Sauropus Androgynus for Increasing Uterine Weight in Menopausal Women: An Experimental Study Using Animal Models

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Abstract: Menopause is a natural phase of life and happens in every woman who reaches a certain age. This condition has many problems, such as endometrial atrophy, which can cause abnormal bleeding. *Sauropus androgynus*, known as Katu Leaf, has isoflavones compound and acts as an estrogen-like agent or phytoestrogen. Isoflavone can induce cell proliferation activity in increasing endometrial thickness and uterine weight. This study was aimed to find how the is flavone compound in *Sauropus androgynus* can increase the uterine weight in menopausal mice as an animal model. Twenty female mice aged two months and one week were given VCD (*4-Vinyl cyclohexane dioxide*) to induce the atresia acceleration of ovarian follicles, then treated with Katu Leaf extract of 0, 15 and 30mg/kgBW over four weeks. The uterine weights were measured and analyzed with One Way Analysis of Variances (Anova) and further tested with Least Significance Difference in 95% confidence interval. The result showed a difference between control and treatments group ($p<0.05$) with 30mg/kgBW as highest dosage, which increase uterine weight as 112.80 $\pm$8.86mg), but this is not higher than normal (116.36$\pm$6.83mg). This study suggests that Katu Leaf extract has an isoflavone compound which can increase uterine weight in menopausal mice.

1 INTRODUCTION

The revolution in healthcare, education, knowledge and income levels has led to higher life expectancy. The increase in life expectancy also causes the number of women who enter the menopausal phase to become higher. Menopause at 45 to 50 years old is caused by decrease of ovary ability, resulting in the decline of estrogen hormone levels (Gold, 2011). The estrogen and others of the nuclear receptor (NRs) superfamily play roles as nuclear transport proteins, cell cycle and transcription factors. The steroid hormones act by binding to nuclear receptors as ligand-inducible transcription factors. Estrogen has many functions in growth, differentiation and other non-reproductive metabolism functions. Estradiol, a kind of estrogen, gives a female characteristics, regulates reproductive cycles, pregnancy, skin, immunity, protects the cardiovascular system, bone mass and brain system (Sunita and Pattanayak, 2011).

Decreased ovarian function in menopause causes hypoestrogenism. Estrogen deficiency during menopause can lead to risk for many health problems, such as atrophy of the female genital tract, hot flushes, sleeping disorders, vaginal dryness, joint pain, mood swings, reduced bone density, cardiovascular disease and more (Cavadas *et al.*, 2010). The American College of Obstetricians and Gynecologists stated that the decline of estrogen levels in menopause causes reduction of endometrium thickness or endometrial atrophy. This condition may cause abnormal bleeding as one of the health problems in menopause.

The classical therapy of reducing vasomotor symptoms, especially hot flushes and urogenital atrophy, in menopausal women is with hormone replacement therapy (HRT). However, recent study shows that the use of HRT is related with increasing breast and endometrial cancer risk; thus, researchers

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have to look for other therapeutic alternatives (Rymer, Wilson and Ballard, 2003).

In recent few years, isoflavones has become known as an alternative to HRT because they are reported safer, to be an estrogen agonist and have weak activity. This action is termed phytoestrogen, which is a definition applied to any plant substance or metabolite that induces biological responses in vertebrates and can modulate the actions of endogenous estrogens, usually with the binding to estrogen receptors (ERs) (Molla, 2001; Sunita, 2010). Isoflavone is the most studied of phytotherapeutic agents, found mostly in soy with its main compounds being genistein, daidzein, biochanin A and formononetin (Setchell, 2001). Phytoestrogen has two hydroxyl (OH) within 11.0 to 11.5A^0 in its core, exactly the same as estrogens. Distance 11A^0 and the OH group are the basic structure of a substrate in order to have estrogenic effects (Boker *et al.*, 2002; Zand, 2000).

Katu Leaf (*Sauropus androgynus* (L.) Merr.) has a potential as a phytoestrogen according to the research results on Katu Leaf content by Ekawati (2009) and Miean (2000), who found that Katu Leaf has flavonoid compounds, flavone, flavonol, and isoflavones and also has a quercetin. Moreover, Santoso (2010) suggests that this leaf contains the active *Androstan-17-one,3,-ethyl-3-hydroxy-5alpha* which has precursor function or intermediate step in steroid hormones synthesis. Boker *et al.*, (2002) reported that the isoflavones and quercetinas phytoestrogens have structural similarity to estradiol in its rings and cause estrogenic activity and are able to bind to estrogen receptors.

The use of phytoestrogens, especially isoflavone and quercetin agents, for menopausal therapy alternatives has been widely studied. Burton (2002) said that use of isoflavones is a safer therapy which increases endometrial thickness and also cancer preventative agents. Goodman, et al. (1997) found that, although there was a negative correlation between dietary fiber intake and the risk of endometrial cancer, there was also an inverse correlation between isoflavones intake and the risk of endometrial cancer. In addition, Bandera *et al.*(2009) said that there is a reduction in endometrial cancer risk with quercetin and isoflavone consumption.

Many studies, especially with animal models, show the action of phytoestrogen intake in uterus or other female genital tracts. However, the action of *Sauropus androgynus* is known to have isoflavones and quercetin compound in menopause. The objective of the present study was to investigate whether the actions of *Sauropus androgynus* compound can increase the uterine weight in menopausal mice as an animal model.

2 METHODS

2.1 Experimental Design and Sampling

This study was an experimental study using completely randomized design with five replications. Menopausal condition was done by giving VCD (4-*vinyl cyclohexane dioxide*) then in group treatment was given with Katu Leaf Extract (KLE) to know the effect. The study groups consisted of K- (control group, normal), K+ (VCD+KLE 0mg/kgBW), P1 (VCD+KLE 15mg/kgBW), P2 (VCD+KLE 30mg/kgBW).

Tools and materials used included female mice, cages, food, Katu leaf, water, VCD (4-*vinyl cyclohexane dioxide*), sesame oil, Giemsa staining, freeze dryer, chloroform, NaCl 0.9% and analytical balance.

2.2 Katu Leaf Extract

Sterilized and shade air-dried *Sauropus androgynus* leaves were extracted with water extract methods and preserved with freeze-drying according to Popp *et al.* (1996) in the Chemistry Laboratory of Muhammadiyah University of Malang.

Powdered Katu leaves were soaked in boiling distilled water. Filtrate were extracted a second time with additional boiling distilled water. The combined filtrate was then freeze-dried, and the sticky precipitate was stored at 4°C. Aliquot of extract residue were weighed and suspended in de-ionized distilled water to a final concentration before use.

2.3 Animals

Twenty female balb/c mice aged two months and one week, 25-30gm, were obtained from the Animal Biosystematic Laboratory of State University of Malang, housed in plastic cages with wooden chip bed, given food and water *ad libitum*, and exposed to a light-dark cycle of 12 hours as its cyrcadian rhythm following Eckel-Mahan and Sassone-Corsi (2015).

Samples treatment was performed in laboratory under controlled and constant condition. Samples used in this study were observed to know if there was a change in body weight during the study.

2.4 Animal Models of Menopause

Menopausal condition was done by giving VCD (4-*vinyl cyclohexane dioxide*) according to the research conducted by Kempen, Milner and Waters (2011) which states the administration of low dosage of 160mg/KgBW for 10 days in 14 days (five times a week within 14 days) intraperitoneally.

2.5 Estrous Cycle Observation

Estrous cycle was determined by observing the results of the vaginal smear and Giemsa staining by Marcondes, Bianchi and Tanno, (2002). The constituent phases determination of the estrous cycle and long-cycle was conducted by comparing nucleated epithelial cells, gore epithelial cells (cornification), leucocytes, mucus and the vaginal smear results.

The normal estrous cycle length in mice is approximately 3-4 days, the cycle change indicated the beginning of menopause. Compared with human, Harlow and Paramsothy (2011) said that menopausal transition begins with variability in menstrual cycle length.

2.6 Data Collection

Animals were terminated on diestrus phase then uterine samples were isolated and weighed using analytical balance, measured in milligrams.

2.7 Statistical Analysis

Collected data were tested with *Shapiro-Wilk* normality test and *Lavene* homogenity test. If the results were normal and homogeneous (P>0.05) then it was continued with 5% one way Anova analysis. If there was a significant change, it was further tested with LSD (Least Significance Difference) 5%.

3 RESULTS

The results obtained from the current study and presented in Table 1 revealed that the group with Katu Leaf extract showed significant ($p<0.05$) increase of uterine weight (91.82 ± 10.43mg versus 107.58 ± 10.40mg and 112.80 ± 8.86). The study groups treated with Katu Leaf extract intake (P1, P2) showed higher weight of uterine than those not treated. Increased uterine weight in mice may be caused by increase of endometrial thickness.

Table 1: Uterine weight in mice treated with different doses of Katu Leaf extract.

Study Groups	Uterine weight (mg)	P
K+ (VCD + KLE 0 mg/kgBW)	91.82 ± 10.43	0.004*
P1 (VCD + KLE 15 mg/kgBW)	107.58 ± 10.40	
P2 (VCD + KLE 30 mg/kgBW)	112.80 ± 8.86	
K- (normal group)	116.36 ± 6.83	

*p <0.05 indicates that there is significant difference between treatment and control groups

Increased uterine weight is directly proportional with the dose of Katu Leaf extract, shown in Figure 1. The highest uterine weight is in normal group, without any treatments.

The average of uterine weight is affected by Katu Leaf treatment known from correlation test between uterine weight (x) and body weight (y). Pearson test shows that there is no relationship between uterine weight and body weight (p>0.05). The mice body weight may be changed by decreasing levels of estrogen, but, in this study, we found that the change of the uterine weight was not affected by the mice body weight.

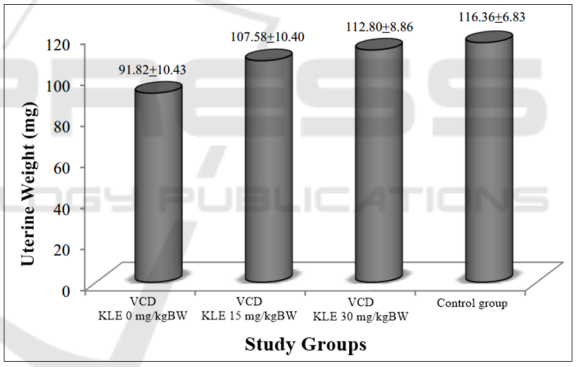

Figure 1: Increase of mice uterine weight treated with different doses of Katu Leaf extract.

4 DISCUSSION

Estrogen action in the body is by binding with estrogen receptors (ER), ER-α or ER-β, which have its own function. Recent studies show that the uterus has two receptors with different function. ERα is for maturation, paracrine and autocrine mitotic activation and function of uterine tissues, while ERβ is expressed in the uterus for genital development, including regulating the development of stromal, myometrial and uterine glands (Dupont *et al.*, 2000). Development of the genital tract is then shown in the uterine weight.

From Table 1, it may be found that miceuterine weight is higher by giving Katu Leaf extract. Previous study by Helmy (2014) shows that the phytoestrogen intake can increase the endometrial thickness and then the uterine weight. This action is modulated by binding of the isoflavone and estrogen receptors. The type of estrogen receptor is ERβ, known from immunostaining, which shows that it is more expressed and isoflavone is known as preferential affinity with ERβ. The complex binding of ligand and receptor induces the expression of estrogen-responsive genes, which ultimately results in increased uterine mass.

In this study, menopause mice condition was with 4-*Vinyl cyclohexane dioxide* (VCD) induction. VCD causes accelerated ovarian follicle atresia through the increased apoptosis protein and may cause declined ovarian ability to produce estrogen and boost uterine growth (Hu *et al.*, 2001). Reduced human blood estrogen concentration causes reduced thickness of the endometrium, then uterine glands are not able to make secretion and results in loss of uterine weight.

Moggs *et al.* (2004) said that animal uterus with ovary disorder is very sensitive to the effect of estrogenic compound. Giving estrogen-like compounds causes hyperemi and water imbibitions into the uterus, with increased regular metabolism degree as a result of increased use of oxygen, the increase in the degree of phosphorus use, the increased glycolicis degree and the increased amount of DNA, RNA and proteins.

Cooke *et al.* (1998) also explain about the mechanism of this process, wherein a phytoestrogen compound will bind with hormone receptors on target cells, thus changing hormone receptor conformation. This conformation change leads to an active phytoestrogen receptor, thus it will be able to perform binding site on the DNA chain, specifically on the acceptor side. The interaction between phytoestrogen receptor and the DNA acceptor side causes the increased gene expression. This gene expression is then catalyzed by an RNA polymerase enzyme, which may lead to increased mRNA. Moreover, tRNA synthesis will also increase and cell material synthesis becomes higher, which may support cell proliferation activity.

Although there was an increase in uterine weight in the treatment groups, as shown in Figure 1, this was not higher than the control group. This result indicates that phytoestrogen intake is safe and does not increase endometrial risk as the proliferation activity is in normal levels. But, as is known there are still pros and cons regarding the use of phytoestrogens for menopausal therapy is, mostly concerning the breast and cancer risk, although many recent studies have concluded that phytoestrogens can even reduce carcinogenesis risk (Patisaul and Jefferson, 2010).The study by Ferraz Carbonel *et al.* (2011) concluded that isoflavones consumption in normal dose is of benefit for the body, including have an uteotropic effect, but that too high a dose of isoflavones can promote endometrial squamous metaplasia. In this study, we found that the dose we use is safe. However, it should be further investigated concerning a high dose of Katu Leaf and how to determine the correct recommended dose of isoflavone or Katu Leaf.

5 CONCLUSIONS

The current study demonstrates the effect of *Sauropus androgynus,* or Katu Leaf, 15 and 30mg/kgBW on menopausal mice uterus and their proliferative effect, which increased the uterine weight.

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