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Ethanol Extract of Cogon Grass Root (*Imperata cylindrica*) Potential as Contraception Agent by Shortening Estrus Cycle in Female Mice

(EKSTRAK ETANOL AKAR ALANG-ALANG (IMPERATA CYLINDRICA) BERPOTENSI SEBAGAI AGEN KONTRASEPSI DENGAN MEMPERPENDEK SIKLUS BERAHI MENCIT BETINA)

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ABSTRACT

The use of herbs as contraception has become the focus of current contraceptive development. In the previous study, the extract ethanol of cogon grass roots (CGG) has the effect of decreasing sperm production and changing metabolite profiles in male mice. However, the effect of this Indonesian well-known herb is still indefinite in female._The purpose of this study was to investigate the effect of ethanol extract of (CGG) roots to the fertility of female mice. The study was conducted at Animal Laboratory of Faculty of Medicine and Central laboratory University of Padjadjaran. Mice were given ethanol extract of CGG roots 90 and 115 mg/kgBW per oral. Vaginal cytology was observed every days before 9 p.m and metabolite profiles were measured before and the end of experiment. The results showed a significant shortening of the estrus phase in 90 and 115 mg/BW but not significant in glucose and cholesterol serum level during pre and post CGG treatment ($p<0.05$)._ This study indicated the extract ethanol of cogon grass roots has potential effect as contraceptive agent because stimulated the estrus phase and the effect is independent from metabolite profiles.

Keywords: cogon grass; *Imperata cylindrica;* estrus cycle phase; metabolite profile

ABSTRAK

Penggunaan herbal sebagai kontrasepsi telah menjadi fokus pengembangan kontrasepsi saat ini. Pada penelitian sebelumnya, ekstrak etanol akar alang-alang (*Imperata cylindrica*) memiliki efek menurunkan produksi spermatozoa dan mengubah profil metabolit pada mencit jantan. Namun, efek akar alang-alang ini masih belum diketahui pada mencit betina. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak etanol akar alang-alang terhadap fertilitas mencit betina. Penelitian dilakukan di Laboratorium Hewan, Fakultas Kedokteran dan Laboratorium Pusat University of Padjadjaran. Mencit diberi ekstrak etanol akar alang-alang 90 dan 115 mg/kg bobot badan per oral. Setelah tiga minggu, sitologi vagina diamati dan profil metabolit diukur.

Hasil penelitian menunjukkan terjadi pemendekan yang signifikan pada fase estrus pada kelompok perlakuan 90 dan 115 mg /kgBB dan kadar glukosa serta kolesterol yang tidak signifikan sebelum dan setelah pemberian ekstrak etanol akar alang-alang (p<0,05). Penelitian ini menunjukkan ekstrak etanol akar alang-alang memiliki efek potensial sebagai agen kontrasepsi yang ditunjukkan dengan kemampuannya dalam memperpendek siklus estrus sebelum dan setelah pemberian perlakuan. Efek yang ditimbulkan tersebut tidak bergantung pada profil metabolit.

Kata-kata kunci: lang-alang; fase siklus estrus; profil metabolit

INTRODUCTION

The worldwide population increases every year, estimated at 83 million people per year and may lead to overpopulation (United Nations, 2015). This condition can lead to an increased of food and settlement demand, and other social problems that have an impact on the deterioration of quality of life. One of the method to control population growth is by using contraception agent.

The deliberate use of artificial methods or other techniques to prevent pregnancy as a consequence of sexual intercourse is imperative. The major forms of artificial contraception are: barrier methods, of which the commonest is the condom or sheath; the contraceptive pill, which contains synthetic sex hormones which prevent ovulation in the female; intrauterine devices, such as the coil, which prevent the fertilized ovum from implanting in the uterus; and male or female sterilization (Oxford 2018).

Female animal, including mouse, estrus cycle is one of parameters to determine fertility performance, beside of hormonal response, and follicle size (Moonmanee and Yammuen-art, 2015). Estrus cycle is a rhythmic reproductive cycle in sexually matured female mammals and is influenced by the release of gonadotropin releasing hormone from the pituitary gland and sex hormones from the gonads. Estrus cycle includes several phases of proestrus, estrus, metestrus and diestrus (Hill, 2018). While female cyclicity characterised by vaginal cytological changes as observed in estrus cycle is an index of good functioning of the neuroendocrine-reproductive system and ovarian activity, the disturbance of the normal oestrus cycle indicates the disruption of ovarian progesterone and oestrogen balance (Auta and Hassan, 2016).

The phases of estrus cycle are closely related to the sex hormone that dominate each phase of estrus phase. The formation of these hormones itself is highly dependent on the levels of metabolites present in the body. Metabolite profile has a close relationship with the activity of folliculogenesis and steroidogenesis in the ovary so it is expected to reflect its activity by looking at the fluctuations in the female mice metabolite profile such as glucose and cholesterol. In research conducted by Barata *et al*. (2013) and Letelier *et al*. (2008) showed that administration of glucogenic mixture orally increase ovulation rate in non diabetic sheep, this effect caused by increase in metabolite hormone such as insulin, insulin-like growth factor 1 (IGF-1), and leptin that stimulate the folliculogenesis and decrease negative feedback of stimulating hormone (Armstrong *et al*,, 2003; Scaramuzzi *et al,*, 2006), cholesterol is a raw material for estrogen and progesterone hormone formation (Yoon *et al*., 2006; Tarumi *et al*., 1988). Therefore, the alteration of metabolite levels can be a reflection of fertility rates in female.

The side effects of oral contraception, such as weight gain, mood changes, etc , that is often undesirable and become the main reason for people for not taking oral contraception. Furthermore, oral contraception requires high discipline for it to work. Therefore, study of natural product based-oral contraception is necessary to increase compliance of contraceptive users.

Cogon grass (*Imperat cylindrica*) is a kind of grass that has a huge population that spread in many parts of the world (CABI, 2018; MacDonald, 2004). Cogon Grass contains flavonoid compound (Mensah *et al*., 2004; Parvathy, 2012). Flavonoid, in general, is also known to have ability to reduce circulated metabolites such as glucose and cholesterol (Salvamani *et al*., 2014; Hsu and Yen, 2007; Kwon *et al*., 2007), this effect caused by inhibiting the transporter protein in the instine and inhibit intestinal transport of glucose and cholesterol (Kwon *et al*., 2007; Nekohashi *et al*., 2014). Flavonoid also has an estrogenic effect that may interfere with hormonal balance throughout

estrus cycle thus altering the folliculogenesis (Miksicek, 1995). In our previous study, extract ethanol of Cogon grass roots has ability to reduce testis weight and reduce epididymal sperm quality (Widyastuti *et al*., 2018; Lubis *et al*., 2018). However, the effect of extract ethanol of Cogon Grass in female reproduction is not known. Thus, the purpose of this study was to investigate the effect of the ethanol extract of cogon grass roots in female estrus cycle.

RESEARCH METHODS

This research was conducted at Animal Laboratory, Faculty of Medicine, Padjadjaran University. The materials in this study were 8-12 weeks old female mice DDY strain from BioFarma, Bandung, giemsa dye, CMC 0.5% , ethanol extract root cogon grass in dose 90mg / kg weight and 115mg / kg weight, 0.9% physiological NaCl, cholesterol and glucose reagents from DiaSys and tools used in this study are disposable syringe 1 cc, oral sonde, microscope, object glass, small size cotton swab, gloves, tissue paper, placemats for slides and capillary tube.

Animal Treatment and Extract Administration

This study was approved by ethical committe of Padjadjaran University (No:326/UN6.KEP/EC/2018). Mice were acclimatized for seven days before experiment. Mice were housed in room with 12h/12h light dark cycle and controlled air and temperature. Mice were given standard chow and water *ad libitum*. mice were divided into three groups with seven mice in each groups. The ethanol extract of cogon grass roots were dissolved in 0.5% CMC solution and given orally for 20 days with dose 0, 90 and 115 mg / kg body weight/day. The dose used in this study was based on previous study (Dhianawaty and Ruslin, 2015). Body weight were measured every three days interval to adjust the dose.

Vaginal Cytology

Vagina Sample collection was conducted with vaginal swab cytology everydays at 9:00 a.m. for looking mice that had five consecutive cycles of 20 days. Only mice with normal and regular cycles were used as samples in this study. Estrus phase in mice can be seen by observing several types of cells, namely neutrophils, nucleated epithelial cells, and anucleated keratinized epithelial cells. The estrus cycle was determined by first looking at the number of observed neutrophils then viewing and identifying the observed epithelial cells. The proestrus phase was marked by the observation of nucleated epithelial cells and observed very little or no observation of neutrophils, estrus phases was marked by the observation of anucleated epithelial cells and the absence of neutrophils, the metestrus phase was characterized by the observed nucleated epithelial cells and the observation of many neutrophils and the diestrus phase was characterized by observed many neutrophils and observed very little until the absence of nucleated epithelial cell (Cora *et al*., 2002). -

Serum Collection and Metabolite Profile Examination

Examination of the metabolite profiles (glucose and cholesterol) were performed before and after the study was conducted. Mice were overnight fasted before blood was collected and identified based on their follicular status: luteal and follicular phase. Blood was collected from sinus orbital of the eye. Blood was incubated in room temperature and then centrifuged 2500 rpm for 30 minutes to collect serum. Serum was stored at -80°C before further used. Serum level of glucose, and total cholesterol was measured based on manufaturer's protocols and compared independently in follicular and luteal phase.

Statistical Analysis

The data were analyzed using paired t-test to determine the effect of extract on female mice metabolite level before and after treatment, and using Kruskal Wallis to analyze between groups. Research data were analyzed using Statistical Package for the Social Sciences (SPSS) version 15 for windows.

RESULTS AND DISCUSSION

Table 1 showed no significant shortening of the estrous phase between treatment and control groups after ethanol extract of cogon grass roots administration. The shortening was statistically significant different between before and after administration of extracts in both groups of treatment ($p<0.05$). The length of estrus cycle were decreased 0.95 days and 0.73 days in group that were given ethanol extract of cogon grass roots of dose 90 mg/kg BW per day and 115 mg/kg BW respectively. Shortening in estrus phase may indicate an increase in estrogen levels (Auta and Hassan, 2016), whether due to increased

Table 1. Estrus cycle length before and after treatments

Groups of Treatment	Duration in days spent in each phase							
	Proestrus		Oestrus		Metestrus		Diestrus	
	before	after	before	after	before	after	Before	after
Control	0.79±0.22	0.97±0.40	1.72±0.74	1.42±0.73	0.4±0.33	0.15±0.20	3.11±1.75	4.19±1.68
90mg/kgBW	0.64±0.25	0.85±0.87	2.37±1.11[a]	1.42±0.65[b]	0.29±0.19	0.31±0.36	4.18±1.16	4.5±1.72
115 mg/kg BW	0.87±0.52	0.63±0.24	2.11±2.31[a]	1.38±0.17[b]	0.21±0.33[a]	0.03±0.08[b]	4.08±2.07	4.77±2.15

Note : Different letter in raw indicates statistically significant in the group at the same cycle phase

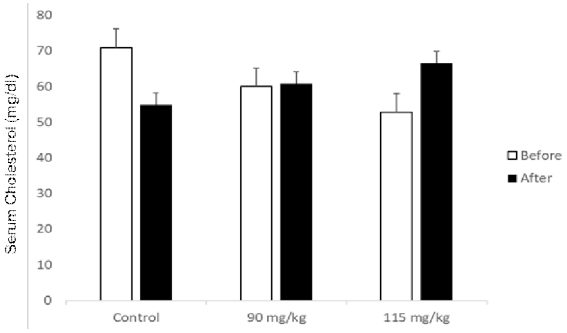

Figure 1. Cholesterol concentration before and after treatment in luteal phase.
Note: Data is not statistically significant

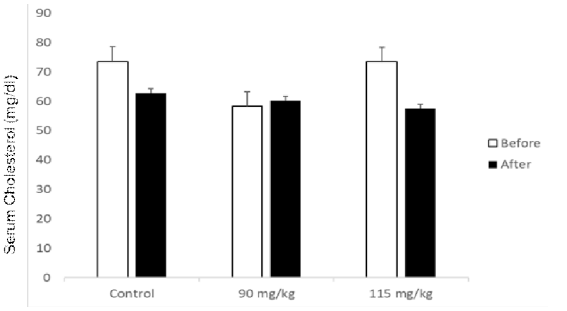

Figure 2. Cholesterol concentration before and after treatment in follicular phase
Note : Data is not statistically signifc

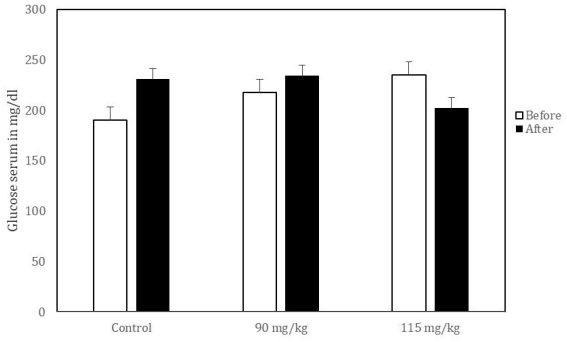

Figure 3. Glucose concentration before and after treatment
Note : Data is not statistically signifcant

estrogen production or from estrogenic substances. This increase in estrogen can induce an ongoing LH surge and induce ovulation then speed up the cycle in this phase. The previous research reported the similar effect of estrus phase shortening after treatment by aqueous wood ash extract of *Azadirachta indica* (neem) and ethanol extract of *Rivea hypocreteriformis* (Auta and Hassan, 2016; Shivalingappa *et al*., 2002). This effect was caused by the presence of phytoestrogens (Hassan *et al*., 2015). Phytoestrogens are a heterogeneous group of polyphenolic plant compounds that based on their chemical structure, can be classified into three subtypes: isoflavones, lignans, and coumestans that mimic the estrogen effects (Lechner *et al*., 2005). Since nutrient profiles can effect hormonal status. We further examined total cholesterol and glucose serum levels

Cholesterol has an important role in the reproductive cycle. This lipid substance plays a role as raw material for the manufacture of gonad hormones such as estrogen and progesterone with the highest level in the blood that occurs in the late estrus phase when the gonadal hormone levels decrease (Yoon *et al*., 2006; Tarumi *et al*., 1988) (Figure 1).

Based on the results obtained, the administration of cogon grass roots extract orally for 20 days caused only an insignficant changes in blood cholesterol levels in luteal phase and follicular phase mice. This condition may imply that there was no increased of cholesterol utilization for hormone production. It might be assumed that the elevation of estrogen level might be caused by external estrogen-like substance such as phytoestrogen.

Glucose acts as the main source of energy in the body so that the levels in the blood symbolize the use of energy in the body. Naturally, the glucose level is low during estrus phase because of the effect of estrogen by increasing insulin sensitivity. Rise in estrogen

level can cause shorter estrus phase by promoting LH surge (Nelson *et al*., 1981).

Figure 2 showed slight decrease of glucose serum in 115 mg/kg BW of cogon grass roots ethanol extract. This condition may be caused by direct antihyperglycemic effect of cogon grass (Syamsunarno *et al*., 2017) and or an indirect effect of increasing estrogen concentration.

The shortening of estrus cycle can also caused by another factor. One of them is the disturbance in circardian rhythm. Circardian rhythm work by sensing the light and processed in Supra Chiasmatic Nucleus (SCN). The SCN control the hypothalamus LH surge secretion and therefore induce ovulation. If this pathway was inhibited than normal estrus cycle could not happen (Miller and Takahashi, 2014).

The level of stress can also influence the estrus cycle. Stress activate the body fight or flight response and suppress the need of reproduction. In stress, body produce glucocorticoid hormone that may interfere in multi level of hypothalamus-pituitary-gonadal axis. In hypothalamus level cortisol inhibit sexual behavior, in pituitary level cortisol interfere LH and FSH secretion and in gonad level glucocorticoid interfere the ovulation process (Whirledge *et al.*, 2010).

CONCLUSION

Cogon grass roots extract have an effect to shorten estrus phase in female mice suggesting this effect might be due to an estrogenic effect of cogon grass roots extract. But cogon grass roots extract has no effect on glucose and cholesterol level in female mice.

SUGGESTION

Further research needs to be carried out related to the effect of cogon grass roots ethanol extract gavage to follicular development.

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