Effect of *Rhizophora sp* mangrove leaf extract on mice blood glucose levels

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Abstract.  
Diabetes mellitus is a disease that occurs due to increased blood glucose levels (hyperglycemia) due to disorders of the pancreas. Mangrove leaves are known as herbal plants that can reduce blood glucose levels because it contains flavonoid compounds that can repair damaged pancreatic β cells and stimulate pancreatic β cells to produce the hormone insulin. This study aimed to determine the effect of *Rhizophora* leaf extract administration in reducing blood glucose levels of mice. This research was an experimental laboratory with the approach the post-test only control group design. This test used 5 groups, consisting of negative control group, positive control group (Glibenclamide 5 mg/kg BW), KP1 (extract 250 mg/kg BW), KP2 (extract 500 mg/kg BW), KP3 (extract 700 mg/kg BW). Measurement of fasting blood glucose levels using glucometer. The results showed that the administration of *Rhizophora* leaf extract in the KP1, KP2, and KP3 treatment groups was able to reduce blood glucose levels compared to before being treated, although the decrease was not yet up to normal limits. The conclusion was that the *Rhizophora* leaf extract was able to reduce fasting blood glucose levels with the highest percentage reduction in KP2 at a dose of 500 mg/kg BW by 29%.

Keywords: extract, *Rhizophora sp* mangrove leaf, blood glucose levels, mice, in vivo

1. Introduction

Diabetes Mellitus (DM) is a disease with a collection of symptoms arising from high blood glucose levels (hyperglycemia) and is a chronic metabolic disease. This is caused by a disturbance in the pancreas, resulting in excess glucose in the blood [1]. Diabetes mellitus is a serious threat to humans and has become the 7th cause of death in the world [2]. The prevalence of people with diabetes in Indonesia is 9.1 million people [3]. The number of diabetes sufferers in 2017 in West Nusa Tenggara (NTB) Province at the age of ≥ 15 years was 25,856 people [4]. Treatment of diabetes mellitus is currently still limited to the use of oral hypoglycemic drugs (OHO) such as the sulfonylurea, glibenclamide, biguanide, thiazolidinedione, and acarbose and insulin injections. Besides having good efficacy, diabetes drugs can also cause various side effects such as diarrhea, headache, nausea and vomiting, weight gain, and hypoglycemia if not treated immediately, coma and even death can occur, other side effects include disorders of the central nervous system in the form of vertigo, Confusion, ataxia, hematologic including leukopenia, thrombocytopenia, agranulocytosis, and aplastic anemia can occur although rarely [5,6,7]. Selection of herbal medicine has been widely practiced in Indonesia, one of the plants that can be used as herbal medicine, especially anti-diabetes treatment is mangroves. *Mangrove Rhizophora sp* is also known to have the potential to reduce blood glucose levels or as an antihyperglycemic agent [8,9]. Based on research conducted by Babuselvam [10] and Abidin [11] *R. mucronata* leaf contain 2- (2-ethoxy ethanol, kau-16-ene and benzophenone, phenolic compounds of the flavonoid group, phenolic acids, tannins, dihydroflavonols, caffeine acid, vanillic acid, p-hydroxybenzoate acid, alkaloids, coumarins, flavonoids, phenols and polyphenols, quinones, resins, saponins, phytosterols, xanthoproteins, pigments (chlorophyll, carotenoids) and sugar [10,11]. Flavonoids function as antioxidants, anti-inflammatory,
antibiotics, prevent bone loss, and increase the effectiveness of vitamin C [12]. Many natural flavonoids play an important role in the prevention of diabetes and its complications [13]. The action of flavonoids as antidiabetic is thought to regenerate (repair) pancreatic beta-cell damage and stimulate pancreatic beta cells to produce insulin [14,15].

The ability of *Rhizophora sp* mangrove fruit as anti-hyperglycemic and the presence of flavonoid compounds that have an antioxidant role is known, but until now there is limited information regarding *Rhizophora sp* mangrove leaf extract in reducing blood glucose levels. Hence, the current study aims to evaluate the effect of mangrove leaf extract *Rhizophora sp* to decrease blood glucose levels of mice.

2. Materials and Methods

2.1. Mangrove Leaf Extracts of *Rhizophora sp*

*Rhizophora sp* mangrove leaf were taken from the sheet area of West Lombok Regency. The leaf obtained are washed thoroughly until all dirt attached to the leaf is gone, then dry them at room temperature for ± 1 month. The dried mangrove leaf are then blended to form a powder. The mangrove leaf powder was weighed as much as 500 grams, macerated using 96% methanol solvent for 1 day at room temperature then filtered with Whatman filter paper to separate the filtrate (liquid) and the residue (residual sample). The residue is macerated again and filtered. This stage is repeated three times. Furthermore, the resulting filtrate is evaporated using a Rotary Vacuum Evaporator, at a temperature of 40 until a thick extract is obtained.

2.2. Preparation of Test Animals and Pathological Treatment (Hyperglycemia)

The test animals (mice) were adapted for 7 days with commercial feed and drinking water, after a long journey at the time of purchasing mice so that the mice did not experience stress. Then the mice were divided into 5 groups. The mice were fasted for 12-16 hours and then measured the blood glucose levels of the mice to determine their initial blood glucose levels. The pathological treatment in mice was carried out by administering alloxan 125 mg/kg body weight to mice which aims to cause the mice to experience hyperglycemia [16]. Alloxan was given to mice once a day for 3 days. After alloxan administration, the mice blood glucose levels were measured on day 5, if the mice blood glucose levels were >200 mg/dl, it was stated that the mice had hyperglycemia [17].

2.3. Antidiabetic Activity Test of Mangrove Leaf Extract *Rhizophora sp*

In this study, the mice used were 2-3 months, weighing 20-30 grams, physically healthy, and had normal activities. Mice were grouped into 5 groups, namely the negative control group (healthy), positive control (Glibenclamide dose 5 mg/kg BW), KP1 (mangrove leaf extract dose 250 mg/kg BW), KP2 (mangrove leaf extract dose 500 mg/kg BW), and KP3 (mangrove leaf extract dose of 700 mg/kg BW). The treatment was given orally, once a day for 7 days. The preparation of the mangrove leaf extract dosage was done by weighing the *Rhizophora sp* mangrove leaf extract according to the required dosage using an analytical balance, then dissolving each dose into 10 ml of Aquadest solution. The diluted mangrove extract was given to the mice orally once a day for 7 days.

2.4. Measurement of Blood Glucose Levels

Measurement of blood glucose levels of mice was carried out after the mice were satisfied for 12-16 hours. Measurement of blood glucose levels was done by taking blood from the tails of mice that had been cleaned using 70% alcohol. The blood in the tail is taken using an auto click and then the blood is dripped on a glucose strip that has been entered into the Autocheck glucometer. The results of the measurement of blood glucose levels will appear after 10 seconds. The results listed on the glucometer are the results of the blood glucose values in mg/dl units. Measurement of blood glucose levels of mice was carried out in all groups, namely on day 1 (D0) as initial blood glucose levels before being treated with test materials, on day 3 (D3), day 5 (D5), and day 8 (D8).
3. Results and Discussion

Table 1. The average measurement of fasting blood glucose levels in mice before alloxan administration and after alloxan administration for 3 days.

| Group | Before Alloxan Administration (mg/dl) | After 3 days of alloxan administration (mg/dl) | Average Range |
|-------|---------------------------------------|-----------------------------------------------|---------------|
| K –   | 95.70±36.61                           | 100.00±26.90                                 | 72.3 – 98.00  |
| K +   | 84.70±3.78                            | 389.30±171.02                                | 240.70 – 389.30|
| KP1   | 98.00±26.21                           | 333.70±210.75                                |               |
| KP2   | 72.30±17.24                           | 286.00±50.03                                 |               |
| KP3   | 76.00±13.00                           | 240.70±66.12                                 |               |
| Average Range | 72.30 – 98.00 | 240.70 – 389.30 | |

Based on the results of the average measurement of blood glucose levels in mice (Table 1) where the average range of initial blood glucose levels of mice (before alloxan administration) is 72.3 - 98.0 mg/dl where the blood glucose levels of mice are still in normal stated by Anwar O H [18] that the normal value of blood glucose in mice is 62 - 175 mg/dl [18]. Then there was an increase in the average blood glucose levels of mice after being induced by alloxan for 3 days of 240-389.3 mg/dl so that it was said that the mice had hyperglycemia or diabetes according to the criteria of diabetic mice according to Alarcon-Aquilera [17] namely diabetic mice blood glucose level >200 mg/dl [17]. Thus it is proven that giving alloxan from the conditions before DM increases blood glucose levels. The increase in glucose levels in the blood is caused by the destruction of the pancreatic β cells which produce insulin due to alloxan which is an oxygen-derived pyrimidine. Alloxan can damage and affect the ultrastructure of pancreatic β cells, which in turn can cause necrotic cell death [19]. Alloxan, which is a derivative of uric acid, can selectively damage pancreatic cells through oxidative stress. Alloxan causes a decrease in hepatic glycogen within 24-72 hours and its cytotoxicity effect is mainly due to the conversion of radical anions which causes pancreatic damage which ultimately decreases insulin levels [20].

Table 2. Average Results of Measurement of Fasting Blood Glucose Levels in mice after treatment.

Table 3. Percentage of Decrease in Average Blood Glucose Levels in mice after treatment.
given drinking water without treatment. In the positive controls (glibenclamide), KP1, KP2, and KP3, there was a decrease in blood glucose levels after administering glibenclamide drug treatment and mangrove extract administration for 7 days compared to blood glucose levels before treatment. However, this treatment has not been able to reduce blood glucose levels to normal limits so that the mice still experience hyperglycemia. Glibenclamide is an oral antidiabetic drug sulfonylurea class where the mechanism of action of the drug is to stimulate an increase in insulin secretion from pancreatic β cells, so that blood glucose levels decrease. In the positive control group (Glibenclamide), the condition of the mice who were still experiencing hyperglycemia probably occurred because the therapy was administered once a day for 7 days, and the half-life of the glibenclamide drug itself was only 4 hours [21].

Giving mangrove leaf extract to the KP1, KP2, and KP3 treatment groups, the conditions of the mice were still experiencing hyperglycemia with a range of average fasting blood glucose levels of respectively 261-321 mg/dl, 192-218 mg/dl, and 228-270 mg/dl. However, giving Rhizophora sp mangrove leaf extract for 7 days showed a significant decrease when compared to before treatment. Based on table 3, it can be seen that the average percentage reduction in fasting blood glucose levels in mice in the KP1 and KP2 groups is 33% and 83%. Meanwhile, in the KP3 group, there was a decrease only on the 5th day by 13%.

Table 4. One Way Anova Test.

| Blood Glucose Levels | Sum of Squares | df | Mean Square | F    | Sig. |
|----------------------|----------------|----|-------------|------|------|
| Between Groups       | 97274.994      | 4  | 24318.748   | 3.024| .042 |
| Within Groups        | 160815.500     | 20 | 8040.775    |      |      |
| Total                | 258090.494     | 24 |             |      |      |

The One Way Anova test results showed a significance value of 0.042 < 0.05, this indicates that the Rhizophora sp mangrove leaf extract had the effect of reducing blood glucose levels in hyperglycemic mice even though the decrease had not reached the normal limit. Post Hoc (LSD) test showed that the negative control group (healthy) was significantly different from the positive control group (glibenclamide) and KP1 dose of 250 mg/kg BW.

The decrease in blood glucose levels in the KP1, KP2, and KP3 treatment groups was due to the content in the Rhizophora sp mangrove leaf extract, which is a flavonoid compound known to have antioxidant activity related to the antidiabetic activity. Flavonoids are known to have antioxidant activity which is believed to protect the body against damage caused by Reactive Oxygen Species (ROS), so that they can inhibit the occurrence of degenerative diseases such as DM [22,23]. Flavonoid compounds have also been proven in vitro to have very strong biological effects as antioxidants. The provision of antioxidants and polyphenol compound components can capture free radicals, reduce oxidative stress in diabetes mellitus [24].

According to research conducted by Mu’nisa A and Rosi KA [9], it is known that mangrove fruit extracts (Rhizophora mucronata) can reduce blood glucose levels of hyperglycemic mice, presumably because mangrove fruit extracts contain bioactive compounds such as flavonoids, steroids, saponins, tannins, and phenols. These compounds are bioactive compounds that can reduce blood glucose levels in mice experiencing hyperglycemia [9]. Red mangrove fruit extract (Rhizophora stylosa) has an antihyperglycemic activity which works as an inhibitor of the α-glucosidase enzyme because red mangrove fruit (Rhizophora stylosa) contains bioactive compounds in the form of flavonoids, phenols, hydroquinones, steroids, and triterpenoids [8]. According to Shinde [25] stated that secondary metabolite compounds that act as inhibitors work by inhibiting the α-glucosidase enzyme found in the small intestine. α-glucosidase enzymes have a role in hydrolyzing oligosaccharides and disaccharides in the small intestine wall. Inhibition of this enzyme action can effectively reduce the digestion of complex carbohydrates and their absorption, thereby reducing the increase in postprandial glucose levels in diabetics [25].

The healing mechanism for diabetes, flavonoids are thought to play a significant role in increasing the activity of antioxidant enzymes and being able to improve the sensitivity of insulin receptors. Flavonoids are also known to regenerate damaged β-pancreatic cells so that insulin deficiency can be overcome, flavonoids also act as alpha-glucosidases which function to delay the absorption of carbohydrates so
that glucose levels in the blood decrease [26,27]. This statement is also supported by research conducted by Lee [28], which shows that there is a positive correlation between antioxidant activity and α-glucosidase enzyme inhibitors. There are several mechanisms of tannins to decrease blood glucose levels, namely tannins reduce nutrient absorption by inhibiting glucose absorption in the intestines, besides inducing the regeneration of pancreatic β cells which affect adipose cells to strengthen insulin activity [28]. Tannins are free radical predators and increase the uptake of glucose in the blood through insulin-mediating activity, thereby reducing glucose in the blood [29].

The condition of diabetes which was still experienced by mice in the positive control group (glibenclamide), KP1, KP2, and KP3, was thought to be due to the influence of alloxan so that pancreatic β cells were still damaged which led to a decrease in insulin receptor sensitivity. In addition, the condition of mice that still had diabetes after 7 days of treatment was thought to be due to the lack of drug therapy or treatment period. This is in accordance with research conducted by Tibrani [30], mice with diabetes and untreated (K-) continue to experience impaired glucose homeostasis and the condition of DM continues. This is thought to be due to the influence of alloxan on the destruction of pancreatic β cells and a decrease in insulin receptor sensitivity that continues to occur [30].

4. Conclusion

The conclusion of this research result is the in-vivo administration of Rhizophora sp mangrove leaf extract was able to reduce fasting glucose levels in mice (Mus musculus). The highest percentage reduction in blood glucose levels occurred in the KP2 treatment group, namely the provision of Rhizophora sp mangrove leaf extract at a dose of 500 mg/kg BW with a decrease percentage value of 29%.

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