

Okra Infusion Water Improving Stress Oxidative and Inflammatory Markers on Hyperglycemic Rats

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Abstract:

Objective: Okra has been known for its properties in improving diabetes markers. Most study used okra extract instead of its infusion water. Okra infusion water (OIW) contains polysaccharide and viscous fiber that delay gastric emptying, and help controlling glucose and HbA1C level. As the glucose low level, so does the inflammation and oxidative stress on hyperglycemic rats. This study aims to investigate the effect of OIW in improving glucose level, HbA1C, SOD and CRP levels. **Materials and methods:** Posttest only control group design was applied to this experimental study using 15 male wistar rats divided randomly into 3 groups: Control, Streptozotocine (STZ), Streptozotocine+okra (OKRA). STZ, and OKRA groups induced with 65 mg/kg BW Streptozotocine and 110 mg/kg BW Nicotinamide on day 8. After the desirable glucose level achieved, OKRA were given 3.6 ml OIW on day 12-28. Data were analyzed using ANOVA in each variable. **Results:** Fasting and Postprandial Glucose level of OKRA (137,40±3,57 mg/dL and 154.58±2.71 mg/dL), STZ (251,77±2,30 mg/dL and 270.18±3.03 mg/dL); HbA1C level of OKRA (7.93±0.25), STZ (21.29±0.65); SOD level of OKRA (55.292±3.77%), STZ (16.472±5.298%); CRP level of OKRA (1,540±0,059 mg/dL), STZ (2,230±0,093 mg/dL). **Conclusions:** OIW able to improve glucose, HbA1C, SOD, and CRP level on hyperglycemic rats.

Keywords: okra infusion water, hyperglycemic, streptozotocine induced rats

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Introduction

Diabetes Mellitus is a degenerative disease that leads to vascular comorbidities like coronary artery disease, detrimental effect on physical and cognitive function, and also death.¹ Type 2 Diabetes Mellitus (DM2) caused by deficiency on insulin production, and insulin resistance.² Diabetes Mellitus marked by hyperglycemia, an elevated blood glucose level beyond normal range.³ Hyperglycemia resulted from imbalance condition between hepatic glucose production during fasting, glucose intake, and periphery tissues resistance on insulin action.¹ Hyperglycemia activate protein kinase C, polyol,

and hexosamine pathways, produce advanced glycosylated end products, leads to mitochondrial dysfunction, endoplasmic reticulum stress, thus promote accumulation of reactive oxygen species, and induce oxidative stress. Hyperglycemia also increase pro-inflammatory expression causing apoptosis.⁴ Chronic hyperglycemia resulted in long term damage, including eyes, kidneys, nerves, heart, and blood vessels.⁵ Therefore, controlling hyperglycemia is mandatory in order to prevent further damage either by administration of oral hypoglycemic agent, or plant with hypoglycemic properties.

Okra (*Abelmoschus esculentus*) is a crop that widely

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known and used from Malvaceae family. Okra is a multipurpose crop used for its pods, seeds, leaves, flowers, stems, and buds on fresh conditions as vegetables in salads, soups, stews. Ethnomedicine also used okra mucilage as plasma replacement, cholesterol binding and so on.⁶ Powder of peel and seed of Okra (*Abelmoschus esculentus*) has been studied for its properties in lowering blood glucose level on streptozotocine induced rats.⁷ Overnight okra infusion water able to reduce blood glucose on rats,⁸ but did not evaluate the effect of its administration on stress oxidative and inflammation markers, therefore this study is needed to fill in the gap.

Materials and methods

Streptozotocine and Nicotinamide Doses

Nicotinamide (NA) 110 mg/kg body weight was injected intra peritoneal to prevent the occurrence of Diabetes Mellitus type 1. Fifteen minutes after NA injection, Streptozotocine (STZ) 65 mg/kg body weight was then injected intra peritoneal. Leave for three days to achieve the desirable serum glucose level.

Preparation of Okra Infusion Water

Green colored okra with smooth and intact surface was purchased from traditional market. Three fresh okra's pod were sliced and infused in 250 ml water. After 12 hours, okra pieces were removed, the already slimy water were then given to rats in OKRA group using oral gauge. Daily consumption of okra in human range 2-3 pods, weigh 20 grams, then infused in 200 ml potable water. Thus, the conversion dose for rats is 3.6 ml Okra Infusion Water (OIW). The okra infusion water was freshly made every day, and given every morning, from day 8 until day 39.

Study design and animals

Posttest only controlled group design was applied to this experimental study. Using 15 male wistar rats, aged 3 months, weighed 200 grams, obtained and maintained in Centre for Food and Nutrition (Pusat Studi Pangan dan Gizi) of Gajah Mada University, Yogyakarta Indonesia. After being acclimatized for 7 days using only standard pellet and distilled water ad libitum, rats were randomly divided into 3 groups as follow:

CONTROL group	:	Received only standard pellet and distilled water, without any streptozotocine and Nicotinamide (STZ & NA) injection, or okra.
STZ group	:	Injected with STZ & NA
OKRA group	:	Injected with STZ & NA, given 3.6 ml okra infusion water

On day 8, STZ, and OKRA group were injected with 110 mg/kg BW Nicotinamide and 65 mg/kg BW streptozotocine intraperitoneal, leave for 3 days with ad libitum diet and distilled water. On day 11, blood glucose were then measured using glucometer. Hyperglycemia obtained if the glucose level > 200 mg/dL and followed by treatment in each group.

After the desirable glucose level was achieved, okra infusion water was given for 28 days in OKRA group. At the end of treatment (day 40), rats were fasted overnight. Blood samples were collected from rats using ophthalmic vein, measured for its glucose (fasting and postprandial), HbA1C, SOD, and CRP level.

The Evaluation of Blood Glucose, HbA1C, SOD and CRP level

Rats were fasted overnight. In the morning, blood serum was collected from ophthalmic vein, measured for fasting glucose level using enzymatic glucometer. Rats were then fed with standard pellet, fasted for 2 hours, and measured for post prandial glucose using the same method as the fasted glucose before. Blood collected were tested for HbA1C, SOD, and CRP using ELISA.

Statistical analysis

Statistical analysis of this study were performed using SPSS 22. Both descriptive (mean, and standard deviation) and inferential test were used to analyze the results. ANOVA was used to compare differences within each variable, considered significance only if $p < 0.05$. **Ethical clearance:** This study was performed after being approved by Health/Medical Research Bioethic Commission, Medical Faculty of UNISSULA (No. 289/IX/2017/Bioethic Commission).

Results

After being acclimatized, rats on STZ, and OKRA group were injected with STZ and NA on day 8. Blood glucose level were analyzed at day 11 (Table 1) to evaluate whether the hyperglycemic induction was succeeded or not.

Table 1. Mean Blood Glucose Level (\pm SD) after injected with STZ & NA

	STZ	OKRA	p
Blood Glucose Level	225.34 \pm 5.11	221.28 \pm 4.78	0.077

The induction was succeeded, blood glucose level was more than 200 mg/dL, and all those three groups showed no differences ($p > 0.05$). The treatment for each groups were then conducted for 28 days. The glucose level (both fasting and postprandial glucose

level), HbA1C, SOD, and CRP level at the end of treatment (day 40) were displayed at Table 2.

Table 2. Mean (\pm SD) Fasting, postprandial glucose level, HbA1C, SOD, and CRP level after treatment (at day 40)

Variable	CONTROL	STZ	OKRA	p ANOVA
Fasting Glucose (mg/dL)	83.68 \pm 0.46	251.77 \pm 1.03	137.4 \pm 3.57	0.000
Postprandial Glucose (mg/dL)	98.48 \pm 3.09	270.18 \pm 3.03	154.58 \pm 2.71	0.000
HbA1C (%)	4.37 \pm 0.41	21.29 \pm 0.65	7.93 \pm 0.25	0.000
SOD (mg/dL)	83.53 \pm 4.07	16.47 \pm 5.3	55.29 \pm 3.77	0.000
CRP (mg/dL)	0.69 \pm 0.54	2.23 \pm 0.09	1.54 \pm 0.06	0.000

The best results for fasting & postprandial glucose level, HbA1C, SOD, and CRP level were found in CONTROL group (p 0.000), followed by OKRA group, and the worst results were on STZ group. Posthoc test was conducted in order to evaluate the significant difference between groups. The differences between fasting, postprandial glucose, and HbA1C level was shown in figure 1, while the CRP and SOD level between groups was shown in figure 2.

Discussion

Streptozotocine and Nicotinamide (STZ-NA) were succeed in diabetic/hyperglycemic induction in this study. Mean glucose level on STZ, and OKRA group after being induced with STZ-NA were significantly higher than in the CONTROL group, which was received no induction at all. Other study using 110 mg/kg BW NA and 65 mg/kg BW STZ was also showed hyperglycemic condition, marked by blood glucose level >200 mg/dL. Streptozotocine known for its high affinity for β cell membrane, generate free radical formation, which causes DNA methylation and breaks, activate poly ADP ribose polymerase (PARP-1), decreases NAD⁺, and leads to energy deprivation and β cell death. Thus impairing insulin production and secretion.⁹ While STZ has a detrimental effect on β cell, Nicotinamide protected it. Nicotinamide acts as an oxygen free radicals scavenger, inhibiting PARP-1, and increasing NAD⁺, thus NA is able to protect β cell's damage from STZ induction, and modulate the occurrence of Diabetes Mellitus type.¹⁰ After successfully induced by STZ-NA, rats on OKRA group were treated with okra. The results for fasting and postprandial glucose level in OKRA group was better than those in STZ group. This results is similar to Sabitha's, administration of Okra's peel and seed powder decreased elevated blood glucose after STZ induction.⁷ Okra's extract also showed reduction in blood glucose of STZ

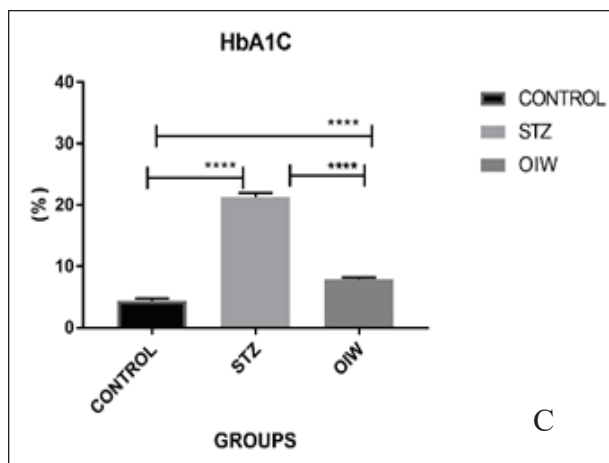
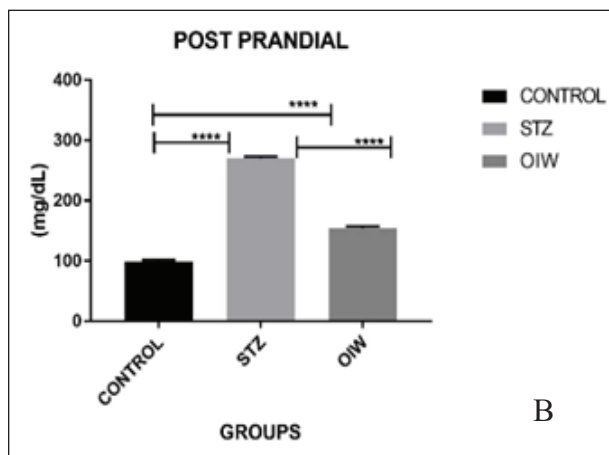
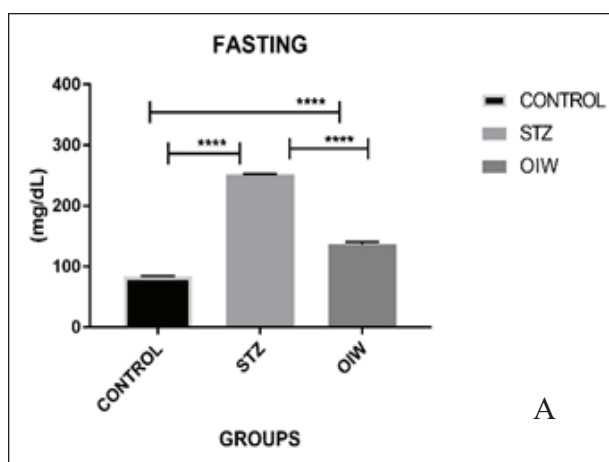


Figure 1. The fasting (A), postprandial glucose (B), and HbA1C (C) level between groups (****p<0.0001) induced diabetic mice.⁸ Okra infusion water contains viscous water soluble dietary fiber.⁶ Administration of viscous water soluble dietary fibers reduced glucose diffusion and postponing the absorption and carbohydrate digestion, therefore reducing postprandial blood.¹¹ Fiber consumption associated with beneficial shifts in gut microbial composition.¹² Dietary fibers resist digestion in intestine, therefore it is fermented to produce short chain fatty acid/

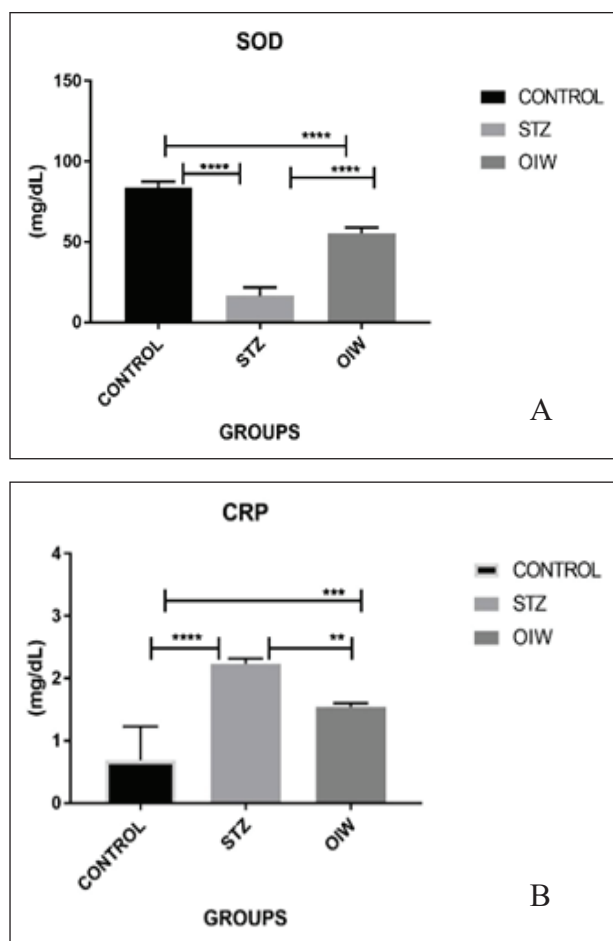


Figure 2. SOD (A) and CRP (B) level between groups (**** $p < 0.0001$)

SCFA. SCFA reducing serum free fatty acids, hence decrease blood glucose levels through competition in insulin-sensitive tissues.¹³ Isoquercetin and quercetin 3-O-gentibioside in okra has ability to inhibit carbohydrate metabolizing enzyme, maltase and sucrose, and act as α -glucosidase inhibitors.¹⁴ Other study found that ethanol extract of Okra administration inhibited Peroxisome Proliferator-activated receptor (PPAR γ), the important regulator in glucose and lipid homeostasis.¹⁵

The present study also showed the HbA1C level in OKRA group was found lower than in STZ group. Study from Sabitha also found that HbA1C in the administration of Okra's peel and seed powder is lower than those in diabetic rats group.⁷ HbA1C is a modified form of hemoglobin, derived from non-enzymatic binding between glucose and hemoglobin via Amadori reaction.¹⁶ The α -glucosidase inhibitors property from okra reducing glucose level,¹⁴ minimizing the binding between glucose and hemoglobin, hence reducing the HbA1C score.

While the glucose parameter shown improvement with Okra's administration, the same pattern also

seen in inflammation marker, C-reactive protein/CRP. Diabetes Mellitus associated with inflammation, marked by an increase in CRP level.¹⁷ CRP in OKRA group was better than CRP level in STZ group. In a study on diabetic patients consuming more dietary fiber also shown a reduction in CRP level.¹⁸ Dietary fiber intake was shown to reduce circulating level of CRP.^{19,20} The possible mechanisms of this reduction is due to its ability to slow down the glucose absorption, regulate insulin sensitivity, and reduce lipid oxidation, hence alleviate inflammation process.²¹ High fiber diet also contains vitamins, minerals, and antioxidants which may reduce inflammation through other pathways.¹⁹ Myricetin in Okra is able to improve carbohydrate metabolism and enhance glucose utilization.²¹ Myricetin inhibit the production of pro-inflammatory mediators. Myricetin suppress NF- κ B and STAT1 activation and Nrf2-mediated HO-1 expression pathways. NF- κ B is a key for inflammatory mediators production.²⁰ Okra contains polyphenols and flavonoids in its pod and seed.²² The SOD level on OKRA group was higher than SOD level on STZ group. This results is in-line with previous study, the administration of okra seed increase SOD level in mice.²³ Okra's pod contains quercetin, myricetin, ascorbic acid.⁶ Quercetin is a potent antioxidant which act as an enhancer for endogenous antioxidant enzymes activity (such as SOD), and also act as an inhibitor for generation of free radical generation.²⁴ Quercetin is able to increase tissue total antioxidant capacity, lowering tissue oxidant level and oxidative stress.²⁵ Ascorbic acid interact with harmful free radical, quenching free radical until became less reactive, and thus increase SOD level.²⁶ Myricetin, also act as an antioxidant if combined with ascorbic acid, by removing central atom of the iron-ascorbic acid complex, reducing oxidative stress, and increasing endogen antioxidant.^{27,28}

Conclusions

Okra infusion water improve fasting glucose level, postprandial glucose level, HbA1C, CRP and SOD level of diabetic induced rats.

Conflict of interest: None

Authors' contribution:

Data gathering and idea owner of this study: Tyagita N, Study design: Tyagita N,

Data gathering: Tyagita N, Utami KP, Zulkarnain FH, Rossandini SM, Pertiwi NP, Rifki MA, Safitri AH.

Writing and submitting manuscript: Tyagita N

Editing and approval of final draft: Tyagita N, Utami KP, Pertiwi NP, Rifki MA, Safitri AH

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