International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 04, 2020
ISSN: 1475-7192

# The Ginger Honey And Cocktail Honey Supplementation Effect On *Estradiol* Hormone Levels In Female *Balb/C* Mice Stress-Induced Through Swimming Activities

Emmasitah, Indah Raya, Andi Nilawati Usman,
Latifah Rahman, Andi Dirpan

***Abstract---*** *This study intends to determine the effect of Ginger Honey and Cocktail Honey on Estradiol (E2) hormone levels, and to assess the magnitude of the difference in the effects of Ginger Honey and Cocktail Honey on Estradiol levels in female Balb/c mice induced stress. A total of 25 female Balb/c mice were used in this study and were divided into 4 groups (5 in each group) with 1 control group (negative control), 2 intervention groups Ginger Honey: Ginger Honey I (containing 50% Honey: 50% ginger extract), Ginger Honey II (33% Honey: 67% ginger extract) a dose of 28mg/20gBW of mice, and Cocktail Honey Group (trigona spp. honey, royal jelly and bee bread) with the distribution of Cocktail Honey I (14mg/20gBW), Cocktail Honey II (28mg/20gBW) given 1 time/day for 14 days. Estradiol levels were examined using the ELISA kit mouse. This study was an in vivo laboratory test with a pretest-posttest design with a control group. The data were displayed as the mean and analyzed using a paired T-test. This study shows that the intervention of Ginger Honey I (50% Honey: 50% ginger extract), Ginger Honey II (33% Honey: 67% ginger extract) at a dose of 28mg/20gBW of mice, and Cocktail Honey (honey, royal jelly and bee bread) with a dose distribution Cocktail Honey I (14mg/20gBW), Cocktail Honey II (28mg/20gBW) have a positive effect and statistically have a significant value on the levels of Estradiol in female Balb/c mice induced stress when compared to control group. The statistical results both shows $P<0.05$. However, between both, the greatest effect on increasing levels of Estradiol is shown by the Cocktail Honey I group of 19,466 ng/ml. This study concluded that among all the intervention groups, both Ginger Honey and Cocktail Honey has great influence on the hormone Estradiol. However, Cocktail Honey I has the greatest effect on Estradiol hormone levels.*

***Keywords---*** *Estradiol levels; Ginger Honey; Cocktail Honey; Reproductive Hormone; female Balb/c mice; swimming activities*

## I. INTRODUCTION

Estrogen is the main female sex hormone, and *Estradiol* (E2) is the most dominant type of estrogen in reproductive women. E2 has a role in regulating the reproductive processes and menstrual cycles in women (Ryan, 1982; Sherwood, 2016). Adverse effects may occur due to estrogen hormone imbalance, where high estrogen exposure in women can trigger ovarian cancer, thyroid cancer, endometriosis and PMS (Premenstrual Syndrome)

E*mmasitah, Midwifery Study Program, Graduate School, Hasanuddin University, Indonesia. E-mail: emmasitah@pasca.unhas.ac.id*
*Indah Raya, Chemistry Department, Faculty of Mathematics and Natural Science, Hasanuddin University, Indonesia. E-mail: indahraya05@gmail.com.*
*Andi Nilawati Usman, Midwifery Study Program, Graduate School, Hasanuddin University, Indonesia.*
*Latifah Rahman, Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Indonesia.*
*Andi Dirpan, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Indonesia.*

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

(Ray, Kushnir, Bunker, Rockwood, & Meikle, 2012). This can also be one of the causes of risk factors that increase breast cancer (Jasienska, 2001; Ray et al., 2012; Rosner, Hankinson, Sluss, Vesper, & Wierman, 2013; Yager & Davidson, 2006; Yu et al., 2003). On the other hand, the impact of estrogen deficiency in young women increases the risk of reduced bone density, especially in women who have POI (Primary Ovarian Insufficiency) (Popat et al., 2009). Low estrogen levels in women are also known to have a correlation with depression (Soyupek, Ayhan, Ceceli, & Yorgancioglu, 2006), and are closely related to hypogonadism that can causes infertility in women (Barut et al., 2018).

The imbalance of estrogen levels in women can be treated by using pharmacological therapies namely HRT (Hormone Replacement Therapy) and non-pharmacological therapy. Alternative substitute for HRT that can be used as a non-pharmacological therapy is honey (Zaid, Sulaiman, Sirajudeen, & Othman, 2010). Kaempferol is a type of flavonoid contained in honey, and is known to have an estrogenic effect in ovariectomy mice (Trivedi et al., 2008). In the use of Manuka Honey which is monitored using proliferation test in culture MCF-7 breast cancer cells, showing the presence of estrogenic activity. This is due to the high concentration of antioxidants from Manuka honey which causes inhibition of cell growth (Henderson, Aldhirgham, Nigam, & Owusu-Apenten, 2016).

Royal Jelly (RJ) is one of the products produced by honey bees that has been extensively studied for its content on estrogen levels in humans and animals. There are four unsaturated fatty acid compounds contained in RJ namely (10H2DA, 10HDA, 2DEA and 24MET) which indicate the activity of estrogen receptor *(ERs) β-binding*. The fatty acids contained in RJ act as intermediaries of estrogen signaling in targeting genes through modulation of *ERα* (Estrogen Receptor alpha), *ERβ* (Estrogen Receptor beta) and co-activators (Moutsatsou et al., 2010; Suzuki et al., 2008). The 10H2DA compound increases ovulation hormone synthesis, maintains lower expression of FSH and LH in young ovarian cells. In addition, it is efficient in increasing hormone regulation, and prevents depletion of follicular assemblages (Takahashi, 2012).

Bee bread is another product of honey bees that is rich in flavonoids and polyphenols as antioxidants (Waykar & Alqadhi, 2016). The content of flavonoids commonly found in bee bread is kaempferol, quercetin, and chlorogenic acid (Kieliszek et al., 2018). Higher levels of polyphenols in bee bread act as reductone in bee bread where these compounds can react with free radicals, then convert them into more stable products and end radical chain reactions (Oh, Jo, Cho, Kim, & Han, 2013).

Ginger is also known to have an effect in maintaining hormone balance. In a study showed that ginger extract significantly reduced the serum concentrations of LH and the hormone estrogen, and increased serum FSH and progesterone levels in the group receiving treatment compared to the PCOS group who did not receive treatment (Atashpour, Kargar Jahromi, Kargar Jahromi, & Maleknasab, 2017; Mokhtari, Reza, & Harfsheno, 2014). Ginger is rich in antioxidants that can protect the body from free radical activity, so it can prevent oxidative stress which is one of the causes of infertility (Fadaki, Modaresi, & Sajjadian, 2017; Rhode et al., 2007; Sook, Young, Kang, & Kyung, 2008).

The number of studies on the benefits of honey and ginger for women, makes researchers interested in conducting research with the treatment of honey with the novelty of using Ginger Honey which is a combination of ginger and honey, and Cocktail Honey which is a combination of honey bee products namely royal jelly, bee bread

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

and honey which is still rarely given as a treatment in maintaining the balance of *Estradiol* levels in both experimental animals and women.

The purpose of this study is to determine the effect of Ginger Honey and Cocktail Honey supplementation on *Estradiol* levels, and to assess the magnitude of the difference in effect on *Estradiol* levels in female *Balb/c* who consume Ginger Honey and Cocktail Honey.

## II. METHODOLOGY

**Research Design**

This study was an *in vivo* laboratory research with a pretest-posttest control group research design.

**1. Ginger extraction**

This study used ginger from Camba District, which is the type of emprit ginger (*Zingiber officinale var.amarum*), as much as 12kg. Ginger was cleaned using running water and roughly chopped. After that, it was dried in a natural way under indirect sunlight until dry and resemble like crackers. Then, ginger was mashed using a standard blender. For the process of extracting ginger, the maceration method was used using 70% ethanol solution for 7 days. Ginger has been extracted then separated between the liquid with solid, then the liquid was evaporated using a rotary evaporator so that is obtained as much as 40g. The ginger maceration process was carried out at the Chemistry Phytoplankton Laboratory of Hasanuddin University, and the ginger evaporation process was carried out at the Biopharmaca Laboratory of Hasanuddin University Research Activity Center.

**2. Ginger Honey Production**

The combination of Ginger Honey is produced from thick ginger extract and *trigona spp.* honey which are then mixed and homogenized using a magnetic stirrer. This process is divided into 2 groups. Ginger Honey I is a mixture of 50% honey: 50% ginger extract, and Ginger Honey II is a mixture of 33% honey: 67% ginger extract. The usual dose of honey given to the experimented rats is 200mg/kgBW, which is then converted to a dose of mice to get 28mg/20gBW.

**3. Cocktail Honey Production**

The production of Cocktail Honey includes mixing *trigona spp.* honey, royal jelly, and bee bread which are then homogenized using a magnetic stirrer. Cocktail Honey is then divided into 2 groups: Cocktail Honey I is a mixture with a low dose (14mg/20gBW), and Cocktail Honey II is a mixture with a moderate dose (28mg/20gBW).

**4. Animal**

Animal subjects were maintained and handled in accordance to the protocol approved by the Hasanuddin University Medical Ethics Committee with protocol number: UH19110935. Mice were obtained from the Biopharmacy laboratory, Faculty of Pharmacy, Hasanuddin University. Before being given the intervention, mice were left to adapt with the group for 7 days. The trial is based on the guidelines from the research guidelines for evaluating the safety and efficacy of herbal medicine. The mice used were female *Balb/c* mice.

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

During the adaptation the mice were in a standard condition with 12 hours cycle of exposure to light and 12 hours in the dark, with an average temperature of 22-28 ± ˚C.

Inclusion criteria were 8-12 weeks old, 20-30 grams of weight, never been pregnant before, healthy and active. Whereas the exclusion criteria were the sick mice during the adaptation or mice died before the last blood draw (end of treatment). The number of mice used was 25 and divided into 4 groups. The mice were given a standard diet and an adequate drink *ad libtum.* Before the initial blood sampling (pretest), examination of vaginal smears was performed to determine the estrous cycle in mice to ensure that the estrous cycle phase of the mice was in the same phase before the blood was taken. The mice were then let to swim prior to the initial blood test (pretest) taken to determine the *estradiol* levels. The mice were then intervened and induced-stress by swimming until they showed signs of stress. The interventions used gastric sonde in accordance to grouping for 14 days with 1 time giving/day. On the 15th day the blood sampling was repeated to determine the level of the hormone *estradiol* (posttest).

**5. Treatment of Research Subjects**

Ginger Honey I: the mice were left to swim until they showed any signs of stress before they were given the Ginger Honey I (50% honey: 50% extract ginger) at a dose of 28mg/20gBW mice once a day for 14 days. Ginger Honey II: the mice were left to swim until they showed any signs of stress before they were given the Ginger Honey II (33% honey: 67% extract ginger) at a dose of 28mg/20gBW mice once a day for 14 days.

Cocktail Honey I: the mice were left to swim until they showed any signs of stress before they were given the Cocktail Honey I (*trigona spp.* honey: royal jelly: bee bread) at a dose of 14mg/20gBW mice once a day for 14 days. Cocktail Honey II: the mice were left to swim until they showed any signs of stress before they were given the Cocktail Honey II (*trigona spp.* honey: royal jelly: bee bread) at a dose of 28mg/20gBW mice once a day for 14 days. Control Group (without interventions): the mice were left to swim until they showed any signs of stress then they were allowed to rest and given standard water and food for 14 days.

## III. ANALYSIS DATA

### 1. Vaginal Swab

Vaginal swab was performed before the pretest examination of *estradiol* levels after posttest blood sampling. It aims to determine the estrous cycle in mice, to ensure mice are not ovulating. Vaginal fluid in mice was taken using a cotton bud that had been moistened with 0.9% NaCL. After that, the cotton bud was smeared on the slide and then dropped with methylene blue and allowed to dry (3-5 minutes). The slide was then rinsed with water, and allowed to dry. After the slide was dry, the vaginal swab was observed using a microscope, and the stage of the reproductive cycle was determined through cytological images.

### 2. Hormone Measurement

Checking the *estradiol* levels for pretest was conducted by taking 0.5ml of blood of mice in the eyes or tail for all groups before giving the intervention. Subsequently, activity and intervention were given for 14 days. On the 15th day 0.5ml of blood from the eyes or tail of all groups was retaken as a posttest. In this study, a test was conducted using the R & D method of the Mouse *Estradiol* Enzyme Linked Immunosorbent Assay

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

(ELISA) to measure *estradiol* levels. The blood collected was then stored in an EDTA tube before it was centrifuged at 4000 RPM for 15 minutes at 24°C.

All equipment and reagents were then prepared, before adding 50µL standard to standard well, 40µL plasma sample to sample well, 10µL anti-E2 antibody to sample well, and 50µL streptavidin-HRP to the sample well and standard well respectively before all the parts were mixed well. The well was closed with the provided adhesive strip. The incubation process was carried out for 1 hour at 37$^{o}$C. Afterwards, the well cover was opened and washed by soaking the well using a wash buffer of ± 0.35ml for 30-1 minutes. The washing process was repeated 5 times. After the final washing, the remnants of the wash buffer were removed and dried using a tissue. Then 50µL of substrate A solution was added to each well, followed by the addition of 50µL of substrate B solution to each well. The incubation was repeated by closing the well using a new cover for 10 minutes at 37$^{o}$C in the dark. Subsequently, 50µL Stop Solution was added to each well, where the blue color will quickly turned yellow. The optical density of each well was then determined using a microplate reader that had been set to 450 nmwithin 10 minutes after the stop solution was added.

### 3. Statistical Analysis

Data was displayed in the form of *mean* and tested using paired t-test. The effect of Ginger Honey, Cocktail Honey, and *estradiol* level was shown in the form of mean ± (standard deviation) with confidence interval (95% CI). Before a statistical test was performed, a normality test was performed first. Data were normally distributed so the bivariate paired T-test was used to see differences in pre and post *estradiol* levels in each Ginger Honey, Cocktail Honey and control groups.

## IV. RESULT

### 1. The Hormone *Estradiol* Levels in Female *Balb/C* Mice

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

| Intervention Day | ESTRADIOL LEVELS | | | | |
|---|---|---|---|---|---|
| | **GH1 (50%Honey: 50%Ginger, dose 28mg/20gBW/Day) Mean ±SD** | **GH2 (33%Honey: 67%Ginger, dose 28mg/20gBW/Day) Mean ±SD** | **CH1 (14mg/20gBW/ Day) Mean ±SD** | **CH2 (28mg/20gBW/ Day) Mean ±SD** | **Control Mean ±SD** |
| ***Pretest*** | 4.142 ±2.970 | 12.507 ±4.169 | 2.875 ±2.678 | 13.142 ±3.725 | 1.969 ±0.936 |
| ***Posttest*** | 22.443 ±1.015 | 28.203 ±2.867 | 22.341 ±1.992 | 29.017 ±2.358 | 6.726 ±4.687 |
| **Different Mean** | 18.300 | 15.696 | 19.466 | 15.874 | 4.756 |
| ****P*** | 0.004 | 0.001 | 0.001 | 0.001 | 0.095 |

****Paired T-test***

Based on the data shown in table I, it shows that among all the intervention groups in the form of Ginger Honey and Cocktail Honey and control that had the most influence on the hormone *Estradiol*, namely the Cocktail

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

Honey I and Ginger Honey I groups. The statistical analyses of both groups showed a P<0.05. However, the highest influence in increasing the *estradiol* levels is shown by the Cocktail Honey I group of 19.466 ng/ml.

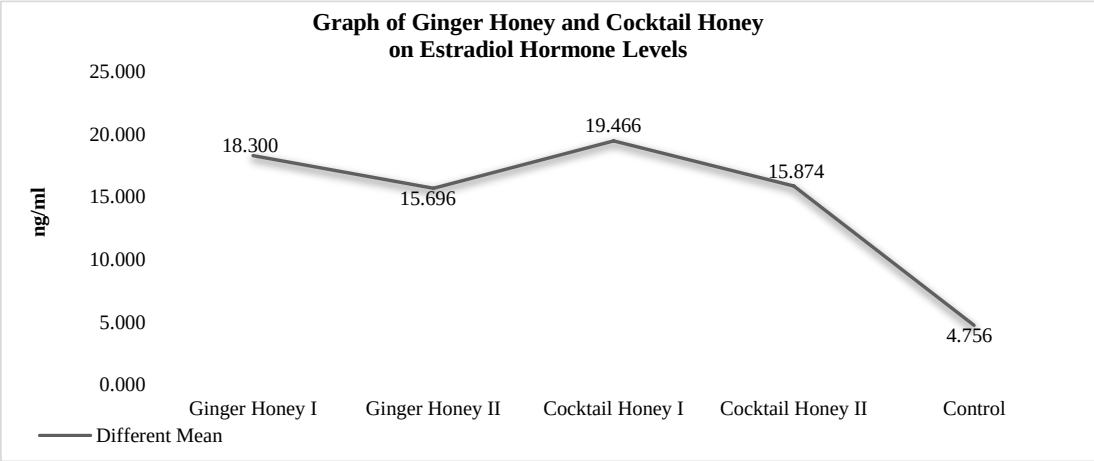
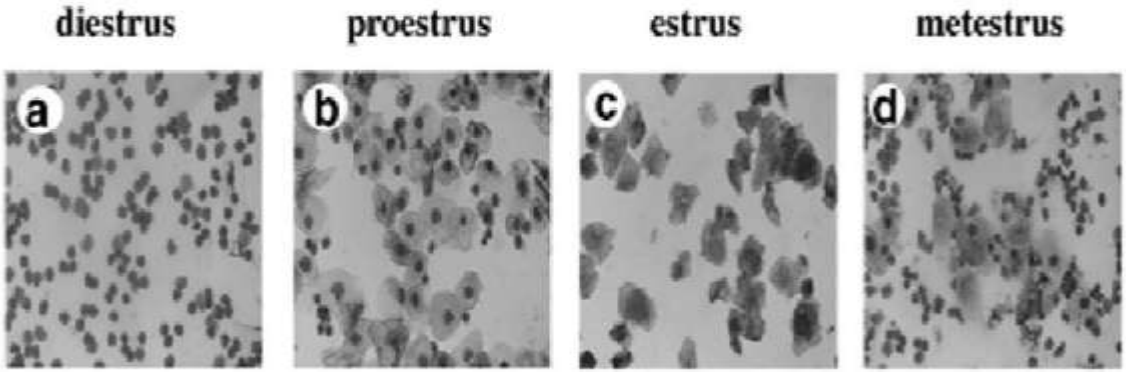



Figure 1. Different Means Graphic on Ginger Honey, Cocktail Honey dan controlled group.

Figure 1 shows the different mean from the effect of Ginger Honey and Cocktail Honey administration on changes in *estradiol* levels in each group, both in the intervention group and the control group. When compared with other groups, the control group is the group that experienced the lowest changes, where there is a decrease in the level of the hormone *estradiol*.

## 2. The Results of Examining The Stages of The Reproductive Cycle on The Subject

In the examination of the reproductive cycle using vaginal smears, 2 cycles were obtained, namely the diestrus cycle and the proestrus cycle. Pretest blood sampling from the group with the proestrus cycle was carried out on the same day as the vaginal swab, while pretest blood taking in the group with the diestrus cycle was performed 2 days after the initial vaginal smear examination. This aims to ensure that all samples are in the stages of the proestrus cycle at the time of the pretest blood collection, so that the samples in this study are in the same cycle, and not in the ovulation cycle.




*** Cytological images stage of the reproductive cycle of the subject**

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

## V. DISCUSSION

It is well known that research on handling *estradiol* (E2) levels by using honey or ginger has been carried out numerous times. However, the use of supplementation by combining ginger and *trigona spp.* honey, and mixing *trigona spp.* honey, royal jelly and bee bread for handling the E2 hormone problem and stress is still rarely done. A study conducted by Atashpour *et al* exhibited how ginger extract had a better effect in increasing *estradiol* levels. This is consistent with the results of this study, where the administration of extracts of ginger and honey showed increased levels of E2 in stressed mice.

Sports and physical activity are body movements that can improve health status and contribute to the prevention of diseases, but excessive physical activity can also increase stress. Stress level depends on the type of exercise, intensity and duration of the exercise. Therefore, exercise is also known as one of the stressors in humans and animals. Stress is known to be physiologically able to cause changes in metabolism, reproduction, cardiovascular system, breathing, digestion, and kidney (Contarteze, Manchado, Gobatto, & De Mello, 2007).

In recent years, around 6-79% of female athletes have reproductive abnormalities, including delayed menarche, amenorrhea, and oligomenorrhea (Frolich MA, Banks C, Warren W, Robbins M, 2016). Additionally, women who engage in more physical activities have lower ovulation rates (Olive, 2010). Female swimmers tend to experience delayed puberty and menstrual disorders (Roosevelt, 2014) which is considered conclusive in exhibiting how swimming is known to have a negative impact on fertility (Jana et al., 2014; Roosevelt, 2014). Therefore, in the study the swimming intervention was given as physical activity in female mice.

In a study conducted by Nazanin *et al*, it was found that swimming for 2 weeks can reduce E2 levels. Thus, swimming for long periods of time can have an adverse effect on fertility. This, in line with the results of research obtained by researchers. In the control group, there was a decrease in levels of E2 in female *Balb/c* mice conditioned in a stressful state by swimming. Meanwhile, in the intervention group both by giving Ginger Honey and Cocktail Honey statistically there is a significant increase in E2 levels. Also noted, low levels of E2 and corticosterone (a hormone that plays a role when stress occurs) synergistically contribute to increasing one's susceptibility to oxidative stress (Al-Rahbi, Zakaria, Othman, Hassan, & Ahmad, 2014; Aschbacher et al., 2014).

Royal jelly is one of the honey bee products that has the potential to treat various diseases in humans. One of aforementioned potentials is the nature of its biological activity as an antioxidant. Research conducted both *in vivo* and *in vitro* shows that royal jelly has estrogenic activity. Components in royal jelly have been shown to interact with estrogen receptors. The main active component contained in RJ is 10-hydroxyl-2-decenoic acid (10H2DA). This compound is known to increase ovulation hormone synthesis, maintain lower FSH and LH in ovarian cells, and improve hormonal regulation. Additionally, it contributes in the prevention of the aging process, and it is an antiaging product (Takahashi, 2012) due to certain lipids (10H2DA, 10HDA, trans-2-decenoic acid and 24-methylenecholesterol) it contains (Mishima et al., 2005; Suzuki et al., 2008). In line with the results of this study, it is found that the Cocktail Honey I group, which is treated with a mixture of honey, royal jelly and bee bread, shows a positive effect on increasing higher E2 levels, 19.466 ng / ml.

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

A study conducted by Mishima *et al* provided evidence that Royal Jelly (RJ) has estrogenic activity with estrogen receptor interactions and is followed by endogenous gene interactions. RJ also has the potential to reduce DNA damage by adding antioxidants. In addition, RJ also rebalances hormone levels in the blood, FSH, and increases estrogen concentrations in old rats. Provision of natural antioxidants can be a preventive measure for several health problems such as inflammation, allergies, diabetes, cardiovascular (Ahmed et al., 2018; Erejuwa et al., 2011; Petelin et al., 2019), as well as increasing estrogen levels in the menstrual cycle when facing large amounts of ROS phase. Estrogen can help collect antioxidants (Mumford et al., 2016), reduce oxidative stress (Azman, Zakaria, Abdul Aziz, & Othman, 2016), and provide a calming and anti-anxiety effect (Fadaki et al., 2017). This study found that the intervention group Cocktail Honey I (a combination of *trigona spp.* honey, RJ, and bee bread) with a dose of 14 mg/day given for 14 days exhibits the most significant increase of E2 levels of all the other intervention groups. In contrast, the control group without intervention shows a decrease in E2 levels.

Ginger (Zingiber officinale) is rich in various bioactive compounds such as phenolic, flavonoids, vitamins, carotene, terpene, polysaccharides, lipids, organic acids, and raw fiber, so that ginger has a role in improving health (Ghasemzadeh, Jaafar, & Rahmat, 2010). Phenolic compounds in ginger especially gingerol, shogaol, and paradol with various bioactivity has health benefits. The pharmacological activity of ginger correlates with its active phytocomponent compounds, 6-gingerol, 6-shogaol, zingerone, phenolics and other flavonoids (Danciu et al., 2015; Ha et al., 2012). The most reported bioactive compound in ginger is 6-gingerol, with various pharmacological effects including antioxidant, analgesic, anti-inflammatory and antipyretic properties (Dugasani et al., 2010; Kundu & Surh, 2009). Several studies have proven that ginger has an effective protection against oxidative stress (Akinyemi, Ademiluyi, & Oboh, 2013; Ji et al., 2017). A study shows that ginger plants with flavonoid content and phenolic compounds can form a natural balance between estrogen and progesterone through its specific pharmacological-physiological effects. Ginger can balance the increase or decrease in sex hormones (Liu et al., 2004).

Intervention of Ginger Honey I (50% honey: 50% extract ginger), and Ginger Honey II (33% honey: 64% extract ginger) with a dose of 28mg/20gBW of mice, and Cocktail Honey (honey, RJ and bee bread) with a dose of Cocktail Honey I (14mg/20gBB), and Cocktail Honey II (28mg/20gBW) with intervention time for 14 days have a positive effect and, statistically, have a significant value on the level of *Estradiol* in female *Balb/c* mice that induced stress.

## VI. CONCLUSION

Through this study, the Ginger Honey and Cocktail Honey show the greatest influence on *Estradiol* levels, both of which shows $P<0.05$. However, the greatest influence is shown by the Cocktail Honey I group, which is 19.466 ng/ml. The results of this study indicate that the intervention of *trigona spp.* honey, royal jelly, bee bread and ginger is known to have antioxidant content, affecting the levels of *Estradiol* in female *Balb/c* mice conditioned under stress for 14 days by swimming. Thus, the supplementation of Ginger Honey and Cocktail Honey can be used as a complementary hormonal therapy, and helps in preventing oxidative stress in women.

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 

## Acknowledgment

The authors would like to thank the laboratory staffs of Hasanuddin University Teaching Hospital where the study took place.

## REFERENCES

[1] Ryan KJ. Biochemistry of Aromatase: Significance to Female Reproductive Physiology. aacrjournals. 1982;42(8).

[2] Sherwood L. FISIOLOGI MANUSIA : DARI SEL KE SISTEM. En: Ong HO, Mahode AA, Ramdhani D, editores. 8.ª ed. Jakarta: ECG; 2016.

[3] Ray JA., Kushnir MM., Bunker A., Rockwood AL., Meikle AW. Direct measurement of free estradiol in human serum by equilibrium dialysis-liquid chromatography-tandem mass spectrometry and reference intervals of free estradiol in women. Clin Chim Acta. 2012;413(11-12):1008-14.

[4] Jasienska G. Lifestyle, hormones, and risk of breast cancer. TT -. BMJ Br Med J. 2001;322(7286):586-7.

[5] Rosner W., Hankinson SE., Sluss PM., Vesper HW., Wierman ME. Challenges to the measurement of estradiol: An endocrine society position statement. J Clin Endocrinol Metab. 2013;98(4):1376-87, doi: 10.1210/jc.2012-3780.

[6] Yager JD., Davidson NE. Estrogen carcinogenesis in breast cancer N Engl J Med 354 (3): 270–282. Find this Artic online. 2006:270-82.

[7] Yu H., Shu XO., Shi R., Dai Q., Jin F., Gao YT., et al. Plasma sex steroid hormones and breast cancer risk in Chinese women. Int J Cancer. 2003;105(1):92-7, doi: 10.1002/ijc.11034.

[8] Popat VB., Calis KA., Vanderhoof VH., Cizza G., Reynolds JC., Sebring N., et al. Bone mineral density in estrogen-deficient young women. J Clin Endocrinol Metab. 2009;94(7):2277-83, doi: 10.1210/jc.2008-1878.

[9] Soyupek F., Ayhan F., Ceceli E., Yorgancioglu R. The evaluation of hand function in women: The important role of estrogen. J Back Musculoskelet Rehabil. 2006;19(4):103-9, doi: 10.3233/BMR-2006-19401.

[10] Barut MU., Çoksüer H., Sak S., Bozkurt M., Ağaçayak E., Hamurcu U., et al. Evaluation of Sexual Function in Women with Hypogonadotropic Hypogonadism Using the Female Sexual Function Index (FSFI) and the Beck Depression Inventory (BDI). Med Sci Monit. 2018;24:5610-8, doi: 10.12659/msm.910304.

[11] Zaid SSM., Sulaiman SA., Sirajudeen KNM., Othman NH. The effects of tualang honey on female reproductive organs, tibia bone and hormonal profile in ovariectomised rats - animal model for menopause. BMC Complement Altern Med. 2010;10, doi: 10.1186/1472-6882-10-82.

[12] Trivedi R., Kumar S., Kumar A., Siddiqui JA., Swarnkar G., Gupta V., et al. Kaempferol has osteogenic effect in ovariectomized adult Sprague-Dawley rats. Mol Cell Endocrinol. 2008;289(1-2):85-93, doi: 10.1016/j.mce.2008.02.027.

[13] Henderson K., Aldhirgham T., Nigam P., Owusu-Apenten R. Evaluation of Manuka Honey Estrogen Activity Using the MCF-7 Cell Proliferation Assay. J Adv Biol Biotechnol. 2016;10(3):1-11, doi: 10.9734/jabb/2016/29887.

[14] Moutsatsou P., Papoutsi Z., Kassi E., Heldring N., Zhao C., Tsiapara A., et al. Fatty acids derived from royal jelly are modulators of estrogen receptor functions. PLoS One. 2010;5(12):1-10, doi: 10.1371/journal.pone.0015594.

[15] Suzuki KM., Isohama Y., Maruyama H., Yamada Y., Narita Y., Ohta S., et al. Estrogenic activities of fatty acids and a sterol isolated from royal jelly. Evidence-based Complement Altern Med. 2008;5(3):295-302, doi: 10.1093/ecam/nem036.

[16] Takahashi Y. Molecular alterations during female reproductive aging: Can aged oocytes remind youth? Embryol - Updat Highlights Class Top. 2012, doi: 10.5772/35938.

[17] Waykar B., Alqadhi YA. Indian Journal of Pharmaceutical and Biological Research ( IJPBR ) Biological Properties and Uses of Honey : A Concise Scientific review. 2016;4(3):58-68.

[18] Kieliszek M., Piwowarek K., Kot AM., Błażejak S., Chlebowska-Śmigiel A., Wolska I. Pollen and bee bread as new health-oriented products: A review. Trends Food Sci Technol. 2018;71:170-80, doi: 10.1016/j.tifs.2017.10.021.

[19] Oh J., Jo H., Cho AR., Kim SJ., Han J. Antioxidant and antimicrobial activities of various leafy herbal teas.

Food Control. 2013;31(2):403-9, doi: 10.1016/j.foodcont.2012.10.021.
[20] Atashpour S., Kargar Jahromi H., Kargar Jahromi Z., Maleknasab M. Comparison of the effects of Ginger extract with clomiphene citrate on sex hormones in rats with polycystic ovarian syndrome. Int J Reprod Biomed. 2017;15(9):561-8, doi: 10.29252/ijrm.15.9.561.
[21] Mokhtari M., Reza ME., Harfsheno S. The effects of alcoholic extract of Marrubum vulgare on hormonal parameters in female rat model of polycystic ovarian syndrome. Med Sci. 2014;24(2):74-80.
[22] Fadaki F., Modaresi M., Sajjadian I. The Effects of Ginger Extract and Diazepam on Anxiety Reduction in Animal Model. 2017;51(3):159-62, doi: 10.5530/ijper.51.3s.4.
[23] Rhode J., Fogoros S., Zick S., Wahl H., Griffith KA., Huang J., et al. BMC Complementary and Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. 2007;9:1-9, doi: 10.1186/1472-6882-7-44.
[24] Sook H., Young E., Kang NE., Kyung W. [ 6 ] -Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. 2008;19:313-9, doi: 10.1016/j.jnutbio.2007.05.008.
[25] Contarteze RVL., Manchado FDB., Gobatto CA., De Mello MAR. Biomarkers of stress in rats exercised in swimming at intensities equal and superior to the maximal estable lactate phase. Rev Bras Med do Esporte. 2007;13(3):169-74, doi: 10.1590/S1517-86922007000300008.
[26] Frolich MA, Banks C, Warren W, Robbins M NT. The Association Between Progesterone, Estradiol, and Oxytocin and Heat Pain Measures in Pregnancy: An Observational Cohort Study. Anesth Analg. 2016;123(2):396-402, doi: 10.1213/ANE.0000000000001259.
[27] Olive DL. Editorial: Exercise and fertility: An update. Curr Opin Obstet Gynecol. 2010;22(4):259-63, doi: 10.1097/GCO.0b013e32833c7227.
[28] Roosevelt SL. in Swimmers : A Distinct. 2014;(July):2740-4.
[29] Jana K., Dutta A., Chakraborty P., Manna I., Firdaus SB., Bandyopadhyay D., et al. Alpha-lipoic acid and N-acetylcysteine protects intensive swimming exercise-mediated germ-cell depletion, pro-oxidant generation, and alteration of steroidogenesis in rat testis. Mol Reprod Dev. 2014;81(9):833-50, doi: 10.1002/mrd.22354.
[30] Nazanin S., Saadat S., Student MD., Mohammadghasemi F., Sajedi HR., Student MD., et al. Ovarian and uterine alterations following forced swimming: An immunohistochemical study. 2016;14(10):629-36.
[31] Al-Rahbi B., Zakaria R., Othman Z., Hassan A., Ahmad AH. Protective Effects of Tualang Honey against Oxidative Stress and Anxiety-Like Behaviour in Stressed Ovariectomized Rats. Int Sch Res Not. 2014;2014:1-10, doi: 10.1155/2014/521065.
[32] Aschbacher K., O'Donovan A., Wolkowitz OM., Dhabhar FS., Su Y., Epel E. Good Stress, Bad Stress and Oxidative Stress: Insights from Anticipatory Cortisol Reactivity. Psychoneuroendocrinology. 2014;38(9):1698–1708, doi: 10.1016/j.psyneuen.2013.02.004.
[33] Mishima S., Suzuki KM., Isohama Y., Kuratsu N., Araki Y., Inoue M., et al. Royal jelly has estrogenic effects in vitro and in vivo. J Ethnopharmacol. 2005;101(1-3):215-20, doi: 10.1016/j.jep.2005.04.012.
[34] Ahmed S., Othman NH., Shamim N., Baig AA., Jabeen S., Liaqat S., et al. Honey as a Potential Natural Antioxidant Medicine: An Insight into Its Molecular Mechanisms of Action. Oxid Med Cell Longev. 2018;2018:1-19, doi: 10.1155/2018/8367846.
[35] Erejuwa OO., Sulaiman SA., Wahab MSA., Sirajudeen KNS., Salleh MSM., Gurtu S. Differential responses to blood pressure and oxidative stress in streptozotocin-induced diabetic wistar-kyoto rats and spontaneously hypertensive rats: Effects of antioxidant (Honey) treatment. Int J Mol Sci. 2011;12(3):1888-907, doi: 10.3390/ijms12031888.
[36] Petelin A., Kenig S., Kopinč R., Deželak M., Černelič Bizjak M., Jenko Pražnikar Z. Effects of royal jelly administration on lipid profile, satiety, inflammation, and antioxidant capacity in asymptomatic overweight adults. Evidence-based Complement Altern Med. 2019;2019, doi: 10.1155/2019/4969720.
[37] Mumford SL., Browne RW., Schliep KC., Schmelzer J., Plowden TC., Michels KA., et al. Serum Antioxidants Are Associated with Serum Reproductive Hormones and Ovulation among Healthy Women. J Nutr. 2016;146(1):98-106, doi: 10.3945/jn.115.217620.
[38] Azman KF., Zakaria R., Abdul Aziz CB., Othman Z. Tualang Honey Attenuates Noise Stress-Induced Memory Deficits in Aged Rats. Oxid Med Cell Longev. 2016;2016, doi: 10.1155/2016/1549158.
[39] Ghasemzadeh A., Jaafar HZE., Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysia young ginger (Zingiber officinale Roscoe). Molecules. 2010;15(6):4324-33, doi: 10.3390/molecules15064324.
[40] Danciu C., Vlaia L., Fetea F., Hancianu M., Coricovac DE., Ciurlea SA., et al. Evaluation of phenolic profile, antioxidant and anticancer potential of two main representants of Zingiberaceae family against

B164A5 murine melanoma cells. Biol Res. 2015;48:1-9, doi: 10.1186/0717-6287-48-1.

[41] Ha SK., Moon E., Ju MS., Kim DH., Ryu JH., Oh MS., et al. 6-Shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. Neuropharmacology. 2012;63(2):211-23, doi: 10.1016/j.neuropharm.2012.03.016.

[42] Dugasani S., Pichika MR., Nadarajah VD., Balijepalli MK., Tandra S., Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J Ethnopharmacol. 2010;127(2):515-20, doi: 10.1016/j.jep.2009.10.004.

[43] Kundu JK., Surh YJ. Molecular basis of chemoprevention with dietary phytochemicals: Redox-regulated transcription factors as relevant targets. Phytochem Rev. 2009;8(2):333-47, doi: 10.1007/s11101-009-9132-x.

[44] Akinyemi AJ., Ademiluyi AO., Oboh G. Aqueous extracts of two varieties of ginger (Zingiber officinale) inhibit angiotensin I-Converting enzyme, iron(II), and sodium nitroprusside-induced lipid peroxidation in the rat heart in vitro. J Med Food. 2013;16(7):641-6, doi: 10.1089/jmf.2012.0022.

[45] Ji K., Fang L., Zhao H., Li Q., Shi Y., Xu C., et al. Ginger Oleoresin Alleviated γ -Ray Irradiation-Induced Reactive Oxygen Species via the Nrf2 Protective Response in Human Mesenchymal Stem Cells. Oxid Med Cell Longev. 2017;2017, doi: 10.1155/2017/1480294.

[46] Liu J., Burdette JE., Sun Y., Deng S., Schlecht SM., Zheng W., et al. Isolation of linoleic acid as an estrogenic compound from the fruits of Vitex agnus-castus L. (chaste-berry). Phytomedicine. 2004;11(1):18-23, doi: 10.1078/0944-7113-00331.

[47] Yao, L., Romero, M.J., Toque, H.A., Yang, G., Caldwell, R.B., Caldwell, R.W. The role of RhoA/Rho kinase pathway in endothelial dysfunction (2010) Journal of Cardiovascular Disease Research, 1 (4), pp. 165-170.

[48] Ozaifa kareem, nasir nisar, nahida tabassum (2017) mu-opioid receptor antagonists and their role in treatment of chronic constipation. Journal of Critical Reviews, 4 (5), 1-6. doi:10.22159/jcr.2017v4i5.22260

[49] Murthy, H.S.N., Meenakshi, D.M., Babaeizadeh, S., Laciar, E.ANN, SVM and KNN classifiers for prognosis of cardiac ischemia-A comparison(2015) Bonfring International Journal of Research in Communication Engineering, 5 (2), pp. 7-11.

[50] Hameroff, S.Orchestrated reduction of quantum coherence in brain icrotubules: A model for consciousness (2007) NeuroQuantology, 5 (1), pp. 1-8.

[51] B.Mahalakshmi, G.Suseendran "An Analysis of Cloud Computing Issues on Data Integrity, Privacy and its Current Solutions", Editor V. E. Balas et al. (eds.), Data Management, Analytics and Innovation, Advances in Intelligent Systems and Computing 839, 2019, pp.467-482, Doi: 10.1007/978-981-13-1274-8_35

[52] Dr. Jafar A. Alzubi, Dr. Omar A. Alzubi, Dr.G.Suseendran, Dr.D.Akila "+A novel Chaotic map encryption methodology for image cryptography and secret Communication with steganography- International Journal of Recent Technology and Engineering, Vol.8(1C2), May 2019, pp.1122-1128

[53] C. Diwaker, P. Tomar, A. Solanki, A. Nayyar, NZ Jhanjhi, et al., "A New Model for Predicting Component-Based Software Reliability Using Soft Computing," in IEEE Access, vol. 7, pp. 147191-147203, 2019. doi: 10.1109/ACCESS.2019.2946862

**DOI: 10.37200/IJPR/V24I4/PR201619**
**Received: 20 Jan 2020 | Revised: 22 Feb 2020 | Accepted: 28 Feb 2020** 