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# Anti Diabetic and Anti Hypercholesterolemia Potential of Abelmoschus Esculentus (Okra) Functional Beverage with Ginger Extract in Streptozotocin-Induced Diabetic Mice

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## Abstract

27 The prevalence of diabetes mellitus is projected to increase. Okra, ginger, and stevia have potential properties to improve the metabolic effect of diabetes. This 21 study aimed to investigate the anti-diabetic and anti hypercholesterolemia effects of okra-functional drinks in diabetic mice. 32 twenty-four male mice were divided randomly into four groups of control, okra, diabetes, diabetes + okra. In this experimental study, diabetes was induced by streptozotocin. Okra juice was combined with stevia and ginger extract in a 75:22:3 ratio and administered orally for twelve days. Bodyweight, blood glucose, and cholesterol total were then measured. Okra beverage significantly decreased blood glucose ( $p < 0.05$ ), and it maintained weight in diabetic mice. However, okra showed no effect on cholesterol. This result indicates that okra seemed to be a blood glucose-lowering agent but not possesses lipid-lowering properties.

**Keywords:** Blood glucose, Diabetes Mellitus, Okra, Ginger, Cholesterol Total

## 20 Introduction

Diabetes Miletus (DM) is a chronic, non-communicable disease that affects a large proportion of the world's population. According to WHO, the prevalence rate of DM in Indonesia is 6.6% and contributes 6% of the major causes of death (Organization, 2017, Organization, 2018). DM is defined by hyperglycemia and clinical symptoms such as polyuria, polyphagia, polydipsia, and unexplained weight loss (Rudianto et al., 2011). In diabetes, excess body weight, family history, and poor dietary habits and lifestyle contribute to risk factors of the disease (Elling et al., 2018). Diabetes is currently managed by antidiabetic drugs usage, nutrition therapy, and exercise (Palsamy and Subramanian, 2008, Dyson et al., 2018). However, drug use is also reported to have adverse effects, which encourages researchers to look for alternative therapies such as herbal medicine and its interactions (Gupta et al., 2017, School of and Related Research, 2003).

Currently, the research focuses on the okra-ginger beverage sweetened with stevia, referred to as a functional okra beverage. Sensory evaluations conducted previously revealed that the functional beverage made with okra, stevia, and ginger in a 75:22:3 ratio received the highest ratings for color, aroma, and viscosity (Azni and Amelia, 2018). Okra, ginger, and stevia have nutritional values and can be used as potential diabetic therapeutic agents. Okra (*Abelmoschus esculentus*) is a vegetable that contains a high concentration of antioxidants and fiber, which has benefits in blood glucose regulation (Arapitsas, 2008, Fan et al., 2013). Stevia leaves can be a sugar substitute with no calories with an anti-diabetic effect (Aghajanyan et al., 2017). In addition, ginger can be added to the beverage formulation to mask the okra's unpleasant aroma and boost the okra functional drink's antioxidant content.

Dyslipidemia condition is commonly found in diabetes (Wu and Parhofer, 2014). Improved glycemic control has a beneficial effect on lipoprotein levels in diabetes, thereby reducing the risk of cardiovascular disease (Maahs et al., 2010, Ray et al., 2009; Permatasari et al., 2021). On diabetic mice, the effect of okra in functional drink form combined with ginger and stevia remains unclear. As a result, it is essential to investigate the effect of okra-functional drink on blood glucose and total cholesterol levels in DM mice.

## Materials and Methods

### Okra preparation

Okra was sorted and washed. After sliced, okra was blanched at 80° C for 15 minutes and dried. The dried okra was blended with water and filtered. Okra juice was formulated with stevia and ginger extract in the ratio of 75:22:3, respectively. Detailed preparation has been published elsewhere (Azni and Amelia, 2018).

### Animal model

Twenty-four Swiss male mice (30 – 50 days, 20-35 gram) were obtained from Animal Laboratory Management Unit, Ministry of Health Republic of Indonesia. The animals were habituated on the condition at 12 hours light-dark cycle in a temperature-controlled room at 25 ± 2 °C and standard diet for ten days. Mice were divided then into four groups (n= 5-7): control group (standard diet), okra group (standard diet + okra 0.52 ml/day), diabetes group (standard diet + STZ injection), diabetes + okra group (standard diet + STZ injection + okra 0.52 ml/day) for 12 days. A standard diet and water were provided ad libitum. The animal experiments were approved by the Research Ethics Committee University of Pembangunan National Veteran, Jakarta (No. B/1949/5/2019/KEPK). The research protocol or use of experimental animals used is based on the Declaration of Helsinki and The Council for International Organizations of Medical Sciences (CIOMS).

### Diabetes Induction

Streptozotocin at a single dose of 55 mg/kg was dissolved in citrate buffer 0.1 mol/L (pH 4.4) and injected intraperitoneally after an overnight fast. The following day, an additional STZ dose of 40 mg/kg was administered. Blood sugar levels were measured three days after the injections. Mice with blood sugar levels greater than 200 mg/dl were classified as diabetic mice for this experiment.

### Weight and Blood Biochemistry Determination

Every three days, body weight and blood glucose levels were determined, while total cholesterol levels were determined before and after treatment. Blood glucose and total cholesterol levels were determined using a glucometer strip and a cholesterol strip (multi-check easy touch GCU meter) from a blood sample taken from the tail of mice.

### Statistical Analysis

The data are presented as the mean ± standard deviation (SD). Paired t-test and independent t-test were performed using SPSS 24 and Graph Pad Prism 8 software. A p-value < 0.05 was considered statistically significant.

## Results

### Effects of Okra Beverage on Body Weight

31 The initial body weight of mice in all groups was similar ( $26.7 \pm 2.90$  gr). Table I presents mice's body weight over twelve days. After treatment, the bodyweight of the control group and okra group gained significantly than that of the other groups ( $p=0.020$  and  $p=0.038$ , respectively). 16 There was no significant difference in body weight between the diabetic group treated and the diabetic group that was not treated with okra. This result indicates that okra had no effect on the diabetic mice's body weight.

Table 1. Dependent paired t test on before and after treatment

Days	Body Weight (grams)			
	Control	Okra	DM	DM+Okra
Day 0	$27.33 \pm 0.58$	$26.67 \pm 1.53$	$25.67 \pm 3.21$	$27.00 \pm 5.57$
Day 3	$28.00 \pm 0.00$	$27.67 \pm 3.06$	$23.33 \pm 2.08$	$27.00 \pm 5.29$
Day 6	$29.33 \pm 0.58$	$29.00 \pm 2.65$	$24.33 \pm 2.08$	$26.33 \pm 5.77$
Day 9	$29.33 \pm 1.15$	$30.00 \pm 1.00$	$24.00 \pm 4.24$	$27.67 \pm 6.35$
Day 12	$29.67 \pm 0.58$	$30.00 \pm 1.73$	$26.67 \pm 3.51$	$26.00 \pm 5.29$
p-value	0.02	0.038	0.225	0.662

Dependent paired t test on before and after treatment, significant on  $p < 0.05$

### Effects of Okra Beverage on Blood Glucose

Next, we measured the blood glucose levels. To confirm the effect of streptozotocin effect on the blood glucose of diabetic mice, a glucose test was performed three days after injection. Diabetes and okra-treated diabetes mice initially had significantly higher glucose levels ( $> 200$  mg/dl) than the control and okra groups, but then returned to near control group levels. After a week, 2 the blood glucose levels of the untreated diabetic group experienced an increase, whereas the DM mice treated with okra maintained a stable blood glucose level. After twelve days, the untreated DM group reached a high level of  $399.33 \pm 198.05$  mg/dl, 23 which was significantly higher than the okra-treated diabetes group ( $p=0.023$ ). This finding suggests that okra may prevent hyperglycemia. 26 No significant difference in blood sugar levels was seen in the control and okra groups (Fig. 1).

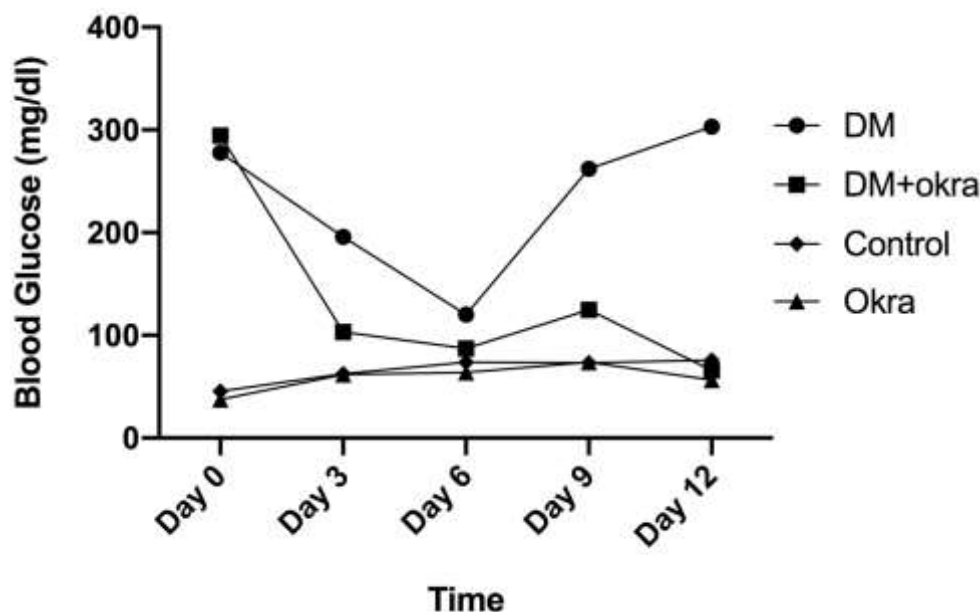


Figure 1. Effect Okra on Blood Glucose

### Effects of Okra Beverage on Cholesterol Total

As illustrated in Figure 2, there was no significant difference in the total cholesterol serum levels between the groups. However, there was a slight reduction in the cholesterol level in okra-treated STZ-diabetic mice compared to the untreated STZ-diabetic group. Even though it is unclear whether okra contributed to the decrease in cholesterol total in the diabetic group, okra may have positive effects if treatment is given for a longer period.

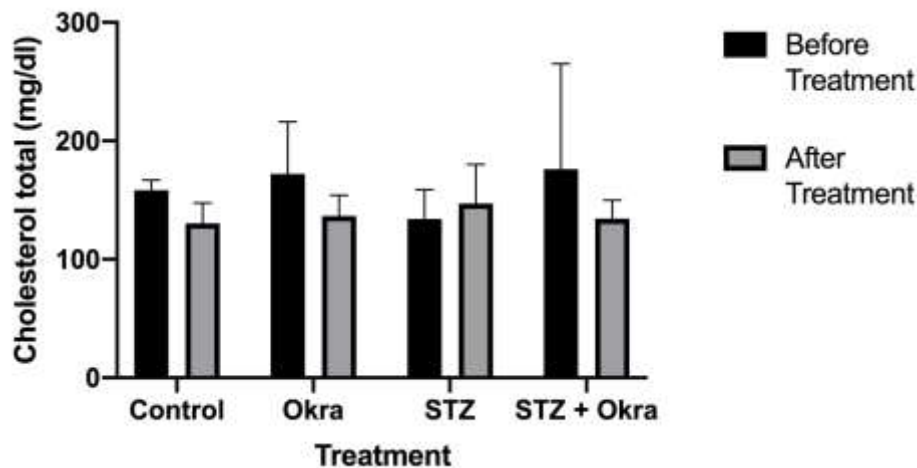


Figure 2. Effect Okra on Cholesterol Total

### Discussion

Overweight and obese people have a higher risk of getting diabetes (Animaw and Seyoum, 2017). The current study found that no significant reduction in body weight of diabetic mice treated with okra. In this study, an okra beverage of 0.52 ml was given once a day. Although there was a slight decrease in body weight in the DM-okra group, the average weight of three other groups tended to increase. It is likely because of the standard daily diet, supporting evidence from previous observation (Anjani et al., 2018). This finding is contrary to that of Fan et al. (2013), who found lower body weight in obese mice treated with a high-fat diet (HFD) and okra extract (Fan et al., 2013). Furthermore, okra is a rich source of fiber, particularly viscous dietary fiber (Kendall and Jenkins, 2004). Artiss et al. (2005) mentioned weight reduction in high-fat diets and a-cyclodextrin as a fiber, indicating fiber may play a role in body weight reduction (Artiss et al., 2006; Gunawan et al., 2021). Together with okra, ginger extract, also contained in the rat drink, could lower the rat's body weight. Sayed et al. study demonstrated that the rat group that received ginger water at a rate of 25% and 50% in their drinking water (ad libitum) possessed an anti-obesity effect compared to the control (Sayed et al., 2020). However, both okra and ginger in this study did not show any significant reduction in body weight. In addition, previous research showed that stevia leaves did not affect body weight reduction (Matias et al., 2021). This inconsistency results may be due to the okra administration dose and the diet given. Existing research indicated that overweight and obese mice, but not normal weight mice, experience a greater bodyweight reduction following okra treatment.

The most exciting finding was that the DM mice group were given okra functional drink decreased blood glucose levels than untreated DM mice (Liu et al., 2016). Similar to our findings, previous studies have demonstrated that okra could reduce blood sugar in STZ-induced diabetic rats (Fauza et

al., 2019, Uadia et al., 2020). There are several possible explanations for this result. Okra plants contain a polysaccharide called rhamnogalacturonan (Liu et al., 2018). Polysaccharides have been shown to possess significant anti-diabetic activity. In streptozotocin-induced diabetic mice,  $\beta$ -D-(1 $\rightarrow$ 6)-glucan can increase insulin levels and hepatic glycogen accumulation while decreasing blood glucose levels (Liu et al., 2016). In accordance with our findings, Erfani et al. have reported a decrease of PPAR- $\gamma$  genes in diabetic rats after okra powder was administered orally for 30 days (Erfani Majd et al., 2018). PPAR- $\gamma$  as glucose regulating genes is involved in glucose transport (Kumar et al., 2016). PPAR- $\gamma$  activation increases adiponectin levels and enhances insulin sensitivity in muscle and liver. As a result, skeletal muscle glucose absorption increases, whereas glucose synthesis and liver fat accumulation in the liver decreases (Meier and Gressner, 2004). Furthermore, ginger, as a mixture of the drink, has been reported can improve plasma glucose level and lipid fraction (Arablou et al., 2014, Mahluji et al., 2013). It can therefore be assumed that the okra-functional drink has a potent hypoglycemic effect on diabetes mellitus.

Diabetes is frequently linked to dyslipidemia. Dyslipidemia is marked by elevated LDL levels, elevated lipid carried by blood lipoprotein, such as cholesterol, triglycerides, or both, and a decrease in HDL levels, contributing to metabolic syndrome (Miller, 2009). Bhowmik et al. identified that high total cholesterol, high triglycerides, and low HDL-C are associated with increase glucose intolerance among the rural population (Bhowmik et al., 2018). In the animal study, Su et al. (2017) reported increasing total cholesterol levels in diabetic rats after a high-fat diet given for a month and STZ intraperitoneal injection compared to the control group (Su et al., 2017). However, the current result has not previously been described. There is no significant difference in total cholesterol after STZ injection in the DM mice group. A possible explanation for this might be because we did not give the mice a high-fat diet which has been known can lead to obesity and hyperlipidemia in rats (Meng et al., 2004, Yang et al., 2006). After obtaining the treatment, the cholesterol total in this study did not show any significant reduction among groups. This finding is contrary to Tian et al. (2015), who found that treatment with okra reduced total cholesterol in gestational diabetes rats (Tian et al., 2015). Another study found that after two weeks on HF diet, C57BL/6 obese mice treatment with okra polysaccharide significantly reduced TC, HLD-C, and LDL-C levels (Fan et al., 2013). Okra's hypolipidemic effect might be mediated by increased cholesterol degradation via CYP7A1 and decreased lipogenesis via SREBP1c and FAS (Wang et al., 2014). Although there appears no considerable difference in total cholesterol between groups, diabetic mice treated with okra beverage had a more significant reduction than others. It suggests that okra beverage may have a potential effect in lower total cholesterol if a high-fat diet and longer duration are used in the future study.

The results showed that an okra-functional drink might improve blood glucose levels and prevent hypercholesterolemia in diabetes mellitus. Based on these findings, future research may examine the effect of okra beverage consumption using a variety of treatment doses and different diets.

## Conclusion

Okra drink with ginger and stevia has a beneficial effect in lowering blood glucose levels and preventing hypercholesterolemia. When blood glucose levels are normal, comorbidities, such as cardiovascular disease and hypertension due to complications of DM, can be prevented

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