

Potential of Okra (*Abelmoschus esculentus* L.) Extract to Reduce Blood Glucose and Malondialdehyde (MDA) Liver in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The study aimed to analyze the potential of okra to reduction blood glucose and MDA liver in streptozotocin (STZ)-induced diabetic rats. Design experiment used in this study was pre and post test controlled group design. The first step of this study was analyzed bioactive compound of okra extract. The next step was administered orally of okra extract to control and diabetic rats induced by streptozotocin 50 mg/kgBW for 14 days. 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. Blood glucose were measured each five days and malondialdehyde (MDA) liver in rats were measured in the end of intervention. The following results show that phenolic and quercetin of purple okra extract were higher (3.60%; 0.45 mg/g) than green okra extract (3.58%; 0.27 mg/g). Administration of GOE I, GOE II, POE I and POE II in diabetic rats showed significant ($P < 0.05$) reduction in blood glucose level (115.25 mg/dl; 86 mg/dl; 180.75 mg/dl; 91 mg/dl) and improve level of MDA. These results suggest that intervention of okra extract based on quercetin compound show an antihyperglycemic potential and improve MDA level.

Keywords: blood glucose, diabetic rats, malondialdehyde, okra extract, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease resulting in an impaired metabolism of carbohydrate, protein and lipid which associated with insufficient secretion of insulin that occurs in type 1 DM or increased insulin resistance as characteristic of type 2 DM (ADA 2014). Type 2 DM represents more than 90% of all cases of DM in the world. According to the International Diabetes Federation (IDF), the number of diabetics reach 382 million in 2013 and this value is predicted to rise to around 592 million by 2035 (IDF 2013).

Hyperglycemia condition can increase oxidative stress by increasing the production of Reactive oxygen species (ROS) by electron transport chains in mitochondria characterized by the changes in glucose biochemistry and end-product of lipid peroxidation indicator i.e malondialdehyde (MDA) (Gupta *et al.* 2009; Aouacheri *et al.*

2014). Increasing oxidative stress be risk factor leads to insulin resistance, dyslipidemia, β cell dysfunction, impaired glucose tolerance and type 2 DM (Tangvarasittichai 2015).

Epidemiological studies suggested that consumption of foods rich in bioactive compounds as catechin in tea, anthocyanin in plants colored red, purple, blue, and quercetin compound can reduce risk of diabetes, heart disease, obesity, hyperlipidemia, stroke and cancer (Xiao *et al.* 2011; Wedick *et al.* 2012; Damayanthi *et al.* 2013). Okra (*Abelmoschus esculentus* L.) also known as lady finger or gumbo, is a tropical vegetable that included in the Mallow family. Okra contains of many flavonoids compounds which have antioxidant activity (Liao *et al.* 2012; Arapitsas *et al.* 2008).

The potential of okra (*Abelmoschus esculentus* L.) as antidiabetic has been demonstrated from some research. Antidiabetic potential of okra by following mechanism: improve uptake

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glucose tissue (Prior *et al.* 2008), improve insulin sensitivity (Sasaki *et al.* 2007), prevent lipid peroxidation (Obloh *et al.* 2014), promote liver and pancreatic cell proliferation and act as α -glucosidase inhibitor (Babu *et al.* 2013). Research conducted by Sabitha *et al.* (2013) have shown that administration of peel and seed okra extract with th dosage of 100 and 200 mg/kgBB significantly lowered in blood glucose levels and increase weight loss and prevent lipid peroxidation in STZ-induced diabetic rats. Purple okra as a results of okra superior cultivation from *Zahira* varieties. Some scientists studied green okra useful in DM and some of them having antioxidant properties, while are studying purple okra useful still rarely done. Antioxidant potential of green okra and purple okra need to be intervention for DM treatment. The aim of the present study was analyzed the effect of green and purple okra extract as anti hyperglycemia agents and improve the level of MDA liver in streptozotocin (STZ) induced diabetic rats.

METHODS

Design, location, and time

Pre and post test controlled group design were used for the experiment. This research held on February until September 2017. It took place in Food Chemistry and Nutrient Analysis Laboratory Community, Nutrition Department, Faculty of Human Ecology and SEAFast Center Bogor Agricultural University. In vivo stage were done at Animal Laboratory Management Unit (UPHL) Faculty of Veterinary IPB. MDA liver measurement were conducted at Pathology Anatomy Laboratory, Medical Faculty in Brawijaya University Malang. This research were got ethical clearance from Animal Ethical Comission LPPM IPB with number 63-2017 IPB.

Materials and tools

The main material used in this research is green okra from *Naila* varieties and purple okra from *Zahira* varieties was obtained from Prof. Muhammad Syukur Department of Agronomy and Horticulture of Bogor Agricultural University. 3 month old male Sprague Dawley rats obtained from the National Food and Drug Administration Center BPOM Jakarta. Material used in the okra extraction is 70% methanol solution. Materials used for proximate analysis include 0.3 ml Folin-Ciocalteu, gallic acid standard solution

for total phenolic analysis and quercetin standard solution for quercetin analysis. Equipments used in the making of okra extraction and proximate analysis were magnetic stirrer Bellco glass. inc (Vineland, USA), vacuum evaporator, freeze dryer, and spectrophotometry Optima SP-300 (Japan), glass (Pyrex®), filter paper Whatman, micropipet 1000 μ L and 10-100 μ L, reaction tube (Schott Duran), centrifuge (Kokusan, Japan), column stationary phase HPLC, vortex, and water bath aquades (Julabo SW22, Japan).

Material and equipment for animal maintenance were individual cage with cage area 33.5 x 27 x 12 cm, husk, drink bottle 250 ml, digital scales Sigma, rats fed standard based on AIN-93 formulation. Material and equipment for diabetes induction were streptozotocin (STZ) from Sigma Chemical Co. (St. Louis, MO, USA), physiological NaCl and syringe intraperitoneal injection (Terumo). Blood glucose were measured using glucometer (GlucoDr Korea) and glucostrip (GlucoDr Korea). Malondialdehyde (MDA) were analyzed using ketamine and xylazine mix solution, scalpel, surgical board for got liver organ.

Procedures

Okra extraction and preparation. 500 g green and purple okra (without separating between skin and seeds) were dried using *freeze dryer* vertical tipe Snijders scientific. Dried okra weighed and smoothed with blender. 10 g powder dried okra added with 500 ml 70% methanol solution in the Erlenmeyer continued with extraction gradually until obtained clearly residue. Filtrate evaporated with vacuum evaporator. Okra extract weighed and analyzed bioactive compound total phenolic using spectrophotometry method (Liao *et al.* 2012) and quercetin compound analyzed using *High Performance Liquid Chromatography* (HPLC) method.

Quercetin analysis using HPLC method. 0.5 g sample were diluted with MeOH 62.5% TBHQ (2 g/l) up to 20 ml. Sample solution were incorporated in Erlenmeyer added with 5 ml HCl 6M then refluxed for 2 hours at 90°C. The extract were allowed to cool then filtered into 25 ml measuring flask. A filtering were performed using 0.45 μ m filter paper whatman and were placed into 25 ml measuring flask diluted with aquades. 10 μ L samples were injected into HPLC 370 nm with comparison of ACN and K₂HPO₄ 0.025M column phase 25:75. Standard quercetin curve were read for 30 min, followed by injecting

10 µl samples into HPLC system for 30 min to obtain quercetin levels of okra extract (Hertog *et al.* 1995).

Streptozotocin (STZ) induction and rats maintenance. 24 rats were adapted for 14 days with standard feeding formulated based on AIN-93 and allowed to drink water *ad libitum*. At day 15 after adaptation, 4 rats were included in normal group (N) and 20 rats were performed intraperitoneal STZ induction. After overnight fasting (12-16 h), rats were injected intraperitoneally with single dose of STZ 50 mg/kgBW. The success of diabetes induction was determined by measuring blood glucose levels after a 3-day STZ induction. When fasting glucose plasma (FPG) levels > 126 mg/dl were included in diabetic model rats groups (Jung *et al.* 2011). The diabetic model rats were divided into 5 randomized treatment groups including diabetes control (DM) ie untreated diabetic model rat, treatment group given green okra extract with the dosage of 5 mg/kgBW quercetin (GOE I) and 10 mg/kgBW (GOE II), treatment group given purple okra extract with the dosage of 5 mg/kgBW quercetin (POE I) and 10 mg/kgBW (POE II). The GOE and POE dissolved with Twin 1% were administered orally to the treatment group animals for 14 days. Administration of okra extract each day limited to maximum of 3.5 ml based on stomach capacity of rats were 10 ml/kgBW (Mc Connel *et al.* 2008). The dosage of 5 mg/kgBW quercetin on okra extract equals with consumption of 263 g fresh okra for humans 70 kg, while the dosage of 10 mg/kgBB quercetin on okra extract was equivalent to the consumption of 526 g fresh okra. The dosage of okra extract was based on quercetin compound present in okra based on Gomes *et al.* (2015) research.

Measurement of fasting plasma glucose (FPG) and malondialdehyde of liver levels. Blood glucose was measured every five days start from 0 day after rats showed diabetic condition by induced STZ. After overnight fasting (rats were deprived of food for 12-16 h but allowed to water), rats were collected blood from vein tail. In the end of intervention (day 15) rats were general anesthesia using ketamine: 75 mg/kgBB and xylazine: 5 mg/kgBB solution. Liver organ were obtained from rats surgery. Measurement of malondialdehyde level on liver organ using thiobarbiturat acid test (TBA) (Aulanni'am *et al.* 2012).

Data analysis

Data was processed and analyzed using Microsoft Excel 2010 and SPSS software version 16.0. Statistical analysis were assessed by one-way analyses of variance (ANOVA). Pre-test and post-test data of results were assessed by paired sample t-test to analyzed differences data between before and after intervention. If there is differences between data sets (significant on $p < 0.05$), further test conducted by Duncan's multiple-range test.

RESULTS AND DISCUSSION

Bioactive compound of green okra and purple okra extract.

Initial stages in this study is to analyze bioactive compound of green okra extract (GOE) and purple okra extract (POE) including total phenolic, and quercetin. GOE contents quercetin compound 0.27 mg/g, while quercetin compound of POE were higher 0.45 mg/g (Table 1). Quercetin is the major flavonoid in Okra where 70% total antioxidant compound in okra comes due to the quercetin derivative (Shui & Peng 2004). Quercetin content in GOE and POE were higher when compared with torbangun leaves extract (*Coleus amboinicus* Lour) 0.02 mg/g (Trini *et al.* 2015).

Table 1. Bioactive compound of GOE and POE

Bioactive compound	GOE	POE
Total Phenolic (%)	3.58	3.60
Quercetin (mg/g)	0.27	0.45

Scientific research about bioactive content of okra extract has been done. Some studies have shown that the skin and okra seeds contain polyphenol components such as hydroxynamic acid and its derivatives, catechins, quercetin derivatives, and flavonols (Shui & Peng 2004; Huang *et al.* 2007). The results showed that total phenolic of POE and GOE are similar almost, while quercetin on POE was higher than GOE. Okra skin contains polyphenols from hydroxynamic acid group and quercetin derivatives in the amount of 0.2 mg/g and 0.3 mg/g samples respectively (Arapitsas *et al.* 2008). In this study using fresh okra without separated between skin and seeds.

The highest polyphenols compound in okra can found in seeds with total phenolic of 29.5%, while in skin 1.25% (Fan *et al.* 2014). α -glucosidase activity in okra leaf extract and okra seed is quite large (IC_{50} =142.69 μ g/ml; 150.47 μ g/ml), while α -amylase activity (IC_{50} =132.63 μ g/ml; 147.23 μ g/ml) (Sabitha *et al.* 2012). α -glucosidase and α -amylase activity using IC_{50} method defined as the ability of okra extract to inhibit 50% breakdown of carbohydrates into glucose, this activity plays important role in the antidiabetic mechanism in managing increased blood glucose.

Effect of okra extract intervention on blood glucose

The mean fasting plasma glucose (FPG) of treatment group at pre-intervention were 210.25 to 285 mg / dl, 14 days after oral administration of okra extract (green and purple) FPG ranged from 100.25 to 125.25 mg/dl (Table 2). 14-days after POE treatment, significantly decreased the FPG in diabetic rats ($p < 0.05$). GOE treatment group also showed a decrease in FPG comparable amount to the normal range although not significant based on different Paired t-test. From preliminary test results of okra extract known quercetin in POE was higher than GOE. The major flavonoid in okra is quercetin showed act as an antidiabetic potency (Fan *et al.* 2014).

Flavonoids have done antioxidant activity by scavenging ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) resulting from diabetogenic compounds ie STZ (Fuhrman & Aviram 2001). The major polyphenolic groups of flavonoids, tannins and phenolic acids play a

role in carbohydrate metabolism by inhibition of α -glucosidase and α -amylase responsible for digestion of starch into glucose, leading to an increased the blood glucose (Iwai 2008; Tadera *et al.* 2006). Decrease in FPG on treatment group of okra extract (green or purple) due to quercetin compound in okra showed antioxidant activity by reduction free radicals from STZ. When free radicals can be scavenge will cause the insulin receptors in beta cells become active and were effectively made glucose can be used by cells as energy and cause decreased gluconeogenesis, as a result FPG levels go through decrease. Regulation of carbohydrate metabolism by polyphenolics is also due to an increase of glycolysis and glucose oxidation in the body that can improve glucose homeostasis and insulin resistance (Bahadoran *et al.* 2013).

In vitro studies showed that polyphenolic quercetin may increase insulin production, glucose availability in muscle cells and adipocytes through GLUT4 glucose transporter translocation to plasma membranes thus inducing the AMP-activated protein kinase (AMPK) pathway (Zhang *et al.* 2011; Park *et al.* 2007). AMPK is an energy cellular sensor as a stimulation of glucose uptake to increase of glucose uptake as an energy store, when AMPK pathway become active is considered a new mechanism in the role of treatment of obesity, type 2 DM, metabolic syndrome (Towler 2007).

POE group with the dosage of 5 mg/kgBW quercetin showed that greatest effect on decreases of FPG compared with other treatment groups. The increasing dosage of okra extract

Table 2. Fasting plasma glucose (FPG) in rats pre and post intervention

Groups	FPG	FPG	p	Δ FPG
	pre intervention	post intervention		
	Mean \pm SEM (mg/dl)	Mean \pm SEM (mg/dl)		
Normal	84.25 \pm 5..	82.75 \pm 5.39	0.678	-1.50 \pm 3.28
DM	319.00 \pm 71.04 ^a	375.00 \pm 70.60 ^b	0.316	54.25 \pm 47.95 ^b
GOE I	215.50 \pm 50.34 ^a	100.25 \pm 8.32 ^a	0.074	-115.25 \pm 42.86 ^a
GOE II	210.25 \pm 32.13 ^a	124.25 \pm 34.26 ^a	0.061	-86.00 \pm 29.45 ^a
POE I	284.50 \pm 50.77 ^a	103.75 \pm 7.09 ^a	0.029*	-180.75 \pm 45.65 ^a
POE II	236.00 \pm 71,89 ^a	145.00 \pm 58.43 ^a	0.009*	-91 \pm 14.80 ^a
p	0.0084**	0.002**		0.009**

*) Differences test using *Paired t-test*, significant difference on $p < 0.05$, **) ANOVA followed by Duncan's multiple range test, significant on $p < 0.05$.

didn't showed better effect on blood glucose levels because of administration high dosage of certain bioactive compounds such as flavonoids can be prooxidant and trigger an increase in ROS by autoresidation and altering redox reactions (Bouayed & Bohn 2010; Iwasaki *et al.* 2011). All of the treatment group had a significant decreases of FPG compared with DM group, but the decrease of FPG between treatment groups was not significantly different or relatively similar and close to FPG range in normal group. The decrease of FPG levels with administration of okra extract was due to quercetin compound in okra. Oral administration of quercetin in STZ-induced diabetic rats has an effect decreasing in serum glucose and viability of pancreatic β cells by increasing the natural antioxidant system and inhibition of lipid peroxidation (Yin *et al.* 2011).

Effect of okra extract intervention on malondialdehyde of liver

In this study different interventions showed that significant changes ($p < 0.05$) on MDA levels rats. MDA levels after 14 days of treatment in DM group were significantly different with mean MDA levels of normal and treatment groups (Table 3).

Table 3. Malondialdehyde (MDA) of liver level on post intervention

Groups	Malondialdehyde (MDA) level
	Mean \pm SEM (ng/ml)
Normal	148.00 \pm 2.22
DM	227.25 \pm 3.07 ^c
GOE I	164.50 \pm 8.87 ^{ab}
GOE II	186.88 \pm 10.04 ^b
POE I	156.50 \pm 10.05 ^a
POE II	182.00 \pm 7.41 ^{ab}
p	0.000*

* ANOVA followed by Duncan's multiple range test, significant ($p < 0.05$)

High levels of MDA in DM control due to the condition of diabetes caused insulin levels low as a result of β -oxidation initiation fatty acids and the accumulation of free radicals in body that produce lipid peroxidation products (Annie *et al.* 2005). The mean levels of MDA in treatment group showed significant improvement af-

ter 14 days the intervention compared to a control group of diabetes, shown that okra extract based on quercetin compound reduced level of MDA in STZ induced diabetic rats. Antioxidant activity on the major flavonoid in okra that is quercetin compound due to an aromatic hydroxyl group which as ROS and RNS scavenger by inhibiting peroxidation reaction which leads to formation of final products in lipids peroxidation (du Thie & Crozier 2000; Moskaug *et al.* 2004).

Polyphenols compounds have antioxidant activity as free radical scavenger by direct interaction with cellular receptors as a key of redox stages resulting in modified redox reactions and triggering a series of redox-dependent reactions (Scalbert *et al.* 2005). In vivo studies conducted by Oboh *et al.* (2014) reported that quercetin significantly inhibited the activity of α -amylase and α -glucosidase which suppressed the rise of blood glucose thus inhibiting lipid peroxidation process and pancreatic cell destruction.

The mean data of MDA levels showed that group receiving GOE or POE intervention with the dosage of 5 mg/kgBW caused the change of MDA level close to the range MDA levels of normal group. The mean level of MDA in GOE and POE treatment group with the dosage of 10 mg/kgBW was significantly different with mean level of MDA in DM group, but the change of MDA level given better effect compared with the dosage of 5 mg/kgBW.

Intervention of POE based on quercetin compound at the dosage of 5 mg/kgBW shows that highest decrease level of MDA compared with other treatment groups after 14 days. Quercetin treatment could act as antioxidant and prooxidant depending on the concentration given. At low concentrations of quercetin given protective effect on DNA damage (in vitro) in human lymphocytes and prevent occurrence of stress oxidative, but high concentration of quercetin with the dosage of 30 μ M caused cytotoxicity effect (Spencer *et al.* 2003; Wilms *et al.* 2005).

CONCLUSION

Intervention of okra extract (green okra and purple okra) based on quercetin compound showed reduction on blood glucose levels and improved level of MDA liver. Purple okra extract significantly lowered blood glucose in streptozotocin induced diabetic rats.

Further research is needed on influence the dosage of okra extract based on quercetin compound in a variety of levels from low dosage up to high dosage (more than 10 mg/kgBW) to know the effects and characters of anti/pro-oxidants quercetin of okra.

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