



## Effects of *Abelmoschus Esculentus* Infused Water on Blood Glucose Levels of *Rattus Norvegicus* in Hyperglycemia Conditions

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### Abstract

Efforts to find alternative medicines or herbal-based interventions for diabetes sufferers are very necessary. The aimed of this study was to find out whether the use of okra fruit infusion (*Abelmoschus esculentus*) with different administration frequencies can reduce the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*), and to find out what frequency of giving okra fruit infusion can reduce blood sugar levels. white rat (*Rattus norvegicus*) tallest. This type of research is true experimental, consisting of 5 treatment groups, (P0-, P0+, P1, P2, and P3), namely negative control, positive control, giving 6 ml of okra fruit infusion water/day given once, giving infused water 6 ml of okra fruit/day was given 2 times a day and 6 ml of okra fruit infusion water was given 3 times a day. Each group consists of 5 repetitions. The research was carried out at the Chemistry Laboratory, Muhammadiyah University of Malang. The data collection method was by measuring the blood glucose levels of white mice that had been treated, then analyzing them using SPSS. Based on the results of data analysis and discussion, it can be concluded that there is an effect of the frequency of administration of okra fruit infusion on reducing blood sugar levels in white rats (hyperglycemia. Treatment P3 is a group of hyperglycemic rats that were given okra fruit infusion water at a dose of 6 ml/head/day, given once a day in the morning and P2, namely the group of hyperglycemic mice that were given okra fruit-infused water at a dose of 6 ml/head/day, given 2x a day in the morning and evening, is the best treatment because it has the same notation as P0 (negative control) or the group of mice that not given water infused with okra fruit and not given alloxan. Giving an infusion of okra fruit at a dose of 6 ml/head given once in the morning or twice (morning and evening) has an effect on reducing the blood sugar levels of hyperglycemic white mice. Okra can be consumed fresh, processed by boiling, or frying, dry, but not made into flour.

**Keywords:** Frequency; okra fruit infusion water; blood sugar; white rats; hyperglycemia

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## INTRODUCTION

Diabetes Mellitus can attack all social and economic levels. From various epidemiological studies in Indonesia, it was found that the infection rate for Diabetes Mellitus was 1.5% - 2.3% in the population aged over 15 years (Deshpande et al., 2008; Indrahadi et al., 2021; Pulungan et al., 2018). The number of people with diabetes, especially type 2 diabetes, is increasing throughout the world, especially in developing countries due to wrong lifestyle changes that cause obesity (Animaw & Seyoum, 2017; Khan et al., 2020; Ong et al., 2023).

In healing, diabetes sufferers are generally advised to take medication combined with diet and exercise (Sasmita et al., 2017). Therefore, diabetes sufferers often limit their consumption of rice because rice is accused of being a hyperglycemic food (Fujiwara et al., 2019). The glycemic index is a ranking of food ingredients according to their effect on blood sugar. The glycemic index values of foodstuffs are grouped into low (<55), medium (55-70),

and high (>70). If diabetics consume foods that have a high glycemic index, their blood sugar levels will increase quickly, and vice versa (Dan Ramdath, 2016; Frost, 2003).

The first sign that can be seen that someone is suffering from diabetes mellitus can be seen directly from the effect of increasing blood sugar levels, where the increase in blood sugar reaches a value of 60-80 mg/dl and urine contains sugar, so the urine is often surrounded by ants. Apart from that, the signs and symptoms that diabetes mellitus sufferers often complain about include feeling thirsty, urinating a lot, eating a lot, body feeling weak, weight loss, itching, frequent tingling, especially in the fingers, blurred eyes, dry skin, if there is a wound that is difficult to heal, and so on (M. Mouri, 2021; Sapra & Bhandari, 2023). The patient's weight decreases drastically, this is because the glucose in the blood cannot enter the tissue cells. As is known, glucose is really needed by the body because it is the main source of energy (El-Zayat et al., 2019; Wineland, 2021).

Consuming vegetables, especially vegetables that are included in the category of vegetables for diabetes, is highly recommended. With sufficient fiber intake, digestion can run smoothly. Apart from that, fiber also functions to inhibit the release of glucose into the blood, so that blood sugar levels can return to near stable levels. Someone who has high blood sugar levels should go on a low energy diet (Alison & Rebecca, 2019; Wang et al., 2016). One of the ideal foods for diabetes patients is okra (*Abelmoschus esculentus*), because it has a low glycemic index. This is supported by research conducted by previous researchers, that okra fruit mucus can reduce the blood sugar levels of hyperglycemic white mice after being treated with okra fruit infusion at a dose of 2 ml, 3 ml and 4 ml per day. The best treatment was obtained by giving an infusion of okra fruit at a dose of 4 ml per day, but the mice's blood sugar had not yet returned to normal. Therefore, it is necessary to carry out further research by increasing the amount (dose) given, namely 6 ml per day with a longer research duration (1.5 months). However, this infusion is given at different frequencies, namely 1, 2 and 3 times a day (Adap et al., 2019; Alblihd et al., 2023; Damayanthi et al., 2018; Elkhaliifa et al., 2021).

Several studies related to okra and its relationship with health have been conducted previously. The results of the research show that giving bokashi vegetable waste can increase the growth and yield of okra plants (Millah et al., 2022). Okra fruit contains phenolic components which play a role in antioxidant activity (Manik et al., 2019). The Okra extract tested contained positive chemical compounds of flavonoids, tannins, saponins, triterpenoids, steroids, alkaloids and carbohydrates, while glycosides were negative (Septianingrum et al., 2018). Giving soaked okra fruit has an effect on reducing cholesterol levels in hypercholesterolemia patients (Jayadi et al., 2021). The okra plant, which contains the main active compounds in the form of polysaccharides and phenolic compounds, is effective as an antibacterial against Gram-positive and Gram-negative bacteria (Ramadan & Sumiwi, 2023). Okra fruit extract has the potential to be used as therapy in diabetes mellitus sufferers (Pasaribu et al., 2022).

Diabetes mellitus is difficult to cure completely or completely. What can be done is to control or maintain sugar levels so that they do not get too high. Apart from taking medication from a doctor, diabetes sufferers limit their consumption of sugar and carbohydrates, but increase their consumption of fiber, which comes from fruit and vegetables (Asif, 2014). Okra is a plant rich in protein and fiber. The oil content in okra seeds is 40%. Okra seed oil is rich in unsaturated fatty acids such as oleic acid and linoleic acid. Okra fruit contains 3.9% protein and 2.05% fat. The energy in 100 grams of okra is 40 kcal. The minerals in okra are potassium (6.68%) and phosphorus (0.77%). Okra is a green vegetable that is rich in dietary fiber. Fiber is very important for the body because it can prevent constipation, obesity, hypercholesterolemia, diabetes and colon cancer (Dantas et al., 2021; Sami et al., 2013).

The main problem of this research is what frequency can reduce blood sugar levels in white mice. It is not yet known the best frequency of okra fruit infusion that can reduce blood sugar levels in white mice. Therefore, it is necessary to carry out research on various frequencies of administration of okra fruit infusion to reduce blood glucose levels in white

mice. Based on the problems above, the aim of this research is to determine whether there is an effect of the frequency of giving okra fruit infusions in reducing the glucose levels of hyperglycemic white rats (*Rattus norvegicus*) and to find out how many times a day giving okra fruit infusions can reduce the blood sugar levels of white rats (*Rattus norvegicus*) the best.

## METHOD

### Research Design and Methods

This type of research is true experimental. This study consisted of 5 treatment groups, each group consisting of 5 repetitions. Calculation of how to determine repetitions according to Kemas (1993). The treatments in this study were as follows: P0 (-): Group of mice that were not given okra fruit-infused water and were not given alloxan (negative control). P0 (+): Group of mice that were not given okra fruit infusion water and were given alloxan (positive control). P1: A group of hyperglycemic rats were given okra fruit infused water at a dose of 6 ml/head/day, given once a day, namely in the morning. P2: Group of hyperglycemic rats given okra fruit infused water at a dose of 6 ml/head/day, given 2x a day, morning and evening, 3 ml each. P3: Group of hyperglycemic rats given okra fruit infused water at a dose of 6 ml/head/day, given 3x a day, namely morning, afternoon, evening, 2 ml each.

### Population and Sample

Population is defined as a generalization area consisting of objects/subjects that have certain qualities and characteristics that are applied by researchers to study and then draw conclusions. Population is the entire object being studied, whether in the form of people, objects, events, values, or things that happen. The population in this study was white rats (*Rattus norvegicus*). The sampling technique used is Simple Random Sampling, namely a random sampling technique so that each sampling unit in the population has the same chance of being selected into the sample. Meanwhile, the research samples were adult white mice weighing 150-200 grams which were obtained from the UMM Chemistry Laboratory. The number of samples in this study was 25 white mice.

### Research Stages

The research stages consist of (1) Preparing research tools and materials; (2) Research Implementation: (a) Prepare 25 male white rats, divide them into 5 cages (for 5 groups), 5 animals each, then adapt them for 1 week; (b) Then the mice were injected with alloxan (except the P0- group), so that the mice became hyperglycemic. Inject mice with alloxan at a dose of 150 mg/kg body weight. Injections were carried out in all mice in the treatment and positive control groups). The injection is carried out intraperitoneally. Next, leave it for 3 days; (c) After 3 days the white mice had their blood sugar levels measured and recorded. Mice that were not given alloxan also had their blood sugar levels checked. Blood sugar levels are measured by taking blood from the slashed tail; (d) Preparing an infusion of okra fruit, made by soaking 100 grams of okra pieces in 100 ml of warm water, then leaving it for 6-8 hours. After leaving it for this period of time, you get an infusion of okra fruit in the form of a thick mucus. This mucus was given to white mice that were already hyperglycemic; (e) Next, white mice were treated according to the design, namely the control group (P0 -), namely mice without alloxan injections and without administration of okra fruit-infused water; The positive control group (P0+), namely mice were injected with alloxan alone without being given okra fruit infused water. P1, rats were injected with alloxan and given 6 ml of okra infused water per day, given once in the morning; P2, rats were injected with alloxan and given okra infused water 6 ml per day, given 2 times per day, 3 ml each in the morning and afternoon; P3, rats were injected with alloxan and given 6 ml of okra infused water per day, given 3 times a day, 2 ml each in the morning, afternoon and evening; (f) After 30 days, male mice were killed by anesthesia. Then the white mice were dissected using a section tool. Surgery starts from the abdomen, placed on the surgical board supine; (g) Then the blood is taken using a 3cc syringe from the aorta of the heart. The blood was centrifuged at 6000 rpm for 20 minutes; (h) Take 10 µl of blood serum

and add 1000 µl of gluco-oxidase and phenol-peroxidase reagent; (i) Mix until homogeneous and leave at room temperature for 20 minutes, then observe the absorbance using a spectrophotometer at a wavelength of 500 nm; (j) Calculating blood glucose levels.

**Data analysis technique**

The data obtained was then analyzed using the SPSS program. The data analysis used was one-way analysis of variance (ANOVA) to determine differences between treatments. Before carrying out the Anava test, the data obtained needs to be tested for normality and homogeneity first. If the data is normally distributed and homogeneous, then the Anava test can be continued. To determine the best treatment, the Duncan Test was carried out.

**RESULTS AND DISCUSSION**

Based on the results of the research that has been carried out, research data was obtained as presented in Table 1 and Table 2.

**Table 1.** Data on White Rat Blood Sugar Levels before treatment (mg/dl)

No	Treatment	Repetition					Average
		1	2	3	4	5	
1	P0 (-)	125	129	110	108	125	118.4
2	P0 (+)	368	600	413	302	582	453
3	P1	306	557	217	485	461	405
4	P2	371	317	513	265	314	356
5	P3	427	371	218	367	251	326.8

**Table 2.** Data on Blood Sugar Levels of White Rats after Treatment (mg/dl)

No	Treatment	Repetition					Average
		1	2	3	4	5	
1	P0 (-)	118	112	115	107	121	114.6
2	P0 (+)	337	486	474	600	345	448.4
3	P1	265	196	251	218	161	218.2
4	P2	170	196	154	157	140	163.4
5	P3	87	122	120	127	141	119.4

The data has been analyzed with the help of SPSS 21 by looking at normality and homogeneity first and continued with hypothesis testing using the ANOVA test. To determine the best treatment, the Duncan Test was carried out. The Normality Test shows that the data is normally distributed. Meanwhile, the homogeneity test results show that the data is not homogeneous. However, because the sizes of the two samples being compared are the same size and relatively equal, the hypothesis analysis can be continued.

Based on the ANOVA test results presented in Table 3, the calculated  $F = 32.533$  was obtained with  $p\text{-value} = 0.000 < \alpha (0.05)$ . Thus,  $H_0$  is rejected, while the research hypothesis is accepted. This means that there is an effect of the frequency of administration of okra fruit infusion on reducing the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*).

**Table 3.** ANOVA test results for blood glucose levels after treatment

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	381718.400	4	95429.600	32.533	0.000
Within Groups	58665.600	20	2933.280		
Total	440384.000	24			

After knowing that there is an effect of the frequency of giving okra fruit infusion on reducing the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*), it is necessary

to know the best treatment, which is carried out using the Duncan Test, where the results are presented in Table 4.

**Table 4.** Duncan Advanced Test Results

Treatment	Average	Notation	
P0 (-)	114.6	a	
P3	119.4	a	
P2	163.4	a	b
P1	218.2	b	
P0 (+)	448.4	c	

Based on Table 4, it is known that treatment P3 is a group of hyperglycemic rats that were given okra fruit infused water at a dose of 6 ml/head/day, given once a day in the morning and P2 is a group of hyperglycemic mice that were given okra fruit infused water at a dose of 6 ml/head / day, given 2x a day in the morning and evening is the best treatment because it has the same notation as P0 (negative control) or the group of mice that were not given okra fruit-infused water and were not given alloxan. The average value for P3 is 119.4 and the average value for P2 is 163.4, both of which are not different from the average for P0 (-) which is 114.6.

Giving an infusion of okra fruit at a dose of 6 ml/head given once in the morning or twice (morning and afternoon) has an effect on reducing the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*). The results of this study confirm previous research, there was an effect of various doses of okra fruit infusion (*Abelmoschus esculentus*) on reducing blood sugar levels in white mice (Khosrozadeh et al., 2016; Nikpayam et al., 2021). This dose is slightly higher than the results of research by other research which showed that a dose of okra fruit mucus filtrate had a more effective effect in reducing glucose levels in the blood of male white rats (Damayanthi et al., 2018).

The results of this study are in line with the results of research by Desthia et al (2015) which showed that the test extract could have the effect of lowering blood glucose levels but was not better when compared to the metformin comparison group, while reducing blood glucose levels was most effective with okra fruit extract at 1.12 g/Kg BW. mice. Also in line with Safitri, (2015) that administration of okra fruit ethanol extract at a dose of 42 mg/30gBW, 84 mg/30gBW, 168 mg/30gBW can reduce blood glucose levels of male white mice (*Mus musculus*) which is significantly different from the negative control and comparable to positive control (glibenclamide).

Glibenclamide is an oral hypoglycemic derivative of sulfonyl urea which works actively to reduce blood sugar levels. Glibenclamide works by stimulating insulin secretion from the pancreas. Therefore, glibenclamide is only useful in adult diabetics whose pancreas is still able to produce insulin when used orally, some of the glibenclamide is absorbed into the extracellular fluid and some is bound to plasma proteins. Giving a single dose of glibenclamide will reduce blood pressure for 3 hours and this level can last for 15 hours. Glibenclamide is excreted in the feces and as a metabolite in the urine. Glibenclamide stimulates the beta cells of the pancreatic islets of Langerhans so that insulin secretion is increased. In addition, the sensitivity of beta cells to blood glucose levels is also increased through their influence on glucose transport proteins. There are indications that this drug also improves the sensitivity of the target organ to insulin and reduces absorption by the liver (Safitri, 2015).

In contrast to glibenclamide and various other synthetic chemical drugs, according to research results from Nurfatwa (2018), okra is not dangerous to health. Based on the research results, there was no significant difference in SGOT or SGPT levels between all groups although there was an increase in levels as the test dose increased. Histopathology did not show significant damage to hepatocytes. Administration of okra fruit extract does not have acute toxic effects on the liver. According to Ajizah (2017), apart from playing a role in reducing

blood glucose levels, okra is also able to reduce liver MDA levels and effectively repair beta cell damage due to streptozotocin induction and is able to activate pancreatic beta cells to produce insulin. Okra extract intervention based on quercetin content has antidiabetic potential.

Furthermore, according to Anjani (2018), the effectiveness of okra in relation to antidiabetes is due to the content of flavonoid compounds which have antioxidant activity which acts as a free radical scavenger. Quercetin is the main flavonoid in okra which has antidiabetic potential through the mechanism of increasing glucose uptake in tissues, increasing insulin sensitivity, preventing lipid peroxidation, liver and pancreatic cell proliferation, and inhibiting  $\alpha$ -glucosidase.

Okra can function as a dietary therapy for hyperglycemia, especially in terms of maintaining a relatively ideal body weight (Erfani Majd et al., 2018; Gomes et al., 2023). Okra can be consumed fresh, processed by boiling, or fried, dried, but not made into flour (Adelakun & Oyelade, 2011; Gomes et al., 2023; Lea, 2013; Nnamezie et al., 2021; Ofori et al., 2020). This is based on the results of research by Astaty and Kasmawati (2017) which showed that there was no effect of giving okra flour on body weight in diabetic Wistar rats between the control group and the treatment group. Another positive side of consuming fresh okra is the high mineral content, which is of course very good for body health. This is in line with Fahmi (2018) that the levels of calcium, potassium and magnesium in fresh okra are higher than the levels of calcium, potassium and magnesium in boiled okra and okra mucus. Consuming dried okra can still be done because the results of research by Ajizah (2017) concluded that dry extract of *Abelmoschus esculentus* at a dose of 200 mg/KgBW/day orally for 28 days had a hyperglycemic effect and improved the apoptotic index of heart cells.

The results of this study are different from the results of research by Prakoso et al (2016) which actually shows that okra fruit does not have the property of lowering blood glucose. In fact, what has the property of lowering blood glucose is okra leaves. However, it should be noted in the research of Prakoso et al (2016) that the results of measuring glucose levels in the group given okra fruit extract were found to decrease but did not reach normal blood glucose levels. This may be due to the insufficient dose given, the extract being given only once and the time only being given until the 24th hour, so the effect of reducing glucose levels is not yet visible.

## CONCLUSION

Based on the results of data analysis and discussion, it can be concluded that there is an influence on the frequency of administration of okra fruit infusion on reducing the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*). Treatment P3 was a group of hyperglycemic rats that were given okra fruit infused water at a dose of 6 ml/head/day, given once a day in the morning and P2 was a group of hyperglycemic mice that were given okra fruit infused water at a dose of 6 ml/head/day, given twice a day in the morning and evening is the best treatment because it has the same notation as P0 (negative control) or the group of mice that were not given okra fruit-infused water and were not given alloxan. Giving an infusion of okra fruit at a dose of 6 ml/head given once in the morning or twice (morning and afternoon) has an effect on reducing the blood sugar levels of hyperglycemic white rats (*Rattus norvegicus*).

## RECOMMENDATION

Okra can be consumed fresh, processed by boiling, or fried, dried, but not made into flour. In this regard, further research is needed regarding the most appropriate form of preparation or consumption so that okra can be utilized more optimally. It is also necessary to compare the effectiveness of the okra plant organs in relation to reducing blood sugar levels. Furthermore, more research is needed to see the best therapeutic effect of okra as a therapy for diabetes mellitus patients. In the future, it is very necessary to conduct research on the long-term effects of okra consumption on blood glucose levels.

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