Antidiabetic potential of purple okra (*Abelmoschus esculentus* L.) extract in streptozotocin-induced diabetic rats

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Abstract. Okra (*Abelmoschus esculentus* L.), a tropical vegetable has been reported to have many important biological properties and has been used extensively as a traditional medicine especially for diabetic. In general, the objective of this study was to analyze the potential of okra extract to body weight and pancreas histopathology in streptozotocin (STZ)-induced diabetic rats and the result will be compared with that of control DM. The experimental design in this study was pre and post test controlled group design. The first step of this study was analyzing the bioactive compound of okra extract. The next step was administering okra extract to control group and diabetic rats induced by STZ 50 mg/kgBW for 14 days group. Sprague dawley rats were divided into six groups: normal control (N), diabetic control (DM), diabetic treated with green okra extract (GOE) with the dosage of 5 mg/kgBW quercetin and 10 mg/kgBW quercetin, diabetes treated with purple okra extract (POE) with the dosage of 5 mg/kgBW quercetin and 10 mg/kgBW quercetin. Body weight was weighed every three days and pancreas histopathology was measured by immunohistochemistry indirect method. The following results showed that IC₅₀, antioxidant capacity, fenolic, and quercetin contents of purple okra extract were higher (316.86 ppm; 417.54 mg/100g; 3.60%; 0.45 mg/g) than green okra extract (326.48 ppm; 341.43 mg/100g; 3.58%; 0.27 mg/g). Administration of GOE I, GOE II, POE I and POE II in diabetic did not give a significant effect to changes in body weight of rats, but effectively could improve repairmen of β cell pancreas destruction due to STZ induction. These results suggest that intervention of green okra extract and purple okra extract based on quercetin compound showed an antidiabetic potency.

1. Introduction
Diabetes mellitus (DM) is a chronic metabolic disease that caused metabolic disorders of carbohydrates, proteins and lipids which is characterized by hyperglycemia. The increasing of blood glucose levels occur due to abnormal insulin secretion in DM type 1 or insulin resistance that occurs in DM type 2 [1]. Insulin resistance followed by inability of beta cells to balancing the insulin resistance and can caused beta cell dysfunction in pancreatic [2]. End stage complications of diabetes associated with the etiology of diabetes mellitus with oxidative stress [3]. Prevalence of diabetes mellitus type 1 is rarely about 7-10% in the world, whereas diabetes mellitus type 2 (DMT2) contribute for more than 90% of all DM cases worldwide [1].

The World Health Organization (WHO) estimated that about 90% of population in developing countries use plants and herbal products as traditional medicine for primary medication [4].
Epidemiological studies recommend that consuming foods that contain many bioactive compounds such as catechins in tea, anthocyanins in red, purple, blue vegetables and quercetin compounds may reduce the risk of diabetes, heart disease, obesity, hyperlipidemia, stroke and cancer [5–7]. Okra (Abelmoschusesculentus L) also known as lady finger or gumbo, is a tropical vegetable that is included in Mallow family. Okra has many flavonoid compounds that have antioxidant activity [8-10].

The main flavonoid content in okra (isoquercetinand quercetin) was reported have antidiabetic activity in vitro studies through the action of increasing glucose uptake in the tissues by stimulating AMPK [11], increasing insulin sensitivity and improving insulin resistance in db/db mice by improving antioxidant status through 0.04% quercetin supplementation in diet [12, 13], proliferation of liver and pancreatic cells, decreased hyperglycemia and increased insulin production in STZ-induced diabetic rats receiving 0.5% quercetin diet for 14 days and quercetin from okra seeds act as α-glucosidase inhibitors [14]. In vitro studies reported that quercetin have antidiabetic properties significantly inhibited α-amylase activity and α-glucosidase concentrations, and prevented lipid peroxidation from homogenates of pancreatic tissue [15]. Research conducted by Sabitha et al. [16] using skin and seeds okra extract at doses of 100 and 200 mg/kgBW significantly lowering blood glucose levels, increasing body weight and preventing lipid peroxidation in STZ-induced diabetic rats.

Purple okra as a result of okra superior seed cultivation from Zahira varieties. The development of purple okra has been expected to have higher bioactive components than green okra. Some studies related to green okra has been done, but the research using purple okra is still rarely done. The antioxidant potency of purple okra extract needs to be intervention in the medication of diabetes based on quercetin compound.

2. Objective
To analyze the bioactive compound of okra extract and analyze the effect of purple okra extract on body weight and histopathology of pancreatic tissues in diabetic rats model, and compared with that of control okra (green okra).

3. Method
3.1. Time and place
This research held on February until August 2017. Ethical Clearance obtained from animal ethics committee of LPPM IPB. Okra extraction was done in the Laboratory of Biochemistry and Nutritional Analysis of the Community Nutrition Department, Faculty of Human Ecology IPB and SEAFAST Center IPB. The analysis of quercetin, antioxidant and total phenol was done at Biopharmaceutical Study Center IPB. In vivo stage were done at Animal Laboratory Management Unit (UPHL) Faculty of Veterinary Medicine IPB.

3.2. Material and equipment
The main material used in this research are greenokra and purple okra that was obtained from Prof. Muhammad Syukur at Department of Agriculture and Horticulture of Bogor Agricultural University and 3 month old male Sprague Dawley rats weighing 180-250 g that were obtained from the National Food and Drug Administration Center BPOM Jakarta. Material used in the okra extraction is ethanol solution. Materials used for proximate and antioxidant activity analysis are DPPH, methanol, cyanidin 3-O-glucosidase and standard quercetin solutions. Tools used in extraction of okra and proximate analyzes of okra extract are stirrers, vacuum evaporator, freeze dryers, trays, digital scales, knives, pans, spectrophotometry and freezers, glass (Pyrex®), stirrer, mortar, petri dish, filter, micropipet of 1000 μL and 10-100 μL, test tube, sieve (Buchner funnel), centrifuge, sonicator, HPLC stationary column, vortex, and water bath aquades.

The required materials and tools to maintenance and rats adaption are individual cagesize 33.5 x 27 x 12 cm, bottles, meal box, analytic scales, standard feeds of rats formulated based on AIN-93. Materials and tools for diabetes induction are streptozotocin (STZ), nicotinamide and intraperitoneal injection syringes. Injections and apparatus for blood-taking and surgery at the end of the intervention
are xylazine and ketamine solutions, 4% formalin solution, cotton, syringe, ependorf tube, centrifuge, scalpel, surgical board, hand gloves, antiseptic soap, mask, alcohol, cotton ball, diaminobenzidine and light microscope.

3.3. Procedure
The first step of this study was analyzing the bioactive compound of okra extract. The next step was administering okra extract to control group and diabetic rats induced by STZ 50 mg/kgBW for 14 days group.

3.3.1. Okra extraction. Fresh okra (green and purple) extraction used methanol extraction method. 500 g green and purple okra were dried using freeze dryer. Dried okra weighed and smoothed with blender. 10 g powder dried okra added with 500 ml methanol solution, continued with extraction gradually until obtained cleary residue. The filtrate were evaporated with vacuum evaporator. Okra extract weighed and analysed bioactive of extract. Extract stored in the freezer 4°C-8°C until intervention time.

3.3.2. Bioactive analysis of okra extract. Bioactive compound of okra extract analyzed included the antioxidant IC<sub>50</sub>, antioxidant capacity, total phenol and quercetin. Measurement of quercetin compound was performed on okra extract using HPLC method of column C-18 [17]. Measurement of antioxidant activity of green and purple okra extract using DPPH method (2,2-Diphenyl-1-picrylhydrazyl). Arrest free radicals indicated as the percentage of control solution of DPPH absorbance. The value of IC<sub>50</sub> is defined as mg/mL sample required for initiation of DPPH decrease by 50% [18, 19]. Analysis of antioxidant capacity was calculated as equivalence with vitamin C performed on samples of green and purple okra extract [20]. Total phenols were determined by using the Folin-Ciocaiteu method. The absorption is measured at spectrophotometry at a wavelength of 765 nm. The results of total phenols compound showed as percentage mg equivalent of gallic acid per mg of sample [9].

3.3.3. Preparation Animal Model and STZ induction. A total of 24 rats were adapted for 14 days with standard feeding of rats that was formulated based on AIN-93 and drinking water ad libitum. On 15th day after adaptation, the rats was induced with STZ intraperitoneally. To obtain diabetic condition for 12-16 hours, then performed single-dose STZ induction of 50 mg/kgBW. Success of induction was determined by measuring the blood glucose levels on day 3 after the induction of the STZ. The condition of diabetic is achieved when blood glucose level of rats >126 mg/dL [21]. Diabetic model rats were divided into six groups: normal control (N), diabetic control (DM), diabetic treated with green okra extract with the dosage of 5 mg/kgBW quercetin (GOE I) and 10 mg/kgBW quercetin (GOE II), diabetes treated with purple okra extract with the dosage of 5 mg/kgBW quercetin (POE I) and 10 mg/kgBW quercetin (POE II). Body weight rats measured every three days during intervention period using the scales.

3.3.4. Histopathology of pancreatic tissues. Histopathology of pancreatic tissues was analyzed by indirect immunohistochemical method. At the end of the study (15th day of post-treatment) the rats were anesthetized by a mixture of Ketamine: 75 mg/kgBW and Xylazin: 5 mg/kgBW. Rats surgery is performed to obtain pancreatic organs pancreatic organs then were fixated in 4% formalin solution and optimally conditioned then cut into 10 μm parts according to standard protocol [22]. Observation parameters of immunohistochemical staining results in the pancreatic tissue of rats observed pancreatic tissue were cell damage. Observations of pancreatic tissue fragments, especially in beta cells visualized by diamin benzidine, were performed by looking at distribution of pancreatic beta cells of Langerhans island in STZ induced diabetic rats [23].
3.4. Design experiment

The research design used was Random Design Group. In this experiment, animals were grouped into six groups: one group of normal control, one diabetes control and four treatment groups. Further experimental animals (rats) were calculated by the formula of Federer [24]:

\[(n-1) \times (t-1) \geq 15\]

Information:
- n: number of samples per treatment
- t: number of treatments

The obtained number of samples for each treatment to be used in this study at least 4 rats with total amount of required are 24 rats. The dosage of okra extract given to treatment groups consisted of two levels, namely green and purple okra extract containing quercetin of 5 mg/kgBW and 10 mg/kgBW based on study of [26] in rats model diabetic.

3.5. Data processing and analysis

Body weight data was processed and analyzed using Microsoft Excel 2010 and SPSS software version 16.0. All data are expressed in average value and standard error means (SEM) with One Way ANOVA statistical analysis. If there is significantly difference, further tests will be conducted using Duncan's multiple-range test. Descriptive data in this study is histopathology of pancreas made by indirect of immunohistochemical.

4. Result and discussion

4.1. The property of antioxidant of green and purple okra extract

Initial stages in the study of antidiabetic potential of okra extract is to analyze the property of antioxidant green and purple okra extract including IC50 of antioxidant activity, total phenol, and quercetin. Based on the analysis of antioxidant activity known IC50 value in purple okra extract is lower than green okra extract (table 1). The low IC50 values indicate high antioxidant activity [26]. The results of antioxidant activity of IC50 extract of green and purple okra that are 326.48 ppm and 316.86 ppm.

| Bioactive compound | GOE       | POE       |
|--------------------|-----------|-----------|
| Antioxidant IC50   | 326.48 ppm| 316.86 ppm|
| Antioxidant capacity AEAC | 341.43 mg/100g | 417.54 mg/100g |
| Total phenol       | 3.58 %    | 3.60 %    |
| Quercetin          | 0.27 mg/g | 0.45 mg/g |

According to [27] antioxidant activity is very active when IC50<50 ppm, active (IC50 50-100 ppm), medium (IC50 250-500 ppm), and relatively weak at IC50>500 ppm). In vitro studies of antioxidant assays on the methanol extract of okra by [28] demonstrated the ability of antioxidants or free radical antidotes by the method of xanthine oxidase and 2-deoxyglycine inhibitory 50% obtained at concentrations of 25 and 43 mL of extract. The results of IC50 antioxidant activity on the okra extract were positively correlated with antioxidant capacity using calculations based on the standard vitamin C curve, which showed that the antioxidant capacity of purple okra extract was higher by 417.54 mg AEAC / 100 g than that of green okra extract 341.43 mg AEAC / 100g, so it can be concluded that the lower the IC50 value is the higher the antioxidant capacity of the extract.

In phytochemical analysis showed that POE contains quercetin compound 0.45 mg/g higher than quercetin of GOE 0.27 mg/g. Quercetin is main flavonoid in okra where 70% of total antioxidant okra...
is a quercetin derivative [29]. Animal studies have shown quercetin reduced blood glucose, protected cells and functions of pancreatic beta cell integrity that can be used in the prevention of diabetic complications [30].

Some research suggests skin and okra seeds contain polyphenol compound such as hydroxynamic acid and its derivatives, catechins, quercetin derivatives, and flavonols [29, 31]. Based on this results, the total phenol and quercetin extract of POE is higher than GOE. Okra seeds have higher bioactive compound with catechins of 2.5 mg/g and flavonol derivatives (quercetin) 3.4 mg/g. Skin okra contains hydroxynamic acid group polyphenols and quercetin derivatives of 0.2 mg/g and 0.3 mg/g samples respectively [10]. This research used whole okra without separated between the skin and seeds.

The total phenol extract of POE showed 3.60% compared to that of GOE 3.58%. The highest content of polyphenols in okra found in seeds with total polyphenols of 29.5%, while in seeds 1.25% [32]. Another study conducted by [33] showed that the total phenolic compounds in pulp extract and okra seed were 10.75 ± 0.02 mg GAE/100g and 142.48 ± 0.02 mg GAE/100g extract. The activity of α-glucosidase in okra and okra seed extracts was considerable (IC₅₀ 142.69 μg/mL; 150.47 μg/mL), while α-amylase activity (IC₅₀ 132.63 μg /mL; 147.23 μg/mL) [34].

4.2. Effect of intervention okra extract in body weight
Average data of body weight started at day 0 trial (first day of rats had diabetes) ranged from 199.50-227.75 g, after 14 days intervention ranged from 237-256 g (table 7). During 14-days intervention period, FPG measurements were taken every three days, i.e 0 day after rats were declared diabetes until the 15th day at the end of intervention. Average weight of rats tended to increase, with weight gain not significantly different between treatment groups (table 2).

Data changes on weight gain after 14 days of okra extract intervention showed weight gain in all treatment groups but not significant, and between treatment groups with normal and DM control groups did not show any significant difference (table 2). Group GOE I showed the greatest weight gain (42.50 ± 19.06 g) compared to other treatment groups and DM control group but lower than normal control group 47.75 ± 9.94 g.

| Groups | Pre intervention | Post intervention | ∆Body weight |
|--------|-----------------|------------------|--------------|
| Normal | 199.50 ± 4.41ᵃ | 247.25 ± 5.66ᵃ | 47.75 ± 9.94ᵃ |
| DM     | 227.75 ± 6.94ᵇ | 256.00 ± 7.52ᵃ | 28.50 ± 15.42ᵃ |
| GOE I  | 215.25 ± 6.11ᵃᵇ| 247.00 ± 5.87ᵃ | 31.75 ± 8.23ᵃ |
| GOE II | 201.25 ± 5.36ᵃᵇ| 243.75 ± 20.29ᵃ| 42.50 ± 19.06ᵃ |
| POE I  | 209.00 ± 1.47ᵃ | 237.00 ± 7.36ᵃ | 28.00 ± 6.36ᵃ |
| POE II | 215.50 ± 3.50ᵃᵇ| 241.50 ± 18.25ᵃ| 26.00 ± 35.5ᵃ |
| p-value | 0.008* | 0.924 | 0.826 |

*)ANOVA further Duncan’s multiple range test, significant on p<0.05

In this study using standard feeding, not specific diet. Okra extract given once a day by oral administration. Standard feeding will have no effect on body weight in adult age [35]. Rats given standard feeding are relatively stable on the body weight changes, although occurs weight gain in the end of study, but body weight changes in all treatment groups are relatively same or not significant. Intervention okra extract did not give a significant effect to changes in body weight of rats, it is suspected because quercetin in okra extract did not give real effect on weight change. In vivo studies used mice and rats with low to high dose ranges showed that quercetin in the okra has no effect on changes in the body weight and body fat. Quercetin only acts to increase transient energy expenditure in the body for 3 weeks [35-37].
4.3. Effect of intervention extract okra in pancreas histopathology

Histopathology observations of pancreatic tissues in rats were done to analyze the protective effect of the okra extract intervention on pancreatic tissue caused induced by STZ induction. Pancreatic histopathology control DM showed that beta cell destruction of pancreas has changed size, number and amount of Langerhans island below (figure 1A).

**Figure 1.** Pancreas histopathology A. DM group, few β pancreas cells which produce insulin (brown), B. Normal group, β cells active produce insulin. (immunohistochemistry indirect method metode, objective 40x).

The changes seen from nucleus cell that irregular changes shape, nucleus cell becomes small (piknosis) and disappears only visible cytoplasm that is experiencing hyperchromatic. Necrosis occurs in pancreas that begins with degeneration pancreatic cell or decreased cell function due to DNA fragmentation showed that damage to pancreatic β cells. In addition to necrosis, based on pathogenesis of type 2 DM, about 60-70% of pancreatic β cells under amyloid deposition [38]. Destruction of pancreatic β cells is reversible causing reduction secretion of insulin characterized by faded and slightly brown color. Induction STZ 50 mg/kgBB selectively damages the pancreas especially β cells so that insulin secretion in the body decreases [39].

The pancreatic histopathology in normal group (figur1B) showed that β cells highly active to produce normal amounts of insulin, there is no change in nuclear cell form. In the pancreatic tissues of normal group of entire island, their cells actively produce insulin can be known from intensity of strong brown color depicts the presence of insulin β cells are spread flat on islets. Specific immunohistochemical staining techniques is coloring β cells in pancreatic tissues and can be used to see whether or not insulin is present in β cells. In normal pancreatic tissue, the number of β cells is estimated to be 65% and α 35%, whereas the diameter of Langerhans islets 150 μm [40].

Pancreatic histopathology in treatment group (figur 2A) showed that alteration β cells. GOE I group which acquaired intervention of GOE dose 5 mg / kgBB showed the number of beta cells in the pancreas island is quite a lot but the amount of insulin produced is still slightly indicated by the lack of intensity of brown color slightly faded such as normal group pancreatic tissue. The histopathologic features of GOEII group (figur2B) also showed that regeneration of β cells previously damaged by STZ induction. Intervention of GOE dose 10 mg/kgBB resulted that regeneration of β cells pancreas characterized by β cells in island already in large quantities and began to actively produce insulin but the amount of insulin contained in island is still slightly characterized by a faded brown color when compared with GOE I group.
Figure 2. Pancreas histopathology. A. DM group + GOE I, β cells have been active to produce insulin, B. DM group + GOE II, the amount of β cells which produce insulin less than GOE I group (Immunohistochemistry indirect method, objective 40x).

Immunohistochemistry pancreatic tissue of diabetic model rats treated with POE I treatment in (figure 3A) showed that β cell have regeneration after 14 days intervention.

Quercetin is known have potential antidiabetic through proliferation pancreatic cells, which is thought to inhibit apoptosis in β cells during the development of diabetes, a mechanism that key to antidiabetic potential of quercetin [41-43]. Decrease apoptosis of β cells can affect blood glucose levels and increase insulin secretion. Phenolic compounds play a role reducing the burden of chronic stimulation of β cells and pressing pressure on β cells, then delaying degradation of pancreatic islets and development of type 2 DM [44]. In this study, induction STZ 50 mg/kgBW did not result in beta cell damage absolute such as in type 1 DM, so this research suggested that intervention of okra extract to controldiabetes could be used in treatment type 2 DM.

5. Conclusion and recommendation

5.1. Conclusion
IC₅₀, antioxidant capacity, fenolic, and quercetin contents of purple okra extract were higher than green okra extract. Administration of GOE I, GOE II, POE I and POE II in diabetic rats did not give a significant effect to changes in body weight. POE with the dosage of 5 mg/kgBW effectively could improve repairment of β cell pancreas destruction due to STZ induction with pancreatic cell activation, and could improve the ability of β cell on insulin production. These results suggest that intervention of
green okra extract and purple okra extract based on quercetin compound showed an antidiabetic potency.

5.2. Recommendation
Further research is needed on the effect of dosing of quercetin on the okra at various levels to determine the effect and anti / pro-oxidant properties of quercetin in okra.

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