Content of Nutritional Honey Moringa Oleifera Nutrition & Glycemic Index Value On Pregnancy Rats (Rattus Norvegicus)

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ABSTRAK

Honey has been widely used as a substitute for sugar, especially during pregnancy that requires food intake with a low glycemic index (GI). The aims of this study to assess the nutritional content and IG of honey Moringa in pregnant wistar rats. The research method is experimental research lab to design Randomized Post Test Only Control Group Design on GI and Proximate analysis and phytochemical analysis of the nutrient content of Moringa honey. The research sample used 24 pregnancy rats into 1 control group (0.22ml) and 3 honey intervention group (0.27ml) consisting 6 animals each. Intervention in 14 days with measurement GI on gestation day 14. Analysis use correlation method AUC to show value of GI and statistical analysis using the One-Way ANOVA, followed by post hoc test analysis test methods LSD. The results study of carbohydrate content honey plus Moringa do not reach SNI, protein and fat reach SNI, for carbohydrate, protein and fat content of Moringa honey reach SNI, while the flavonoid content be higher in honey and polyphenol content is higher in Moringa honey. The results animal studies of GI honey (61), honey plus moringa (62.07) and honey moringa (65.05) are medium category (55-70). The results significant differences in food group reference to the intervention group. The results of post hoc LSD was no significant difference between the natural honey (105.17), Honey plus moringa (126.50) and honey moringa (132.83) in comparison Glycemic value. Honey, honey plus moringa and honey moringa can control the GI in pregnancy.

INTRODUCTION

World Health Organization (WHO), in 2013 reported that more than 80% of deaths due to Diabetes Mellitus (DM) occurred in low-middle income countries (Guariguata et al., 2014). It was also shown in the Asian region that those with diabetes had 13.2% to 5.5% in 3 years (Ramachandran et al., 2012). Indonesia, according to WHO in 2012, is the second largest country after India which has the most DM sufferers, 8,426,000 people at the Southeast Asian level, and is estimated to increase to 21,257,000 by 2030 (WHO, 2013).

Indonesia ranks 7th in the number of diabetes mellitus in the world with 8.5 million people with diabetes mellitus with top positions are China (98.4 million), India (65.1 million) and America (24,4 million), Brazil (11.9 million), Russia (10.9 million), Mexico (8.7 million) (International Diabetes Federation, 2013). Whereas in 2017, the incidence of diabetes mellitus in Indonesia increased to 6th with 10.3 million sufferers with first place in China (114.4 million), second in India (72.9 million) and third in America (30.2 million) (International Diabetes Federation, 2017). Then in Riskesdas data (2013) the prevalence of diabetes was 2.1% and Riskesdas (2018) the prevalence of diabetes was 1.5% (Kemenkes RI, 2013) (Kemenkes RI, 2018).

Diabetes mellitus can also occur in pregnancy, because during the second and third trimesters, there is an increase in insulin resistance up to 80%. Increased insulin resistance as pregnancy increases, can cause maternal glucose levels to increase. This situation can increase the risk of gestational diabetes mellitus. According to the American Diabetes Association (ADA) in 2000, gestational diabetes mellitus occurs 7% in pregnancy each year. The prevalence of gestational diabetes varies from 1-14%.

In Indonesia, the prevalence of gestational diabetes mellitus ranges from 1.9 to 3.6% in general pregnancy (Soewondo et al., 2011). Impacts that occur on pregnant women with gestational diabetes mellitus are mothers at risk of excess weight gain, preeclampsia, eclampsia, cesarean section, while their impact on high-risk infants is affected by macrosomia, hypocalcemia, hyper bilirubinemia, respiratory distress syndrome, polistemia, obesity and type 2 DM (Perkins et al., 2007).

The case of Diabetes Mellitus in Indonesia is increasing in accordance with the lifestyle that tends to adopt the lifestyle of a western country that is consuming fast food which is high in carbohydrate and fat content but low in fiber. Therefore it is necessary to maintain blood sugar levels close to normal blood sugar. The effect of food consumption on blood glucose levels over a certain period is called the glycemic response. A good understanding of the glycemic response in healthy people is needed to prevent DM as well as those with DM in maintaining blood glucose levels, so it is necessary to choose the type, form of intake and amount of carbohydrate food consumed (Sheard et al., 2004).

Carbohydrates with a low glycemic index trigger a slight increase in blood sugar levels, when those with a high glycemic index trigger high blood glucose. The glycemic index for most honey with a portion of 25 grams is low and some types are in the moderate range. Consumption of types of honey that have a low glycemic index has the advantage of physiological effects. Thus, honey with low / moderate GI values may be useful for inclusion in the diabetic diet as a substitute for table sugar (Khan et al., 2018).

Significantly the proportion of women in the high-glycemic index group had a higher increase in insulin resistance compared to women in the low-glycemic index group. In addition, 47% of women in the high glycemic index diet group who met the criteria for increased insulin resistance avoided the diet by switching to

a low glycemic index diet (Moses et al., 2009).

Honey is a supernatant liquid sugar. Honey has a sugar content in the form of fructose and glucose which is a type of monosaccharide sugar that is easily absorbed by the intestine (Erejuwa et al., 2012). This honey also reduces the digestion of starch in the digestive tract, reducing the glycemic response of the blood (Javier et al., 2016).

In addition to honey, in the study of Moringa oleifera or Moringa have large polyphenols and flavonoids that have a hypoglycemic effect, although hypoglycemic effects are involved in stimulating pancreatic Beta cells and subsequently increasing insulin secretion (Tende et al., 2011). Moringa leaf extract contains polyphenolic compounds, flavonoids and phenols as antioxidant components that kill free radicals (Sreelatha et al., 2009).

Controlling glycemic during pregnancy, nutritional content analysis can be done and the glycemic index assessment in the honey honey intervention. Analysis of the nutritional content of Moringa honey in the form of macro nutrients (Carbohydrates, Proteins, Fat) and Antioxidants (Flavonoids and Polyphenols) and the calculation of Honey Glycemic Index, honey plus Moringa and Moringa honey in pregnant white wistar strain rats.

The purpose of this study was to assess the nutritional content and glycemic index of honey, honey plus Moringa and Moringa honey which were intervened in pregnant wistar rats.

METHOD

Research Design and Location

The study was conducted in August to September 2019 with ethical approval from the Hasanuddin University Faculty of Medicine. Nursing and intervention of experimental animals were carried out at the Immunology and Molecular Biology Laboratory, Microbiology Section, Hasanuddin University Medical School.

Population and Sample

This study used 28 experimental white male Wistar rats (Rattus Norvegicus strain wistar) and 28 pregnant female mice aged between 10-12 weeks and body weight ranging from 110-150 grams reproduced 1: 1 in one cage (1 female mouse: 1 male mouse). The sample consisted of four groups: one control group (reference food) and three treatment groups with a total sample of six wistar rats per group. Doses given in the control group (Reference Food) 0.23 ml / head / day as well as honey, honey plus Moringa and Moringa honey with a dose of each 0.27 ml / head / day of oral administration (sonde) for 14 days of pregnancy. Then the fasting blood was taken for 12 hours in the tail vein of experimental animals on the 14th day of pregnancy with the duration of blood taking for 0 minutes, 30 minutes, 60 minutes and 120 minutes. The data used in the form of quantitative data on the value of the glycemic index and the results of proximate analysis and phytochemical analysis from testing the nutritional content of Moringa honey.

Data Analysis

Data analysis using SPSS version 20 with normality test using Kolmogorov-Smirnov. If the data is normally

distributed (P> 0.05) then One-Way Anova Test is performed to compare the mean / average value of the group, after that the analysis continues with the Posh Hoc Test LSD method if significant differences are found (p <0.05) to calculate the group value different.

RESULTS

Table 1. The content of nutrients in honey, honey plus Moringa, Moringa honey and SNI

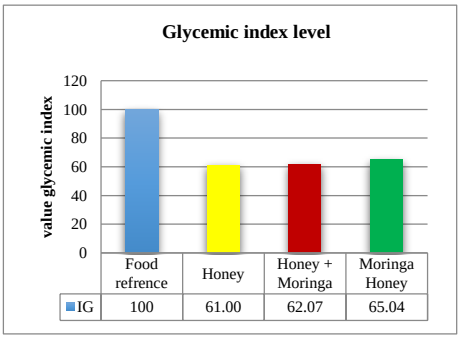
Parameter	Unit	Honey Sampel Code			
		Moringa Honey	honey + moringa	Honey	SNI
Viscosity	Cp	1093.2	720	3067	
Water	(%)	26.59	42.09	21.14	Max 22
Ash	(%)	0.19	0.50	0.27	Min 0.5
Crude Protein	(%)	0.99	0.83	1.10	Min 0.05
Crude Fat	(%)	0.06	0.02	0.01	Min 0.01
Polifenol	(%)	0.13	0.02	0.11	
Carbohydrate	(%)	72.17	56.560	-	Min 60
Total Acid	mEq/Kg	0.01	0.010	0.004	0.431
Flavonoid	Ppm	0.028	0.027	14.346	
Antioxidant	Ppm	130.060	123.27	343.27	
Vitamin C	Cps	278.62	418.21	-	
Beta Caroten	Ppm	118.24	111.84	-	
pH	Ppm	5.40	5.90	6.1	3.9
P	(%)	0.02	0.01	20	1.9-6.3
K	(%)	0.07	0.05	1570	
Fe	Ppm	175	227	-	60- 1500
Zn	Ppm	12	35	-	
Ca	Ppm	998	847	632	
Na	Ppm	65	1321	361	
Mg	Ppm	163	73	216	120- 350

Tabel 2.Results of Glycemic Difference Analysis of pregnancy Rats

Variable	GD 0’	GD 30’	GD 60’	GD 120’
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Food Reference	101.50±23.132	184.83±30.354	191.00±43.781	133.67±32.414
Honey	91.83±8.519	105.17±13.615	102.67±19.613	95.00±20.465
Honey + Moringa	79.00±27.590	126.50±27.833	106.33±25.982	82.17±13.527
Moringa Honey	85.00±22.812	132.83±26.248	107.17±16.229	90.50±17.558
*p**	0.341	0.000	0.000	0.003

* one-way anova

The results of the analysis of the nutritional content of Moringa honey are presented in the form of characteristics in Table 1. The results obtained in the form of 56.56% carbohydrates, 0.83% protein, 0.02% fat, antioxidants 1303060 ppm, flavonoids by 0.027 ppm, Polyphenols by 0.02 ppm contained in honey plus honey Moringa. While the results of Moringa honey in the form of 72.17% carbohydrates, 0.99% protein, 0.06% fat, antioxidants at 123.27 ppm, flavonoids at 0.013 ppm, polyphenols at 0.02 ppm.



Graph 2. Graph of glycemic index level Food reference, natural honey, honey plus Moringa and Moringa honey

In the glycemic index calculation the AUC method (Area under curva) is shown in graph 2 where the results of the glycemic index food Reference are 100, while the honey glycemic index is 61.00, honey plus Moringa has IG 62.07, Moringa honey has IG 65.04 which is classified in the moderate IG category (55 <x> 70).

The results of the statistical analysis of the glycemic differences between the reference food groups and the intervention groups are presented in table 2. The Kolmogorov-Smirnov test shows that all data are normally distributed and the data variants are equal to p values> 0.05 so that one-way Anova test of the reference food groups can be tested and intervention group. The results of the different test analyzes in table 2 show that the p value = 0,000 in GD 30 'and GD 60, and the p value = 0.003 in GD 120' which means there is a glycemic difference in the reference food group with the Wistar rat intervention group between groups (p> 0.05). The statistical test results of the Posh Hoc Test LSD method in table 3 there is no significant difference between natural honey (105.17 ± 13.615), honey plus Moringa (126.50 ± 27.833) and Moringa honey (132.83 ± 26.248) in the glycemic ratio. The final result of this study is the assessment of honey plus Moringa glycemic index and Moringa honey can control glycemic in pregnancy.

Table 3.Results of Glycemic LSD Posh Hoc Test 30', 60' and 120'

	Group	FR	H	H+M	MH
Glycemic 30'	Food Reference		.000	.001	.002
	Honey	.000		.160	.073
	Honey + Moringa	.001	.160		.670
	Moringa Honey	.002	.073	.670	
Glycemic 60'	Food Reference		.000	.000	.000
	Honey	.000		.826	.787
	Honey + Moringa	.000	.826		.960
	Moringa Honey	.000	.787	.960	
Glycemic 120'	Food Reference		.007	.001	.003
	Honey	.007		.327	.728
	Honey + Moringa	.001	.327		.522
	Moringa Honey	.003	.728	.522	

DISCUSSION

The nutritional content of honey plus Moringa and Moringa honey contains carbohydrates, proteins and fats. While the intervention of pregnant wistar rats by assessing the glycemic index of the reference food group has a value of 100 with a high category (> 70), the natural honey group has a value of 61 then the honey plus Moringa group has a value of 62.07 and the Moringa honey group has a value of 65.04 which means this glycemic index in the moderate glycemic index food category (55-70). Then, there is a significant difference between natural honey, honey plus Moringa and Moringa honey with reference food in glycemic pregnant rats.

According to SNI no. 3543: 2013 in 2013 where the quality requirements of honey are reducing sugar content of at least 65%, then honey plus Moringa has not reached minimum standard and Moringa honey has reach minimum standard of SNI honey quality (Standar Nasional Indonesia, 2013). The protein content of honey plus Moringa and Moringa honey is sufficient RDA (0.5%). The amount of protein in honey, including a little, the highest of 0.5% consisting of enzymes and amino acids, but protein is needed in the body (Alvarez-Suarez et al., 2010). Fat content in honey plus Moringa and Moringa honey is lower than in previous studies where the lipids in honey samples ranged from 0.83 to 0.89 and 1.04-1.31% in centrifuged and pressed honey. The lipid content of honey samples in this study was lower than those reported (Almeida-Muradian et al., 2013) who found 0.37-0.39% of lipids in wild honey, and (Escuredo et al., 2013) who found the mean values average 0.1%.

The quality of honey is influenced by the strength of the colony, the stronger the colony the productivity of the queen in laying eggs is higher, the population of the colony increases, and the number of erosion in the hive increases. The influence factor of honey maturity or a lot of at least seals of honey in honeycomb combs is influenced by the interaction of several variables including the number of cells that need to be sealed, number of colonies, temperature (climate), origin of flowers (influence of sugar and nectar water ratio), number of colonies, evaporation rate of worker bees, and humidity in the hive (Eyer, 2016).

The antioxidant value of honey plus Moringa and Moringa honey is higher than that of Natzir et al (2014) which is 94.83 ppm and 81.37 ppm which is known that dorsata honey and trigona honey have antioxidant compounds in the category of "moderately active". The flavonoid content of honey plus Moringa and Moringa honey is lower than that of Alvarez-Suarez et al (2010) which is 43.1 mg CE / kg. While in Moniruzzaman et al (2014) the total flavonoid content in the honey samples studied ranged from 11.46 to 116.67 mg CE / kg. The polyphenol content of honey plus Moringa and Moringa honey is lower than Alvarez-Suarez et al (2010) which is 757.2 mg GAE.

The studies report that the appearance and functional properties of honey depend on the total content of polyphenols. However, variations in the content of polyphenols, flavonoids, and anthocyanins might be caused by flower types, climatic

conditions, types of bee species, and harvest periods (Cimpoiu et al., 2013). In another study said that among the honey produced by bee cerana, millefera, and dorsata it was found that honey from dorsate bees had the highest phenolic and flavonoid acid content (Mohamed et al., 2009).

The glycemic index value of the reference food, has a value of 100 which means this reference food (pure glucose) can raise blood glucose levels quickly and have a high glycemic index value (> 70) and as a reference food in measuring the glycemic index. This is because the reference food is pure glucose which has sucrose content which needs to be digested in the body with the help of the insulin hormone produced by the pancreas gland, then absorbed as energy (Tim Karya Tani Mandiri, 2010).

The other studies have shown results that there was a sharp increase in blood glucose response to standard glucose and glucose amylopectin as food to reach a relatively high average value (5.13 mmol / L) 15 minutes after the start of feeding animals to experimental animals . Fifteen minutes later (at 45 minutes), the glucose response began to slow down to reach the highest values of 5.63 and 6.4 mmol / L for glucose and amylopectin food respectively (Thannoun, 2010).

Determination of the index value on natural honey has a value of 61 then honey plus Moringa has a value of 62.07 and Moringa honey has a value of 65.04, which means that the glycemic index of honey plus Moringa is included in the category of moderate glycemic index food (55-70). Then the results of statistical analysis there are significant differences between natural honey, honey plus Moringa and Moringa honey with reference food after glycemic examination. This is because honey has carbohydrate content, namely fructose and glucose which has a hypoglycemic effect. The highest content of honey is carbohydrate, which is around 95%, which mostly consists of fructose and glucose (Erejuwa et al., 2012). Fructose in honey can increase hepatic glucose absorption as well as glycogen synthesis and storage thereby increasing glycemic control in diabetes mellitus (Eteraf-Oskouei et al., 2013). In the study of Khan et al (2018) statistical analysis revealed that GI was significantly and inversely correlated with three variables; AA (r = -0.8539, p: 0.0017) (Khan et al., 2018).

The results of this study indicate that the glycemic index of honey is highly dependent on antioxidants. The level and activity of these antioxidants is inversely proportional to the GI value. The role of moringa in honey plus Moringa intervention products and Moringa honey also plays a role in the low glycemic index. In Steve's (2015) study the glycemic index of functional foods there was no significant difference (> 0.05) between the GI values of the formulation, but the values were significantly (<0.05) lower when compared to the reference value (glucose). The low GI and GL observed in this study could be due to the extraordinary incorporation of moringa leaf flour into formulated functional foods. For example, it is well known that plant-based foods have a lower glycemic index compared to vegetable food products (Du et al., 2008).

In the results of posh hoc tests, there were no significant differences between natural honey, honey plus Moringa and

Moringa honey in the glycemic ratio. This is because the amount of antioxidants in natural honey is greater than the antioxidants of honey plus Moringa with Moringa honey, especially the amount of flavonoids and polyphenols. Flavonoids further stimulate glucose uptake in peripheral tissues, regulate the activity and expression of enzymes involved in carbohydrate metabolic pathways and act like insulin, by influencing the mechanism of insulin signaling. The difference in antioxidants can affect the glycemic index of honey which is between 32 to 87 GI values (Foster-Powell et al., 2002).

The role of polyphenol compounds is very important in the glycemic index, especially epigallocatechin gallate (EGCG) (Bhattacharya et al., 2013). The EGCG content is thought to be able to act as an antihyperglycemia through a mechanism of increasing insulin sensitivity, protecting damage to pancreatic beta cells from the effects of oxidation and increasing glucose uptake in adipose tissue (Potenza et al., 2007).

The results of antioxidants honey plus Moringa and Moringa honey is lower than natural honey due to the short process of harvesting Moringa honey which is 1 week. Climate variations along with the diversity of nectar flowers from various regions affect the antioxidant activity / potential of honey. Phenolic and flavonoid compounds isolated from honey reveal beneficial activities against several disease conditions including diabetes (Alvarez-Suarez et al., 2010; Bertoncelj et al., 2007; Ferreira et al., 2009).

CONCLUSION AND SUGGESTION

The nutritional content of carbohydrate honey plus Moringa does not reach the SNI honey, for the protein content and honey fat plus Moringa reach the SNI honey. While the nutritional content of carbohydrates, proteins and fat of Moringa honey reach the SNI honey. The antioxidant content of flavonoids was found to be higher in honey and polyphenol antioxidants were higher in moringa honey. Intervention of natural honey, honey plus Moringa and Moringa honey as much as 0.27 ml / head / day in pregnant Wistar strain rats (rattus nurvegicus) with 14 days of pregnancy shows the glycemic index value of natural honey in pregnant Wistar rats is 61, honey plus Moringa is 62.07 and Moringa honey in pregnant wistar rats was 65.05 with all three intervention groups included in the moderate glycemic index category with a significant glycemic difference in the reference food, which meant that they could control insulin resistance in pregnancy. In the results of further statistical tests, there were no significant differences between natural honey, honey plus Moringa and Moringa honey in the glycemic ratio.

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