The Effectiveness of Pare Extracts (*Momordica Charantia L*) in Lowering the Level of Blood Glucose on Wistar Rat (*Rattus Norvegicus*) with Hyperglycemia

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Abstract: Pare (Momordica charantia L.) is one of the traditional herbal plants used to cure several diseases, one of which is to decrease the blood glucose level. This research aims to examine the effectiveness of the ethanol extract in pare to decrease blood glucose level on male Wistar rat (Rattus norvegicus). This was an experimental research which used experimental animals with pretest-posttest control group design. The sample of this research were 40 male mice (Rattus norvegicus)which were divided randomly into 5 groups of treatment; 2 groups were control groups and 3 groups were given pare extract. All of the male mice were induced by 10% glucose solution to increase their blood glucose level. In order to examine the response, the level blood glucose was observed in 30 minutes, 65 minutes, 95 minutes, 125 minutes, and 155 minutes after the glucose induction. The result of blood glucose level was analyzed using the Anova test and Duncan test. The result of this study shows that pare extract with 200mg/kgBB group, compared to other dose level, had faster onset to decrease the blood glucose level of the male Wistar mice that was induced by 10% glucose solution. In conclusion, pare extract effectively decreases the blood glucose level in the glucose-induced male mice.

1 INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder. One of the pathological conditions found in DM is hyperglycemia. Hyperglycemia leads to various organs' disorders. Therefore, diabetes mellitus usually aims to overcome the hyperglycemic condition. Some herbal plants are thought to be able to cure the hyperglycemic condition.

DM treatment can be done in several ways; i.e. diet (nutritional therapy), insulin, and oral hypoglycemic drugs (OHO). The long-term usage of OHO not only requires big budget but also causes some side effects. The side effects of OHO depend on the type of the drug and the way it works. Therefore, people often use herbal remedies that have not been standardized yet, e.g. pare (Momordica charantia L).

Previous research has shown that pare extract can lower the blood glucose levels of alloxan-induced mice to destruct pancreatic beta cells (type 1 diabetes). However, there is no single study that examines the direct effects of pare on hyperglycemia. This study will examine the effectiveness of pare as anti-hyperglycemic on glucose-induced mice.

2 RESEARCH METHODOLOGY

This was a laboratory experimental research using Completely Randomized Design (RAL) with *pretest posttest control group design* method. The maintenance of sample animals and blood sampling were done at the Faculty of Veterinary Unsyiah; the Herbarium Test were conducted at Biology Laboratory; while the pare extract (Momordica charantia.L.) were processed in the Chemical Laboratory of Faculty of Math and Natural Sciences (FMIPA). The study was conducted in September to October 2017. The sample in this study were wistar mice (Rattus norvegicus). The Inclusion Criteria are:

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male; age 3-4 months; body weight 150-200 grams; healthy condition (active and not disabled); The Exclusion Criteria: mice have been used for other studies; and Drop Out Criteria: mice died during the study. The subject size for each group was measured using Lameshow formula and got 9 mice per group.

2.1 Research Instruments and Materials

For maintenance and treatment, there were: cage and pad, gastric sonde, animal scales, lancet, GlucoDR glucometer, GlucoDR glucose test strip. For the manufacture of pare extract, there were: knifes, blenders, filters, measuring cups, dropper, reaction tube, tube rack, stir bar, reaction tube shelf, and vacum rotary evaporator. For the glucose induction, there was: 1 cc syringe.

For maintenance and treatment, there were: male wistar mice (Rattus norvegicus), rat blood, water, and pellets. For the production of pare extracts, there were: pare (Momordica charantia.L) and ethanol 70%. For the induction of glucose, there was: 10% glucose.

2.2 Research Procedures

The Preparation and Maintenance of Sample*s*

Prior to the treatment, the mice were given an adaptation period for one week, then they were fasted for 12 hours. During the adaptation process, their weight and activities were continuously observed. The mice moved actively and none of their body weighed less than 200 grams during and after the adaptation process. Therefore, no samples were released. Mice were placed in cages that had previously been dried in the sun to be sterilized and were given the husk pads. The cage is cleaned and the husk pad is replaced at least every 3 days.

The mice were fed twice a day and given aquadest with ad libithum. The pellet used was T79-4P, with the composition as follows: fish meal, soybean meal, wheat fraction, rice bran, vitamin A, vitamin D3, vitamin E, vitamin K, vitamin B2, vitamin B6, vitamin B12, niacin, calcium D, panthethonate, choline chloride, minerals, and antioxidants. The nutrient content of pellets were protein (16-18%), fat (4%), fiber (8%), ash (12%), moisture content (12%) (Smith, 1988). The pellet was given using gastric sonde by inserting a dull pointed oral needle or cannula into the mouth, then slowly launched through the ceiling toward the back until the esophagus into the stomach. During the adaptation process, the weight and activities of the mice continuously observed.

Herbarium Test

The herbarium test was conducted at the Research Laboratory of Department of Biology at Faculty of Mathematics and Natural Sciences (FMIPA). The herbarium test was performed to confirm the taxonomy of pare (Momordica charantia L) and the following is the taxonomic result:

Kingdom : *Plantae*
Division : *Magnoliophyta*
Class : *Magnoliopsida*
Order : *Cucurbitales*
Family : *Cucurbitaceae*
Genus : *Momordica*
Species : *Momordica charantia L*

The Preparation and Extraction of Pare

The pare extract was made from 5000 grams of fresh pares which were crushed using mortal. After that, the thanol solvent was added, put into the container, and closed. It was left for two days, isolated from the sunlight and stirred, then filtered for maserate. The mixture was macerated using ethanol with the same procedure, the maceration was performed until a clear maserate is obtained. Then, all ethanol maserates were mixed and evaporated using a rotary vacuum evaporator at a temperature of ± 40 ° C until a thick ethanolic extract was obtained.

The Determination of Pare Extract Dose

Medical Administration Volume (VAO) is the volume of dosage administered to animals in ml units. In a previous study, Adewole et. Al. used doses at the intervals of 50 mg/kgBW to 400 mg/kgBW. Therefore, it was obtained that the volume of drug administration (VAO) for pare extract to be administered is 20 mg/ml, 40 mg/ml, and 80 mg/ml.

The Determination of Metformin Dose

Human Equivalent Dose (HED) is used to determine the dose conversion factors used from animals to humans, using the Body Surface Area (BSA). The ultimate effect of Metformin are to reduce the production of liver glucose (gluconeogenesis), and improve peripheral glucose uptake (Perkeni, 2015). Metformin has a dose of 1500-2500 mg/dL. The side effects that occur are dyspepsia, diarrhea and lactate

accidosis. Therefore, to reduce complications, the initial oral dose of metformin given is 1500 mg (Suherman, 2009).

The Determination of Glucose Volume Given to the Samples

The volume of glucose given was based on the VAO recommendation. A 10% glucose solution was given after the initial blood glucose measurement was performed. 2 ml of glucose solution was orally given once. Previous research by Sari proved that the 2 ml of 10% glucose solution can increase blood glucose levels. The peak glucose level will occur for 30 minutes to 1 hour and return to normal within 2 hours.

The Treatment of Sample Animals

The white-mouse treatment group was divided into 5 groups. The first group of mice were given 10% glucose and aquadest. The second group of mice were given 10% glucose, and comparative drugs (metformin). The third group of mice were given 10% glucose, and the pare extract at a dose of 100 mg/kgBW. The fourth group of mice were given 10% glucose and the extract at a dose of 200 mg/kgBW. The fifth group of mice were given 10% glucose and the extract at a dose of 400 mg/kgBB referring to the dose range in the previous study by Adewole[36]. The post test were conducted done 4 times, that is at minute 65, minute 95, minute 125 and minute 155.

Data Analysis

The data of how blood glucose works was being tested for its normality and homogeneity, to determine whehther the data is normally distributed or not. The normally-distributed data was then proceeded using the Anova test with Duncan's multiple-range test.

Research Ethics

The ethics of this study were based on the highest respect to the sample animals as one of God's creatures. Referring to the research ethics, the research guarantees was submitted to the Ethics Commission of Faculty of Medicine Unsyiah to obtain the etchical clearance prior to the research. The ethical clearance of this research is written in letter Number: 42/KE/FK/2017.

3 RESULT AND DISCUSSION

The data on the average blood glucose level was measured and the results can be seen in the table 1 and figures

Table 1 The Average Blood Glucose Level of the Sample Animals

Group	Minutes					
	0	30	65	95	125	155
P0	85,2[a]	172,5[a]	159,7[a]	158.5[a]	159,4[a]	143,7 [a]
P1	86,2[a]	196,8[a]	151[a]	117,6[b]	98,6[b]	83,6 [b]
P2	82,4[a]	174,3[a]	147,3[a]	135,5[a]	119,1[c]	110,5[c]
P3	89,2[a]	176,6[a]	126,4[b]	117,4[b]	105,8[b]	95,1[b]
P4	85,5[a]	182,5[a]	150[a]	115,3[b]	103,6[b]	85,5[b]

Legend:
P0 : negative control
P1 : Metformin
P2 : extract 100 mg/kgBB
P3 : extract 200 mg/kgBB)
P4 : extract 400 mg/kgBB)

Notes :
1. The value of blood glucose levels in units of mg/dl
2. Different Superscripts shows significant differences

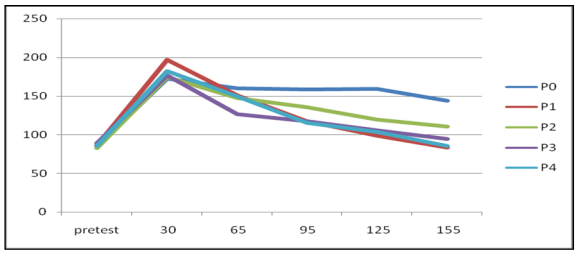

Figure 1 Graph of average decrease in blood glucose level of the sample animals

In this study, the blood glucose level of the fasting mice ranged between 82.4-86.2 mg/dl. This shows that all mice had normal fasting blood glucose levels. After 10% glucose was inducted, there was a 125% increase in mice's blood glucose level. This situation indicates that the induction of glucose had successfully made all mice experiencing the hyperglycemic conditions. Proven that all mice have hyperglycemic condition, the intervention was done by giving metformin, pare extract with dose 100 mg/kgBB, 200 mg/kgBB, and 400 mg/kgBB.

In the first 30 minutes after the intervention by pare extract at minute 65, a significant decrease was found in blood glucose levels in the group with 200mg/KgBB of pare extract. This was a significant reduction compared to not only the negative control group, but also with the standard treatment group, i.e. metformin. This suggests that among all the doses tested, the dose of 200mg/kg ($P = 0.016$) has the most rapid effect in lowering blood glucose levels, as compared with metformin.

After 60 minutes intervention of pare extract at minute 95, there was a significant difference of blood glucose level in metformin group, 200 mg/KgBB and 400mg/KgBB of pare extract compared to the negative control group. However, there was no significant difference among the three groups, which indicates that the three groups had the same capacity to lower blood glucose levels.

In the 125th minute, there was a significant difference between P2 (100mg/kgBW) and metformin ($p = 0.009$), but the potential was not as big as metformin P3 (200mg / kgBB) and P4 (400mg / kgBW) ($p = 0.000$).

In the 155th minute, blood glucose levels decreased in all treatment groups and metformin was the lowest, but the difference was not significant with P3 (200mg/kgBB) and P4 (400mg/kgBW), so it was considered the same.

The Duncan test results also showed the average decreased of blood glucose levels, the negative control group was the smallest and not statistically significant. This is because the aquadest given to this group have no effect on the decrease of blood glucose. Changes in blood glucose levels that occur in the negative control group tended to be result of the body's physiological response to the increased glucose intake. After the glucose was given, the glucose levels will increase initially but will return to normal within 2 hours (Price, 2006).

Momordica charantia L extract given to the sample animals decrease their blood glucose level, i.e. male mice (Rattus norvegicus) induced by glucose compared to the negative control. The decrease in blood glucose levels in sample animals was due to the active compounds that contribute to decreased blood glucose levels in pare extract, they are alkaloids, saponins, flavonoids and charantin.

Charantin works by activating AMP-activated protein kinase (AMPK) which increases the synthesis of glycogen and also increases the glucose uptake in liver and muscle cells (Bagchi, 2012).

Alkaloids lowers blood glucose by inhibiting the glucose absorption in the intestine, increasing glucose transport in blood, stimulating glycogen synthesis and inhibiting glucose synthesis by inhibiting glucose 6-phosphatase enzyme, 1.6-bisphosphatase fructose, and increasing glucose oxidation through 6-phosphate glucose. The 6-phosphatase glucose and 1,6-biphosphatase fructose are enzymes that play a role in gluconeogenesis. Inhibition of these two will decrease glucose formation from substrates other than carbohydrates (Tachibana, 2001).

Saponins works in the same way as the class of α-glucosidase enzyme inhibitors to prevent the absorption of glucose, so that the amount of monosaccharides absorbed by the intestine decreases. Saponin compounds that act as antihyperglycemia is triterpene saponins, which prevents the glucose transport to the small intestine which is a place for glucose absorption; therefore preventing the rise of glucose level in blood (Mohammady Elatar, 2012).

Another compound in Momordica charantia L that plays a role in lowering the blood glucose level is flavonoids. Flavonoids have the hypoglycemic effects with several mechanisms, i.e. by inhibiting the glucose absorption, increasing glucose tolerance, acting like insulin, increasing the glucose uptake by peripheral tissues and regulating enzymes that play a role in carbohydrate metabolism (Brachmachari, 2011).

From the above description, it can be seen that Momordica charantia L has active substances that potentially act as anti-hyperglycemia. However, in this study we did not isolate each of the active

ingredients.

Concerning the effective dose in lowering blood glucose levels, it was found that the doses of P3 (200mg/kgBB) and P4 (400mg/kgBW) can lower blood glucose levels inthe same way of certain anti-hyperglycemic, i.e. metformin. In addition, the 200mg/kgBB extract has the most rapid onset of action compared to other doses, including metformin. The 200mg/kgBB can lower blood glucose levels at minute 65, while metformin at 95 minutes. It was noted that at minute 65, the blood glucose level was significantly decreased in hyperglycemic mice. At the same time, however, there were no significant decrease in the blood glucose levels of the other groups.

4 CONCLUSION

Based on the research results and discussion above, it can be concluded that the ethanol extract of pare (Momordica charantia L) effectively decrease the blood glucose level of male Wistar mice (Rattus norvegicus) induced by glucose. The most effective dose for the pare ethanol extract (Momordica charantia L) to lower the blood glucose level of Wistar (Rattus norvegicus) male mice induced by glucose was 200 mg/kgBW.

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