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ANTIDIABETIC EFFECTS OF EXTRACT ETHANOL *MOMORDICA CHARANTIA* (BITTER MELON) ON GLUCOSE BLOOD IN DIABETEC RATS

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ABSTRACT
This study aims to determine the content of active compounds from the ethanolic extract of bitter melon and the effect of bitter melon extract on blood glucose levels of diabetic rats induced by alloxan. This study was conducted in vivo to determine the decrease in blood glucose levels during therapy with ethanol extract of bitter melon. Mice were conditioned hyperglycemic by using alloxan. This study used 18 male white rats which were divided into 6 groups, namely normal control, positive control, and 4 dose variation controls. The dose used is 1 mL; 0.80 mL; 0.60 mL; 0.45 mL/200 g BW. Therapy was carried out for 14 days by measuring blood glucose levels on days 2, 4, 5, 6, 8, 10, 12, and 14 after therapy. Phytochemical test results of ethanol extract of bitter melon contains alkaloids, tannins, saponins and terpenoids. Therapy with ethanol extract of bitter melon showed the effect of treatment time on reducing blood glucose levels of alloxan-induced rats. The time needed is 14.6 days to reach blood glucose levels when they become normal.

KEYWORDS: Bitter melon, aloxan, mice, glucose blood.

1. INTRODUCTION
Diabetes mellitus (DM) is a metabolic disease characterized by symptoms of hyperglycemia as a result of impaired insulin secretion. Lack of insulin hormone that functions to utilize glucose as an energy source and synthesize fat is caused by the pancreas no longer being able to secrete insulin, while the relative lack of insulin is caused by insufficient insulin production to meet the body's needs. According to the World Health Organization, worldwide, about 10% of all cases of diabetes are type 1, and the remaining 90% are cases of type 2, the number of people with diabetes worldwide has increase from 171 million in 2000 to 366 million in 2030.[1] In various countries DM is one of the main threats to human life. Health experts usually recommend controlling the patient's blood sugar level through a combination of medication, diet control, and regular physical exercise.[2-4]

However, there is no available drug that has the ability to completely cure type 2 diabetes. Treatment of diabetes mellitus is currently still limited to the use of Oral Hypoglycemic Drugs (OHO) such as biguanides, sulfonylureas, glinides, thiazolidinediones, and acarbose as well as insulin injections. These diabetes drugs can cause various side effects such as diarrhea, dizziness, headaches, nausea and vomiting, weight gain and hypoglycemia and if not treated immediately, coma and even death can occur.[5] The use of traditional medicine is generally considered safer than the use of modern medicine (chemical drugs). This is because traditional medicine has relatively fewer side effects than chemical medicine.[6]

One type of plant that is widely used as traditional medicine is bitter melon (Momordica charantia). Bitter melon (Momordica charantia) is one type of plant that has the potential to be developed because it has high economic value as a food plant and traditional medicinal ingredient. Bitter melon contains flavonoids, saponins, and polyphenols.[7] The content of bitter melon that is useful for lowering blood glucose is charantin, polypeptide-P insulin, and lectins. The content of saponins, flavonoids, polyphenols, and vitamin C of bitter melon serves as an antioxidant that aims to ward off free radicals that can interfere with the survival of Leydig cells due to diabetes mellitus.[8] Bitter melon has a hypoglycemic effect by lowering blood glucose levels by inhibiting gluconeogenesis in the liver, protecting pancreatic -cells, increasing insulin sensitivity, and reducing oxidative stress.[6]

In their research, Deepak and Anurekha,[9] used the extract of M. charantia Linn. To reduce blood glucose levels of male white rats of Wistar strain, alloxan was injected intraperitoneally. After 72 hours of alloxination, the rat blood glucose level increased to 361.40 mg/dL. Giving M. charantia extract at a dose of 400 mg/kgBW

for 14 days orally, can reduce blood glucose levels to 230.80 mg/dL. In the study of Pasupuleti[10] reported that within 48 hours the blood glucose levels of albino rats induced with alloxan 120mg/kgBW intraperitoneally increased to 356.5mg/dL. Then the rats were given hydroalcoholic extract of M.charantia at a dose of 300mg/dL. kg body weight for 21 days orally. On day 21, blood glucose levels dropped to 203.8mg/dL. Phytochemical screening showed that the hydroalcoholic extract of M. charantia contained alkaloids, tannins, phenolic groups, proteins and amino acids, saponins, carbohydrates, steroids, triterpenoids, and flavonoids.

Kolawole, et al[11] reported that the methanol extract of bitter melon showed a hypoglycemic effect in normal rats and an antihyperglycemic effect in alloxan-induced diabetic rats. The results showed that 600mg/kg of methanol extract of bitter melon can reduce blood glucose from 192.5±5.3 to 122.2±3.9 and produce an antihyperglycemic effect of 36%. The decrease in blood glucose levels in this study was not too significant and ineffective because high doses and long durations could only reduce blood glucose levels by 36%. This indicates that methanol solvent is not effective in extracting antidiabetic compounds in bitter melon fruit.

A number of clinical studies have shown that bitter melon is a vegetable that contains flavonoid compounds, polypeptides and is used to control diabetes naturally.[12] Polypeptide-p or p-insulin is an insulin like hypoglycemic protein, shown to lower blood glucose in gerbils, langurs and humans when injected subcutaneously.[13] P-insulin works by mimicking the action of human insulin in the body, so it can be used as a substitute for plant-based insulin in patients with type-1 diabetes.[14] Recently, Wang et al[15] have cloned and expressed the gene sequence encoding 498 bp for the polypeptide p gene in bitter melon and have also proven the hypoglycemic effect of the polypeptide recombinant in alloxan-induced diabetic rats1. Oral administration of bitter melon seed extract produced a hypoglycemic effect in streptozotocin (STZ)-induced type 1 diabetic rats.[16] This shows that compounds in bitter melon besides p-insulin are also effective in the treatment of diabetes. Based on the description above, it clearly shows that bitter melon and its extract can regulate blood glucose through two mechanisms. First, it can regulate how much glucose is absorbed by the intestines into the blood after a meal and second, it can stimulate the absorption of glucose into skeletal muscle cells like insulin. This study aims to improve the anti-diabetic properties of bitter melon fruit by using a ethanol extract from bitter melon fruit.

2. MATERIALS AND METHODS

2.1 Material and apparatus

The materials needed are Momordicha charanthia L, NaCl 0.9%, HCl 2%, HCl 1N, chloroform, FeCl3 1%, Anhydrous Acetic Acid, Aquades, Mg, H_2SO_4, Dragendrorff's reagent, and Meyer's reagent, alloxan, and white rats Rattus norvegicus male species in good health, The apparatus needed are: a balance, a blender, a 60 mess sieve, an oven, and a glassware, syringe, gastric probe and gloves. scissors, mask, DR glucometer, and glucose strip.

2.2 Bitter gourd extract

A total of 10 kg of bitter melon, washed with running water, then cut into thin slices, dried in an oven at a temperature of 50-60 C for 24 hours. Dried bitter melon fruit is mashed with a grinder until it becomes powder. 1 kg of bitter melon fruit powder was macerated with 7 L of 96% ethanol for 48 hours. Then filtered using filter paper to obtain the filtrate. Furthermore, the filtrate was evaporated using a rotary vacuum evaporator (RVE) to produce a thick extract of 500 grams of bitter melon.The maceration results were tested for phytochemicals to see the content of secondary metabolites contained therein.

2.3 Preparation of test animals

This study used 18 male white rats (Rattus norvegicus) wistar strain 2-3 months old with a weight of ± 200 g. The condition of the rat is in a healthy state which is characterized by active movement. Mice were acclimatized in the laboratory for 1 week in a special cage to uniform the way of life, feeding and conditions of the experimental cage. All mice were fed commercial feed and water ad libitum.

2.4 Induction of test animals with alloxan

The 2% alloxan monohydrate solution was made fresh. After the mice fasted for 18 hours, the mice were injected intraperitoneally with 2% alloxan monohydrate solution at a dose of 200 mg/kg body weight. After 1 week, fasting blood glucose (FBG) levels of mice were measured and only mice with FBG levels between 13-20 mmol/L were used for the experiment. Diabetes is characterized by blood glucose levels of more than 200 mg/dl. Then the diabetes mellitus rats were allowed to stand for 5 days and then therapy was carried out.

2.5 Test of blood glucose levels in mice given alloxan,
Mice that had experienced hyperglycemic conditions were divided into 6 groups. Group 1 was given a dose of bitter melon fruit extract at a dose of 0.4 mL/200 g BW, group 2 was given a bitter melon extract at a dose of 0.6 mL/200 g BW, group 3 was given a bitter melon extract at a dose of 0.8 mL/200 g BW, group 4 was given a bitter melon extract at a dose of 1 mL/ 200 g of body weight 4, group 5 was given water and group 6 was given glibenclamide as a comparison. Furthermore, blood glucose checks will be carried out at any time every 2 days, namely on days 0, 2, 4, 6, 8, 10, 12, and 14.

2.6 Data analysis
The data obtained were analyzed to determine the effect of length of time on decreasing blood glucose levels during therapy. The data obtained were tested using normality and homogeneity tests, then one-way ANOVA (Analysis of Variance) and Mann-Witnay tests were used to determine the effect of length of time on therapy on decreasing blood glucose levels. The decrease in blood glucose levels is then searched for the value of the regression equation.

3. RESULTS AND DISCUSSION

3.1 Phytochemical test
Phytochemical tests were carried out to determine the secondary metabolite compounds contained in the infusion of bitter melon which are thought to have antidiabetic properties. Phytochemical test is a qualitative analysis that only identifies the presence of compounds without determining the levels. The results of phytochemical tests are shown in Table 1

Table 1: Phytochemical test results of bitter melon fruit extract.

No	Parameter	Result
1	Alcaloid	+
2	Flavonoid	+
3	Terpenoid	+
4	Steroid	-
5	Tanin	+
6	Saponin	+

2.2 Test of rat blood glucose levels before induction with alloxan
Blood glucose levels of rats before rats were induced with alloxan were measured using a Glucometer. Figure 1 shows the average rat blood glucose levels in each group

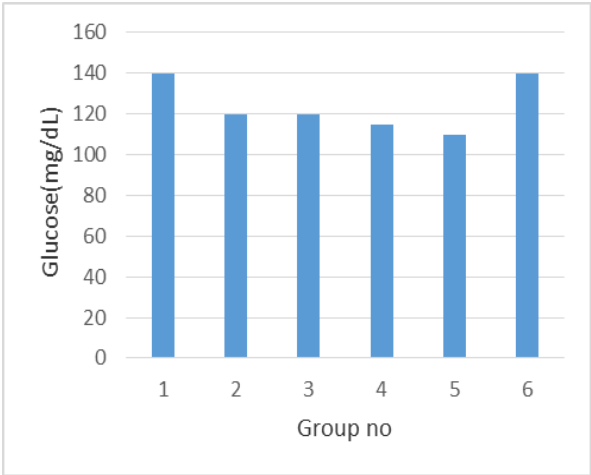

Fig. 1: Mouse blood glucose before alloxan induction.

Figure 1 shows that the results of measuring blood glucose levels of all rats before alloxan induction were under normal conditions, namely below 200 mg/dL. Although the blood glucose levels obtained vary widely. This is because of the biological variations possessed by each experimental animal so it is not possible to obtain the same blood glucose levels.

3.3 Test of rat blood glucose levels after induction with alloxan
Blood glucose levels of rats after rats were induced with alloxan were measured using Glucometer. Figure 2 shows the average rat blood glucose levels in each group. Alloxan has caused rats to experience an increase in blood glucose levels > 200 mg/dL or it can be called diabetes mellitus. However, the level of diabetes is not the same, this is because the immune system of each foreign mouse is not the same in responding to alloxan injected in each mouse. So that the increase in blood glucose levels in rats before and after alloxan injection was not evenly distributed.

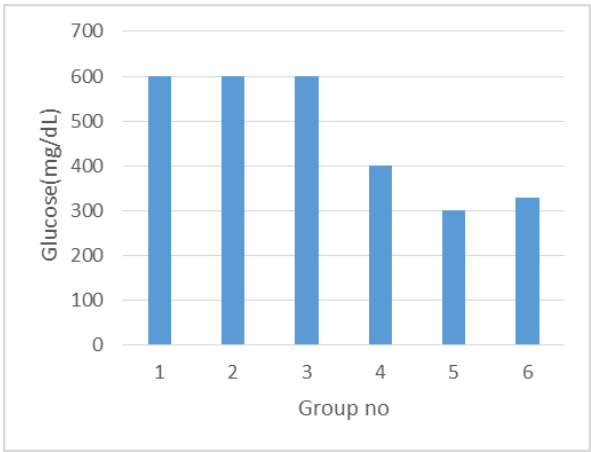

Fig. 2: Mouse blood glucose after alloxan induction.

Alloxan reacts by destroying essential substances in pancreatic cells, resulting in reduced insulin-carrying granules in pancreatic cells. Alloxan causes oxidative stress on pancreatic cells. The mechanism of increasing

oxidative stress in diabetes mellitus is formed through three processes, namely non-enzymatic glycation of proteins, polyol-sorbitol pathway and glucose auto-oxidation.Glycation is a reaction that increases aldehydes in proteins, causing the accumulation of chemical modifications of proteins in tissues, especially in the kidneys, liver and others. In the polyol-Sorbitol pathway, glucose is converted to sorbitol with the help of the enzyme aldose reductase. Under normal circumstances, the concentration of sorbitol in cells is very low. However, in hyperglycemic conditions, the concentration of sorbitol increases. Sorbitol degradation is very slow so that it accumulates in cells and causes an increase in osmotic pressure which results in cell damage. These three reactions cause an increase in oxidative stress in diabetic rats.

3.4 Therapeutic test of diabetic rats with ethanol extract of bitter melon

Oxidative stress in diabetic rats can be inhibited with antioxidants. Antioxidants are molecules that can neutralize free radicals by donating unpaired electrons. Research results from Raish[17] (2017), Tan and Gan[18] and [Hani19] show that bitter melon fruit (Momordica charantia L.) has activity antioxidant Figure 3 shows a graph of blood glucose levels after being treated with ethanol extract of bitter melon.

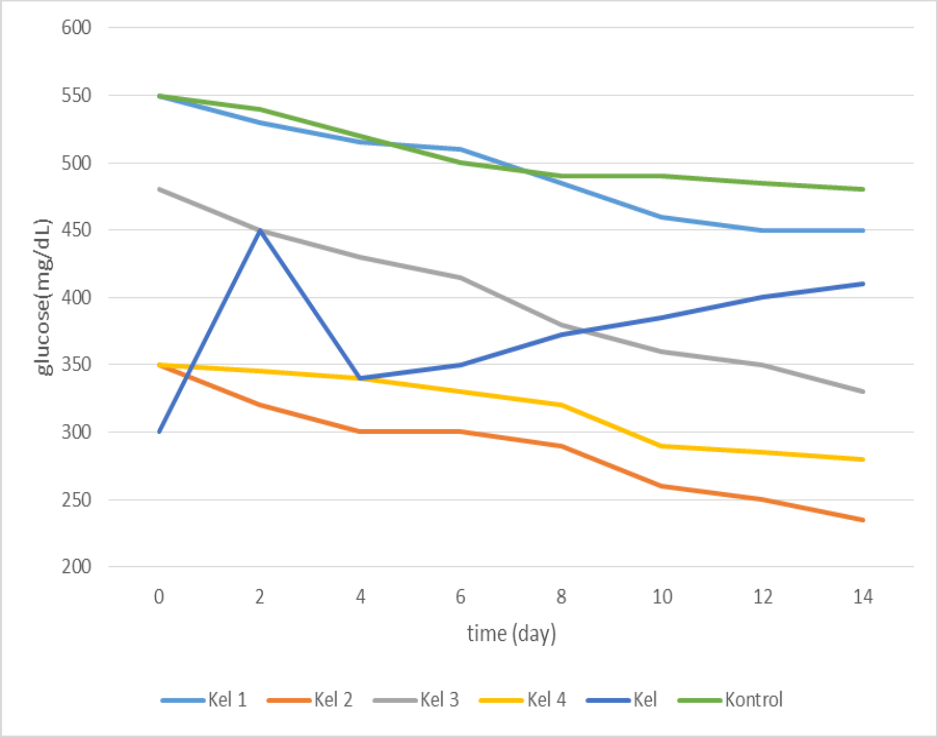

Fig. 3: Effect time on glucose concentration.

Figure 3 shows that the longer the treatment time can reduce the blood glucose levels of diabetic rats. The decrease that occurred on the 2nd and 4th day after therapy did not show a significant decrease. This is based on the Kruskal Wallis test with = 0.06 which shows the value of sig. 2nd day is 0.238 and sig. day 4 is 0.088. Kruskal Wallis test was performed because the blood glucose level data obtained were not homogeneous and on the 2nd day the blood glucose level data was not normally distributed.

Measurement of blood glucose levels on day 6 showed a decrease in blood glucose levels which was supported by the results of the Kruskal Wallis test with a sig value. 0.038. This indicates that there is a significant effect of the duration of therapy for the ethanol extract of bitter melon on the reduction of alloxan-induced rat blood glucose levels. To determine the effect, the Mann-Witnay test was carried out. The results obtained that all doses of bitter melon ethanol extract had an effect when compared to normal controls. This is also the same with the effect of the duration of therapy on 8th, 10th, 12th and 14th days. The results of the Kruskal Wallis test with = 0.06 obtained a sig value. respectively, namely 0.055, 0.044, 0.33 and 0.33. It can be concluded that the duration of therapy have an effect on reducing blood glucose levels in diabetic rats. However, the dose variation did not have any effect on decreasing blood glucose levels in diabetic rats.

4.7 Relationship of Active Compounds with Blood Glucose Levels Concentrated infusion Therapy of bitter melon can maintain blood glucose levels in normal conditions in rats with diabetes mellitus, presumably due to the influence of active compounds contained in concentrated infusion of bitter melon. It is possible that the active compound can prevent oxidation in pancreatic beta cells so that damage can be minimized. The active

compounds include alkaloids, saponins, terpenoids and tannins.

Saponins function as antihyperglycemic agents by preventing or inhibiting gastric emptying either by promoting glucagon secretion or by inhibiting its degradation. In addition, saponins function to stimulate the release of insulin from pancreas and it could be due to decreased degradation of glucagon-like peptides.

Tannins have hypoglycemic activity by increasing glycogenesis. In addition, tannins also function as astringents or chelators that can shrink the epithelial membrane of the small intestine thereby reducing the absorption of food juices and as a result inhibiting sugar intake and the rate of increase in blood sugar is not too high.

Alkaloids have the ability to efficiently stop free radical chain reactions. Radical compounds derived from these amine compounds undergo a very long termination stage. According to Tayyab[13] alkaloids 78 and tannins can inhibit the absorption of glucose in the intestine. So the presence of alkaloids has a beneficial effect on diabetes mellitus.

Terpenoid compounds are antioxidants that can capture free radicals below the cell surface so that cell damage can be minimized. The following is the reaction of terpenoids with free radicals in the body. Catching free radicals that occur due to the presence of active compounds will be able to minimize cell damage and even regenerate damaged cells. If the regeneration of cells is in the pancreas, it can increase insulin secretion. By increasing the permeability of cells to glucose, insulin will work to increase the transfer of glucose from the blood into cells and used as an energy producer. The liver and muscles will also convert glucose into glucogen which will then be stored for later use. This can cause blood glucose levels in the rat's body to decrease slowly.[20]

4. CONCLUSION

The test results showed that the ethanol extract of bitter melon fruit can reduce blood glucose levels of rats induced with alloxan. The results of observations for 14 days showed that the blood glucose levels of rats decreased from 550 mg/dL to 240 mg/dL. Blood glucose levels are still above normal because of the amount of bioactive compounds, especially flavonoids low antioxidant power.

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