

Original Research Paper

Effect of Dayak Onion (*Eleutherine bulbosa* (Mill.) Urb) on Uterine wall and Lipid Profiles of Ovariectomized Rat

Amalia Purnamasari and Anton Bahtiar

Department of Pharmacology and Toxicology,
Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia

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Corresponding Author:

Anton Bahtiar

Department of Pharmacology
and Toxicology, Faculty of
Pharmacy, Universitas
Indonesia, Depok, Indonesia
Tel: +62-21-7864049
Fax: +62-21-7863433
Email: anton.bahtiar@farmasi.ui.ac.id

Abstract: Menopause is a physiologic condition, in which women no longer experience menstruation. Decreased estrogen during and after menopause causes structural, physiological and biochemical changes that alter the health of women in general, both in organs and non-reproductive organs. Dayak onion contains eleuterinol, an naphtoquinone derivative that can be as SERM. Therefore, we evaluated the effects of dayak onions on lipid profiles and uterine wall. This study used female white rats of Sprague-Dawley. All groups, except the sham group, is ovariectomized to obtain the conditions of hypoestrogen. Rats were divided into 6 groups. SHAM (CMC Na 0,5%), OVX (CMC Na 0,5%), TAM (tamoxifen), DOE dose 1 (dayak onion extract 8 mg/200), DOE dose 2 (dayak onion extract 12 mg/200 g) and DOE dose 3 (dayak onion extract 18 mg/200 g). Mouse model of ovariectomy given 3 doses of extract, from giving each extract it was seen that the weight of uterine DOE dose 1, DOE dose 2 and DOE dose 3 respectively 55,24, 53,38 and 61,85 mg had lower uterine weight when compared with Positive control given tamoxifen 72,2 mg. The effects of onion on uterine weight reduction are greater than that of tamoxifen on the animal model menopause.

Keywords: Dayak Onion, Ovariectomy, Uterine Size, *Eleutherine Bulbosa*

Introduction

The menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Oestrogen and progesterone receptors are found throughout the urogenital tract and are sensitive to any hormonal changes that occur around the menopause. The characteristic changes that occur to all tissues are epithelial thinning, reduced vascularity, decreased muscle bulk and increased fat deposition.

Hormone Replacement Therapy (HRT, a combination of estrogen and progestin) or Estrogen Replacement Therapy (ERT) has been used for many years as a gold standard for treating menopausal symptoms (Gennari *et al.*, 2010). However long-term use may increase the risk of breast cancer, endometrium and deep vein thrombosis (Jordan *et al.*, 2001; Gennari *et al.*, 2010).

There are several alternatives to HRT, one of which is Selective Estrogen Receptor Modulators (SERMs). SERMs are a class of compounds that interact with Estrogen Receptors (ER) in target organs as both agonists and antagonists. SERMs compounds have no

estrogen steroid structure, but have tertiary structures that can bind to ER α and/or ER β (Gennari *et al.*, 2010).

Estrogen is the women sex hormone that secreted by ovarian (Sherwood, 2010). Estrogen works in the development of female secondary sex characteristics during puberty. In addition, estrogen also affects stimulation of bone formation and limits bone resorption, affecting lipid profile and vascular endothelial, liver and cardiovascular system (Corwin, 2008). The menopause marks the end of reproductive life and occurs after 12 consecutive months of amenorrhoea, for which no other pathological or physiological cause can be established (O'Neill and Eden, 2017). Symptoms associated with the menopausal transition occur in up to 85% women and include vasomotor, vaginal dryness, dyspareunia, urinary frequency, urgency and a number of other symptoms, with varying degrees of severity and disruption to their lives (O'Neill and Eden, 2017).

The most effective treatment for relief of menopausal symptoms (hot flushes, night sweats and urogenital atrophy) is oestrogen with or without progestogen. Most estrogenic responses in tissues are mediated by Estrogen

Receptors (ERs), ER α or ER β , which involve ligand-dependent transcription factors. As postmenopausal hormone replacement has become an ever more complex issue, women have sought non estrogen alternatives to treat their menopausal symptoms and improve their overall health. Selective Estrogen Receptor Modulators (SERMs) have been developed to preserve the benefits of traditional hormone therapy while avoiding unwanted side effects (De Oliveira *et al.*, 2013). SERMs are ER ligands that in some tissues act like estrogens, but block estrogen action in others through competitive inhibition of estrogen binding to ERs (Pinkerton and Thomas, 2014). The resulting biologic action can vary according to the specific type of ER, cofactors, responses and ligands leading to tissue specific agonist and antagonist activity. The ideal SERM would treat such symptoms as hot flashes, vaginal dryness and mood changes while protecting women from osteoporosis and heart disease. These benefits would be met without substantially increasing the risk of breast or endometrial cancer (De Oliveira *et al.*, 2013).

Dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb.) is from Iridaceae family, it contains eleutherinol compounds that was *in silico* study have been shown to bind to estrogen receptors (Amelia *et al.*, 2014). This plants empirically has been used to cure breast cancer, high blood pressure, lowering blood glucose, lowering cholesterol, drugs boils, colon cancer, stroke and abdomen pain after delivered a baby (Galingging, 2009). Therefore, the aim of this study is to evaluate the effects of dayak onion buls extract on uterine as reproductive organ and lipid production as non reproductif organ.

Materials and Methods

Plant and Chemical Materials

Eleutherine bulbosa (Mill.) Urb. were collected from Palu, Central celebes, Indonesia. Determined by the Research Center for Natural resources of Tadulako University (Certificate of Determination No. 206/IPH.1.01/If.07/I/2016). Extraction of Dayak onion bulbs waas carried out in the laboratory of Indonesian Center for Spices and Medicinal Plants Research, Bogor. The extractive value of methanol from dried powders was calculated as % w/w yield and was found to be 3.77%. The identification of the extracts was conducted in the laboratory of the Center for the Study of Biofarmaka, Bogor Agricultural University.

Animals

Female Sprague–Dawley rats, aged 42 days, were purchased from National Institute of Health Research and Development (NIHRD). The animals were grouped

and housed in polyacrylic cages with one animal per cage and maintained under standard laboratory conditions (temperature 25±2°C) with dark and light cycle (12/12 h) and allowed free access to commercial pellet diet and water ad libitum.

Rats were acclimatized to laboratory condition for 1 week before commencement of experiment. At 50 days of age, bilateral ovariectomy was performed via a dorsal midline incision under ketamine injection (Raso *et al.*, 2009). All rats were allowed controlled access to a commercial standard pellet and free access to deionized water. Normal (sham-operated) and OVX rats were sacrificed under light anesthesia to determine the baseline at 21 days after ovariectomy. after checking the success of ovariectomy, animals were assigned to experimental groups, SHAM, OVX, OVX-Tamoxifen, DOE dose 1, DOE dose 2 and DOE dose 3, with six animals per group. Group of DOE dose1 to DOE dos 3 are groups who received 70% ethanolic extracts of Dayak Onion bulbs (DOE)/*Eleutherine bulbosa* suspended in 0,5% CMC with doses 8; 12; and 18 mg/200 g BW of rats, respectively. Tamoxifen and dayak onion bulbs extracts was given orally using a stomach tube for 3 weeks. The food intake of all rats was measured everyday.

On the day after the last dose, the rats were blood collected from orbital plexus and sacrificed under light anesthesia (Joslin, 2009). The uterus was removed and the wet weight and subject for histology preparation.

Determination of Lipid Profiles

Cholesterol, Triglyceride, LDL, HDL levels were determined by the enzymatic colorimetric method using kit (DiaSys, Germany) and evaluated using spectrophotometer UV-Vis Shimadzu 1800 (Japan) (Xu *et al.*, 2014).

Histological Analysis

The uterine was fixed in 10% buffered formalin for 48 h. Uterine were cut for three cross section per area. All samples were embedded in paraffin and 3 μ m thick sections were cut, mounted and stained with Hematoxylin and Eosin (H&E) for microscopic analysis (López-Belmonte *et al.*, 2012).

Statistical Analysis

Data from the animal experiments were expressed as the mean ± S.E.M. The statistical significance of differences between the groups were assessed with a one-way ANOVA, followed by Bonferroni or LSD post-hoc test analysis using software SPSS. p values of less than 0.05 were considered to indicate significant differences.

Results

Identification of Naphtoquinone of the Dayak Onion extracts by TLC

We have evaluated naphtoquinone compound by TLC as a core of eleutheronol Fig. 1. We did not have standart of eleutheronol therfore we evaluated naphtoquinone. Using 10% of potassium hydroxide in ethanol spray, we identified the naphtoquinone at Rf 0.87 after eluted extract by chloroform: Methanol (7:1) (Insanu *et al.*, 2014).

The Effects of Dayak Onion Bulbs Extract on Serum Lipid Profiles

Table 1 showed that ovariectomized rats have increased of all the lipids except for HDL, this result similar with previous research taht showed increased of lipid plasma in ovariectomy (Kitson *et al.*, 2015). All treatment using tamoxifen and dayak onion bulb extracts could reduce lipid profiles in ovariectomized rat. The treatment by the dayak onions bulb extracts has better result compared with tamoxifen treatment, especially on DOE dose 3.

We then evaluated the body weight of rats and found that ovariectomized increased body weight. The

treatment of ovx rats by tamoxifen have not changed the body weight that increased by ovx. But the treatment of ovx rats by dayak onions bulbs ektracts for 3 weeks can be decreased the increasing weight of ovx rats especially on DOE dose 2 and DOE dose 3.

The Effects of Ovariectomy to Uterine Size and Uterine Weight

We then evaluated the extract on uterine size and weight as shown in Fig. 2 and Table 3. Figure 2 indicated that ovariectomy decrease the size of uterine and the administration of tamoxifen and three doses of extract could not recover the size of uterine.

Table 3 indicated that ovariectomy decreased the weight of uterine and also the thickness of uterine and endometrium walls significantly, the administration of Tamoxifen and dayak onions bulb extracts in three different doses can not recover the changed of uterine.

We then evaluated the thickness of uterine wall and endometrium wall as shown in Fig. 3 and Table 2.

Figure 3 indicated that ovariectomy decreased the thickness of uterine wall and endometrium wall.

Table 1: Measurements of Lipid profile of ovariectomized rats after 3 weeks treatment by dayak onion bulbs extracts

| Groups | Triglyceride (mg/dL) | Total Cholesterol (mg/dL) | HDL (mg/dL) | LDL (mg/dL) |
|------------------|----------------------|---------------------------|-------------|----------------|
| Sham | 145.76±32.70# | 232.43±56.37 | 60.99±5.65 | 142.28±57.25# |
| Negative control | 270.02±18.08* | 394.31±51.33* | 65.19±19.81 | 275.12±36.58* |
| Positive control | 214.74±55.07* | 309.17±16.61* | 55.89±4.57 | 210.33±25.57#* |
| DOE dose 1 | 207.02±54.31#* | 330.16±10.09* | 63.13±11.29 | 225.63±13.83* |
| DOE dose 2 | 188.21±26.33# | 270.74±16.49* | 60.95±15.09 | 172.16±22.36# |
| DOE dose 3 | 160.63±28.94# | 233.07±17.78 | 56.27±2.71 | 144.68±15.63# |

Note: Sham = CMC 0,5%, negative control = CMC 0,5%, positive control = tamoxifen 0,36 mg/200 g BW, DOE dose 1 = E. bulbosa extracts bulb lb 8 mg/200 g BW, DOE dose 2 = 12 mg/200 g BW, DOE dose 3 = 18 mg/200 g BW

Table 2: Body weight of ovariectomized rats after 3 weeks treatment by dayak onion bulbs extracts

| Groups | Before Treatment (g) | After Treatment (g) | Weight difference (g) |
|------------------|----------------------|---------------------|-----------------------|
| Sham | 130,35±11,73 | 140,02±13,16 | 9,67±2,91 |
| Negative control | 145,18±18,54 | 160,88±29,93 | 15,70±15,60* |
| Positive control | 140,88±10,45 | 155,25±11,21 | 14,37±9,26* |
| DOE dose 1 | 141,97±24,77 | 153,32±28,68 | 11,35±10,55* |
| DOE dose 2 | 123,93 ±11,80 | 134,57±11,50 | 10,63±4,65# |
| DOE dose 3 | 145,35±18,82 | 154,33 ±16,52 | 8,98±11,88# |

Note: Sham = CMC 0,5%, negative control = CMC 0,5%, positive control = tamoxifen 0,36 mg/200 g BW, DOE dose 1 = E. bulbosa extracts bulb 8 mg/200 g BW, DOE dose 2 = 12 mg/200 g BW, DOE dose 3 = 18 mg/200 g BW, (*) = p<0.05 compared with negative control, (#) = p<0.05 compared with sham

Table 3: The effects of ovariectomy on the weight of uterine

| Groups | Uterine weight (g) | Endometrium wall thickness (μm) | Uterine wall thickness (μm) |
|------------|--------------------|---------------------------------|-----------------------------|
| Sham | 129.62±37.12 | 45.01±6.38 | 94.01±6.71 |
| OVX | 42.23±6.79* | 20.56±4.17* | 38.22±5.15* |
| TAM | 54.21±5.92* | 22.64±2.33* | 44.35±2.95* |
| DOE dose 1 | 66.93±26.58* | 21.58±4.34* | 45.27±2.43* |
| DOE dose 2 | 61.86±13.47* | 20.35±4.34* | 46.55±3.22* |
| DOE dose 3 | 63.48 ±13.06* | 22.85±4.56* | 42.55±4.33* |

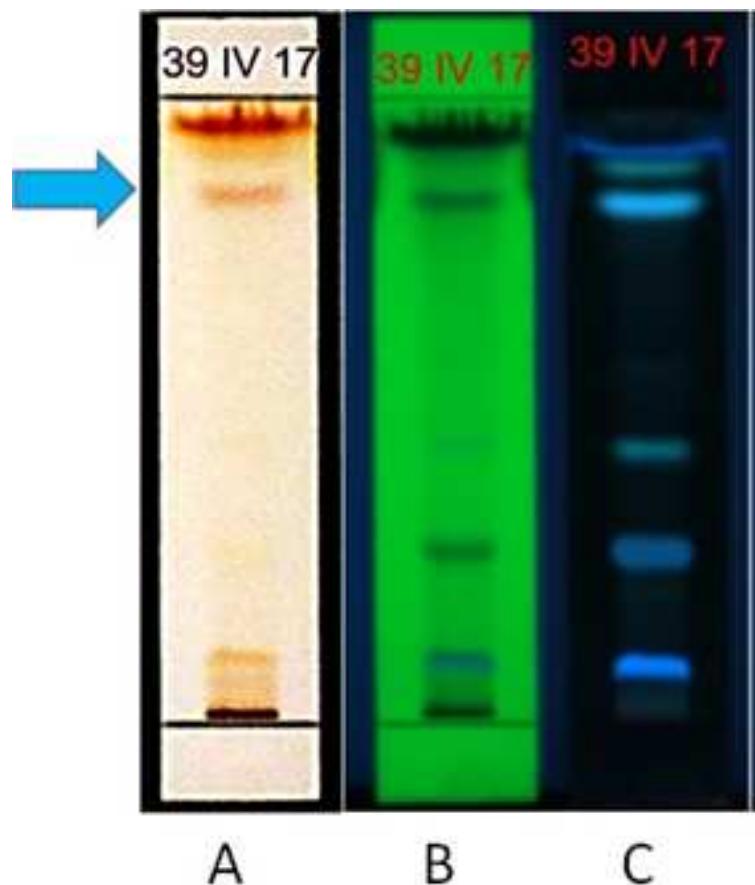


Fig. 1: TLC of the Dayak onions extracts. A. Spray by KOH; B. UV 256 nm; C. UV 366 nm. Blue arrow is naphtoquinone

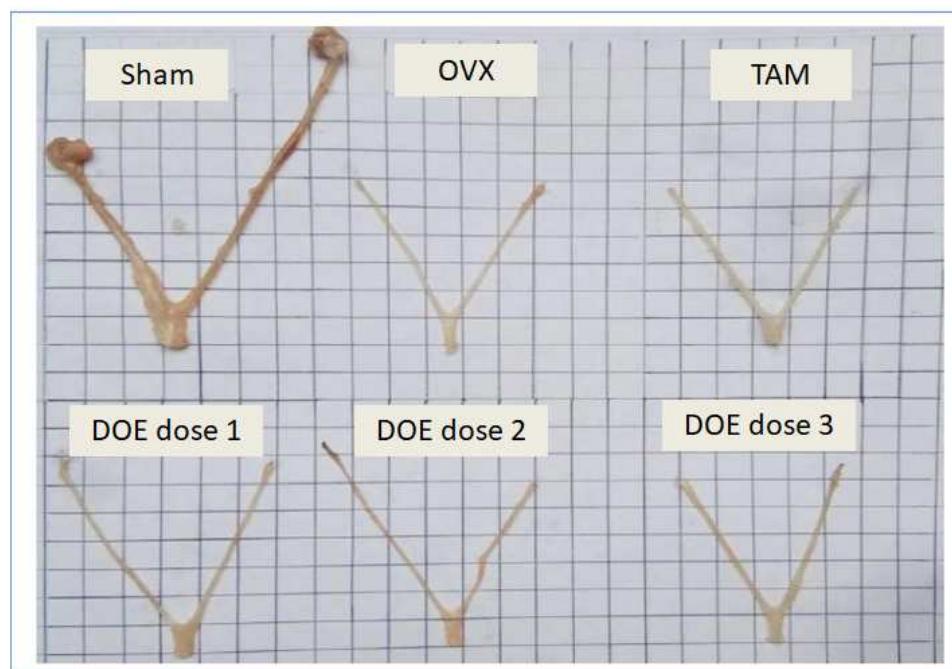


Fig. 2: The effects of ovariectomy on uterine size

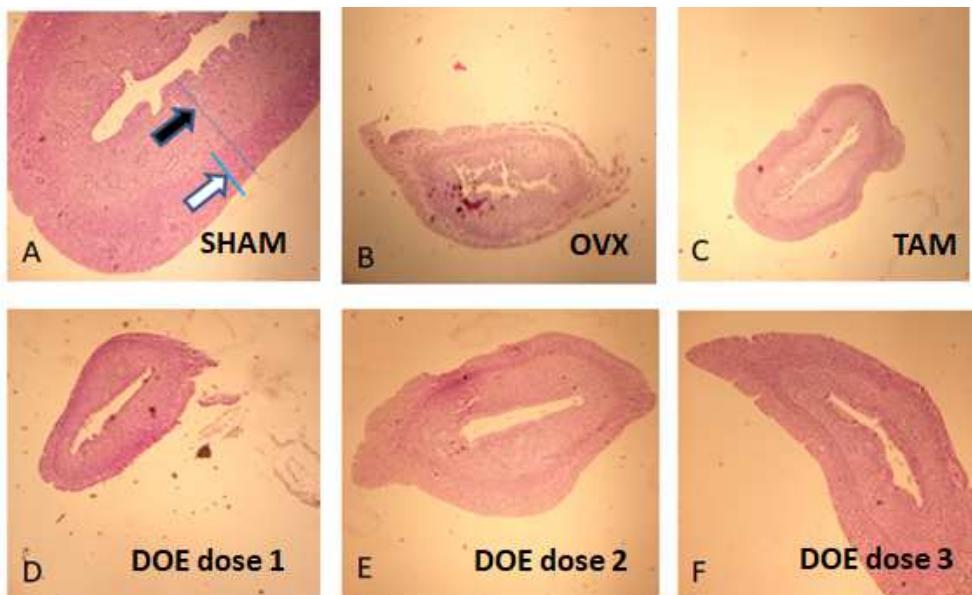


Fig. 3: The effects of Ovariectomy on the histology of the uterine walls

Discussion

The result of TLC profile 70% of ethanol extract of Dayak Onion bulb (DOE) gave Rf value 0,10, 0,28, 0,44 and 0,87. These results show similarities with Rf from the previous study using the same eluent. To confirm whether DOE contain naphtoquinone we then observed TLC by 10% KOH spray in and showed orange spot TLC plate with Rf 0.87. This reaction very unique for naphtoquinone (Kotakemori and Okada, 1966).

There was a significant difference in triglyceride levels between SHAM and OVX ($p<0.05$), it could be seen that the ovariectomy caused an elevated triglyceride levels (Kitson *et al.*, 2015). Administration of DOE at various doses could decrease triglyceride levels in ovx rats. In mice, ovariectomy increased adipose triglyceride lipase signaling, resulting in increased nonesterified fatty acids and glycerol, which were attenuated by E2 treatment (Wohlers and Spangenburg, 2010). Treatment of female mice with E2 leads to decreased expression of lipogenic genes in adipose tissue (Bryzgalova *et al.*, 2008).

The data on HDL levels for each group have tendency to increase in OVX compared with the SHAM. There was a tendency to decrease in HDL levels in tamoxifen treated group and DOE in various dose. Estrogen in rat plays a role in lowering both LDL and HDL in the blood.

LDL concentrations between SHAM showed statistically differences with the OVX group ($p<0.05$). This suggests that the condition of hypoestrogen would increase LDL levels in the blood, this result similar with previous study (Xu *et al.*, 2014). There was a significantly difference between tamoxifen and DOE group compared with OVX group ($p<0.05$). This

suggests that Dayak onion bulb extract could decrease LDL levels to normal levels such as SHAM.

From the data obtained, it could be seen that the administration of dayak onion bulbs extract could decrease the lipid profile of hypoestrogenic rat. This was due to the compounds contained in the dayak onion bulb extract of eleutherinol could bind to the estrogen receptor and caused some estrogenic effects, which estrogen could increase the activity of LDL receptors in the liver and increase lipolysis. Giving tamoxifen in animal showed that tamoxifen did not give significant effect to decrease of total cholesterol, LDL and triglyceride and decrease in blood pressure both systolic and diastolic. This result is relevant with previous study which proves that tamoxifen does not significantly reduce total cholesterol, LDL and triglyceride levels in the blood because they have oncoprotein MUC-1 that can resistance to tamoxifen. Therefore does not significantly affect the cardiovascular system (Poirot *et al.*, 2012).

The effects of DOE on uterine size, weight and the thickness of uterine and endometrium wall has been evaluated by weighing and histology of uterine. In all measurement, OVX reduced size, weight and the thickness of the uterine. Administration of tamoxifen and DOE could not recover size, weight and the thickness of the uterine.

Conclusion

Based on the research that has been done, it can be concluded that dayak onion bulbs extract has an effect on lipid profiles and has no effects on uterine organ.

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Author's Contributions

Amalia Purnamasari: Designed the research plan, contributed to the writing of the manuscript and organized the study.

Anton Bahtiar: Coordinated the data-analysis and coordinated the mouse work.

Ethics

This research had been certified by ethical certification of Faculty of Medicine, University of Indonesia (UI FM No. 44/UN2.F1/Ethics/2017) for the use of animals in experiments.

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