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The Effect of Sambiloto Extract on the Expression of β Estrogen Receptors in Ovaries and Lust Cycle in Mice Model PCOS - Insulin Resistance

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Abstract

Infertility was a serious problem in the reproductive period, one of the caused of infertility were common in women of childbearing age group is Polycystic Ovary Syndrome (PCOS).Sambiloto (Andrographis paniculata Nees), had been already known contain flavonoid and lactones. The purpose of this research had to determine the effect of various doses of sambiloto extract with the expression of β estrogen receptor and estrus cycle in the mice model of PCOS - insulin resistance. This research was an animal model experimental laboratory research with a completely randomized design (CRD).The results showed that sambiloto extract therapy in treatment group had an improvement estrus cycle compared with control group. Positive control group, almost 90% had persistent anestrus condition. In treatment group 1 and 2 in the last vagina smear obtained anestrus condition as much as 60% in diestrus and medestrus phase. Whereas treatment groups 3 all experimental animals in a estrus state by proestrus and estrus phase. β Estrogen receptor expression had tested by Kruskal Wallis test for overall treatment, obtained significantly different results ($p \leq 0.002$) followed by Mann - Whitney, showed that immunoreactive cells score from highest to lowest occurred in each group Negative control (KN), P3, P2, P1 and Positive Control (KP).It could be concluded that sambiloto extract at dose 18mg/kgbb, 36mg/kgbb, and 72mg/kgbb had been given an overview of the differences expression of â estrogen receptor, could change the estrus cycle of female mice models of insulin resistance PCOS.

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INTRODUCTION

Infertility is a serious problem during reproduction, the etiology of infertility is 40% of the incidence is male, 40% of the incidence is contributed by female factors, and 10% of the incidence is contributed by both factors, while the rest is unknown (unexplained factor) (Kasdu, 2002). One of the most common causes of infertility in the age group of fertile women is Polycystic ovary syndrome (PCOS), which is an endocrine disorder that occurs in women during reproductive years with a prevalence of 4-12% (Sheehan, 2004).

PCOS is associated with 75% of all anovulatory disorders that cause infertility, 90% of women with oligomenorrhea, more than 90% with hirsutism and more than 80% with persistent acne PCOS is also associated with insulin resistance, obesity, metabolic disorders, and infertility. (Dunaif A, 1997). The disruption of insulin action causes hyperinsulinemia which increases the secretion of androgens in the ovaries, which is accompanied by abnormal follicle development, which causes ovarian function disorders (Dunaif A, 1997).

The administmiceion of Testosterone Propionate (TP) dose of 1 mg / 100 gBB for 14 days will result in a condition that resembles PCOS with the characteristics absence of a corpus luteum, shownpolycystic ovaries, hypertecosis in the stroma and atression of granulosa cells. Administmiceion of TP for 21 days began to get a state of insulin resistance. TP for 28 days was more significant in the state of insulin resistance. Hyperandrogens can affect the insulin resistance index and free fatty acid levels in serum. The longer the exposure to androgens is given, the insulin resistance index and free fatty acid levels will increase (Muttaqin et al., 2008).

It is believed that insulin resistance and / or insulin's abnormal response to glucose stimuli are the principal underlying etiologic factors of PCOS (Legro, 2001; and Hopkinson, 1998). According to Samsulhadi, 2008 insulin resistance was one of the biggest influences in the pathogenesis of PCOS (69%).

Sambiloto (Andrographis paniculata Nees) contains andrographolide, which is a diterpenoid glycoside that can be used as a diuretic, antipireutic, analgesic and antiulserogenic Yulinah, et al. (2001). The ethanol extract of sambiloto herb had the effect of reducing blood glucose in alloxan-induced diabetic mice at a dose of 2.1 g / kg BW. The results of chemical research, it is known that sambiloto contains saponins, flavonoid, dan tanin (Winarto, 2004)

One of the functions of giving sambiloto leaf extract is as a diuretic. Hoped that it can reduce insulin levels in the blood so insulin resistance does not occur. Decreasing androgen levels makes the aromatization process of the androgen hormone converted into estrogen. So that, folliculogenesis can occur and eventually the mices has lust cycle.

This research aims to determine the effect of sambiloto extract with various doses on the expression of estrogen receptors and the lust cycle in mice with PCOS - insulin resistance models.

METHODS

This type of research is true experimental labomiceory with a completely randomized design method. Using female mice (Micetus novergicus) age 3month with 100-150 grams of body weight. Divided into 5 groups, each group consisting of five experimental animals. To avoid sample shortages due to death during treatment, the sample size of each group was added more two samples, so that the total sample was 35 mices.

The variables in this research consisted of independent variables, the dose of sambiloto extract 18mg / kgBW, the dosage of sambiloto extract 36mg / kgBW, the dose of sambiloto extract 72mg / kbBB. And the dependent variableexpression of â estrogen receptors in the ovaries, changes in the lust cycle.

RESULT

Lust Cycle

The effect of giving sambiloto extract on lust cycle shown in this Table 1.

Based on the result,sambiloto extract as sample therapy shown better improvement lust cycle compare with control group subject. Folicular phase on vaginal swab shown proestrus cycle and estrus. Than on lutheal phase shown metestrus and diestrus cycle. Control group positive almost 90% under persistent anestrous, where at the end of vaginal swab examination 4 experimental animals were in a diestrous condition which was the longest last period in the lust cycle. In this phase, there are many leukocyte cells and towards the final phase of diestrus, there will be a few epithelial cells in the vaginal swab prepamiceions. Whereas in treatment group 1, 60% of the first vaginal swabs were in

Table 1 Data on vaginal swab results

Repeat	Vagina Swab														
	KN			KP			P1			P2			P3		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1	E	D	D	D	D	D	P	M	M	M	D	M	D	M	E
2	D	E	P	D	D	D	P	D	D	M	D	D	D	M	E
3	D	D	P	D	D	P	P	D	P	M	D	D	D	D	E
4	D	E	M	P	D	D	D	M	D	D	M	E	D	D	P
5	D	D	D	P	D	D	D	M	E	D	D	E	D	D	P

Information:
I = before treatment testosteron propionat
II = after treatment testosteron propionat
III = after treatment extract sambiloto
Fase Folikuler/Estrus : P = Proestrus, E = EstrusFase
Luteal/ Anestrus : M= Medestrus, D=Diestrus

estrous conditions, and the rest were in anestrous conditions. The experimental animals that are still in anestrous condition are waiting for the cycle to rotate again to uniform the cycle to a minimum of proestrus then after being given a testosterone propionate injection, on the second vaginal swab all are in anesthetic condition then after being treated with sambiloto extract dose of 18mg / kgbw for 15 days, still Anestrous conditions were obtained as much as 60%, in the diestrus and medestrus phases. Medestrus is the phase that occurs after estrus is complete. The vaginal swab showed the presence of leukocytes and some cornified epithelium. Diestrus is the longest last period in lust cycle. In this phase, there are many leukocyte cells and towards the final phase of diestrus, there will be a few epithelial cells in the vaginal swab prepamiceions. In treatment group 2, before treatment all experimental animals were in anestrous conditions, so that the treatment was started after the cycle returned to estrous conditions (uniformity of the cycle to a minimum of proestrus) and then injection of testosterone propionate for 28 days given and shown the results of the vaginal swabs of the two experimental animals were in anestrous conditions. After being treated with sambiloto extract at dose of 36mg / kgbw for 15 days, 60% of the anestrous condition was still obtained, in the diestrus and medestrus phases, where medestrus phase that occurs after the estrus is complete. The vaginal swab showed the presence of leukocytes and some cornified epithelium. Diestrus is the longest last period in the lust cycle. In this phase, there are many leukocyte cells and towards the final phase of diestrus, there will be a few epithelial cells in the vaginal swab prepamiceions. In group 3 treatment, before treatment all experimental animals were in anestrous conditions, so that the treatment was started after the cycle turned back to estrous conditions (uniformity of the cycle to minimum

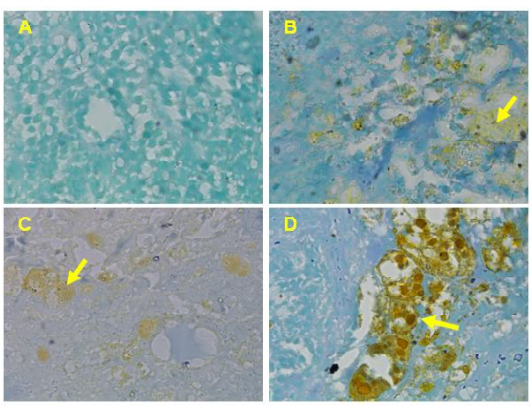

Figure 2 Expression of estrogen (arrow) on immunoreactive cells in all treatment groups, estrogen tended to be expressed on garanulosa cells and some theca cells with varying intensities from negative (slide A), weak (slide B), modemicee (slide C) and strong (slide D) at 1000x magnification.

Information :
L : leukocytes
B : Basal Cell
K : Cells undergo cornification

Table 2 Results the Mann-Whitney test for estrogen receptor expression

Kelompok	Median	Minimum	Maksimum	Mean	SD
KP	1[b]	0	2	1.2	± 0.837
KN	4[a]	3	6	4.0	± 1.225
P1	1[b]	1	2	1.4	± 0.548
P2	2[b]	2	4	2.6	± 0.894
P3	3[a]	2	4	3.2	± 0.837

Different superscripts in the same column indicate that there are significant differences
KN : Negative control without treatment
KP : Positive control with induction of testosterone propionate 1mg / 100gBW
P1 : Treatment 1 was given 18 mg of sambiloto extract for 15 days
P2 : Treatment 2 with 36 mg of sambiloto extract for 15 days
P3 : Treatment 3 was given 72 mg of sambiloto extract for 15 days

proestrus) and then injected with testosterone propionate for 28 days and results the vaginal swabs of the two experimental animals were in anestrous conditions. After being treated with sambiloto extracts at dose of 72mg / kgbw for 15 days, all experimental animals were in estrous conditions with proestrus and estrous phases. The description of each period can be seen in Figure 1.

β Estrogen Receptors Expression

All data were then tested using the Kolmogorov Smirnov test to determine whether the data was normally distributed or not and the results showed that the data were normally distributed (p> 0.05).

The analysis calculation results carried out by the Kruskal Wallis test on the overall treatment obtained significantly different results (pd”0.002). Then from the analysis results a comparison between treatments with the Mann - Whitney test. Based on Table 2, it can be seen that there are significant differences in the expression of â estrogen receptors between the negative control group and the positive control group (induction of testosterone propionate 1mg / kgBW), treatment 1 (18 mg of sambiloto extract for 15 days). While the positive control group had a significant difference in the expression of β estrogen receptors with treatment group 2 (giving 36 mg of sambiloto extract for 15 days) in treatment group 3 (giving 72 mg of sambiloto extract for 15 days).

In group 1treatment (giving 18 mg of sambiloto extract for 15 days) showed a significantly different expression of β estrogen receptors with group 2 treatment (36 mg of sambiloto extract for 15 days) and treatment 3 (72 mg of sambiloto extract for 15 days). However, between treatment group 2 (sambiloto extract 36 mg for 15 days) did not show a significant difference in the expression of â estrogen receptors with treatment 3 (sambiloto extract 72 mg for 15 days).

For more details, the differences in the expression of β estrogen receptors in each group can be shown in Figure 2 below.

Based on Figure 2, the results of this research indicate that the immunoreactive cell scores from the highest to the lowest occurred in the Negative Control (KN), P3, P2, P1 and Positive Control (KP) groups, respectively.

DISCUSSION

Lust Cycle

This research shows that hyperandrogenic condition with insulin resistance after being given Sambiloto extract for 15 days turned out to change the hyperandrogen condition, which initially stopped the estrous cycle (anestrus) to be running again in the treatment group whereas in the positive control group as a pathological condition the cycle did not run because it was not given extract Sambiloto as therapy, so that the mice are still under stress, and show a state of persistent anestrus. The higher therapeutic dose in group treatment is expected to accelemicee the process of improving the condition of insulin resistance so that the estrous cycle can run again.

Table 1 shows the changes in the phase ofthe estrous cycle that occurred in each individual mice for a period of 43 days represented by 3 vaginal

swab examinations, the first before giving testosterone propionate, second examination after giving testosterone propionate for 28 days. This examination is to ensure that the animal's estrus is in the anestrous phase (medestrus or diestrus phase). Then the last vaginal swab examination was carried out after administration of sambiloto extract for 15 days. It hoped that with this therapy, the estrous cycle can occur again in experimental animals with indications that they are in proestrus and estrous phases.

It can be seen that negative and positive control groups shown differences, where the negative control group runs normally, while the positive control group cycles cannot return to normal, because they have been given testosterone propionate induction as a model of PCOS insulin resistance, so that all experimental animals experience persistent anestrus describing anovulatory condition. Increase in testosterone levels will suppress the secretion of SHBG by the liver, resulting in increased levels of testosterone and free estradiol (sex active form of steroids hormone is the free form). The increase in estrone and estradiol levels will provide a positive feedback to LH, so that LH levels increase even more. The increase in LH levels stimulates synthesis of androgens, the increase in the levels of androstenedione is converted by fat / muscle tissue to estrogen. Meanwhile, the FSH level remains low but there is still follicle growth until the anthral stage with approximately 8 mm. There was an accumulation of small follicles lining in ovary, but never enlarged, and ovulation. This image occurred in positive group control. The group treatment was given sambiloto therapy with various doses, there was a change in the pattern of the estrous cycle after this treatment was actually closely related to the changes occurred in ovaries. The estrous cycle is a repetitive process that describes changes in reproductive hormone levels caused by ovarian activity under of pituitary hormones. Changes in reproductive hormone levels, cause structural changes in constituent tissues. Where after given sambiloto extract therapy which is thought to have an important role in this case is andographolid and isoflavone, which are phytoestrogens cause anti-estrogenic effects when high estrogen concentration senvironment, and vice versa cause estrogenic effects when low estrogen concentration senvironment. This estrogenic effect can result decreasing androgens. The follicle is able to change androgen dominant environment into estrogen dominant. The growth period of follicle until it reaches maximum development is the proestrus phase in mice. The FSH hormone initiates development of ovarian follicles and increases number of granulosa cells, in addition, increase number of theca cells is influenced by LH which in turn can increase estrogen production and progesterone synthesis (Brook and Marshall, 1995).

Estrogen β Receptor Expression

ER β immunohistochemical examinate female rats ovaries with ER â monoclonal antibody showed that micea-micea expression of estrogen receptor β (ER β) ovaries of female rats in the group treatment (sambiloto extract) was higher when compared to group control. This research has proven that bitter extract contains compounds similar to estradiol, and administration to female rats caused significant differences to the control group (Table 2). Furthermore, Harris (2007) stated that ER β has a sizable role in ovaries, cardiovascular system and brain (Wang et al, 2006).

Phytoestrogens fully actat beta estrogen receptors, they have a high affinity for â estrogen receptors (Kuiper et al, 1998). After hormone bound with receptor, then biological effect tissue emerge. Estrogens can carry out biological actions through extranuclear receptors by interacting directly with other growth factors, namely the EGF receptor or through ER membrane. Furthermore, Razandi et al (2003) stated that like estrogen receptors in nucleus, the receptors on plasma membrane will form dimers to action support of rapid signal transduction and can affect physiological functions.

Non-genomic action will cause increase in genomic action activity or convergent (Bjornstrom, 2005) or there is an integration between non-genomic and genomic actions in influencing gene expression (Pedram et al., 2002). Biological actions of both genomic and non-genomic estrogens can activate factors. Another growth, in this case IGF-I, causes cross-talk between IGF-I and Estrogen (Kato et al, 2000). This means, the stronger ERâ expressionthat occurs in group treatment, the more it can activate IGF-1 so that there can be an overlap between hormone functions between IGF-1 and estrogen.

The regulatingmechanism action of hormones at the cellular level can be regulation number of receptors. Receptors can be more or less sensitive

to a hormone depending on cell differentiation. Cells can even lose their capacity to respond hormones due to loss of cellular differentiation and recovery of membrane receptors. This is what happened to IGF-1. When insulin levels are very high, and attach to receptors. Receptors and insulin are directly degraded by the lysosomes, resulting in down regulation of insulin receptors. This will reduce receptors number on cells so that high insulin response remains normal.

Phytoestrogens are natural generally derived from plants, which is estrogen-like subtances (called phytoestrogens or herbal estrogens) (Tapan. 2003). Phytoestrogens generally contained in bitter plant are triterpenes glycosides and isoflavones. Increasing the dose,more phytoestrogen content in the blood and tissue of mice. So that on examination through the immunohistochemical method, the results are higher dose, higher β estrogen receptor expression as shown in Figure 2.

In addition, it is possible that there other factors that may have an effect, this research uses natural ingredients form extracts instead of certain bioactive compounds from the isolation of sambiloto so that, there is a possibility that other compounds in sambiloto extractaffect estrogenic activity, it also affectsestrogen receptors â.expression.

CONCLUSION

1. Sambiloto extract with doses of 18mg / kgbw, 36mg / kgbw, and 72mg / kgbw had been given to micees, had shown differences â estrogen receptors expression, where the highest to the lowest figures respectively occured in the negative control group (KN), P3, P2, P1 and Positive Control (KP).
2. Sambiloto extract were starting at a dose of 36mg / kgbw, and 72mg / kgbw could improved female mice lust cycle insulin resistance PCOS models to re-estrus with the optimum dose in this research is 72mg / kgbw. However, sambiloto extract dose of 18mg / kgBW had not been able to improve this condition.

SUGGESTION

Further research is still needed, regarding other factors that may affect the improvement of infertility conditions and insulin resistance in addition to the lust cycle and also β estrogen receptors expression. Then further research can find the optimum dose without causing negative effects or causing toxicity so that later it can be applied to humans.

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