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UTERINE PERFORMANCE OF RATS EXPOSED TO CIGARETTE POST-THERAPY ETHANOL EXTRACT OF KEBAR GRASS *(Biophytum petersianum Klotzsch)*

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KEYWORDS

Biophytum petersianum Klotzsch; Uterus; Kebar Grass

ABSTRACT

This study aims to determine the effect of giving ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) on the uterine performance of Rattus norvegicus rats exposed to cigarette smoke. The experimental design used was a completely randomized design (CRD). The results obtained were analyzed by Analysis of Variance (ANOVA) then continued with Duncan's test at a significant level of α = 0.05 using SAS software. The sample consisted of 12 rats, which were divided into 4 groups. The rat group (+), 0.067, 0.0135, decreased uterine function by exposure to 10 cigarettes/head/day for 28 days. Mice (-) were the negative control group, namely rats that were not exposed to cigarette smoke and ethanol extract of coriander grass. Mice (+) are the positive control group, namely rats exposed to cigarette smoke 10 cigarettes/head/day for 28 days. Groups 0.067 and 0.135 were exposed to cigarette smoke 10 cigarettes/head/day for 28 days and then given 0.067 mg of coriander grass ethanol extract for the 0.067 group and a dose of 0.135 mg for the rat group 0.135. The results showed that there was an effect of increasing the performance of the rat uterus after administration of ethanol extract of coriander grass. It can be concluded that administration of the ethanol extract of the grass Biophytum petersianum Klotzsch kebar can increase uterine weight and uterine length of rats, with a good dose of 0.135 mg.

INTRODUCTION

Disorders of the uterus can be caused by chemical compounds either from food or air pollution such as cigarette smoke (Talakua & Unitly, 2020). Cigarette smoke contains a lot of complex toxic compounds and if inhaled it can cause a decrease in reproductive function which causes disorders of the reproductive organs resulting in difficulty having children, menstrual disorders, impaired fetal development and menopause (Febriyeni, 2010). Cigarette smoke contains free radicals which are dangerous oxidants because they have electrons from other molecules, which can damage the molecular components of cells (the structure and function of cell membranes), which can result in cell death and affect hormone production (Fitria et al., 2013). Nicotine, carbon monoxide, tar, nicotine in cigarette smoke affect nerve cells in the brain inhibiting the secretion of the hormone estradiol (Lestari & Demartoto, 2011), and causes a decrease in the concentration of estrogen in the blood that causes uterine atrophy.

Estrogen plays a role in reproduction by stimulating uterine growth by increasing endometrial and myometrial masses, stimulating uterine contractility, proliferation and differentiation of the vaginal epithelium, stimulating the development of mammary gland ducts and influencing the development of secondary genitalia (Firmawati et al., 2021). According to Gadjahnata (1989) in Unitly (2008) to stimulate the development of the uterus, estrogen is needed, which plays a role in the proliferation of uterine cells. Estrogen can also cause hypertrophy and hyperplasia of the endometrium and myometrium, causing the uterus to gain

weight due to water retention (Hardjoprajoto, 1995). Reducing the concentration of estrogen in the blood can cause uterine atrophy so that endometrial thickening does not occur and the uterine glands are in a state of not secreting secretions so that the uterus shrinks and its weight decreases (Suparni & Yuli, 2016).

The importance of the role of estrogen on the uterus, it is necessary to have other estrogen intake from outside the body (exogenous estrogen) which is relatively safe (Rahandity et al., 2021). One of the plants that is suspected to have an exogenous estrogen effect and contain phytochemicals that are able to neutralize cigarette smoke free radicals is kebar grass (*Biophytum petersianum* Klotzsch) which is found in Kebar sub-district, Manokwari district, West Papua. Based on the description above, it is suspected that the phytochemical content of coriander grass such as triterpenoids, saponins, steroids, flavonoids, Vitamin A and vitamin E can affect the development of the uterus of female rats exposed to cigarette smoke. To prove this, it is necessary to conduct a study that aims to determine the effect of giving ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) on the uterine performance of *Rattus norvegicus* rats exposed to cigarette smoke. This study aims to determine the effect of giving ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) on the uterine performance of Rattus norvegicus rats exposed to cigarette smoke.

RESEARCH METHOD

Working Method

The study was conducted at the Zoology Laboratory, Department of Biology, FMIPA, University of Pattimura using a laboratory experimental method with a completely randomized design (CRD) consisting of four treatments, each of which was repeated three times. The research stages are as follows:

Preparation phase

The rats used were 10-week-old adult female female rats with a body weight of ± 200 gr. Mice were obtained from a zoology laboratory majoring in biology, Faculty of Mathematics and Natural Sciences, Pattimura University. 12 female rats were acclimatized in the cage for 7 days. Each cage is lined with husks and covered with wire and then given food and water regularly.

Cigarette Smoke Exposure Stage

Cigarette smoke exposure to rats was carried out every day. One cigarette from the start lit until it runs out takes 10-12 minutes. Exposure to red kretek cigarette smoke from Gudang Garam Merah was carried out at a dose of 10 cigarettes/ head/ day twice a day at 09.00 and 15.00 WIT. There have been previous studies using 10 cigarettes namely (Ghanbari et al., 2007). The first stage of exposure to cigarette smoke is to prepare the equipment used, namely the smoking chamber. The smoking chamber has two holes, where the function of the first hole is as a path for expelling the exposed smoke, while the function of the second hole is to insert the tip of the burned cigarette. The cigarette smoke is exhaled repeatedly with the help of a syringe until the cigarette burns out.

Stages of Making Kebar Grass Ethanol Extract

Kebar grass is taken as much as 1 kg and air-dried then the Kebar grass is mashed using a blender. After obtaining kebar grass powder, it is then followed by the extraction process using the maceration method. The manufacturing procedure is as follows: a) Weigh as much as 250g of coriander grass powder and put it into the Erlenmeyer. b) After that, 1 liter of 70% ethanol was added and allowed to stand for 24 hours. c) After 24 hours, it was filtered using a 0.2 Wartman filter paper to obtain a liquid extract of coriander grass. The extraction residue was repeated 3 times. d) Liquid extract of kebar grass that has been obtained, then concentrated

with a rotary evaporator. e) From the concentration results, a concentrated ethanol extract of kebar grass is obtained.

Stage of Dosing of Kebar Grass Ethanol Extract

The first dose was 0.067 mg/head/day, and the second dose was 0.135 mg/head/day. The administration of coriander grass ethanol extract was carried out to rats that had been exposed to cigarette smoke, to see the differences in the doses of coriander grass ethanol extract given to rat models:

(-) : The negative control group was rats that were not exposed to cigarette smoke and ethanol extract of coriander grass

(+) : The positive control group was rats that were exposed to cigarette smoke for 28 days

0.067 : The rat group which was exposed to cigarette smoke for 28 days was then given 0.067 mg/head/day of kebar grass ethanol extract for 28 days.

0.135 : The rat group which was exposed to cigarette smoke for 28 days was then given 0.135 mg/head/day of ethanol extract of kebar grass for 28 days.

Uterine Analysis Stage

To analyze the uterus, the rats were first anesthetized using ether until they died, then the rats were dissected and the uterus removed. The uterus that has been removed, the left and right lengths are measured, then weighed to obtain a wet weight, then the uterus is extracted in ethanol 1 x 24 hours and ether alternately 2 x 24 hours intended to free fat from the uterus, then weighed to obtain a wet weight free of fat (BBBL).

Data analysis

The results of changes in uterine performance (uterine weight, left uterine length, right uterine length, total uterine length, and uterine fat-free wet weight) were analyzed by Analysis of Variance (ANOVA) and continued with Duncan's test with a 95% confidence interval (α = 0.05) using SAS software.

RESULTS AND DISCUSSION

Results

The results showed that administration of coriander grass ethanol extract caused changes in uterine performance (uterine weight, left uterine length, right uterine length, total uterine length, uterine fat-free wet weight) in rats exposed to cigarette smoke which are presented in Table 1. Results of uterine weight analysis showed that administration of ethanol extract of coriander grass (*Biophytum petersianum* Klotzsch) was not significantly different (P>0.05) on the uterine weight of rats exposed to cigarette smoke. Rat uterine weight on exposure to cigarette smoke reached 0.70gr ,dose 0.067 ie0.87gr, whereas at a dose of 0.135, it is 0.92gr. Meanwhile, the results of left uterine length analysis showed that administration of ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) dose of 0.135 was significantly different (P<0.05) to left uterine length of rats exposed to cigarette smoke, at doses of 0.067 and dose 0.135 was not significantly different (P<0.05), and in the negative control rat treatment group and the dose of 0.135 was not significantly different (P<0.05). The left uterine length of rats in the cigarette smoke exposure treatment group reached 2.07cmand at a Dose of 0.067 ie3.60cm, while at a dose of 0.135, it is 4.90cm.

Table 1. Average bobt uterus, left uterine length, right uterine length, total uterine length, and uterine fat-free wet weight (BBBL) of rats exposed to cigarette smoke after being treated with ethanol extract of kebar grass (biophytum petersianum klotzsch)

Parameter	Control (-)	Control (+)	Dosage 0.0675	Dosage 0.135
Uterine Weight (gr)	0.50 ± 0.35a	0.70 ± 0.08a	0.87 ± 0.26a	0.92 ± 0.33a
Left Uterine Length (cm)	3.53 ± 1.58ab	2.07 ± 0.66a	3.60 ± 1.55ab	4.90 ± 0.79b
Right Uterine Length (cm)	3.43 ± 1.68ab	2.00 ± 0.65a	3.27 ± 1.27ab	5.07 ± 0.73b
Total Uterine Length (cm)	4.87 ± 0.77a	4.47 ± 0.87a	5.12 ± 0.57a	5.45 ± 0.64a
Uterine Fat-Free Wet Weight (BBBL) (gr)	0.26 ± 0.02ab	0.20 ± 0.02a	0.28 ± 0.06b	0.40 ± 0.03c

Information: Different superscripts in the same column show significant differences (P<0.05) between treatments

Based on the results of the analysis showed that the administration of ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) was significantly different (P<0.05) to the length of the right uterus of each treatment rat, but in the negative control treatment group and dose 0.067 was not significantly different. The length of the rat's right uterus on exposure to cigarette smoke reached 2.00cm, at a dose of 0.067 ie3.27cm while at a dose of 0.135, it is 5.07cm.

The results of the total uterine length analysis showthat administration of ethanol extract of coriander grass (*Biophytum petersianum* Klotzsch) was not significantly different (P>0.05) on total uterine length of rats. The total uterine length of rats on exposure to cigarette smoke reached 4.47cmand at a dose of 0.067 ie5.12cm and at a dose of 0.135 which is 5.45cm. Based on the results of the analysis of rat uterine fat-free wet weights, it was shown that administration of ethanol extract of coriander grass (*Biophytum petersianum* Klotzsch) was significantly different (P<0.05) to negative controls and positive controls, where the rats' uterine fat-free wet weights on exposure to cigarette smoke reached 0.26gr.at a dose of 0.067 ie0.28gr and at a dose of 0.135 which is 0.40gr. Based on the results of the analysis of the mean for each treatment, it is presented in graphical form below:

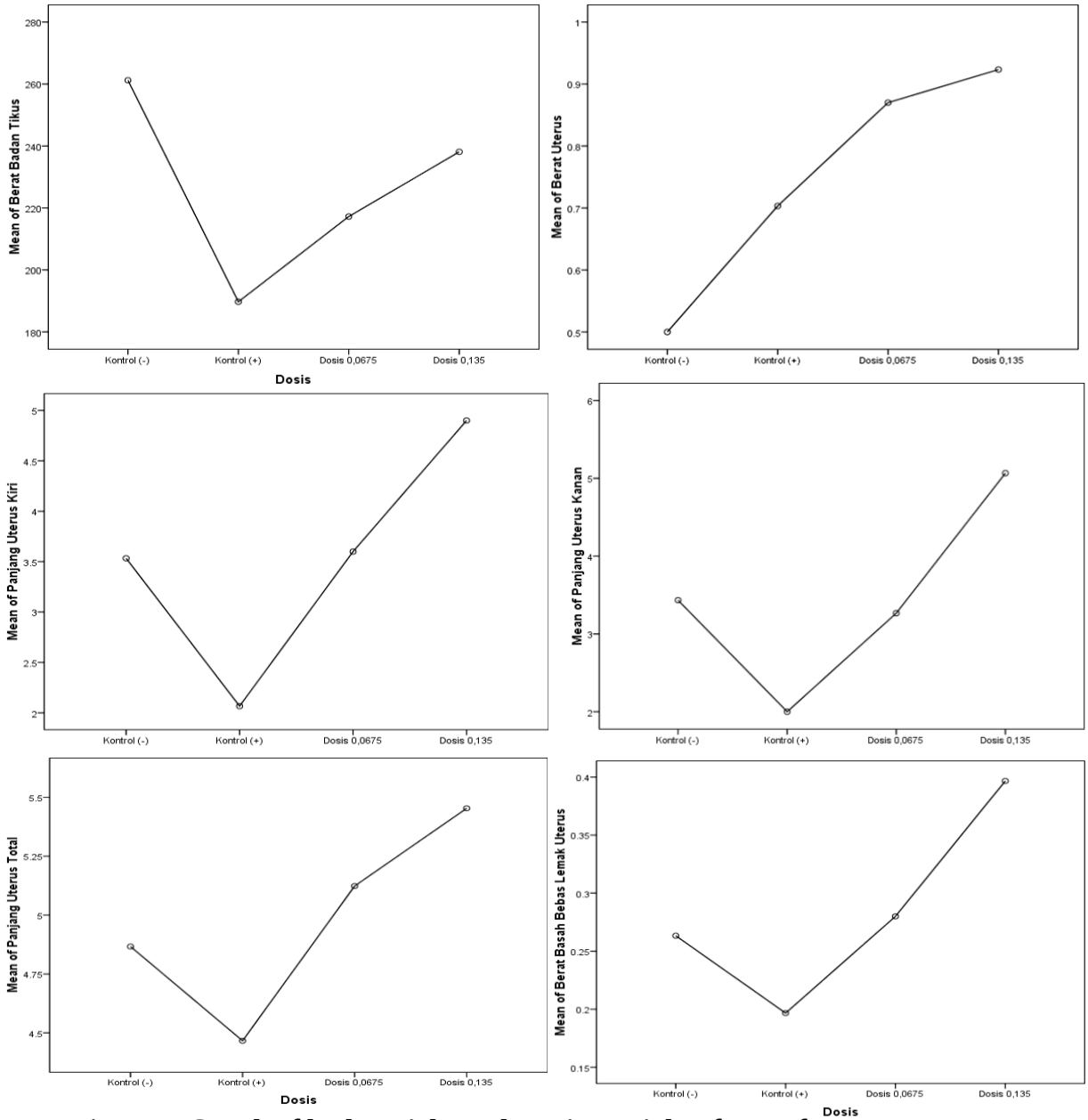

Figure 1. Graph of body weight and uterine weight of rats after treatment

Discussion

The results of the analysis of uterine weight and uterine length in the treatment group of rats that were given kebar grass ethanol extract after exposure to cigarette smoke showed that the treatment at doses of 0.067 and dose 0.135 experienced an increase in uterine weight and uterine length. This shows that there was an improvement in the rat uterus which resulted in an increase in uterine weight resulting in a change or increase in uterine weight as well as uterine length. This is presumably due to the content of vitamin E in coriander grass which is an antioxidant so that it can neutralize free radicals, where these compounds will enter through the bloodstream and then bind to chemical partners of incoming free radicals thereby preventing damage caused by toxins and maintaining the health of the reproductive organs (Sadsoeitoeboen, 2005).

Kebar grass also contains chemical compounds belonging to the alkaloid group, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids and glycosides (Sembiring & Darwati, 2014). The content of active compounds contained in Kebar grass can be used as a phytoestrogen that can replace the role of estrogen (Unitly et al., 2022). The increase in uterine weight and length is also caused by the phytoestrogen content in kebar grass which acts as a substitute for estrogen through the estrogen receptor. Estrogen receptors (ERs) are synthesized by several cell types in two isoforms, namely ERα and ERβ. The distribution of ERα and ERβ is in the uterus. The amount of estrogen in the body can have a very broad effect on organs and tissues, especially in the reproductive organs such as the uterus (Sembiring & Darwati, 2014). Flavonoids, namely isoflavones, are a substitute for estrogen. This is in line with Unitly et al. (2015) states that isoflavones have estrogen activity which can cause increased estrogen production and stimulate endometrial thickening so that the uterus enlarges and the uterine weight increases. Unity et al. (2015) added that estrogen can stimulate the development of tissues involved in reproduction where the vaginal epithelium proliferates and differentiates, the uterine endometrium proliferates then the uterine glands experience hypertrophy and elongate.

The administration of ethanol extract of coriander grass at a dose of 0.067 mg and a dose of 0.135 mg had a different change in the increase in uterine weight and length where at a dose of 0.135 mg it had a weight and length exceeding the 0.067 mg dose. This shows that the higher the dose of ethanol extract containing phytoestrogens causes an increase in the weight and length of the rat uterus. Hafez (2000) states that estrogen can increase vascularity and greater uterine mitotic activity so that the organ gains weight. It can be said that estrogen isoflavones are uterotropic due to nitrogen retention which causes increased cell proliferation. The effect of estrogen on the uterus is very evident in the presence of tissue weight gain caused by the process of endometrial and myometrial hypertrophy and hyperplasia. In addition, uterine elongation shows spacing for increasing the number of embryos where the estrogen produced affects the development of good uterine tissue to prepare for the implantation process and embryo development (Santell et al., 1997).

CONCLUSION

The results of the analysis of uterine weight and uterine length in the treatment group of rats that were given kebar grass ethanol extract after exposure to cigarette smoke. It showed that the treatment at doses of 0.067 and dose 0.135 experienced an increase in uterine weight and uterine length. Thus, based on the results of the research conducted, it was concluded that the administration of the ethanol extract of kebar grass (*Biophytum petersianum* Klotzsch) canmeincrease the weight of the uterus and the length of the rat uterus, with a good dose of 0.135 mg.

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