

Optimization of Estrogenic Activities of Kebar Grassextract (Biophytum Petersianum) on White Female Mice (Mus musculus)

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Abstract: Menopause occurs caused by the decline of estrogen hormone due to the depletion of the ovarian egg. As estrogen levels decrease, the risk of osteoporosis, bone fractures, heart disease and even cancer increase. The kebar grass (*Biophytum petersianum*), which has long been used as a traditional fertility medicine by Papuans, is suspected to have an estrogenic activity. The purpose of this study was to determine the estrogenic activity of kebar grass extract on female mice (*Mus musculus*). Five experimental female mice groups were used in this study. Each group consisted of 5 mice. The negative control group was administered with aquadest; the positive control was administered with the estrone conjugated estrogen 0.625 mg; the first group (P1) was administered with 3% kebar grass extract; the (P2) group was administered with 5% kebar grass extract and the (P3) was administered with 10% kebar grass extract. The experiment was carried out for 7 days. The results of the study showed that the kebar grass extract has an estrogenic effects on female mice include to shorten the estrous cycle, prolong the estrous phase, improving the vascularity of ovary and uterus, and increase the weight of ovary and uterus. The highest estrogenic activity was reached when the mice was administered with 10% kebar grass extract..

Index Terms: Kebar grass, estrogenic activity, white female mice.

I. INTRODUCTION

The psychological and physical function decline in are closely related to the aging process in human body. In women, the aging process was triggered by the lack of estrogen production in the ovarium during menopause or late reproductive phase [1]. Menopause is the normal, natural transition in life that begins between the ages of 35-55. During this time, fertility declines, the eggs are depleted, the ovaries get smaller and stop producing the hormones estrogen and progesterone that control the menstrual cycle [2].

The subside of estrogen production result in various common syndromes such as hot flashes, sweating profusely, insomnia, depression and irritability. The lack of estrogen also leads to osteoporosis, heart disease and even cancer risk [3]. The estrogen replacement therapy or hormone replacement therapy (HRT) using synthetic hormone such as

estradiol was the common method used to treat estrogen deficiency, but the long-term use of synthetic estrogens associated with various side effect such as hyperplasia, uterine cancer, and breast cancer [4].

The use of plant materials containing phytoestrogens is an option that can be used as a substitute for synthetic estrogens and it is believed that natural estrogens are safer because they carry less risk of bleeding and have fewer side effects [5]. The kebar was known by local Papuan people as a medicine to enhance women fertility. The local name of this grass is banondite which means many children. Previous research revealed that the kebar grass contains the fertility enhancing active compounds [6]. Phytochemical study of Sembiring and Darwati (2014) shows that kebar grass contains alkaloids, saponins, tannins, phenolic, flavonoids, triterpenoids, steroids and glycosides [7].

The study of [8] had proved that the infusion of grass at a concentration of 5% significantly increase the spermatogenesis activity. However, this study only reveals the activity of spermatogenesis, while for oogenesis has not been done.

According to research of [9], administration of 0.135 mg/g body weight of kebar grass extract to white female mice can prolong estrus length, increase the number of embryo, increase body weight, the number of child and a weight of newborn child.

This study was conducted to determine the activity of kebar grass extract to the estrogenic activity, duration of the estrous cycle, duration of estrous phase, vascularity and ovarian and uterine weight in female white mice. A research on the activity of kebar grass extract in the form of liquid preparations in the hope that it can shorten the estrous cycle, extend the estrous phase, vascularization and increase ovarian weight and uterine weight with dose reference. The benefits of the results of this study can be used in community service as information about plants that estrogen as estrogenic to the community, especially mothers who have stepped on the age of 40-50 years.

II. MATERIAL

A. Apparatus

Juicer, analytical scales, funnel, dropper, sonde apparatus, beaker glass, measuring cup, spatula, plastic tub, mice feeding bottle, syringe (mL), microscope, object glass, cotton bud and dissecting kit.

Revised Manuscript Received on April 25, 2019.

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B. Chemicals

Physiological NaCl, giemsa dye, aquadest, esthero conjugated estrogen 0.625 mg,

Ketamin xylazin and methanol 10%, and other supporting materials such mice forage.

C. Animal

Twentyfive female mice *Mus musculus* aged 2-3 months, weighed 20-25 g were acclimatized for 1 week. All the mice had free access to tap water and standard pellet diet. The acclimatization procedure was performed according to the international guidelines for laboratory animal use and care. After 1 week, the mice were divided into 5 treatment group that are K-, K+, P1, P2, and P3. Each group consisting of five mice.

- (K-) negative control, administered with aquadest
- (K +) positive control, administered with esthero conjugated estrogen 0.625 mg
- (P1) administered with kebar grass extract at concentration of 3%.
- (P2) administered with kebar grass extract at concentration of 5%.
- (P3) administered with kebar grass extract at concentration of 10%

To stimulate whitten effect or synchronous estrus on female mice, the cage of male mice was placed next to the female cage. administered for 3 days, this method is done by cage male mice placed on the cage of female mice.

III. METHOD

A. Extraction Procedure

The kebar grass was sorted, cleaned, washed under running tap water and dried in room temperature. The grass (500 gr) was grinded using juicer machine then filtered using Whatman paper. The extract obtained was measured using measuring cup [10].

B. Determination of Estrogenic Activity

The parameters measured to determine the estrogenic activity of kebar grass were the duration time of estrous phase, the ovary and uterus vascularity and the weight of ovary and uterus.

C. Measurement of Estrus Cycle Time

Estrus cycle time was measured by observing physical change of vaginal mice (vaginal preparation method/vaginal smear). Preparation of twice daily in the morning and at night during treatment and after giving each treatment for 7 days, while looking at macroscopic signs of estrus covering the condition of the vulva and vagina [11]. Observing the length of the estrous cycle (proestrus, estrus, metestrus and diestrus) can be investigated by calculating the time (hour) of the estrous cycle of the mouse starting from the end of the estrous phase (the presence of cornification cells and the presence of leukocytes) up to the early signs of the estrous phase characterized by the presence of chromosomal epithelial cells).

D. Ovary and Uterus Vascularity

The ovary and uterus vascularity was observed by dissection methods. Dissection process was performed to the estrous phase mice during treatment and after treatment, then dissected for ovaries and uterus removed, mucosal colors were seen in the ovaries and uterus of mice. Assessment and observation of vascularization is expressed by scoring, in accordance with modification of the Rugh method [12]

E. Measuring the Ovary and Uterus Weight

The weight of ovaries (left and right ovaries and uterine) were measured separately. The ovary and uterus were taken from the mice, then washed using physiological NaCl, placed on filter paper and weighed in wet condition using analytical balance [13]

IV. RESULTS AND DISCUSSION

A. Kebar Grass Extract

The 62 mL concentrate extract (100 %) was obtained from 500 g fresh kebar grass. The concentrate extract then diluted with aquadest to obtain 3%, 5% and 10% of kebar grass extract solution. The effects of kebar grass extract administration to the estrus cycle in female mice during and after treatment were shown in Table 1 and Table 2.

Table 1. Female Mice Estrus Cycle During Administration of Kebar Grass Extra

Repeating	Estrus Cycle Period (hours)				
	K-	K+	P1 3%	P2 5%	P3 10%
1	36	48	48	60	0
2	96	0	0	0	0
3	120	48	84	36	0
4	132	36	36	60	0
5	96	0	60	-	-
Sum	480	132	228	156	0
Average	96 ^c	26,4 ^{ab}	45,6 ^{bc}	39 ^b	0 ^a

Table 2. Female Mice Estrus Cycle After Administration of Kebar Grass Extract

Repeating	Length of Estrus Cycle (hours)				
	K-	K+	P1 3%	P2 5%	P3 10%
1	108	48	72	108	48
2	144	108	72	24	24
Sum	252	156	144	132	72
Average	126	78	72	66	36

Data presented in Table 1 showing that, based on the Duncan test, the administration of kebar grass extract at various concentrations has a very significant different effect in shortening the estrous cycle time ($P < 0.01$). The most effective dose of kebar grass extract to shorten estrus cycle period was 5% (P2), but it's still longer than the administration of positive control (K+). Increasing the dose to 10% caused estrus phase to continue without pause.

The development and ability of the ovaries to produce the estrogen hormone can be seen indirectly through the estrogenic activity on the changes of vaginal epithelial cytology. The results of this study prove that administration of kebar grass extract can shorten the length

of the estrous cycle of female mice.

Table 2 shows that there was a decrease in the length of the estrus phase that occurred in all treatments. Aquadest administration as a negative control resulted in the longest estrus phase duration among the other treatments, which was reach 126 hours, while administration of 10% (P3) kebar could shorten the length of estrus cycle to 36 hours. grass extract still showed the longest estrus phase with length of 36 hours. Positive control resulted in an estrus phase time of 90 hours, which was shorter than the administration of with a concentration of 3% (P1) and a concentration of 5% (P2) each produced an estrus length of 96 hours and 102 hours. These results prove that administration of biophytumpetersianum is still giving an estrogenic effect even though after 7 days the treatment is stopped.

This results probably due to the presence of secondary metabolites contained in the grass kebar. According to the research [13], kebar grass plants contain chemical compounds class of steroids and saponins. Saponins are the basic ingredient for the synthesis of steroid hormones (gonadotropic). Estrogen is classified as a steroid hormone that has 18 C atoms and one of the sex hormones that plays an important role especially in women. The active compound of flavonoids found in the turmeric grass can bind to the estrogen receptor (ER α and ER β).

B. Effect of Kebar Grass Extract Administration to the Length of Estrus Phase

The data obtained confirmed that the administration of kebar grass extract has an effect to the length of estrus phase. Table 3 and table 4 show that the difference of kebar grass extract concentration significantly (P < 0.01) affected the length of estrus phase. The result of Duncan test stated that giving of grass seeds showed very different result with negative control (Iadest), while the positive control (Esthero) gave relative influence with 3% concentration of P3 concentration (P1), 5% concentration (P2)) and concentration of 10% (P3). So it can be stated that the concentration of 10% grass (P3) is the most effective concentration, while the 3% concentration is the most efficient.

According to [14], the average duration of estrous phase in mice is 12-14 hours, but during long estuary biophytum petersianum to 168 hours (7 days), this indicates that the grass extract is capable prolong the duration of the estrous phase. The result of this research shows that the tendency of

increasing the dosage of grass extract will increase the average of estrus length. According to [14], the longer the estrus phase will provide greater opportunities for animals.

Table 3. The Length of Estrus Phase During Administration of Kebar Grass Extract

Repeating	Length of Phase Estrus (Hours)				
	K-	K+	P1 3%	P2 5%	P3 10%
1	132	120	120	108	168
2	72	168	168	168	168
3	48	120	84	132	168
4	36	132	132	108	168
5	72	168	108	-	-
Sum	360	708	612	516	672
Average	72 ^a	141,6 ^{bc}	122,4 ^b	129 ^{bc}	168 ^c

Table 4. The Length of Estrus Phase After Administration of Kebar Grass Extract

Repeating	Length of Phase Estrus (Hours)				
	K-	K+	P1 3%	P2 5%	P3 10%
1	60	120	96	60	120
2	24	60	96	144	144
Sum	84	180	192	204	264
Average	42	90	96	102	132

ovulate. This will prove that grass kebar essence has estrogenic activity. Based on observations of estrogenic activity of grass cleansing essence after 7 days of treatment, Table 6 shows that there was a decrease in estrus phase time occurring in all treatments. The giving of aquadest as a negative control resulted in the shortest duration of the estrus phase among other treatments of only 42 hours, while giving 10% (P3) grass extract still showed the longest estrous phase of 132 hours. Positive controls resulted in a 90-hour estrus phase, which was shorter than that of 3% (P1) and 5% (P2) concentrations of grass flowered grasses each yielded a duration of 96 hours and 102 hours. This result proves that giving kebar grass still gives estrogenic effect even after 7 days treatment is stopped.

C. Effect of Kebar Extract Administration to the Vascularity of Ovary and Uterus

The vascularity of ovary and uterus was affected by administration of kebar grass extract as shown in Table 5 and Table 6. The data showed that the administration of 10% kebar grass extract increased ovarian and uterine vascularization of mice with an average score of 3 marked with the very red color of ovarian and uterus mucosa.

Table 5. The Ovary and Uterus Vascularity During the Administration of Kebar Grass Extract

Repeating	Vascularity Scores Ovaries and uterus				
	K-	K+	P1 3%	P2 5%	P3 10%
1	3	1	2	2	3
2	1	2	2	2	3
3	1	1	3	-	-
Sum	5	4	7	4	6
Average	1,67 ^{ab}	1,33 ^a	2,33 ^{ab}	2 ^{ab}	3 ^b

Table 6. The Ovary and Uterus Vascularity After the Administration of Kebar Grass Extract

Repeating	Length of Phase Estrus (Hours)				
	K-	K+	P1 3%	P2 5%	P3 10%
1	3	1	2	1	3
2	2	2	2	2	3
Sum	5	3	4	3	6
Average	2,5	1,5	2	1,5	3

The data obtained by statistical analysis using SPSS 24. The result of analysis showed that giving kebar grass as estrogenik gave no significant effect on ovarian vascularization and uterine mice (P > 0,05). The results of the follow-up test using Duncan test showed that giving concentration of 10% (P3)

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concentration of the grass concentration (P3) showed different result with negative control (ladest).

The results of this test show that giving kebar grass (Biophytum petersianum) has an estrogenic activity that leads to increased secretion of estrogen hormones and will lead to ovarian and uterine vascularization. Vascularization is seen from the color of the mucosa to red due to increased blood vessel activity to the uterus. According to [15] an increase in the number of blood vessels to the uterus due to increased secretion of estrogen. Occurrence of vascularization of the uterus will accelerate blood flow to the uterus. This is reinforced by the opinion of [16], that estrogen can cause an increase in blood flow indirectly because there is also an increase in prostaglandin which causes vasodilation of blood vessels in the endometrium and myometrium. After 7 days treatment showed that grass cleansing with 10% concentration (P3) still got scores with average 3 and was the highest score compared to other concentration and positive control having score score 2.5, whereas negative control had the lowest scoring average score and the equivalent of 5% concentration of 5% concentration (P2) grass extract, resulting in an average score of 1.5. During 7 days of grass extract giving various concentrations showed the giving of negative control in the form of aquadest resulted the average value of the lowest ovarian weight that is 0,0480 g, meanwhile the highest mean value of weight is on giving kebar grass extract with concentration of 3% (P1) was 0.1088 g. The result of data analysis using SPSS 24 showed that administration of grass kebar extract has no significant effect ($P > 0,05$) on the ovarium weight of female mice.

Result of Duncan test that is giving of grass concentration of 3% (P1) concentration has the same effect with concentration of 10% (P3). While giving positive control (Esthero) gives the same effect with negative control and grass extract concentration 5% (P2). From the results of further testing can be seen that giving grass kebar 3% (P1) and 10% concentration is the best concentration to increase ovarium mice weight. After 7 days, the treatment of grass extract was carried out again on the weighting of ovarium mice. The result is a decrease in ovary weight after 7 days of treatment at all concentrations except on positive control (Esthero) increased ovarium weight. Positive control (Esthero) gave the highest ovarium weight after 7 days treatment of 0.0698 g, then giving 10% (P3) grass sari grass gave ovary weight which is not much different from positive control that is 0,0615 g. The lowest ovarium weight after 7 days of admission was negative control of 0.0348. This result indicates that giving 10% (P3) of aqueous grass concentration to maintain ovarium weight in mice and its effect is equivalent to positive control (Eshthero) both when treatment is given and 7 days after treatment. For 7 days, the result showed that the grass extract of 3% (P1) of concentration of parsed grass yielded 0.3213 g of the uterus, not significant from the 10% concentration of 10% concentration of the grass of the concentration of the grass, which resulted in 0.3151 g uterine weight. Uterine weight data was then analyzed using SPSS 24 statistic, the result is that all treatments gave the same effect ($P > 0,05$) but giving 3% kebar grass (P1) yielded **the most optimum value compared to other treatment.**

The results obtained after 7 days of treatment showed a decrease in uterine weight in all treatments except the provision of positive control (Esthero), while the average weight produced was the lowest on the negative control (ladest) only 0.1086 g.

Kebar grasses have chemical compounds that can improve reproductive performance [17]. The active compounds that are estrogenic work in the same way as estradiol. After 7 days of treatment still affect vascularization and ovarian-uterine weights. On the positive control and grass extracts of 10% (P3) concentration of saliva still showed good results from the scores of ovarian and uterine. In the estrus phase the de Graaf follicle enlarges and maturation occurs. This phase of estradiol derived from mature de Graaf follicles, will cause changes in the female reproductive tract and more secrete the esrogen hormone. During the estrous cycle time will occur the process of growth, development and maturation of follicles and produce a number of estradiol from ovaries stimulated by FSH. The shorter the time it takes 1 estrus cycle will speed up follicular maturation. The mature follicle will be stimulated by LH and ovulation will occur. Unovulated follicles will have atresia [13].

This can be the basis of estrogenic activity in the kebar grass extract which can shorten the estrus ccess, extend the estrous phase, vascularization and increase ovarium-uterine weight. Based on the results of [13] research, grass seed karar extract can increase follicular development because it contains saponin which is the basic material for synthesis of steroid hormone that can improve reproduction system performance.

Turmeric grass can be called phytoestrogens because it has estrogenic activity. The mechanism of estrogenic activity can make vascularization and the addition of ovarium-uterine weights are described according to [18], estrogen-targeted tissue is a network of cells containing estrogen receptors. Estrogen secreted by the ovary functions in the enlargement of the uterine organ at puberty because the uterus has a high response to estrogen. According to [19], estrogen receptor ($ER\alpha$ and $ER\beta$) play a role in maintaining the physiological function of the uterus. The role of $ER\alpha$ is as a marker receptor of estrogenic effects on increased uterine weight and proliferation of luminal epithelial cells, whereas $ER\beta$ is more instrumental in modulating uterine function.

V. CONCLUSION

From this research can be concluded:

1. Kebar grass extract an estrogenic activity in female white mice (*Mus musculus*).
2. **The most effective** dose of kebar grass extract to shorten estrus cycle, prolonged the duration of estrus phase, increasing vascularization, and increasing the weight of ovaries and uterine weight in female mice was at a concentration of 10%.

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