

The Changes of Mice Reproductive Hormones (*Mus musculus*) Caused by Mangosteen (*Garcinia mangostana* L.) Skin Extract

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ABSTRACT

Background. The population in Indonesia has been increasing from 2016 to 2018 and will continue to increase in 2019. The efforts of the Indonesian government to reduce the rate of population growth by establishing a family planning program for couples of childbearing age with the most widely used methods are injections and pills. However, the acceptor of birth control pills risk 17.2 times to cause hypertension, so it is necessary to develop alternative contraceptives. Mangosteen peel extract contains compounds that can inhibit the fertilization process.

Objective. This study aims to determine the effect of mangosteen peel extract on the process of folliculogenesis in female mice.

Methods. This study is a true experimental study with a post-test only - control group design. Mice were divided into five groups, the negative control group; a positive control group was mice given the combined contraceptive pill; group treated with three different doses of group, P1 was given mangosteen peel extract 9.8 mg / day; P2 was given mangosteen peel extract 19.6 mg / day; P3 was given mangosteen peel extract 39.2 mg / day for 21 days. Each group consists of 6 mice.

Results: This study shows that giving mangosteen peel extract at a dose of 9.8; 19.6 and 39.2 mg / 20 gBB had no effect on Luteinizing Hormone levels estradiol levels, progesterone levels ($p = 0.172, 0.195, 0.260$).

Conclusion: It can be conclude that mangosteen peel extract had no effect on reproductive hormones.

Keywords: *Garcinia mangostana* L, antifertility, folliculogenesis, reproductive hormones

BACKGROUND

The population in Indonesia has been increasing from 2016 to 2018 - 258,705,000, 261,890,900, and 265,015,313 residents and will continue to increase in 2019. The large population in Indonesia has not been matched by the quality of human resources (HR). High population growth also results in increasing demand for fulfilling needs, decreasing population quality, decreasing adequate consumption, decreasing work productivity, causing crucial problems related to employment.¹

The efforts of the Indonesian government to reduce the rate of population growth by establishing a family planning program for couples of childbearing age which functions to prevent pregnancy. The family planning method used in Indonesia is by injecting as much as 62.77% and 17.24% by pill. However, users of birth control pills are 17.2 times more at risk of developing hypertension.² so research is needed on contraceptive

alternatives that continues to be developed is the use of natural ingredients or medicinal plants as raw materials for making contraceptive drugs.

Mangosteen peel (*Garcinia mangostana* L.) is an abundant source of anti-oxidants and phenolic natural including xanthone and its derivatives, benzophenone, flavonoids, anthocyanins, and tannins compounds contained in mangosteen peel extract can inhibit the process of fertilization by interfering with the function of the ovaries, uterus, or vagina. Saponins have the effect of causing abortion in farm animals, lowering the surface tension of body fluids and hemolysis of blood cells.³ Tannin has a toxic effect on developing eggs cells. Xanton can disrupt the endocrine system by inhibiting the release of GnRH (Gonadotropin-releasing hormone), causing disruption in the release of FSH (follicle stimulating hormone) and LH (luteinizing hormone).FSH and LH play a role in stimulating the ovaries to release the hormones estrogen

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and progesterone which play an important role in the process of folliculogenesis and endometrial proliferation^{4,5}.

METHOD

This study is a true experimental study with a post-test only control group design. This research uses test animals namely mice (*Mus musculus*) which are divided into 5 groups. The negative control group (K-) is healthy mice; positive control group (K+) is mice given the combined contraceptive pill; group P1 was given mangosteen peel extract 9.8 mg / day; P2 group was given mangosteen peel extract 19.6 mg / day; group P3 was given mangosteen peel extract 39.2 mg / day for 21 days. Each group consists of 6 mice. Samples were executed using chloroform in the estrous phase. The treatment of mice was carried out at the Biology Laboratory of Universitas Negeri Semarang, and then blood was taken from the heart organ as much as 3 cc for examination of reproductive hormones namely luteinizing hormone (LH), estrogen hormone, and progesterone hormone conducted at the GAKI Laboratory Universitas Diponegoro Semarang.

RESULTS

Table 1. Effects of Mangosteen Skin Extract on Luteinizing Hormone Levels in Test Animals

Groups	n	LH levels (Mean ± SD) ng / L	ρ value
K-	5	50,17 ± 8,16	0,172
K+	5	46,64 ± 10,4	
P1	5	36,48 ± 10,0	
P2	5	47,52 ± 12,7	
P3	5	37,91 ± 9,50	

The results of the bivariate analysis with Anova test showed that there was no effect of mangosteen peel extract on LH levels in the estrous phase of mice with a ρ value of 0.172. The normal LH hormone parameter of mice used was the negative control group that was 50.17 ng / L and the parameter for the effect of infertility was the positive control group that was 46.64 ng / L. The level of positive control LH and the treatment group (Δ) showed a tendency to decrease the level of estrous phase LH, but these results did not show a significant effect. In the treatment group the dose one and three reached the cut-point of the positive control group as an effect of infertility although it had not shown significant results.

Table.2. Effects of Mangosteen Skin Extract on Estrogen Hormone Levels in Test Animals

Groups	n	Estrogen Levels (Mean ± SD) ng/L	ρ value
K-	5	20,62 ± 2,77	0,195
K+	5	14,23 ± 8,16	
P1	5	19,99 ± 1,89	
P2	5	17,79 ± 6,34	
P3	5	13,70 ± 9,50	

The results of bivariate analysis with the Anova test showed that there was no effect of mangosteen peel extract on estrogen levels in the estrous phase of mice with a ρ value of 0.195. The normal estrogen hormone parameters of mice used were the negative control group with 20.62 ng / L and the parameter for the effect of infertility was the positive control group with 14.23 ng / L. Estrogen hormone levels between groups showed a decrease in results when it was compared with the negative control group, but only in the dose three group that reached the cut-point of the positive control group as

an effect of infertility. Although there was a decrease in estrogen levels in the estrous phase, the difference in the dose of mangosteen peel extract which was given statistically had no effect on estrogen levels in mice.

Table.3. Effect of Mangosteen Skin Extract Against Progesterone Hormone Levels in Test Animals

Groups	n	Progesterone levels ng/ml (Mean ± SD)	ρ value
K-	5	4,45 ± 0,87	0,615
K+	5	4,87 ± 0,85	
P1	5	4,44 ± 0,45	
P2	5	5,41 ± 0,83	
P3	5	5,10 ± 0,82	

The results of bivariate analysis with Anova test showed that there was no effect of mangosteen peel extract on the level of the hormone progesterone in the estrous phase of mice with ρ value of 0.615. The normal progesterone hormone parameters of mice used were the negative control group at 4.45 ng / L and the parameter for the effect of infertility was the positive control group at 4.87 ng / L.

DISCUSSION

Luteinizing hormone levels

Surgery in this study was carried out in the estrous phase. In the estrous phase, the maturation happened to de graaf follicles and the process of ovulation in mice. The process of ovulation is the result of the mechanism of the hypothalamus, pituitary and ovary. The hypothalamus begins the process of ovulation by discharging Gonadotropin Releasing Hormone in a pulsatile manner. This release of pulsatile causes the anterior pituitary, releasing LH and FSH which then acts on the ovaries. Ovary growth occurs in 3 essential cells, namely theca cells, granulosa cells, and oocytes. LH stimulates theca cells to produce androstenedione. Androstenedione is then converted to estradiol through aromatase produced by granulosa cells. The conversion process to produce the hormone estrogen is stimulated by the hormone FSH. After reaching a critical concentration of estradiol, feedback stimulates the anterior pituitary to increase the LH (LH wave) which normally results in a decrease in estrogen.⁶ One of the times when high LH levels occur in the body is the midcycle phase or when ovulation occurs.⁷ This study illustrates the highest LH levels in the negative control group (normal mice). This is closely related to the reproductive cycle of mice under normal conditions so that when the execution is carried out in the estrous phase the LH level of the negative control group is classified as the highest level.

Decreased levels of LH occur in the negative control group given the combined contraceptive pill. Giving exogenous estradiol administration will trigger GnRH pulsation disorders in the hypothalamus, which interferes with the reproductive cycle. Exogenous estrogen in combined contraceptive pills has a mechanism to suppress follicle stimulating hormone and luteinizing hormone.

The treatment groups given mangosteen peel extract had various results. Giving a mild dose of 9.8 mg / mouse can reduce to near the cut of point effect of infertility (positive control group), but the middle dose of 19.6 mg / mouse above the cut of point had effect of infertility. This is according to the analysis of researchers; there are other factors that affect these varied results. But based on

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the analysis of phytochemical content of mangosteen peel, tannins and saponins were found. If tannin and saponin are consumed in large quantities, it will have effects that are not good for health.⁸ Saponin has an antiestrogenic effect which causes disruption of estrogen secretion so that it interferes with follicular development and ovulation processes. Saponins and tannins also have a cytotoxic effect on developing cells, for example ovum cells by damaging cell membranes, increasing cell permeability, and inhibiting granulosa cells.

Giving mangosteen peel extract in small doses can improve the reproductive function of test animals in male test animals.^{9,10} The dose given is 7 mg / kg or equivalent to 0.14 mg / mouse for 7 days can increase the number of spermatozoa, but with a dose of 100, 150 mg / kg can reduce the number of spermatozoa.⁹ Meanwhile other studies describe that giving of a dose of 25 mg / kg or equivalent to 0.5 mg / mouse for 35 days can improve sperm quality compared to 50 and 100 mg / kg.

Estrogen hormone levels

Research with rat illustrates that normal levels of the hormone progesterone were taken in the estrous phase are 5.7 ng / ml.¹² The results of the study of progesterone hormone levels in the second dose intervention group had the highest hormone levels of 5.41 ng / ml and the lowest hormone levels of 4.44 ng / ml in the first treatment group. The results showed that it did not vary in all groups and close to the normal progesterone values in test animals.

Progesterone along with estrogen is a steroid hormone that plays a role in the regulation of female sex hormones. Progesterone is also an indication of ovulation wherein the level of the hormone progesterone starts to rise compared to the follicular phase and reaches its peak in the luteal phase.^{4,7,10} Examination in humans shows progesterone levels around 1 ng / ml in the follicular phase and rises to around 4-5 ng / ml during ovulation and reaches the highest levels around 10-20 ng / ml during the luteal phase.⁷

Based on the analysis of researchers, this study shows that most groups have progesterone levels reaching 4 - 5 ng / ml, so it can be estimated that the execution is carried out in the estrous phase and ovulation occurs on average, this is evidenced by the presence of tertiary follicles formed during analysis of the number of follicles.

Research on the effects of mangosteen peel extract on progesterone levels is still limited. Research on mangosteen peel affecting influences that affect the reproduction of test animals is associated with pregnancy and saponin compounds. Phytochemically mangosteen peel contains saponins.^{11,12}

Saponins are compounds that play a role in cell defense. Saponins have a bitter taste, so they can irritate the lining of the mouth and stomach so that they can interfere with absorption of nutrients. Saponins can also stimulate abortion and zygote growth failure.⁵

Pre-clinical studies illustrate that progesterone levels decrease with the consumption of monosodium glutamate (MSG). Glutamate contained in MSG will naturally be stored in neurons when in excess conditions. This can stimulate the state of damage or neurotoxic. Such damage can interfere with hypothalamic and pituitary activity so that it can disrupt the reproductive system.¹³

CONCLUSIONS

Providing mangosteen peel extract (*Garcinia mangostana* L.) at a dose of 9.8; 19.2 and 13.2 mg / 20 g BW had no effect on;

- LH levels in female mice (*Mus musculus*) with a ρ value of 0.172. The average LH level was highest in the positive control group 50.172 + 8.16 ng / l and the lowest was in the two-dose treatment group with 36.482 + 10.08 ng / l.
- Estradiol hormone levels in female mice (*Mus musculus*) with a ρ value of 0.195. The average level of the highest estradiol hormone in the positive control group was 20,626 + 2,778 ng / l and the lowest was in the two-dose treatment group with 13,704 + 5.77 ng / l.
- Progesterone levels in female mice (*Mus musculus*) with a ρ value of 0.260. The average estradiol hormone levels were highest in the dose two treatment group 5.408 + 0.836 ng / ml and the lowest in the dose one treatment group with 4.440 + 0.456 ng / l.

So that further research is needed to determine the effectiveness of the mangosteen peel methanol extract (*Garcinia mangostana* L.) as an antifertility agent, it is recommended to increase the dosage of mangosteen peel extract by first doing the toxicity test. As well as modifying the treatment of research by marrying female mice with males to determine the effect of infertility in couples and determine the number of conditions produced by puppies.

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