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

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Abstract--- *This study aims to investigate the effect of ginger honey and cocktail honey on decreasing cortisol hormone levels and to determine the difference in the effect of ginger honey (GH) and cocktail honey (CH) on decreasing cortisol hormone. This study is an in vivo study using the pre-test-post-test control group method. The sample used in this study were 25 Balb/c female mice which were divided into 5 groups (each group consist 5 mices); 1 control group and 4 intervention groups. The Intervention group divided based on different combination for ginger honey and different dose for cocktail honey; GH I (28 mg/20g BW/day), GH II (28 mg/20g BW/day), CH I (14 mg/20g BW/day) and CH II (28 mg/20g BW/day). All of group were given oral intervention using oral sonde for 14 days. Cortisol levels were examined using the ELISA method. Data were analyzed using statistical analysis T-test paired test. In this study it was found that the group of GH II given for 14 days could significantly reduce cortisol levels (p= 0.005, p <0.05) by -1,418 ng/ml and CH II can significantly reduce cortisol hormone levels (p = 0.005, p <0.05) by -0.806 ng/ml compared to the control group. However, among the two most influential on decreasing cortisol levels was GH II. This study concludes that the administration of ginger honey and cocktail honey can have an effect on reducing cortisol hormone levels and can be used as a supplement to deal with stress*

Keywords--- *Ginger honey; Cocktail honey; Cortisol; Stress*.

I. INTRODUCTION

Stress is a non-specific body mechanism for changes in the body caused by environmental changes (stressors) to maintain homeostasis from the body (Holmqvist-Jämsén et al., 2017; Koolhaas et al., 2011). One of the stress assessments can be done by measuring cortisol levels which can be done through serum or saliva (Greff et al., 2018; Holmqvist-Jämsén et al., 2017).

State of stress or depression that occurs in women can result in several reproductive problems such as menstrual disorders, infertility, miscarriage and so on. Psychological stress before and during pregnancy can cause miscarriage or abortion(C. D Lynch, Sundaram, Maisog, Sweeney, & Buck Louis, 2014; Courtney. D Lynch, Sundaram, & Buck Louis, 2018). In addition, stress can affect fertility in women(Qu et al., 2017).

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There are some treatments that can be done to deal with stress by providing several types of foods containing antioxidants that can deal with stress. One of the herbal plants that contains flavonoids and polyphenols is ginger (Prasad & Tyagi, 2015; Syafitri, Levita, Mutakin, & Diantini, 2018).Previous research conducted on experimental animals (mice) found that giving as much as 200 mg / kg BW of ginger extract can significantly reduce anxiety symptoms and can replace diazepam(Fadaki, Modaresi, & Sajjadian, 2017). In addition, other studies conducted on animals given ginger resulted in an increase in the total antioxidant serum capacity(Khaki, Khaki, Hajhosseini, Golzar, & Ainehchi, 2014).

Honey is widely used as an alternative treatment for several diseases (Miguel, Antunes, & Faleiro, 2017)honey containing flavonoids, phenolic acids and α-tocopherols which are antioxidants are believed to be able to handle stress(O. O Erejuwa et al., 2010; Omotayo O Erejuwa, Sulaiman, & Wahab, 2012; Pasupuleti, Sammugam, Ramesh, & Gan, 2017).Previous studies on experimental animals by giving Tualang honey as much as 200 mg / kg BW diluted with 1 ml sterile water and given to rats for 14 days were proven to protect against memory loss due to exposure to stress resulted by sound and/or aging through the increased mPFC and hippocampal morphology. which may reduce oxidative stress in the brain and increase BDNF concentrations and the cholinergic system(Azman, Zakaria, Abdul Aziz, & Othman, 2016).

In addition to honey, there are several other products produced by honey bees such as royal jelly and bee bread where each of which has its own properties. Royal jelly contains antioxidants related to phenolic compounds, proteins and peptides(Kocot, Kiełczykowska, Luchowska-Kocot, Kurzepa, & Musik, 2018; Maqsoudlou et al., 2019). Previous studies that used royal jelly in post-menopausal women can reduce menopausal symptoms, one of which is anxiety(Asama et al., 2018).

Bee bread is one of the products of honey bees that began to be used for apitherapy(Habryka & Kruczek, 2016). Bee bread also contains natural antioxidants, such as flavonol glycoside derivatives and shows antioxidant activity and is effective against gram-positive bacteria and fungi (Bakour et al., 2019; Kieliszek et al., 2018).

Since many benefits are produced from honey, researchers are interested in using honey as a therapy in dealing with stress during the preconception. The novelty of this research is that in this study the researchers used processed honey in the form of ginger honey and cocktail honey. Ginger honey consists of ginger and honey while cocktail honey consists of honey, bee bread and royal jelly. The compounding of processed honey that researchers used is rarely given as a treatment for stress. This research is a basic research that uses experimental animals as an intervention medium.

The purpose of this study is to determine the effect of giving ginger honey and cocktail honey on decreasing cortisol hormone levels, and to find out the effectiveness level of giving ginger honey and cocktail honey on decreasing cortisol hormone.

II. METHODOLOGY

This research is an in vivo laboratory research on female *Balb/c* mice. In this study, the researchers used a pretest-posttest control group design.

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Product Preparation

Ginger Extraction

The ginger used in this research is the type of emprit ginger (*Zingiber officinale var Amarum*) obtained from Camba area as much as 12 kg, then cleaned with running water and cut into small pieces, then dried in a natural way that is aerated until dry like crackers. After the ginger is dried, it is blended using a blender, and then the ginger is extracted for 7 days using maceration method and 70% ethanol solution, and then the extracted ginger is separated between the liquid with solids, and then the liquid is evaporated with a rotary evaporator so that a thick ginger extract is obtained as much as 40 grams. The process of maceration of ginger was carried out at the Laboratory of Phytoplankton MIPA Chemistry at Hasanuddin University and the process of evaporation of ginger extracts was carried out at the Biopharmaca Laboratory of Hasanuddin University Research Activities Center.

Ginger honey (GH)

The resulted thick ginger extract was put into a porcelain cup and weighed the extract weight then mixed with trigona honey *spp*, and then it was homogenized using a magnetic stirrer. The making of ginger honey is divided into 2 groups of GH I (50% honey combine with extract ginger), and GH II (33% honey combine with extract ginger). The usual dose of honey in rats is 200 mg/kg BW, and then it is converted to mice is 28 mg/20g BW. Therefore, the dose given for the ginger honey group was 28 mg/20g BW per day for 14 days.

Cocktail honey (CH)

Cocktail honey is a mixture of royal jelly, bee bread and honey which is then homogenized using a magnetic stirrer. Cocktail honey is divided into 2 groups namely CH I given in 14 mg / 20g BW / day and CH II given in a dose of 28 mg/20g BW/day.

Animal

The number of 25 female *Balb/c* mice (8-12 weeks old) was obtained from the biopharmacy laboratory of Hasanuddin University. The mice used are female mice which have never been pregnant with a body weight of 20-35 grams. Whereas the exclusion criteria are the sick mice during adaptation, and mice that die before the last blood draw (end of treatment).The research subjects were stored in a laboratory with the lights in the room set 12 hours on and 12 hours turned off at a temperature of 22-28oC. Before being given the intervention, mice were adapted with their groups first for 7 days, and they were then given the activity in the form of swimming, and then taking an initial blood (pretest) was conducted to determine the level of the cortisol hormone on the next day which was the 1st day of the administration of mice interventions by getting the subjects to swim until they seemed stressful. After that, interventions were given using a gastric sonde in accordance to the grouping for 14 days. On the 15^{th} day, a repeated blood extraction was taken to determine the level of the hormone cortisol (post-test). This study has received an ethics permission from the Ethics Committee of the Faculty of Medicine, Hasanuddin University with protocol number UH19110934, Makassar.

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Treatment of research subjects

The treatment was carried out by dividing 25 mice into 1 control group and 4 intervention groups. The control group was given swimming activity to show symptoms of stress then rested and given water + standard feed for 14 days. Ginger Honey I group was given swimming activity and Ginger Honey I group as much as 28 mg / 20g body weight of mice / day. Ginger Honey II group was given swimming activity and Ginger Honey II group as much as 28 mg / 20g body weight of mice / day. Cocktail Honey I group was given swimming activity and Cocktail Honey I intervention (a mixture honey, bee bread and royal jelly) as much as 14 mg / 20g body weight of mice / day. And the Cocktail Honey II group were given swimming activity and Cocktail Honey II group (a mixture of honey, bee bread and royal jelly) as much as 28 mg / 20g body weight of mice / day.

ELISA measuring of Cortisol Hormone Levels

Hormone measurements for cortisol levels were carried out on day 0 (pretest) and on day 15 (post-test) in each group by taking 0.5 ml of blood on the eyes or tail of the mice. In this study, a test was conducted using the R & D method of the enzyme linked Immunosorbent Assay (ELISA) Mouse to measure cortisol levels. The sample used is a plasma sample that has a centrifuge with a speed of 4000 RPM for 10 minutes at 24^{o}C. After the plasma was taken and then put at a temperature of -20^{o}C until an examination using mouse cortisol ELISA kit was conducted. The plate has been coated by COR mouse antibodies. The COR in the sample was added and bonded to antibodies that have been coated properly. Then, the biotinylated COR mouse antibodies were added and bonded to the COR in the sample. Then, Streptavidin-HRP was added and bonded to the biotinylated COR antibody. After incubation, non-participating Streptavidin-HRP would be washed during the washing process. The substrate solution was then added, and the color change will match the number of COR mouse. The reaction was stopped by adding the acidic stop solution, and then absorption was measured. The determination of the optical density of each subject by using a microplate reader set it to 450 nm within 10 minutes after adding a stop solution.

Statistical Analysis

Data on the effect of ginger honey, cocktail honey, and cortisol levels are displayed in mean ± SD (standard deviation) with confidence intervals (95% CI) and tested using paired T-test samples to see the differences of pretest and post-test cortisol levels between groups.

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III. RESULTS

Table 1. Analysis of cortisol hormone levels before and after intervention in female *balb/c* mice

Treatment Time	Cortisol Hormone Levels (ng/ml)				
	Control Group Mean±SD	Ginger Honey I Mean±SD	Ginger Honey II Mean±SD	Cocktail Honey I Mean±SD	Cocktail Honey II Mean±SD
Pretest	8.123±0.623	8.383±0.177	9.265±1.109	8.515±0.497	9.028±0.444
Posttest	12.062±3.541	9.896±0.718	7.846±1.217	9.797±2.357	8.222±0.438
Mean Difference	+3.938	+1.512	-1.418	+1.281	-0.806
P value*	0.095	0.025	0.005	0.264	0.005

**** Paired sample T-test***

Based on the data shown in table 1, the statistical results of the paired t test show that there are 3 groups that show p <0.05, namely Ginger Honey I (P = 0.025) and Ginger Honey II (P = 0.005) and Cocktail Honey II (P = 0.005). However, among all groups of intervention both in the form of Ginger Honey and Cocktail Honey, and Controls which is able to reduce the cortisol hormone are Ginger Honey II and Cocktail Honey II where statistical results show that both significantly effect (p = 0.005, p <0.05). However, among the two, the most significant decrease is shown by the Ginger Honey II group of -1,418 ng / ml.

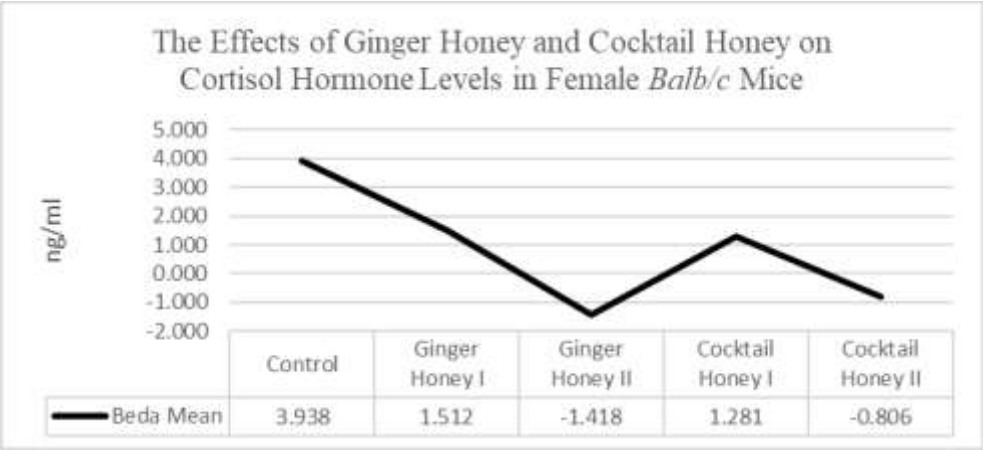

Figure 1. The graph of the effect of ginger honey and cocktail honey on cortisol levels in *Balb/c* female mice based on different mean

Based on Figure 1, it can be concluded that the group administration is only in the Ginger Honey II and Cocktail Honey II groups which show a significant decrease in cortisol levels for 14 days by -1.418 ng/ml and -0.806 ng/ml.

This study finds out that after being given an intervention there are differences in the levels of the hormone cortisol in female *Balb/c* mice between the Ginger Honey group, the Cocktail Honey group and the control group. In this study it is found that some ginger honey and cocktail honey have decreased cortisol hormone levels and some have increased cortisol hormone levels while the control group have increased cortisol hormone levels. After

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conducting statistical tests there are 3 groups which have p <0.0, but only 2 groups show a decrease in cortisol hormone levels after swimming activity and are given honey intervention groups namely Ginger Honey II and Cocktail Honey II with a decrease in each each as much as -1.418 ng/ml and -0.806 ng/ml.

Secretion of the cortisol hormone is influenced by the body's response to stressors, one of which is physical activity that can cause stress (Hannibal & Bishop, 2014). Provision of swimming activities can increase levels of the corticosterone or cortisol hormone in mice(Jones, 2007). Therefore, the swimming activity is given as a stress induction.

Previous studies have concluded that the administration of honey whether combined with sports activities or not in mice can reduce serum cortisol levels or corticosterone levels (Azman et al., 2016; Mosavat, Ooi, & Mohamed, 2014).Other studies have also concluded that the administration of ginger extract in mice and rats can affect cortisol levels and can deal with stress or depression (Fadaki et al., 2017; Khosravi, Modaresi, & Sajjadian, 2018).This is suitable with what is obtained from this study where there is a decrease in cortisol hormone levels of -1.418 ng / ml after swimming activity is given, and ginger honey with a combination of ginger honey II and given as much as 28 mg/ 20gBW/day for 14 days is given as well.

Previous studies that mixed honey with royal jelly given to male athletes could significantly reduce cortisol levels(Büyükipekçi, Sarıtaş, Soylu, Mıstık, & Silici, 2018).This research proves that the mixture of honey, royal jelly and bee bread given as much as 28 mg/20gBW/day for 14 days can reduce cortisol levels in mice by -0.806 ng/ml.

Although in this study the two intervention groups have an influence on decreasing cortisol hormone levels, the greater amount of decrease is in ginger honey II showing that this combination has good synergy to influence the decrease in cortisol hormone levels, and the influence of ginger is more dominant.

IV. CONCLUSION

This study concludes that the administration of ginger honey and cocktail honey can influence the decrease in cortisol hormone levels and can be used as a supplement to deal with stress. In this study it is found that ginger honey II at a dose of 28mg / 20gBW / day has a positive effect on reducing the cortisol hormone by -1.418 ng/ml. In cocktail honey II at a dose of 28mg/20gBW/day, it has a positive effect on reducing the hormone cortisol by -0.806 ng/ml. Ginger honey has more effect on decreasing the hormone cortisol level than cocktail honey.

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