



DIFFERENCES IN ESTROGEN LEVELS BEFORE AND AFTER GIVING CALLANGGI TEA TO WISTAR STRAINING WHITE FEMALE RATS (RATTUS NORVEGICUS) IN MEDROXYPROGESTERONE ACETATE (DMPA) INDUCTION

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ABSTRACT

Data from the 2017 Indonesian Demographic and Health Survey (IDHS) show that hormonal injection contraception has been the most common choice taken by women in more than 90 countries worldwide since 1967. Hormonal contraception uses essential ingredients from synthetic hormones used to regulate pregnancy. The 3-month injection KB (Depo-medroxyprogesterone acetate/DMPA) has the highest rating, 46.84%. DMPA thickens the cervical mucus, interfering with the ovulation process. The affects couples of childbearing age to get offspring quickly, causing stress where women often experience anxiety, depression, and prolonged fatigue that affect hormones and female reproductive organs.

Midwifery care has been carried out by combining conventional and complementary midwifery services, which have become an essential part of midwifery practice. Decree of the Minister of Health Number 1109/Menkes/Per/IX/2007 concerning Alternative Complementary Medicine. Complementary is complementary, perfecting. Complementary therapy is carried out to complement conventional medical treatment and is rational, and does not conflict with health values and laws in Indonesia. There are several methods of accelerating fertility recovery, one of which is using medicinal plants as an alternative, namely clover. The community widely uses clover for daily consumption. In this study, the method used as clover in a tea preparation is to be induced orally in female Wistar rats. Clover belongs to the water spike group, which contains isoflavones, antioxidants, and anti-inflammatories. The content of isoflavones in clover leaves can balance female hormones. The results of this research are beneficial for the development of science and technology, especially complementary therapy regarding the potential of clover as a natural ingredient that helps restore fertility.

Keywords: Depo Medoroxinprogesteron Asetat, Estrogen, Wistar Strain White Female Rat Model

INTRODUCTION

Family planning (KB) is one of the primary and main preventive health service efforts to support the achievement of maternal and infant health (BKKBN, 2015). Family planning plays a role in reducing the risk of death by preventing pregnancy, delaying pregnancy, spacing, limiting pregnancies, and determining when you want to get pregnant (WHO, 2018). According to the Government Regulation of the Republic of Indonesia Number 87 of 2014, to optimize the benefits of family planning for the health and welfare of the community, its services must integrate with existing reproductive health services. It is done because contraceptive use must consider various factors, namely health status, potential side effects, consequences of failure, and the risk of complications in reproductive health.

Data from the 2017 Indonesian Demographic and Health Survey (IDHS) show that hormonal injection contraception has been the most common choice taken by women in more than

90 countries worldwide since 1967. Hormonal contraception uses essential ingredients from synthetic hormones to regulate pregnancy, including one month of birth control, three-month birth control pills, and implants. The highest interest in acceptors for 3-month injectable birth control, namely 46.84% (BKKBN, 2015). The 3-month injectable birth control is DMPA or Depo Provera, which has a chemical structure like progesterone in the form of microcrystals suspended in an aqueous solution. The content of DMPA is a gestagen derivative of progesterone stored in the body in adipose tissue (Baziad, 2008). The dose is 150 mg, given every three months by intramuscular injection. This family planning works by thickening the cervical mucus (cervix), so it is difficult for sperm cells to reach the uterus and cannot fertilize the egg with 99% effectiveness.

DMPA contraception has limitations in its use, including changes in menstrual bleeding, weight gain, and fertility restoration on average 7-9 months after discontinuation. The effects of these limitations include the occurrence of serum lipids, decreased bone density, decreased libido, headaches, and vaginal dryness (JNPKKR, 2004). The mechanism of DMPA in controlling conception prevents ovulation by inhibiting follicular maturation and development. After bonding with the receptor, progestin will slow down the frequency of GnRH release from the hypothalamus. It will suppress pre-ovulatory LH, thereby preventing follicular maturation and inhibiting ovulation due to uncomposed LH, affecting cervical mucus thickening. DMPA causes the viscosity of cervical mucus to increase so that sperm penetration is disrupted. Changes also occur in the endometrium, namely undergoing an abortive secretory transformation which gradually becomes atrophic due to the transformation of the endometrial epithelium so that it does not allow implantation to occur. It also inhibits the transport of gametes by the tubes (slow motility) (Bakry, 2010).

Long-term use of DMPA causes a condition of hypoestrogenic, which causes a decrease in plasma antioxidant levels and an increase in lipid peroxidation, reducing estradiol levels in the early follicular phase compared to the normal premenopausal cycle (Faddah, 2005). It is due to the inhibition of gonadotropin release and inhibition of estrogen production in the ovaries (Melmed, 2011). Estrogen has one of the actions of protecting lipoproteins and oxidation. Estrogen in lipid peroxidation can affect like an antioxidant (Comelli, 2013). Prolonged use of DMPA causes an increase in oxidative stress, characterized by lipid peroxidation and decreased antioxidant defences in the body. Oxidative stress is associated with the cause of various diseases in humans, and administering high doses of progesterone is a source of oxidants for the body (Valko, 2007). Long-term use of DMPA disrupts estrogen, progesterone, FSH, and LH levels, which causes increased oxidative stress and decreases endogenous antioxidant defences' ability to inhibit ROS (Schieber, 2014).

Every couple has a plan for the right time to have children. The choice to become a 3-month injectable family planning acceptor is part of delaying having children to ensure a sufficient age gap after the birth of the previous child. Fertility will return after the acceptor stops using birth control injections, but it will not be immediately. The body needs time to restore fertility. It takes about 8-12 weeks for the progestogen to leave the body and 6-12 months for regular menstrual cycles. Hormonal birth control acceptors have a lower chance of getting pregnant than non-hormonal family planning acceptors. Conventionally, what needs to be done includes consuming a balanced nutritional diet, intake of vitamins, regular rest, and exercise (JNPKKR, 2004). In 2004 the Food and Drug Administration (FDA) placed DMPA contraception into a black box, but WHO gave a different statement, namely by providing age restrictions that are allowed use, namely the age group of 18-45 years.

Currently, the paradigm of health services has combined conventional and complementary services. Complementary medicine is generally regulated in the Decree of the Minister of Health Number 1109/Menkes/Per/IX/2007 concerning Alternative Complementary Medicine. Complementary is complementary, perfecting. Complementary therapy is carried out to complement conventional medical treatment and is rational, and does not conflict with health values and laws in Indonesia. There are several methods of accelerating fertility recovery after family planning acceptors, one of which is using medicinal plants as an alternative, namely clover. Red clover contains many isoflavone compounds such as genistein, daidzein, formononetin, and biochanin. A. Isoflavones are functional foods that include phytoestrogens. Isoflavone compounds act like female hormones, namely estrogen, which plays a vital role in reproductive health (Catherine, 2002). Based on research (Anisa, 2017) states that giving red clover extract at a dose of 30 mg/kg BW given for ten days gives a fundamental change in estrogen levels, supported by research (Carmignani, 2010) shows that giving it for 12 weeks at a dose of 90 mg /kg bb isoflavone significantly improved the histological structure of the vagina and administration of 70 mg/kg BW red clover ethanol extract for 15 days affected the estrus cycle (Mahardika, 2016). Estrogen protects against lipid peroxidation, such as antioxidants, and protects lipoproteins from oxidation. However, DMPA decreases estrogen levels, causing an increase in lipid peroxides, whose access can be seen by increased serum MDA levels (Cornelli, 2013).

Based on this background, I am interested in conducting research entitled "The Effect of Clover Tea on Estrogen Serum Levels, Estrus Cycles in Wistar Strain Female White Mice (*Rattus Norvegicus*) Induced by DPMA (Depo-medroxyprogesterone Acetate).

LITERATURE REVIEW

Fertile Period After Use of 3 Months Injection Contraceptive Devices (DMPA)

Family Planning (KB) is a government program for planning pregnancy. Married couples are encouraged to participate in this program for the common good, especially after having 1 (one) child, or to maintain a spacing between childbirths. However, sometimes there is a desire to stop for specific reasons. The question is how to fertilize the womb after using the family planning program (BKKBN, 2015).

Based on research (Hassan, 2007), the effect of using DMPA in experimental animals given an injection dose of 2.7 mg/BB affects side effects on reproductive health, one of which is ovulation disorders. The return of fertility can be known by menstruation returning regularly. The 3-month injectable birth control contains the hormone progestin, which prevents pregnancy by thickening the cervical mucus and preventing ovulation. During DMPA contraceptive injections, menstrual disorders can occur, such as irregular menstruation, spotting, excessive bleeding, or irregular menstruation (Baziad, 2008).

The return of fertility will be marked by regular menstrual cycles after stopping injecting birth control. Several factors affect when a woman can get pregnant again after stopping birth control. Of course, it will have an effect. In using a 3-month injection of KB (DMPA), regular menstrual cycles will return within six months - 1 year after the use of KB injections is stopped. The longer family planning is used before, the longer it will take to fertilize the womb again (Baziad, 2008).

The Effect of Clover on Fertility Recovery

Clover belongs to the class of phytoestrogens, and clover contains a lot of isoflavone compounds, such as formononetin, biochanin, and genistein. This isoflavone compound will act like a female hormone, namely estrogen, which can relieve various symptoms or side effects of the premenstrual period. In addition, some compounds act as anti-inflammatory or anti-inflammatories in the body, namely phosphorus compounds. The isoflavone compounds in clover act as a substitute for the hormone estrogen in the body, which helps relieve several symptoms in women, such as reducing anxiety and depression, facial redness, and several other symptoms.

Based on research (Anisa, 2017) states that giving red clover extract at a dose of 30 mg/kg BW given for ten days gives a fundamental change in estrogen levels, supported by research (Carmignani, 2010) shows that giving it for 12 weeks at a dose of 90 mg /kg bb isoflavone significantly improved the histological structure of the vagina and administration of 70 mg/kg BW red clover ethanol extract for 15 days affected the oestrous cycle (Mahardika, 2016).

METHODS

This study used an analytic research type with a pure experimental design on experimental animals of female white rats (*Rattus norvegicus*) who were given clover tea. Type of research design pre-post-test group design (Notoatmodjo S, 2012). She was giving intervention to white rats in DMPA induction. A sampling of hormone levels was carried out at the UMP Laboratory. The research period is eight months. The population of this study was *Rattus norvegicus* female white rats. The sample for this study was female white rats of the Wistar *rattus* Norwegians strain obtained from the UGM Faculty of Medicine Laboratory.

The tools and materials used in this study were rat cages, where each cage could accommodate ten rats. Rat food was given in all groups three times a day with the same amount and type.

The ingredients used are DMPA and clover tea. Wistar strain female white rats (*Rattus norvegicus*) aged at least eight weeks were kept in cages with sufficient humidity and light. Mice were divided into two groups randomly. Researchers measured levels using the ELISA method using blood serum samples. Data are presented as the mean (SD) if it is usually distributed and will be presented in quartile form if it is not normally distributed. Data were processed and analyzed using SPSS with a meaning limit of $p < 0.05$. The Shapiro-Wilk test was used to assess whether the samples were generally distributed because the number of samples was less than 50.

RESULTS AND DISCUSSION

The results of the study are as follows:

Univariate Analysis

Table 1. Estrogen Levels Before and After Intervention

Examination	Groups	n	Mean Eestrogen (pg/ml)	t	P
Pretest	Control (P0)	10	14.96±2.0468	0.654	0.361
	Treatment (P1)	10	13.21±2.2905		
Posttest	Control (P0)	10	14.07±1.7851	-10.277	0.000
	Treatment (P1)	10	29.33±2152		

Table 1 shows that the t-value of estrogen levels before the intervention was 0.0654, and the t-value after the intervention was -10.277.

Bivariate Analysis

Table 2. Estrogen Levels Between Control and Experiment Groups

Subject Group	Estrogen Level (pg/ml)		Mean Difference	t	P
	Pretest	Posttest			
Control (P0)	14.96±2.1468	14.07±1.7851	-0.89	-0.125	0.795
Treatment (P1)	13.21±2.2905	29.33±2152	-16.12	-14.195	0.000

Based on table 2, the t-value of estrogen levels in the control group is -0.125, and the t-value of estrogen levels in the intervention group is -14.195.

Clover tea can increase LH secretion, increasing estrogen levels in the blood. Semanggi tea contains isoflavones which stimulate it as a source of estrogen (phytoestrogens), namely non-steroidal compounds whose structure and function are similar to estrogen and derived from plants. It still needs to be determined how the mechanism of action of phytoestrogens is in increasing estrogen levels in the body. However, other studies suggest that providing foods containing phytoestrogens increases estrogen levels in the blood. The mechanism of action of phytoestrogens is to increase LH secretion. In this study, the experimental animals used were female Wistar rats who were given clover tea which has an estrogen agonist effect and increases blood levels of estrogen. Phytoestrogens are components that come from plants and have a structure and function similar to estrogen, so they can play a role in preventing menopausal symptoms. Phytoestrogen can be prevented as an alternative to hormone replacement therapy that shows low estrogen. There are many physiological functions of the hormone estrogen for the body, so the potential for phytoestrogens begins to develop at the specified concentration and dosage.

The ratio of progesterone to estrogen in DMPA injections is high, and the effect is to cause intermittent bleeding of varying duration. The endometrial lining receives signals from estrogen with fluctuating levels. Estrogen will trigger the proliferation of the endometrium to achieve abnormal and fragile immunity. The hormone estrogen only triggers the growth of the endometrial lining without the effect of progesterone which functions to maintain endometrial stability. Venous capillaries in conditions of endometrial proliferation will increase and dilate, causing bleeding. Progesterone discharge bleeding occurs in the endometrial lining exposed to both exogenous and endogenous phytoestrogens. The amount and duration of bleeding can vary according to the level and duration of estrogen stimulation in endometrial proliferation. During ovulation, estrogen production decreases to its lowest point. However, due to the influence of the hormone FSH, it will increase to 50pg/ml, continue to increase in line with the maturation of the ovum, and will reach a peak of 250-300pg/ml. In the luteal phase, estrogen levels will decrease to 125pg/ml. Progesterone produced by the corpus luteum and estrogen will provide negative feedback on the hypothalamus and anterior pituitary. Estrogen hormones that can be examined include estrone, estradiol, and estriol. Estrogen levels are checked to determine the ovarian axis, ovulation timing, menopause, and monitoring of fertility treatment.

CONCLUSION

Based on the results and discussion above, it is necessary to know that hormonal contraception uses the essential ingredients of synthetic hormones used to regulate pregnancy. There are several methods of accelerating fertility recovery, one of which is using medicinal plants as an alternative, namely clover. The community widely uses clover for daily consumption. The water spike group, of which clover is a member, includes plants that contain isoflavones, antioxidants, and anti-inflammatories. Clover leaves have a high isoflavone content, which helps to maintain hormonal equilibrium in women. Clover tea has the potential to act as an isoflavone, which has been shown to raise oestrogen levels in female rats. The use of natural materials can be developed in women's reproductive health efforts. It is necessary to research other reproductive parameters.

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