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Short Communication

HYPOGLYCEMIC AND HYPOLIPEMIC EFFECTS OF B-GLUCAN DERIVED FROM *AUREOBASIDIUM* IN STZ-INDUCED DIABETIC RATS

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ABSTRACT

The primary objective of the present study was to determine the effects of β-glucan derived from *Aureobasidium* on diabetes and diabetic hyperlipemia in an animal model of streptozotocin (STZ)-induced diabetes. β-glucan was orally administered to STZ-induced diabetic hyperlipemic Sprague-Dawley (SD) rats for 4 weeks beginning at 25 days after STZ administration (late diabetic hypoglycemic and hyperlipemia model). Changes in body weight were recorded throughout the study and blood glucose and serum low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and total cholesterol (T-CHOL) levels were measured at 28 days after β-glucan administration. The β-glucan results were compared with those from rats that received Simvastatin (10 mg/kg). Following the occurrence of STZ-induced diabetes, the STZ control group exhibited decreases in body weight and serum HDL levels in conjunction with increases in blood glucose, serum LDL, triglyceride, and T-CHOL levels compared to the intact control group. However, serum LDL, triglyceride, and T-CHOL levels dramatically decreased in the STZ groups that received β-glucan (62.5 and 125 mg/kg). The present results suggest that β-glucan derived from *Aureobasidium* does not exert hypoglycemic effects in an SD rat model of STZ-induced diabetes. However, β-glucan did have favorable effects in terms of attenuating complications related to diabetic hyperlipemia at a dose of 62.5 mg/kg/day or more. Therefore, β-glucan may be a candidate as a novel hypolipemic agent designed specifically to treat diabetic complications related to hyperlipemia regardless of the severity of disease.

Keywords: Hypoglycemic effect, hypolipemic effect, β-glucan, rats.

INTRODUCTION

Diabetes mellitus is a chronic disorder characterized by high blood glucose levels and either insufficient or ineffective insulin activity (Malaguti-Boyle, 2016). This disorder is associated with a variety of complications including atherosclerosis, myocardial infarction, and nephropathy, among others (Kim *et al*., 2012). Numerous studies have shown that obesity, physical inactivity, high-fat diets, and diets rich in saturated fatty acids increase the risk of diabetes (Hu *et al*., 2001).

A variety of oral antidiabetic medications, such as the thiazolidinediones and metformin, are currently being used or developed for the treatment of diabetes, because such drugs improve insulin resistance. However, the currently available pharmacological agents for treating diabetes or diabetes-related obesity tend to have a number of limitations, including adverse side effects and high rates of secondary failure (Norris *et al*., 2001). Based on these considerations, diabetic patients and healthcare professionals have increasingly been considering complementary and/or alternative approaches for the treatment of diabetes, such as the use of medicinal herbs. Thus, the present study aimed to determine the effects of β-glucan derived from *Aureobasidium* on blood glucose levels and diabetic hyperlipemia in a rat model of streptozotocin (STZ)-induced diabetes.

Diabetes mellitus refers to a heterogeneous group of metabolic disorders characterized by hyperglycemia and glycosuria, as well as the possibility of related events such as ketoacidosis and the loss of minerals, nitrogen, and body weight (Kuzuya *et al*. 2002). All of these events eventually lead to coma and death if treatment is not instituted. Two major categories of diabetes are recognized in humans: insulin-dependent diabetes, which is also known as type 1 or juvenile-onset diabetes, and non-insulin-dependent diabetes, which is also known as type 2 or mature-onset diabetes. Patients with type 1 diabetes have an absolute insulin deficiency, while those with type 2 diabetes often have below- or

above-normal plasma insulin levels in a fasting state and may also exhibit an impaired insulin response to a glucose load (Kuzuya *et al*., 2002). The following symptoms are commonly monitored in diabetes patients regardless of their type: hyperglycemia, glycosuria, diuresis, decreased carbohydrate utilization, increased fat and protein catabolisms, weight loss and polyphagia, loss of resistance to infection, bilateral cataracts, coma, and death.

Despite the beneficial effects associated with good glycemic control, antihypertensive therapies, and the widespread use of agents that block the renin-angiotensin system, a significant proportion of diabetes patients continue to progress in terms of diabetic nephropathy, hyperlipemia, and liver damage (Howland *et al*., 2006). Therefore, additional therapies and agents that attenuate the degree and effects of diabetic hyperlipemia should be developed. Simvastatin is a cholesterol-lowering 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) inhibitor that inhibits the production of cholesterol by the liver and lowers overall and low-density lipoprotein (LDL) cholesterol blood levels (Pedersen and Tobert, 2004). LDL cholesterol is thought to be the "bad" cholesterol that is primarily responsible for the development of coronary artery disease, because lowering LDL levels slows and may even reverse the progression of this disease (Gardner *et al*., 1996). It is also generally accepted that Simvastatin has favorable effects in patients with diabetic hyperlipemia and, thus, this drug is often selected as a control drug in models of diabetic hyperlipemia (Sheu *et al*., 2001).

β-glucan a fiber-type complex sugar (polysaccharide) derived from the cell walls of baker's yeast, oat, barley, and mushrooms, is a key factor in the cholesterol-lowering effects of oat bran (Othman *et al*., 2011). Like other soluble fiber components, the binding of cholesterol (and bile acids) by β-glucan, and the subsequent elimination of these molecules via the feces, is very helpful for reducing blood cholesterol levels. Double-blind trials investigating either oat- or yeast-derived β-glucans have found typical reductions of approximately 10% and 8% in total cholesterol (T-CHOL) and LDL cholesterol levels, respectively, in conjunction with increases in high-density lipoprotein (HDL) cholesterol levels ranging from 0–16% after at least 4 weeks of use (Bell *et al*. 1999).

Thus, the primary objective of the present study was evaluate the effects of β-glucan (62.5 and 125 mg/kg; Glucan Corp. Ltd., Busan, Korea) derived from *Aureobasidium* on blood glucose levels and diabetic hyperlipemia in a rat model of STZ-induced diabetes. Additionally, the possibility that the severity of diabetes would alter the effects of β-glucan was evaluated using a single dose (62.5 mg/kg).

MATERIALS AND METHODS

Test articles and formulation: The β-glucan solution containing 2.5% β-glucan and a brownish-sticky substance was obtained from Glucan Corp. and stored in a refrigerator at 4ºC to protect it from light and degeneration. The β-glucan was prepared following the method of Seo *et al*. (2002). Briefly, while shaking at 150 rpm at 30°C for 5 days in a 500-mL glass flask, *Aureobasidium pullulans* SM-2001, a UV-induced mutant of *A. pullulans* ATCC 42023, was cultured in 150 mL of liquid medium (5.0 g/L K_2HPO_4, 1.0 g/L NaCl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.6 g/L $(NH_4)_2SO_4$, 2.5 g/L yeast extract, and 0.5 % (w/v) sucrose). The supernatant of the *A. pullulans* culture was separated by centrifugation at 8,000×*g* for 20 min. A double volume of 95.0% ethanol was added to and mixed with the separated supernatant and then stored at 4°C overnight. The ethanol and supernatant mixture was centrifuged at 8,000×*g* for 20 min to obtain the crude β-glucan precipitate, which was washed twice with 95.0% ethanol. We placed the washed β-glucan precipitate in a dialysis membrane (exclusive MW = 14,000; Spectrum Labs, USA) for 2 days and changed the distilled water five times. The β-glucan powder was prepared using a freeze-drier.

The β-glucan solution was diluted with distilled water and administered via oral gavage using a sonde attached to a 3 mL syringe, at doses of 62.5 and 125 mg/kg for 4 weeks beginning 25 days after STZ administration. Additionally, the possibility that the severity of diabetes would alter the effects of β-glucan on blood glucose levels was evaluated using a single dose (62.5 mg/kg) with the severity of diabetes classified as follows: severe (HC), middle (vehicle control), and slight (LC). Animals in the HC and LC groups received a 62.5 mg/kg injection of β-glucan while the Simvastatin (Sigma, St. Louis, MO, USA) group received the drug in a solution of distilled water at 10 mg/kg. All test articles including Simvastatin were dosed at 5 mL/kg (Table 1).

Animals and husbandry: The present study included 100 6-week-old female Sprague-Dawley (SD) rats (Shizuoka Laboratory Center Inc., Hamamatsu, Japan). After acclimatization for 8 days, the animals were housed three per polycarbonate cage in a room with a controlled temperature (20–25°C) and humidity level (30–35%), a light/dark cycle of 12:12 h, and ad libitum feed (Samyang Corp., Seoul, Korea) and water. Of the 100 rats, 88 received administrations of STZ and the remaining 12 rats were used as shams. To institute the late diabetic model (Therapeutic Model), β-glucan treatment was initiated 25 days after STZ administration and each group was treated once per day for 4 weeks (Qd × 28). Approximately half of the STZ and sham animals (five or six per group) were selected for blood glucose sampling (Table 2) and the samples and body weight measurements

were obtained 21 days after STZ administration. The animals were divided into none groups with five animals. All animal procedures were performed in accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee of Silla University (Busan, Korea; approval no. SUACUC-2016-020).

Administration of the test article: The β-glucan solution was given daily between 09:00 and 10:30 for 4 weeks, beginning at 25 days after STZ administration. For each animal, the actual treatment dose was calculated based on the most recent body weight. Additionally, prior to the collection of the blood samples, STZ administration, initial administration, and/or termination, each animal was fasted for at least 12 h; water was not restricted.

STZ administration: In the control and test groups, a single intraperitoneal injection of STZ (60 mg/kg/5 mL) dissolved in 50 mM of citrate buffer was administered to each animal. An equal volume of vehicle was administered to the sham animals using the same methods.

Measurements of body weight: Body weight was measured at STZ administration, initial dosing of β-glucan, and at 7, 14, 21, 27, and 28 days after β-glucan dosing (at sacrifice) using an automatic electronic balance (Sartorius Co., Ltd., Bohemia, NY, USA). Prior to blood collection, STZ administration, initial β-glucan dosing, and termination, the experimental animals were fasted overnight for 12 h (water was freely available) to reduce the erratum aroused by feeding. Gains in body weight (g) were calculated as follows:
(Equation 1)
Change I: change during the STZ induction period from pre-administration to 25 days after STZ administration
Change II: change during the observation period from days 0–28 of the test article administration

Measurements of blood glucose levels: To determine blood glucose levels, blood samples were collected from the orbital plexus 1 day before STZ administration, 21 days after STZ administration, and then 28 days after administration (at sacrifice); the samples were deposited in an NaF glucose vacuum tube (Becton Dickinson; Franklin Lakes, NJ, USA) and the plasma was separated. Blood glucose level (mg/dL) and serum chemistry analyses were performed with an automated blood analyzer (200 FR; Toshiba, Tokyo, Japan) and changes during the induction period (from the normal baseline to 21 days after STZ administration) and the dosing period (from 21 days after STZ administration to sacrifice) were calculated to reduce the erratum arouse due to individual differences, at the initiation of grouping, as follows:
(Equation 2)
Change I: [Serum levels $_{\text{at 21 days after STZ administration}}$ − Serum levels $_{\text{at 1 day before STZ administration}}$]
Change II: [Serum levels $_{\text{at sacrifice}}$ − Serum levels $_{\text{at 21 days after STZ administration}}$]

Measurements of serum lipid levels: To assess the levels of serum LDL, HDL, triglyceride, and T-CHOL, blood samples were collected from the orbital plexus at sacrifice (28 days after dosing) and the serum was separated using general methods. The serum LDL, HDL, triglyceride, and T-CHOL levels were detected using an automated blood analyzer (Toshiba 200 FR) with enzymatic methods and the serum chemistry analyses were conducted at E-won Clinical Laboratory (Seoul, Korea).

Statistical analyses: All data were analyzed based on the mean and standard deviation (mean ± SD). All statistical analyses were conducted with the Mann-Whitney U/Wilcoxon rank sum W test (MW test) using SPSS for Windows software (ver. 6.1.3.; SPSS Inc., Chicago, IL, USA). Additionally, the percentage of change relative to the vehicle control (including HC and LC or sham) was calculated using the following equations:
(Equation 3) Percentage changes vs. sham (%) = [((Data $_{\text{vehicle controls}}$−Data $_{\text{Sham}}$)/Data $_{\text{Sham}}$) × 100]
(Equation 4) Percentage changes vs. vehicle controls including HC and LC (%) = [((Data $_{\text{test groups}}$ − Data $_{\text{vehicle controls}}$)/Data $_{\text{vehicle controls}}$) × 100]

RESULTS AND DISCUSSION

Changes in body weights: When an individual suffers from diabetes, the ability to use nutrients in the body (i.e., the capacity for glucose metabolism) decreases even though food intake remains the same or increases (Kuzuya *et al*. 2002). In general, body weight decreases as diabetes progresses (Kamalakkanan *et al*., 2003; Kim *et al*., 2006; Kim *et al*., 2013) and, in terms of experimental studies, Kim *et al*. (2012) demonstrated weight loss in an animal model of STZ-induced diabetes despite the fact that the animals did not decrease their food intake. Thus, a reduction in weight may be seen as a characteristic symptom of diabetes.

The changes in body weight after the administration of STZ and the test article are summarized in Table 3. Although there were significant decreases in body weight gains during the induction periods of STZ-induced diabetes and diabetic hyperlipemia ($p < 0.01$ or $p < 0.05$), no meaningful or significant changes in body weight gains were detected in any of the test article groups compared to the vehicle control. There were slight increases in body weight gains in some dosing groups relative to the vehicle control group during the observation periods. However, no significant changes were observed in any of the dosing groups, including the Simvastatin group, except for the β-glucan 62.5 mg/kg group, which exhibited a significant ($p < 0.05$) increase in body weight gains during the dosing periods compared to

the vehicle control. Additionally, no meaningful changes in body weight gains were detected in the β-glucan subgroups of the severe (HG) and slight (LG) hyperlipemia groups compared to those of the HC and LC groups, respectively.

Blood glucose levels: Hyperglycemia is the primary sign associated with diabetes and STZ-induced diabetic rats exhibit this symptom (Kim *et al*., 2006). During the progression of diabetes, either hypertrophy or hyperplasia of the endocrine pancreatic cells is related to insulin resistance (Terauchi *et al*., 2007). Moreover, the ratio of insulin- to glucagon-producing cells in the endocrine pancreas in STZ-induced diabetic rats decreases due to the destruction of insulin-producing β-cells and increases in glucagon-producing α-cells (Kim *et al*., 2006). Because the most critical issue associated with hyperglycemia is typically increased blood glucose levels, the efficacy of hyperglycemic agents is generally evaluated based on decreases in blood glucose (Kim *et al*., 2006; Kim *et al*., 2013).

Blood glucose levels after STZ administration and test article administration are summarized in Table 4. Significant increases in blood glucose levels were detected during the induction period of STZ-induced diabetes in all test groups compared to the sham group. Additionally, a significant increase in blood glucose levels (256.17%) was observed in the vehicle control group at sacrifice relative to the sham group. In contrast, similar blood glucose levels were found in all dosing groups compared to the vehicle control group at sacrifice. Although small decreases or increases in blood glucose levels were detected in some β-glucan groups, no dose-dependent or significant effects were detected in the present study (Table 3).

Serum levels of LDL, HDL, triglyceride, and T-CHOL: Serum LDL and HDL levels after STZ administration and test article administration are summarized in Table 4. In general, the most critical problems associated with hyperlipemia are increased serum LDL levels and decreased HDL levels (Inkeles and Eisenberg. 1981). LDL is a major lipoprotein, in which each particle contains a single apolipoprotein B-100 molecule (Elovson *et al*. 1988). LDL can transport the content of lipid molecules into artery walls and attract macrophages, thus driving atherosclerosis (Ross, 1999). As a result, LDL cholesterol has now largely replaced T-CHOL as a risk marker and the primary treatment target in patients with hyperlipemia (Wadhera *et al*., 2016). HDL cholesterol is the smallest lipoprotein particle and its abundant apolipoproteins include apo A-I and apo A-II (Swaney and O'Brien, 1978). The functions of HDL include the transport of cholesterol to the liver or steroidogenic organs, such as the glands, ovaries, and testes, via direct and indirect pathways. HDL levels can be increased by eating fewer carbohydrates, engaging in aerobic exercise, losing weight, and ingesting magnesium (Mensink *et al*., 2003; Spate-Douglas and Keyser 1999; Hausenloy and Yellon 2008; Rosanoff and Seelig, 2004).

The efficacy of a hypolipemic agent is generally evaluated based on decreases in serum LDL levels in conjunction with increases in serum HDL levels. In the present study, there were significant increases in serum LDL levels, and significant decreases in serum HDL levels, in the vehicle control group at sacrifice compared to the sham group (LDL: 105.88%; HDL: -32.97%). However, there were significant increases in serum HDL levels, and significant decreases in serum LDL levels, in all dosing groups relative to the vehicle control group at sacrifice. These same changes were also observed in the HG and LG groups compared to the HC and LC groups, respectively.

All β-glucan groups in the present study exhibited significant decreases in serum LDL and significant increases in serum HDL. These findings suggest that β-glucan has relatively favorable effects in terms of inhibiting the alterations in serum LDL and HDL levels that are induced by diabetic hyperlipemia, regardless of the severity of diabetes. Although somewhat slight or similar efficacies were observed in the β-glucan groups compared to the Simvastatin group (in terms of lowering serum LDL levels), β-glucan had more favorable effects with respect to increasing serum HDL than Simvastatin.

Serum triglyceride and T-CHOL levels after STZ administration and test article administration are summarized in Table 5. In general, some of the most critical problems associated with hyperlipemia include increases in serum triglyceride and T-CHOL levels[16] (Inkeles and Eisenberg. 1981). T-CHOL is the sum of LDL, HDL, and very-low-density lipoprotein (VLDL), but most hyperlipemia is due to increases in LDL. Therefore, as LDL increases, T-CHOL increases. Triglycerides, formed by the combination of glycerol with three fatty acids, are then used for energy by the body; however, excessive increases in triglyceride levels in the blood raise the risk of heart disease and stroke (Ordovas, 2006). Moreover, high blood triglyceride levels are induced by obesity, diabetes, hypothyroidism, kidney disease, high calorie intake, and high levels of alcohol consumption (Silva *et al*., 1987; McCarty 2004; Pejic and Lee 2006).

The efficacy of a hypolipemic agent is generally evaluated based on its ability to decrease serum triglyceride and T-CHOL levels. In the present study, increases in serum triglyceride and T-CHOL levels were observed in the vehicle control group at sacrifice compared to the sham group (triglyceride: 2,784.55%; T-CHOL: 59.73%). However, there were significant decreases in serum triglyceride and T-CHOL levels in all dosing groups compared to the vehicle control group at sacrifice, including the Simvastatin group. These same

changes were also detected in the HG and LG groups compared to the HC and LC groups, respectively.

In the present study, all β-glucan groups showed significant decreases in serum triglyceride and T-CHOL levels. Therefore, β-glucan likely exerts relatively favorable effects, in terms of inhibiting the changes in serum triglyceride and T-CHOL levels that are induced by diabetic hyperlipemia regardless of severity. Somewhat slight or similar efficacies were found in the β-glucan group compared to the Simvastatin group with respect to the attenuating effects on serum triglyceride and T-CHOL levels.

Hyperglycemia is the most critical problem associated with diabetes and, thus, the effects of hypoglycemic effects and consequent decreases in urine glucose excretion have been considered an essential characteristic of anti-diabetic agents (Kim *et al*., 2006; Ojewole and Adewunmi 2003; Maiti *et al*., 2005). Although the mechanisms underlying the reduced excretion of glucose in patients with diabetic hyperlipemia remain unclear, it is thought that the favorable effects of β-glucan are mediated by various mechanisms with complex interactions. Further research will be necessary to fully elucidate the mechanisms of action associated with β-glucan treatment.

In the present study, STZ-induced diabetes resulted in dramatic decreases in body weight and serum HDL levels in conjunction with increases in blood glucose, serum LDL, triglyceride, and T-CHOL levels in the STZ control group relative to the intact control group. These changes associated with diabetic hyperlipemia were reversed following treatment with β-glucan (62.5 and 125 mg/kg) but there were no dose-dependent or significant effects. Based on these results, it was concluded that β-glucan has favorable effects in terms of ameliorating diabetic complications related to diabetic hyperlipemia at doses of 62.5 mg/kg or higher. Therefore, β-glucan may have potential as a novel hypolipemic agent intended for treating diabetic complications related to hyperlipemia regardless of the severity of disease.

Clinical studies have examined the effects of β-glucan from oats and yeast on serum lipids. For example, oat β-glucan reduced the serum LDL cholesterol in humans (Wolever *et al*., 2010); oat β-glucan significantly reduced the total and LDL cholesterol in subjects with elevated cholesterol (Braaten *et al*., 1994; Queenan *et al*., 2007); and a yeast-derived-glucan fiber changed the plasma lipids in hypercholesterolemic patients (Nicolosi *et al*., 1998). However, no clinical study has examined the effects of β-glucan derived from *Aureobasidium* on diabetic hyperlipidemia. Therefore, further research is needed.

Table 1. Group identification and composition of test articles.

Group		Dose	N	Group ID	Vehicle	Route	Schedule
Sham	Sham	5ml/kg	6	G0			
	Moderate Control	5ml/kg	6	G1			
	Simvastatin	10mg/kg/5ml	6	G2			
	β-Glucan	62.5mg/kg/5ml	6	G3	Injectable distilled water	Oral	Once a day for 4 weeks
STZ[1)] dosing	β-Glucan	125mg/kg/5ml	6	G4			
	Severe Control	5ml/kg	5	HC			
	β-Glucan	62.5mg/kg/5ml	5	HG			
	Slight Control	5ml/kg	5	LC			
	β-Glucan	62.5mg/kg/5ml	5	LG			

[1)] Streptozotocin (60 mg/kg/5 mL) was administered via a single intraperitoneal injection. All test articles and vehicle doses were administered for 4 weeks via a gastric gavage beginning at 25 days after STZ administration.
G0, sham: sterilized and distilled water (5 mL/kg)-treated group; G1, control: moderate diabetes control group; G2: glybenclamide (3 mg/kg)-treated group or Simvastatin (10 mg/kg)-treated group; G3: β-glucan (62.5 mg/kg)-treated group; G4: β-glucan (125 mg/kg)-treated group; HC: severe diabetes control group; HG: β-glucan (62.5 mg/kg)-treated group after severe diabetes; LC: severe diabetes control group; LG: β-glucan (62.5 mg/kg)-treated group after slight diabetes

Table 2. Changes in body weight after STZ administration and test article administration.

Body weight	At STZ[1)]	At Dosing	Weeks after dosing				At Sacrifice[1)]
			1 week	2 weeks	3 weeks	4 weeks	
G0	171.67± 7.12	223.00± 11.90	234.17± 13.57	241.50± 20.70	247.83± 14.61	253.17± 9.91	236.83± 9.68
G1	169.33± 4.55	184.67± 10.50*	190.83± 13.11*	193.67± 17.15*	194.50± 21.91*	190.83± 26.87*	165.50± 22.54*
G2	168.67± 8.52	180.17± 14.78*	194.00± 22.93*	194.67± 23.12*	202.17± 22.96*	187.67± 26.53*	173.17± 28.84*

G3	165.33± 8.02	180.50± 16.50*	199.33± 22.92*	203.83± 22.57*	210.17± 18.23*	210.33± 17.68*	179.67± 16.48*
G4	168.33± 7.45	181.50± 12.57*	188.00± 16.96*	192.67± 19.53*	196.83± 22.36*	204.50± 17.71*	175.33± 14.21*
HC	165.20± 8.84	173.80± 8.58*	180.40± 8.65*	176.20± 19.45*	184.40± 14.96*	186.80± 13.72*	162.60± 14.17*
HG	163.80± 7.53	171.40± 12.58*	176.40± 20.19*	177.40± 20.92*	179.00± 23.44*	184.80± 23.59*	162.80± 17.92*
LC	169.40± 10.04	200.80± 7.95**	219.60± 9.76	225.40± 7.44	225.80± 7.98**	232.60± 6.66*	201.20± 6.14*
LG	169.40± 6.80	196.40± 5.59*	208.00± 12.81**	220.00± 13.91	227.60± 10.16**	231.00± 16.08	209.60± 17.33**

Changes	Changes I	Changes II
G0	51.33±7.69	30.17±5.38
G1 [PCA][2)]	15.33±10.97* [-70.13]	6.17±20.61** [-79.56]
G2 [PCB][3)]	11.50±9.07* [-25.00]	7.50±21.75** [21.62]
G3 [PCB]	15.17±11.87* [-1.09]	29.83±9.17**,# [383.78]
G4 [PCB]	13.17±15.60* [-14.13]	23.00±5.76 [272.97]
HC [PCA]	8.60±8.56* [-83.25]	13.00±10.22** [-56.91]
HG [PCB]	7.60±15.57* [-11.63]	13.40±19.35 [3.08]
LC [PCA]	31.40±14.48** [-38.83]	31.80±9.60 [5.41]
LG [PCB]	27.00±9.11* [-14.01]	34.60±15.81 [8.81]

N = 6; (mean ± SD, g), group identification as listed in Table 1. [1)]Overnight fasted; Change I: change during induction periods (from pre-administration to 25 days after STZ administration), Change II: change during observation periods (from day 0 to sacrifice). [2)]PCA, percentage (%) change vs. sham (G0). [3)]PCB, percentage (%) change vs. vehicle controls (G1, HC, or LC); * $p < 0.01$ and ** $p < 0.05$ compared to the sham group using an MW test; # $p < 0.05$ compared to the vehicle control groups using an MW test.
G0, sham: sterilized and distilled water (5 mL/kg)-treated group; G1, control: moderate diabetes control group; G2: glybenclamide (3 mg/kg)-treated group or Simvastatin (10 mg/kg)-treated group; G3: β-glucan (62.5 mg/kg)-treated group; G4: β-glucan (125 mg/kg)-treated group; HC: severe diabetes control group; HG: β-glucan (62.5 mg/kg)-treated group after severe diabetes; LC: severe diabetes control group; LG: β-glucan (62.5 mg/kg)-treated group after slight diabetes

Table 3. Changes in blood glucose levels after STZ administration and drug administration.

Blood Glucose	Base Lines (Before STZ-dosing)	At 21 days after STZ-dosing	At 28 days after test article-dosing (Sacrifice)
G0	91.83±8.08	102.33±7.42	105.33±12.23
G1 [PCA][1)]	90.33±5.65 [-1.63]	333.67±28.30* [226.06]	375.17±28.35* [256.17]
G2 [PCB][2)]	92.67±7.97 [2.58]	335.50±30.87* [0.55]	177.83±48.89**,# [-52.60]
G3 [PCB]	89.33±6.15 [-1.11]	335.50±12.94* [0.55]	374.00±28.18* [-0.31]
G4 [PCB]	92.00±8.37 [1.85]	325.00±19.83* [-2.60]	371.50±25.63* [-0.98]
HC [PCA]	88.80±3.49 [-3.30]	377.40±9.53* [268.79]	382.20±47.93* [262.85]
HG [PCB]	90.60±2.88 [2.03]	382.40±15.82* [1.32]	378.20±19.84* [-1.05]
LC [PCA]	86.00±5.15 [-6.35]	147.80±28.68* [44.43]	295.00±78.57* [180.06]
LG [PCB]	86.20±7.50 [0.23]	146.80±9.52* [-0.68]	292.40±93.18* [-0.88]
Simvastatin[3)]	88.17±12.56 [-2.40]	332.67±28.94* [-0.30]	375.50±22.44* [0.09]
Captopril[3)]	91.33±14.12 [1.11]	335.00±22.62* [0.40]	376.17±28.64* [0.27]
Silymarin[3)]	91.17±8.50 [0.92]	332.17±16.07* [-0.45]	259.83±39.01*,# [-30.74]

Changes	Changes I	Changes II
G0	10.50±9.75	3.00±10.18
G1 [PCA]	243.33±24.81* [2217.46]	41.50±21.43* [1283.33]
G2 [PCB]	242.83±34.94* [-0.21]	-157.67±55.89*,# [-479.92]
G3 [PCB]	246.17±11.63* [1.16]	38.50±25.82* [-7.23]
G4 [PCB]	233.00±18.92* [-4.25]	46.50±31.24** [12.05]
HC [PCA]	288.60±8.02* [2648.57]	4.80±51.61 [60.00]

HG [PCB]	291.80±15.06* [1.11]	-4.20±24.89 [-187.50]
LC [PCA]	61.80±32.24* [488.57]	147.20±75.43** [4806.67]
LG [PCB]	60.60±12.03* [-1.94]	145.60±88.76 [-1.09]
Simvastatin[3)]	244.50±28.77* [0.48]	42.83±31.07* [3.21]
Captopril[3)]	243.67±26.47* [0.14]	41.17±27.86* [-0.80]
Silymarin[3)]	241.00±11.52* [-0.96]	-72.33±53.25*,# [-274.30]

N = 6; (mean ± S.D.), mg/dL; Group identification as listed in Table 1. [1)]PCA, percentage (%) change vs. sham (G0). [2)]PCB, percentage (%) change vs. vehicle control groups (G1, HC, or LC). [3)]Percentage (%) changes were calculated vs. vehicle control (G1); Change I: during induction periods (from pre-administration to 21 days after STZ administration), Change II: during observation periods (from Day 0 to Sacrifice); * $p < 0.01$ and ** $p < 0.05$ compared to the sham group using an MW test; # $p < 0.01$ compared to the vehicle control groups using an MW test.

G0, sham: sterilized and distilled water (5 mL/kg)-treated group; G1, control: moderate diabetes control group; G2: glybenclamide (3 mg/kg)-treated group or Simvastatin (10 mg/kg)-treated group; G3: β-glucan (62.5 mg/kg)-treated group; G4: β-glucan (125 mg/kg)-treated group; HC: severe diabetes control group; HG: β-glucan (62.5 mg/kg)-treated group after severe diabetes; LC: severe diabetes control group; LG: β-glucan (62.5 mg/kg)-treated group after slight diabetes

Table 4. Changes in serum LDL and HDL levels after STZ administration and drug administration.

Serum Chemistry I	Serum LDL levels (mg/dl)	Serum HDL levels (mg/dl)
G0	5.67±2.16	56.62±9.49
G1 [PCA][1)]	11.67±3.67** [105.88]	37.95±6.43** [-32.97]
G2 [PCB][2)]	6.17±0.75# [-47.14]	46.17±3.60**,## [21.65]
G3 [PCB]	6.50±1.87## [-44.29]	52.02±9.60## [37.07]
G4 [PCB]	6.00±2.37## [-48.57]	52.47±8.86## [38.25]
HC [PCA]	30.60±10.64* [440.00]	27.12±14.80* [-52.10]
HG [PCB]	14.00±6.00**,## [-54.25]	54.96±20.83 [102.65]
LC [PCA]	12.40±4.39** [118.82]	40.32±6.16** [-28.78]
LG [PCB]	6.40±2.07## [-48.39]	51.92±4.19## [28.77]

N = 5 or 6; (mean ± SD), mg/dL; Group identification as listed in Table 1; [1)]PCA, percentage (%) change vs. sham (G0); [2)]PCB, percentage (%) change vs. the vehicle control groups (G1, HC, or LC); * $p < 0.01$ and ** $p < 0.05$ compared to the sham group using an MW test; # $p < 0.01$ and ## $p < 0.05$ compared to the vehicle control groups using an MW test.

G0, sham: sterilized and distilled water (5 mL/kg)-treated group; G1, control: moderate diabetes control group; G2: glybenclamide (3 mg/kg)-treated group or Simvastatin (10 mg/kg)-treated group; G3: β-glucan (62.5 mg/kg)-treated group; G4: β-glucan (125 mg/kg)-treated group; HC: severe diabetes control group; HG: β-glucan (62.5 mg/kg)-treated group after severe diabetes; LC: severe diabetes control group; LG: β-glucan (62.5 mg/kg)-treated group after slight diabetes

Table 5. Changes in serum triglyceride and T-CHOL levels after STZ administration and drug administration.

Serum Chemistry II	Serum Triglyceride levels (mg/dl)	Serum T-CHOL levels (mg/dl)
G0	18.33±7.45	62.50±14.27
G1 [PCA][1)]	528.83±291.47* [2784.55]	99.83±12.06* [59.73]
G2 [PCB][2)]	183.50±83.50*,## [-65.30]	54.17±7.41# [-45.74]
G3 [PCB]	217.50±137.14* [-58.87]	59.00±19.45# [-40.90]
G4 [PCB]	177.50±98.13*,## [-66.44]	60.17±13.04# [-39.74]
HC [PCA]	815.20±409.06* [4346.55]	162.00±48.35* [159.20]
HG [PCB]	330.20±184.62*,## [-59.49]	99.00±27.01** [-38.89]
LC [PCA]	316.20±149.51* [1624.73]	72.80±11.03 [16.48]
LG [PCB]	76.00±19.99*,# [-75.96]	71.40±5.55 [-1.92]

N = 5 or 6; (mean ± SD), mg/dL; Group identification as listed in Table 1; [1)]PCA, percentage (%) change vs. sham (G0); [2)]PCB, percentage (%) change vs. the vehicle control groups (G1, HC, or LC); * $p < 0.01$ and ** $p < 0.05$ compared to the sham group using an MW test; # $p < 0.01$ and ## $p < 0.05$ compared to the vehicle control groups using an MW test.

G0, sham: sterilized and distilled water (5 mL/kg)-treated group; G1, control: moderate diabetes control group; G2: glybenclamide (3 mg/kg)-treated group or Simvastatin (10 mg/kg)-treated group; G3: β-glucan (62.5 mg/kg)-treated group; G4: β-glucan (125 mg/kg)-treated group; HC: severe diabetes control group; HG: β-glucan (62.5 mg/kg)-treated group after severe diabetes; LC: severe diabetes control group; LG: β-glucan (62.5 mg/kg)-treated group after slight diabetes.

Conclusion: Although the present study found that β-glucan did not exert significant hypoglycemic effects in a rat model of STZ-induced diabetes, it attenuated diabetic complications related to diabetic hyperlipemia at doses of 62.5 mg/kg/day or more. Therefore, β-glucan may be a novel hypolipemic agent that could be used for the specific treatment of diabetic complications related to hyperlipemia, regardless of the severity of disease. Because similar or slight efficacies were detected in the β-glucan groups compared to the Simvastatin group – in terms of ameliorating the altered LDL, triglyceride, and T-CHOL levels – and because the most favorable effect occurred in terms of changing HDL levels, the effective dose of β-glucan should be considered as being below 62.5 mg/kg/day, regardless of the severity of diabetic hyperlipemia.

Conflict of interest: The authors declare no conflict of interest.

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