Effect Of Red Spinach Leaf Infusa *(Amaranthus Tricolor)* On Reducing Blood Glucose Levels In Mice In Vivo

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Abstrak

This study was conducted as an effort to preserve natural materials that have the potential as antidiabetics, namely red spinach leaves *(Amaranthus tricolor)*, containing chemical compounds such as flavonoids, tannins, phenols, alkaloids and saponins that can reduce blood glucose. This study aims to determine the effect of red spinach leaf infusa in reducing blood glucose levels and the most effective concentration to reduce blood glucose. Conducted at the Pharmacy Laboratory of Muhammadiyah University of Palopo in August 2023. It is a type of experimental research with the stages of sampling, making infusa of red spinach leaves *(Amaranthus tricolor)*, making 1% na CMC solution, making glibenclamide suspension, making glucose solution as an inducer of test animals. It takes 3 days in the treatment of mice by measuring blood glucose levels. The results of blood glucose reduction obtained are in negative control at 15.40 mg/dL, positive control at 57.40 mg/dL, 20% infusion at 42.13 mg/dL, 25% infusion at 58.20 mg/dL and 30% infusion at 58.27 mg/dL. From the results of the one way ANOVA test and continued with the Duncan test, the results varied or significantly different between the negative control and the treatment groups (positive control, 20%, 25% and 30% infusion). It was also concluded that the most effective in reducing blood glucose levels was 30% infusa with an average value of 58.27 mg/dL.

Keywords: Keywords: *Antidiabetic, Red spinach (Amaranthus tricolor), Infusa, In Vivo*

1. Introduction

Diabetes mellitus is a serious chronic disease that occurs either when the pancreas does not produce enough insulin or when the body is unable to use the insulin produced effectively. Diabetes mellitus is a chronic metatabolic disorder in which insulin deficiency leads to impaired metabolism of fats, proteins and carbohydrates. In general, diabetes mellitus means a condition where the body cannot make insulin as needed or the body is unable to properly utilize the insulin hormone obtained, resulting in an increase in blood sugar levels above normal [1].

One of the diseases suffered by many people is diabetes mellitus. The triggering factors are genetic factors, unbalanced diet, lack of physical activity, and this disease is characterized by increased glucose levels in the blood. Patients with diabetes mellitus are needed to regulate their diet and use treatment both pharmacologically and non-pharmacologically, very many people choose non-pharmacological treatment, namely herbal drinks because they are easily available and cheap. Herbs come from natural ingredients that can be found around us such as spices, vegetables and leaves. The use of herbal treatment needs to be examined regarding changes in blood sugar levels so that a decrease in blood glucose is known both before and after herbal treatment [2].

One of the plants used by some people for diabetes mellitus is red spinach *(Amaranthus tricolor)* which has many benefits, namely lowering cholesterol, improving digestion, and being antidiabetic and can reduce the risk of cancer. Red spinach *(Amaranthus tricolor)* has antioxidant activity because it has flavonoid compounds. in research [3] suggests that red spinach *(Amaranthus tricolor)* has antioxidant content and can be used in the management of diabetes, hyperlipidemia and diabetes affiliated with dyslipidemia.

Red spinach *(Amaranthus tricolor)* is proven to have a variety of main benefits, namely lowering cholesterol, improving digestion, as antidiabetes, lowering LDL, antihyperlipidemia, and can reduce the risk of cancer [4]. The compound content of red spinach leaves *(Amaranthus tricolor)* as antidiabetes is phenol compounds and their derivatives (flavonoids), saponins and tannins which are antidiabetic compounds that can inhibit carbohydrate hydrolysis and glucose absorption, regenerate beta cells so that they can increase insulin release, inhibit aldose reductase, control glucose levels, prevent some beta cell damage and interfere with sugar absorption (Rahmawati & Retnaningrum, 2021). This study uses the drug glibenclamide as a positive control because it has the same mechanism of action as flavonoids as antidiabetics contained in red spinach leaves *(Amaranthus tricolor)*, namely stimulating the secretion of the pancreatic β-cell hormone insulin [5].

Based on the results of observations of the community in Balo-Balo village empirically utilizing red spinach leaves *(Amaranthus tricolor)* as antidiabetes by taking red spinach leaves *(Amaranthus tricolor)* then boiling and taking the water to drink. Based on the above problems, researchers are interested in conducting research on the effectiveness of red spinach leaf infusa *(Amaranthus tricolor)* as an antidiabetic to reduce blood glucose levels in mice.

2. Methodology

Type of Research

Using experimental research (experiment)

Tools and Materials

The tools used in this study include handscoon, mask, spoit for oral, cannula, beaker glass, stirring rod, thermometer, funnel, three legs, bunsen, wire gauze, measuring cup, tweezers, horn spoon, mortar, pestle, digital scales, analytical scales, mice cage, glucometer, stopwatch. The materials used in this study are red spinach leaves *(Amaranthus tricolor)*, distilled water, Na.CMC, glucose, glibenclamide, aluminum foil, blood sugar strips, parchment paper.

Research Procedures

Sampling

Taken red spinach leaves *(Amaranthus tricolor)* in Balo-balo village, then separated from dirt and foreign particles attached to red spinach leaves and washed thoroughly using running water, then chopped.

Preparation of Red Spinach Leaf Infusa *(Amaranthus tricolor)*. Made infusa from red spinach leaves *(Amaranthus tricolor)* with three concentrations of 20%, 25%, 30%.

Preparation of Red Spinach Leaf Infusa (Amaranthus tricolor)

Made infusa from red spinach leaves *(Amaranthus tricolor)* with three concentrations of 20%, 25%, 30%.

Preparation of Na. CMC 1% Solution

Na. CMC 1% was weighed as much as 1 gram and then added little by little to 100 mL of hot distilled water while stirring using a stirrer until it thickened [6].

Preparation of glibenclamide suspension

Glibenclamide tablet powder was weighed as much as 0.14 grams, then added with 10 mL of 1% Na.CMC solution then stirred until smooth and sufficient volume using 1% Na.CMC solution to 100 mL [6].

Preparation of Glucose Solution

Weighed 15 grams of glucose and put into a 100 mL volumetric flask, added distilled water little by little while stirring until dissolved and homogeneous and then increase the volume to 100 mL [6].

Treatment of test animals

This study used test animals, namely mice *(Mus musculus)* with a body weight of 20-30 grams used 25 heads which were divided into 5 treatment groups adapted for 3 days with regular feeding and drinking. After that, each mice was fed for 8 hours. After 8 hours of fasting, blood glucose levels were measured as the initial blood glucose levels of mice and then induced glucose solution as much as 1 mL/KgBb to raise the blood glucose levels of mice. After 60 minutes each group received treatment, namely:

- Group 1 as a negative control was given aquadest
- Group 2 as a positive control was given glibenclamide
- Group 3 concentration of 20% was given infusion of red spinach leaves *(Amaranthus tricolor)*
- Group 4 concentration of 25% given red spinach leaf infusa *(Amaranthus tricolor)*
- Group 5 concentration of 30% given red spinach leaf infusa *(Amaranthus tricolor)*

Data Analysis

Data analysis used in this study used ANOVA. The One Way ANOVA test is used to determine the results that vary or differ significantly from the five groups on the decrease in blood glucose levels [7].

Result and Discussion

Result

Tabel 1. ***Uji One Way ANOVA***

Penurunan

	Sum Squares	Df	Mean Square	F	Sig.
Between Groups	6900.928	4	1725.232	5.284	0.005
Within Groups	6530.445	20	326.522		
Total	13431.372	24			

Tabel 2. Uji Duncan

Penurunan

Duncan[a]

Perlakuan	N	Subset for alpha = 0.05 1	2
Kontrol Negatif	5	15.40	
Infusa 20%	5		42.13
Kontrol positif	5		57.40
Infusa 25%	5		58.20
Infusa 30%	5		58.27
Sig.		1.000	0.210

Discussion

This study was conducted with the aim to see the effect of giving infusa of red spinach leaves *(Amaranthus tricolor)* on reducing blood glucose levels in glucose-induced male white mice. Red spinach leaves *(Amaranthus tricolor)* used as samples are red spinach leaves that are still fresh and not rotten, red spinach leaves obtained from Balo-balo Village, Belopa District. Red spinach leaves are often used traditionally by the community to treat diabetes.In addition, this red amaranth leaf is also one of the plants that can be used to treat diabetes.

which is easy to obtain because it is easy to cultivate.

Flavonoid compounds contained in red spinach leaves are known to be one class of compounds that can reduce blood glucose [8]. Flavonoid compounds in red spinach leaves have

an effect that can stimulate insulin secretion and regenerate beta cells in the pancreas [9].. And has phenol compounds as antioxidants that are thought to be able to protect a number of β cells that remain normal, thus allowing the regeneration of existing β cells through the mitotic process [10].

In this study, white male mice *(Mus musculus)* were used. Mice *(Mus musculus)* used for research are mice that have never been treated with drugs and mice that are declared healthy with the criteria that are not physically disabled and visually show normal behavior. The selection of the sex of mice is based on the consideration that male mice have a more stable hormonal condition than female mice, this is because female mice experience changes in hormonal conditions at certain times such as during the estrus cycle, pregnancy and lactation so that it can affect the psychological condition of the test animals which is feared to affect the test results. In addition, the stress level in female mice is higher than male mice which may interfere with research.

Before testing the mice were acclimatized first for 3 days. The purpose of this acclimatization is to adapt mice to their environment and not stress, for each mice are given food and drink as usual (BPOM, 2022). Mice were grouped into 5 groups, in 1 group consisting of 5 mice. Group 1 as a negative control was given aquadest, group 2 as a positive control was given glibenclamide, Groups 3, 4 and 5 as treatment groups were given infusion of red spinach leaves *(Amaranthus tricolor)* with concentrations of 20%, 25% and 30%.

After acclimatization for 3 days and has met the requirements, then the mice are first fed for 8 hours. This aims to empty the mice's digestive tract so that it will not affect drug absorption. In addition, the purpose of the mice is to avoid the possibility of factors that can affect the test results arising from the food given to the mice. Before the mice were given the test preparation, the blood glucose level of the mice was measured first to determine the initial glucose level of the mice, then the mice were given a glucose solution with a concentration of 0.15gr b/v (15 grams of glucose in 100mL of distilled water) orally with the volume of administration according to the mice's BW then after 60 minutes of glucose administration, the blood glucose was measured again, then the mice were given the test preparation as well as distilled water and glibenclamide as a comparison. This method is the Test of Oral Glucose Tolerance (TTGO) method. TTGO is a test to measure blood glucose levels after consuming glucose [11].

In this study, the inducer used was glucose. The reason for choosing glucose as an inducer of mice is because glucose is one of the compounds that can increase blood glucose levels. This is adjusted to the condition of people who experience excess glucose in their bodies. Glucose induction in mice orally has successfully raised the blood glucose levels of mice. The results obtained from this glucose induction are blood glucose levels exceeding or equal to 126 mg/dL [5].

Aquadest was used as a negative control. The use of distilled water in the negative control is only as a comparison to see the increase or decrease in blood sugar levels with positive controls and samples given red spinach leaf infusa red spinach leaves *(Amaranthus tricolor).* After giving aquadest decreased blood glucose levels, this is in line with research [12] which explains that most likely the mice used in the negative control are stressed because

of the surrounding environment and many activities of other mice.

In the positive control used is glibenclamide. The results showed that after the administration of glibenclamide, the blood sugar levels of mice decreased due to the mechanism of action of the drug glibenclamide in reducing blood glucose levels, which stimulates the secretion of the hormone insulin from the granules of β-cells of the islands of Langerhans pancreas. Its interaction with the ATP-sensitive K channel on the β-cell membrane causes membrane depolarization and this situation will open the Ca channel. After the opening of the Ca channel, Ca2+ ions will enter the β-cells then stimulate the granules containing insulin and insulin secretion will occur so that it can reduce blood glucose levels [13]

In table 1, the results of the ANOVA used Data analysis in this study is using One Way ANOVA or one-way ANOVA which is used to determine the results that vary or differ significantly from the five groups on lowering blood glucose levels [7]. In this test using a significant value of 0.05, if the value> 0.05 is not continued with the Duncan test and if the value is> 0.05, the test is continued. <0.05 followed by Duncan's test which is in line with research [7] which explains that this test is carried out to find out from the five groups that have varying or significantly different results so that from the test the most effective infusa concentration can be obtained in reducing blood glucose levels in mice. Based on table 4.7 one-way ANOVA test results between the five treatments obtained a significant value of 0.005 <0.05, meaning that there is a decrease in blood glucose levels that vary or differ significantly from the five treatments. This also indicates that the five treatments have varying or significantly different effects in reducing blood glucose levels in mice.

Based on table 2, it is known that the negative control group given aquadest is significantly different from the positive control group, the 20%, 25% and 30% infusion treatment groups, characterized by the negative control being in a different subset from the other groups. While the positive control given glibenclamide with 20%, 25% and 30% infusa treatment groups is not significantly different, this is characterized by the positive control group being in the same subset as the 20%, 25% and 30% infusa treatment groups, which means that the negative control with the treatment group (positive control, 20%, 25% and 30% infusa) can reduce blood glucose levels in mice. And with the Duncan test obtained infusa with a concentration of 30% which has the highest average of 58.27 mg/dL, the most effective in reducing blood glucose levels in mice.

Conclusion

Based on the results of the research conducted, it can be concluded that the administration of red spinach leaf infusa *(Amaranthus tricolor)* has an effect on reducing blood glucose levels tested on white mice *(Mus musculus)* males induced glucose orally. *One-way ANOVA* testing between the five groups showed a significant value of 0.005 <0.05, which means there is a decrease in blood glucose levels that vary from the five treatments.

Of all the treatment groups can reduce blood glucose levels and after the Duncan test which has the highest average of 58.27 mg/dL in reducing blood sugar is in the 30% concentration treatment group.

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