The Superiority of Purple Okra (*Abelmoschus esculentus*) to Green Okra on Insulin Resistance and Pancreatic β cell improvement in Diabetic Rats

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Abstract

Introduction: The use of antidiabetic plants on the diabetes treatment gets more preferable since they are assumed to have minimum side effects. Okra is a plant containing quercetin, which may induce pancreas regeneration and have antidiabetic effect. Based on many researches, purple okra contains more quercetin than green okra.

Objective: This research proves the advantages of purple okra over green okra on the diabetic markers improvement in diabetic rats.

Materials and methods: Fifteen male 2-month old wistar rats were injected with 65mg streptozotocine and 110mg niacinamide intraperitoneal. The blood glucose level was tested three days after injection. The induction was succeed if the glucose level was higher than 250mg/dL, and considered as diabetic rats. The diabetic rats were divided into 3 groups: Acarbose group, POP

and GOP groups were respectively administered with purple and green okra powder for 28 days. Blood serum was taken to examine fasting blood glucose (FBG), insulin, HOMA-B and GLUT-4 levels. Pancreas was examined for damage using hematoxylin eosin staining.

Results: The FBG, insulin, HOMA-B and GLUT-4 levels of diabetic rats administered with POP ($p<0.05$) were better than GOP. The least damage ($p<0.05$) to pancreatic beta cells takes place with the POP group.

Conclusion: Purple okra was superior to green okra on the improvement of diabetic markers on rats.

Keywords: purple okra, pancreatic beta cell, blood glucose, HOMA-B, insulin

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a serious threat to human's health throughout the world. The World Health Organization (WHO) states that there are 422 million diabetic patients on 2014, significantly increasing from 108 million patients in 1980[1]. The elevation of diabetic patients also increases the complication risk. The damage to the world's Gross Domestic Product (GDP) resulting from the direct and indirect effects of DM2 is 1.7 trillion USD. T2D affects microvascular and macrovascular caused polyneuropathy, retinopathy, diabetic nephropathy, coronary heart disease, atherosclerosis and stroke[2]. The success on T2D management is then important to

minimize any damage arising from it, one effort of which is to use an oral hypoglycemic drug or plant with hypoglycemic effect, such as okra (*Abelmoschus esculentus*).

Inadequate pancreatic β cell cause dysfunction of insulin secretion and lead to T2D. Insulin secretion function may be restored if blood glucose level is decreased, with antidiabetic drug or any other therapy[3]. Acarbose is an antidiabetic drug which works through inhibiting alpha glucosidase inhibitor enzyme, that inhibit glucose absorption from intestine, and prevent postprandial hyperglycemia[4]. Acarbose is actually able to restore the function of pancreatic beta cells and insulin resistance[5]. On the other hand, okra is often used by the people on diabetes management.

Okra is a Malvaceae plant and has some variants, such as green and purple okra, which may serve as an hypoglycemic agent, since it is able to inhibit alpha glucosidase[6]. Okra contains some advantageous nutrients and substances such as high fiber, but low calorie and fat. It also contains protein, minerals of phosphor, zinc, copper, potassium, magnesium, calcium, manganese, and vitamins A, B2, B3, B6, C, and K[7]. Okra contains antioxidants such as polyphenol, hyperoside, quercetin, coumarin scopoletin, uridine, and phenylalanine compounds[8]. Okra peel and seed powder used on diabetic rat evidently decreases blood glucose level[9]. Okra infusion water also evidently decreases blood glucose level[10]. Administration of okra peel powder and okra seed powder evidently decreases diabetic rat's blood glucose level[9]. Many studies have been conducted on green okra, but not on purple okra. Purple okra has higher phenolic and antioxidant content than green okra. Quercetin contained in purple okra is higher than green okra, thus it is expected to have higher potential antidiabetic effect than green okra[11].

Oleanolic acid, beta sistosterol, myricetin and kaempferol are okra's main contents with antidiabetic effect. Beta sistosterol may inhibit target protein diabetes[12]. Quercetin and isoquercetin may inhibit maltase and sucrase intestinal enzymes. These two compounds are similar to oral hypoglycemic drug, α-glucosidase inhibitors[13]. Quercetin may increase insulin secretion and protect pancreatic beta cells from death[14]. In addition, quercetin may also protect pancreatic beta cells from damage because of H_2O_2 and production of interleukin1β-induced nitrite[15]. Myricetin may also protect pancreatic beta cells from apoptosis through stress inhibition on endoplasmic reticulum[16]. Administration of 200mg/kg green okra powder may down regulate PPAR-γ gen, regulator of cell proliferation and glucose homeostasis[17].

The objective of this study was to confirm whether purple okra superior than green okra, on the diabetic markers improvement on rats.

MATERIAL AND METHODS

This research was conducted to the ethical standards and approved by Ethical Committee, of Faculty of Medicine, UNISSULA Semarang, Indonesia number 6/I/2019/Komisi Bioetik.

This research was done on 15 male 12-week old wistar rats, with body weight of 200-250gram. The research was conducted in the Center of Food and Nutrition Studies, Gadjah Mada

University, Yogyakarta. All rats were given with standard feed and distilled water and undergo acclimatization for 7 days before induction.

Purple and Green Okra Powder Preparation

Washed purple and green okra powders were dried using cabinet dryer at 40ºC and mashed up to form fine powder. Forty milligrams of purple, and green okra powders were dissolved in 50ºC distilled water until become homogenous and given once daily to the POP and GOP groups using oral gauge.

Induction of experimental diabetes mellitus and experimental design

Diabetes induction on rats was conducted using intraperitoneal injection of niacinamide 110mg, followed by streptozotocine 65mg, 15 minutes after niacinamide injection. The niacinamide is from Sigma-Aldrich, St. Louis, MO, USA Lot #BCBS3492V, and the streptozotocine is from Nacalai Tesque, Inc., Kyoto Japan. The behavioral change of rats was observed after diabetes induction, and distilled water was provided ad libitum. Fasting blood glucose was checked on the third day after induction. Rats were considered diabetic if only the fasting blood glucose level over 250 mg/dl.

The diabetic rats were randomized and divided into 3 groups: Acarbose group were given acarbose 6 mg. POP group were given purple okra powder 40mg/200g BW, and GOP group were given green okra powder 40mg/200g BW. The treatment was done for 28 days. Fasting blood sample was taken from ophthalmic vein.

Measurement

The fasting blood glucose (FBG) level was measured using GOD-PAP enzymatic photometric test. The pre-post data was taken during the treatment and ΔFBG was calculated for analysis. The levels of serum insulin, GLUT-4 and IGF-1 were examined using ELISA. Homeostasis model assessment of β-cell function (HOMA-β) was measured using the formula [Fasting insulin (μIU/ml) x 20]/[Fasting glucose (mmol/L) – 3.5][18]. The damage of pancreatic beta cells was measured by calculating the number of necrosis and apoptosis cells using optical microscope.

Data Analysis

The mean levels of fasting blood glucose, fasting serum insulin, HOMA-B and GLUT-4 of pancreatic beta cells were analyzed using one way ANOVA, followed with post-hoc LSD using software SPSS ver. 16.0 and Graph pad ver. 8.2. The data were declared significant if $p < 0.05$.

RESULTS

FASTING BLOOD GLUCOSE LEVEL

The mean level of fasting blood glucose (FBG) after streptozotocin-niacinamide induction (pre-treatment) on the three rats groups is higher than 250mg/dL (Fig.1), and is comparable for the three groups ($p > 0.05$).

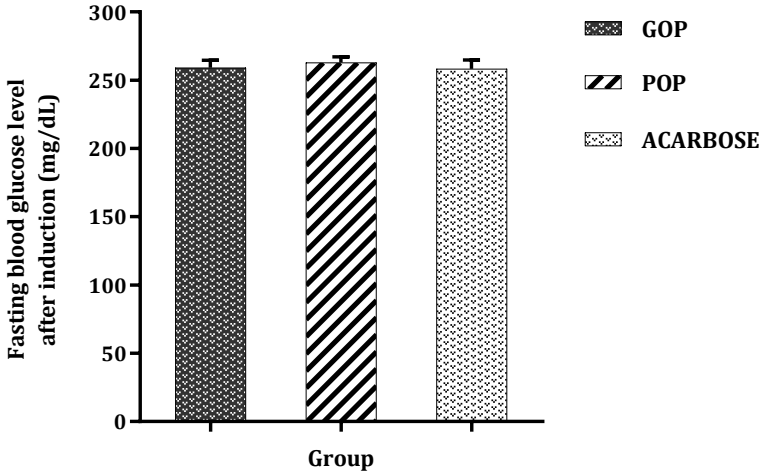

Figure 1.Fasting blood glucose level pre-treatment

The GOP group has the highest mean level of fasting blood glucose (135.94 mg/dL) of between the three groups. The fasting blood glucose level of POP (112.58 mg/dL) and acarbose (112.69 mg/dL) groups is tested at the end of treatment with post-hoc LSD and found not significantly different (p>0.05) (Fig.2).

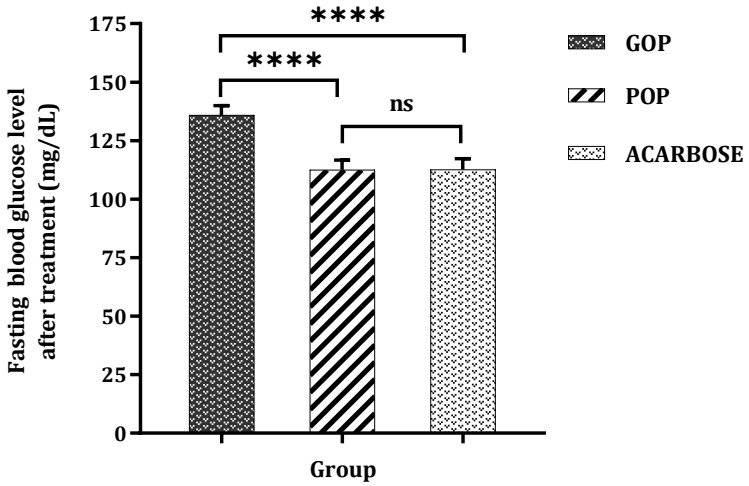

Figure 2.Fasting blood glucose level after treatment (ns: not significantly differ, p>0.05)

The same trend is also found on ΔFBG. GOP has the lowest ΔFBG (-123.13 mg/dL). ΔFBG between POP (-150.28 mg/dL) and acarbose groups (-145.706 mg/dL) is not significantly different ($p>0.05$) (Fig.3).

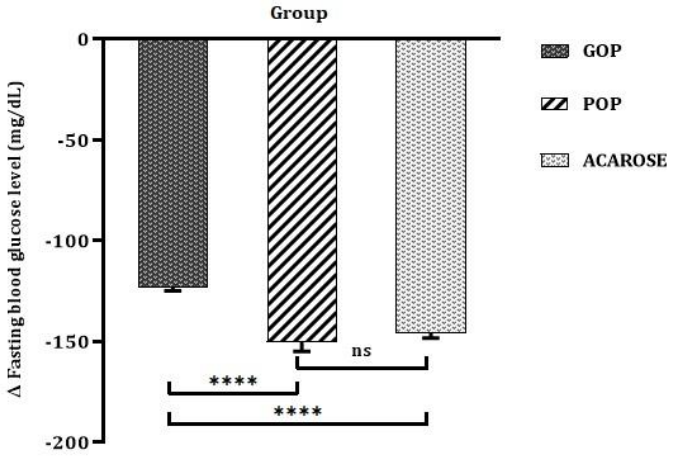

Figure 3. Δ Fasting blood glucose level after - before treatment (ns: not significantly differ, $p>0.05$)

FASTING SERUM INSULIN LEVEL

The highest mean fasting serum insulin level was found on the POP group (16.6μIU/mL). The fasting serum insulin level between the POP and acarbose groups (16.01 μIU/mL) was not significantly different ($p>0.05$) after test using post-hoc LSD (Fig.4).

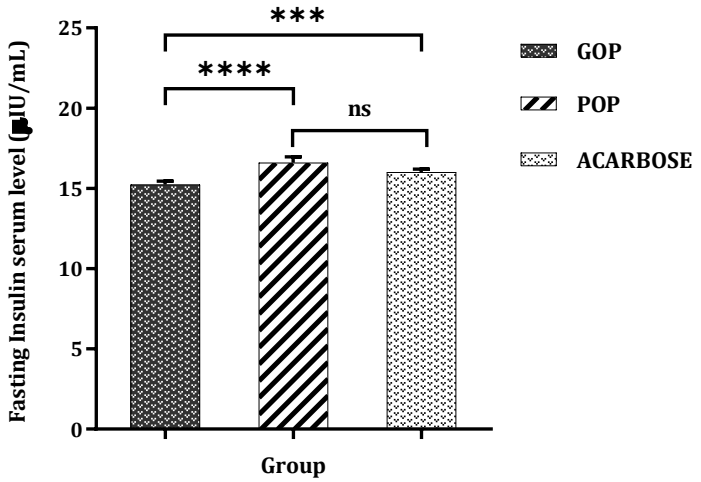

Figure 4. Fasting serum insulin level all groups (ns: not significantly differ, p>0.05)

HOMA-B

The differences of HOMA-B between POP (49.69) and acarbose groups (47.76) was not significantly different (p>0.05). GOP group has the lowest HOMA-B (36.9) between the groups (Fig.5).

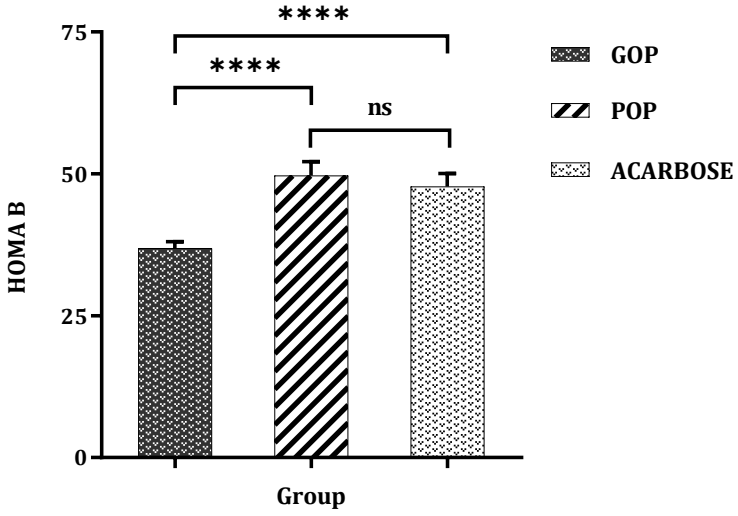

Figure 5. HOMA-B (ns: not significantly differ, p>0.05)

GLUT-4 LEVEL

The same pattern also occurs with GLUT-4. GLUT-4 between POP (10.76 ng/mL) and acarbose groups (10.98 ng/mL) is also found not significantly different ($p>0.05$), while GOP group has the lowest GLUT-4 (9.73 ng/mL) between the groups (Fig.6).

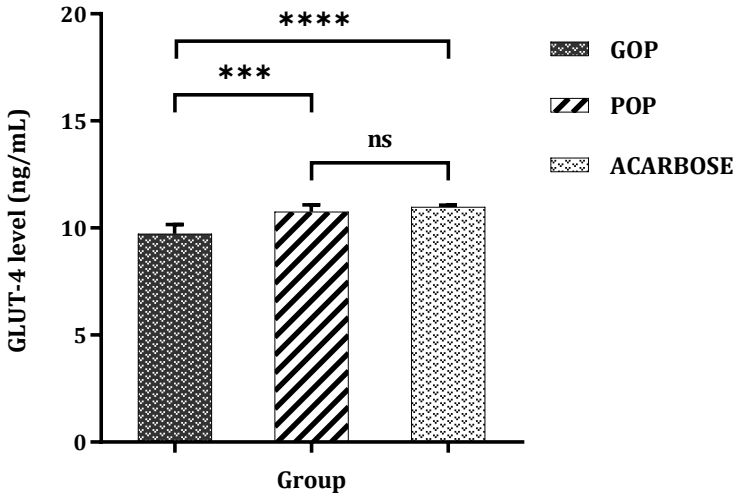

Figure 6. GLUT-4 level (ns: not significantly differ, $p>0.05$)

IGF-1 LEVEL

A different pattern was reflected in the IGF-1 result. The highest IGF-1 level was found on the GOP group (296.49 pg/mL). In this case, the GOP and POP groups were not significantly different ($p>0.05$). The lowest IGF-1 level was found in the acarbose group (258.95 pg/mL) (Fig.7).

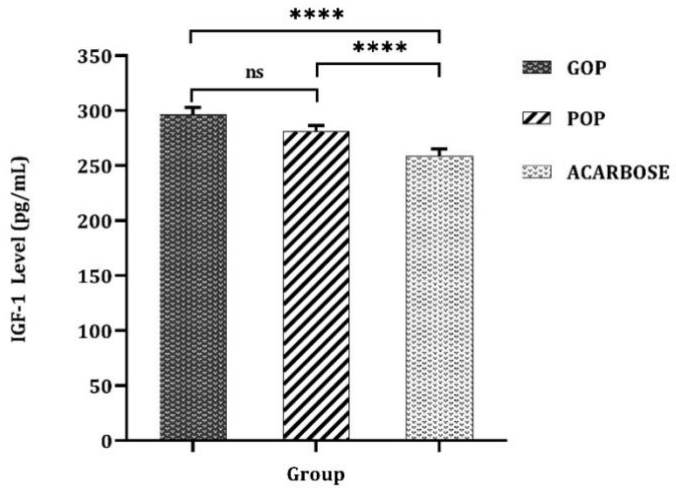

Figure 7. IGF-1 level (ns: not significantly differ, p>0.05)

NUMBER OF DAMAGED PANCREAS β-CELLS

The lowest number of damaged pancreatic β cells was on the acarbose group (15.2). On the contrary, the number of damaged pancreatic β cells between GOP (26.36) and POP (26.48) groups were not significantly different (p>0.05) (Fig.8)

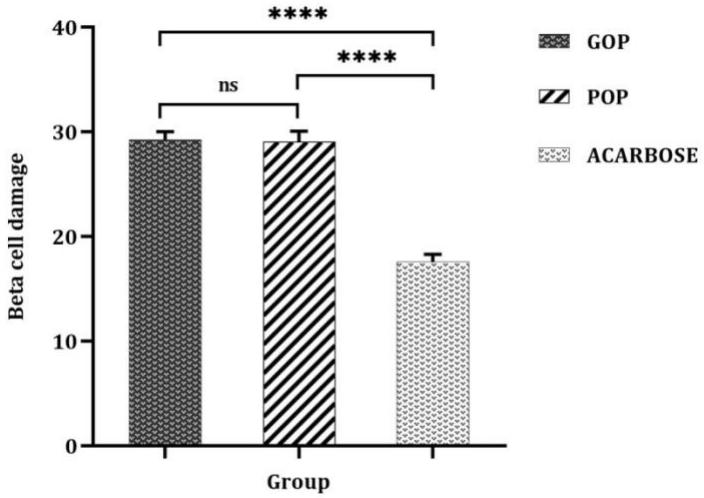

Figure 8. Number of damaged beta cells (ns: not significantly differ, p>0.05)

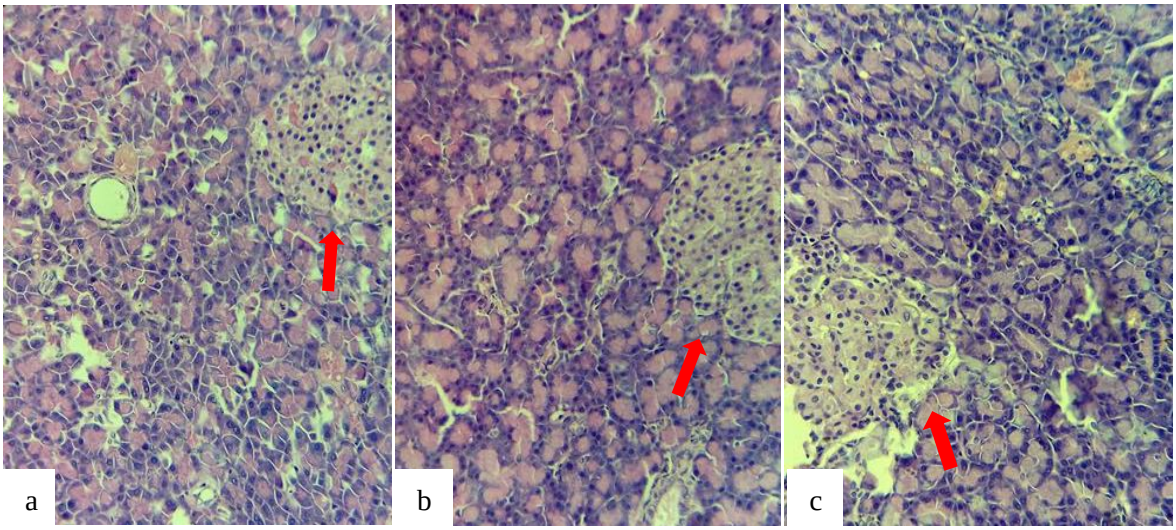

Figure 9. Pancreas with Hematoxylin Eosin staining, 400x magnification. (a). GOP (b). POP (c). Acarbose

DISCUSSION

The injection of 65mg streptzotocine-110mg niacinamide was successfully induced diabetes on the rats, as proven that the fasting blood glucose level was over 250mg/dL. The same result was also presented by Pari's research (2006) with a procedure similar to that in this research[19]. Streptozotocin (STZ) disturbs the work of pancreatic β cells in glucose oxidation and decrease insulin synthesis. Niacinamide (NA) was given with STZ injection to decrease the cytotoxic activity of STZ. The antioxidant effect of NA scavenge free radicals formed from STZ reaction on cells, thus it will reduce disturbance and necrosis on pancreatic β cells[20].

Mean FBG level after treatment on POP was higher than on GOP. Delta (Δ) FBG after and before treatment on POP was also higher than on GOP. This result were same with the previous study, the fasting blood glucose level was lower on group given with purple okra than with green okra, due to the quercetin content in POP was higher than in GOP[21]. Quercetin inhibits sucrase and maltase intestinal enzymes and GLUT-2, thus it inhibits glucose absorption in small intestine and reduce blood glucose[6,22]. The results also show interesting findings that the mean FBG level after POP treatment was not significantly different from Acarbose. This findings supports the theory that okra and acarbose, they both have the same mechanism as alpha glucosidase inhibitors[6,23]. Another content of okra was myricetin which able to improve carbohydrate metabolism and drives glucose utilization[24]. Dietary fibers and polyphenols contained in okra have hypoglycemic effect. Kaempferol which is also contained in okra evidently returns plasma glucose level to normal[22]. Mean fasting serum insulin level on POP is higher than on GOP. Mean fasting serum insulin level of POP is not significantly different from mean fasting serum insulin level of acarbose. Previous research found that group given with okra has lower insulin level than without okra[25]. Quercetin given to diabetic rats may improve insulin level. Quercetin modulate Ca^{2+} in insulin secretion[26]. Purple okra's higher quercetin level evidently improves insulin level better than green okra. Previous studies also find that rats administered with kaempferol with high fat diet and low dose streptozotocine injection have their insulin level improved[27]. Kaempferol which is contained in *Cyathea phalerata* Mart. even has insulin mimicking action effect[28]. On the other hand, myricetin injection thrice daily on rats with insulin resistance evidently improves insulin performance. Myricetin may reinforce insulin performance, thus it may improve insulin sensitivity[29]. Purple okra is superior than the green ones in improving insulin level due to its higher

quercetin. Thus, a further study in determining kaempferol and myricetin contained in purple okra is mandatory to established the superiority of purple okra in improving insulin level on diabetics.

HOMA-B of POP group is also better than that of GOP group. HOMA-B reflects the function of pancreatic β cells and insulin secretion. Another research which uses quercetin from *Psidium guajava* also shows improvement of HOMA-B[30]. Higher quercetin content in purple okra gives HOMA-B higher score to rats given with purple okra than green okra. In line with the results on glucose levels, HOMA-B on POP is not significantly different from HOMA-B on acarbose. Acarbose administration on diabetes patient with medium HOMA-B may evidently improve pancreatic β cells function[31]. Improved blood glucose level improves insulin sensitivity and secretion, thus it may improve residual function of pancreas β cells[32]. Okra and acarbose have the same mechanism in ameliorating glucose toxicity in inhibiting postprandial glucose uptake. A different condition is found with the number of pancreatic β cells damaged. The number of pancreatic β cell damage on POP group is not different from that on GOP group. Kaempferol and myricetin, contained in purple okra were also supposed to be higher than in green okra, also has effect in giving a higher score in HOMA-B on POP group. Kaempferol, which is contained in okra, is protective against pancreatic damage[27]. Myricetin acts as glucagon-like peptide 1 receptor (GLP-1R) agonist. Long-term administration of myricetin may improve pancreatic islets because of its insulinotropic effect[33].

Flavonoid on purple and green okra has the same ability in improving IGF-1 secretion, as shown with insignificant difference on GOP and POP. Low IGF-1 level is related to DM2 progressivity. IGF-1 increases serum insulin level and decreases blood glucose level[34]. Flavonoid

supplementation to sheep feed also increases sheep's IGF-1 serum level[35]. Both IGF-1 and insulin receptors are highly homologous in structure and function[36]. IGF-1 synthesis is also regulated by insulin[37]. Okra delayed gastric absorption of glucose, influenced insulin secretion, and thus influenced IGF-1 synthesis. Kaempferol serve as glucosidase inhibitor[38]. Kaempferol in purple okra is thought to be higher than in green okra, resulting in lower blood glucose level, better insulin and IGf-1 level in purple okra group, than those in green okra group.

GLUT-4 level on POP was not significantly different with acarbose and lower than GOP. This result is thought to have correlation with the higher content of quercetin, kaempferol, and myricetin in purple okra, comparing to green okra. The quercetin on the purple okra increase GLUT-4 expression. The elevation of GLUT-4 expression was also obtained on cultured rat L6 skeletal muscle cells given with quercetin for 18 hours[39]. Another research also finds that quercetin administration to L6 myotube also increases glucose uptake via translocation of glucose transporter type 4 (GLUT4)[40]. Translocation and expression of GLUT-4 on skeletal muscle are stimulated by quercetin administration, through adenosine monophosphate-activated protein kinase (AMPK) activation[22] which will increase glucose uptake with GLUT-4 translocation to cell membrane[39]. Myricetin injection for 14 days increases GLUT-4 expression of rat's membrane fraction of soleus muscles[41]. Myricetin also improves sensitivity of IRS-1-associated PI3-kinase with translocation of glucose transporter subtype 4 (GLUT 4) on soleus muscles with insulin resistance[29].

CONCLUSION

Purple okra powder was superior to green okra powder on the improvement of diabetic markers on diabetic rats. Purple okra powder has even better potential than acarbose on the improvement of fasting blood glucose, insulin, HOMA-B, and IGF-1 levels.

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