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ANTIESTROGENIC EFFECT OF TUBER EXTRACT OF *CYPERUS ROTUNDUS* L. ON THE ENDOMETRIAL THICKNESS OF MICE (*MUS MUSCULUS* L.)

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ABSTRACT

Uterine receptivity for implantation of an embryo is regulated by ovarian hormone estrogen. When function of the hormone is disrupted, for example by anti-estrogenic substances, the implantation process may also be disrupted. Some types of plants, including nut grass, have been known to contain anti-estrogenic compounds. The research aimed to determine the anti-estrogenic effect of tuber extract of *Cyperus rotundus* on the endometrial thickness of mice (*Mus musculus* L). By using a completely randomized design, 18 of healthy female mice, aged 3-4 months, weighing 30-40 g were grouped into three each consisted of six mice. Group-1 is mice that are given distilled water as a control. Group-2 is mice treated with anti-estrogenic drug tamoxifen of 0.16 mg/40g body weight. Group-3 is mice given tuber extract of nut garss at a dose of 135mg/40g body weight. The treatment were given once daily for 14 days. On the day 15 all mice were sacrificed, the womb was dissected to be made histological slides of uterus. The result is either tamoxifen 0.16mg/40g body weight as well as tuber extract of nut grass 135 mg/40g body weight are significantly reduce the thickness of endometrium compared with that of

control. Thus it can be concluded that the tuber extract of *C. rotundus* has anti-estrogenic effects on the endometrial thickness of mice.

KEYWORDS: Cyperus rotundus, nut grass, tamoxifen, antiestrogenic effect, endometrial thickness

INRODUCTION

Implantation involves interaction between the blastocyst and the uterine endometrium that goes through a series of unique stages[1] on a right time and place.[2] The end stages of the implantation is the invasion of blastocyst into the maternal tissues, from which the embryo obtain nutrients essential to life and development.[3] Uterine receptivity for implantation of an embryo is regulated by ovarian hormones estrogen and progesterone. Several cytokines and growth factors also play an important role in embryo implantation under the influence of ovarian hormones. As some of the molecules involved in the interaction between the embryo and maternal tissue during the implantation process takes place. It has also been known that, if the function of these molecules during implantation failure, the implantation is also impaired, and even lead to infertility.[4] Biochemical and molecular aspects of the endometrium is strongly associated with the ability of embryo implantation. In an endometrial biopsy there was molecules known to be associated with the endometrial response. A number of factors could cause interference with the implantation, low receptivity of endometrium will lead implantation failure.[5,6] One of the causes of the implantation failure is biochemical and molecular disruption in endometrium.[7] In addition, malfunctioning of the endometrium can be caused by antiestrogenic compounds that known to be contained in the nut grass *Cyperus rotundus.*[8] Antiestrogenic drugs often used in the treatment of breast cancer, because physiologically estrogen stimulate normal breast growth. Antiestrogen acted by modifying or antagonize the action of estrogen and among the antiestrogenic drugs is tamoxifen.[9] A study to compare the effect of estrogen with antiestrogen reported by Papaconstantinou et al.[10] showed that the estrogen compounds causing increases the weight of uterus, on the contrary anti-estrogen made the uterine weight decreased. Antiestrogen is substances that fight or lessen the effects of estrogen and able to eliminate a part or the whole action of estrogen.

To ascertain the antiestrogenic effects of crude extract of nut grass plant (Cyperus rotundus) on the uterine wall thickness, methanolic tuber extract of the plant has been applied to female mice for 14 days.

MATERIALS AND METHODS

Plant Samples and Extraction

The samples of nut grass plant (Cyperus rotundus L.) were collected from suburbs Bandar Lampung. To prepare simplicia, fresh tubers of the grass which has been previously washed were sun dried. Once dry, the tubers were ground to be a powder form. Then, by using Soxhlet apparatus the simplicia was extracted using methanol solvent at the temperature of 35^{o}C and the rotation of 60 rpm for 60 minutes.

Experimental Mice and Treatment

Female Swiss albino mice (Mus musculus L.) aged 3-4 moths, weighing 30-40 grams were used for the study. The animals and the food pellets were obtained from Lampung Veterinary Center, Indonesia. Mice were housed in a room at the temperature of 25°C and 12:12-hour light-dark cycle with free access to water and pellets *ad libitum*. All animal care and treatment procedures were approved by the Ethics Committee, Faculty of Medicine, University of Lampung, Indonesia.

By using a completely randomized design, the animals grouped into three consisted of six rats each. Group-1 is mice that are given distilled water as a control. Group-2 is mice treated with anti-estrogenic drug tamoxifen of 0.16 mg/40g body weight. Group-3 is mice given tuber extract of nut grass at a dose of 135mg/40g body weight. The treatment were given once daily for 14 days. By the day 15, after being deeply anesthetized using chloroform, all mice were sacrificed, the womb was dissected to be made histological slides of uterus.

Study Parameters and Data Analysis

Study parameters assessed in this study is the thickness of endometrial layers of the uterine wall. The data are presented as Mean ± SD and analyzed statistically using a one-way ANOVA. Least Significance Difference (LSD) test was used as the post hoc test. All of the statistics that were applied are programmed in SPSS version 21.

RESULTS NAD DISCUSSION

The descriptive data of the effects of tamoxifen and tuber extract of *C. rotundus* on thickness of endometrial layers of mice uterine wall are presenterd in Table 1. The results of analysis of variance (ANOVA) of the data in the Table 1 are tabulated in Table 2.

Table 1: The descriptive data of the effects of tamoxifen and tuber extract of *C. rotundus* on thickness of endometrial layers of mice uterine wall.

N	Control	Tamoxifen	Extract
1	203.5	154	145.2
2	196.5	147	138.1
3	189	140.5	131.5
4	182.9	133.5	124.5
5	175	126	117
6	168.5	119.5	110.5
Mean	185.90	136.75	127.85
SD	13.13	12.96	13.03

Table 2: The results of Analysis of Variance.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	11743	5871.4	34.5	0.000
Error	15	2552	170.1		
Total	17	14295			

As the F-value of the ANOVA (=34.5) and the P-value <0.01 it can be suggested that the difference between groups of treatment is highly significant. By using Least Significant Difference (LSD) against the above descriptive data resulted in the lower bound of LSD=16.046 and the upper bound=22.191. The notation of the difference of mean values between the treatments are presented in Table 3.

Table 3: The difference of mean thickenss of endometrium between treatment group of mice.

Treatments	Mean	Difference	
		Control	Tamoxifen
Control	185,90		
Tamoxifen	136,75	49,15**	
Extract	127,80	58,10**	8,95^{ns}

Notes: ** mean highly significant different at P<0.01

'ns' mean not significant different at P>0.05

Based on the results of statistical test above, it can be affirmed that both tamoxifen and tuber extract of nut grass effectively reduce the thickness of the endometrial layers of uterine wall of mice.

The endometrial surface consists of mucosal epithelial cells, which its main function to facilitate implantation of an embryo.[11] The endometrial receptivity determined by the molecular and genetic markers in the form of cytokines, growth factors, transcription factors

and ovarium hormones, such progesterone, the most essential for implantation and pregnancy of all mammals, as well as the specific estrogen hormones.[12, 4] In this study, tuber extract of C. rotundus showed antiestrogenic effect on reducing thickness of the endometrial lining and caused no proliferation on endometrial epithelial cells. It was allegedly due to the absence of estrogen influence on the endometrium which imply that tuber extract of C.rotundus possess an anti estrogenic activity. Estrogen is required for the proliferation of uterine epithelial cells and enhance the action of progesterone through the induction of progesterone receptor genes. The progesterone plays a role in controlling proliferation, differentiation, maintenance of endometrial stroma, glandular and myometrial cells.[12]

The syntesis of progesterone by corpus luteum will stimulate proliferation and diffentiation of stroma cells. Furthermore, implantation of an embryo the will promote secretion of estradiol-17β that in turn stimulate proliferation and differentiation of the uterine epithelial cells.[13]

It was also suggested that the endometrium may undergo histological changes, such as vascularization of the endometrium, development of endocrine glands and pinopod to be luminal surface of the epithelium.[14] These changes are known as morphological changes due to the influence of the endometrial estrogen and progesterone.[15] A series action of estrogen and progesterone of endometrium will result in secretion of epithelial gland and a series of decidual transformation in the stroma cells.[16] Estrogen and progesterone are important for regulating the uterine receptivity to embryo development and the success of pregnancy. The differentiation of stromal cells into decidual cells is responded by the progesterone hormone during the decidualization process, which is characterized by morphological changes and prolactin secretion.[17] Finally endometrium undergo morphological and functional changes, including growth, differentiation and desquamation. Finally endometrium undergo morphological and functional changes, including growth, differentiation and desquamation. Simultaneously physiological changes initiated in preparation for endometrial receptivity and control the invasion of cells tropoblas in the mid-secretory phase of implantation process.[18]

CONCLUSION

Either tamoxifen as well as tuber extract of nut grass equally give effect in reducing endometrial thickness of mice. Thus it can be concluded that tuber methanolic extract of C rotundus allegedly possess antiestrogenic activities and potentially as an antiimplatation drug.

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