PalArch's Journal of Archaeology of Egypt / Egyptology

EVALUATION OF THE INSULIN LEVELS INCREASING EFFECT OF TRIGONA HONEY IN MICE DIABETES MELLITUS

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Tri Damayanty Syamsul, Rosdiana Natzir, Suryani As'ad, Veni Hadju, Mochammad Hatta, Sutji Pratiwi, Andi Tenriola. Evaluation Of The Insulin Levels Increasing Effect Of Trigona Honey In Mice Diabetes Mellitus-- Palarch's Journal Of Archaeology Of Egypt/Egyptology 17(4), 168-177. ISSN 1567-214x

Keywords: Trigona Honey; Local Honey; Plasma Insulin Levels

ABSTRACT

Objective: Many herbal medicines can be used for the therapy of several diseases, infection diseases and metabolic disease. Honey is natural sweet, bee product. It is main components are glucose, fructose, protein, amino acids, vitamins, enzymes, main carbohydrates, minerals and bioactive component such as flavonoid, phenol, tannin, triterpenoid. Trigona honey contains vitamins that function as antibiotics, antitoxins, and antioxidants and increase the immune system. To analyze effects of trigona honey (*Tetragonula sp*) on plasma insulin levels in diabetes mellitus mice.

Method: True experimental pre and post to investigate the effectiveness of trigona honey. *Result:*Showed, $P < 0.05$, there were differences in the effect of trigona honey (*Tetragonula sp*) on plasma insulin levels.

Conclusion: Daily consumption of trigona honey has a remarkable potential to increase plasma insulin, thus it can contribute to the prevention of the diabetes mellitus development.

INTRODUCTION

Indonesia has many herbal plants, various type of plants used in herbalism and some of these plants have medicinal activities. Some plants, consider as a important source of nutrition,has potential effect plants can be therapeutic values.[1] Several traditional and herbal medicine have been reported as effect in infectious diseases, metabolic diseases and aging. Indonesia is a country in southeast Asia has a diverse flora that potential as herbal medicine. Herbal medicine are known to have anti inflammatory, antimicrobial and antioxidant potential.[2]

Many herbal medicines can be used for the therapy of several diseases, infection diseases and metabolic disease. These medicines in several diseases such as *Curcuma xanthorriza Sygium aromaticu,* Musa L.paradisiaca L (MPL) fruit, Red fruit (*Pandanus conoideus*), *Curcuma longa*, Miana Leaves (*Coleus scutellariodes* (L), Andaliman fruit (*Zantoxylum acanthopodium* DC), Ajwa dates and Thallasia hemprichi grass.[3–10]

Honey is natural sweet, bee product. It is main components are glucose, fructose, protein,amino acids, vitamins, enzymes, main carbohydrates, minerals and bioactive compononent such as flavonoid, fenol, tannin, triterpenoid. Flavonoid components can be found in honey.[11] Trigona honey is rich in phenolic compounds because it is a food collected by bees from plants.[12]

Alternative approaches to diabetes therapy include the use of herbal preparations, food components or supplements and other natural product therapies such as honey. Honey is a natural substance produced by bees from nectar. Honey has been used as a traditional natural therapeutic agent to improve the immune system and prevent various diseases.[13] Honey decreases GDP (4.2%) and CRP (3.2%) and increases HDL cholesterol (3.3%).[14]

Trigona honey contains vitamins that function as antibiotics, antitoxins, and antioxidants and increase the immune system. It also (*Teragonula Biroi*) contains protein, fat, carbohydrates, sugar, energy, vitamin C, beta carotene, calcium, magnesium, zinc, flavonoids, and polyphenols because trigona bees can collect nectars from the deepest part of flowers.[15]

MATERIALS AND METHODS

METHODS

This research is a true experimental pre and post to investigate the effectiveness of trigona honey.

Honey 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.

Preparation and adaptation for animal experiment

This stage is for the preparation and adaptation of animals experiment, Mice are placed in cages made of wire with a floor size of 30 cm x 50 cm x 15 cm. The density of each cage contains six tails. Mice are given 300 g/day/head of feed (feed), enough drink, and the cage is cleaned every day. To maintain a stable environmental atmosphere, mice are placed in a room with sufficient air circulation according to room temperature at standard conditions (28 ± 2^0C) with 50 ±10 % humidity. The room light is also maintained with a 12 h cycle the light is turned on and 12 h extinguished. This procedure is carried out for 1 week. After that, the randomization is carried out. All mice were grouped into 3groups randomly, namely, the negative control group, group 1 (negative control), group 2 (trigona honey 0,2 ml/kg BW/day), and group 3 (trigona honey 0,4 ml/kg BW/day). The intervention was conducted for 21 days. After 21 days.

Animals

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 three groups (n=5). three of the groups were introperitoneally streptozotocin. Negatif control (aquadest), and two treatment groups (honey a dose 0.2 ml/Kg.body weight and honey a dose 0.4 ml/Kg body weight).[7,8] Then the initial blood glucose level was measured and insulin levels was measured after being fasted for 10 hours.[16,17]

Diabetes Induction

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. three of groups, Negatif control, and two treatment groups. All mice were induced intraperitoneal streptozotocin 0,13 ml x 105 ml/CFU.[16,17]

Addition of trigona honey

Trigona honey in this research was from the beekeeping in *Bontocane* Village, *Kahu* Subdistrict, *Bone* Regency of *South Sulawesi*. The trigona honey dose per day given 0,2 ml/kg BW mice and 0,4 ml/kg BW mice. Trigona honey therapy is given 3 days after induction streptozotocin. Trigona honey is dissolved in distilled water and given through the nasogastric sonde for 21 days.

Experimental design

This research is a true experimental pre and post to investigate the effectiveness

of trigona honey.

Measurement of plasma insulin levels

The measurement of plasma insulin levels from blood samples taken earlier was done after the end of observation. Blood samples for measurement of plasma insulin levels were taken from the lateral vein of the tail using a *Pasteur pipette* after 1.5 hours of mice being fed. Blood samples obtained were centrifuged at 4˚ C at a speed of 3000xg for 10 minutes and a plasma sample containing insulin (antigen) was obtained. Then the plasma sample is reacted with a *monoclonal anti-mouse insulin (antibody)* that has been coated at the bottom *of microplate wells* and reagents available in the *ELISA kit mouse insulin*. After going through some of these reactions, the sample was measured with a *microplate reader* at a wavelength of 450 nm.

STATISTICAL ANALYSIS

The data obtained was processed using SPSS version 21 for windows and Insulin plasm was statistically analyze the value of with a significance level of < 0.05. was considered as a statistically significant. The difference test conducted was using one-way analysis of variance "ANOVA" followed Post hoc Bonferroni multiple comparison test.

RESULTS AND DISCUSSION

The results of measurement results of insulin levels can be seen in Table 1.

Table 1. Measurements on Plasma Insulin Level of Each Group Before Treatment, After Induction, and During Treatment.

Groups	Insulin level (ng/ml) in the day		
	1	6	22
Negative control	286.23	170.32	102.71
	312.79	182.40	91.84
	365.92	95.47	59.24
	352.64	122.03	48.38
	320.04	141.35	20.61
Mean Scores	327.52	142.31	64.56
SD	31.95	35.35	33.25
Positif control (Metformin)	336.94	85.81	300.72
	328.49	108.75	276.57
	294.68	130.48	252.42
	333.32	159.46	213.79
	358.67	149.80	237.94
Mean Scores	330.42	126.86	256.29
SD	23.08	30.06	33.70

Honey 0.2 ML	274.15	97.94	162.23
	251.53	125.33	183.67
	297.97	53.89	136.04
	286.06	139.61	165.81
	238.44	68.17	189.62
Mean Scores	269.63	96.99	167.47
SD	24.46	36.43	21.04
Honey 0.4 ML	338.45	84.84	172.95
	313.44	112.23	146.76
	264.63	46.74	122.94
	320.59	131.28	177.71
	257.49	150.33	175.33
Mean Scores	298.92	105.08	159.14
SD	35.83	40.61	23.78

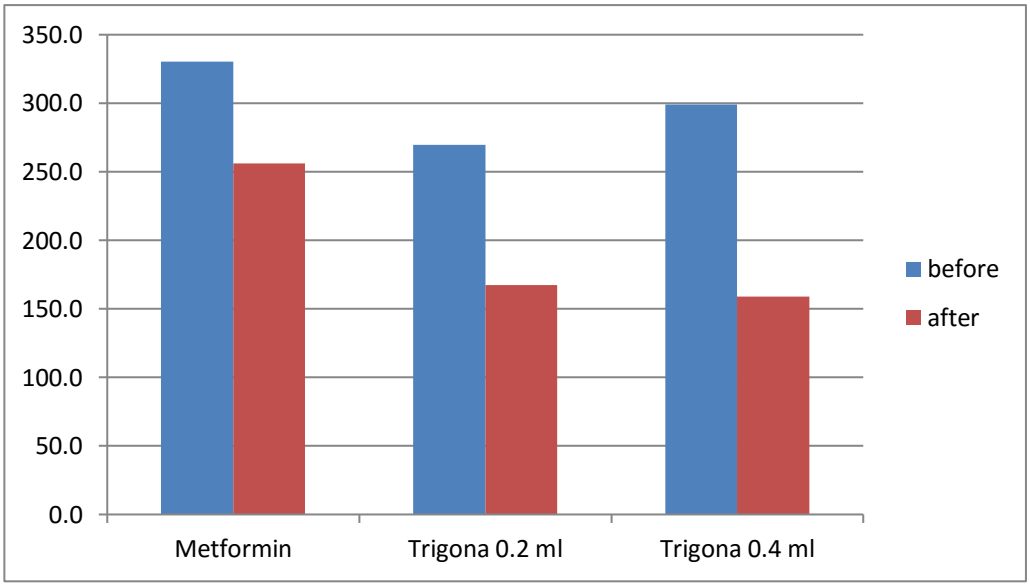

Figure 1. Comparison of insulin levels Plasma Before and After Giving Trigona Honey

The results of testing the effect of trigona honey on mice plasma insulin levels DM conditions with aquadest dose 0 mg / KgWH negative control, Metformin 0.13 ml / KgWH positive control, Trigona honey 0.2 ml / KgWH treatment 1, Trigona honey 0.4 ml / KgWH of treatment 2 for 21 days, showed that trigona honey affects the blood glucose levels of mice with diabetes mellitus. The average and the results of statistical analysis of plasma insulin levels in mice Diabetes mellitus condition after observation for 21 days can be seen in Figure 1.

Figure 1 shows that the average plasma insulin levels of the treatment group after being given trigona honey for 21 days have differences. The average

plasma insulin level of mice in the negative control group showed the smallest compared to the metformin and trigona honey groups.

The average plasma insulin level of this negative control group shows that insulin secretion from pancreatic β cells continues to decrease because the effect of streptozotocin on these cells continues**.** The effect of increasing insulin levels occurs due to active compounds contained in trigona honey such as alkaloids, flavonoids, triterpenoids and polyphenols. Alkaloids are proven to have the ability to regenerate damaged pancreatic β cells. There is an improvement in the pancreatic tissue, there will be an increase in the amount of insulin in the body so that blood glucose will enter the cell and cause a decrease in blood glucose levels in the body and an increase in insulin levels. The average plasma insulin levels of the metformin and trigona honey groups (positive control, Trigona Honey Treatment group with a dose of 0.2 ml and the Trigona Honey group with a dose of 0.4 ml) showed that metformin and trigona honey were able to inhibit the effect of Streptozotocin on β cells pancreas.

Based on Anova results, mice plasma insulin levels between treatment groups experienced differences (0.000< 0.05) with a significance of 0.05. This means that the administration of trigona honey has a different effect on the dose of trigona honey 0.2 ml / KgBB and trigona honey 0.4 ml / KgBB for 21 days. Increased plasma insulin levels in this study indicate that Honey has a stimulating effect on insulin secretion and also increases insulin sensitivity thereby reducing glucose levels. Honey produces *hydrogen peroxide* which has an insulin-like effect. Honey has *nitric oxide*taboliteand can stimulate *NO synthase* and increase NO production. *Nitric oxide* is stimulating insulin release. Honey can reduce plasma and urinary levels at several prostaglandins such as PGE2, PGF2 and TXB. Prostaglandins are inhibitors of insulin secretion. *Zinc* and copper are the normal constituents of honey. So honey administration increases serum zinc and copper levels which can play an important role in insulin and glucose metabolism. Honey fructose can reduce the hyperglycemic response of honey glucose content. The results of previous studies that the dose of low-fructose honey given with glucose decreases the glycemic response to the healthy glucose load of individuals and type 2 diabetic patients. This indicates that fructose acts by stimulating *glucocinase translocation*. So honey stimulates *glucokinase* to take glucose to the liver.[18,19]

Alkaloids are proven to regenerate damaged β cells of the pancreas. Improvement in pancreatic tissue can increase the amount of body insulin so that blood glucose will enter the cells decreasing its level in the body.

Several studies are in line with the current research, including the one conducted by (Majid Muhammad, et al. 2014) which examined the effects of natural honey on blood glucose and fluid profiles for young adults in Pakistan.[18] The randomized control trial was conducted at Army Medical College, Rawalpindi, Pakistan for 4 weeks on 70 young adults divided into two groups (control group and intervention group) of 35 people randomly receiving 70 grams of honey every day for 4 weeks. Consumption of natural honey during the period was significantly effective in reducing glucose, TG, total cholesterol, and LDL and in increasing HDL in healthy young adults. Therefore, healthy individuals

should include honey in their diet to increase glucose and lipid profiles, and to prevent metabolic diseases such as diabetes, cardiovascular disease, hyperlipidemia, and obesity.

In line with this study, using a different sample, Enginyurt, et al (2017) used a pre-experimental method to investigate the role of pure honey in the treatment of diabetes mellitus.[20] It was carried out on 64 randomly selected individuals consisting of 32 people with type II diabetes categorized as group 1 and the rests were 32 healthy individuals put in group 2. In the polyclinic of Family Medicine, Hospital, University of Ordu, they underwent checks on their fasting-prandial blood glucose, HbA1c, total cholesterol, LDL, HDL, and triglycerides with different honey doses intervention (5 gr, 15 gr, 25 gr) and the rest, combined with the drug metformin for 8 weeks. It showed that honey can clean reactive oxygen radicals, improve oxidative stress which can lower blood glucose by decreasing blood lipid levels, and increase the value of HbA1c. Honey reduces hyperglycemia in infants, diabetic rats, and humans.

Honey consists of fructose and glucose. The hypoglycemic effect of honey is believed to result from fructose. Glucose and fructose exert synergistic effects on the digestive system and pancreas and their effects are believed to increase insulin release. Results have shown that fructose increases hepatic glucose uptake as well as glycogen synthesis and storage.

The results of this study are following previous research by (Nuray, et al, 2005).[21] Proving that the administration of honey brings positive results in the form of a significant reduction in GDP levels of 55.6 mg/dl. Consuming a small portion of fructose has a positive effect to lower the blood glucose due to the increase of glucose uptake by the liver, stimulation of the hexokinase enzyme, and an increase in insulin concentration. In this study, it was found that honey can significantly reduce the blood glucose and lipids (cholesterol, triglycerides, and VLDL).

In line with the research of Majid et al. (2014), honey produces hydrogen peroxide which has an insulin-like effect. Honey has nitric oxide metabolites thus it can stimulate NO synthase and increase NO production. Nitric oxide stimulates insulin release.[18] Honey has been found to lower plasma and urinary levels of several prostaglandins such as PGE2, PGF2, and TXB. Prostaglandins are inhibitors of insulin secretion. Zinc and copper are normal constituents of honey. Therefore, giving honey can increase serum zinc and copper levels which play an important role in insulin and glucose metabolism. Honey fructose can decrease the hyperglycemic response to honey glucose content. It has been found that low doses of fructose administered with glucose decrease the glycemic response to the glucose load in healthy individuals and type 2 diabetic patients. This indicates that fructose acts by stimulating the translocation of glucokinases. So honey stimulates glucokinase to take up glucose to the liver.
Research conducted by Atayoglu, Timucin Ali, et al, (2016) which examines the glycemic index value of monofloral Turkish honey and the effect of consumption on glucose metabolism showed that the values of serum insulin and C-peptide after honey consumption were relatively lower than those after eating or consuming glucose.[22] After 120 minutes, serum insulin levels were

significantly higher, whereas a significant reduction was observed after the consumption of chestnut honey.

Alkaloid has been proven able to regenerate the damaged pancreas β cell. Improvement in pancreas tissue can lead to an increased level of insulin in the body so that blood glucose will enter cells reducing its level in the body and increasing the insulin level.

There are some related studies that were carried out earlier like Majid Muhammad, et al. (2014) investigating the effect of natural honey on blood glucose and profile of body fluids of young adults in Pakistan.[18] The randomized controlled trial was performed in Army Medical College, Rawalpindi, Pakistan, during 4 weeks involving 70 young adults divided into control and intervened groups. Subjects in the intervened group were randomly given 70-gram honey for weeks. The findings showed that consuming natural honey for four weeks is significantly effective to reduce glucose, TG, total cholesterol, and LDL, while HDL experienced an increase in healthy young adults. Therefore, healthy individuals are suggested to include honey in their diet to improve their glucose and lipid profiles, to prevent metabolic diseases like diabetes, cardiovascular, hyperlipidemia, and obesity.

This study found that honey can clean reactive oxygen radicals and improve oxidative stress that can reduce blood glucose, lipid levels in the blood, and increase the HbA1c. Honey can lower hyperglycemia in babies, diabetic mice, and humans. Honey contains fructose and glucose. The hypoglycemia effect of honey is believed to result from fructose. Glucose and fructose give a synergic effect on digestion and the pancreas and the effect is estimated possibly boosting insulin secretion. Results proved that fructose can increase the absorption of liver glucose and glycogen synthetic and their storage.

CONCLUSION

Based on the results of research and discussion, it can be concluded that trigona honey has an effect in reducing blood glucose levels, increasing body weight and increasing plasma insulin levels.

ACKNOWLEDGMENT

The authors would like to thank my supervisor in Hasanuddin university for warm support, LPDP, and the Ministry of Research Technology and Higher Education of Republic Indonesia, for providing the scholarship during this study.

CONFLICT OF INTEREST

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

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.

Funding support

This paper received grant thesis of the Ministry of Research Technology and Higher Education of Republic Indonesia.

REFER

Rasool Hassan BA. Medicinal plants (importance and uses). Pharm Anal Acta. 2012;3(10):2153-435.

Jafriati J., Hatta M., Yuniar N., Junita AR., Dwiyanti R., Sabir M., et al. Mini Review: Thalassia hemprichii Seagrass Extract as Antimicrobial and Antioxidant Potential on Human. J Biol Sci. 2019:363-71, doi: 10.3923/jbs.2019.363.371.

Karo MB., Tambaip T., Hatta M., Simanjuntak TP., Irmawaty L., Rina T., et al. A mini review of Indonesian medicinal plants for vulvovaginal candidiasis. Rasayan J Chem. 2017;10(4):1280-8.

Kamelia E., Islam AA., Hatta M., Kaelan C., Patellongi I. The effect of administration of ethanol extract from musa paradisiaca-l (MPL) fruit on the caspase-3 mRNA gene expression in rat amyloid beta induced, an alzheimer's disease model. Asian J Pharm Clin Res. 2018;11(4):298-302.

Tambaip T., Karo MB., Hatta M., Dwiyanti R., Natzir R., Massi MN., et al. Immunomodulatory effect of orally red fruit (Pandanus conoideus) extract on the expression of CC chemokine receptor 5 mRNA in HIV patients with antiretroviral therapy. Res J Immunol. 2018;11(1):15-21.

Simanjuntak TP., Hatta M., Rauf S., Prabandari SA., Siagian CM., Dwiyanti R. Tumor Necrosis Factor-alpha Levels and Histopathology Finding after Intervention with Curcuma longa Extract. J Med Sci. 2018;18(2):56-62.

Karo M., Hatta M., Salma W., Patellongi I., Natzir R. Effects of miana (Coleus Scutellariodes (L) Benth) to expression of Mrna Il-37 in Balb/C mice infected Candida albicans. Pharmacogn J. 2018;10(1).

Sirait LI., Massi MN., Hatta M. The effects of extract andaliman fruit (Zanthoxylum acanthopodium Dc) to CAMP mRNA expression and bacterial load in mice balb-C after Gardnerella vaginal Infection. Indian J Public Heal Res Dev. 2018;9(11):607-11.

Royani I., As'ad S., Mappaware NA., Hatta M., Rabia. Effect of Ajwa Dates Consumption to Inhibit the Progression of Preeclampsia Threats on Mean Arterial Pressure and Roll-Over Test. Biomed Res Int. 2019;2019, doi: 10.1155/2019/2917895.

Usman AN., Syam Y., Natzir R., Rahardjo SP., Hatta M., Dwiyanti R., et al. The effect of giving trigona honey and honey propolis trigona to the mRNA Foxp3 expression in mice Balb/c strain induced by Salmonella typhi. Am J Biomed Res. 2016;4(2):42-5, doi: 10.12691/ajbr-4-2-3.

Syam Y., Usman AN., Natzir R., Pratiwi S. Nutrition and pH of Trigona Honey from Masamba, South Sulawesi, Indonesia. Int J Sci Basic Appl Res. 2016;27(1):32-6.

Samarghandian S., Farkhondeh T., Samini F. Honey and health: A review of recent clinical research. Pharmacognosy Res. 2017;9(2):121.

Mushtaq R., Mushtaq R., Khan ZT. Effects of natural honey on lipid profile and body weight in normal weight and obese adults: a randomized clinical trial. Pak J Zool. 2011;43(1):161-9.

Amsyah UK., Hatta M., Tahir H., Alam G., Asmawati A. Expression of IL-10 in A. actinomycetemcomitans Induced Rat Treated by Purple Miana Leaves. Biomed Pharmacol J. 2019;12(04):2099-104, doi: http://dx.doi.org/10.13005/bpj/1845.

Syamsul TD., Natzir R., As'ad S., Hadju V., Hatta M., Pratiwi S., et al. The Chemical Composition of Trigona Honey in Bone, South Sulawesi. Indian J Public Heal Res Dev. 2020;11(9):258-63.

Ridwan R., Natzir R., Rasyid H., Patellongi I., Hatta M., Linggi EB. Decreased Renal Function Induced by High-Fat Diet in Wistar Rat: The Role of Plasma Angiotensin Converting Enzyme 2 (ACE2). Biomed Pharmacol J. 2019;12(3):1279-87.

Hatta M., Surachmanto EE., Islam AA., Wahid S. Expression of mRNA IL-17F and sIL-17F in atopic asthma patients. BMC Res Notes. 2017;10(1):202.

Majid M., Younis MA., Naveed AK., Shah MU., Azeem Z., Tirmizi SH. Effects of natural honey on blood glucose and lipid profile in young healthy Pakistani males. J Ayub Med Coll Abbottabad. 2013;25(3-4):44-7.

Rivera-Mancía S., Trujillo J., Chaverri JP. Utility of curcumin for the treatment of diabetes mellitus: evidence from preclinical and clinical studies. J Nutr Intermed Metab. 2018;14:29-41, doi: https://doi.org/10.1016/j.jnim.2018.05.001.

Enginyurt O., Cakir L., Karatas A., Cankaya S., Kaya Y., Tugcu H. The role of pure honey in the treatment of diabetes mellitus. Biomed Res. 2017;28(7).

Öztaşan N., Altinkaynak K., Akçay F., Göçer F., Dane Ş. Effects of mad honey on blood glucose and lipid levels in rats with streptozocin-induced diabetes. Turkish J Vet Anim Sci. 2005;29(5):1093-6.

Atayoglu AT., Soylu M., Silici S., Inanc N. Glycemic index values of monofloral Turkish honeys and the effect oftheir consumption on glucose metabolism. Turkish J Med Sci. 2016;46(2):483-8.