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RESEARCH ARTICLE

The Effect of 70% Ethanolic Extract of Dayak Onion Bulbs and Cowpea on Cardiovascular Parameters of Hypoestrogen Model Rats

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Abstract

Introduction: Eleutherinol has strong affinity to bind with Estrogen Receptor alpha (ER-α) and can be found in Dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb.). The cowpea (*Vigna unguiculata* (L.) Walp) contain Daidzein that acts on estrogen receptors beta (ER-β). This study was aimed to investigate the effect of both extracts on ER-α and ER-β by estimating the blood pressure and serum lipid profile of hypoestrogen model rats. **Material and Methods**: Thirty-six of female *Sprague-Dawley* were randomly assigned to eight groups as followed, SHAM, OVX, RAL (Raloxifene 1 mg/200 g BW), Cowpea 100 mg/200 g BW with Dayak onion bulbs: 36 mg/200 g BW (D1); 18 mg/200 g BW (D2); 9 mg/200 g BW (D3); 4.5 mg/200 g BW (D4) and single dose of cowpea 100 mg/200 g BW (D5). All groups, except the SHAM,were ovariectomized to obtain the conditions of hypoestrogen. The extracts were given orally for 28 days. Blood pressure and lipid profile were measured after 28 days of treatment. **Results**: Combination of extracts at D2 (18 mg/200 g BW Dayak onion bulbs and 100 mg/200 g BW cowpea) significantly decreased blood pressure and serum lipid profile of hypoestrogen model rats. The combination extracts was also better than a single dose of 100 mg/200 g BW cowpea. **Conclusions**: The results indicated that a combination extract of Dayak onion bulbs and cowpea could be used as a drug for menopause woman in the future.

Keywords: Blood Pressure, Cowpea, Dayak Onion, Hypoestrogen, Lipid Profile

1. Introduction

Menopause is defined as the permanent cessation of ovulation due to no response of oocytes on the ovary, with a decrease in estrogen and progesterone hormones[1]. In postmenopause women, a decrease in estrogen level results in decreased Estrogen Receptor binding in which estrogen receptors are spread in organs and tissues namely alpha and beta Estrogen Receptors (ERα and ERβ). Alpha Estrogen Receptors are predominantly distributed in the uterus, breast, bone, adipose tissue, liver, skeletal muscle, digestive tract's tissue; whereas ERβ is predominantly found in the central nervous system, immune system and spinal cord[2].

The bonding of estrogen with ERα will produce an effect on the vascular endothelium, whereas the result of estrogen binding to ERβ stimulates nitric oxide production. Both effects cause hypotensive effects with vascular dilatation[3]. The decrease of Estrogen Receptor bonds can also cause weight gain and impaired lipid metabolism[4]. Estrogen levels are directly proportional to High-density Lipoprotein (HDL) levels but are inversely proportional to total cholesterol, triglyceride and Low-density Lipoprotein (LDL) levels in plasma[5]. The effects of estrogen in the liver is an increase of apolipoproteins A synthesis, which is a component of HDL[6]. Dominant Estrogen Receptor that works on hepatocyte cells is ERα. If estrogen binds to ERα, there will be an increase of lipolysis, insulin clearance, glycogen storage, cholesterol removal. It will also decrease lipogenesis, lipid uptake, gluconeogenesis and cholesterol synthesis[7].

Hormone Replacement Therapy (HRT) are desirable as postmenopausal therapy with agonist activity in all

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Estrogen Receptors in the body but are not selective, thereby causes an increased proliferation in all cells that have Estrogen Receptors. Postmenopausal therapy has switched to SERMS class medicine. Selective Estrogen Receptor Modulators (SERMS) are ligands of Estrogen Receptors that have both agonist and antagonist effects on specific tissues. In postmenopause, bazedoxifen is used to prevent osteoporosis by blocking estrogen stimulation in the breast and uterus[8]. The example of SERMS are tamoxifen for treatment of positive Estrogen Receptor breast cancer; raloxifene for treatment of osteoporosis in postmenopausal women and bazedoxifene as medication to prevent osteoporosis by blocking estrogen stimulation in breast and uterus[9].

Several studies have reported that that plant based products also exhibit SERMS-like effects as an alternative to postmenopausal treatment. Previous studies have proved that cowpea and Dayak onions have SERMS-like effect. Ethanol extract of Dayak onion bulbs can reduce blood pressure in hypoestrogen induced rat. Eleutherinol, a naphthoquinone derivative contained in Dayak onion bulbs has been known to bind with alpha Estrogen Receptors (ERα). Furthermore, the ethanol extract of cowpea contains daidzein, a compound that has been known to bind with beta Estrogen Receptor (ERβ)[10–12]. The combination of Dayak onion bulbs extract and cowpea would act ERα and ERβ. Also, this study would will give insight to whether, the combined use of Dayak onion bulbs extracts and cowpea can increase its effectiveness. Such a combined study will layout the criterion for an ideal SERMS compound and its limit in the evaluation of blood pressure and lipid metabolism. Hence, the current syudy was carried out to study blood pressure changes and lipid profiles as test parameter for Dayak onion bulbs extract combined with cowpea extract as a postmenopausal treatment or hypoestrogenic condition.

2. Materials and Method

2.1 Plant Materials

Fresh Dayak onion bulbs were collected from Palu, Central Sulawesi, Indonesia and were identified by The Technical Service Unit of Biology Resources, Tadulako University, Central Sulawesi. The cowpea was collected and identified by The Plant Conservation Center Bogor Botanical Garden, West Java.

2.2 Preparation of Materials

Dayak onion bulbs (5 kg, dried) were macerated with 70% ethanol and evaporated in a rotary evaporator at 50°C to obtain the extract. Cowpea (1 kg, dried) were macerated with 70% ethanol and evaporated in a rotary evaporator at 50°C to obtain the extract.

2.3 Animal Study

Thirty-two white female Sprague-Dawley rats used for the study were obtained from IPB University, weighing approxitmately 100 g and 42 days of ages. The animals were acclimatized for a week before treatments in standard animal cages to adapt with a new environment and also to reduce stress. Permission and approval for animal studies were obtained from the ethics committee Faculty of Medicine, Universitas Indonesia. (Approval No. 0037/UN2.F1/ETIK/2018)

2.4 Ovariectomy Method

The ovariectomy procedure was performed by removing both ovaries in female rats except the sham group. All rats were weighed first before ovariectomy, then anesthetized with ketamine 120 mg/kg BW of rats intraperitoneally. The rats were positioned in the left lateral position and the hair on the left side was shaved. Bilateral ovariectomy (left and right ovary removal) was performed through a midline caudal incision, then an incision in the skin 2 cm long from the umbilicus in the caudal direction was made using a scalpel. The wound was treated with povidone iodine. The same procedure was also performed on the right side of the rat to remove the right ovary. Single cage was prepared for each rat, dialed with tissue and clean paper. Ovariectomized rats were placed in these single cages. Wounds were treated with povidone iodin daily and the tissue layer in the rat cage were replaced daily to maintain the cleanliness of the cage during rat recovery.

2.5 Dosage and Treatment Duration

Selection of dose and duration of treatment provision was based on the results of previous studies. The combination of Dayak onion bulbs and cowpea extract were administered for 28 days after 28 days of ovariectomy. The dose treatment is as shown in Table 1.

Table 1. Variation of combination extract dose

Dosage	Dayak onion bulbs extract	Cowpea extract
Dose 1	36 mg/200 g BW	100 mg/200 g BW
Dose 2	18 mg/200 g BW	100 mg/200 g BW
Dose 3	9 mg/200 g BW	100 mg/200 g BW
Dose 4	4.5 mg/200 g BW	100 mg/200 g BW
Dose 5	-	100 mg/200 g BW

2.6 Animal Grouping

Thirty-two female Sprague-Dawley rats were weighed and divided randomly into 8 groups as follows: SHAM (performed surgery without ovaries removal); OVX (ovariectomized rats as negative control); RAL (ovariectomized rats admistered with raloxifene as positive control); and 5 combination variation dose groups (D1, D2, D3, D4 and D5) as shown in Table 2. After 28 days ovariectomized, all rats were checked for ovariectomy success at day 28, followed by the administration of the test substance for 28 days orally. Blood pressure of all rats was measured weekly during treatment and serum lipid profile was analyzed after treatment.

2.7 Blood Pressure Measurement

Blood pressure measurement was performed by non-invasive blood pressure CODA. The occlusion and VPR cuff were mounted on the tail of rats and performed to get the result of systolic and diastolic blood pressure. Blood pressure measurements were carried out in all groups of rats and were carried out in the second, third and fourth weeks after ovariectomy and first, second, third and fourth weeks during the treatment.

2.8 Serum Lipid Profile Analysis

Rat blood was collected after 28 days of treatment from eye veins to the blood collection tube without anticoagulant. Blood was centrifuged at 3000 rpm for 15 minutes. The supernatants were transferred into clean and dry microtubes, then kept in the freezer at -30°C. Serum was used for analysis of total cholesterol, triglyceride, HDL and LDL by colorimetric method. The result of reaction serum with kits was measured with Spectrophotometry UV-vis against the blank at 500 nm of wavelength.

Table 2. Animal treatment

Group	Number of rats	Treatment
SHAM	4	urgery without ovaries removal + CMC 0.5% solution 2 ml/200 g BW per ora
OVX	4	Ovariectomy + CMC 0.5% solution 2 ml/200 g BW per oral
RAL	4	Ovariectomy + raloxifene 1.08 mg/200 g BW in 2 ml of CMC 0,5% solution per oral
D1	4	Ovariectomy + combination extract at Dose 1 in 2 ml of CMC 0,5% solution per oral
D2	4	Ovariectomy + combination extract at Dose 2 in 2 ml of CMC 0,5% solution per ora
D3	4	variectomy + combination extract at Dose 3 in 2 ml of CMC 0,5% solution per oral
D4	4	Ovariectomy + combination extract at Dose 4 in 2 ml of CMC 0,5% solution per oral
D5	4	Ovariectomy + combination extract at Dose 5 in 2 ml of CMC 0,5% solution per oral

2.9 Data Analysis

Data obtained from this experiment were analyzed statistically using SPSS. Analysis conducted was normality (Shapiro-Wilk test) and homogeneity (Levene's test). To see the relationship between all groups, one-way Analysis of Variance (ANOVA) was conducted followed by an analysis of Significant Difference test (LSD). Differences between means will be considered significant at 5% level of significance i.e. $P < 0.05$.

3 Results

3.1 Extraction and Identification

Each Dayak onion bulbs and cowpea were extracted in the Research Institute of Spices and Medical Plants of Indonesia by using maceration method with 70% ethanol solvent. The yield of Dayak onion bulbs 70% ethanolic extract and cowpea 70% ethanolic extract was found to be 188.7 g (3.77%) and 105.8 g (10.58%) respectively.

The 70% ethanolic extract of Dayak onion bulbs contained alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids and glycosides and the 70% ethanolic extract of cowpea contained alkaloids, saponins, tannins, phenolics, flavonoids and glycosides. These extracts were also identified by LC-MS. Five compounds that were detected in Dayak onion bulbs extracts were 2,4,7-Trihydroxy-9,10-dihydrophenanthrene, Cuspidatumin A, Dendromonisilide E, Liquiritigenin, and Natsudaidain.2-Monolinolein, Glycerol-β-stearate, Momor-cerebroside I, Trigonelline and Daidzein were detected on cowpea extract.

3.2 Blood Pressure Measurement

The effect of the combination of 70% ethanolic extract of Dayak onion bulbs and cowpea on blood pressure are presented in Table 3. The administration of combination extract can decrease systolic and diastolic blood pressure close to normal which is evident form D2 and D4 group as compared with the SHAM and OVX group. The D2 and D4 group significantly different from OVX group, but not significantly different from the SHAM group. These groups also has the largest percentage decrease in systolic and diastolic blood pressure.

3.3 Serum Lipid Profile Analysis

Measurement of total cholesterol, triglycerides, HDL and LDL levels were determined using enzymatic colorimetric methods. Serum lipid levels including total cholesterol, triglycerides, HDL, and LDL levels are as shown in Table 4.

4 Discussion

This work was aimed to investigate the effects of the combination of Dayak onion bulbs and cowpea extracts on blood pressure and serum lipid profile. Previous studies have proved that cowpea and Dayak onion bulbs have SERMS-like effect. Ethanol extract of Dayak onion bulbs also reduces blood pressure in hypoestrogen induced rat. Based on LC-MS studies, Dayak onion bulbs extract has Cuspidatumin A and eleutherinol is reported to have an estrogenic effect by selectively binding to ERα. Furthermore, the ethanol extract of cowpea contains daidzein, a compound has the potential as a SERMS by binding with beta Estrogen Receptor (ERβ)[10, 12, 13].

The significant effect of the combination of extracts on blood pressure can be seen from the last week of treatment in each group, where systolic and diastolic blood pressure differed significantly against the negative control group ($P < 0.05$) and didn't differ significantly with the SHAM group ($P > 0.05$). Based on blood pressure measurement data in Table 3, systolic and diastolic blood pressure at D2 showed a significant difference in the negative control ($P < 0.0001$) and did not differ significantly with the SHAM group ($P = 0.792$). Systolic and diastolic blood pressure at D4 also showed a significant difference in the negative control ($P < 0.002$) and did not differ significantly with the SHAM group ($P = 0.792$). The largest percentage of systolic blood pressure and diastolic blood pressure reduction was observed in D2 by 25, 70% and D4 groups by 17.37% respectively. When compared to a single dose of cowpea extract (D5), the combination of extracts were better. This is presumably due to the

Table 3. Blood pressure of rats after treatment

Group	Systolic blood pressure		Diastolic blood pressure	
	After treatment (mm Hg)	% decrease (%)	After treatment (mm Hg)	% decrease (%)
SHAM	117.75 ± 8.77*	4.78 ± 7.83	95.00 ± 8.72*	0.55 ± 7.63
OVX	149.75 ± 3.77#	0.37 ± 6.76	126.25 ± 2.22#	-13.27 ± 12.94
RAL	136.00 ± 17.07#	13.30 ± 12.60	107.25 ± 10.34#*	9.76 ± 8.74
D1	137.50 ± 12.23#	10.49 ± 6.05	111.00 ±6.68#*	5.04 ± 12.24
D2	115.50 ± 6.40*	25.70 ± 7.02	95.25 ± 5.56*	16.90 ± 7.64
D3	126.75 ± 20.81	19.35 ± 10.59	111.75 ± 12.76#*	3.42 ± 8.46
D4	120.00 ± 9.70*	22.74 ± 8.11	104.50 ± 4.51*	17.37 ± 1.04
D5	137.00 ± 6.48#	11.66 ± 5.99	108.50 ± 6.45*	9.91 ± 6.28

Note: values are means ± s.e.m., n = 4 rats. within a column, values with a superscript are significantlydifferent: # p, 0.05 compared with sham rats; *p < 0.05 compared with ovx rats.

Table 4. Serum lipid profile of rats after treatment

Group	Serum lipid profile parameters (mg/mL)			
	Total Cholesterol	Triglyceride	HDL	LDL
SHAM	60.131 ± 7.51*	14.059 ± 1.13*	29.788 ± 4.27*	51.418 ± 7.91*
OVX	75.054 ± 13.65#	21.188 ± 6.63#	20.699 ± 4.56#	65.081 ± 13.15#
RAL	45.425 ± 9.41#*	15.743 ± 1.22	29.810 ± 3.95*	38.238 ± 7.44#*
D1	56.209 ± 5.56*	10.891 ± 5.14*	35.515 ± 4.30*	48.049 ± 5.14*
D2	59.477 ± 3.97*	10.198 ± 4.69*	29.965 ± 4.37*	51.715 ± 3.90*
D3	58.932 ± 14.72*	12.772 ± 5.76*	30.628 ± 7.16*	49.888 ± 12.40*
D4	56.536 ± 10.18*	17.079 ± 7.17	24.525 ± 0.86	49.415 ± 11.86*
D5	70.153 ± 4.54	10.743 ± 1.25*	16.895 ± 2.07#	61.926 ± 5.14

Note: values are means ± s.e.m., n = 4 rats. within a column, values with a superscript are significantlydifferent: # p, 0.05 compared with sham rats; *p < 0.05 compared with ovx rats.

eleutherinol in Dayak onion bulbs which are agonists on alpha (ERα) Estrogen Receptors in the heart and blood vessels, which can reduce systolic and diastolic blood pressure in hypoestrogen rats[6,14].

Effect of the combination of 70% ethanolic extract of Dayak onion bulbs and cowpea on serum lipid profile shows decreased levels of total cholesterol, triglycerides, LDL and also an increase in HDL levels against negative controls. In the group dose combination of Dayak onion bulbs and cowpea extracts (D1, D2, D3 and D4), a statistical significant lower total cholesterol levels was observed when compared to negative controls (OVX). The combination dose groups (D1, D2, D3 and D4) did not show significant difference to the SHAM group. Hence, it can be confirmed that the total cholesterol levels of ovariectomized rats were close to normal. When compared with a single dose of cowpea extract (D5), the combination extract groups showed a better reduction in cholesterol levels. Statistically D5 group did not have a significant difference with the negative control group (Table 4). It may be due to the presence of eleutherinolin Dayak onion bulbs extract which bind to ERα in adipose and liver tissue, thereby decreasing cholesterol synthesis decreases and increasing cholesterol elimination[7].

On triglyceride level, the combination of Dayak onion bulbs and cowpea extract at D2 had the lowest level compared to all groups and showed significant difference to the negative control group (OVX) with P value 0.002. The D2 group was also better than single dose of cowpea extract (D5). May be due to the binding of eleutherinol to ERα and daidzein to Erβ; thus increasing the effect of decreasing serum triglycerides. Adipose tissue has both estrogen receptors, ERα and ERβ. A decrease in triglycerides occurs because Estrogen Receptor activation stimulates the alpha 2-adrenergic receptors that act as antilipolysis. Triglycerides in adipose tissue will not break down into free fatty acids and glycerol that can be released into the circulation[15,16].

Decreased HDL in the negative control group indicates that estrogen can lower serum HDL levels. The combination of Dayak onion bulbs and cowpea extract can increase serum HDL levels, which were significantly different from the negative control and better than a single dose of cowpea extract (D5). The combination of Dayak onion bulbs and cowpea extract can decrease serum LDL levels, which were significantly different from the negative control and better than a single dose of cowpea extract (D5).

5. Conclusion

It can be concluded that the combination of 70% ethanolic extract Dayak onion bulbs and cowpea affects cardiovascular system. It can be seen from the decrease of systolic and diastolic blood pressure and also in serum lipid profile. The best effect was obtained at dose 2 (18 mg/200 g BW of rats Dayak onion bulbs extract and 100 mg/200 g BW of rats cowpea extract) than a single dose of 100 mg/200 g BW of rats cowpea extract. The results indicates that this combination extracts can be used as a drug for menopause woman in the future.

6. Acknowledgment

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7. Abbreviation:

ERα: Estrogen Receptor α; **CMC:** Carboxymethylcellulose; **BW:** Body Weight; **HRT:** Hormone Replacement Therapy; **SERMS:** Selective Estrogen Receptor Modulators; **OVX:** Ovariectomy

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