

The Impact of the Ethyl Acetate Fraction of Bitter Melon Fruit (*Momordica charantia* L.) on Insulin Hormone Levels of Diabetic Rats (*Rattus norvegicus*) Using the Enzyme-Linked Immunosorbent Assay Method

Parawansah^{1,3}, Tien², Amiruddin Eso², Muhammad Junaid Azis², Nuralifah³

¹ Department Pharmacology, Faculty of Medicine, Halu Oleo University, Kendari, Indonesia

² Faculty of Medicine, Halu Oleo University, Kendari, Indonesia

³ Department Pharmacology, Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia

Corresponding author e-mail: parawansah@uho.ac.id

Background: The World Health Organization (WHO) has forecast a rise in the prevalence of diabetes mellitus from 2.8% in 2000 to 4.4% in 2030. Numerous investigations have been undertaken to identify alternate avenues for antidiabetic treatment. *Momordica charantia*, sometimes referred to as bitter melon, is recognized for its possession of mimic insulin components. The ethanol extracts derived from *Momordica charantia* have been found to exhibit favorable outcomes in the reduction of blood glucose levels and the stimulation of pancreatic beta cells for the secretion of insulin hormone. **Purpose:** The purpose of this research is to investigate the impact of bitter melon extract on insulin levels in rats induced with streptozotocin. **Method:** The present study employed various methodologies to investigate the research question. The present investigation comprised three distinct sets of tests, specifically streptozotocin-induced rats, streptozotocin-induced rats administered with an ethyl acetate fraction of *Momordica charantia* extract, and non-induced streptozotocin rats. The measurement of insulin levels is conducted using the enzyme-linked immunosorbent assay (ELISA) technique. If the p-value was more than 0.05, an unpaired T-test was employed for data analysis. **Result:** The research findings indicate that there is no statistically significant distinction in the insulin levels between streptozotocin-induced rats and streptozotocin-induced rats administered with the ethyl acetate fraction of the *Momordica charantia* extract group ($p = 0.081$). The insulin levels of streptozotocin-induced rats administered with an ethyl acetate fraction of *Momordica charantia* extract were found to be statistically similar to those of non-induced streptozotocin rats ($p = 0.505$). **Conclusion:** There is no discernible distinction in the insulin levels among streptozotocin-induced rats, streptozotocin-induced rats administered with an ethyl acetate fraction of *Momordica charantia* extract, and non-induced streptozotocin rats.

Keywords : diabetes mellitus; insulin hormone; *Momordica charantia*; streptozotocin

INTRODUCTION

Diabetes mellitus (DM) encompasses a range of physiological illnesses characterized by disturbances in glucose, lipid, and protein metabolism, among other etiological factors. Diabetes mellitus is distinguished by elevated levels of blood glucose that beyond the standard thresholds as a result of heightened gluconeogenesis and glycogenolysis (1).

Based on statistics provided by the World Health Organization (WHO), it is anticipated that the global prevalence of DM

would rise from 2.8% in 2000 to 4.4% in 2030, with a continued upward trajectory in the next years. Indonesia ranks fourth globally in terms of the prevalence of diabetes, following India, China, and the United States. In 2000, the country had approximately 8.4 million individuals diagnosed with diabetes. However, projections indicate that this figure is expected to rise significantly to approximately 21.3 million by the year 2030 (2).

Diabetes mellitus is one of the most concerning systemic diseases in Indonesia. This is because DM has a high incidence and mortality rate. Diabetes mellitus is a condition where the concentration of glucose in the blood is chronically higher than the normal value due to insulin deficiency or ineffective insulin function (3,4).

The presence of insulin target cells that fail to respond to insulin normally will cause insulin resistance resulting in an excessive increase in hepatic glucose production. Thus, at the onset of type 2 diabetes, cells were found to have impaired insulin secretion in the first phase, this indicates insulin secretion failed to compensate for insulin resistance. If this is allowed to continue, there will be progressive destruction of pancreatic cells, resulting in insulin deficiency. In general, people with type 2 diabetes are found both, namely, insulin deficiency and insulin resistance (5,6).

The use of plants as herbal medicines is starting to be found in today's society. According to research (7) who used noni fruit extract were found at a dose of 125 mg/kg to reduce blood glucose levels. In addition, one of the plants that has been reported to be efficacious in lowering blood glucose levels is bitter melon extract (8).

From the results of phytochemical tests, the ethanolic extract of bitter melon contains secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and triterpenoids (9). In research it was found that administration of ethanol extract of bitter melon at a dose of 400 mg/kg in streptozotocin-induced DM rats (STZ)

showed optimal results in reducing blood glucose levels in hyperglycemic rats (10,11).

In the study of (12) with the induction of alloxan 100 mg/kg and administration of bitter melon extract at a dose of 150 mg/kg and 300 mg/kg found that the charantin content of bitter melon stimulates pancreatic beta cells to secrete the hormone insulin. The difference in the metabolite content of bitter melon in each fraction became the basis of the interest of researchers to conduct research on insulin hormone levels in the serum of diabetic rats (*Rattus norvegicus*) using the ethyl acetate fraction of bitter melon (*Momordica charantia* L.). This study aims to determine effect of bitter melon extract on insulin levels in streptozotocin-induced rat (13).

METHODS

This research was carried at the Laboratory of the Faculty of Medicine, University of Halu Oleo, Laboratory of the Faculty of Pharmacy, University of Halu Oleo and Laboratory of the Teaching Hospital of Hasanuddin University. This study used rats (*Rattus norvegicus*) with a sample of 36 which were divided into 3 groups, namely a group of rats induced by STZ, a group of rats induced by STZ and then given the ethyl acetate fraction of bitter melon extract and a group of rats that were not induced by STZ.

To determine the difference in insulin hormone levels in the serum of rats (*Rattus norvegicus*) induced by STZ with a group of rats induced by STZ and then given the ethyl acetate fraction of bitter melon extract (*Momordica charantia* L.) and to determine the difference in serum insulin levels of rats

(*Rattus norvegicus*) the group the group of rats induced by STZ was then given the ethyl acetate fraction of bitter melon extract (*Momordica charantia* L.) with the group of rats that were not induced by STZ.

Bitter Melon Extraction

Bitter melon extraction process is done by maceration method (soaking). 628 grams of bitter melon simplicia was macerated using ethanol for 3 X 24 hours at room temperature while stirring and filtered. The maceration process is repeated several times while changing the same solvent. The liquid extraction was concentrated using a rotary vacuum evaporator at a temperature of 50°C for 6 hours and baked at a temperature of 50°C for 24 hours to obtain a thick ethanol extract of 28.4 grams.

Ethyl Acetate Fractionation of Bitter melon Fruit Extract

The residual fraction of bitter melon extract (*Momordica charantia* L.) was added to 75 ml of ethyl acetate. The mixture is put into a separatory funnel while being shaken slowly and exhaust gases from the separatory funnel. Let stand for a while until the separation process occurs which produces the ethyl acetate fraction and the extract residue fraction. Repeat 3 times and vaporize until thick in the vial. The final yield of the ethyl acetate fraction was 3.5 g.

Preparation of streptozotocin solution

The STZ solution was made by dissolving 0.401 grams of STZ into 10 ml of Na citrate buffer solution pH 6 then shaking until dissolved and then storing the STZ solution in a dark glass container.

Test Animal Treatment

The sample used in this study was male white rat wistar strain (*Rattus norvegicus*) with a body weight of 150-200 grams. After acclimatization for 7 days, the measurement of blood glucose levels of rats was carried out before giving STZ until the last day of sampling before being given FEA treatment of bitter melon. The administration of bitter melon fruit FEA in rats was done orally and STZ was administered subcutaneously. The administration of 10% dextrose was carried out after the administration of STZ.

The rat surgery was carried out after giving the treatment for taking blood samples in the rat heart organ. Centrifuge the sample for 15 minutes at 2000-3000 rpm at 2 - 8 °C within 30 minutes after collection.

ELISA test

Prepare all reagents, standard solutions and samples. This test was carried out at room temperature. In this test using 96 wells. 50µl standard in 5 wells, then in the Blank section (F1) filled with 50µl standard diluent. Add 40µl of sample then add 10µl of anti-INS antibody. Add 50µl of Streptavidin-HRP to standard wells and samples except for the Blank section (F1). Cover with sealer.

Incubate for 60 minutes at 37 °C. Then open the sealer and wash it using 350µl wash buffer and then leave it for 1 minute and discard it. Repeated for 5 times. Tap on the tissue after the wash buffer is removed.

Add 50µl of substrate solution A to each well then add 50µl of substrate solution B to each well. Incubate with the new sealer for 10 minutes in a dark place/incubator. Add 50µl stop solution to each well. Insert it

into a microplate reader at 450 nm in 10 minutes.

The use of experimental animals in this study used the 3R principle according to the research protocol, namely Replacement, Reduction, and Refinement (14). The data obtained were analyzed using the unpaired T statistical test to see differences in insulin hormone levels by comparing between group 1 and group 2 and comparing between group 2 and group 3 with an alpha value of 0.05 ($\alpha < 0.05$) and 95% Confidence Interval.

RESULT

Based on Figure 1, it can be seen that the serum insulin levels of rats in group 1 that were only induced by STZ 0.4832 mIU/L were lower than that of group 2 induced by STZ and given the ethyl acetate fraction of bitter melon extract 0.6511 mIU/L.

The serum insulin level of mice in group 2 was 0.6511 mIU/L lower than that in group 3 as control was 0.7141 mIU/L.

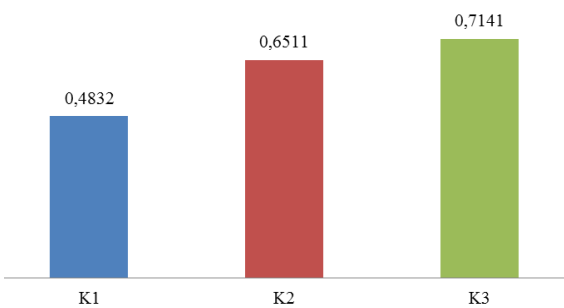


Figure 1. Graph of average insulin levels.

The mean value of HOMA-IR group 1: 0.290, group 2: 0.144 and group 3: 0.203 with statistical tests there is a significant difference in groups 1 and 2 with p value < 0.05 , and there is no significant difference

in groups 2 and 3 with p values > 0.05 . According to Swaminathan et al (2019), the normal value for HOMA-IR is < 2.6 , while the optimal cut-off value for HOMA-IR to identify DM is 1.37 and the best cut-off value for HOMA-IR for detecting insulin resistance is 3.8. From the results of the calculation of the mean HOMA-IR, each group has not been identified as DM and insulin resistance. Based on research regarding the relationship between IGF-1 and insulin resistance as assessed by the results of HOMA-IR 1 and and HOMA-IR 2, it shows an increase in HOMA-IR > 2.5 which indicates the occurrence of insulin resistance. This results in an increase in serum IGF-1 concentrations (15).

Table 1. Results of the analysis of differences in insulin levels between group 1 and group 2

Group	Sample (n)	Median (minimum-maximum)	p Value
1 (Diabetes)	9	0,4832 (0,2546-0,7097)	0,081
2 (Treatment)	9	0,6512 (0,4328-0,9650)	

Table 2. Results of the analysis of differences in insulin levels between group 2 and group 3

Group	Sample (n)	Median (minimum-maximum)	p Value
2 (Treatment)	9	0,6512 (0,4328-0,9650)	0,505
3 (Normal)	9	0,7140 (0,4118-1,0204)	

Meanwhile, based on table 1 and table 2, the results of the unpaired T statistical test showed that there was no statistically significant difference between the serum insulin levels of rats in group 1 and group 2, as well as between group 2 and group 3 there was no significant difference. This can be seen in table 1 and table 2, namely p value 0.05.

DISCUSSION

Diabetes mellitus is caused by disorders of carbohydrate, fat and protein metabolism associated with decreased insulin secretion or increased insulin resistance (16,17). The body's failure to control insulin regulation, storage and breakdown of glucose causes an increase in blood glucose levels (18). The results of this study found that there were significant differences in blood glucose levels between K1 and K2. However, there was no significant difference in insulin levels in each group.

The role of STZ in destroying pancreatic cells causes a decrease or reduction in insulin synthesis and administration of bitter melon FEA is expected to reduce blood glucose levels and increase insulin secretion. However, in this study, there was no significant difference in the decrease in insulin levels in K1 compared to K3 as control and the increase in insulin levels in K2 was lower than in the control group. The results of this study are not in line with the research of (19) that after administration of bitter melon extract in rats fed a 45% high fat diet at week 12 were able to control hyperglycemia and hyperinsulinemia by significantly reducing

blood glucose levels and increasing insulin in rats (20).

STZ reaction to pancreatic β -cells is accompanied by characteristic changes in blood insulin and glucose concentrations that cause hyperglycemia and decreased blood insulin levels. Streptozotocin affects glucose oxidation and decreases insulin biosynthesis and secretion. This results in a decrease in peripheral insulin receptor sensitivity so that it has an impact on increasing insulin resistance and increasing blood glucose levels (21,22).

Although statistically no significant difference was found in insulin levels, this study showed that bitter melon had an effect on STZ-induced rats. Administration of STZ at a dose of 30-40 mg/kg can induce hyperglycemia, blood glucose levels increase on days 1-7 will return to normal levels in 10 days and insulin secretion will return to normal in 3 months. In contrast, administration of STZ >40 mg/kg results in long-term, stable hyperglycemia without an insulin response to glucose within 3 months.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that there is no difference between insulin hormone levels in the group of rats induced by STZ, the group of rats induced by STZ given FEA extract of bitter melon and the group of rats that were not induced by STZ.

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