

Effect Of Bangun-Bangun Leaves Ethanol Extract On Lipid Profile Ovariectomized Female Rats

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Abstract. Menopause was a condition of the body when ovaries stopped producing ovum; ovum is the main producer of estrogen hormone. Menopause can increasing lipid profile in blood; it causes any problems in the cardiovascular system. People of North Sumatra have long used Bangun-Bangun leaves as a breastfeeding agent; it contains flavonoid bioactive compounds, which are be expected to replace lost estrogen hormone. This study determined the estrogenic activity of Bangun-Bangun leaves ethanol extract (BBLEE) on lipid levels of ovariectomized female rats as a menopausal model. BBLEE has obtained by maceration techniques with 96% ethanol and extract used on 24 ovariectomized rats which were divided into 6 groups. Group 1 (normal), group 2 (positive) was treated by estradiol at dose of 0.18 mg/kg BW, group 3 (negative) was treated by 0.5% Na-CMC and group 4, 5, 6 were treated by BBLEE at dose of 30, 60, 90 mg/kg BW given orally for 14 days. The data were analyzed by ANOVA and Post Hoc Tukey HSD test. The result showed that effective dose of BBLEE was 90 mg/kg BW which improved lipid profile (TC = 55.75±0.47; TG = 63.00±0.40; HDL = 37.00±0.81; LDL = 30.50±0.86) in blood of ovariectomized female rats as a menopausal model. BBLEE at the dose of 90 mg/kg BW can ameliorate the lipid profile in the blood of ovariectomized female rats as a menopausal model.

Keyword: Plectranthus amboinicus (Lour.) Spreng, Phytoestrogens, Ovariectomy, Lipid Profile, 17β-estradiol

Abstrak. Menopause adalah suatu keadaan tubuh ketika ovarium berhenti memproduksi ovum, ovum merupakan penghasil utama hormon estrogen. Menopause dapat memperburuk profil lipid dalam darah, menyebabkan masalah pada sistem kardiovaskular. Bangun-Bangun telah lama digunakan oleh masyarakat Sumatera Utara sebagai agen peningkat ASI, mengandung senyawa bioaktif flavonoid yang diharapkan dapat menggantikan hormon estrogen yang hilang. Tujuan penelitian ini adalah untuk mengetahui aktivitas estrogenic EEDBB pada kadar lipid tikus betina yang diovariectomi sebagai model menopause. EEDBB diperoleh dari teknik maserasi dengan pelarut etanol 96% dan ekstrak digunakan pada 24 ekor tikus ovariektomi yang dibagi menjadi 6 kelompok. Kelompok 1 (normal), kelompok 2 (positif) estradiol dosis 0,18 mg/kg BB, kelompok 3 (negatif) 0,5% Na-CMC dan kelompok 4, 5, 6 EEDBB dosis 30, 60, 90 mg/kg BB diberikan secara oral selama 14 hari. Data dianalisis dengan ANOVA dan uji Post Hoc Tukey HSD. Dosis BBLEE terbaik adalah 90 mg/kg BB yang memperbaiki profil lipid (TC = 55,75±0,47; TG = 63,00±0,40; HDL = 37,00±0,81; LDL = 30,50±0,86) dalam darah tikus betina yang diovariectomi sebagai model menopause. EEDBB dosis 90 mg/kg BB dapat meningkatkan profil lipid dalam darah tikus betina yang diovariectomi sebagai model menopause.

Kata kunci: Plectranthus amboinicus (Lour.) Spreng, Fitoestrogen, Ovariectomi, Profil Lipid, 17β-estradiol

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1. Introduction

Menopause is a reproductive aging process that occurs in women. Menopause can be a constraint on women's health [9]. Symptoms that often occur in women in transition-menopause (MT) include hot flashes and night sweats which can significantly affect the quality of life [5]. Menopause based on formed is grouped into natural menopause and surgical menopause. Natural menopause can be defined as the process of stopping menstruation without any medical-surgical cause for 12 consecutive months [15], where the average age of natural menopause is around 51 years [3]. However, the age of menopause can be different for each woman, and some women experience menopause before the age of 40 years (premature). Others experience menopause between 40-45 years (early) [14]. Women who undergo surgery to remove one of the ovaries will have a long-distance between stopping menstruation and stopping ovarian function. Still, women who undergo surgical removal of both ovaries will immediately experience menopause because of the cessation of menstruation which coincides with the cessation of ovarian function [8].

Estrogen and progesterone synthesis mostly occurs in the ovaries, and a small part occurs in the adrenal glands and adipose tissue. Estrogen is produced together with the release of an egg from the ovary; estrogen through two receptors (α estrogen and β estrogen) can affect tissue growth, differentiation, and function [13]. Two types of estrogen are active in nonpregnant women, namely estrone and estradiol, while pregnant women have estriol in large amounts as the active type of estrogen. There is a decrease and increase in physiological hormones throughout life that can determine cognitive and organic changes in the female body. One of them is a decrease in blood estradiol levels which can cause cognitive complaints at menopause [2].

2. Material and Methods

2.1 Material

Plant and Extract Preparation

Leaves of the Bangun-Bangun plant were collected from Sada Perarih village, Karo Regency, North Sumatra, and identified at Herbarium Medanese (MEDA), Faculty of Mathematics and Natural Sciences, University of Sumatera Utara. The leaves are dried and mashed to form simplicia powder and immersed in 96% ethanol for seven days. The immersion was filtered with Whatman filter paper no.2 and separated between ethanol and extract with a Heidolph® rotary evaporator at 40°C. The extract was dried at room temperature and stored in a refrigerator.

2.2. Animal Preparation

Twenty-four female rats aged eight weeks weighing 150-200g and acclimatized for one week in a good cage to adapt to their environment.

2.3. Rat Ovariectomy

The rat was anesthetized with Ketamine Hameln Combiphar® at a dose of 10 mg/kg BW, then the hair on the rats' stomachs was shaved and cleaned with 70% alcohol. The rat was a plate on the surgical board then stretched legs and arms; GEA® scalpel No. 10 are slowly rubbed into the abdomen until the ovaries are visible. The oviduct is clamped and tied and then cut to take the ovary, done on the left and right ovaries. Postoperative wounds are sutured and given an antiseptic [19]. The rat was kept for two weeks to heal postoperative wounds and induce menopausal effects after ovariectomy.

2.4. Animal Grouping

After two weeks, the rats were grouped into six groups where each group contained four rats, with group details:

Group I: Non-ovariectomized rats + 0.5% Na-CMC suspension (normal group)

Group II: ovariectomized rats + suspension of 17- β estradiol valerate dose 0.036 mg/200g BW (positive group)

Group III: ovariectomized rats + 0.5% Na-CMC suspension (negative group)

Group IV: ovariectomized rats + EEDBB suspension 30mg/kg BW (EEDBB group 30mg/kg BW)

Group V: ovariectomized rats + EEDBB suspension at a dose of 60 mg/kg BW (EEDBB group 90 mg/kg BW)

Group VI: ovariectomized rats + EEDBB suspension at 90 mg/kg BW (EEDBB group 90 mg/kg BW)

2.5. Phytochemical Screening Results

Phytochemical screening was carried out to determine which secondary metabolites had biological activity in the leaves ethanol extract. Phytochemical screening is carried out to examine the class of alkaloid compounds, flavonoids, tannins, glycosides, saponins, and steroids/triterpenoids. The results of the EEDBB phytochemical screening are shown in Table 1.

Table 1. BBLEE Phytochemical Screening Results

No	Secondary Metabolites	Results
1	Alkaloids	Negative
2	Tannins	Positive
3	Saponins	Positive
4	Triterpenes/Steroids	Positive
5	Flavonoids	Positive
6	Glycosides	Negative

2.6 Measurement of Lipid Levels

Measurement of lipid levels is done by measuring total cholesterol levels, triglyceride levels, HDL levels, and LDL levels using the following techniques:

1. Total Cholesterols

The cholesterol reagent was measured as the measurement standard. Then the supernatant was measured by mixing 10 μ l and 1 ml of cholesterol reagent, incubated for 20 minutes, and then measured at a wavelength of 546 nm with a 300 microlab spectrophotometer [12].

2. Triglycerides

The triglycerides reagent was measured as the measurement standard. Then the supernatant was measured by mixing 10 μ l and 1 ml of cholesterol reagent, incubated for 20 minutes, and then measured at a wavelength of 546 nm with a 300 microlab spectrophotometer [12].

3. HDL

HDL reagent is measured as the measurement standard. Then the supernatant was measured by mixing 10 μ l and 1 ml of HDL reagent, incubated for 20 minutes, then measured at a wavelength of 546 nm with a 300 microlab spectrophotometer [12].

4. LDL

LDL reagent is measured as the measurement standard. Then the supernatant was measured by mixing 10 μ l and 1 ml of LDL reagent, incubated for 20 minutes, then measured at a wavelength of 546 nm with a 300 microlab spectrophotometer [12].

2.7 Statistical Evaluation

The statistical evaluation begins with a normality test carried out using the Lilliefors Significance Correction test and is continued with the Shapiro-Wilk test. Then performed the homogeneity test followed by the ANOVA test and proved by the Tukey HSD test as a Post Hoc Test.

3. Results and Discussion

3.1 Results

Profile of lipid levels (lipid profile) examined total cholesterol, triglyceride, HDL, and LDL levels in mg/dL units; data were analyzed by SPSS 22 and displayed in Mean \pm SEM values. The data are shown in Table 2.

Table 2. Mean Value of Total Cholesterol, Triglyceride, HDL, and LDL Levels in Blood (values in Mean \pm SEM)

Group	Cholesterols	Triglycerides	HDL	LDL
	(Mean \pm SEM)			
I	50.25 \pm 0.85	59.50 \pm 0.64	36.25 \pm 0.75	28.25 \pm 0.47
II	54.00 \pm 0.91	61.75 \pm 0.85	38.75 \pm 0.62	32.00 \pm 0.70
III	76.50 \pm 0.64	88.00 \pm 0.70	29.25 \pm 0.85	38.25 \pm 0.47
IV	63.00 \pm 0.70	69.50 \pm 0.64	32.50 \pm 0.64	35.50 \pm 0.64
V	56.50 \pm 0.64	67.25 \pm 0.85	35.00 \pm 0.40	35.00 \pm 0.40
VI	55.75 \pm 0.47	63.00 \pm 0.40	37.00 \pm 0.81	30.50 \pm 0.86

Comparison of total cholesterol, triglyceride, HDL, and LDL levels between groups can be seen more clearly in Figure 1.

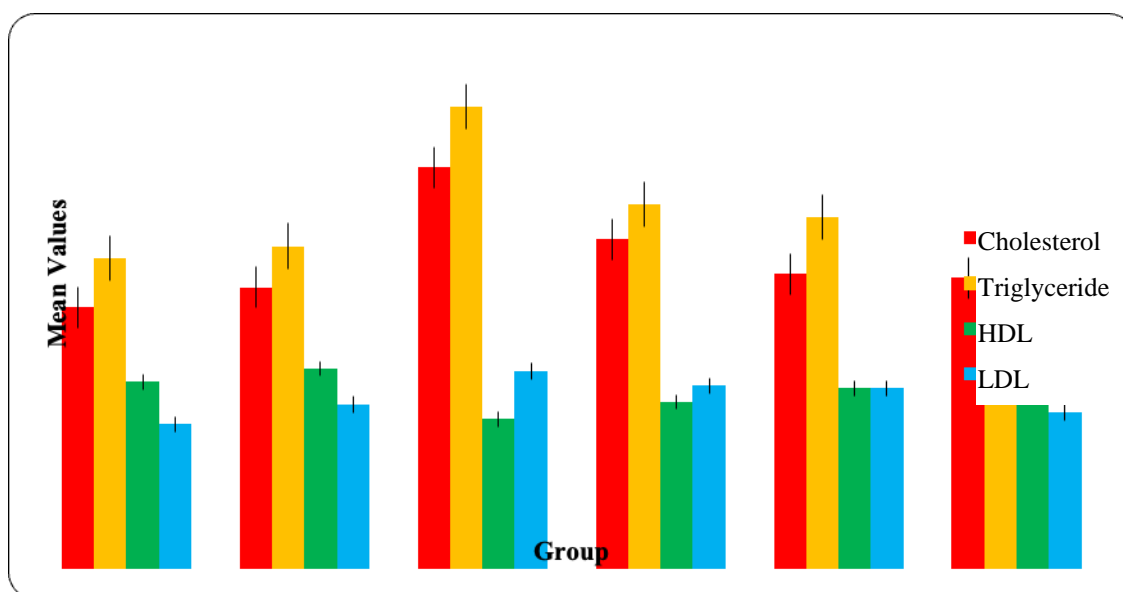


Figure 1 Mean Value Graphic of Total Cholesterol, Triglyceride, HDL, and LDL Levels in Blood (values in Mean \pm SEM)

As shown in Figure 1, it can be seen that the lowest mean of total cholesterol was in group I (50.25 \pm 0.85), and the highest mean of total cholesterol value was in group III (76.50 \pm 0.64). Group I has the lowest triglyceride mean (59.50 \pm 0.64), and the highest mean of triglyceride was in group III (88.00 \pm 0.70). The mean of HDL in group III (29.25 \pm 0.85) was lower than the mean of group I (36.25 \pm 0.75), and the mean of LDL in group III (38.25 \pm 0.47) was higher than the mean of group I (28.25 \pm 0.47). With the given of BBLEE doses of 30, 60, and 90 mg/kg BW significantly ($p < 0.05$) can reduce cholesterol levels (55.75 \pm 0.47), lower triglyceride levels (63.00 \pm 0.40), increasing HDL levels (55.75 \pm 0.47), and reducing LDL levels (30.50 \pm 0.86) in the blood than group III as a negative control. BBLEE at a 90 mg/kg BW dose showed comparable effectiveness with 17- β estradiol valerate (group II) in improving lipid profiles.

3.2 Discussion

The results obtained in this study indicate that the tested BBLEE can reduce total cholesterol levels, lower triglyceride levels, increase HDL levels, and reduce LDL levels in the blood of ovariectomized female rats as a menopausal model. Ovariectomy, which is done by taking the ovaries of female rats, can cause an increase in total cholesterol levels [16, 17], increase triglyceride levels [16,11], and reduce HDL levels [16,20] and increasing LDL levels [16,17] in the blood of ovariectomized female rats. Increased levels of total cholesterol, triglycerides, and LDL, and decreased HDL levels in the blood of ovariectomized female rats occurred due to the inactivity of the ovaries in producing ovum, which also produces the hormone estrogen. The hormone estrogen is a hormone that affects the work system in a woman's body. The decrease in the hormone estrogen that occurs in menopausal women can have many negative effects on women's health, increasing blood fat. Increased fat in the blood can cause diseases that attack the cardiovascular system, including atherosclerosis. It causes the need for women to consume synthetic hormones or hormone replacement therapy (HRT), which plays a role in replacing the estrogen hormone that is no longer produced in the body of menopausal women. However, HRT that menopausal women consume can also cause many negative effects, including hyperplasia. The use of plant-based estrogen hormones (phytoestrogens) is starting to attract menopausal women due to the lack of negative effects it causes. Flavonoids contained in plants can replace the hormone estrogen that has been lost in menopausal women [7]. The Bangun-Bangun plant has been known to the people of North Sumatra for increasing breast milk in women who are breastfeeding. It is due to the regulated gene expression of prolactin receptors [22], and there is also a flavonoid content that has a strong correlation with antioxidant activity [21]. Judging from the effect of BBLEE in improving the lipid profile in the blood of female rats, it is due to the effect of flavonoids which can convert androgens into estrogen [7]. Estrogen binds to the G-protein-coupled receptor GPR30, activating fast kinase signaling pathways such as PI3K and MAPK [10]. With the change in genes through the ER nucleus, it can be shown that EEDBB is estrogenic, which plays a role in improving the lipid profile in the blood of ovariectomized female rats as a model of menopause.

3. Conclusion

Our data show that EEDBB at doses of 30 mg/kg BW, 60 mg/kg BW, and 90 mg/kg BW can improve lipid profiles in ovariectomized female rats as a model of menopause. The dose of 90 mg/kg BW is the best in improving the lipid profile in ovariectomized female rats as a model of menopause.

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