



Dayak Onions (*Eleutherine bulbosa* (Mill.) Urb.) Bulbs Extracts Reduce the Blood Pressure of Ovariectomized Rats

Anton Bahtiar* and Delvika Yessi Chumala

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia;
anton.bahtiar@ui.ac.id

Abstract

Background: *Eleutherine bulbosa* (Mill.) Urb. extract has eleutherinol that could bind with estrogen receptor alpha selectively, therefore it's a candidate for selective estrogen receptor modulator drugs. **Objective:** To determine the influence of 70% of ethanol extract of *E. bulbosa* (Mill.) Urb., bulbs on blood pressure of ovariectomized rats. **Methods:** To analyze the content of the *E. bulbosa* (Mill.) Urb., bulbs, thin layer chromatography of the extracts were performed using chloroform : methanol (7 : 1, v/v) as the mobile phase. Thirty-six female Sprague-Dawley rats were used for this study, they were divided into six groups: SHAM and ovariectomized both given vehicle; tamoxifen (1.8 mg/kg BW.) as the positive control; three doses of extract at 40, 60, and 90 mg/kg BW were administered respectively. All groups were ovariectomized on day 1, except the SHAM group. Measurement of blood pressure was performed on the day before ovariectomy, every week after ovariectomy until 28 days before treatment by the extract and then every week after treatment by the extract. **Results:** Three weeks after treatment of ovariectomized rats by the dose of 90 mg/kg BW of *E. bulbosa* (Mill.) Urb., extracts, could reduce the systolic blood pressure level to 28.06%, the diastolic to 30.47%. **Conclusion:** 70% of ethanolic extracts *E. bulbosa* (Mill.) Urb., could recover the blood pressure of ovariectomized rats, particularly at 90 mg/kg.

Keywords: Blood Pressure, Dayak Onions, Eleutherinol, Naphthoquinone, Ovariectomized

1. Introduction

Menopause is a condition of the cessation of menstrual cycles in women due to decreased estrogen hormone production. Reduced estrogen levels lead to increased production of TNF- α , so that osteoclast formation increases and osteoporosis develops. In the plasma lipid profile, estrogen levels are directly proportional to High-Density Lipoprotein (HDL). Moreover, inversely with the total cholesterol, triglycerides and Low-Density Lipoprotein (LDL) in plasma. In ovariectomized conditions, the total cholesterol, triglycerides, and LDL increase, while plasma HDL levels decrease. The increasing of lipid profile may increase the risk of atherosclerosis. The occurrence of atherosclerosis will also increase the risk of hypertension^{1,2}.

Women who have menopause are prone to hypertension as a result of reduced estrogen hormone. Based on research conducted by the Joint National Committee (JNC 7), women who have postmenopause have a higher systolic pressure of 4–5 mmHg than women who are still in premenopausal phase.

The most often applied therapy in treating postmenopausal symptoms is by using Hormone Replacement Therapy (HRT) which has agonist activity on all estrogen receptors in the body and is not selective, resulting in effects on unwanted organs such as breasts, and other reproductive organs³. The further treatment is by using Selective Estrogen Receptor Modulators (SERMs) that work agonists on receptors in non-reproductive organs and antagonists in reproductive organs. Examples of widely used SERMs are raloxifene and tamoxifen⁴. The

*Author for correspondence

ideal SERMs have anti-estrogen activity in the breast and endometrium, and estrogen activity can reduce the risk of osteoporosis and reduce total cholesterol, triglycerides, LDL, increase HDL, and improve cardiovascular function⁵. So it is encouraging to find an alternative treatment that has an ideal estrogenic effect, that is, agonists on estrogen receptors in non-reproductive organs and antagonists of estrogen receptors in the reproductive organs.

Dayak onion bulbs which are from the Iridaceae family, contain eleutherinol compounds which have been reported through *in silico* study that it can bind to estrogen receptors⁶. Considering the traditional use of this plant which has also been used to cure breast cancer, hypertension, diabetes mellitus, decreasing cholesterol, and stroke⁷, we studied Dayak onion bulbs as an alternative herb on the treatment of postmenopausal symptoms with parameters of blood pressure.

2. Material and Methods

2.1 Chemicals and Reagents

Dayak onion bulbs extracts was obtained from Indonesian Spice and Medicinal Crops Research Institute (BALITRO), Bogor, Indonesia. Tamoxifen was obtained from PT. Kalbe Farma, Indonesia, Lipid Reagents Kit was bought from DiaSys, Germany. Distilled water, ethanol 70%, Carboxymethylcellulose (CMC), ether, and povidone-iodine was obtained from Brataco Chemical, Indonesia. Ketamine was obtained from MMC hospital, Jakarta.

2.2 Plant Materials

Dayak onion bulb as the primary material, was obtained from Palu, Central Sulawesi and authenticated by the Research Center for Natural resources of Tadulako University (No. 65/UN.28.UPT-SDHS/LK/2016). This plant was harvested from Palu forest in Central Sulawesi, Indonesia.

2.3 Dayak Onion Bulbs Extract Preparation

The Dayak onions were sliced and were dried in an oven at 45°C, and milled to powder. The extraction of dried powder of Dayak onions bulbs was done using 70% of

ethanol by maceration. The rotary evaporator was used to concentrate extract.

2.4 Eleutherinol Identification by TLC

The sample (10 µL) was spotted on a silica gel 60 F254 plates, and eluted by chloroform : methanol (7 : 1, v/v) as the mobile phase. The elution process was performed until the mobile phase reaches the finish line. The spot then was identified using 10% KOH in ethanol to find naphthoquinone derivate.

2.5 Animals

Pre-pubertal albino female Sprague-Dawley rats aged 28 d were purchased from Indonesia National Institute of Health Research and Development⁸. In the pre-pubertal rat, it is estimated that rats do not have a normal hormonal cycle that will affect the results⁸. Animals were grouped and maintained under standard laboratory conditions [room temperature] with 12 h dark and 12 h light cycle. The rats were allowed free access to a food and water. The experiment was approved and certified by the Ethics Committee of the Faculty of Medicine (44/UN2.F1/Ethics/2017).

In this study, 36 female rats were divided into six groups. Group 1 is SHAM; group 2 to group 6 were ovariectomized and divided as follows: group 2 is a negative control group which received 0.5% CMC; group 3 is a positive control group that received a dose of tamoxifen (1.8 mg/kg BW of rats); group 3, 4, and 5 are treatment groups which received 70% ethanolic extracts of *E. bulbosa* (Mill.) Urb., bulbs suspended in 0.5% CMC with doses of 40 mg; 60 mg; and 90 mg/kg BW of rats for 21 d respectively.

Estrous cycle was done before and after ovariectomy, to evaluate estrous cycle of rat. Pre-pubertal rat does not have estrous cycle. While pubertal rat has estrous cycle, this is similar with SHAM group. Ovariectomized rat does not have estrous cycle.

2.6 Measurement of Blood Pressure

Blood pressure measurements were performed with a non-invasive blood pressure gauge CODA® (Kent Scientific Corporation, USA), CODA® tail-cuff blood

pressure system utilizes Volume Pressure Recording (VPR) sensor technology to measure the rat tail blood pressure. Ovariectomy would increase venous tone in the female Spontaneously Hypertensive Rat (SHR) via autonomic mechanisms⁹.

2.7 Statistical Analysis

All the data that get in this experiment was processes using statistics software for statistical analysis. The data represented the mean \pm SD. To analyze the different between the group, One-way ANOVA followed by multiple comparison tests was used to analyze the difference in a group. A p -value of <0.05 was considered to be statistically significant.

3. Results

3.1 Naphthoquinone Content Analysis

The active component of Dayak onion bulb extract was confirmed by TLC, and the result is shown in Figure 1.

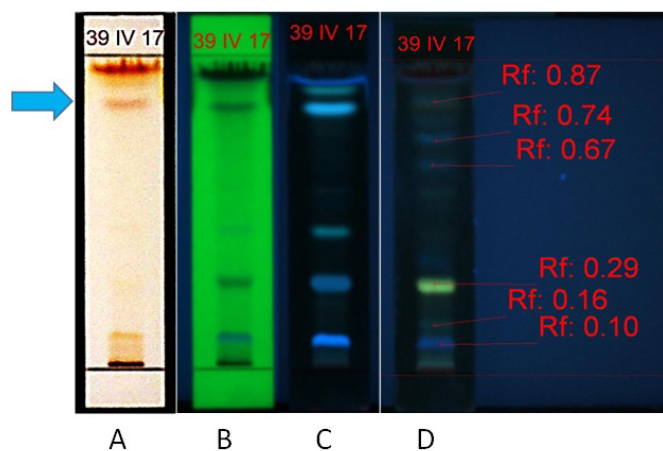


Figure 1. TLC of *E. bulbosa* extracts. The naphthoquinone (blue arrow) was identified by 0.5 N KOH spray and given an orange band at the Rf 0.87. **(A)** After sprayed by 0.5 N KOH by visible. **(B)** Before spray by UV 254 nm. **(C)** Before spray by UV 366 nm. **(D)** After sprayed by 0.5 N KOH by UV 366 nm.

The spot corresponding to the extract gives a retardation factor of 0.87. Identification of the TLC profile of ethanol extract 70% of Dayak onion bulbs showed six bands at Rf values 0.10, 0.16, 0.29, 0.67, 0.74 and 0.87. The addition of 10% KOH spray reagent in ethanol showed orange spot on TLC plate

with Rf 0.87 as seen in Figure 1. This data indicated that the extract contained naphthoquinone derivate, eleutherinol.

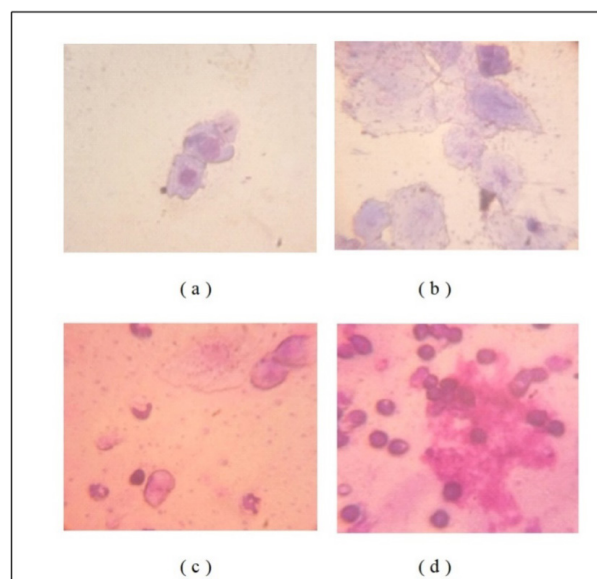


Figure 2. Evaluation of vaginal swabs of SHAM for an estrous cycle. Note: **(a)** Day 1 of the proestrous phase. **(b)** day 2 of the estrous phase. **(c)** Day 3 of the metestrus phase. **(d)** Day four diestrus phase. Observations were made at 40 x magnification.

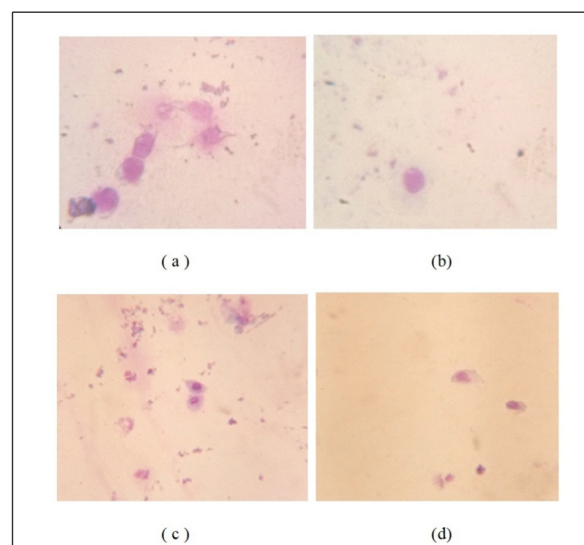


Figure 3. Evaluation of vaginal swabs of the ovariectomized group for the estrous cycle after ovariectomy. Note: Observation of the vaginal swab for four consecutive days of the ovariectomized group showed no difference in phases **(a)** day 1. **(b)** day 2. **(c)** day 3. **(d)** day 4. Observations were made at 40x magnification.

3.2 Ovariectomy of Rats

The evaluation of ovariectomy in the rats is as shown in Figure 2 to 4. Figure 2 shows that in SHAM, the estrous cycle has 4 phase as a normal condition. This does not appear in ovariectomy rat as shown in Figure 3.

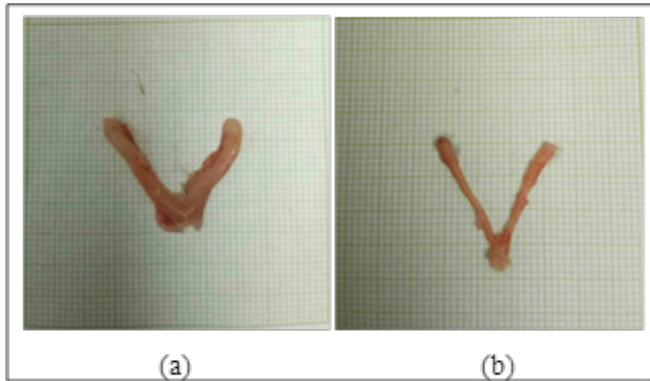


Figure 4. (a) The size of uterine of SHAM group. (b) Ovariectomized group.

The size and the weight of the uterus were recorded (Figure 4 and Table 1). The ovariectomized rat has smaller and lighter of uterus compared with SHAM group. This data indicates the confirmation of ovariectomy.

Table 1. The weight of uterine of SHAM group and ovariectomized group

	SHAM (g) Mean \pm SD	OVX (g) Mean \pm SD
Uterine weight (g)	1.51 \pm 0.18	0.34 \pm 0.03
Body weight gain (g)	35.58 \pm 11.35	50.58 \pm 13.35

3.3 The Effects of Dayak Onion Bulbs Extract on Blood Pressure

The ovariectomy could have increased systolic and diastolic blood pressure, four weeks after ovariectomy systolic and diastolic blood pressure as compared with SHAM as shown in Table 2 and Tabel 3.

Table 2. The alteration of systolic pressure after three weeks of treatment

Groups	Systolic Pressure (mmHg)		The decrease in systolic BP
	Before	After	
SHAM	120.83 \pm 10.48	114.67 \pm 4.93	-1.18 \pm 8.17
Negative control	175.33 \pm 5.82*	169.67 \pm 5.12*	1.93 \pm 2.02
Positive control	161.00 \pm 5.69*	141.17 \pm 1.60*	16.48 \pm 2.61
Dose I	153.33 \pm 9.48*	139.00 \pm 4.56#	10.28 \pm 5.07
Dose II	159.33 \pm 2.73*	133.00 \pm 1.67#	19.43 \pm 4.13
Dose III	164.67 \pm 5.89*	123.17 \pm 4.83#	28.06 \pm 5.66

Note: SHAM = CMC 0.5%, negative control = CMC 0.5%, positive control = tamoxifen 0.36 mg/200 g BW, Dose I = *E. bulbosa* extracts bulb 8 mg/200 g BW, Dose II = 12 mg/200 g BW, Dose III = 18 mg/200 g BW, (*) = p < 0.05 compared with SHAM, (#) = p < 0.05 compared with OVX.

Table 3. The alteration of diastolic pressure after three weeks of treatment

Groups	Diastolic Pressure (mmHg)		The decrease in diastolic BP
	Before	After	
SHAM	73.83 \pm 9.11	87.67 \pm 8.16	-17.60 \pm 18.95
Negative control	130.00 \pm 27.28*	141.33 \pm 14.29*	7.26 \pm 10.80
Positive control	125.17 \pm 15.84*	119.83 \pm 8.23#	15.94 \pm 10.85
Dose I	126.00 \pm 16.54*	111.17 \pm 8.68#	7.11 \pm 26.31
Dose II	127.67 \pm 16.07*	115.50 \pm 2.51#	8.22 \pm 12.88
Dose III	132.67 \pm 10.69*	91.83 \pm 12.32#	30.47 \pm 13.82

Note: SHAM = CMC 0.5%, negative control = CMC 0.5%, positive control = tamoxifen 0.36 mg/200 g BW, Dose I = *E. bulbosa* extracts bulb 8 mg/200 g BW, Dose II = 12 mg/200 g BW, Dose III = 18 mg/200 g BW, (*) = p < 0.05 compared with SHAM, (#) = p < 0.05 compared with OVX.

There was a significant difference in systolic blood pressure between extract treatment group and negative control group at one week after treatment while there was no significant difference between diastolic blood pressure between the treatment group and negative group. There was a difference in both systolic and diastolic blood pressure after two weeks of treatment compared to a condition before treatment. The result on Table 2 and Table 3 showed that in the third week after treatment there was no significant difference in systolic and diastolic blood pressure between the groups given Dayak bulb extract (90 mg/kg) and SHAM group.

4. Discussion

4.1 Analysis of Naphthoquinone by Thin Layer Chromatography

The TLC method successfully identified the content of the Dayak onion extracts. Naphthoquinone was separated by methanol : chloroform (7 : 1, v/v) and identified by chromogenic spray of 0.5 N Potassium hydroxide⁸. When sprayed with alcoholic potassium hydroxide, the green fluorescence changed to an orange fluorescence.

4.2 Analysis of Blood Pressure

At one week after treatment there was a significant difference in systolic pressure between Dayak onion bulbs extract and OVX, while there was no significant difference in diastolic pressure between Dayak onion bulbs extract and OVX. Both systolic and diastolic pressure in the treatment group had a significant difference ($p < 0.05$) with the SHAM at two weeks after treatment. In the third week after treatment, there was no significant difference in systolic and diastolic blood pressure between the groups treated by Dayak onion bulbs extract (90 mg/kg) and SHAM. This suggests that administration of 90 mg/kg dose of Dayak onion bulb extract could lower both systolic and diastolic blood pressure to normal blood pressure after three weeks of treatment. Ovariectomized made blood pressure

increases due to an increase in vasoconstrictor NPY and decrease of vasodilator neuropeptides¹⁰.

Furthermore, the decrease in blood pressure both systolic and diastolic occurred due to the presence of eleutherinol compounds contained in bulbs extract of Dayak onions which are estrogen agonist. The decrease in blood pressure was thought to be due to a decrease in blood lipid profile, preventing atherosclerosis and decreasing blood pressure in ovariectomized rat¹¹.

5. Conclusion

The 70% ethanolic extract of Dayak onion bulbs could reduce blood pressure by reducing lipid profile of ovariectomized model rat, particularly at 90 mg/kg.

6. Acknowledgement

This research was supported by Directorate Research and Community Service, Universitas Indonesia 2017.

7. Reference

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