Anti-hyperglycemic effect and glucose tolerance of guajava (*Psidium guajava* L.) leaf ethanol extract in diabetic rats

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Abstract. Traditionally guava (*Psidium guajava* L) leaf is used for treatment of various ailments like diarrhea, wounds, rheumatism, anti-allergy, ant-spasmodic, etc, as folk medicine. The aim of this research is to know the effect of hypoglycemia and glucose tolerance of ethanol extract of guava leaf against male white rat. The guajava leaf was obtained from Balitro Bogor. Preparation of guajava leaf extract was done by cold maceration extraction technique using ethanol 70%. Male albino rats were made into diabetics using the alloxan method. Rats were divided into 6 groups, as a comparative drug for anti-hyperglycemic used glibenclamid and as a comparative drug for glucose tolerance used acarbose. The result of blood glucometer test showed that ethanol extract 70% of guajava leaf had effect as anti-hyperglycemic and glucose tolerance with no significant difference with glibenclamid drug as anti-hyperglycemic and acarbose as glucose tolerance drug.

1. Introduction

Diabetes is a chronic health problem with devastating, yet preventable consequences. It is characterized by high blood glucose levels resulting from defects in insulin production, insulin action, or both. Globally, rates of type 2 diabetes were 15.1 million in 2003, the number of people with diabetes worldwide is projected to increase to 36.6 million by 2030. Diabetes is on the rise. No longer is a disease of predominantly rich nations, the prevalence of diabetes steadily increasing everywhere, most markedly in the world's middle-income countries [1].

Insulin therapy has been shown to benefit the prognosis in patients with type 2 diabetes, but its initiation and intensification is often delayed through concerns about hypoglycemia and weight gain. In addition, weight gain is linked to the pathophysiology of type 2 diabetes and contributes to the overall risk for adverse cardiovascular outcomes. So are other synthetic Diabetes medicines that have many adverse side effects. Therefore it is necessary to find a drug that comes from nature that has a smaller side effect [2].

Psidium guava. L. is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. All parts of this tree, including fruits, leaves, bark, and roots, have been used for treating stomachache and diarrhea in many countries. Leaves, pulp and seeds are used to treat respiratory and gastrointestinal disorders, and as an antispasmodic, anti-inflammatory, as a cough sedative, anti-diarrheic, in the management of hypertension, obesity and in the control of diabetes mellitus. It also possesses anticancer properties. The seeds are used as antimicrobial, gastrointestinal, anti-allergic and anticarcinogenic activity [3]. The important active constituents of guava are essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters, and aldehydes etc. This paper explains the Evidence-based information regarding the phytochemistry and pharmacological activity of this plant [4].

2. Materials and Method

2.1. Preparation of the extract

Fresh guava leaves were made into a slurry using a blender with 70% ethanol. Then immersed in a glass

beaker with a height of 70% ethanol there were 2.5 cm above the surface of the slurry. The maceration process is carried out for 3 hours while stirring. Then filtered using cotton to filter the dregs. This process is done repeatedly until no more extracts are extracted, which is marked with a clear color of solvent. The obtained filtrate was collected, filtered using filter paper and evaporated ethanol until a viscous ethanolic extract was obtained. A test solution was then prepared and administered to the test animal.

2.2. Preparation of experimental animals

An acclimatization of test animals was performed to male albino rats of 7-9 weeks age, weighing 170-190 g, were used. The animals were kept in clean and dry plastic cages, with light-dark at room temperature. The animals were fed with standard pellet diet and water was given ad libitum. This study was carried out in the animal house of State Islamic University, Syarif Hidayatullah, Jakarta and this study was approved by the Institutional Ethical Committee. Animals were divided into 6 groups and each group had 5 rats.

2.*3 Administration of sample and determination of rat blood glucose level*

Rats are fasted for 16 hours. Prior to treatment, rat blood was taken through the rat tail vein and measured as fasting glucose using glucometer. Then the rat was given extract with high doses using gastric sonde. 30 minutes after administration, the mice were given 50% glucose solution at a dose of 1 g / kg BW, then immediately took the blood of the mouse and its glucose level was measured as blood glucose level at minute 0. Furthermore, rat blood was taken at 30, 60, 90, 120, 150 and 180. The data obtained is the result of oral glucose tolerance test. Then the rats were again fed and drank normally, with the administration of high-dose extracts daily. On day 3, 8 and 14 after oral glucose tolerance test, rat blood was taken and measured glucose levels.

2.4. Statistical analysis

Prior to the ANOVA test, first test of normality and Levene homogeneity test was performed. If the normality test and Levene homogeneity test is worth ($p > 0.05$), then it is continued with ANOVA test. However, if the significance of the test for normality and homogeneity test Levene value of less than 0.05, ANOVA test cannot be performed, so proceed with Kruskal Wallis test.

3. Results

The results of this study give results that the highest effect of hypoglycemia extract of guava leaf ethanol (*Psidium guajava* L.) with alloxan diabetes test method is 1,300 mg / kg BW which can decrease blood glucose level up to 31.66%, indicated by no significant difference between test dose With normal and positive control, and a significant difference between the test dose and the negative control ($P < 0.05$). As shown in table 1.

The highest effect of hypoglycemia extract of guava ethanol extract (*Psidium guajava* L.) by oral glucose tolerance test method was proven to inhibit glucose absorption, indicated by no significant difference between 1300 mg / kg BW dosage with normal and positive control, and presence A significant difference between the test dose and the negative control ($P < 0.05$). As shown in table 2.

Table 1. Effect of ethanolic (70%) leaf extract of guava as anti-hyperglycemic on blood glucose (mg/dL) alloxan-induced diabetic rats

Time (Days)	NC	C(-)	C(+) Glibenclamid	Low Doses	Middle Doses	High Doses
Day 0, BI	84.75	85.5	83.75	84.75	86.25	82.75
Day 0,AI	88.5	114.25	172.25	135.25	134.5	139.5
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Day 8, AI	104.25	158.25	77	139	163.5	179.5

Day14, AI	90.5	152	78.5	110.75	96.25	97

Note: BI = Before Induction; AI = After Induction; NC = Normal Control; C(-) = Negative Control; C(+) = Positive Control (Glibenclamid)

Table 2. Effect of ethanolic (70%) leaf extract of guava on test of glucose tolerance (mg/dL) alloxan-induced diabetic rats

Time (Minutes)	NC	C(-)	C(+) Acarbose	Low Doses	Middle Doses	High Doses
Fasting	88.5	122.75	108.25	135.25	136.5	156.25
Minute 30 AGA	95.75	161.75	114.25	294	213.5	146.75
Minute 60 AGA	92.75	280.75	103.75	252.5	161.75	154.5
Minute 90 AGA	86.5	285.25	118.5	203	155.25	134.75
Minute 120 AGA	93.25	244.5	106.5	169.5	128.75	127.5
Minute 150AGA	94.5	237	110.5	162.5	120.5	116.25
Minute 180 AGA	115	229.5	132.25	154.25	140.75	131.25

Note: AGA = After Glucose Administration; NC = Normal Control; C(-) = Negative Control; C(+) = Positive Control (Acarbose)

4. Discussion

Bhanu et al [5] conducted a study of the the antihyperglycemic and antihyperlipidemic effects of guava on alloxan-induced diabetic rats by making extracts of guava with 96% ethanol at hot temperatures, but they did not test the glucose tolerance of guava against alloxan-induced diabetic rats.The results of this study for antihyperglycemic are similar to the results of research conducted by Bhanu S, et al, [5].

In this research, preparation of guava leaf extract (*Psidium guajava* L.) using cold way extraction method by maceration and using ethanol 70% as solvent. Cold-way extraction has the advantage in the total extraction process, which minimizes the likelihood of damage to the thermolabile compounds present in the sample. Most of the compounds can be extracted by cold-way extraction, although there are some compounds that have solubility limitation to the solvent at room temperature. The maceration method is chosen because the process is easy, the equipment used is fewer and simpler, and does not require any special skills. While 70% ethanol is used because ethanol is commonly used in total extraction. The advantage of using ethanol is that most of the lipophilic and polar compounds can be extracted. Guava leaf used is a bit old leaves because it contains the most tannins with the characteristics of dark green leaves, clear and hard leaf bones, the top surface is slippery and has a little stiff.

From the results of decreased blood glucose levels showed that the greater the dose of thick guava leaf extract test the greater the ability to lower blood glucose levels. The result of percentage of glucose decrease after 14 days given test preparation is as follows: low dose 162,5 mg / kg BW equal to 18,11%, 325 mg / kg BW equal to 28,49%, and 1300 mg / kg BW equal to 31,66 %.

Blood glucose data obtained from each measurement is then processed statistically. The statistic method used is One Way Variance Analysis (ANOVA) to blood glucose level. First, normality test and homogeneity test are done. Such testing is a common practice before a statistical method is applied. The method used in the normality test is by Kolmogorov-Smirnov test, and its homogeneity uses Levene test.

Based on the normality test showed that blood glucose levels throughout the initial animal test group before treatment were normally distributed. While homogeneity tests showed blood glucose levels throughout the initial animal test group before homogenized treatment

In the alloxan diabetes test, the one-way Variance Analysis Test showed the 8^{th} day blood glucose level after treatment between the 162.5 mg / kg BW dose group and the normal control did not differ significantly, while the blood glucose level between the 325 mg / kg BW dose group and the dose of 1300 mg / kg BW with normal control differed significantly at the 0.05 test level. Blood glucose level

on day 14 after treatment showed between group 325 mg / kg BW and dose 1.300 mg / kg BW with normal control and positive control did not differ significantly while there was significant difference at test level 0.05 between group 162.5 mg / kg BW with normal control and positive control. The decrease in blood glucose level of the group given glibenclamide was caused by increased insulin stimulation but decreased blood glucose levels of the group which were given test of viscous ethanol extract of guava leaf (*Psidium guajava* L.) unknown mechanism of action.

In the oral glucose tolerance test, fasting blood glucose levels across all animal groups differed significantly. This is because blood glucose levels throughout the test group had hyperglycemia, whereas normal blood glucose control levels remained within the normal range. Blood glucose levels between the low-dose assay groups with normal control and positive control varied significantly in the 30^{th}, 60^{th}, 90^{th} and 120^{th} minutes, but at minutes 150 and 180 did not differ significantly at the 0.05 test level. Blood glucose levels between the moderate-dose test group and the normal control and positive control varied significantly in the 30^{th} minute, but at 60, 90, 120, 150 and 180 minutes did not differ significantly at the 0.05 test level. However, the highest inhibitory ability was obtained in the high dose test group. This was demonstrated by statistical results indicating that blood glucose levels between high dose test groups with normal control and positive control in the 30^{th} to 180^{th} minutes did not differ significantly at the 0.05 test level.

Inhibition of group blood glucose absorption inhibited by acarbose caused by inhibition mechanism of α-glucosidase enzyme. While the inhibition of glucose absorption in the group that was given test of viscous ethanol extract of guava leaf (*Psidium guajava* L.) was allegedly caused because of one of the compounds contained in guava leaf viscous extract which can inhibit the absorption of glucose. Guava leaf extract (*Psidium guajava* L.) contains tannins. Based on previous research, tannin compound is astringent that can minimize skin surface pores [6].

5. Conclusion

Ethanol extract of 70% guava leaf (*Psidium guajava* L.) has the potential to lower blood glucose levels, either by alloxan diabetes test or oral glucose tolerance test.

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Nilai Pengusul =				27

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on day 14 after treatment showed between group 325 mg / kg BW and dose 1.300 mg / kg BW with normal control and positive control did not differ significantly while there was significant difference at test level 0.05 between group 162.5 mg / kg BW with normal control and positive control. The decrease in blood glucose level of the group given glibenclamide was caused by increased insulin stimulation but decreased blood glucose levels of the group which were given test of viscous ethanol extract of guava leaf (*Psidium guajava* L.) unknown mechanism of action.

In the oral glucose tolerance test, fasting blood glucose levels across all animal groups differed significantly. This is because blood glucose levels throughout the test group had hyperglycemia, whereas normal blood glucose control levels remained within the normal range. Blood glucose levels between the low-dose assay groups with normal control and positive control varied significantly in the 30th, 60th, 90th and 120th minutes, but at minutes 150 and 180 did not differ significantly at the 0.05 test level. Blood glucose levels between the moderate-dose test group and the normal control and positive control varied significantly in the 30th minute, but at 60, 90, 120, 150 and 180 minutes did not differ significantly at the 0.05 test level. However, the highest inhibitory ability was obtained in the high dose test group. This was demonstrated by statistical results indicating that blood glucose levels between high dose test groups with normal control and positive control in the 30th to 180th minutes did not differ significantly at the 0.05 test level.

Inhibition of group blood glucose absorption inhibited by acarbose caused by inhibition mechanism of α-glucosidase enzyme. While the inhibition of glucose absorption in the group that was given test of viscous ethanol extract of guava leaf (*Psidium guajava* L.) was allegedly caused because of one of the compounds contained in guava leaf viscous extract which can inhibit the absorption of glucose. Guava leaf extract (*Psidium guajava* L.) contains tannins. Based on previous research, tannin compound is astringent that can minimize skin surface pores [6].

5. Conclusion

Ethanol extract of 70% guava leaf (*Psidium guajava* L.) has the potential to lower blood glucose levels, either by alloxan diabetes test or oral glucose tolerance test.

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