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Original Article

The Evaluation of Dietary Black Soybean and Purple Sweet Potato on Insulin Sensitivity in Streptozotocin - Induced Diabetic Rats

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ABSTRACT

Introduction: Hyperglycemia mediates the production of excess free radicals and reduces endogenous antioxidant in type 2 diabetes mellitus (T2DM). Black soybean (BSB) is rich in antioxidant mainly from isoflavones, whereas the antioxidant of purple sweet potato (PSP) comes from high anthocyanin. The study aimed to evaluate the efficacy of BSB, PSP, and its combination on malondialdehyde (MDA), superoxide dismutase (SOD) concentrations, insulin and insulin receptor substrate-1 (IRS-1) expression in T2DM rats. **Methods:** T2DM induced by high-calorie diet for five weeks and then injected with a low dose of streptozotocin (30 mg/kg BW) intraperitoneally. The DM rats then treated with black soybean (DM + BSB), purple sweet potato (DM + PSP) and the combination of BSB and PSP 1:3, 2:2, 3:1 respectively (DM + C1-3). Treatments were given for thirty days. The effect of BSB, PSP and its combination evaluated by measuring SOD and MDA, necrosis in pancreas evaluated through hematoxylin-eosin (HE) and insulin and IRS-1 expression in pancreas through immunohistochemistry-fluorescence (IHC-F). **Results:** Our result indicated that there were no significant differences of BSB and the combination in decreasing MDA concentrations. The ratio of BSB and PSP combination of 2:2 increase SOD towards near normal, decrease necrosis, and improve insulin and IRS-1. **Conclusion:** The combination of BSB and PSP had the potential to improve insulin sensitivity through the increase of SOD, reduce necrosis, and improve insulin and IRS-1 expression.
Keywords: Antioxidant, Malondialdehyde, Necrosis, Superoxide Dismutase, T2DM.

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is characterized by insulin resistance and metabolic disturbance accompanied by an increase in blood glucose level.[1] T2DM is a heterogeneous disease characterized by an abnormality in carbohydrate, lipid and protein metabolism consisting of pancreatic β-cell function disorder and insulin resistance. Insulin resistance causes chronic hyperglycemia and in a severe long-term lead to complication.[2] Lifestyle changes such as sedentary lifestyle and unhealthy diet take great responsibility causes T2DM.

Hyperglycemia is a common diabetic marker that decreases the concentration of endogenous antioxidant and facilitates reactive oxygen species (ROS) overproduction.[3] ROS will trigger the formation of malondialdehyde (MDA) through lipid peroxidation process. Lipid peroxidation process will increase MDA concentration and initiate the various degenerative diseases. Furthermore, high MDA concentration can be used as a marker of oxidative damage.[3]

Pancreatic β-cells is known susceptible against oxidative stress due to a smaller amount of antioxidant enzyme.[4] Moreover, pancreatic β-cells are essential to maintain glucose homeostasis via insulin regulation.[5] In DM condition, superoxide dismutase as an endogenous antioxidant fails to suppress the overproduction of free radical.[6] An ever-increasing free radical causes a decrease in insulin sensitivity and leads to dysfunction in pancreatic β-cell and hyperglycemia.[7]

Dietary natural source is growing interested in recent past year due to its beneficial effects for health.[8] Black soybean (BSB) contains a large number of antioxidant in the seed coats or the whole seeds and has been used in China as a good source of diet to prevent various diseases.[9] However, the use of BSB in Indonesia is not optimal and only limited to soy sauce production. BSB has the antioxidant capacity and total phenolic compound higher than yellow soybean,[10] capable of suppressing free radicals,[11] and improving the quality of spermatozoa in mice given with high-fat diet.[12] In addition, soybean is a source of protein but low in carbohydrate, thus it will need other dietary source, such as purple sweet potato (PSP).

PSP is an agricultural product that rich in carbohydrate, fiber, vitamin and mineral.[13] PSP rich-anthocyanin content which played a role as a strong free radical suppressor.[14] In addition, PSP has a low glycemic index thus it cannot increase blood glucose significantly.[15] PSP from Kawi Mountain is a local sweet potato produced in Kawi Mountain, Malang Regency and contains anthocyanin of 100-220 mg/100 g.[16] However, information on the effect of BSB and PSP combination on DM model experimental animals is still limited. Antioxidant

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content in BSB and PSP is expected to work synergistically to suppress free radical, increase the antioxidant concentration and improve insulin sensitivity in T2DM rats.

MATERIALS AND METHODS

Reagents

Chemical materials used were Streptozotocin (41910012-3: Bioworld), insulin (sc-8033: Santa Cruz), IRS-1 (sc-8038: Santa Cruz) and Goat anti-Rat IgG-FITC (sc-2011: Santa Cruz).

Black Soybean and Purple Sweet Potato Preparations

Black soybean *var.* Detam-1 was obtained from Indonesian Legumes and Tuber Crop Research Institute (ILETRI), Karangploso, Malang. Purple sweet potato was obtained from a plantation in Kawi Mountain. Both BSB and PSP were clean washed, air dried and grounded into flour. The process was done at the Balai Materia Medika, Batu City.

Streptozotocin Injection Diabetes Mellitus-Model Rats

Thirty-five male *Wistar* rats were placed in plastic cages, three rats each cage, in a room with a temperature of 22-25°C and the light-dark cycle of 12/12 h. Rats were adapted for a week by giving normal diet and drink *ad libitum*. Rats were made obese by giving high-calorie diet using Hi-Gro of Pokphand 551.[17] Five weeks after diet manipulation, rats were fasted overnight and then injected intraperitoneally with a low dose of Streptozotocin (STZ) (30 mg/kg BW in 0.1 citrate-buffered salines, pH 4.5).[18] After a week, STZ injection was repeated once again and then the glucose was checked every week after the injection of STZ. Rats with blood glucose level >200 mg/dL after the diet manipulation and STZ injection were considered DM.

Experimental Animals Treatment

Rats were divided into two large groups, N (healthy rats) and DM. DM rats were randomly divided into five groups, DM (T2DM); DM + BSB (T2DM + black soybean), DM + PSP (T2DM + purple sweet potato), DM + C1-3 (T2DM + combination of BSB and PSP 1:3, 2:2, 3:1). After 30 days treatments, rats were sacrificed using diethyl ether followed by cervical dislocation. The blood is taken from the heart and kept for 30 min in room temperature to precipitate the blood cells. After that, blood sample was centrifuged at 3000 rpm for 15 min. Blood serum was collected and stored at -20°C until ready to use. All animal welfare had been approved by the ethical committee of the Brawijaya University with approval number 878-KEP-UB.

Malondialdehyde Measurement

Serum sample of 100 µL added with 100% TCA of 100 µL, Na Thio 1% of 100 µL, HCl 1 N of 250 µL and distilled water of 450 µL and then homogenized using vortex. The next stage was heating at a temperature of 100°C for 10 min and centrifuged at 3000 rpm for 5 min. 700 µl supernatant was taken and added with distilled water until 2800 µL. The measurement was conducted using Spectrophotometer UV-1601 at 532.8 nm and 1,1,3,3-tetramethoxypropane (TMP) solution was used as a standard.

SOD Measurement

Serum sample of 100 µL put into a test tube and added with 100 µL xanthine, 100 µL xanthine oxidase, 100 µL NBT and 600 µL PBS. After that, the mixed added with PBS until 2000 µL and homogenized using vortex. The tube was covered with parafilm and incubated in a waterbath at a temperature of 30°C for 30 min. After heating, the centrifuge at 3500 rpm for 10 min and supernatant taken and removed to new test tubes. Sample absorbance measured using spectrophotometer of Shimadzu UV-visible spectrophotometer UV-1601 at 580 nm.

Histopathological Assessment

The pancreas was cleaned using PBS solution three times and kept in 10% formalin. Pancreas tissues were cut transversely, processed as per routine procedure and dehydrated using alcohol. Next, the pancreas was cleaned using xylene and embedded using paraffin. Pancreatic paraffin block tissues were cut in 3 µm of thickness using microtome and stained using hematoxylin-eosin (HE). The examination of pancreatic histopathological changes conducted in microscopic using Olympus Type BX21 with 400x magnification. The percentage of pancreatic β-cells percentage was calculated from 100 pancreatic β-cells observed in five respective fields.

Immunohistochemistry of Pancreas

Pancreas embedded with paraffin were cut in 3 µm of thickness and put on poly-L-Lysine slides and incubated in the oven at a temperature of 40°C overnight. Pancreatic slides rehydrated and deparaffinated using xylol and alcohol. The slides then treated with 10 mM citrate buffer pH 6.0 and incubated in the oven at a temperature of 120°C for 15 min. The slides blocked with BSA 2% (in PBST pH 7.4) for an h at room temperature and washed with PBST three times. The slides were given with primary insulin antibody (cat: SC-8033) or IRS-1 (sc-8038: Santa Cruz) in BSA2% (1:500) and then incubated for an h at room temperature. The slides then washed with PBST for three times and then dripped with secondary antibody Goat anti-Rat IgG-FITC (Cat: sc-2011) in dark condition and incubated for an h then washed with PBST for three times for 10 min each. The preparations were dried and dripped with 10% glycerol and covered with cover glass. Preparations observed using a fluorescence microscope (Olympus IX81) connected with Olympus Fluoview Software and the calculation of intensity/mm^2 of insulin and IRS-1 expression in pancreatic β-cells with 100x magnification.

Statistical Analysis

Data obtained were analyzed using one-way ANOVA with Microsoft Excel. If a significance occurred ($p < 0.05$), the test followed by a post hoc test of Duncan Multiple Range Test (DMRT). Data presented as the mean ± standard deviation (SD).

RESULT

Comparison of MDA concentration between treatments with BSB, PSP, and the combination of both in rat T2DM model

Our result indicated that MDA concentration in DM groups was significantly increased ($p < 0.05$) compared to normal group (Figure 1). BSB treatment and the combination of BSB and PSP were not significantly decrease the MDA concentration compared to the DM group. Interestingly, PSP treatment alone significantly increased the MDA concentration compared to the DM group (Figure 1).

Comparison of SOD concentration between treatments with BSB and PSP and the combination of both in rat T2DM model

Our result indicated that SOD concentration significantly decreased ($p < 0.05$) in DM groups compared to normal group. Treatment with combination of BSB and PSP in a ratio of 2:2 increased SOD concentration towards near normal (Figure 2). Interestingly, treatment with BSB and PSP alone were failed to increase SOD concentration compared to the DM group.

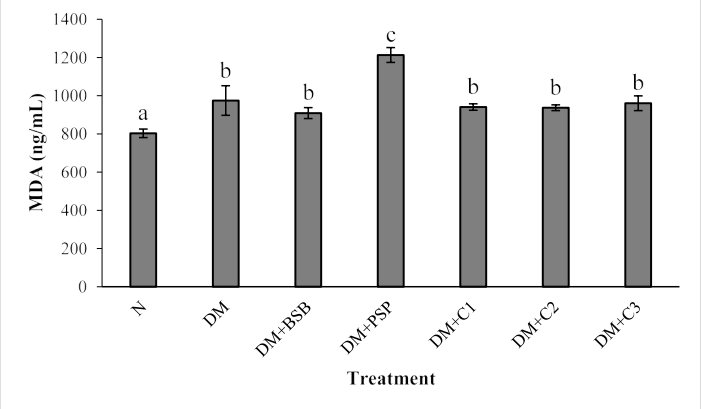

Figure 1: Treatments with BSB, PSP, or the combination of both did not significantly decreased MDA concentration in T2DM-model rats. Data represented as mean ± SD. The different letter indicates a significantly different ($p < 0.05$) based on the DMRT post hoc test. Note: N = normal rats; DM = T2DM rats; DM + BSB = T2DM rats + black soybean; DM + PSP = T2DM rats + purple sweet potato; DM + C1 = T2DM rats + combination of BSB and PSP (1:3); DM + C2 = T2DM rats + combination of BSB and PSP (2:2); DM + C3 = T2DM rats + combination of BSB and PSP (3:1).

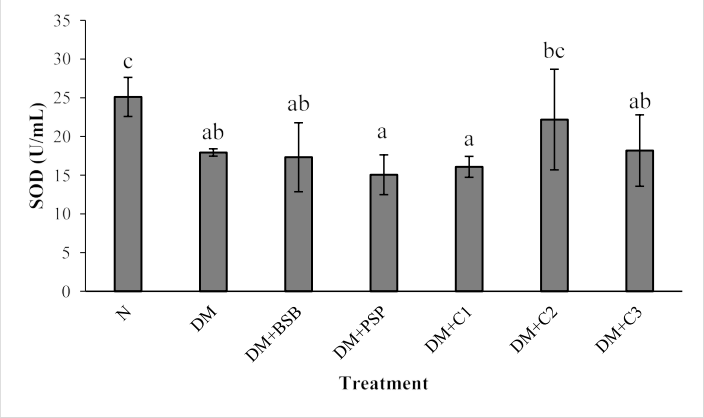

Figure 2: Treatment with combination of BSB and PSP in ratio 2:2 was able to increased SOD concentration on T2DM-model rats. Data represented as mean ± SD. The different letter indicates a significantly different ($p < 0.05$) based on the DMRT post hoc test. Note: N = normal rats; DM = T2DM rats; DM + BSB = T2DM rats + black soybean; DM + PSP = T2DM rats + purple sweet potato; DM + C1 = T2DM rats + combination of BSB and PSP (1:3); DM + C2 = T2DM rats + combination of BSB and PSP (2:2); DM + C3 = T2DM rats + combination of BSB and PSP (3:1).

Comparison of pancreatic necrosis between treatments with BSB and PSP and the combination of both in rat T2DM model

Our result indicated that necrosis percentage in DM groups was significantly increase ($p < 0.05$) compared to normal group (Figure 3A-B). Treatments with BSB and combination of BSB and PSP were able to improve necrosis (Figure 3A). Interestingly, treatment with combination of BSB and PSP in a ratio of 2:2 improved pancreatic damage better than other combinations (Figure 3B).

Comparison of insulin and IRS-1 expression between treatments with BSB and PSP and the combination of both in rat T2DM model

Our result indicated that insulin expression in DM group was significantly increased ($p < 0.05$) compared to normal group (Figure 4A). Treatments with BSB, PSP and the combination of BSB and PSP were able to decrease insulin expression. Treatment with combination of BSB and PSP in a ratio of 2:2 improved insulin towards near normal. Interestingly, BSB treatment alone decreases the insulin expression lowest than other treatments (Figure 4C).

Our result indicated that IRS-1 expression in DM group was significantly increase ($p < 0.05$) compared to normal group (Figure 4B). Treatments with BSB, PSP and the combination of BSB and PSP decline IRS-1 expression. Treatment with combination of BSB and PSP in a ratio of 2:2 improved receptor insulin towards near normal. Interestingly, treatment with BSB alone decreases the IRS-1 expression lowest than other treatments (Figure 4C).

DISCUSSION

Hyperglycemia causes oxidative stress development through an increase in the production of free radicals such as ROS.[7] ROS bind with Polyunsaturated Fatty Acid (PUFA) to form PUFA peroxide. PUFA peroxide not only produces oxyradical but also react with H^+ and form lipid hydro-peroxide. Further, oxyradical and lipid hydroperoxide will form MDA.[3,19] MDA concentration has been used as indicator of oxidative damage in the presence of free radicals.[20]

MDA plays essential role in cellular damage development and causes chronic diseases, such as diabetes.[3,20] Recent advances suggest that dietary antioxidant decreased the MDA concentration and increased antioxidant status.[21,22] Our present study suggests that BSB and PSP combination not significantly decrease MDA concentration in DM rats. BSB contains isoflavones genistein and daidzein which acts as a scavenger to suppressing ROS production.[23] Furthermore, BSB active compound suppresses oxidative damage in DNA by neutralizing free radical in cytosol and nucleus.[24] In addition, PSP rich-anthocyanins contents prevent free radical of 2,2-diphenyl-1- picrylhydrazyl (DPPH) and superoxide radical.[25] The combination of BSB and PSP reduce MDA concentration in DM rats. Otherwise, in our study, PSP alone showed the increase of MDA concentration compared to the DM group. We assumed that the antioxidant capacity of PSP depends on its variety.[25,26]

Next, our result showed that the combination of BSB and PSP (2:2) enhance SOD concentration compared tothe DM group. The previous study reported that isoflavone-rich content in soybean enhances the SOD activity through preventing superoxide anions to form H_2O_2.[27] Furthermore, genistein may promote Nrf2, which is main transcription factor for antioxidant. Activation of Nrf2 signaling leads to production of an antioxidant enzymes such as SOD, catalase and GSH, which in turn improve the free radical-mediated tissue damaged.[22] Anthocyanin also reported enhancing antioxidant enzymes such as SOD, GPx and catalase via Nrf2-Keap1 complex.[21] Further, anthocyanin restores SOD function in DM condition.[28] We assumed that BSB and PSP might act synergistically in response to intracellular damage by ROS *via* enhanced the Nrf-2 signaling to produces antioxidant.

In line with our result, the decline of MDA and improving SOD are followed by the amelioration of necrotic cell in the pancreas. Long-term hyperglycemia promotes β-cells dysfunction and insulin signaling.[29] Consequently, cells fails to recompensate insulin, promotes impaired glucose tolerance and leads to excessive mitochondrial ROS production. These condition trigger cellular damage and disturb IRS-1 function.[30] Our previous study suggests that BSB and PSP combination decline blood glucose level in T2DM rats.[31] In line with the result, the BSB and PSP combination improved cellular damage through the decrease of necrotic cells in the pancreas.

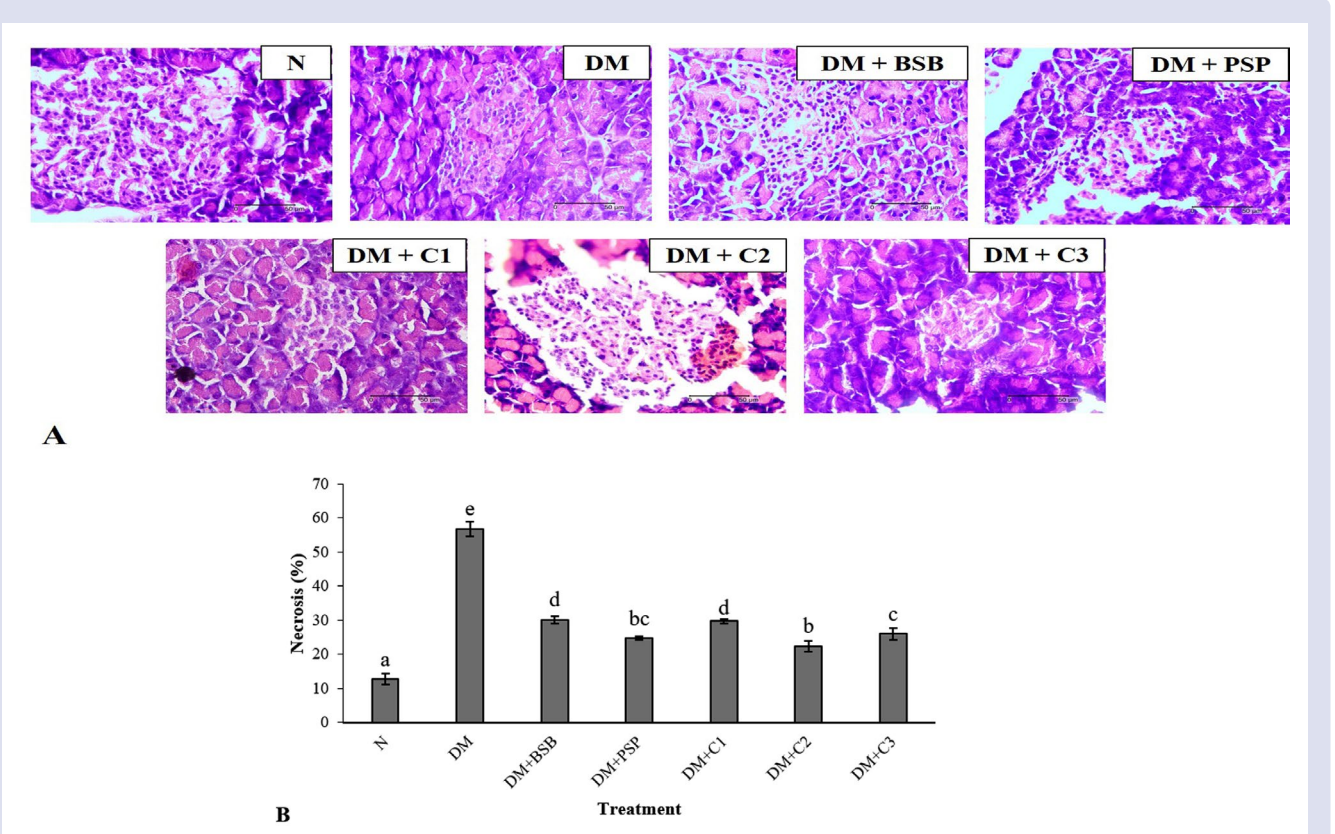

Figure 3: Treatments with BSB, PSP and the combination of both were able to decrease necrosis in T2DM-model rats. (A) A cross-section of the pancreas with HE staining in 400x magnification. (B) Pancreatic necrosis percentage represented as mean ± SD. Data represented as mean ± SD. The different letter indicates a significantly different ($p < 0.05$) based on the DMRT post hoc test. Note: N = normal rats; DM = T2DM rats; DM + BSB = T2DM rats + black soybean; DM + PSP = T2DM rats + purple sweet potato; DM + C1 = T2DM rats + combination of BSB and PSP (1:3); DM + C2 = T2DM rats + combination of BSB and PSP (2:2); DM + C3 = T2DM rats + combination of BSB and PSP (3:1).

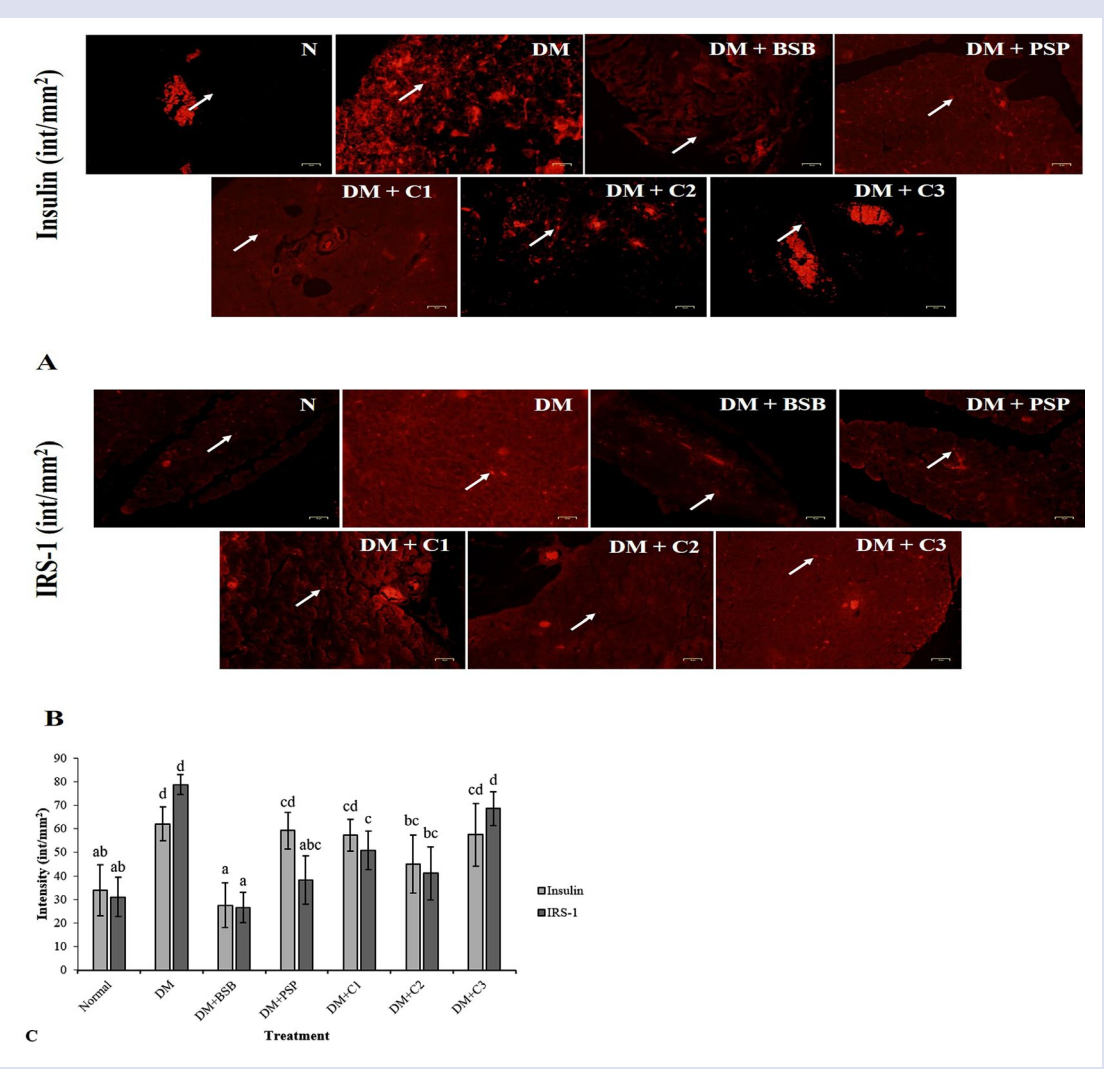

Figure 4: Treatments with BSB only and combination of BSB and PSP were able to improved insulin sensitivity. (A) A cross-section of the pancreas with IHC-Fluorescence insulin staining. (B) A cross-section of the pancreas with IHC-Fluorescence IRS-1 staining. (C) The intensity of insulin and IRS-1 in pancreas represented as mean ± SD. The different letter indicates a significantly different ($p < 0.05$) based on the DMRT post hoc test. Note: N = normal rats; DM = T2DM rats; DM + BSB = T2DM rats + black soybean; DM + PSP = T2DM rats + purple sweet potato; DM + C1 = T2DM rats + combination of BSB and PSP (1:3); DM + C2 = T2DM rats + combination of BSB and PSP (2:2); DM + C3 = T2DM rats + combination of BSB and PSP (3:1).

Furthermore, the improves in pancreatic cells are followed by the improves of IRS-1 function. Isoflavone-rich content in soybean modulate the glucose metabolism enzyme through AMPK activation and promotes insulin sensitivity.[32] BSB also rich in anthocyanin (cyanidin 3-glucoside; C3G) and procyanidin, which up-regulate the GLUT4 expression in the muscle tissue and down-regulate the gluconeogenesis.[33] PSP may act to restore the glucose metabolism through the regulation of leptin/AMPK signaling pathway.[34] These results support our previous finding that BSB and PSP combination restores the sperm quality[31] because improving insulin sensitivity is essential to support normal spermatogenesis.[35,36] We assumed that BSB and PSP combination might act synergistically to restores insulin sensitivity.

CONCLUSION

Our present study suggests that BSB and PSP combination not significantly decreased MDA concentration. Therefore, the BSB and PSP combination (2:2) restores SOD concentration, improve pancreatic β-cells damage, and restored insulin sensitivity in T2DM rats. Consumption of BSB and PSP is expected to be used as one of the easy, cheap and simple alternatives for T2DM therapy. Further research is required to find out the insight mechanism from BSB and PSP to validate the combination of both can be applied as therapy for diabetes mellitus.

ACKNOWLEDGEMENT

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CONFLICTS OF INTEREST

The authors stated that there is no conflicts of interest.

ABBREVIATIONS

BSB: Black Soybean; IRS-1: Insulin Receptor Substrate-1; MDA: Malondialdehyde; PSP: Purple Sweet Potato; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; STZ: Streptozotocin; T2DM: Type 2 Diabetes Mellitus.

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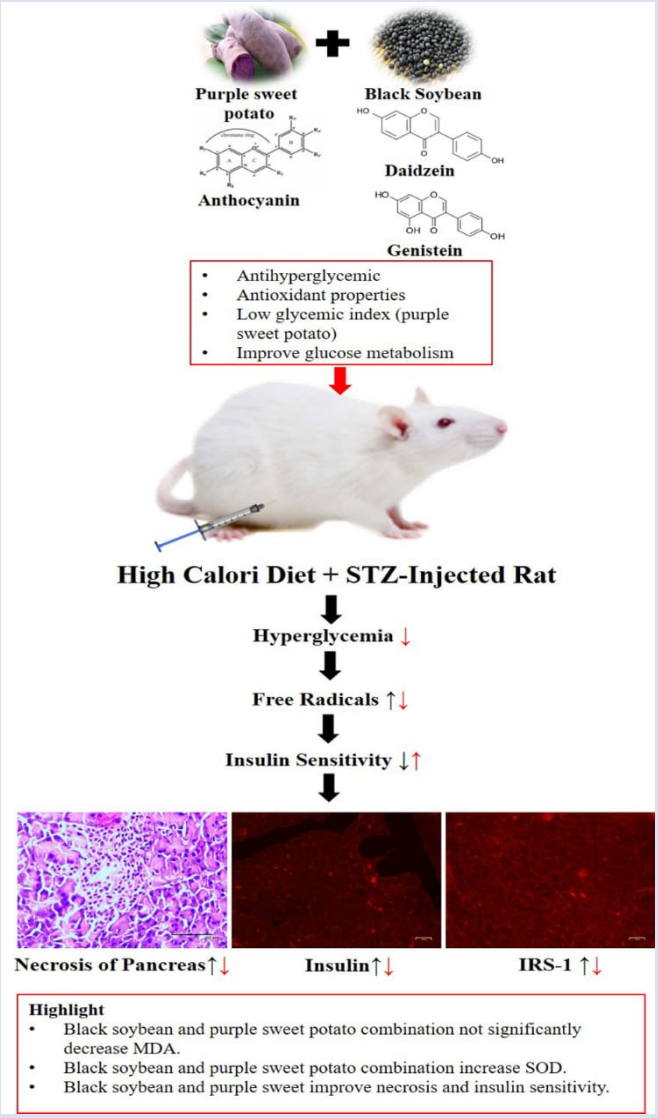
GRAPHICAL ABSTRACT



SUMMARY

Hyperglycemia mediates the production of excess free radicals and reduces endogenous antioxidant in type2 diabetes mellitus (T2DM). Black soybean is rich in antioxidant mainly from isoflavones, whereas the antioxidant of purple sweet potato comes from high anthocyanin. The study aimed to evaluate the efficacy of black soybean, purple sweet potato and its combination on malondialdehyde (MDA), superoxide dismutase (SOD) concentrations, insulin and insulin receptor substrate-1 (IRS-1) expression in T2DM rats. Our research suggests that there were no significant differences of BSB and the combination in decreasing MDA concentrations. The ratio of BSB and PSP combination of 2:2 increase SOD towards near normal, decrease necrosis, and improve insulin and IRS-1. In conclusion, combination of black soybean and purple sweet potato had the potential to improve insulin sensitivity through the increase of SOD, reduce necrosis and improve insulin and IRS-1 expression.

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