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Original Article

Superiority of Purple Okra (*Abelmoschus esculentus*) to Green Okra in Insulin Resistance and Pancreatic β Cell Improvement in Diabetic Rats

Nurina Tyagita[1], Endang Mahati[2], Azizah Hikma Safitri[1]

[1] *Department of Biochemistry, Faculty of Medicine, Universitas Islam Sultan Agung (UNISSULA), Semarang, Indonesia*
[2] *Department of Pharmacology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia*

Correspondence author: Azizah Hikma Safitri, Universitas Islam Sultan Agung, Semarang, Indonesia; E-mail: azizah.safitri@unissula.ac.id

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Abstract

Introduction: Antidiabetic medicinal plants are increasingly used in the treatment of diabetes as they are generally assumed to produce minimal side effects. Okra is a quercetin-containing plant which can induce pancreas regeneration and has antidiabetic effect. There has been a lot of research that demonstrate that purple okra contains more quercetin than green okra.

Aim: To demonstrate 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 intraperitoneally with 65 mg streptozotocin and 110 mg niacinamide. Their blood glucose levels were measured three days after the injection. The induction of diabetes was deemed successful if the glucose level of the rats got higher than 250 mg/dL, and then such rats were considered diabetic. The diabetic rats were divided into three groups: an acarbose group, a purple okra powder group, and a green okra powder group. The latter two were given, respectively, purple and green okra powder for 28 days. Blood serum was taken to examine the fasting blood glucose, insulin, HOMA-B and GLUT-4 levels. Pancreas was examined histologically for damage using hematoxylin eosin staining.

Results: Fasting blood glucose, insulin, HOMA-B, and GLUT-4 levels of diabetic rats that received purple okra powder ($p<0.05$) were better than those of the rats that received green okra powder. The least damage ($p<0.05$) to pancreatic beta cells was found in the purple okra powder group.

Conclusions: Purple okra is superior to green okra in terms of improving the diabetic markers of rats.

Keywords

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

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a major global health threat. The World Health Organization (WHO) estimates for 2014 showed that there were 422 million diabetic patients compared to 108 million in 1980.[1] The growing number of diabetic patients also increases the risk of diabetic complications. The global loss of Gross Domestic Product (GDP) due to the direct and indirect effects of T2D is estimated to be around 1.7 trillion dollars. Type 2 diabetes is a disease that is strongly associated with both microvascular and macrovascular complications, including polyneuropathy, retinopathy, diabetic nephropathy, coronary heart

disease, atherosclerosis and stroke.[2] T2D is considered successfully managed if we could minimize any damage it causes, one possible approach to this issue being the use of an oral drug or plant such as okra (*Abelmoschus esculentus*) with hypoglycemic effect.

Inadequate pancreatic β cells cause dysfunction of insulin secretion which leads to T2D. Insulin secretion function can be restored if blood glucose level is decreased by either an antidiabetic drug or some other therapy.[3] Acarbose is an antidiabetic drug which works through inhibiting the alpha-glucosidase inhibitor enzyme that inhibits glucose absorption from intestine, and prevents postprandial hyperglycemia.[4] Acarbose is actually able to restore the function of pancreatic beta cells and insulin resistance.[5]

Okra is often used by people on diabetes management. Okra is a Malvaceae plant and has some variants, such as green and purple okra, which may serve as a 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 in diabetic rats 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. The amount of quercetin contained in purple okra is higher than that in 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 ingredients 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 drugs, 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 the production of interleukin 1β-induced nitrite.[15] Myricetin may also protect pancreatic beta cells from apoptosis through stress inhibition on endoplasmic reticulum.[16] Administration of 200 mg/kg green okra powder may down regulate PPAR-γ gen, regulator of cell proliferation and glucose homeostasis.[17]

AIM

The objective of this study was to investigate whether purple okra is better than green okra in improving diabetic markers in rats.

MATERIALS AND METHODS

This research was conducted in full compliance to the ethical standards and approved by the Ethical Committee of Faculty of Medicine, UNISSULA Semarang, Indonesia (No 6/I/2019/Komisi Bioetik).

This study included 15 male 12-week-old Wistar rats (200-250 g). It was conducted in the Center of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. All rats were given standard food and distilled water, and acclimatized for 7 days before induction.

Purple and green okra powder preparation

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

Induction of experimental diabetes mellitus and experimental design

Diabetes induction in rats was implemented using intraperitoneal injections of niacinamide 110 mg, followed by streptozotocin 65 mg, 15 minutes after niacinamide injection. The niacinamide was provided by Sigma-Aldrich, St. Louis, MO, USA Lot #BCBS3492V, and the streptozotocin – by 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 measured three days after induction. The rats were considered diabetic if the fasting blood glucose level was over 250 mg/dl.

The diabetic rats were randomized and divided into 3 groups: one group was given 6 mg of acarbose, another was given purple okra powder 40 mg/200 g b.w. and a third group was given green okra powder 40 mg/200 g b.w. The treatment was administered for 28 days. Fasting blood sample was taken from the ophthalmic vein.

Measurements

The fasting blood glucose (FBG) level was measured using GOD-PAP enzymatic photometric test. The pre-post data was taken during the treatment and delta FBG 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)×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 an optical microscope.

Statistical 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 SPSS ver. 16.0 and GraphPad ver. 8.2. Data were considered significant if $p<0.05$.

RESULTS

Fasting blood glucose level

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

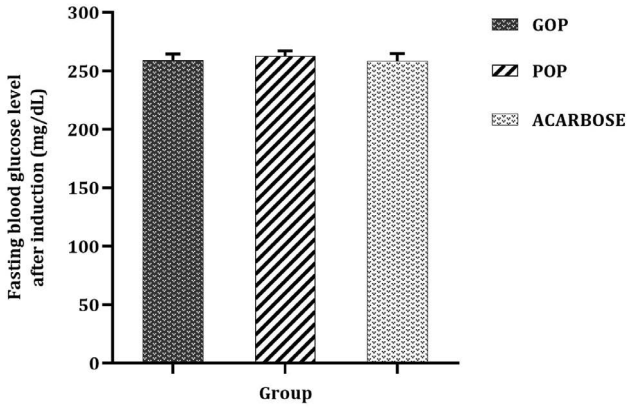

Figure 1. Fasting blood glucose level pre-treatment.

The GOP group had the highest mean FBG level (135.94 mg/dL) of the three groups. The FBG level of POP group (112.58 mg/dL) and acarbose group (112.69 mg/dL) was measured at the end of treatment with post-hoc LSD and found to be not significant ($p>0.05$) **(Fig. 2)**.

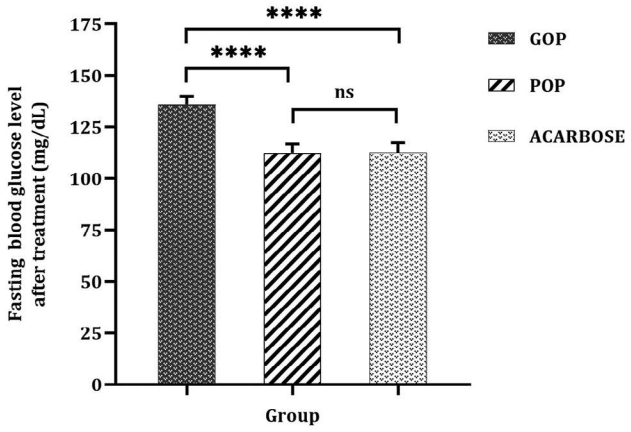

Figure 2. Fasting blood glucose level after treatment (ns: non-significant, p>0.05).

The same trend was also found for delta FBG. GOP had the lowest delta FBG (-123.13 mg/dL). Delta FBG between POP (-150.28 mg/dL) and acarbose groups (-145.706 mg/dL) was not significantly different ($p>0.05$) **(Fig. 3)**.

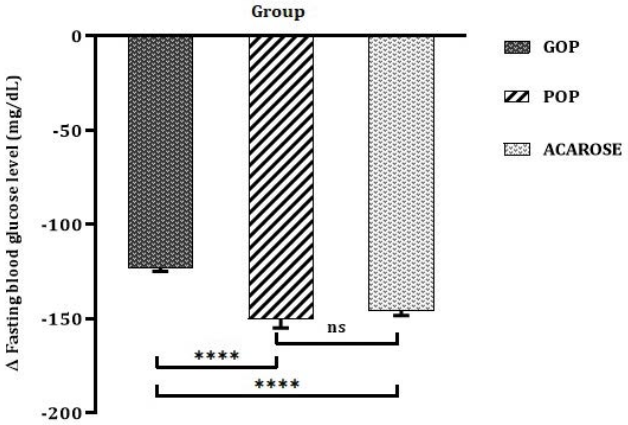

Figure 3. Delta fasting blood glucose level after/before treatment (ns: non-significant, p>0.05).

Fasting serum insulin level

The highest mean fasting serum insulin level was found in the POP group (16.6 µIU/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)**.

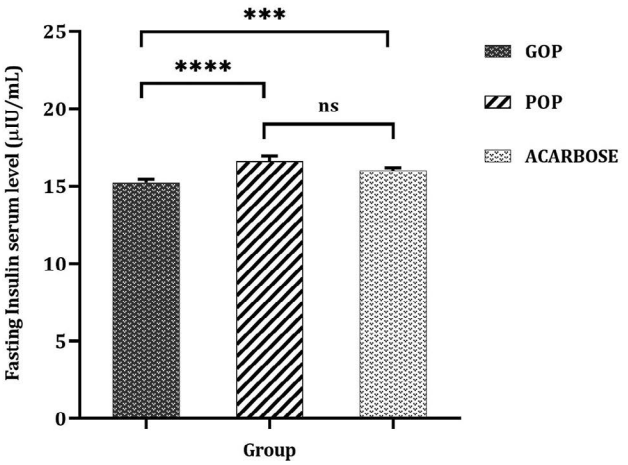

Figure 4. Fasting serum insulin level all groups (ns: non-significant, p>0.05).

HOMA-B

The differences in HOMA-B between POP (49.69) and acarbose groups (47.76) were not significantly different ($p>0.05$). The GOP group had the lowest HOMA-B (36.9) of the three groups **(Fig. 5)**.

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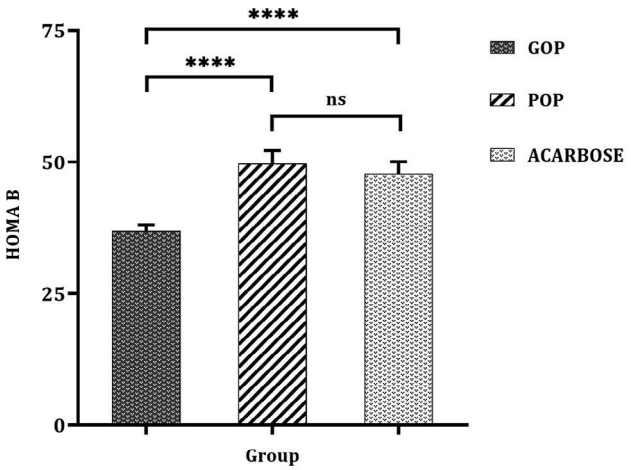

Figure 5. HOMA-B (ns: non-significant, p>0.05).

GLUT-4 LEVEL

The same pattern also occurred with GLUT-4. GLUT-4 between POP (10.76 ng/mL) and acarbose groups (10.98 ng/mL) was also found to be not significantly different (p>0.05), while the GOP group had the lowest GLUT-4 (9.73 ng/mL) of the three the groups **(Fig. 6)**.

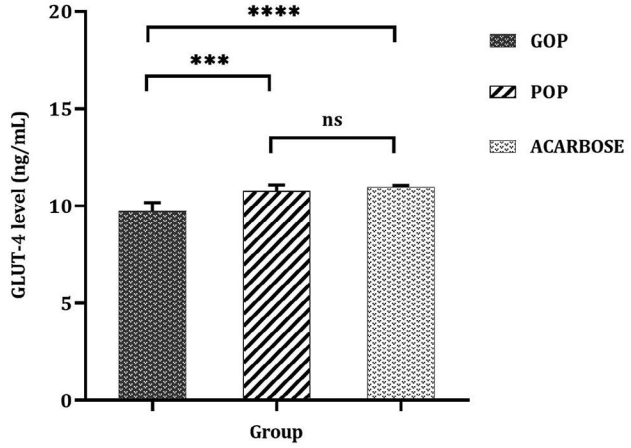

Figure 6. GLUT-4 level (ns: non-significant, p>0.05).

IGF-1 LEVEL

A different pattern was seen in the IGF-1 result. The highest IGF-1 level was found in 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)**.

Number of damaged pancreatic β cells

The lowest number of damaged pancreatic β cells was found in the acarbose group (15.2). Conversely the difference between GOP (26.36) and POP (26.48) groups in the number of damaged pancreatic β cells did not reach statistical significance (p>0.05) **(Fig. 8)**. The histological images show differences between groups **(Fig. 9).**

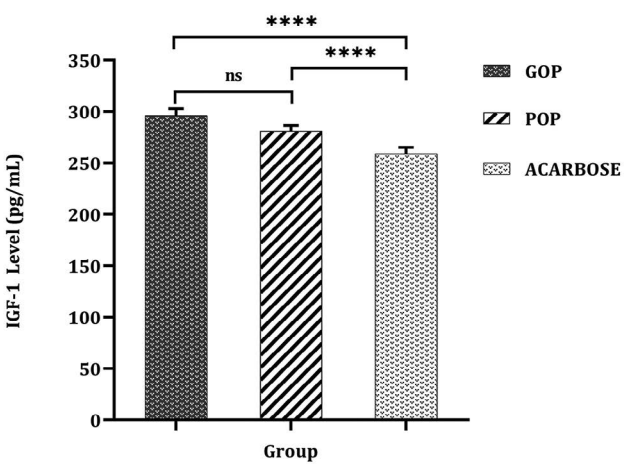

Figure 7. IGF-1 level (ns: non-significant, p>0.05).

DISCUSSION

By administering 65 mg of streptozotocin and 110 mg of niacinamide we successfully induced diabetes in the rats as proven by their FBG level (>250 mg/dL). These results were consistent with the results reported by Pari et al. using a procedure similar to that in the present study.[19] Streptozotocin (STZ) disrupts the work of pancreatic β cells in glucose oxidation and decreases insulin synthesis. Niacinamide (NA) was given with STZ injection to decrease the cytotoxic activity of STZ. The antioxidant effect of NA scavenges the free radicals formed from the STZ reaction on cells and therefore reduces the disturbance and necrosis in pancreatic β cells.[20]

The mean FBG level after treatment of POP group was higher than that of GOP rats. Delta FBG after and before treatment of POP was also higher than that of GOP. This result is the same as that in a previous study – the fasting blood glucose level was lower in the group given purple okra than that in the group receiving green okra, which was due to the greater concentration of quercetin in POP than in GOP.[21] Quercetin inhibits sucrase and maltase intestinal enzymes and GLUT-2, thus it inhibits glucose absorption

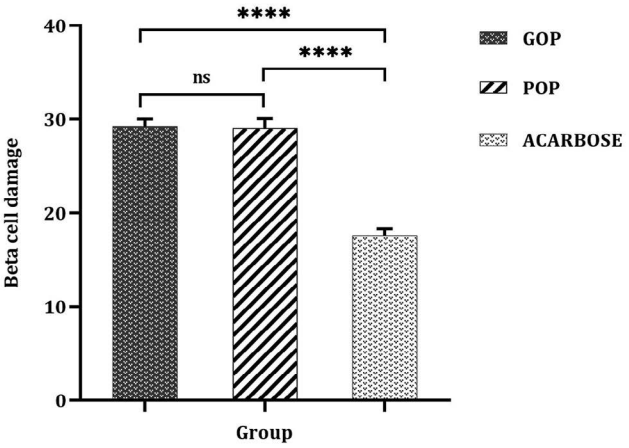

Figure 8. Number of damaged beta cells (ns: non-significant, p>0.05).

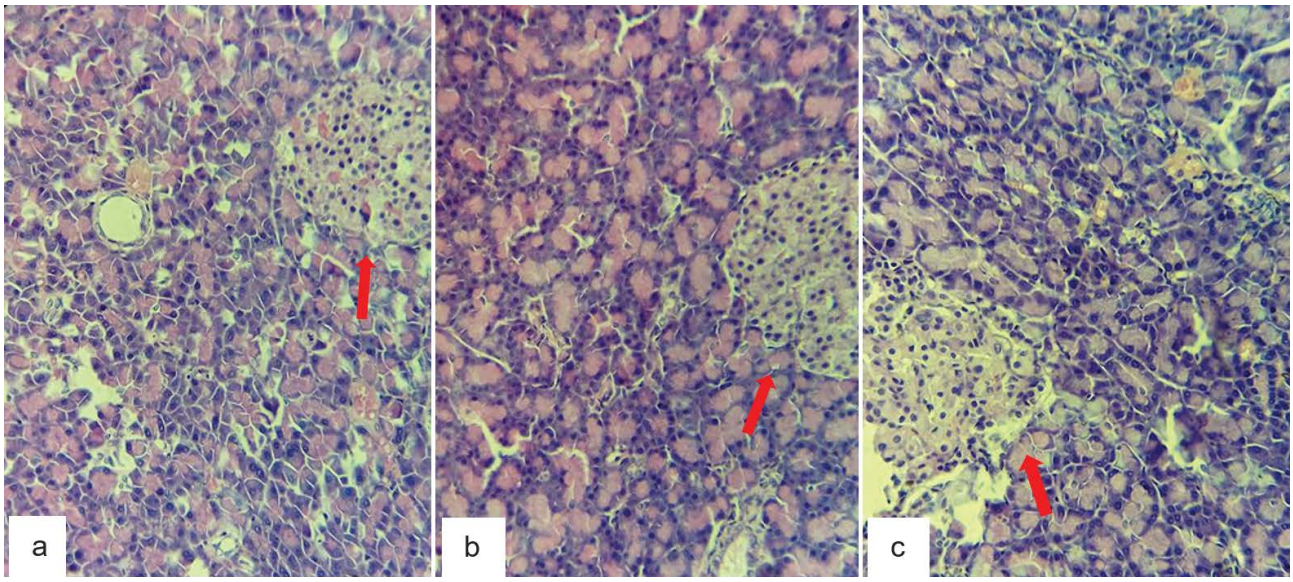

Figure 9. Histological examination of pancreas: a. Green okra powder treatment; b. Purple okra powder treatment; c. Acarbose treatment; (hematoxylin and eosin staining, magnification, ×400).

in small intestine and reduces blood glucose.[6,22] Another interesting finding is that the mean FBG level after POP treatment was not significantly different from that in acarbose treatment. This findings supports the reports that okra and acarbose have the same mechanism of action as that of alpha glucosidase inhibitors.[6,23] Another ingredient of okra is myricetin, which is able to improve carbohydrate metabolism and drive glucose utilization.[24] Dietary fibers and polyphenols contained in okra have a hypoglycemic effect. Kaempferol, another constituent of okra, returns plasma glucose level to normal.[22] Mean fasting serum insulin level in POP treatment was higher than that in GOP. Mean fasting serum insulin level of POP was not significantly different from the mean fasting serum insulin level of acarbose. Previous research found that the okra-treated group had a lower insulin level than without okra.[25] Quercetin given to diabetic rats may improve insulin level. Quercetin modulates Ca^{2+} in insulin secretion.[26] Purple okra's higher quercetin level evidently improves insulin level better than green okra does. Previous studies also have found that rats which received kaempferol with a high-fat diet and low dose streptozotocin injection had 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 in rats with insulin resistance improves insulin performance. Myricetin may reinforce insulin performance, thus it may improve insulin sensitivity.[29] Purple okra is superior to the green one in improving insulin level due to the higher concentration of quercetin. Thus, a further study in determining kaempferol and myricetin contained in purple okra is mandatory to establish the superiority of purple okra in improving insulin level on diabetics.

HOMA-B of POP group was 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 purple okra than green okra. In line with the results on glucose levels, HOMA-B on POP was not significantly different from HOMA-B at acarbose treatment. Acarbose administration on diabetes patient with medium HOMA-B may evidently improve pancreatic β cells function.[31] Improved blood glucose level boost insulin sensitivity and secretion, thus it may improve the residual function of pancreatic β cells.[32] Okra and acarbose have the same mechanism in ameliorating glucose toxicity in inhibiting postprandial glucose uptake. A different condition was found with the number of damaged pancreatic β cells. The number of pancreatic β cell damage on POP group was not different from that in GOP group. Kaempferol and myricetin, contained in purple okra, were also supposed to be higher than in green okra, also has effect of giving a higher score in HOMA-B on POP group. Kaempferol, which is contained in okra, protects 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 in purple and green okra has the same ability to improve IGF-1 secretion, as shown with insignificant difference on GOP and POP. Low IGF-1 level is associated with the progress of T2D. 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.

Kaempferol concentration 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.

The GLUT-4 level in POP treatment 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 compared to green okra. The quercetin in purple okra increases GLUT-4 expression. The elevation of GLUT-4 expression was also obtained on cultured rat L6 skeletal muscle cells given quercetin for 18 hours. Another research also found that quercetin administration to L6 myotube also increased glucose uptake via translocation of glucose transporter type 4 (GLUT4). Translocation and expression of GLUT-4 on skeletal muscle are stimulated by quercetin administration through adenosine monophosphate-activated protein kinase (AMPK) activation[22] which increases 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. 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]

CONCLUSIONS

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

Acknowledgments

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Превосходство пурпурной бамии (*Abelmoschus esculentus*) над зелёной бамией в резистентности к инсулину и улучшении содержания β-клеток поджелудочной железы у диабетических крыс

Нурина Тягита[1], Енданг Махати[2], Азизах Хикма Сафитри[1]

[1] *Кафедра Биохимии, Факультет медицины, Исламский университет Султана Аганга, Семаранг, Индонезия*

[2] *Кафедра Фармакологии, Факультет медицины, Дипонегоро Университет, Семаранг, Индонезия*

Адрес для корреспонденции: Азизах Хикма Сафитри, Исламский университет Султана Аганга, Семаранг, Индонезия; E-mail: azizah.safitri@unissula.ac.id

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Резюме

Введение: Противодиабетические лекарственные травы всё чаще используются при лечении диабета, потому что обычно считается, что они вызывают минимальные побочные эффекты. Бамия – это растение, содержащее кверцетин, которое может стимулировать регенерацию поджелудочной железы и обладает противодиабетическим действием. Есть много исследований, которые показывают, что пурпурная бамия содержит больше кверцетина, чем зелёная бамия.

Цель: Продемонстрировать преимущества пурпурной бамии по сравнению с зелёной бамией с точки зрения улучшения диабетических маркеров у диабетических крыс.

Материалы и методы: Пятнадцати двухмесячным самцам крыс линии Вистар внутрибрюшинно вводили 65 мг. стрептозотоцина и 110 мг. ниацинамида. Уровни глюкозы в крови измеряли после инъекции в течение 3 дней. Индуцирование диабета считалось успешным, если уровень глюкозы у крыс превышал 250 мг/дл. Крысы с диабетом были разделены на три группы: группу, получавшую акарбозу, группу, получавшую пурпурную бамию, и группу, получавшую зелёную бамию. Последним двум давали порошок пурпурной и зелёной бамии соответственно в течение 28 дней. Сыворотка крови была взята для измерения уровней глюкозы в крови натощак, инсулина, HOMA-B и GLUT-4. Поджелудочную железу исследовали гистологически на наличие повреждений с помощью окрашивания гематоксилин-эозином.

Результаты: Уровни глюкозы в крови натощак, инсулина, HOMA-B и GLUT-4 у диабетических крыс, получавших порошок пурпурной бамии ($p<0.05$), были лучше, чем у крыс, получавших порошок зелёной бамии. Наименьшее повреждение ($p<0.05$) бета-клеток поджелудочной железы было обнаружено в группе, получавшей пурпурную бамию.

Заключение: Пурпурная бамия лучше, чем зелёная бамия, с точки зрения улучшения диабетических маркеров у крыс.

Ключевые слова

глюкоза в крови, HOMA-B, инсулин, пурпурная бамия, бета-клетки поджелудочной железы