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THE EFFECTIVITY OF BELIMBING WULUH FRUIT ETHANOLIC EXTRACT ON DECREASING 2-HOUR POST PRANDIAL BLOOD GLUCOSE LEVELS OF DIABETIC MALE RATS

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by blood glucose levels greater than normal - hyperglycemia (fasting blood glucose ≥ 126 mg / dl or post prandial blood glucose ≥ 200 mg / dl or blood glucose ≥ 200 mg / dl) caused by insulin deficiency and or insulin resistance. One way to control blood glucose levels can be done in a traditional way using natural ingredients. Belimbing wuluh fruits (*Averrhoa bilimbi* Lin.) has been widely used by the community because containing many active substances, including flavonoids. Flavonoids found in Belimbing wuluh fruit are dihydromyricetin. The purpose of this study was to assess the effectivity of belimbing wuluh fruit ethanolic extract on decreasing 2-hour post prandial blood glucose of male rats compared to acarbose. This type of study is in vivo laboratory experimental (pre and posttest only). The research was done from July to September 2020 in the Biochemistry Laboratory, Biotechnology Laboratory and Animal House, Faculty of Medicine, Sriwijaya University, Palembang. The population of study were white male rats. Statistical data analysis used SPSS version 22. Based on the results of the study, it was concluded that the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) was effective on decreasing 2-hour post prandial blood glucose levels of diabetic male white rats.

Keywords: diabetes mellitus, blood glucose, belimbing wuluh

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by blood glucose levels greater than normal - hyperglycemia (fasting blood glucose ≥ 126 mg / dl or post prandial blood glucose ≥ 200 mg / dl or blood glucose ≥ 200 mg / dl) caused by insulin deficiency and or insulin resistance. Classification of diabetes mellitus consists of type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus and other types of diabetes mellitus. Type 1 diabetes mellitus is the destruction of beta cells, usually leading to absolute insulin deficiency such as autoimmune and idiopathic. Type 2 diabetes mellitus is caused by insulin resistance. Gestational diabetes mellitus is a condition of glucose intolerance that is found during pregnancy, usually in the second or third trimester. Other type of diabetes mellitus is caused by genetic factors of beta cell function, genetics of insulin work, exocrine diseases, endocrinopathy, infections, immunology.[1]

Based on 2018 basic health research by ministry of health, the prevalence of non-communicable diseases especially diabetes mellitus has increased compared to 2013. It is based on blood glucose tests; diabetes mellitus has increased from 6.9% to 8.5%. The increase in the prevalence of diabetes mellitus is related to lifestyle including smoking, consumption of alcohol, lack of physical activity and lack of consumption

of fruits and vegetables. These data indicate that cases of diabetes mellitus in Indonesia are increasing and need to be addressed.[2] Management of diabetes mellitus is effective in the early stages before symptoms or prediabetes appear.

One way to control blood glucose levels can be done in a traditional way using natural ingredients. One of the traditional plants containing an antidiabetic effect is belimbing wuluh. Belimbing wuluh fruit (*Averrhoa bilimbi* Lin.) has been widely used by the community. This is because this plant contains many active substances including flavonoids. The flavonoids found in belimbing wuluh fruit is dihydromyricetin (5, 7, 3′, 4′, 5′-Pentahydroxyl Flavanonol).[3] In addition, belimbing wuluh fruit contains several vitamins (riboflavin, thiamin, niacin, ascorbic acid, carotene, retinol) and the minerals (phosphorus, calcium and iron). [4]

Research by Kurup and Mini in 2014 was said that 50 mg / kg body weight oral use of belimbing wuluh fruit extract significantly reduced blood glucose levels and increased lipid metabolism in streptozotocin-induced rats.[5] Previous research conducted by Stefani Chandra in 2012 proved that belimbing wuluh fruit extract at a dose of 0.25 g / kg body weight can reduce blood glucose levels of alloxan-induced wistar rats (*Rattus norvegicus*) [6] In addition, another similar study conducted by Rahmawati in 2015 showed that giving belimbing wuluh fruit juice (*Averrhoa bilimbi* Linn.) at dose of 2 mL / 200 gram body weight can affect blood glucose levels in rats experiencing hyperglycemia.[7] The purpose of this study was to assess the effectivity of belimbing wuluh fruit ethanolic extract on decreasing 2-hour post prandial blood glucose of male rats compared to acarbose.

2. METHOD

This type of study is in vivo laboratory experimental (pre and posttest only). The research was done from July to September 2020 in the Biochemistry Laboratory, Biotechnology Laboratory and Animal House Faculty of Medicine Sriwijaya University Palembang. The population of study were white male rats. The samples of study were male white rats that fill the inclusion criterias (male wistar rats, minimum 2 months old, 150-200 grams body weight, healthy and clean) and the exclusion criterias (rats that appeared sick, had anatomical abnormalities or rats that died before research). The way this research works is to take blood from each group to determine the 2-hour post prandial blood glucose level. Dose of 160 mg / kg body weight alloxan monohydrate as diabetogen was intraperitoneally injected in each group of rats. After 10 days of exposure, 2-hour post prandial blood glucose levels were remeasured for each alloxan induced rat. Rats with 2-hour post prandial blood glucose levels greater than 180 mg / dl were used for further treatment. Rats with 2-hour post prandial blood glucose levels more than 180 mg / dl were left for 2 weeks to drink and eat sufficiently. Each day was given oral treatment. [5] Group 1 was given 1.35 mg / 200-gram body weight acarbose suspension. Group 2 was only given 1% Na CMC suspension. Group 3 was given suspension of ethanolic extract of belimbing wuluh fruit in dose of 50 mg / kg body weight. Group 4 was given suspension of ethanolic extract of belimbing wuluh fruit in dose 100 mg / kg body weight. Group 5 was given suspension of ethanolic extract of belimbing wuluh fruit in dose 200 mg / kg body weight. On day 14 the rats were measured for 2-hour post prandial blood glucose levels.

3. RESULT

Total of 3000 grams of belimbing wuluh fruit was carried out by maceration using 96% ethanol solvent for 3 x 24 hours for 2 times soaking. Then filtered using cloth and filter paper then followed by

evaporation with a rotary evaporator and finally obtained a yield of 24.03 %. Based on the tests that have been carried out, the results show that the ethanolic extract of belimbing wuluh fruit contains five groups of compounds, namely alkaloids, flavonoids, triterpenoids, saponins and tannins. Statistical analysis of SPSS version 22 was carried out for data on 2-hour post prandial blood glucose levels. The homogenity data of 2-hour post prandial blood glucose levels before alloxan induction showed value of p = 0.120 (> 0.05), which means that the 2-hour post prandial blood glucose levels rats before alloxan induction was homogeneous. Homogenity data of pretest 2-hour post prandial blood glucose levels showed the value of p = 0.022 (< 0.05) which means that the pretest 2-hour post prandial blood glucose levels rats were not homogeneous. Homogeneity data of posttest 2-hour post prandial blood glucose levels showed value of p = 0.044 (< 0.05), which means that the posttest 2-hour post prandial blood glucose levels rats were not homogeneous.

The normality data of 2-hour post prandial blood glucose levels before alloxan induction showed p value = 0.483 (> 0.05), which means that the data on 2-hour post prandial blood glucose levels before alloxan induction were normally distributed.

The normality data, pretest 2-hour post prandial blood glucose level showed value of p = 0.474 (> 0.05), which means that the pretest 2-hour post prandial blood glucose levels were normally distributed. The normality data of posttest 2-hour post prandial blood glucose levels showed value of p = 0.214 (> 0.05), which means that posttest 2-hour post prandial blood glucose level were normally distributed.

Due to the numbers of data are 30, the Shaphiro wilk value is used. The results of the effectivity of the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) on 2-hour post prandial blood glucose levels for before (pretest) and after (posttest) research were analyzed using paired sample t-test (before-after). 2-hour post prandial blood glucose levels decreased significantly in all groups (p value < 0.05). The decreasing of 2-hour post prandial blood glucose before and after treatment is presented in figure 1.

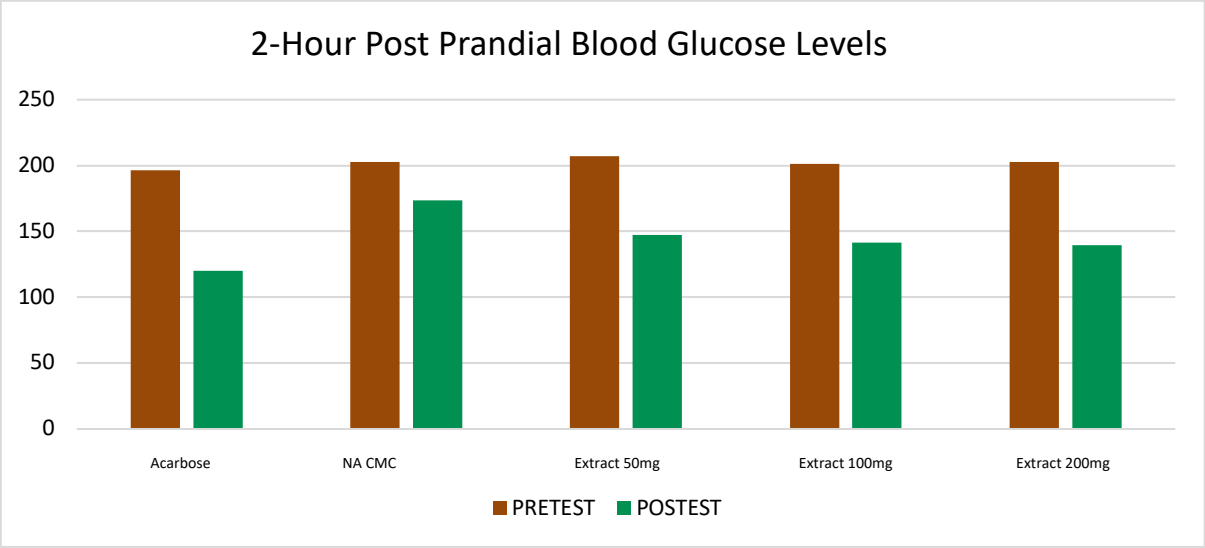

Figure 1. Decreasing of 2-hour post prandial blood glucose levels

Comparison of the effectivity of the ethanolic extract belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) between groups on 2-hour post prandial blood glucose levels after treatment starting from acarbose group, Na CMC group, 50 mg / kg body weight extract group, 100 mg / kg body weight extract group and 200 mg / kg body weight extract group carried out on the 14th day after treatment (posttest) using an independent sample t-test (after-after) analysis. The acarbose group compared to the 50 mg / kg body weight extract group obtained value of $p < 0.05$. It means that there is a significant difference in the effectivity of 2-hour post prandial blood glucose levels after treatment (posttest) between the acarbose group and the 50 mg / kg body weight extract group. In 50 mg / kg body weight extract group compared to 100 mg / kg body weight extract group obtained value of $p = 0.078$ (> 0.05). It means that there is no significant difference in the effectivity of 2-hour post prandial blood glucose levels after treatment (posttest) between the 50 mg / kg body weight extract group compared to 100 mg / kg body weight extract group. In the 100 mg / kg body weight extract group compared to 200 mg / kg body weight extract group obtained value of $p = 0.464$ (> 0.05). It means that there is no significant difference in the effectivity of 2-hour post prandial blood glucose levels after treatment (posttest) between the 100 mg / kg body weight extract group compared to the 200 mg / kg body weight extract group.

This study used three doses of the extract, namely a small dose (50 mg / kg body weight), a medium dose (100 mg / kg body weight) and a large dose (200 mg / kg body weight). Furthermore, the suitability of the extract dosage on the 2-hour post prandial blood glucose levels was described using the LSD post-hoc test. The dosage suitability test for 2-hour post prandial blood glucose levels in the acarbose group was compared with Na CMC and all extracts obtained p value = 0.000 (<0.05). It means that there was significant difference in the 2-hour post prandial blood glucose levels between the acarbose group against Na CMC and all extracts. The 50 mg / kg body weight extract group compared to the 100 mg / kg body weight extract group obtained value of $p = 0.158$ (> 0.05). It means that there was no significant difference in the 2-hour post prandial blood glucose levels between the 50 mg / kg body weight extract group compared to the 100 mg / kg body weight extract group. The 50 mg / kg body weight extract group was compared with the 200 mg / kg body weight extract group obtained value of $p = 0.055$ (> 0.05). It means that there was no significant difference in 2-hour post prandial blood glucose levels between the 50 mg / kg body weight extract group compared to the 200 mg / kg body weight extract group. The 100 mg / kg body weight extract group compared to the 200 mg / kg body weight extract group obtained value of $p = 0.583$ (> 0.05). It means that there was no significant difference in the 2-hour post prandial blood glucose level between the 100 mg / kg body weight extract group compared to the 200 mg / kg body weight extract group.

4. DISCUSSION

This study used 30 wistar strain male white rats as the subject. Alloxan induced rats as diabetogen. This is consistent with previous studies using alloxan doses of 160 mg / kg body weight. [8] Alloxan is a hydrophilic and unstable compound that can rapidly damage the pancreas. The mechanism of action begins with the rapid uptake by pancreas beta cells. Furthermore, alloxan undergoes a reduction cycle to dialuric acid which is then oxidized to alloxan form. This alloxan and dialuric acid determine the redox cycle to generate superoxide radicals which are subsequently dismutated into hydrogen peroxide which occurs spontaneously and possibly catalyzed by superoxide dismutase. One of

the targets of reactive oxygen is the DNA of the langerhans islet in pancreas. [9]

The effectivity of the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) and acarbose on the 2-hour post prandial blood glucose levels were tested based on the average 2-hour post prandial glucose levels rat before being treated (pretest) and after being treated (posttest). The acarbose group, 50 mg / kg body weight extract group, 100 mg / kg body weight extract group and 200 mg / kg body weight extract group obtained value of $p < 0.05$, which means there was a significant decrease on 2-hour post prandial blood glucose levels before being given treatment (pretest) and after being given treatment (posttest) in all groups. This is in accordance with previous research that gave 100 ml of belimbing wuluh fruit extract (*Averrhoa bilimbi* Linn.) for 14 days had an effect on fasting blood sugar levels before and after the intervention in the treatment group. [10] Another study conducted explained that giving belimbing wuluh fruit juice at a dose of 2 mL / 200 gram body weight for 14 days can significantly reduce blood glucose levels in hyperglycemic rats. [7]

Comparison of the effectivity of the ethanolic extract of belimbing fruit (*Averrhoa bilimbi* Linn.) between groups on 2-hour post prandial blood glucose levels after treatment starting from the acarbose group, Na CMC group, 50 mg / kg body weight extract group, 100 mg / kg body weight extract group and 200 mg / kg body weight extract group. In the acarbose group compared to the Na CMC group, extract 50 mg / kg body weight extract group, 100 mg / kg body weight extract group and 200 mg / kg body weight extract group obtained value of $p < 0.05$ means that there is a significant difference in the effectivity of 2-hour post prandial blood glucose levels after treatment (posttest) between the acarbose group, Na CMC group, 50 mg / kg body weight extract group, 100 mg / kg body weight extract group and 200 mg / kg body weight extract group. This means that the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) is effective on decreasing blood glucose levels of male white rats. Based on previous research conducted, it was stated that giving belimbing wuluh fruit extract for 14 days reduced blood glucose levels better than aquadest but not better than metformin. [6] Research conducted states that giving belimbing wuluh fruit juice provides a reduction in fasting blood glucose to adult women. [10]

The dosage suitability test for 2-hour post prandial blood glucose levels used post-hoc test LSD analysis. The acarbose group compared to Na CMC and all extract group values of $p = 0.000$ (<0.05. It means that there was a significant difference in 2-hour post prandial blood glucose levels between the acarbose group against Na CMC group and all extract groups. From this dose suitability test, it can be concluded that the potential of ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) is a flat dose because there is no significant difference in each increasing dose of ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.).

These results indicate that increasing the extract dose is not always accompanied by a significant decrease in blood glucose levels. The relationship between extract dosage and its effects will be explained based on the pharmacodynamics of the drug. A drug can have an effect when there is a binding with the receptor to form a drug-receptor bond. According to the receptor occupancy theory put forward by Alfred Joseph Clark, the relationship between drug dose and its effect is proportional to the number of receptors occupied by the drug which is represented as a hyperbolic graph. There is Emax, which is the maximum effect caused by a high dose concentration. If Emax has been achieved, increasing the dose of the drug will be meaningless because according to the principles of the receptor occupancy theory. At this stage all the existing receptors have been occupied by

the drug. It is possible that the three doses in this study have given rise to Emax. The receptor occupancy theory also applies to drug side effects. [11]

Because the three treatment groups had the same effectivity as acarbose in reducing 2-hour post prandial blood glucose levels, it could be concluded that the first treatment dose was the most effective compared to the other groups. This is because by using the smallest dose, the results of reducing blood glucose are just as effective. In addition, using the smallest dose can minimize the side effects of the drug. Furthermore, these results can be used as a temporary basis in determining the dose of ethanolic extract of belimbing wuluh fruit which is most effective in reducing blood glucose levels of white male rats due to alloxan induction, namely by using a dose of 50 mg / kg body weight / day. In order for these results to be recommended to humans, the dose is converted by multiplying the conversion factor from rats to humans. The conversion factor from rats weighing 200 grams to humans weighing 70 kg is 56 so that the required dose is 560 mg / 70 kg body weight / day. [12]

There was a significant decrease in Na CMC group because Na CMC group was included in dietary fiber which can lower post prandial blood glucose. This is based on the mechanism of action of dietary fiber. The first is increasing the viscosity of the contents of the small intestine and avoiding glucose diffusion. The second is binding glucose and decreasing glucose concentration in the small intestine. The third is slowing down alpha amylase through starch and enzyme encapsulation and directly inhibiting the enzyme.[13] Increasing the viscosity of Na CMC will further reduce blood glucose levels.[14]

The oral administration antidiabetics of type 1 diabetes mellitus as a positive control is based on research which states that giving oral administration antidiabetics will reduce insulin use and increase cost effectiveness as pharmacacoeconomic perspective. [15]

The results showed that the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) has a hypoglycemic potential in alloxan-induced diabetic rats. This hypoglycemic effect is thought to be due to the presence of the main antihypergylchemic agent, namely flavonoids. Flavonoids are polyphenol components found in plants. Inhibition of alpha glucosidase by flavonoids results in the failure of the process of breaking down carbohydrates into monosaccharides so that they cannot be absorbed by the intestine. The principle of this inhibition is similar to that of acarbose which has been used as a diabetes mellitus drug by producing a delay in carbohydrate hydrolysis. This is because the flavonoids react to inhibit the alpha glucosidase enzyme inhibitor, namely 3 '4'-dihydroxy on the B ring and 3-OH on the C ring. The 3-OH group on the C ring serves to maintain binding to the flavonoid molecule. [16]

5. CONCLUSION

Based on the results of research on the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.), it was concluded that the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) was effective on decreasing 2-hour post prandial blood glucose levels of diabetic male white rats. Further research is needed to determine the pure compounds of the ethanolic extract of belimbing wuluh fruit (*Averrhoa bilimbi* Linn.) which play a role on decreasing 2-hour post prandial blood glucose levels.

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