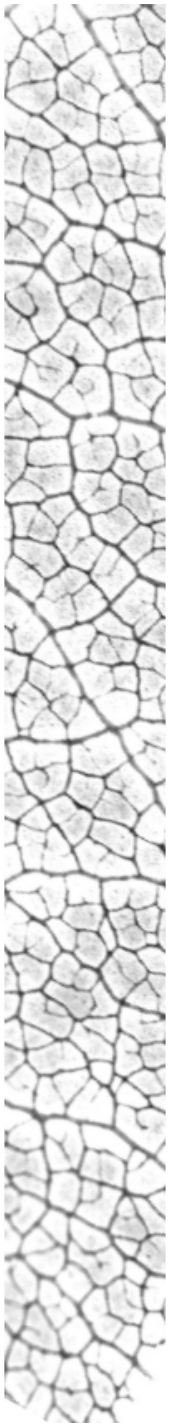

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The antidiabetic effect of bitter melon (*Momordica charantia* L.) extracts towards glucose concentration, langerhans islets, and leydig cells of hyperglycemic mice (*Rattus norvegicus*)

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Abstract

Purpose: This study was aimed to prove the fruit extract of bitter melon (Momordica charantia L.) towards blood sugar levels, of Langerhans islets cells, and Leydig cells of hyperglycemic white mice (Rattus norvegicus).

Methods: Twenty-five mice were divided randomly into 5 groups. They all were induced intraperitoneally by alloxan with dosage of 150 mg/kg in order to damage to the pancreas. From all treatments group, three groups (P1, P2, and P3) were treated with various doses of bitter melon extract with dosage 29, 50, and 59 mg/1 ml/day, respectively. As a comparative group, –negative control group (P0 +) were given with CMC-Na 0,5% 1ml/day, whereas the positive control group (K+) were given Glibenclamide® 0.126 mg/1 ml/day. Bitter melon extract was given for 21 days. In the first day of treatment, blood glucose level of mice was examined after 2 hours, 4 hours, 6 hours, 8 hours after treatments. The blood glucose examinations were subsequently continued at days 7th, 14th and 21st after treatment. After 21 days, the pancreas and testes of mice were taken for histopathological preparations made.

Results: Bitter melon (Momordica charantia L.) extract had antidiabetic effects that can lower blood glucose level, improved pancreatic beta cell damage, and increased the Leydig cells number in a dosage of 50 mg/1 ml/day on the 21st days after treatment.

Conclusion: the extract of bitter melon fruit (Momordica charantia L.) at a dosage of 50 mg/kg/1ml/day can lower blood glucose levels and increased the number of Langerhans islets and Leydig cell of hyperglycemia mice.

Keywords: hyperglycemia, melon extract, blood sugar levels, pancreas cells, leydig cells

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by symptoms of hyperglycemia as a result of impaired secretion of insulin. Deficiency of insulin hormone functions to utilize glucose as a source of energy and synthesize fat caused pancreas is no longer able to secrete insulin while the relative deficiency of insulin caused by insufficient insulin production by the body needs (Larry et al. 2009). DM is categorized as a dangerous disease since it can not be cured, therefore, the drugs consumption is the main choice. DM drugs mostly contain the synthesis-chemically substance from sulfonylurea and biguanide class. Therefore, the long period of anti-diabetic drugs can cause serious unwanted side effects. One of the alternatives to reduce the cost of treatment of diabetic patients is consume the bitter melon fruit to decrease blood glucose levels because it contains charantin, polypeptide-P insulin, and lectins, which are useful substances to decrease blood glucose level. Saponins, flavonoids, polyphenols, and vitamins C from the bitter melon acts as antioxidants that prevent the free radicals that can interfere with the

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Table 1. The average (mean) blood glucose levels of mice after bitter melon extract treatment

Treatment	Blood glucose levels	Blood glucose level after given aquadest and bitter melon extract (mg/dl)						
	Before treatment (mg/dl)	0 hours	2nd hours	4th hours	6th hours	8th hours	7th days	14th days
K+	72.4^{a} ± 7.43	431.3^{a} ± 39.44	383.0^{a} ± 32.90	362.9^{a} ± 39.09	251.9^{a} ± 24.58	184.1^{a} ± 19.12	169.5^{a} ± 8.98	163.5^{a} ± 10.29
P0	69.7^{a} ± 10.51	468.7^{b} ± 49.18	452.2^{b} ± 56.21	416.2^{b} ± 53.21	413.3^{b} ± 59.49	393.8^{b} ± 77.88	385.9^{b} ± 60.27	316.1^{b} ± 13.32
P1	75.5^{a} ± 12.44	461.2^{bc} ± 44.60	461.6^{bc} ± 41.31	420.3^{bc} ± 27.80	407.1^{bc} ± 16.35	333.6^{c} ± 44.12	296.6^{c} ± 48.71	270.5^{c} ± 24.31
P2	79.6^{a} ± 9.82	437.1^{c} ± 38.95	411.2^{c} ± 46.39	383.2^{c} ± 20.03	360.8^{c} ± 27.36	313.8^{c} ± 30.58	273.4^{c} ± 35.19	253.4^{c} ± 12.62
P3	74.2^{a} ± 10.70	413.3^{a} ± 41.61	388.2^{a} ± 25.41	360.1^{a} ± 35.68	258.9^{a} ± 21.76	202.8^{a} ± 15.17	192.9^{a} ± 12.83	181.2^{a} ± 9.77

Different superscripts in the same column indicate significant differences at the level of α = 0.05

presence of Leydig cells due to DM disease (Meles et al. 2017, Subahar et al. 2004).

Pancreatic β-cells damage in mice are induced by alloxan can cause hyperglycemia which is an early symptom of DM (Rho et al. 2000). This hyperglycemic condition will increase the ROS which can trigger the oxidative stress in the body. Those can lead the changes of body metabolism, included sexual function because it can disturb the secretion of serum total testosterone as a result of Leydig cell dysfunction (Wurlina et al. 2017).

DM patients often experience the decreased levels of Follicle Stimulating Hormone (FSH), Interstitial Cell Stimulating Hormone (ICSH), Insulin-like Growth Factor 1 (IGF-1), and Stem Cell Factor (SCF) because of impaired insulin receptor sensitivity (Ballester et al. 2004). Increased blood glucose will affect the decline ICSH which decreased Leydig cells response to secrete testosterone due to impaired insulin receptor sensitivity (Pitteloud et al. 2005). Disturbance sensitivity of insulin receptors also decreased the SCF. Therefore, the decreasing testicular Leydig cell replication that can cause testicular interstitial tissue loses its density and a decrease in the number of Leydig cells per volume of the interstitial space (Ballester et al. 2004). Based on the above background problems, the research about the effect of bitter melon extract (*Momordica charantia* L.) towards blood sugar levels, Langerhans cells islets, and Leydig cells in mice (*Rattus norvegicus*) hyperglycemia was conducted.

EXPERIMENTAL

Twenty-five male rate (*Rattus norvegicus*) weighed 200 gram aged 2-3 months old mice were used as experimental animals. Mice were divided randomly into 5 treatment groups: negative control (K), the treatment 0 (P0), and treatment 1, 2, and 3 (P1), (P2), (P3). The treatments were repeated 5 times in each group. Mice adapted to the conditions and the feed during the 7 days prior to treatment. Each group was placed in a separate enclosure and each group was given different treatments. During the study, mice were fed differently according to the treatments.

1. K+ (hyperglycemia rat) fed with Glibenclamide® 0.126 mg /1 ml/rat/day
2. P0 (hyperglycemia rat) fed with CMC-Na 0,5% 1 ml/rat/day
3. P1 (hyperglycemia rat) fed withbitter melon extract 29 mg /1 ml/rat/day
4. P2 (hyperglycemia rat) fed with bitter melon extract 50 mg /1 ml/rat/day
5. P3 (hyperglycemia rat) fed with bitter melon extract 59 mg /1 ml/rat/day

Bitter melon extract and Glibenclamide were given orally once a day using the gastric tube for 21 days. In the first day of treatment, blood glucose level of mice was examined after 2 hours, 4 hours, 6 hours, 8 hours after treatments. The blood glucose examinations were subsequently continued at 7th, 14th and 21st days after treatment. After 21 days, the pancreas and testes of mice were taken for histopathological preparation to determine the number of Langerhans and Leydig cell quantity.

RESULTS

The Effect of Bitter Melon Extract (*Momordica Charantia* L.) towards Blood Glucose Levels

The blood glucose level increase at zero point treatment of the positive control group (K+), P0, P1, P2, and P3 was > 200 mg/dl. Otherwise, the average blood glucose level of before-treatment mice was <100 mg/dl. The response shown by mice towards alloxan induction was influenced by specific species. Hyperglycemia indication in diabetic alloxan-induced mice occurred due to the alloxan toxic effects that damaged the insulin receptor which followed by pancreatic β-cells destruction.

Measurement of blood glucose levels on the fifth day after the mice induced by alloxan (0 h), shows that the group K+ has significant differences ($p<0.05$) compared with P0, P1, P2 and P3). However, between K+ with P2 and between P0 with P1 has no significant difference ($p>0.05$) (**Table 1**). In spite of differences of significances, it shows that alloxan-induced mice had suffered hyperglycemia or diabetes with glucose levels> 200 mg / dl on the fifth day after induction.

Measurement of blood glucose levels of mice on the 2 hours, 4 hours, and 6 hours after treatment with bitter melon extract bitter melon extract obtained that the group K+ has significant differences compared with the group (P0, P1, P2 and P3) ($p<0.05$). However, there are no significant differences between (K+)- P3 and P0-P1 ($p>0.05$).

The blood glucose measurement on the 8 hours after treatment obtained that the group K+ has significant differences compared to the group (P0, P1, P2, and P3) ($p<0.05$). However, between (K+)-P3 and P1-P2 have no significant differences ($p>0.05$). At 8 hours after bitter

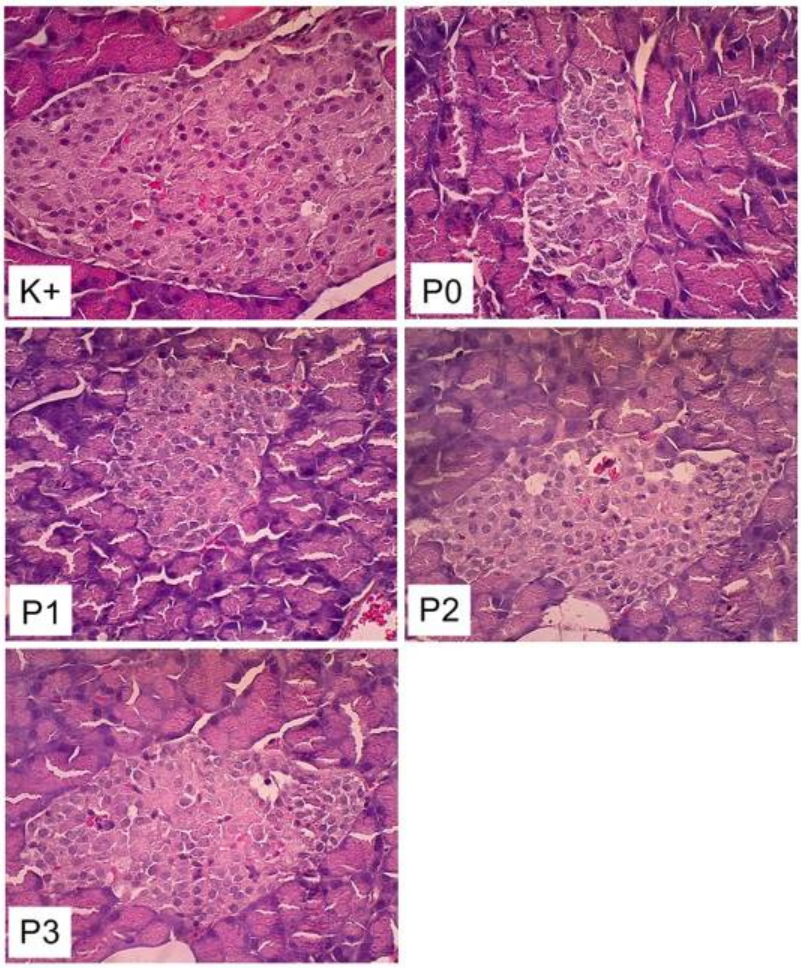

Fig. 1. Langerhans islet cells of hyperglycemia mice stained with Haematoxylin-Eosin staining (M=400x)

melon extract treatment of bitter melon extract, there are no significant differences between K+ and P3 ($p>0.05$). Even though P3 had blood glucose levels higher than 202.8 mg/dl, but the result was no statistically different with K+. This indicated that at 8 hours after treatment of bitter melon extract (59 mg/kg BW) had normalized the glucose levels of mice.

The measurement of blood glucose levels on 7th, 14th, and 21st days after the treatment obtained that there is significant differences between K+ and treatment group (P0, P1, P2 and P3) ($p<0.05$) (**Table 1**). However, there are no significant differences between K-P3 and P1-P2 ($p>0.05$). On the 7th days, there was no significant differences between group (K+) and P3, which the blood glucose levels was decreased into <200 mg/dl. This is indicated that on the 7th days after bitter melon extract treatment (59 mg/kg) could decrease the blood glucose of mice into the normal level (<200 mg / dl).

The Bitter Melon (*Momordica Charantia* L.) Treatment Effect Blood Glucose Levels and the Number of Langerhans Cells and Leydig Cells of Mice (*Rattus Norvegicus*)

Group K+ was treated with standardized diabetes drugs, Glibenclamide, that works by stimulating the release of insulin secretion due to stimulation of glucose (Wurlina et al. 2017). Glibenclamide is expected to improve beta cell insulin secretion.

Table 2. The average of Langerhans cells, blood glucose levels, and Leydig cells

Treatments	Total Langerhans cell	Blood sugar levels	Total Leydig cell
K+	240,89^{a} ± 3,96	148,20^{a} ± 5,80	14,6^{a} ± 2,40
P0	137,20^{b} ± 5,26	261,20^{b} ± 10,03	10,60^{ab} ± 2,70
P1	166,60^{c} ± 11,73	215,40^{c} ± 9,39	18,80^{b} ± 1,30
P2	203,20^{d} ± 19,44	203,2^{c} ± 15,53	25,2^{c} ± 4,54
P3	243,80^{a} ± 13,49	152,80^{a} ± 4,76	26,40^{c} ± 5,22

Different superscripts in the same column indicate significant differences at the level of $\alpha = 0.05$

Group P0 had a lower average number of Langerhans cells than other. This was because of the mice in the group P0 were induced with alloxan and CMC-Na without therapy at first, therefore it caused damage of Langerhans cells and insulin secretion was decreased. According to Szkudelski, alloxan pancreatic β-cell damage through the formation of reactive oxygen species that is preceded by a reduction of alloxan.

The average number of the Langerhans cells of K+, P2, and P3 groups were higher than P0 group (**Table 2**,

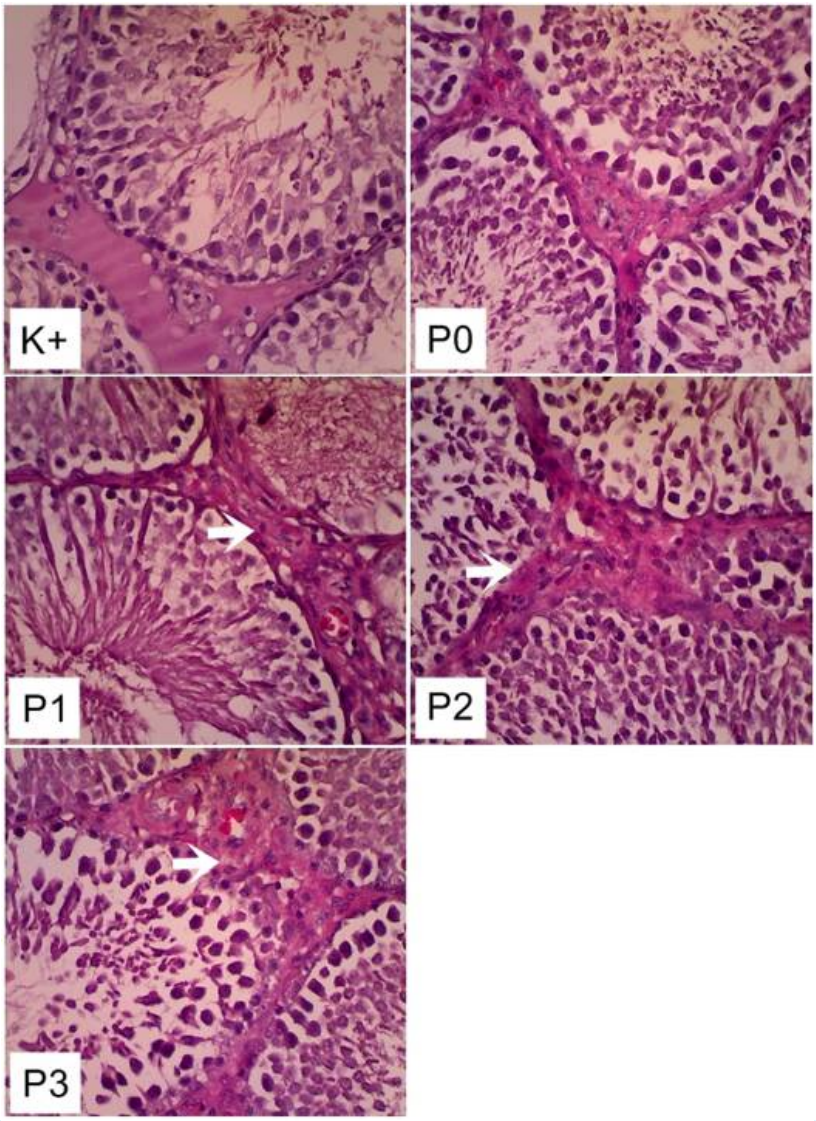

Fig. 2. Leydig cells of hyperglycemia mice stained with Haematoxylin-Eosin staining (M=400x)

Fig. 1). It indicates that there was an improvement the Langerhans cells after treatment.

Bitter melon extract treatment towards P3 group for 21 days was able to lower blood glucose levels back to normal (<200 mg/dl). This is due to the substances contained in the bitter melon extract is a powerful antioxidant that can lead insulin to produce for decrease blood glucose levels until lower than 200 mg/dl. The blood glucose decrease may be related to the compound in the bitter melon extract, which has properties effect similar to insulin. In addition, the substance is able to stimulate the process of glycogenesis, the conversion of excess glucose into fat, and inhibits gluconeogenesis.

The analysis of the average number of Leydig cells quantitatively proves that bitter melon extract was able to increase the number of Leydig cells. The highest result was obtained by P3 group which had no significant difference compared to P2 group. Increased dosage of bitter melon extract was able to improve the Leydig cells number of mice. P1 group Leydig cells were significantly higher than K+ group. This suggests that the ability to increase the Leydig cells number by bitter melon extract is more effective in than Glibenclamide®.

The Leydig cells calculation towards P0 group that given with CMC-Na 0.5% 1 ml, obtained the lowest result compared with the average amount of Leydig cell from other groups (K+, P1, P2, and P3) (**Fig. 2**). This result made sense since P0 group were only given with CMC-Na 0.5%, without bitter melon extract. CMC-Na 0.5% acted as a solvent and not as a drug of diabetes mellitus that could not overcome the mice testicular damage. The decrease of Leydig cells average number in the P0 group was caused by pancreatic β-cells damage. The cells were damaged due to alloxan induction that decreased the insulin secretion and increased blood glucose levels.

The average number of Leydig cells of K+ group, which were given with Glibenclamide® 0.126 mg, obtained that the cells were higher than K- group. Otherwise, the Leydig cell average number of K+ group was lower than other groups (P1, P2, and P3). The results indicated that the ability Glibenclamide® to increase the number of Leydig cells is lower than bitter melon extract. Where actually the case, Glibenclamide® is the oral antidiabetic drugs belongs sulfonylurea class that works to improve insulin secretion of pancreatic β-cells.

The Leydig cells average number of P1 group were higher than K+ group. But, the Leydig cell average number of P1 group was lower than other treatment groups (P2 and P3). Bitter melon extract treatment at a dosage 29 mg/kg have not been sufficiently effective for the purpose of increasing the Leydig cells number of hyperglycemic mice. In addition, the Leydig cell average number P2 group were higher than K+ and P1 ($p<0.05$).

The result of Leydig cells improvement in P2 group was not significantly different with P3 group. However, the therapeutic dosage of bitter melon extract on the P2 group was considered as the best of bitter melon extract treatment in this study. It was proven could increase the Leydig cells number of mice significantly compared to other treatment.

Based on the research, it can be conclude that the extract of bitter melon fruit (*Momordica charantia* L.) at a dosage of 50 mg/kg/1ml/day can lower blood glucose levels and increased the number of Langerhans islets and Leydig cell of hyperglycemia mice.

DISCUSSION

Hyperglycemia indication in diabetic alloxan-induced mice occurred due to the alloxan toxic effects that damaged the insulin receptor which followed by pancreatic β-cells destruction. As a damage result of insulin receptor and pancreatic β-cell, the insulin can not be produced normally. Therefore, the blood glucose can not be retrieved and convert into energy, so that glucose levels in the blood is increased. Pancreatic β-cell damage is alloxan-induced due to the addition of hydroxyl radical superoxide by alloxan-induced (Meleset al. 2009, Szkudelski 2008).

According to Watskin et al., alloxan is toxic to pancreatic β-cells. The natural toxic of alloxan triggers free radicals formation that can damage cell membranes by increased lipid peroxidation that causes disruption of essential ion transport from and within the cell, which causing the cell death. The effects of free radicals by alloxan induced can cause oxidative stress in pancreatic β-cells and lead disturbance to the oxidation phosphorylation process. This mechanism increasing the free radicals production. Furthermore, antioxidants substance in the body is no longer able to change the reactive oxygen (O) into a neutral compound O_2 (Watkins et al. 1964).

Alloxan will react with two SH-groups of the glucokinase enzyme dimer and inactivate the insulin enzyme. Therefore, it disturbs the insulin secretion and damage the β-cell, which is lead to diabetic risk (Szkudelski 2008).

Ayoub et al. (2013) mentioned that the pancreas treated with bitter melon extract had a significant increase in the size, number, and regeneration of Langerhans islet cells. The bitter melon extract can suppress the blood glucose levels, prevent the intestine from absorbing the glucose, and improves pancreatic β-cells to produce insulin.

Bitter melon extract as an antioxidant source can protect the cell components and cell membranes of pancreatic β-cell from free radicals oxidation formed by toxic compounds of alloxan. The bitter melon extract also blocks the influence alloxan induction. Therefore, pancreatic β-cells are able to control the fat by delivering hydrogen peroxide into a reaction that can change the outcome peroxidase lipid peroxyl become less reactive radical. Furthermore, it is not able to disturb fatty acids chain and protect pancreatic β-cells from damage (Winarsi 2007).

Saponins, flavonoids, polyphenols, and vitamins C that contained in bitter melon fruit are acts as antioxidant that works to change ROS into H_2O in order to prevent excessive ROS production that can reduce oxidative stress. Bitter melon fruit antioxidant compounds are aimed to counteract the free radicals caused by diabetes mellitus (Subahar et al. 2004). Antioxidants are compounds of the electron donor, but biologically, the antioxidant is a compound that can reduce the negative impact of oxidants including enzymes and metal binding proteins (Halliwell and Gutteridge 2007).

Basically, the living creatures have a very specific defense mechanisms such as antioxidants to neutralize the effects of oxidative stress. Antioxidants are compounds that are soluble in water (water soluble) or soluble in fat (lipid soluble). Antioxidants are essential and non-essential (Yeum et al. 2009). The vitamin C content in bitter melon fruit serves to maintain the survival and improvement of mice (*Rattus norvegicus*) Leydig cells as a result of free radicals of diabetes mellitus. Mayes suggested that flavonoids can suppress the Reactive Oxygen Species (ROS) by transferring electrons to ROS. Furthermore, the ROS are converted into non-radical compounds that are not harmful to the cell membrane (Mayes 2002).

Dellman and Carithers mentioned that the function of insulin hormone is to stimulate the formation and storage of glycogen in the liver and muscles. Insulin is responsible for the conversion of glucose into fat that will be stored in the body tissue. Obviously, the insulin inadequacy can increase blood glucose levels (Dellman and Carithers 1996).

CONCLUSION

The extract of bitter melon fruit (*Momordica charantia* L.) at a dosage of 50 mg/kg/1ml/day can lower blood glucose levels and increased the number of Langerhans islets and Leydig cell of hyperglycemia mice.

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