THE EFFECT OF POLYSACCHARIDES KRESTIN BIOACTIVITY FROM Coriolus versicolor TO ESTRADIOL LEVELS ON Mus musculus ESTROUS CYCLE

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ABSTRACT

This study aimed to determine the activity of polysaccharide krestin (PSK) from Coriolus versicolor extract to the duration of estrous cycle and estradiol levels. PSK was administrated for 24 days in female Mus musculus, strain Balb/C, 10 weeks old, weight about 25-30 grams. Polysaccharide krestin given in sub-chronic dose. There were 4 groups, i.e K0 (0 mg/kg BW), P1 (15 mg/kg BW), P2 (30 mg/kg BW), and P3 (60 mg/kg BW). Each treatment group contained 7 replications. Data were analyzed using One Way ANOVA and Duncan test at α = 5%. The duration of estrous cycle was determined by vaginal smear methods. The estradiol levels were measured using ELISA kit. The results show that the administration of PSK decreased the duration of estrous cycle with an average of 4.4 days. Polysaccharide krestin dose of 15 and 60 mg/kg BW did not affect the estradiol levels. Decrease of estrous cycle duration was still in the range of normal estrous time between 4-6 days. PSK dose of 15 and 60 mg/kg BW can be used as a therapeutic dose.

Keywords: polysaccharides krestin (PSK), Coriolus versicolor, estrous cycle, estradiol

INTRODUCTION

The usage of mushroom as anti-viral and anti-cancer has been proven. More than 50 species have been tested on animals. Coriolus versicolor mushroom or yun zhi been known at the time of the Ming Dynasty of China. The mushroom is classified in sub-class Homobasidiomycetes and family Polyporaceae (Ho et al., 2006).

Polysaccharopeptide can be obtained from C. versicolor, known as Coriolus versicolor polysaccharides (CVP) or polysaccharides krestin. The main component of PSK is β-glucan (Zhang et al., 2001). Polysaccharides krestin have many pharmacological activities, as an adjuvant in the immune system (Noguchi et al., 1995), increasing the number of leukocytes and macrophages (Wahyuningsih, 2006).

With the facts and the description above, the benefits of PSK from the mushroom C. versicolor is not in doubt. Nevertheless, PSK component is not necessarily safe. Excessive use may cause a bad influence. According Murtini et al. (2010), all the substances that enter into body would potentially be toxic materials. The toxicity of the material depends on the dose consumed and the long period of usage.

PSK is beneficial to increase the production of cytokines, such as interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-7 and IL-8 (Noguchi et al., 1995). Cytokines IL-1, IL-2, and IL-6 give a signal to the brain to activate the hypothalamic-pituitary-adrenal (HPA) axis in response to an immune stress. HPA axis actively stimulates the secretion of adrenocorticotropic hormone (ACTH). The ACTH stimulates the secretion of glucocorticoid. If the use of PSK in the long run, it will increase the secretion of gluco-corticoids and will suppress the HPG axis at a rate supra-pituitary. It will suppress gonadotropin-releasing hormone (GnRH) produced by the anterior pituitary. A decrease of GnRH will reduce production of the reproduction hormone, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). GnRH suppression can reduce the production of FSH and LH. Decrease of FSH production will inhibit the ovarian follicles maturation, thus it also inhibits ovulation. If ovulation is inhibited, it will extend the duration of the estrous cycle. LH decline will also affect the production of the hormone estrogen or estradiol (Kirby et al., 2009).

The estrous cycle is a period that is physiologically can receive a stud or a series of events related to the preparation of the uterus for the reception and implantation of the ovum. The estrous cycle can be divided into four stages: proestrus, estrus, metestrus, and diestrus. This cycle coincides with the maturation of ovarian follicles stimulated by FSH. FSH production stimulated by GnRH (Freeman, 1994; Sarkar et al., 1976).

Estrogen is a female reproductive hormone in animals. This hormone is mainly secreted by the granulosa cells of ovarian follicles constituent. Estrogen takes role on GnRH regulation. On the basis of this study wanted to test the effect of extracts of PSK from C. versicolor against the estrous cycle length and estradiol levels in mice (Mus musculus).
The Effect of Polysaccharides Krestin Bioactivity from Coriolus versicolor to Estradiol Levels on Mus musculus Estrous Cycle

METHODS

Animal materials
This research used female mice (Mus musculus), Balb/C strain, aged 10 weeks, weight about 25-30 g. Mice were acclimatized for 7 days for adjustment to the environment the animal cages.

Polysaccharide krestin extraction

Fruit body of C. versicolor can be foud grows on dead wood, or trees that were not productive during the rainy season. Fungal fruiting body was dried and made a coarse powder in a blender (Wahyuningsih et al., 2009). Coriolus versicolor mushroom extract production was done according to the method of Cui and Chisti (2003) and Wahyuningsih et al. (2009). This step would get dried mushroom extract. Mushroom extracts precipitated with 90% ammonium sulphate. In this process would produce a rough polysaccharopeptide. Further, it was diluted with PBS solution. The solution was dialyzed for 24 hours. This process would produce a solution of PSK (Wahyuningsih et al., 2009).

PSK treatment in experimental animals

PSK solution was given to female mice orally with repeated doses. Polysaccharides krestin was given for 5 times the estrous cycle or 24 days. There are four groups for treatment, ie 1. The control group (K, 0 mg/kg BW), 2. PSK group of 15 mg/kg BW (P1), 3. PSK group of 30 mg/kg BW (P2), and 4. PSK group of 60 mg/kg BW (P3).

Observation of animal estrous cycle length

The estrous cycle length was observed by vaginal smear per hour 8-10 am for 5 times from the time of the estrous cycle or 24 days (Cooper et al., 1993). Results obtained are determined phase and counted how many times the period of one full cycle at each repetition. Furthermore, the data were averaged.

Isolation of blood serum

Blood was collected from the heart. Blood put in a centrifuge and placed oblique. After that, it stored at 4°C overnight. Then centrifuged at 3000 rpm at temperature of 4°C for 15 minutes and taken the serum. Serum was stored at -20°C until the measured levels of estradiol.

Estradiol level measurement

Estradiol levels were measured using ELISA Kit (17β-estradiol mice, Elabscience). Serum estradiol levels were measured with a microplate reader, a wavelength of 450 nm. After knowing the value OD, then the standard curve was made. Estradiol levels were measured by standard curve.

Data Analysis

Data were analyzed statistically. Prior to the statistical test, all the data was tested first distribution normality and homogeneity of its variants. Data of normal and homogenous were tested by using one-way ANOVA. Differences between treatments were analyzed by Duncan test.

RESULTS

The mouse estrous cycle is 4-6 days. The information below refers to determining the stage of the estrous cycle in the mouse by the appearance of the vagina. Result of observation estrous cycles including changes in the vaginal epithelium of each phase can be seen in Figures 1, 2, 3, and 4.

Proestrus (Figure 1) was showed by the presence of nucleated epithelial cells that are round. However, sometimes these cornify rapidly, especially in mice. Estrus (Figure 2) was characterized by non-nucleated, cornified epithelial cells. Metestrus typically (Figure 3) had a low cell number, often with a lot of cell debris. Diestrus (Figure 4) contained mostly leukocytes.

Figure 5 shows the length of the estrous cycle time. Vaginal epithelium looks determined phase and then the data results of the phase count how many times the period of a full cycle. The study of the estrous cycle length showed shorter cycles. Analysis of the estrous cycle length showed cycles were getting shorter. In the control group and the treatment P1 showed estrous cycle length of 4.8 days, whereas the treatment group P2 and P3 long-cycle produces about 4.7 and 4.4 days. The statistical results showed that the treatment group was not significantly different from P3 to P2 group, but significantly different with group K0 and P1. The length of the estrous cycle in this research shortened in the treatment group P3, but it is still in the range of a normal cycle in mice. According Caligioni and Franci (2002), the length of the estrous cycle of a normal cycle in mice was between 4-5 days

Figure 6 shows the concentration of serum estradiol. Results of a study of 17β estradiol levels indicated that treatment group K0, P1, P2 and P3, respectively produced estradiol was 26094.92, 25162.89, 15609.56, and 21609.51 pg/mL. Measurement of 17β-estradiol levels in the control group did not differ significantly by treatment group P1, P2, and P3. This showed that there was no effect on female mice estradiol hormone levels due to treatment.

DISCUSSION

Research on the toxicity of polysaccharide peptide sub-chronic been done by Jian et al. (1999) in Cheng and Leung (2008) with a dose of (0, 1.5, 3, and 6 mg/kg BW) showed no toxic symptoms or no death in experimental animals. Sub-chronic effects of C. versicolor on organs, tissues, and cells had been widely studied by Cheng and Leung (2008). However, not much research related to the effects of C. versicolor specific toxicity to reproductive organs, particularly in the female reproductive organs.

Polysaccharide krestin of the mushroom C. versicolor was used as immuno-potential, can increase the production of cytokines, such as IL-1, IL-2, IL-4, IL-6, IL-7 and IL-8 (Noguchi et al., 1995). Interleukin-6 (IL-6) has been known to have several functions, among others regulate differentiation, stimulation, and activation of immune cells and some inflammatory reaction (Arzt, 2001, Gautron et al., 2003). Recent studies have also shown that IL-6 affects the secretion of various hormones and worked at
various levels of the HPA axis during the local inflammatory process (Bethin et al., 2000; Gautron et al., 2003). Interleukin-6 is mainly stimulates the release of the hormone prolactin (PRL), growth hormone (GH), ACTH, FSH and LH from the anterior pituitary (Renner et al., 1996). In addition, IL-6 regulates the function of the ovaries, including regulation of the LH receptor (LHR) on granulosa cells (Tamura et al., 2000) and also has an important role in folliculogenesis and oocyte maturation (Loret et al., 1998).

Mechanism of IL-6 in regulating the function of the ovaries and LHR is through the increase of the hormone FSH. FSH induces the expression of LHR mRNA through the Janus kinase (JAK) (Tamura et al., 2000). PSK as immuno-potential may increase the secretion of IL-6. Increased IL-6 will result in an increase in FSH and LHR mRNA. Follicle stimulating hormone is a hormone essential to the development of ovarian follicles (Ulloa-Aguirre et al., 2003). If this hormone increases will accelerate the estrous cycle and folliculogenesis (Hamburg, 2005). Increased LHR may cause increased excretion of LH by the anterior pituitary (Ascoli and Puett, 2008). Luteinizing hormone that increases will result in the acceleration time of ovulation (Pratap & Sameer, 2011).

Acceleration time of the phases of folliculogenesis and ovulation was what causes the shortening of the length of time the estrus cycle that occurs on the results of this study (Figure 5). It showed that the PSK in doses of...
15-60 mg / kg body weight increased the levels of IL-6 and FSH, which could shorten the estrus cycle. This is similar to study Tebar et al. (1998), the injection of FSH and LH increase levels of estradiol and shortening the length of time of the estrous cycle in mice. Although the estrous cycle on the research there was a decrease, but it was still within normal limits.

In this study, estradiol levels did not change after the administration of PSK from C. versicolor extract. This was in line with research Jian et al. (1999) that the provision PSK sub-chronic dose did not affect blood and serum biochemistry.

Based on data showed that at administration of PSK does not affect the length of estrous cycle. The research dose used PSK was a safe dose. It was a sub-chronic doses or a dose below the LD50. According to Chen and Leung (2008), the LD50 value of PSK was 26-300.36 mg/kg for administered by intraperitoneal injection. The highest daily tolerant dose was over 18-20 g/kg for mice.

Base on research, PSK dose of 15-60 mg/kg body weight did not affect the estrous cycle time, which was still within the normal range between 4-5 days. PSK dose of 15-60 mg/kg body weight did not affect the hormone levels of serum estradiol mice. It showed that PSK dose 15-60 mg/kg body weight was the optimal dose that could be used without affecting the reproductive system. If this dose used for therapy in humans, it should be tested with advanced testing and doing research on another reproductive parameters with higher doses for long term or equal to this study.

ACKNOWLEDGEMENT

Author would like to thank Amelia Permata Sari Putri who helped the research. The research was funded by DIPA BOPTN Airlangga University Research Activities Decentralisation through fiscal year 2014.

REFERENCES


