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Effect of Trigona honey on Blood glucose levels Diabetes Mellitus in Balb/c Mice

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ABSTRACT

Introduction Diabetes Mellitus (DM) is a multicultural metabolic disease characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both. Abnormalities in insulin secretion or action cause problems in the metabolism of carbohydrates, fats, and proteins. DM Type II is characterized by the occurrence of insulin resistance in body tissues. Trigona honey is rich in phenolic compounds because it is food collected by bees from plants. The total phenolic content in honey is highly correlated with antioxidant activity. In this study, we aimed to see the different effects of trigona honey (*Tetragonula* sp) on plasma insulin and blood glucose levels in mice with diabetes mellitus. **Methods**. Balb/c mice (n= 28) were randomly assigned into the control group (n=7), negative control, positive control, and intervention group which received a daily intake of trigona honey (n= 14). **Results.** showed that administration of trigona honey can increase administration of the metformin. This is caused because honey contains high antioxidants and contains bioactive compounds such as alkaloids, flavonoids, triterpenoids, and phenol compounds. **Conclusion**. Daily consumption of trigona honey has a remarkable potential to decrease the blood glucose, thus it can contribute to the prevention of the diabetes mellitus development.

Keywords: Diabetes Mellitus, Trigona Honey (*Tetragonula biroi*), Blood Glucose Levels, Bioactive Content.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common chronic diseases in most countries and tends to increase in number and significance due to changes in lifestyles which have led to the reduction of physical activities [1]. Diabetes mellitus is a metabolic disease characterized by hyperglycemia which has consequences on the occurrence of multisystem abnormalities in insulin secretion, insulin action, or both. Abnormalities in insulin secretion or action cause abnormalities in the metabolism of carbohydrates, fats, and proteins. Diabetes mellitus type II is characterized by the occurrence of insulin resistance in body tissues [2]. Insulin resistance plays an important role in the pathophysiology of diabetes mellitus. It is characterized by the inability of target organs such as the liver, muscles, and fat cells to respond to the effects of insulin, so they fail to take up glucose from the blood causing hyperglycemia [3].

The prevalence of diabetes in the world is increasing rapidly. In 2013, the world population suffered from DM was as many as 221 million, and it is estimated that by 2030 the number of sufferers will surge to 439 million [4]. This percentage increases to 69% of people in developing countries and 20% in developed countries [1]. Indonesia is at the 4th rank of countries with the highest number of diabetics which is after India, China, and the United States. The number of sufferers was 8.4 million in 2000 and it is expected to increase to 21.3 million in 2030 [5]. Some alternative approaches to diabetes therapy include herbal preparations, food components or supplements, and other natural product therapies such as honey. Honey is a natural substance produced by bees from nectar. It has been widely used as a traditional natural therapeutic agent to improve the immune system and prevent various diseases. Honey can decrease GDP by 4.2% and CRP by 3.2% and can increase HDL cholesterol by 3.3% [6].

Trigona honey obtained from South Sulawesi was identified to have 27 volatile compounds divided into several groups including hydrocarbons (46.06%), imines (21.83%), ketones (19.22%), acids (7.06%), amines (2.37%), phenols (1.53%), alcohol (0.83%), oxime (0.72%), and aldehydes (0.38%), and there are some compounds that have potential as antihyperglycemic agents. Trigona honey is rich in phenolic compounds because it is collected from plants. The total phenolic content in honey is highly correlated with antioxidant activity [7]. Research about diabetes mellitus (DM) on animals can be carried out using diabetogenic chemical compounds, surgery by pancreatomy or genetic manipulation [8]. Experimental research on DM using animal models is based on the pathogenesis of the disease in humans that is chronic or persists. There have been many studies using animal models that are pathologically made to suffer from DM. Pathological conditions in animal models are made for prevention, identification of the pathogenesis

of the disease, determination of the diagnosis, and therapy used in the management of DM disease. In animal models, DM is often caused intervention of *streptozotocin* which can cause damage to *pancreatic beta cells Langerhans* [9].

The use of diabetogenic chemical compounds is an easy way to induce experimental animals into DM, chemical compounds causing necrosis and degeneration of β cells pancreas. These chemical compounds have a diabetogenic effect if treated parenterally (intravenously, ip and subcutaneously) [10]. The administration of STZ has a diabetogenic effect that is initiated by *reactive oxygen species* (ROS) through direct toxic effects on GLUT-2 [8, 10] *Streptozotocin (*STZ) is a *nitrosourea derivate* isolated from *Streptomyces achromogenes*, has anti-neoplasm and broad-spectrum antibiotic activity. *Streptozotocin* can directly damage the critical *pancreatic β cells*. *Streptozotocin* (STZ) or *2-deoxy-2- [3- (methyl-3-nitrosoureido) -Dgluko pyranose]* obtained from *Streptomyces achromogenes* which can be used to induce DM both type 1 and type 2 in tested animals [10, 11]

The *Streptozotocins* compounds have a long half-life and are not easily oxidized. *Streptozotocin* works by forming highly reactive free radicals that can damage cell membranes, proteins, and *deoxyribonucleic acid* (DNA), thus causing impaired insulin production by *pancreatic beta langerhans cells*, as *streptozotocin* enters *pancreatic β cells* through *glucose transporter* (GLUT-2) and will cause *alkylation of deoxyribonucleic acid* (DNA) resulting in DNA damage. DNA damage will later activate *poly adenosine diphosphate (ADP) -ribosylation*. This process will result in the *nicotinamide adenine dinucleotide* elimination of cellular (NAD+), further there will be a reduction in *adenosine triphosphate* (ATP) and will ultimately inhibit insulin secretion and synthesis [12].

MATERIALS AND METHODS

Materials and Methods

This research was conducted after obtaining approval from the ethics committee. The material used was Tetragonula *Biroi* Trigona honey from Bone, South Sulawesi. In phytochemical screening, the following ingredients are needed, namely hydrochloric acid p.a. (Merck), sulfuric acid p.a. (Merck), acetone P p.a. (Merck), boric acid P, oxalic acid P, ether P, anhydrous acetic acid p.a. (Merck), chloroform (Brataco), Dragendroff reagents, Mayer reagents, 10% iron (III) chloride solution. The tools used are drip pipettes, stirring rods, measuring pipettes, horn spoons, porcelain cups, measuring cups, erlenmeyers, beaker cups, test tubes, electric scales (ADAM AFP-360L), ovens (BINDER).

Sample Collection

Honey sampling was repeated three times. The livestock was obtained from the beekeeping in *Bontocane* Village, *Kahu* Subdistrict, *Bone* Regency of *South Sulawesi*. Trigona honey is taken directly from beehives in the village of Kahu Bontocane sub-district, Bone Regency, South Sulawesi. The honey was subjected to an extraction using ethanol as the solvent in accordance with a method reported. The trigona honey was used at doses of 0.2 ml.kg body weight and 0.4 ml/kg body weight.

Animal preparation

The research has been approved by the Research Committee on the Ethical use of Human and Animal, medicine faculty University of Hasanuddin, Makassar, Indonesia with registration number UH19020081, 11 april 2020. The treatment of test animals BALB / c mice (aged 10-14 weeks, weighing 30-40 grams; n=15) were maintenance in the molecular biology and immunology laboratory, Microbiology department faculty of medicine, Hasanuddin University (Makassar, Indonesia). The mice were acclimatized for 2 weeks, then divided into four groups (n=5). four of the groups were introperitoneally streptozotocin. Negatif control (aquadest), positif control (Metformin) and two treatment groups (honey a dose 0.2 ml/Kg.body weight and honey a dose 0.4 ml/Kg body weight).[18.19] Then the initial blood glucose level was measured and insulin levels was measured after being fasted for 10 hours. The model was Diabetes mellitus made by inducing mice with a streptozotocin dose of 40 mg / kg body weight. Blood glucose is measured after 72 hours or 3 days after streptozotocin induction [13, 14].

Measurement of Blood glucose levels

Measurements of blood glucose levels of tested animals were on day 1 (before streptozotocin induction), day 6, and day 22. Before measuring them, the mice had fasted for 8 hours. An enzymatic measurement method using *Glucotest Autocheck* was performed by means of a blood sample that had been taken in the lateral vein of the tail, dripped on a strip that had been attached to the instrument, and awaited for 3-5 seconds, then blood glucose levels appeared on the monitor screen. In addition to fasting blood glucose levels, measurements of the bodyweight of mice were also carried out using electronic scales.

Statistical analysis

All data were presented as means±SE. They were analyzed using ANOVA signed-rank test. All statistical analyses were carried out using SPSS 16.0 software. The *p* value less than 0.05 was regarded as statistically significant.

RESULTS

The results of test on the effect of trigona honey on blood glucose levels in DM conditions with negative aquadest control, Metformin 0.13 ml / Kg.Bw positive control, Trigona honey 0.2 ml / Kg.Bw treatment 1, Trigona honey 0.4 ml / Kg.Bw in the second treatment(at the 21st days)show that honey trigona affects blood glucose levels in mice with diabetes mellitus. The average and the results of statistical analysis of blood glucose levels in mice Diabetes mellitus condition after observation for 21 days can be seen in Figure.1

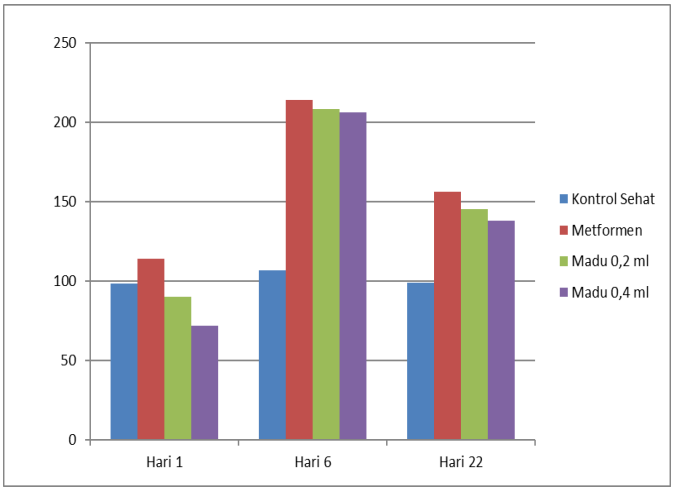

Figure 1: Changes in the average value of blood glucose levels

Table 1: Effects of Trigona Honey on blood glucose levels

Groups	Blood Glucose		
	1	6 (Before)	22 (After)
Negative control (Group 1)	98.6 mg/dl	106,8 mg/dl	99.2 mg/dl
Positive Control Metformin	114 mg/dl	214,2 mg/dl	156,2 mg/dl
Trigona honey (dose 0,2 ml)	84.8 mg/dl	208,2 mg/dl	145,2 mg/dl
Trigona honey (dose 0,4 ml)	72 mg/dl	204,2 mg/dl	138.2 mg/dl

Based on table 1 above, it can be explained that the glucose level at the initial examination (first day) obtained an average value = 92.35 while in the second examination (sixth day), it doubled to 183.35. In the third examination (22nd day), the average value reduced to 134.7

DISCUSSION

The group which received Honey Trigona (*Tetragonula biroi*) before the intervention got the mean value of 92.35 at the first examination. In the second examination (sixth day), it doubled to 183.35, but in the third examination (22nd day) it reduced to 134.7. This shows a linear relationship between the decrease in blood glucose levels with the administration of trigona honey. The effect of the blood glucose level reduction was caused by the presence of bioactive compounds contained in trigona honey such as alkaloids, flavonoids, triterpenoids, and polyphenols. Alkaloids work by stimulating the hypothalamus to increase the secretion of Growth Hormone Releasing Hormone (GHRH), so that the secretion of Growth Hormone (GH) in the pituitary increases. High GH levels will stimulate the liver to secrete insulin-like growth factor-1 (IGF-1). IGF-1 has an effect in inducing hypoglycemia and decreasing gluconeogenesis so that blood glucose levels and the need for insulin decreases. IGF-1 through the negative feedback system will normalize GH levels again (1).

Flavonoids and various phenolic compounds in honey are the most pharmacologically important compounds. These compounds have been proven to be able to ward off free radicals, protect lipids and other compounds (vitamin C) that are easily oxidized. These antioxidants can protect lipoproteins serum from oxidation. The antioxidant properties are produced by anti-radical activity (alkoxy radical and suppress its expansion, superoxide) and inhibit the effect of copper ion (cuprous ion) which is the initiator of oxidation in low density lipoproteins [5]. Flavonoids derived from 1,3-diphenylpropan are a group of natural products that are widespread in honey. The color is yellow and mostly found in honey, leaves, flowers, and fruit. Most of them are quercetin. They are compounds that have the potential as antidiabetic that are able to protect the body against nDNA and mtDNA damage caused by the reactive oxygen (ROS) [15].

Polyphenols contain antioxidant compounds that can reduce oxidative stress by preventing changes on the chain from superoxide (O2) to hydrogen peroxide (H2, O2), and the reaction of habe wells and fentors will form hydroxid radicals (OH). Polyphenols donate hydrogen atoms from the aromatic hydroxyl (-OH) group to bind to free radicals and remove them from the body through the excretion system [16]

In addition to phenolic compounds and flavonoids, honey also contains vitamin C which is an antioxidant compound. It is the main antioxidant in plasma against free radical attack (ROS) and also plays a role in cells. As a free radical scavenger, vitamin C can react directly with superoxide and hydroxyl anions, and various fat hydroperoxides. Meanwhile, as an antioxidant chain reaction breaker, it is possible to regenerate a reduced form of vitamin E.

Antioxidants in honey can be enzymatic and non-enzymatic. The enzymatic antioxidants are catalase, glucose oxidase, and peroxidase, while non-enzymatic antioxidants are ascorbic acid, flavonoids, acids and proteins. Trigona honey has antioxidants that can reduce blood glucose levels by preventing excessive oxidation (so that damage on pancreatic β cells can be prevented) and maintaining insulin content in it [17].

Based on the results of the current study (Figure 2) it can be seen that there were significant differences in plasma insulin levels before treatment (P1) and 3 days after induction of streptozotocin (P). This shows that the administration of streptozotocin before DM affects to pancreatic β is reduced which is the beginning of diabetes mellitus. This shows that the induction of streptozotocin can directly damage the critical *pancreatic β cells*. *Streptozotocin* (STZ) or *2-deoxy-2- [3-(methyl-3-nitrosoureido) -Dgluko piranose]* obtained from *Streptomyces achromogenes* can be used to induce DMs both type 1 and type 2 DM in test animals.

Streptozotocin works by forming highly reactive free radicals that can cause damage to cell membranes, proteins, and *deoxyribonucleic acid* (DNA), thus causing disruption of insulin production by *beta langerhans pancreatic cells*. *Streptozotocin* enters *pancreatic β cells* through *a glucose transporter* (GLUT-2) and will cause *alkylation of deoxyribonucleic acid* (DNA) resulting in DNA damage. DNA damage will later activate *poly adenosine diphosphate (ADP) -ribosylation*. This process will result in the depletion of *nicotinamide adenine dinucleotide* cellular (NAD +), which further, there will be a reduction in *adenosine triphosphate* (ATP) and will ultimately inhibit insulin secretion and synthesis which finally increases glucose levels in the blood.

CONCLUSION

Based on the findings and discussion above, it can be concluded that trigona honey has an effect in reducing blood glucose levels. Daily consumption of trigona honey has a remarkable potential to decrease the blood glucose, thus it can contribute to the prevention of the diabetes mellitus development.

Ethical Clearance

The research has been approved by the Research Committee on the Ethical use of Human and Animal, medicine Faculty University of Hasanuddin, Makassar, Indonesia with registration number UH19020081.

Conflict of Interest

The authors of this paper declare that there are no conflicts of interest

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Author's Contribution

TS, RN, MH and HK initiated and designed the study. TS, RN, MH, BB, HK, and JJ drafted the manuscript. TS, RN, MH, BB, HK, JJ and WW supervised the field activities and the microbiology work. TS, RN, MH, BB, HK, JJ, and WW helped to collect isolates. All authors have read and approved the final manuscript.

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