the increase in triglycerides and LDL-c as well as the decrease in HDL-c (Farbstein and Levy, 2012). G6 and G8 rats had significantly lower cholesterol and LDL-c compared to G5 and G7, respectively (Table 2). The level of triglycerides and cholesterol levels are improved with the consumption of high fiber diet in diabetic patients (Chen et al., 2016). The exact mechanism of how the DF lowers the total cholesterol level is not yet clear, however, it was suggested that soluble fiber may hinder the lipid and/or bile acid metabolism (Theuwissen and Mensink, 2008).

Liver function parameters

Total protein and albumin were decreased in G3 rats compared to G1, G2, and G4 rats (Table 3). An increased fat diet may result in fatty liver syndrome (Guo et al., 2018). Therefore the decreased albumin level in G3 could be attributed to decreased synthesis of albumin in the liver. In G5 rats, the total protein and albumin were significantly lower compared to G1 rats. The hyperglycemia results in endothelial dysfunction with subsequent increase in the endothelial permeability and increased permeability to albumin which explains the albumin decrease in diabetic

Table 2 Effect of feeding different experimental diets on cholesterol, triglycerides, HDL-c and LDL-c of the experimental male albino rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
G1	82.93 ^d ± 1.50	78.52 ^f ± 2.05	47.50 ^a ± 2.60	19.73 ^c ± 2.32
G2	82.40 ^d ± 2.95	86.46 ^e ± 2.77	45.01 ^a ± 2.63	20.10 ^c ± 5.03
G3	120.13 ^a ± 1.60	126.38 ^b ± 4.16	31.83 ^{bc} ± 2.57	63.02 ^{ab} ± 3.30
G4	107.40 ^{bc} ± 6.20	117.06 ^c ± 4.05	33.20 ^{bc} ± 1.3	50.86 ^c ± 5.73
G5	100.51 ^c ± 7.41	99.17 ^d ± 2.65	34.01 ^{bc} ± 2.33	46.60 ^c ± 9.36
G6	87.54 ^d ± 4.50	97.96 ^d ± 2.33	35.48 ^b ± 1.90	32.47 ^d ± 5.38
G7	124.62 ^a ± 2.44	132.48 ^a ± 1.83	26.98 ^d ± 1.83	71.15 ^a ± 3.90
G8	110.32 ^b ± 6.16	129.04 ^{ab} ± 4.77	29.77 ^{cd} ± 3.57	54.77 ^{bc} ± 8.43

Means of each raw followed by the same letter are not significantly different at the 5% level

rats (Scalia et al., 2007). Other factors may contribute to serum albumin level decline in diabetic patients such as an altered liver function or protein-losing enteropathy or nephrotic syndrome. Insulin deficiency was found to decrease liver albumin synthesis (Feo et al., 1991). In our study, G6 rats had high albumin compared to G5 due to feeding a 30% okara whey diet. High dietary fiber and protein can control hyperglycemia in diabetic patients which in turn would lower the complications associated with hyperglycemia with a subsequent improvement of endothelial permeability, liver, and kidney function. On the other hand, a high-fat diet has aggravated the albumin concentration in rats of G7. The feeding of a 30% okara whey diet to G8 rats resulted in a significant increase in albumin and total protein compared to G7 (Table 3). A high-fat diet harms liver function which together with hyperglycemia would result in low serum albumin. On the reverse, the use of high DF improved the albumin concentration accentuating the role of fiber in lowering the blood glucose level since it was suggested that increased and regular consumption of soluble DF improves blood glucose levels significantly (Chen et al., 2016).

Any injury to hepatocytes will result in an elevated ALT, AST, and AP as a result of increased cell membrane permeability which causes excessive leakage of these transaminases. The AP, AST, and ALT activity were found to be significantly higher in G3 and G4 compared to G1 followed by a significant increase in G5 compared to G1, G2, G3, and G4. Hyperglycemia, as evidenced in previous studies, can result in deterioration of liver and kidney functions which explains the increase in the activity of these enzymes in G5 (Bai et al., 2013). In the present study, the activity of transaminases significantly decreased in G6 compared to G5 indicating that high fiber diet and high protein content have improved the liver function (Cantero et al., 2017). The highest activity of transaminases was recorded in G7 having a high-fat diet which might be due to the development of hepatic steatosis as observed in histopathology. Hepatic steatosis is associated with obesity, diabetes, and metabolic syndrome (Guo et al., 2018). The

addition of 30% okara whey diet to the high-fat diet in G8 resulted in a significant decrease in the activity of these enzymes compared to G7 which further emphasizes the role of lifestyle interventions in the management of non-alcoholic fatty liver disease (Thoma et al., 2012).

Histopathological findings

The microscopy of the pancreas of G1 and G2 rats showed normal histology with moderate-sized islets of Langerhans (Fig. 1A). The pancreatic islets of G3 rats were also of normal histology but were large-sized (Fig. 1B). Moderately sized islets were observed in the pancreas of G4 rats. The chronic administration of a high-fat diet leads to a significant increase in islet areas as observed in a previous study which is similar to our observation in G3 (Ickin et al., 2015). The microscopy of the pancreas of G5 rats revealed vacuolation of islet cells, small-sized atrophied hypocellular islets of Langerhans (Fig. 1C) which is similar to previous studies indicating that the number of pancreatic islets and the number of β -cells in these islets are reduced besides being relatively small, atrophic with irregular shape in STZ treated rats (Yazdanparast et al., 2005). On the reverse to G5, the rats in G6 fed a 30% okara whey diet showed a remarkable improvement in the pancreas histology in which there was only slight vacuolation of islet cells (Fig. 1D). It was demonstrated before that some plant extract might have a regenerative effect on pancreatic cells (Shanmugasundaram et al., 1990). Although it is not the case in our study, however, it could be suggested that the okara whey diet might have regenerative potential. The pancreas histology of G7 rats revealed vacuolation, hypocellular, atrophied islets of Langerhans which was more prominent compared to G5 rats (Fig. 1E). On the other side, the pancreas histology of G8 rats showed only slight vacuolation of islet cells (Fig. 1F). The positive effect in this group might be attributed to the high level of fiber in okara which would hinder the absorption of carbohydrate or reduce the macronutrient absorption (Cruz-Requena et al., 2016).

Table 3 Effect of feeding different diets on total protein, albumin, the serum activity alkaline phosphatase, AST, and ALT of male albino rats

Groups	T. Protein (g/dl)	Albumin (g/dl)	GOT/AST (IU/L)	GPT/ALT (IU/L)
G1	6.74 ^a ± 0.40	4.13 ^{ab} ± 0.15	38.2 ^f ± 2.52	20.43 ^c ± 0.71
G2	6.90 ^a ± 0.42	4.39 ^a ± 0.42	38.73 ^f ± 1.75	19.03 ^c ± 1.34
G3	4.88 ^b ± 0.13	3.65 ^c ± 0.35	76.85 ^{cd} ± 2.90	36.53 ^c ± 0.57
G4	5.20 ^b ± 0.26	3.81 ^{bc} ± 0.19	74.68 ^d ± 2.82	34.55 ^{cd} ± 1.26
G5	5.11 ^b ± 0.23	2.92 ^{ef} ± 0.16	82.85 ^b ± 3.09	41.84 ^{ab} ± 1.33
G6	5.32 ^b ± 0.42	3.50 ^{cd} ± 0.28	50.07 ^d ± 1.56	33.16 ^d ± 2.34
G7	4.20 ^c ± 0.21	2.69 ^f ± 0.10	88.08 ^a ± 1.33	44.48 ^a ± 1.53
G8	4.94 ^b ± 0.09	3.16 ^d ± 0.13	79.75 ^{bc} ± 2.01	39.54 ^b ± 2.42

Means of each row followed by the same letter are not significantly different at the 5% level

Fig. 1 Pancreas, Rat. (A) Normal histology of islet of Langerhan in the control group receiving a basal diet (G1). (B) A hyperplastic islet of Langerhan in the group receiving a high-fat diet (G3). (C) A small sized islet of Langerhans with few vacuolated cells in the diabetic group (G5). (D) A normal islet of Langerhans in the diabetic group

receiving basal diet and 30% okara whey diet. (E) Hypocellular small-sized islet of Langerhans in the diabetic group receiving a high-fat diet. (F) A normal medium-sized islet of Langerhan in the diabetic group receiving a high-fat diet and 30% okara whey diet (H and E X 200)

The liver microscopy of G1, G2, and G4 rats showed normal histology (Figs. 2A, B). The liver of rats in G5 had sinusoidal dilatation with leukocytosis, Karyopyknosis, and atrophied hepatocytes (Fig. 2C). The liver of G6 rats exhibited normal histology (Fig. 2D). The liver histology of G7 rats revealed a remarkable degeneration in hepatocytes in which cells had micro and macrovesicular steatosis

on the reverse of G8 (Figs. 2E, F). It was proposed that hepatic fatty acids will be stored in the liver as triglycerides when it exceeds the capacity of the liver to remove it. Therefore, consuming a high-fat diet would result in greater delivery of dietary fat to the liver compared with a low-fat diet (Parry and Hodson, 2017). The gel-forming properties and viscosity of soluble fiber as that of okara are

Fig. 2 Liver, Rat. (A) Normal liver histology in the control group receiving a basal diet and (B) in the group receiving a high-fat diet. (C) Sinusoidal dilation with leukocytosis, Karyopyknosis in hepatocytes, and few centrilobular necrotic cells in the diabetic group. (D) Normal liver histology in the diabetic group receiving basal diet

and 30% okara whey diet. (E) Vacuolar degeneration of hepatocytes in the diabetic group receiving a high-fat diet. (F) Normal liver histology in the diabetic group receiving a high-fat diet and 30% okara whey diet (H and E X 200)

involved in the reduction of macronutrients absorption, slow gastric emptiness, reduction of postprandial glucose response and total cholesterol and LDL-c with a subsequent decrease in dietary fat delivery to liver and improvement of liver steatosis (Cruz-Requena et al., 2016).

Immunohistochemical findings of insulin staining

In the control groups (G1, 2, 3, and 4) which received different kinds of diets, the pancreas demonstrated brown insulin-positive beta cells which were arranged in a continuous cord-like manner in the islets of Langerhans whereas other types of cells didn't stain brown (Fig. 3A).

Fig. 3 Pancreas, Rat. (A) Brown insulin-positive Beta cells which are arranged in continuous cords in the islet of Langerhans in the control group (G1). (B) A small-sized islet of Langerhans with few light Brown Beta cells in the diabetic group (G5). (C) Increased number of Beta cells in the islet of Langerhans in the diabetic group receiving basal diet and 30% okara whey diet (G6). Note the presence of positive cells at the top left corner. (D) Few Beta cells in the islet of

Langerhans in the diabetic group receiving a high-fat diet (G7). (E) A large-sized islet of Langerhan with a high number of beta cells in the diabetic group receiving a high-fat and 30% okara whey diet (G8). (IP stain for insulin X 200). (F) The area percent of positive brown beta cells in the pancreas in the control group (G1) and in diabetic groups (G5, G6, G7, and G8)

The area percent of insulin-positive beta cells were significantly higher in these groups (14.96 ± 2.93) in comparison to the diabetic groups. In G5, the number of beta cells was remarkably decreased compared to control groups. Besides the area percent of these cells were

decreased (1.17 ± 0.402) (Fig. 3B). These results are comparable to a previous study which showed that STZ induced diabetic rats had islet cells degeneration and weak insulin immunohistochemistry (Coskun et al., 2005). On the other hand, the number of beta cells was increased in

the islet of Langerhans with the moderate restoration of its arrangement in G6 (Fig. 3C) and a significant increase in area percent of insulin stained beta cells (5.20 ± 1.37). This could be due to the stimulation of β cells to produce more insulin (Khan et al., 1990) or due to the presence of beneficial nutrients in okara that led to the regeneration of beta cells (Shanmugasundaram et al., 1990; Yazdanparast et al., 2005). Furthermore, there were solitary cells that stained positive for insulin outside the islet around the ductal epithelium in G6. The presence of cells in the pancreas which can regenerate and replace the lost cells under certain circumstances have been reported and were termed endogenous stem cells (Jiang and Morahan, 2014). In G7, there was a remarkable decrease in area percent of brown beta cells (2.24 ± 0.44) which was similar to the diabetic group (Fig. 3D). There was a slight increase in the number of beta cells and their area percent in G8 (2.83 ± 0.67) (Fig. 3E). The insulin stained beta cells were significantly higher in G6 compared to G5 and G7 (Fig. 3F).

In general, diabetes results in cell membrane damage, alteration of lipid profile, and different serum biochemical parameters. This adverse effect was ameliorated by feeding an okara whey diet which reversed hyperglycemia, hyperlipidemia, and hypoproteinemia in addition to the alleviation of histopathological lesions of liver and pancreas.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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