Expression of receptor advanced glycation end products (RAGE) and histological picture of pancreatic β-cells of streptozotocin-induced diabetic rats after yellow soybean powder suspension (*Glycine max*) administration

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ABSTRACT

Diabetes mellitus is a multisystemic disease characterized by hyperglycemia due to defects in insulin secretion and action, or both. *Hyperglycemia* is the most important factor in the onset and progress of diabetic complications. Hyperglycemia increases the expression of receptor for advanced glycosylation end products (RAGE) which leads to pancreatic β-cells damage. Yellow soybean (*Glycine max*) is reported to contain isoflavones which have various biological properties including antidiabetes. Dietary soybean can prevent the progression of diabetic complications. This study was conducted to investigate the effects of yellow soybean powder suspension on expression of RAGE and pancreatic β-cells damage of diabetic rats. Thirty streptozotocin (STZ)-induced diabetic Sprague Dawley male rats aged 11-12 weeks with body weight 200-250 g were used in this study. The rats were divided into 5 groups with 6 rats in each group. Group 1 was non diabetic rats. Group 2 was diabetic rats without treatment. Group 3-5 were given yellow soybean powder suspension of 400, 800 and 1600 mg/kg BW for four weeks, respectively. At the end of the experiment, pancreases tissues were removed for examination of RAGE expression and pancreatic β-cells. The results showed that yellow soybean powder suspension ingestion signifantly decreased blood glucose level of diabetic rats toward normality ($p < 0.05$). Moreover, the percentage of RAGE expression on Group 3 ($50.01 \pm 2.75\%$) and Group 2 ($53.03 \pm 4.02\%$) were not significantly different ($p > 0.05$). Meanwhile, the percentage of RAGE expression on Group 4 ($42.43 \pm 4.08\%$) and Group 5 ($40.62 \pm 3.42\%$) were significantly lower than Group 2 ($p < 0.05$). The percentage of pancreatic β-cells on Group 2 ($10.04 \pm 1.56\%$) was not significantly different compared to Group 3 ($8.61 \pm 0.81\%$) ($p > 0.05$), whereas the percentage of pancreatic β-cells in Group 4 ($16.78 \pm 7.79\%$) and in Group 5 ($22.03 \pm 11.51\%$) were significantly higher than Group 2 ($p < 0.05$). In conclusion, yellow soybean powder suspension can decrease RAGE expression and prevent pancreatic β-cells damage on STZ-induced diabetic rats.

ABSTRAK

Diabetes melitus adalah penyakit multisistemik ditandai dengan hiperglikemia akibat kerusakan sekresi dan atau aksi insulin. Hiperglikemia merupakan faktor paling penting bagi timbulnya dan perkembangan komplikasi diabetes. Hiperglikemia dapat meningkatkan ekspresi *receptor for advanced glycosylation end products* (RAGE) dan menyebabkan kerusakan sel β pankreas. Kedelai kuning (*Glycine max*) dilaporkan mengandung isoflavon yang mempunyai berbagai aktivitas biologis

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termasuk antidiabetes. Diet kedelai dapat mencegah perkembangan komplikasi diabetes. Penelitian ini dilakukan untuk mengkaji efek suspensi bubuk kedelai kuning terhadap ekspresi RAGE dan kerusakan sel-β pankreas tikus diabetes. Tigapuluh tikus jantan Sprague Dawley diabetes yang diinduksi streptosotosin (STZ) berumur 11-12 minggu dengan berat 200-250 g digunakan dalam penelitian ini. Tikus dibagi 5 kelompok, masing-masing kelompok 6 tikus. Kelompok 1 adalah tikus normal. Kelompok 2 adalah tikus diabetes tanpa perlakuan. Kelompok 3-5 adalah tikus diabetes diberi suspensi bubuk kedelai kuning berturu-turut dosis 400, 800 dan 1600 mg/kg BB selama 4 minggu. Di akhir penelitian, jaringan pankreas diambil untuk pemeriksaan RAGE dan sel β pankreas. Hasil penelitian menunjukkan bahwa pemberian suspensi bubuk kedelai kuning menurunkan secara nyata kadar gula darah tikus diabetes hingga normal (p < 0,05). Selain itu, persentase ekspresi RAGE Kelompok 3 (50,01 ± 2,75%) dengan Kelompok 2 (53,03 ± 4,02%) tidak berbeda nyata (p > 0,05). Sedangkan, persentase ekspresi RAGE Kelompok 4 (42,43 ± 4,08%) dan Kelompok 5 (40,62 ± 3,42%) lebih rendah secara nyata dengan Group 2 (p < 0,05). Persentase sel β pankreas Kelompok 2 (10,04 ± 1,56%) dengan Kelompok 3 (8,61 ± 0,81%) tidak berbeda nyata (p > 0,05). Sedangkan persentase sel β pankreas Kelompok 4 (16,78 ± 7,79%) dan Kelompok 5 (22,03 ± 11,51%) lebih tinggi secara nyata dari Kelompok 2 (p < 0,05). Dapat disimpulkan, suspensi bubuk kedelai kuning dapat menurunkan ekspresi RAGE dan mencegah kerusakan sel-β pankreas pada tikus diabetes yang diinduksi STZ.

Keywords: pancreatic β cells - Langerhans islets - streptozotocine - diabetic rats - soybeans

INTRODUCTION

Diabetes mellitus (DM) is a multi-systemic disease characterized by hyperglycemia as the result of defects in insulin secretion, action, or both.[1] The prevalence of diabetes worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes was projected to rise from 171 million in 2000 to 366 million in 2030. In Indonesia, the people with DM reached 8.4 million in 2000 and it will be 21.3 million in 2030. Indonesia is the fourth country with the highest people with diabetes in the world after India, China dan USA.[2]

Hyperglycemia is the most important factor in the onset and progress of diabetic complications. Hyperglycemia is generally accepted to be the major cause of diabetic microvascular complications including nephropathy, neuropathy and vision disorders such as retinopathy, glaucoma, cataract and corneal disease. Moreover, hyperglycemia also plays an important role in the development of macrovascular diseases including cardio-vascular disease, stroke and peripheral vascular disease which can lead to ulcers, gangrene and amputation.[3,4]

Hyperglycemia causes cells oxidative damage by generation of reactive oxygen species (ROS) produced mainly through the glycation reaction resulting in advanced glycosylation end products (AGEs), a non-enzymatic reaction product of glucose.[5] Advanced glycosylation end products have been implicated in an increasing number of diabetic complications that result from, and in, oxidative damage due to ROS generation.[6,7] Advanced glycosylation end products are capable of damaging DNA either directly or by the generation of ROS through the activation of receptor for AGEs (RAGE).[7,8] Advanced glycosylation end products may directly influence the structural integrity of the vessel wall and underlying basement membranes through excessive cross linking of matrix molecules such as collagen and disruption of matrix-matrix and matrix-cell interactions.[9,10] In addition, binding of AGEs to RAGE in many cell types provokes a range of pathophysio-logical responses linked to the downstream activation of NFkB and other signalling

pathways that lead to ROS generation and certain pro inflammatory responses.[11,12]

Prevention and regeneration of further pancreatic β-cells damage are important in the management of DM to prevent its complications. The management of DM is a long term treatment to control hyperglycemia and hypoinsulinemia. The development and application of natural product are expected to control hyperglycemia, prevent pancreatic β-cells damage, repair insulin synthesis and secretion, and improve insulin resistance. Several studies have been conducted to evalute effect of natural products to treat DM in animal model including black cumin (*Nigella sativa*),[13,14] cinnamon (*Cinnamomum zeylanicum*),[15] mahogany (*Swietenia mahagoni*),[16] and soybean (*Glycine max*).[17]

Glycine max or yellow soybean, locally known as *kedelai kuning*, is one of legumes used empirically to prevent and treat diseases associated with DM. Elevated intake of soybean legumes has been linked to a decreased risk of glucose intolerance[18] and type 2 diabetes.[19,20] Soyabean is reported to contain isoflavones which have various biological properties including antidiabetes. Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in diabetic rats.[21] Moreover, dietary soybean can prevent the progression of diabetic rats and therefore neprhopathy can be prevented.[22] The objective of this study was to investigate the effects of a yellow soybean powder suspension ingestion on RAGE expression and on pancreatic β-cells damage of streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Preparation of the yellow soybean powder suspention

Yellow soybeans were purchased from a local market in Yogyakarta District. Soybean seeds were washed with water and dried in an oven at 60°C for 30 minutes. Dried soybean seeds were then powdered with a blender. Soybean powder suspentions were then prepared in two mL water of each dose. Three different doses of suspentions were prepared for each rat group i.e. 400, 800 and 1600 mg/kg BW for this study.

Animals and induction of diabetes

Thirty male Sprague Dawley rats (*Rattus novergicus*) aged 11-12 weeks with body weight 200-250 g obtained from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta were used in this study. The rats were housed at room temperature under 12 hour cycles of dark and light, were fed a standard food and provided an access to water *ad libitum*. After an acclimatization period of one week, rats were divided into 5 groups with 6 rats in each group. Group 1 as normal contol was non diabetic rats. Group 2 as negative control was diabetic rats without treatment. Group 3-5 as treatment groups were given yellow soybean powder suspension of 400, 800 and 1600 mg/kg BW for each group, respectively.

Diabetic rats were induced by a single intraperitoneal injection of STZ at dose 60 mg/kg BW in 0.01 M at pH 4.5. Blood samples were collected from orbital sinus and blood glucose level was determined by using GOD PAP method before (day 0) and three days after STZ induction (day 3). Diabetic rats were confirmed if blood glucose level was >200 mg/dL. Two weeks after diabetic rats were confirmed, yellow soybean powder suspension were then ingested for four weeks.

Rats were sacrified four weeks after the suspension ingestion and after blood glucose level determination (day 52). The rats were anesthetized using diethyl ether. A transverse

abdominal incision was made and pancreases and deudenom were removed from the rat. The pancreases tissues were then divided into two parts, the first part for immunohistochemical analysis of RAGE expression and the second part for histological examination of pancreatic β-cells. Fixed pancreases tissues were processed routinely for paraffin embedding. Serial sections of 5 ìm for each pancreas tissue sample were cut and placed on a poly L-lysine coated glass microscope slide.

Immunohistochemical analysis of RAGE expression

The pancreas tissue sections were deparaffinized, rehydrated using a sequence of xylene, graded ethanol, water then washed in PBS. Briefly, antigen unmasking was performed by incubating the sentions with a 50% trypsin : 50% vercene solution for 2 minutes. After being washed in water for 20 minutes, the sections were permeabilized in 0.1% Triton X-100 for 20 minutes, and rinsed in PBS (phosphate buffered saline solution). The sections were then blocked with endogenous peroxidase by incubating the slides with a 3% H_2O_2 : 0.01% avidin for 5 minutes and washed in water. The next day, the sections were washed again in PBS and then incubated with primary monoclonal antibody anti-mouse RAGE (25 µg/mL) overnight at 4°C. Following the incubation, the sections were washed in PBS and then incubated with a biotinylated secondary antibody for 30 minutes and washed again in PBS. The sections were incubated with a streptavidine peoxidase for 30 minutes at room temperature, washed in PBS and incubated with diamino-benzidine for 5-10 minutes. The sections were then lightly countersatined by haematoxylin, incubated for 30 second at room temperature and washed in water. The sections were then dried and coverslipped.

All sections were then examined and evaluated using light microscope. The RAGE expression in pancreatic β-cells of Langerhans islets was identified by a brown color of the cell, while a blue color of a cell indicated no expression of the RAGE. The RAGE expression was observed on five Langerhans islets and percentage of the RAGE expression was calculated using Cemek[23] formula after modification as follows:

$$\text{Percentage of Rage expression in Langerhans islets} = \frac{\text{Total RAGE} +}{\text{Total cells}} \times 100$$

Histological examination of pancreatic β-cells

The pancreas tissue sections were deparaffinized and incubated with a mordant solution overnight at 37°C. The sections were rinsed in water and treated with an acidified potassium permanganate solution for 3 minutes. After being washed again in water, the sections were treated with 2-5% sodium bisulphite for 1 minute, washed well in water and in 70% alcohol. The sections were then stained with the Victoria Blue solution for 15 minutes at room temperature, washed in water and differentiated with 70% alcohol. The sections were then washed in running tap water fo 1 minute, dehydrated with 30% alcohol, cleared with xylol solution I, II and III and coverslipped.

All sections were then examined and evaluated using light microscope. The existence of pancreatic β-cells in Langerhans islets was demonstrated by blue cells. The existence of pancreatic β-cells was observed on five Langerhans islets and its percentage was calculated using Cemek[23] formula after modification as follows:

$$\text{Percentage pancreatic } \beta \text{ - cells} = \frac{\text{Total b cells} +}{\text{Total cells} +} \times 100$$

Statistical analysis

Data were presented as mean ± standard deviation (SD) and analyzed using SPSS. The difference between blood glucose levels, RAGE expression in Langerhans islets and pancreatic â cell numbers between treatment groups with control groups were analyzed using Kruskal-Wallis test. The significant differences between groups were analyzed with Mann-Whitney test. A p value <0.05 was accepted as statistically significant.

RESULTS

Blood glucose level of normal (Group 1), diabetic (Group 2), yellow soybean powder suspension diabetic (Group 3-5) groups on day 0, 3, and 52 after STZ induction are shown in TABLE 1. Before STZ injection (day 0), the blood glucose level of rats in all groups were in normal level. However, three days after STZ induction (day 3) rats in all groups had blood glucose level higher than 200 mg/dL indicating that rats were diabetic. Yellow soybean powder suspension ingestion for four weeks (Group 3-5) signifantly decreased the blood glucose level of diabetic rats toward normality (< 200mg/dL) (p<0.05), whereas the blood glucose level of diabetic rats (Group 2) did not significantly decrease on day 52 after STZ injection (p>0.05).

TABLE 1.   Blood glucose level of normal, diabetic, yellow soybean powder suspension rat groups on day 0, 3, and 52 after STZ induction

Groups	n	Blood glucose level (Mean ± SD mg/dL)		
		Day 0	Day 3	Day 52
1. Normal rats + aquadest	4	74.79±1.00	75.43 ±1.30	78.40±1.31
2. Diabetic rats + aquadest	3	76.05±1.80	219.63±2.40	212.40±15.70
3. Diabetic rats + suspension 400 mg/kg BW	5	76.38+1.40	213.36+6.80	188.61+1.80
4. Diabetic rats + suspension 800 mg/kg BW	5	75.21±1.70	219.61±5.90	184.70±1.50
5. Diabetic rats+ suspension 1600 mg/kg BW	3	74.22±0.90	220.41±1.30	179.32±2.30

An example of RAGE expression in Langerhans islets from normal rats (Group 1), diabetic rats without treatment (Group 2), and diabetic rats after ingestion of yellow soybean powder suspension (Group 3-5) is shown in FIGURE 1. The positive RAGE expression was identified by a brown color of the cell (arrow heads). Mean percentage of RAGE expression in Langerhans islets from each group is presented in FIGURE 2. The lowest of RAGE expression was observed in Group 1 (5.71± 1.45%), while the highest was observed in Group 2 (53.03±4.02%). The mean percentage of the RAGE expression in Group 3 (50.01±2.75%) was lower than Group 2, however it was not significantly different (p>0.05), whereas, the mean percentage of the RAGE expression in Group 4 (42.43±4.08%) and Group 5 (40.62±3.42%) were significantly lower than Group 2 (p<0.05). No significant difference in the mean percentage of the RAGE expression between Group 4 and Group 5 was observed in this study.
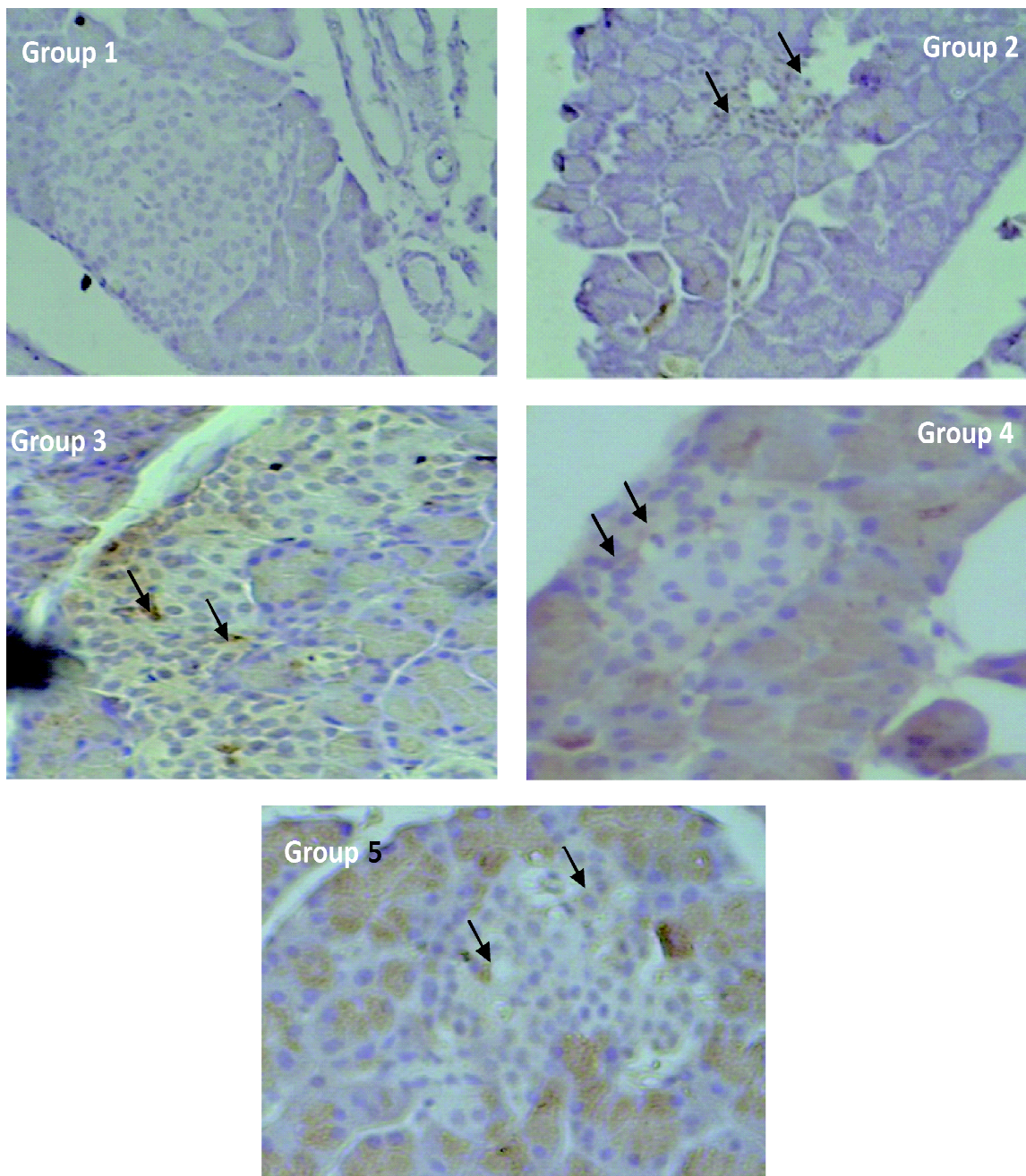
FIGURE 1. RAGE expression in Langerhans islets in normal rats (Group 1), diabetic rats without treatment (Group 2), and diabetic rats after ingestion of yellow soybean powder suspension (Group 3-5). Positive expression (arrow heads) is shown by brown staining. Magnification of 270x
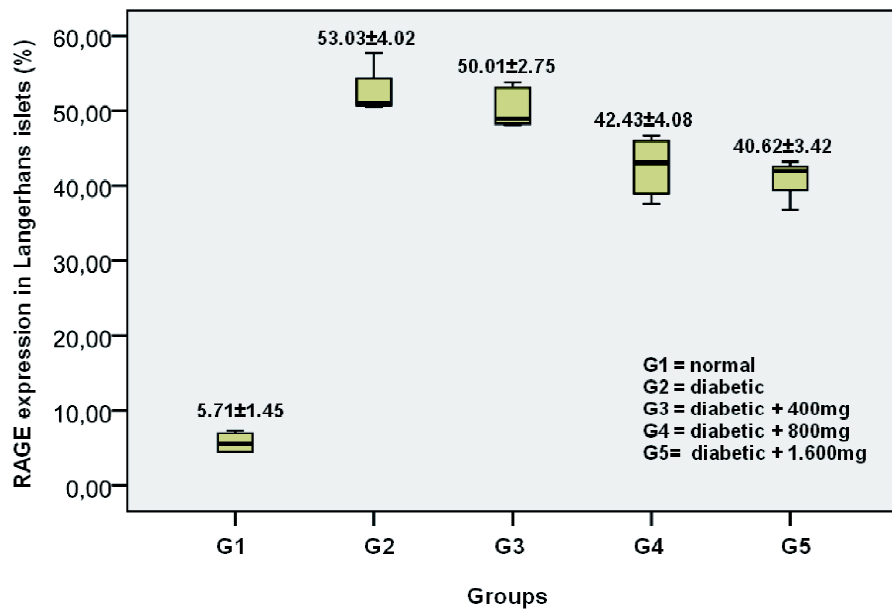
FIGURE 2. Percentage of RAGE expression in Langerhans islets in normal and diabetic rats without and after ingestion of yellow soybean powder suspension

An example of histology of pancreatic β-cells in Langerhans islets after Victoria Blue staining in normal rats (Group 1), diabetic rats without treatment (Group 2), and diabetic rats after ingestion of yellow soybean powder suspension (Group 3-5) is shown in FIGURE 3. Positive β-cells (arrow heads) is shown by blue staining. The highest of mean percentage of pancreatic β-cells was observed in Group 1 (40.21±8.68%), while the lowest was observed in Group 3 (8.61±0.81%). However, it was not significantly different compared to Group 2 (10.04±1.56%) as control diabetic rats without ingestion of yellow soybean powder suspension (p>0.05). Meanwhile, the mean percentage of pancreatic β-cells in Group 4 (16.78±7.79%) and in Group 5 (22.03±11.51%) were significantly higher than Group 2 (p<0.05). No significant difference in mean percentage of pancreatic β-cells between Group 4 and 5 was observed in this study.

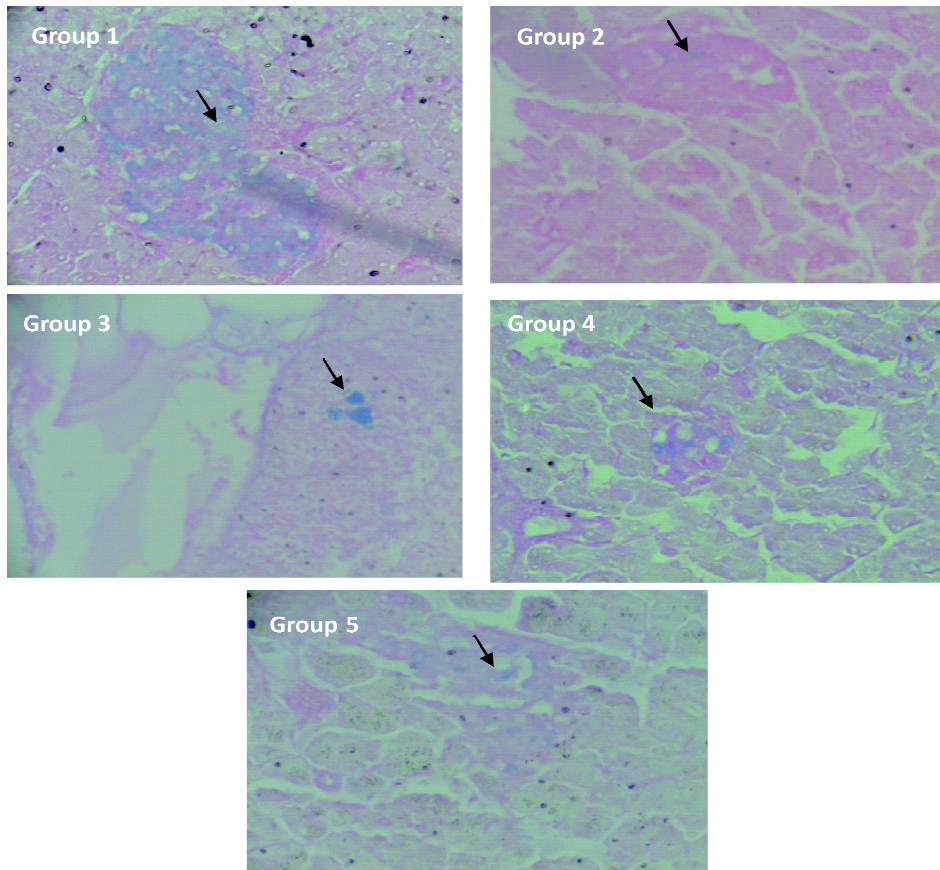FIGURE 3.   Histology of pancreatic β-cells in Langerhans islets after Victoria Blue staining in normal rats (Group 1), diabetic rats without treatment (Group 2), and diabetic rats after ingestion of yellow soybean powder suspension (Group 3-5). Posistive β-cells (arrow heads) is shown by blue staining. Magnification of 540x
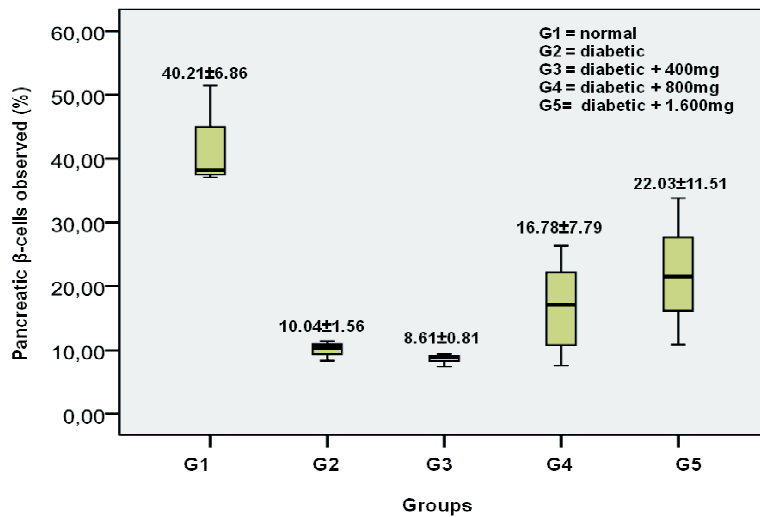

FIGURE 4.   Pancreatic β-cells observed in Langerhans islets in normal and diabetic rats without and after ingestion of yellow soybean powder suspension

DISCUSSION

In this study, it was shown that yellow soybean powder suspension ingestion at dose of 800 and 1600 mg/kg BW for four weeks significantly decreased the expression of RAGE in pancreatic β-cells of STZ-induced diabetic rats. Moreover, this suspension can prevent the â-cells damage due to STZ-induction. The effect of this suspension on inhibition of RAGE expression and prevention of β-cells damage can improve glycemic control as demonstrated with the decrease of blood glucose level of the diabetic rats.

The effect of yellow soybean on glycemic control both in animal model and human has been reported by some authors. Zimmermann *et al.*[24] reported that consumptions of soybean can improve glucose heomeostasis and prevent the progression of diabetes in the db/db mice. Choi *et al.*[25] also reported that soybean may improve glycemic control and prevent the progression of diabetic nephropathy in rats. A soybean diet may prevent the weight loss and morphological disruption of the kidney associated with diabetes in rats. In addition, Xu *et al.*[26] suggested that soybean fibers can control blood glucose level, blood-lipid level by improving their metabolisms. Therefore, it can protect liver and renal damage of diabetic mice.

Soybean is reported to contain rich isoflavones which are well known as antioxidant with various biological properties including antidiabetes. Glyceollins, soy isoflavone phytoalexins, improve oral glucose disposal in prediabetic rats by increasing both the insulin-mediated and the basal, insulin-independent, glucose uptake by adipocytes.[27] Biochanin A, another soy isoflavone, showed antihyperglycemic effect on STZ-diabetic rats by decreasing activities of gluconeogenic enzymes such as glucose 6-phosphatase and fructose 1,6-biphosphatase and by increasing glucokinase, glucose 6-phosphate dehydro-genase.[28]

Possible mechanisms underlying the effect of the yellow soybean on inhibition of RAGE expression and preservation of pancreatic β-cell damage are thought through antioxidant activities of soy isoflavones. Hyperglycemia causes the pancreatic β-cells oxidative damage by generation of ROS produced either directly or through the glycation reaction resulting in advanced glycosylation end products (AGEs). Moreover, AGEs are capable of damaging the pancreatic β-cells either directly or by the generation of ROS through the activation of RAGE.[7,8] As antioxidant, soy isoflavones can neutralize ROS produced under hyperglycemic conditions and maintain the integrity of cell membranes, and therefore prevent the pancreatic β-cells oxidative damage.[29]

CONCLUSION

In conclusion, yellow soybean powder suspension ingestion for four weeks can significantly decrease the expression of RAGE of pancreatic β-cells on STZ-induction diabetic rats. Moreover, yellow soybean powder suspension can prevent the pancreatic β-cells damage due to STZ-induction.

ACKNOWLEDGEMENTS

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