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Efficacy of β-glucan extracted from Pediococcus parvulus F1030 in an acute model of diabetes: hindrance of oxidative stress and atherogenic index of pancreatic cell degradation

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Abstract

Many health problems are associated with diabetes. Beta-glucan has been reported to be associated with many health-promoting effects, as it can improve the glycemic index of meals, antioxidant, cholesterol-lowering effect, antibacterial, and control of cancer. This paper aimed to investigate the prophylaxis ability of natural products, β-glucan (βG) Streptozotocin (STZ)-induced diabetic rats. The comparative effectiveness of bacterial and botanical sources of β-glucan on physiological, genetic, and histological responses in diabetic rats was determined. The forty-two Westar rats in the experiment were divided into 7 groups' six rats for each. The 1st served as a control group. The 2nd served as a diabetic group, induced by a single dose of streptozotocin (STZ) (55 mg/kg b.w i.p). The 3rd (Diabetic + Met) treated with M Metformin 90 mg/kg b.w. The 4th (Diabetic + Baβ-GluL) was treated with a low dose of β-glucan from the bacterial source (100 mg/kg b.w.). The 5th (Diabetic + Baβ-GluH) was treated with a high dose of β-glucan from the bacterial source (500 mg/kg b.w.). The 6th (Diabetic + Boβ-GluL) was treated with a low dose of β-glucan from a botanical source (100 mg/kg b.w.). The 7th (Diabetic + Boβ-GluH) was treated with a high dose of β-glucan from the botanical source (500 mg/kg b.w.). Results showed significant pathological alteration for oxide-nitrosative stress markers, lipid profile, neural and hormonal stress parameters, DNA fragmentation, and histopathological alteration in the diabetic group. In contrast, treated groups with a high dose of the β-glucan from a bacterial source or botanical source ameliorated most pathological changes. Nevertheless, a low dose of β-glucan in the bacterial source showed mild amelioration but the low dose of botanical source didn't show any significant amelioration. Data revealed that β-glucan in afforded diabetic rats with prophylactic ability to decrease pathological alteration inflicted by STZ. On the other hand, β-glucan prepared from a bacterial source showed a high affinity for treated diabetic rats from most common symptoms in comparison with a botanical source.
Keywords: β-glucan; Pediococcus parvulus F1030; Diabetes; Streptozotocin; DNA Pancreas; Rats.

1. Introduction

The term diabetes includes many disorders in the process of building and destruction of carbohydrates. Diabetes is the name specified to a range of various circumstances in which there is too much glucose in the blood. Or that the pancreas cannot produce the insulin or insulin its products is not enough and cannot function accurately. Insulin without doing its work, blood glucose accumulates, leading to elevated levels of glucose in the blood that cause health problems associated with diabetes [1]. The body wants a special sugar termed glucose as its principal source of fuel and energy. The body produces glucose from foods that contain carbohydrates. Glucose is transferred throughout the body by the blood. The level should not be too high or too low. When glucose rises above a certain level, some of it must come out of the blood into the tissues of the body to provide the energy you needed to keep the body working properly **[2]**. *Streptozotocin* (STZ) is a toxic chemical for beta cells in the mammary pancreas. This substance is used in medicine to treat certain types of cancers that affect the Langerhans islands (such as island cell tumor), in medical research to produce an animal model of hyperglycemia when used in high doses, as well as for the production of type I diabetes by a few frequent

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doses **[3]**. In type 1 diabetes, symptoms are often abrupt, life-threatening, and therefore usually diagnosed rapidly. In type 2 diabetes, numerous people have no symptoms at all, while other signs are not observed because they are seen as part of the 'big'. Therefore, while the symptoms are observed, diabetes obstacles may already exist. Common symptoms include feeling thirsty, frequent urination, hunger, slow itching, skin irritation, weight loss, headaches, mood swings, and dizzy **[4]**. *β-glucan* is one of the soluble dietary fiber forms of sugars found in cell walls of bacteria, fungi, yeasts, algae, and plants such as oats, barley, mushroom, and wheat. *β-glucan* is a soluble fiber, it slows down food passage in the intestines **[5]**. This means that it makes the body take longer times to digest food. Slow digestion means that the body does not absorb sugar quickly, which reduces the likelihood of high blood sugar and keeps blood sugar levels stable. When *β-glucan* dissolves in the digestive system create a thick gel material, this gel is associated with an excess of cholesterol and thus helps the body to prevent absorption**.** There is strong evidence that can promote heart health. The food and drug administration organization has agreed that foods containing high levels of *β*-glucan are beneficial for heart health [6]. Many studies have described the association between the molecular structure of β-glucan and its functionality [7]. Chu [8] reported that the biological activities of β-glucans are differed according to shape structure and molecular weight. β-glucan extracted from bacteria and cereal plants are different in structure, shape, and molecular weight and hence in function. The structure of β-glucan extracted from Barley is Linear chains of β-d-glucopyranosyl units linked via (1 → 3) and (1 → 4) linkages [9]. Figure 1 represents backbone structure of β-glucan extracted from cereal [8]. de Palencia et al.[10] investigated (1,3)-β-d-glucan-producing bacterium *Pediococcus parvulus* 2.6 fights gastrointestinal stress, adheres to Caco-2 cells and prompts making of inflammation-related cytokines by separated macrophages.

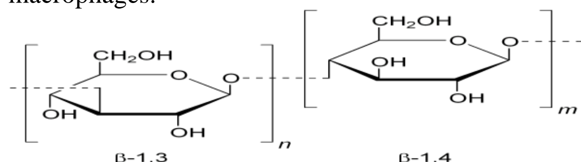

Figure 1 Backbone structure of β-glucan extracted from cereal [11].

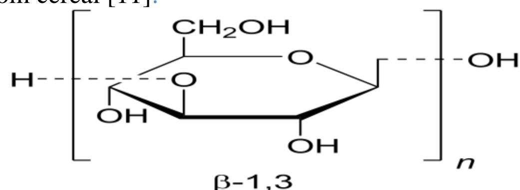

Figure 2 Backbone structure of β-glucan extracted from bacteria [8].

Pediococcus is lactic acid bacteria that produce pediocin that antimicrobial peptide against the foodborne pathogen *L. monocytogenes* [12]. *P. parvulus* 2.6 produces an exopolysaccharide of b-1,3–linked glucan with b-1,2 branching **[13]**, that lowers cholesterol in human volunteer test subjects **[14]**. Several health-enhancing effects, when consumption of foods containing *P. parvulus* 2.6 due to produce fiber in the colon, and also has inhibitory activity against various types of gram-positive bacteria [15]. *Pediococcus parvulus* DSM 28875 is used in the production of silage that is assumed safe for consumers of products from animals fed treated silage [16].

The present study aims to evaluate the beneficial role of *β*-glucan in minimizing diabetic complications and compare the bacterial and plant sources of *β*-glucan and its doses in minimizing diabetic and prophylactic effects.

2. MATERIAL AND METHODS

Bacteria

Pediococcus parvulus F1030 was isolated from Boza (a drink made by fermenting various grains such as barley in Egypt) and identified according to previous researches [17,18]

β- Glucan:

β- Glucan is extracted from *Pediococcus parvulus F1030* and identified according to Abd El Ghany et al [17].

β- Glucan standard

β-D-Glucan extracted from barley was purchased from Sigma Aldrich

Animals

Adult male Sprague Dawley rats, weighing 150-170 g each, were brought from the animal house at the National organization for drug control and research (NODCAR, Giza, Egypt). All animal handling procedures, sample collection, and disposal were according to the regulation of Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University **of** Sadat City, Egypt, under approval number VUSC-001-3-16.

Animals were kept in normal condition and kept for one week for adaptation before the experiment. They were fed a standard diet; water was provided ad libitum and free access to water.

Experimental design

Rats were divided into 7 groups (6 rats each) and treated as follows; the 1st (**Control**) served as a control group. The 2nd (Diabetic) served as a diabetic group, induced by a single dose of streptozotocin (STZ) (55 mg/kg b.w i.p) (i. p.) dissolved in 0.01M citrate buffer anhydrous (pH 4.5). The 3rd (Diabetic + Met) treated with *Metformin* 90 mg/kg b.w. The 4th (Diabetic + Baβ-GluL) was treated with a low dose of *β-glucan* extracted from *Pediococcus parvulus F1030* (bacterial source) as (100 mg/kg b.w.). The 5th

(Diabetic + Baβ-GluH) was treated with a high dose of *β-glucan* from the *Pediococcus parvulus F1030* (bacterial source) (500 mg/kg b.w.). The 6th (Diabetic + Boβ-GluL) was treated with a low dose of *β-glucan* from the botanical source (barely *beta-glucan*) brought from sigma Aldrich (100 mg/kg b.w.). The 7th (Diabetic + Boβ-GluH) was treated with a high dose of *β-glucan* from the botanical source (500 mg/kg b.w.). The experiment lasted for 60 days, meanwhile, *Metformin*, *β-glucan* from bacterial/botanical sources (low/ high dose) were started 30 days before and 30 days after induction of diabetes with the single dose of STZ (30th day). Diabetes was identified by measuring blood glucose concentration 72 hours after STZ injection.

Blood and tissues samples collection

Animals were sacrificed after 33 days of treatments, blood samples were gathered from the retro-orbital plexus. Blood was collected and permits clotting to isolated serum. The serum was used for the determination of glucose, MDA, GSH, GSSG, TAC, T.C, T.G, HDL, LDL, AIP, Hb, Insulin, and HOMA-IR.

Tissues samples collection

Pancreases samples were removed at the time of sacrifice from 6 rats from each group. The pancreases were directly excised, preserved in 10% neutral buffered formalin until processing for histopathological examination

Body weight and biochemical analysis

Body weight was documented individually for animals at the end of the experiment and calculated with mean.

Biochemical parameters

The biochemical parameters carried out in this study were summarized in Table (1).

DNA Comet Assay

Comet assay of DNA was assessed according to the classic alkaline single-cell electrophoresis protocol [31]**.** Samples were stained with ethidium bromide (Sigma, Germany) and analyzed by Comet Score 1.5 software. Percent of DNA in comet tails was considered as the marker of genotoxic effect.

Table 1: Methods and kits used to quantify the different biochemical analyses of blood and liver homogenate

Parameters	Method	Company	Reference
MDA (nmol/g tissue)	HPLC	Standard of 1, 1, 3, 3 tetraethoxypropane (Sigma)	[19]
GSH & GSSG (μmol/g tissue)	HPLC	Standard of 1, 1, 3, 3 tetraethoxypropane (Sigma)	[20]
TAC (nmol H2O2 equivalent / mg protein)	Colorimetric	Chemical reaction	[21]
Cholesterol (mg/dl)	Colorimetric	Stanbio Cholesterol LiquiColor® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA.	[22]
Triglycerides (mg/dl)	Colorimetric	Stanbio Cholesterol LiquiColor® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA.	[23]
HDL. Chol (mg/dl)	Colorimetric	Stanbio Cholesterol LiquiColor® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA.	[24]
LDL. Chol (mg/dl)	Calculated	= TC-(TG/5)-HDL	[25]
AIP	Calculated	AIP = log [triglyceride (mg/dl)/HDL-C (mg/dl)].	[26]
Hb	Colorimetric	Stanbio Cholesterol LiquiColor® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA.	[27]
Glucose	Colorimetric	Stanbio Cholesterol LiquiColor® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA.	[28]
Insulin	ELISA	Meso Scale Discovery A division of Meso Scale Diagnostics, LLC. 9238 Gaither Road Gaithersburg, MD 20877 USA	[29]
HOMA-IR	Calculated	= fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5.	[30]

Histopathological examination

Samples were acquired from pancreatic of rats in diverse groups and fixed in 10% neutral buffered formalin for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56^0C in the hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sled microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stains [32], for histopathological examination through the electric light microscope.

Statistical analysis: The values were expressed as the mean ± SE for the 6 rats in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using SAS. Statistical analysis of the obtained data was performed using the general linear model (GLM). Significant differences among means were evaluated using Duncan's Multiple Range Test.

3. RESULTS

In the present study, *β-glucan* isolated from the bacterial and botanical source has proven to be a valuable compound in the development of novel drugs for diabetic patient. As shown in Table (2) and Fig (3) STZ markedly decreases final b.w and BWG compared with the control group. In contrast, *β-glucan* at the high dose of bacterial and botanical sources showed a marked increase of b.w compared with a diabetic group. *Metformin* and the low dose of *β-glucan* of bacterial source showed mild amelioration of body weight and BWG but markedly ameliorate treated rats in comparison with a diabetic group. The low dose of *β-glucan* showed insignificant amelioration for bodyweight parameters compared to the diabetic group.

Table 2: Hypoglycemic effect of *β-glucan* at different levels in B.W and Glucose of STZ induced diabetic rats in comparison with *Metformin*.

Groups	Parameters		
	Initial B.W / g	**Final B.W / g**	**BWG /g**
Control	158.5 ± 4.32	317 ± 8.92	158.5 ± 4.41
Diabetic	166.1 ± 4.42	182.7 ± 5.14a	16.6 ± 0.46a
Diabetic + Met	155.9 ± 4.27	202.7 ± 5.69ab	46.8 ± 1.25ab
Diabetic + Baβ-GluL	161.2 ± 4.3	209.6 ± 5.87ab	48.4 ± 1.3ab
Diabetic + Baβ-GluH	158 ± 4.26	284.3 ± 8.06ac	126.4 ± 3.47abc
Diabetic + Boβ-GluL	161 ± 4.57	209.4 ± 5.56a	48.4 ± 1.36ab
Diabetic + Boβ-GluH	150 ± 4.12	225.1 ± 6.17abc	75.1 ± 1.97abc

Data are expressed as Mean ± S.E.M for 6 rats /group,
a significant difference from **the control** group,b significant difference from **Diabetic,**c significant difference from **Diabetic + Met** at the same column with one-way ANOVA at P < 0.05.

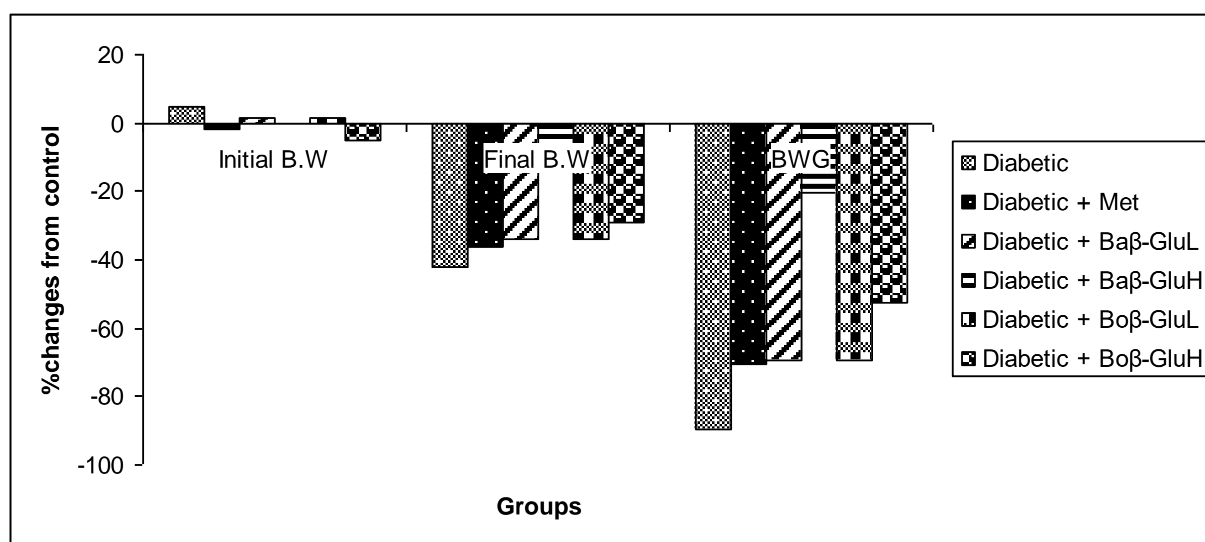

Fig. 3: Percentage changes from the control initial body weight, final body weight, and bodyweight gain for diabetic rats treated with Met, *β-glucan* at different levels (Low/high) from different compared with the control group.

Table 3: Hypoglycemic effect of *β-glucan* at different levels in MDA, GSH, GSSG, and TAC of STZ induced diabetic rats compared with *Metformin*.

Groups	Parameters			
	MDA nmol/l	**GSH µmol/l**	**GGSG µmol/l**	**TAC /l**
Control	74.2 ± 2.04	14.4 ± 0.381	0.477 ± 0.013	28.8 ± 0.823
Diabetic	265.71 ± 7.29a	5.25 ± 0.139a	1.71 ± 0.049ab	10.51 ± 0.283ab
Diabetic + Met	200.59 ± 5.7ab	9.23 ± 0.261ab	1.29 ± 0.035ab	18.46 ± 0.523ab
Diabetic + Baβ-GluLB	224.61 ± 6.2abc	7.37 ± 0.205abc	1.44 ± 0.038ab	14.74 ± 0.396abc
Diabetic + Baβ-GluH	124.13 ± 3.45abc	12.9 ± 0.345abc	0.8 ± 0.022abc	25.79 ± 0.696abc
Diabetic + Boβ-GluL	255.2 ± 6.99abc	6.22 ± 0.167abc	1.64 ± 0.047abc	12.45 ± 0.336bc
Diabetic + Boβ-GluH	153.27 ± 4.09abc	9.84 ± 0.268ab	0.99 ± 0.027abc	19.69 ± 0.519ab

Data are expressed as Mean ± S.E.M for 6 rats /group,
a significant difference from **the control** group, b significant difference from **Diabetic**, c significant difference from **Diabetic + Met** at the same column with one-way ANOVA at P < 0.05.

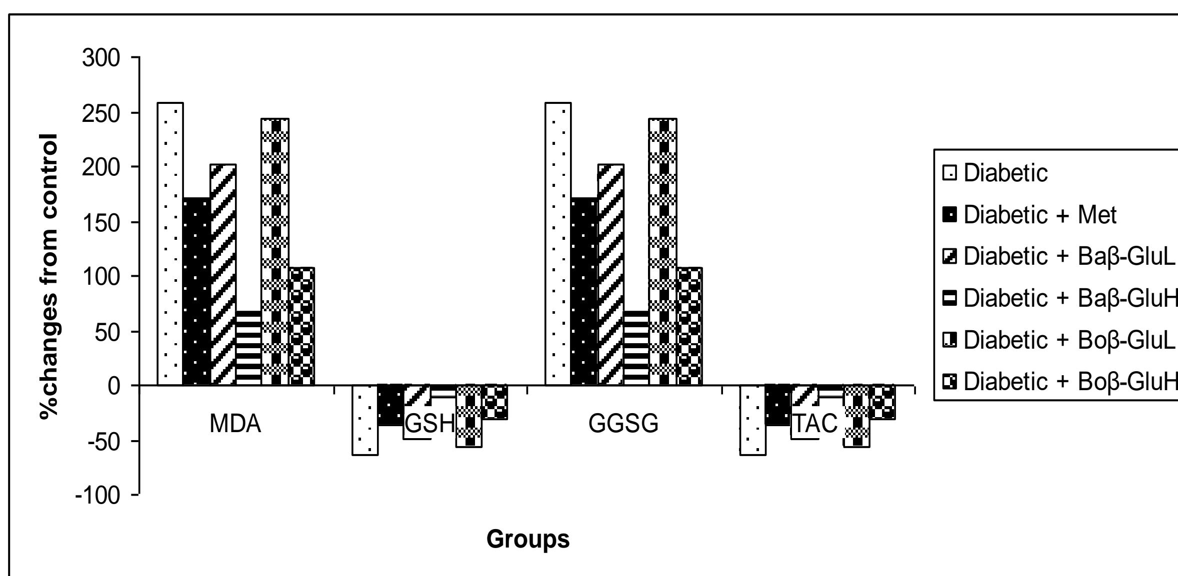

Fig. 4. Percentage changes in the control of oxidative stress markers for diabetic rats treated with Met, *β-glucan* at different levels (Low/high) from different sources compared with the control group.

As shown in Table (3) and Fig (4) *Streptozotocin* STZ markedly accelerate oxidative stress markers such as increasing *malondialdehyde* MDA, *glutathione disulfide* GSSG, and decreasing *glutathione* GSH ad total antioxidant capacity TAC compared with the control group. In contrast, *β-glucan* at the high dose for bacterial and botanical sources showed a markedly decrease of MDA, GSSG, and increase GSH and TAC compared with a diabetic group. Meanwhile, *Metformin* and the low dose of *β-glucan* of bacterial source showed mild amelioration of oxidative stress markers but the low dose of BoβG showed insignificant amelioration for oxidative stress parameters compared to the diabetic group.

As shown in Table (4) and Fig (5) STZ markedly increases lipid profile such as increasing total cholesterol T.C, triglyceride T.G, low-density lipoprotein LDL, very low-density lipoprotein VLDL, *alkaline phosphates* e AIP and decreasing HDL compared with the control group. In contrast, *β-glucan* at the high dose for bacterial and botanical sources showed markedly amelioration of lipid profile compared with a diabetic group. Meanwhile, *Metformin* and the low dose of *β-glucan* of bacterial source showed mild amelioration of lipid profile but the low dose of BoβG showed insignificant amelioration for lipid profile parameters compared to the diabetic group.

Table 4: Hypoglycemic effect of *β-glucan* at different levels in T.C, T.G, HDL, LDL, and AIP of STZ induced diabetic rats compared with *Metformin*.

Groups	Parameters					
	T.C mg/dl	T.G mg/dl	HDL mg/dl	LDL mg/dl	VLDL	AIP
Control	92.9 ± 2.57	72.4 ± 2.02	41.4 ± 1.13	37.02 ± 1.03	14.48 ± 0.39	0.243 ± 0.007ab
Diabetic	220 ± 6.24ab	188 ± 5.18ab	15 ± 0.42ab	167.4 ± 4.77ab	37.6 ± 1.05ab	1.098 ± 0.031ab
Diabetic + Met	160.87 ± 4.49ab	137.31 ± 3.89ab	25.33 ± 0.69ab	108.07 ± 2.9ab	27.46 ± 0.78ab	0.734 ± 0.021ab
Diabetic + Baβ-GluLB	182.17 ± 5.06abc	158.71 ± 4.44abc	21.27 ± 0.6ab	129.15 ± 3.43abc	31.74 ± 0.85ab	0.873 ± 0.024ab
Diabetic + Baβ-GluH	92.46 ± 2.63abc	80.58 ± 2.25abc	36.14 ± 1.01abc	40.2 ± 1.1abc	16.12 ± 0.44abc	0.348 ± 0.009abc
Diabetic + Boβ-GluL	195.09 ± 5.31abc	178.37 ± 4.73abc	18.38 ± 0.51abc	141.04 ± 3.95abc	35.67 ± 0.98abc	0.987 ± 0.003ab
Diabetic + Boβ-GluH	132.52 ± 3.6abc	116.5 ± 3.31abc	27.47 ± 0.74ab	81.75 ± 2.29abc	23.3 ± 0.64abc	0.627 ± 0.017ab

Data are expressed as Mean ± S.E.M for 6 rats /group,
a significant difference from **the control** group,b significant difference from **Diabetic**, c significant difference from **Diabetic + Met** at the same column with one-way ANOVA at P < 0.05.

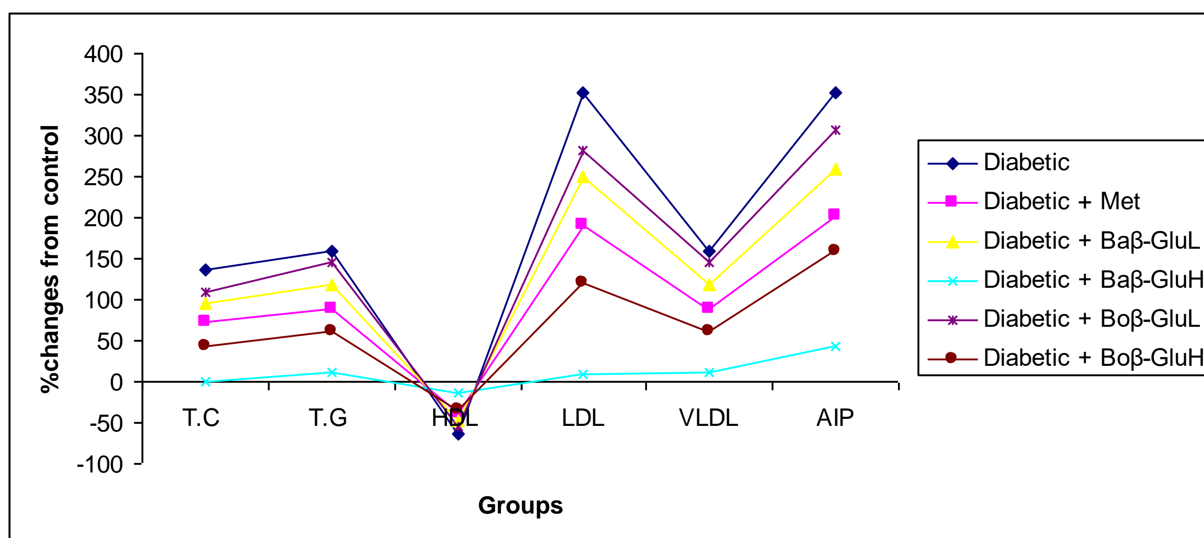

Fig. 5: Percentage changes from the control of lipid profile for diabetic rats treated with Met, *β-glucan* at different levels (Low/high dose) from different sources compared with the control group.

Table 5: Hypoglycemic effect of *β-glucan* at different levels in Hb, Insulin, and HOMA-IR of STZ induced diabetic rats compared with *Metformin*.

Groups	Parameters			
	Hb g/dl	Glucose mg/dl	Insulin pmol/l	HOMA-IR
Control	12.2 ± 0.344	88.7 ± 2.35	58.9 ± 1.627	14.9 ± 0.403
Diabetic	8.54 ± 0.23ab	297.6 ± 8.18a	21.65 ± 0.615ab	1.6 ± 0.043ab
Diabetic + Met	9.61 ± 0.254ab	188.3 ± 5.19ab	37.91 ± 1.067ab	4.5 ± 0.128ab
Diabetic + Baβ-GluLB	8.66 ± 0.23ab	251.2 ± 6.83abc	31.66 ± 0.87ab	2.8 ± 0.077ab
Diabetic + Baβ-GluH	10.54 ± 0.278abc	164.3 ± 4.38abc	50.39 ± 1.426ab	6.9 ± 0.188ab
Diabetic + Boβ-GluL	8.4 ± 0.226ab	278.4 ± 7.74abc	23.83 ± 0.655ab	1.9 ± 0.053ab
Diabetic + Boβ-GluH	9.14 ± 0.26ab	193.51 ± 5.32 ab	37.47 ± 1.018ab	3.8 ± 0.103ab

Data are expressed as Mean ± S.E.M for 6 rats /group,
a significant difference from **the control** group ,b significant difference from **Diabetic** ,c significant difference from **Diabetic + Met** at the same column with one way ANOVA at P < 0.05.

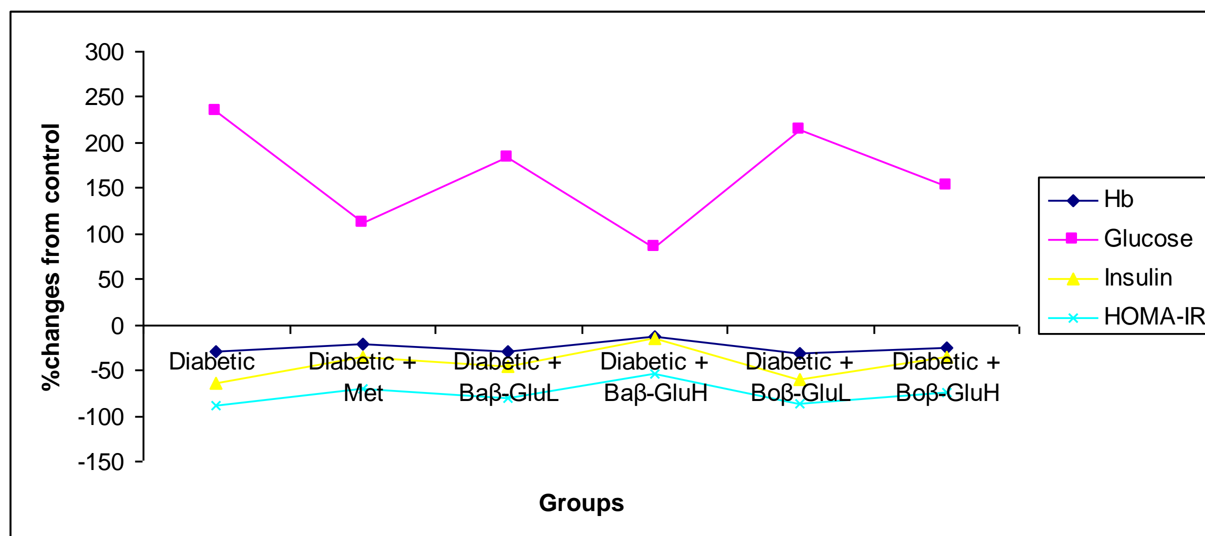

Fig. 6: % changes from the control of oxygen-carrying capacity (Hb), glucose, insulin, and HOMA-IR for diabetic rats treated with Met, *β-glucan* at different levels (Low/high) from different sources compared with the control group.

As shown in Table (5) and Fig (6) STZ markedly impaired hemoglobin Hb, insulin homeostatic model assessment of insulin resistance HOMA-IR and increased glucose compared to the control group. In contrast, *Metformin*, *β-glucan* at the high dose for bacterial and botanical source showed markedly amelioration of Hb, glucose, insulin, and. HOMA-IR compared with the control group. Meanwhile, a low dose of *β-glucan* of bacterial source showed mild amelioration of pancreatic function, hemoglobin, and glucose but the low dose of BoβG showed insignificant amelioration for pancreatic function compared with a diabetic group.

As shown in Table (6) and Fig (8). STZ markedly impaired cell structure and function and increase apoptotic markers (%DNA in the tail, Tail moment, and tail length) compared with the control group. In contrast, *β-glucan* at the high dose for bacterial and botanical sources showed a marked decrease of pancreatic comet level compared with the diabetic group. Meanwhile. *Metformin* and the low dose of *β-glucan* of bacterial source showed mild ameliorate of pancreatic tissue but the low dose of BoβG showed insignificant amelioration for comet assay compared to the diabetic group.

Table 6: Hypoglycemic effect of *β-glucan* at different levels in comet DNA of STZ induced diabetic rats compared with *Metformin*.

Groups	Parameters		
	T. DNA%	**T. Moment (units)**	**T. Length µm**
Control	14 ± 0.383	0.74 ± 0.021	1.1 ± 0.031
Diabetic	40.2 ± 1.092ab	2.7 ± 0.075ab	3.9 ± 0.112ab
Diabetic + Met	30.3 ± 0.807ab	2 ± 0.055ab	3 ± 0.082ab
Diabetic + Baβ-GluL	34.4 ± 0.958ab	2.3 ± 0.064ab	3.4 ± 0.091abc
Diabetic + Baβ-GluH	17.2 ± 0.478abc	1.1 ± 0.032abc	1.7 ± 0.048abc
Diabetic + Boβ-GluL	37.4 ± 0.997abc	2.5 ± 0.07abc	3.7 ± 0.104abc
Diabetic + Boβ-GluH	26.1 ± 0.725abc	1.7 ± 0.048ab	2.6 ± 0.07abc

Data are expressed as Mean ± S.E.M for 6 rats /group,
a significant difference from **the control** group, b significant difference from **Diabetic,**c significant difference from **Diabetic + Met** at the same column with one-way ANOVA at $P < 0.05$.

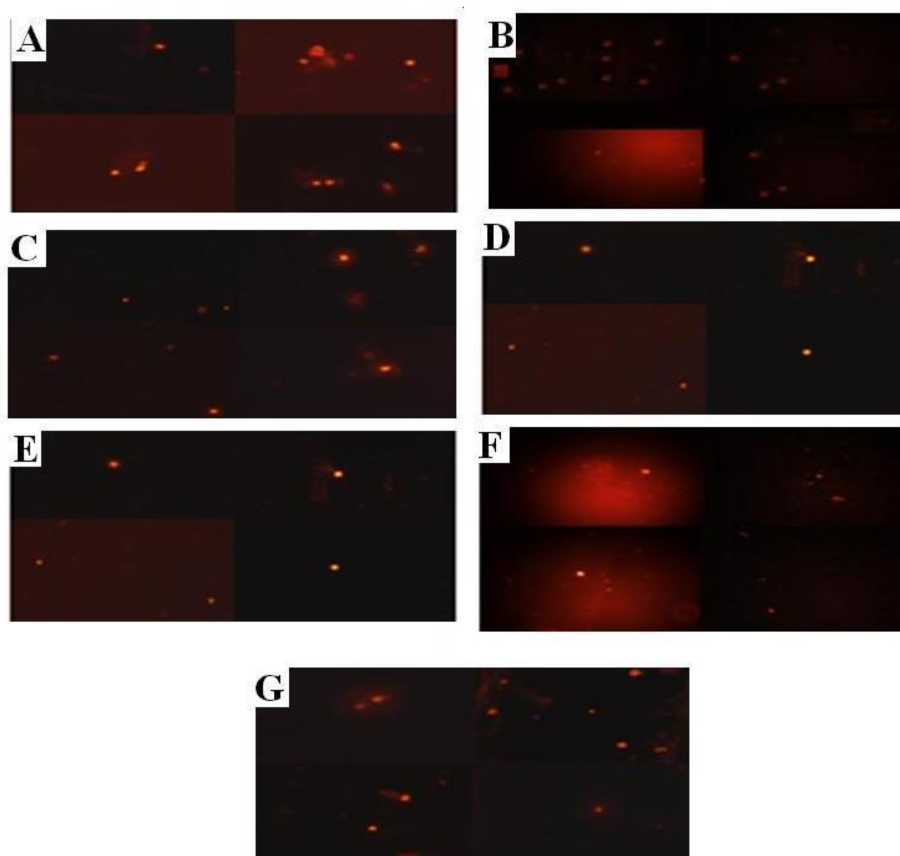

Fig. (7): Representative micrographs of pancreatic tissue stained with ethidium bromide didn't show comet of the control group meanwhile the other diabetic groups treated with different treatment showed comet with different grades: **A.** Pancreatic tissue of the control group didn't show any comet or tailing resembling DNA damage. **B.** The pancreatic tissue of the diabetic group showed a strong comet score resembling DNA damage, increase tail length, and tail moment. **C.** The pancreatic tissue of the diabetic group treated with **Met** showed moderate comet score resembling DNA damage, mild tail length, and tail moment. **D.** The pancreatic tissue of the diabetic group treated with **Baβ-GluL** showed moderate comet score resembling DNA damage, mild tail length, and tail moment. **E.** The pancreatic tissue of the diabetic group treated with **Baβ-GluH** showed a mild comet score resembling mild DNA damage, a significant decrease in tail length, and tail moment and nearly equal to the control group. **F.** The pancreatic tissue of the diabetic group treated with **Boβ-GluL** showed a strong comet score resembling DNA damage and didn't decrease tail length or tail moment. **G.** Pancreatic tissue of diabetic group treated with **Boβ-GluH** showed mild comet score resembling mild DNA damage, significant decrease tail length, and tail moment.

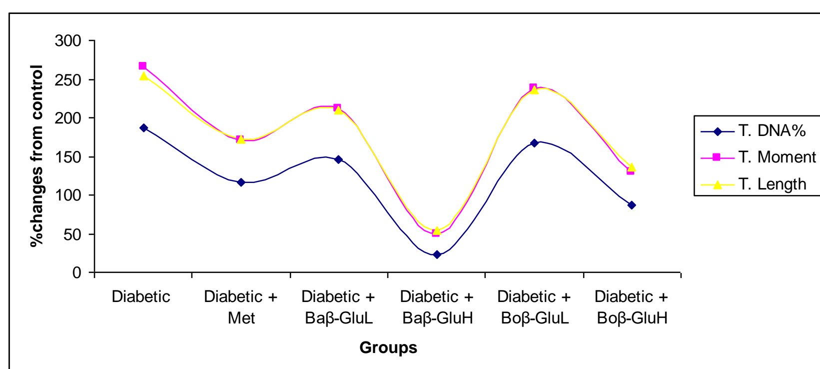

Fig. (8). Percentage changes from the control of comet pancreatic tissue for diabetic rats treated with Met, *β-glucan* at different levels (Low/high) from different sources compared with the control group.

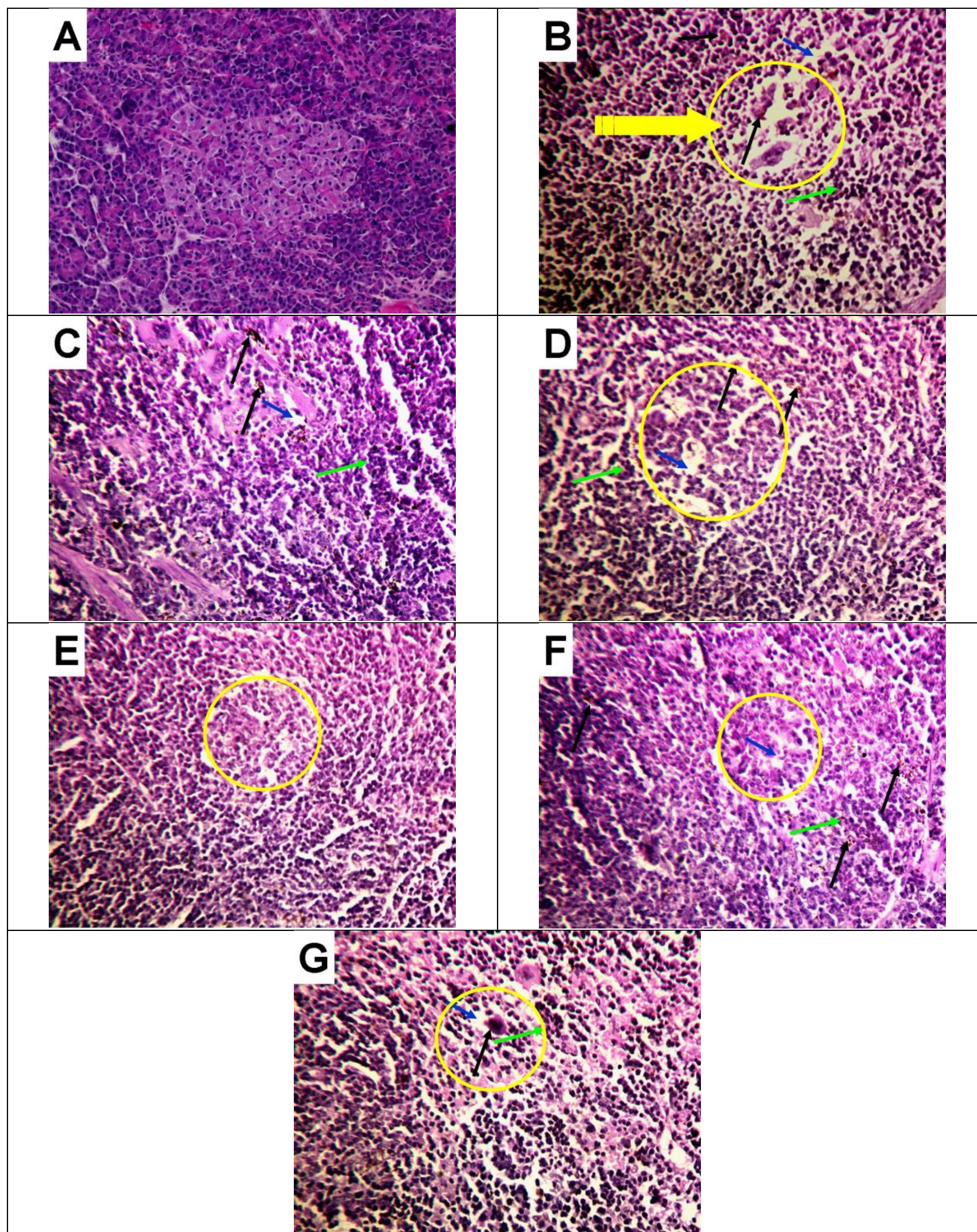

Fig. (9) . Pancreatic tissue of rat induced diabetes mellitus with STZ and treated with *β-glucan* using H&E showed that; (a) pancreatic tissue of control group showed normal intact pancreatic islet, and pancreatic acini; (b) STZ group showed destruction and evacuation of pancreatic islet, congestion, hemorrhage (Black arrow) , edema (Blue arrow) and infiltration between the acini and the peripancreatic area (Green arrow); (C) STZ+Met showed mild destruction of pancreatic islet, congestion, hemorrhage (Black arrow), edema (Blue arrow) and infiltration between the acini and the peripancreatic area (Green arrow); (D) STZ+Baβ-GluL showed mild amelioration and still containing destruction congestion, edema and mild infiltration; (E) STZ+Baβ-GluH showed improvement of cell function and decrease all negative alteration; (F) STZ+Boβ-GluL showed mild amelioration and still containing destruction congestion, edema, and mild infiltration; (G) STZ+Boβ-GluH showed major amelioration for all negative alteration.

Discussion

Glucans are present in fungi, plants, bacteria, and algae. *β-Glucan* is one of the major cell wall components of *Saccharomyces cerevisiae,* which can be able to stimulate immune functions. Glucans are glucose polymers with an α-/β-type glycoside chain. The role of *β-glucan* is in the maintenance of yeast cell wall shape and rigidity [34]. *β-glucan* in recent studies not only reduces the chance of any disease but also daily prove an increase of the health benefits [34].

Diabetes causes many changes in the body of the patient. The patient may change significantly with the disease, and some of these changes may result in serious health complications, dysfunction of the body organs. Revealed data of STZ group showed many diabetic complications such as body weight loss and decrease in daily gain weight compared to control group. The decrease in body weight for the STZ group may be due to the excessive breakdown of tissue proteins **[35]**. Brown and Gordon [36] stated that diminished body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Amplified catabolic reactions that lead to muscle wasting might also be the cause for the reduced weight gain by diabetic rats [37]. Hence, STZ or stress may increase cortisol production, and subsequent cortisol secretion this works to increase blood glucose levels. In addition, cortisol hormone is produced by the body to withdraw glucose from fat in the body, or in the case of the need for glucose for muscles of the body. Owing to the withdrawing of glucose from fat and muscle of diabetic group the body loses weight significantly. Oxidative stress or free radical is a very active oxygen atom that lacks an electron. It is unstable and tries to settle down by finding this missing electron by attacking any molecule of the cells started with the cell wall and finally with DNA fragment [38]**.** Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes **[39].** Maki et al. [40] reported that the STZ markedly increases oxidative stress markers and decreases endogenous antioxidant enzymes. In the same way, obtained data showed a marked increase of MDA, GSSG, and markedly decrease of GSH and TAC. The increase of oxidative stress markers may be due to STZ which inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus. Both effects can be attributed to its specific chemical properties, namely its alkylating potency and due to NO donation. Both high blood glucose and low insulin levels contribute to high cholesterol, triglycerides, LDL, and low level of HDL. As a whole, increasing levels of cholesterol showed a harmful effect on the liver and muscles. High triglyceride levels are caused by a lack of glycemic control in diabetic patients, low levels of thyroid hormone secretion, or chronic diseases with liver or kidney function. Likewise, the conversion of fatty acids into cholesterol in the liver due to STZ along with an excess of triglycerides formation may be discharged into the blood in the form of lipoproteins. Hypertriglyceridemia in the diabetic group showed an increase in hepatic LDL overproduction and impaired catabolism of triglycerides. Overall, the degradation of triglycerides from the bloodstream is decelerated by insulin depletion and activates lipoprotein lipase which is the main enzyme to decelerate them from the body system and ameliorate homeostasis condition. Accomplished data are inconsistent with Chen et al. [41] who reported that consuming beta-glucan (soluble dietary fiber) didn't reduce LDL-cholesterol. In addition, 3 g/d of beta-gLucan didn't reduce the T.Chol or LDL of 62 healthy, middle-aged men and women. A possible explanation for these inconsistent results may be owing to differences in solubility and molecular weight of the *beta-glucan* used in the different studies, which can result in different gut viscosities. In short, *beta-glucan* exerts an anti-hyperlipidemic effect which may be due to a change of enzymatic activity of cholesterol biosynthesis and/or lipolysis level which are under the control of insulin **[42]**.

In addition, obtained data showed that the *beta-glucan* group decreased cholesterol level and this finding may be due to increasing fecal sterol excretion. Our data consist with Kerckhoffs et al. [43], which unveiled the reduction of plasma and hepatic cholesterol levels in hamsters fed by mushrooms, with *beta-glucan*. On another hand, the decrease of cholesterol level may return to the increase of hepatic cholesterol excretion. In other words, *β-glucan* can inhibit the increase of MDA and increase endogenous antioxidants via immunostimulatory properties. In this way increasing the immune response of the body stimulates the anti-oxidants such as GSH which act as cell membrane protective and subsequently decrease MDA production according to a decrease of cell destruction. Obtained data are in agreement with Pourahmad [44] who reported that *β-glucan* reduced MDA and stimulate the glomerulus stimulating hormone. The reduction of MDA production implied a reduction in lipid peroxidation for cell injury and protection of the liver against acetaminophen-induced oxidative damage in mice. Previous studies have shown the effect of *β-glucan* on the decreased MDA and NO levels, and increasing GSH-Px and SOD activities these may owe to the potent antioxidant capacity which may lower limb ischemia-reperfusion injury **[45]**. The mechanism of action by demonstrating potent anti-oxidants properties, preventing damage by ROS, and via immune-antioxidant activity related to glucan which proliferates bone marrow stem cells. According to the hematopoietic properties of *β-glucan* which stimulate hemoglobin and another oxygen-carrying capacity, *β-glucan* ameliorate STZ dysfunction around the normal level. In the same mechanism, De Rooij *et al* [46]

reported that *β-glucan* increases hemoglobin and RBCs by raising the erythropoietic activity in both bone marrow and spleen. The reached diet with soluble or insoluble fibers, pectin, and methylcellulose fibers may improve lowering or flattening of insulin and glucose levels [47]**.** Reveled data are in agreement with Nag *et al*. [48] reported that soluble oat fibers have a beneficial effect in lowering insulin resistance and lower blood glucose concentrations with type 2 diabetes. This action may own inverse relation between the *beta-glucan* and glucose plasma level. Another mechanism for *beta-glucan* combated STZ side effects may be delaying or reducing the absorption of carbohydrates from the gut [34]. Lastly, obtained data suggested that the amelioration of insulin and decrease of glucose may attribute to include digestion resistance and improvement in glucose tolerance [47]**.** Obtained data showed significant confirmation for the amelioration of *beta-glucan* from bacterial sources compared to treatment with *Metformin*, *beta-glucan* botanical source via histological examination and comet assay of a pancreatic cell which relevant to DNA fragmentation and stability or mutation of cell structure and function which perform the roles to maintain the physiological performance of the body system.

Conclusions: Revealed data suggested that β-glucan prepared from bacterial source showed high affinity for treated diabetes and other complications attributed to ameliorate body weight, lipid profile, oxidative stress, insulin, and glucose level, DNA fragmentation resembling in comet assay and finally ameliorate Langerhans islands of pancreatic cell rats in comparing with a botanical source.

Conflict of interest: all authors declare that no conflict of interest

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Authors contribution: A-F OA, KAG, performed the lab work/experiment. RAH, HAM, HAH, analyzed the data, wrote the manuscript, TMA, AAS performed the tools of experiments, All authors contribute in reviewed the manuscript.

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