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The Effect of Ginger Honey and Cocktail Honey Supplementation on Glutathione Levels in *Balb/C* Female Mice Induced Stress

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Abstract--- *This study aims to determine the effect of Ginger honey and Cocktail honey supplementation on increasing glutathione levels and to assess the difference in the effect of Ginger honey and Cocktail honey administration on increasing glutathione levels in female Balb/c mice. The intervention in this research is conducted at the Biopharmaca Laboratory of the Faculty of Pharmacy, Hasanuddin University, and the examination of glutathione levels is carried out at the Microbiology Laboratory of Hasanuddin University Education hospital. The method of this research is in vivo research with the pretest-post-test control group. The samples in this study are 25 female Balb/c mice and are divided into five groups (each 5) with one positive control group (swimming activity) and four intervention groups. The provision of intervention in the form of ginger honey is as much as 28 mg/gramBW sample once every day for 14 days. The provision of cocktail honey (containing honey, royal jelly, and bee bread) as for the division of groups is distinguished from the administration dose of 14 mg/gramBW a day on cocktail honey I and 28 mg/ gramBW a day on cocktail honey II. Glutathione levels are examined using the ELISA method, statistical analysis using paired sample T-test. In this research, it is found that administration of ginger honey II containing 33% honey and extract ginger at a dose 28 mg/gramBW ($p = 0.031$) and cocktail honey II at a dose of 28 mg/gramBW ($p = 0.044$) given once a day for 14 days can significantly increase glutathione levels with a value of $p < 0.05$. This study concludes that the administration of ginger honey and cocktail honey have an effect on the increase in glutathione levels, which are statistically significant*

Keywords--- *Glutathione levels; Ginger honey; Cocktail Honey; Balb/c female mice*

I. INTRODUCTION

Glutathione (GSH) is a master of all antioxidants, detoxification experts and immune system maestros, and one of the main endogenous antioxidants produced by cells that participate directly in neutralizing free radicals and reactive oxygen species and maintain exogenous antioxidants such as vitamin C and vitamin E in the reduced form (Ahmed et al. 2018).

Glutathione is a water-soluble antioxidant that works to protect cells that are protected by water. Glutathione is an important component for the production of glutathione S-transferase, which is an enzymatic antioxidant that is useful in protecting the body from toxicity (Lingga 2012). As an antioxidant, glutathione also protects cells from

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oxidants, electrophilic compounds, and xenobiotics. In vitro, GSH can react with hydroxyl (OH), hypochloric acid (HOCL), carbon center radicals, and singlet oxygen, which then produce thynil radicals (GS) (Suhartono 2016).

The ratio of GSH to disulfide oxidized (GSSG) determines the cell's redox cell status. Healthy cells at rest have a GSH/GSSG ratio of> 100, while the ratio drops to 1 to 10 in cells exposed to oxidant stress. Glutathione is also known as a thiol buffer that maintains the sulfhydryl group of many proteins in a reduced form. Glutathione is produced exclusively in the cytosol and is actively pumped into the mitochondria (Pizzorno 2014).

Lack of levels of glutathione in the body can result in high free radicals caused by a lifestyle so that the amount of free radicals exposed is more than the production of antioxidants (glutathione). Antioxidant intake is needed by utilizing natural ingredients such as honey. Honey is also a conventional therapy as a new antioxidant to relieve many diseases that are directly or indirectly related to oxidative stress (Khalil, Sulaiman, and Boukraa 2010).

In this research, in vivo reveals that honey is able to stimulate antioxidant defense systems in mice tissue (pancreas, serum, kidney, and liver), specifically increasing the activity of cell antioxidant enzymes, such as superoxide dismutase, catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), and increasing glutathione levels (Abdulmajeed et al. 2015; Adeoye et al. 2018; Ahmed et al. 2018; Omotayo O Erejuwa, Sulaiman, and Wahab 2012). Giving honey as much as 1.2 g / kgBW increases the amount and activity of antioxidant agents such as glutathione reductase(Miguel and Faleiro 2017; Yan, Jia, and Gao 2013). In addition to honey, ginger is an herbal plant that is believed to be an antioxidant because it contains 6-GF, which has been shown to increase glutathione activity (Abolaji et al. 2017).

The content of honey is rich in antioxidants, so researchers are interested in conducting research with new innovations using honey combined with herbal plants in the form of ginger and several other honey bee products, namely royal jelly and bee bread that are processed into ginger honey and cocktail honey. This product will be associated with research glutathione levels.

II. METHODOLOGY

This research was conducted in four places namely, Microbiology Laboratory of Hasanuddin University Education Hospital for measuring glutathione levels, Unhas Halal Center for honey processing, Biopharmacy Laboratory of Faculty of Pharmacy of Hasanuddin University, for the process of mice adaptation until the end of treatment, Biopharmaca Laboratory of Faculty of Pharmacy of Hasanuddin University for the extraction process with maceration which would be conducted in November 2019 - December 2019.

Research design

This research was an in vivo laboratory research with a research design using the pretest-post-test control group method. In this study, the researchers conducted a pre-test to see levels of glutathione before being given an intervention then post-test to measure the effect of the intervention by comparing the intervention group with the control group.

Materials and equipment

The materials used in this study were the ginger emprit (Zingiber officinale var arum), Trigona spp honey which had been evaporated obtained from the Halal Center of Hasanuddin University, royal jelly, bee bread, beast test animals *Balb /c*, pellets (pellets), *aquadest*, 70% ethanol, technical ether, filter paper, and alcohol. The equipment used were

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experimental animal cages, digital scales, elutic glutathione kits, gloves, mixers, micropipets, mouse sonde stomach, 1 ml spoit, capillary pipes, vacutainer tubes, safety boxes, cool boxes, closed glass jars, scissors, thermometers, aluminum foil, ether solution, magnetic. stirrer bar, Elisa raider, centrifuge, stirring rod, a cylindrical glass tube (diameter 20 cm height 50 cm), rotary evaporator, and blender.

Extraction

Ginger (Zingiber officinale var.amarum) was obtained from Camba Village of Bone, as much as 12 kg of fresh ginger, then cleaned from the soil attached to the ginger rhizome with running water until it was clean, then the sample was thinly cut and then dried in a natural way under the sun indirectly to dry resembles crackers and slightly brownish-colored. After drying, the ginger was mashed with a standard tool (blender), so that small pieces were obtained. Furthermore, the ginger was soaked with 70% Ethanol for seven days.

Ginger honey

Ginger honey was a mixture of thick ginger extract with honey that was homogenized using a magnetic stirrer. The making of ginger honey was divided into two groups, namely Ginger honey I (50% honey and extract ginger) and Ginger honey II (33% honey and extract ginger). The dose was given to Mice based on the conversion from the usual dose in rats to mice (0.14) so that the dose obtained in mice was 28 mg/20gramBW for 14 days of intervention.

Cocktail honey

Cocktail honey was made by mixing basic ingredients that were used such as, royal jelly, bee bread, and honey, which were then homogenized using a magnetic stirrer. Cocktail honey was grouped into Cocktail Honey I The combination of mixing honey, bee bread and royal jelly using a dose according to the conversion with a low dose of 14mg/20gramBW, and Cocktail Honey II was the combination of mixing honey, bee bread and royal jelly using a moderate dose of 28 mg/20gramBW.

Treatment of research subjects

Research permission from the Faculty of Medicine Ethics of Hasanuddin University Makassar with number: 1152 / UN4.6.4.5.31 / PP36 / 2019 with protocol number UH19110936.

The population of this research was 25 female *Balb/c* mice with 3-4 months old at the Biopharmacy Laboratory of the Faculty of Pharmacy, Hasanuddin University, Makassar. The sample used in this study was *Balb/c* mice, which had a weight ranging from 20-35 grams as many as 25 mice randomly grouped to avoid bias due to the age. Grouping in the sample was divided into five groups and *Balb/c* mice are kept in the Biopharmacy laboratory of the Faculty of Pharmacy Hasanuddin University for 7 (seven) days so that the physical and psychological condition of the mice was stable in the room with sufficient air circulation and maintained at room temperature 12-28 ^{o}C. The light was on for 12 hours and was off for 12 hours as well. Mice are placed in cages made of wire with a size of 60x30 cm. During the maintenance, the mice were given standard feed and adequate drinking through ad libitum.

The trial was conducted based on the Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine following World Health Organization (WHO) standards of at least five individuals. Before the given animal intervention, it was adapted with each group for seven days. Initial blood sampling (pre-test) was after the mice swam until they showed signs of stress before being given an intervention to see the level of glutathione.

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Before the intervention of the group, mice were conditioned in a stressful state by being relaxed until they showed symptoms of stress. Provision of intervention used gastric sonde for 14 days. On the 15th day, a repeat blood taking was conducted to determine the level of glutathione. The interventions in each group were as follows: The ginger honey I group was intervened with a combination of 50% honey and extract ginger given 28 mg/20gBW/day. Ginger honey II groups were intervened with a combination of 33% honey and extract ginger with a dose of 28 mg/20gBW/day. Cocktail honey I group (honey, beebread, royal jelly) at a dose of 14 mg/20gBW/day. Cocktail honey II groups (honey, beebread, royal jelly) at a dose of 28mg/20gBW/day. The control group was only swimming until it showed symptoms of stress, then dried with a towel and given standard water and food every day.

Measuring glutathione hormone

ELISA kit glutathione animal was used to measure glutathione levels before and after the intervention was given to each intervention group and control group. Measurements were taken after the intervention on the 15th day by taking 0.5 ml of blood from the eyes or tail of the mice. After the blood was taken, it was stored in the EDTA tube, and it was then centrifuged at 24 ° C at 4000 RPM for 15 minutes and stored at -20 ° C until the blood sample was tested.

In this research, an examination using the R&D method of the Enzyme-Linked Immunosorbent Assay (ELISA) mouse system to measure the levels of glutathione. Testing Procedure Prepare all reagents, standard solutions, and samples according to instructions. Bring all reagents to room temperature before use. Tests carried out at room temperature. Determine the number of strips needed for testing. Insert a strip in the frame to use. Unused strips should be stored at 2-8 ° C. Add the 50µl standard to the standard well. Note: Do not add antibodies to the standard properly because standard solutions contain biotinylated antibodies. Add 40µl of sample to sample well and then add 10µl of anti-GR antibody to sample well, then add 50µl of streptavidin-HRP to standard well and well samples (No blank well control). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37 ° C. Remove the sealer and wash the dishes five times with the washing buffer. Soak the well with at least 0.35 ml of washing buffer for 30 seconds to 1 minute for each wash. For automatic washing, aspirate all wells and wash five times with washing buffer, wells that are overfilled with washing buffer. Blot plates to paper towels or other absorbent material. Add 50µl A substrate solution to each hole then add 50µl B substrate solution to each hole. Separate plates covered with a new sealer for 10 minutes at 37 ° C in the dark. Add 50µl Stop Solution to each well, and the blue will immediately turn yellow. Determine the optical density (OD value) of each well immediately using a micro-plate reader set to 450 nm within 10 minutes after adding the stop solution.

Data analysis

Data were processed and analyzed using the Microsoft Excel program with statistical data. The effect of giving ginger honey, cocktail honey, glutathione levels were displayed in the form of mean ± SD (standard deviation) with confidence intervals (95% CI). Before the statistical test was done, the normality test was done first. The data were normally distributed, so the bivariate test used was paired sample T-test to see differences in glutathione levels in the ginger honey, cocktail honey, and control groups.

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III. RESULTS

The research on the effect of ginger honey and cocktail honey supplementation on glutathione levels in stressed *Balb/c* mice has been conducted. This research was conducted in November - December 2019. The research sample used female *Balb/c* mice, which were divided into five groups: two ginger honey intervention groups, two cocktail honey intervention groups, and one positive control group with swimming activity to show stress symptoms. From these studies, the following results were obtained:

Table 1. Differences in glutathione levels before and after intervention in *Balb/c* mice with control groups.

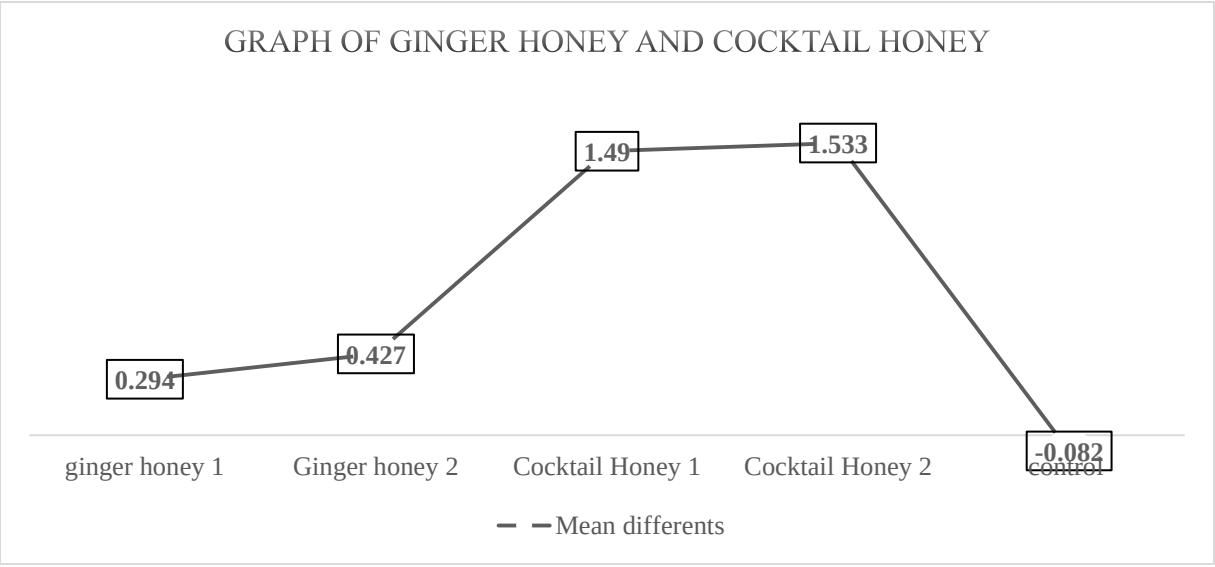
	Glutathione levels (ng / ml)				
Treatment time for	**Ginger Honey I Mean±SD**	**Ginger Honey II Mean±SD**	**Cocktail Honey I (Dose 14mg/day) Mean±SD**	**Cocktail Honey II (Dose 28mg/day) Mean±SD**	**Control Mean ±SD**
Pre	0,747 ± 0,262	0,451 ±0.241	0,436 ±0,241	0,327 ±0,299	1,069 ±0,876
Post	1,0413 ± 0,820	0,878 ±0,210	1,926 ±1,394	1,860 ±1,360	0,987 ±0,767
Different Mean	0,294	0,427	1,49	1,533	-0,082
***P* Value**	0,538	0,031	0,077	0,044	0,792

***Paired Sample T-Test**

Table 1 shows the overall data of statistical test results in the ginger honey pretest-posttest group with the control group (given swimming activity) on female *Balb/c* mice with significant value ($p < 0.05$; $p = 0.03$) on ginger honey II and the cocktail honey pretest-posttest group with significant value ($p < 0.05$; $p = 0.044$) in cocktail honey II.

There is an increase in the difference between the pretest-posttest intervention group and the control group. The greater increase in the cocktail honey II group was 1.533 ng/ml ($p=0.044$). The next highest increase is the ginger honey II group by 0.427 ng/ml ($p=0.031$) while in the ginger honey I and cocktail honey I group there is an increase, but statistically does not have a significant value of $p>0.05$. The control group decreases by -0.082 ng/ ml ($p = 0.792$) $p> 0.05$ which is statistically not significants.

Figure 1. The graph of ginger honey and cocktail honey effect on glutathione levels



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The data in Figure 1 shows that there is an increase in the level of glutathione (ng/ml) in the ginger honey I group with a mean difference of 0.294 ng/ml (p = 0.538) <0.05 statistically having a non-significant value, while in ginger honey II it is 0.427 ng/ml (p = 0.031) statistically p-value <0.05 which means it has a significant value. The control group experienced a decrease in glutathione levels of -0.082 (p = 0.792) with a value of p> 0.05 statistically the results were not significant and the data shows that there is an increase in glutathione levels (ng / ml) in the cocktail honey 1 group by 1.49 ng / ml (p = 0.077) <0.05 statistically had insignificant value, while in cocktail honey II by 1,533 ng / ml (p = 0.044) statistically p value <0.05 which means it has a significant value. The control group experiences a decrease in glutathione levels of -0.082 (p = 0.792) with a value of p> 0.05 statistically; the results were not significant.

IV. DISCUSSION

Heavy activities carried out continuously can affect oxidative stress, which can trigger antioxidant defense mechanisms in cells (Kruk et al. 2019). Research conducted by (Bilski et al. 2019) says that doing activities (treadmills) can increase oxidative stress in mice. One other form of strenuous activity is swimming, so in this study, swimming was done to trigger oxidative stress.

Ginger honey and cocktail honey interventions are expected to increase antioxidant (glutathione) and overcome oxidative stress. Previous studies explained that Manuka honey had the effect of maintaining antioxidants both enzymatic (GPx and SOD) and nonenzymatic (GSH and NO) (Almasaudi et al. 2016). Based on the results of the Paired T-test in Table 1, it shows that in the ginger honey group glutathione levels increases as seen from the pretest-posttest value with a mean difference in ginger honey 1 of 0.294 ng/ml (p = 0.538) <0.05 but statistically it has insignificant value, while in ginger honey II of 0.42 ng/ml (p = 0.031). Whereas in the control group, which was only given swimming activity until signs of stress were seen, it decreased with a mean difference of -0.082 ng/ml (p = 0.792) p> 0.05, meaning that it was not significant.

This study is in line with previous studies that combined metformin or glibenclamide which with honey significantly increasing CAT, GPx, and Glutathione. This shows that combining honey with conventional treatment can provide additional antioxidant effects so as to reduce oxidative damage that is mediated by stress in the kidneys diabetes in rats(Omotayo Owomofoyon Erejuwa et al. 2011).

Other studies also combined gelam honey and ginger given to diabetic rats induced by STZ compared with diabetic control mice showed a significant reduction in SOD and CAT activity (P <0.05) and MDA levels, while GSH levels and GSH/ GSSG ratios significantly increased (P <0.05) (Abdul Sani et al. 2014).

In addition to honey and ginger, this study also provided a cocktail honey intervention with a mixture of honey, royal jelly, and bee bread. Royal jelly increases total antioxidant capacity, SOD activity and reduce GSH, and lipoperoxidation in the liver. Thus, RJ supplementation restores corticosterone levels and the liver antioxidant system in stressed mice (Caixeta et al. 2018).

Table 1 shows the results of cocktail honey, as seen from the pre-posttest values, showing that there was an increase in each group given a different dose. This proves that cocktail honey can increase glutathione levels with a mean difference in cocktail honey I of 1.49 ng/ml (p = 0.077) <0.05 at a dose of 14 mg/day, whereas in cocktail

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honey II by 1.533 ng/ml (p = 0.044) at a dose of 28 mg/day. It is in line with other studies showing that the royal jelly enzyme has high potential as an antioxidant agent that can be used in human and animal food. Enzymes in royal jelly can significantly increase the antioxidant enzyme activity of superoxide dismutase (SOD) and the level of the antioxidant glutathione (GSH) at certain doses (Gu et al. 2018).

This study is in line with previous studies that combined gelam honey with ginger in diabetic rats concluded that the combination had a significant effect on increasing glutathione levels by intervening for 3 weeks a dose of 6 g/kg ginger and 2g/kg honey doses (Abdul Sani et al. 2014).

Besides honey and ginger, royal jelly which is another bee product, has a significant protective effect on the liver and kidneys in reducing the level of lipid peroxidation (MDA), increasing the level of GSH, and increasing the activity of GST, GSH-Px, and SOD (Karadeniz et al. 2011). Administration of royal jelly 200 mg / 14 days effectively increases Glutathione reductase in the cerebral cortex and striatum (Teixeira et al. 2017). In line with what was revealed by Asadi et al. 2019, the administration of royal jelly significantly increased catalase (CAT), Glutathione Px, and reduced MDA levels in mice given varicoceles as triggers for oxidative stress. Therefore, in addition to being an herbal ingredient, royal jelly is also recommended as a protection against oxidative stress. Royal Jelly supplements enhance the antioxidant defense system, reduce lipid peroxidation, and rebuild the glutathione system in stressed rat brains. Furthermore, royal Jelly decreases corticosterone levels which can be associated with an increase in the antioxidant system in the brain, so royal jelly is also called an anti-depressant (Teixeira et al. 2017).

V. CONCLUSION

This study concludes that the administration of ginger honey and cocktail honey has an effect on increasing levels of glutathione, which has a statistically significant value with a dose of 28 mg / 20gBW/day in ginger honey II (33% honey combine with extract ginger). In the other intervention group, cocktail honey increases the most at high doses (28 mg/20gBW/day) while in the ginger honey I and cocktail honey I group, there is a statistically insignificant increase with p> 0.005.

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