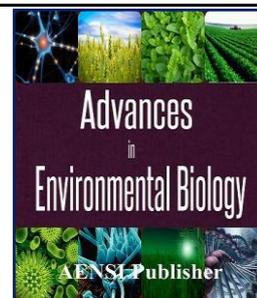




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Effect of Extract Black Cumin (*Nigella Sativa*) on The LDL/HDL Ratio of Ovariectomized White Mouse (*Rattus Novergicus Strain Wistar*)

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ABSTRACT

Background : Changes in fat metabolism by estrogen deficiency is one of the factors increasing the risk of CHD in postmenopausal women. Black cumin (*Nigella sativa*) is a spice that can be used as a medicinal plant. From several studies, *Nigella sativa* could show the effects of estrogen. **Objective:** to determine the effect of black cumin (*Nigella sativa*) against changes on LDL/HDL ratio of the ovariectomized white mouse (*Rattus novergicus wistar strain*) **Method :** True Experimental design with the post test only control group design with 25 female mouse divided into five groups : 2 controle group positive and negative, and 3 treatment groups of black cumin extract at a dose of 1.25, 2.5 and 5 mg/kg BW /day. **Results :** One way ANOVA test showed the non significant value ($p > 0.05$) of weight changes ($p: 0.055$), HDL level ($p : 0.326$) and LDL level ($p : 0.659$). At Kruskal-Wallis test showed the non significant value of LDL/HDL ratio ($p : 0.152$ ($p > 0.05$)). **Conclusion :** Extract of black cumin (*N. sativa*) could not significant affect on LDL/HDL ratio of the ovariectomized white mouse (*Rattus novergicus wistar strain*)

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INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in women, especially those aged over 50 years. Changes in fat metabolism by estrogen deficiency is one of the factors increasing the risk of CHD in postmenopausal women. Epidemiological studies prove that menopause is associated with increased total cholesterol and low density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C). [1,2]

The importance of the role of estrogen on female reproductive organs, encourage researchers to experiment to find the source of estrogen from outside the body (exogenous estrogen) as endogenous estrogen replacement that are relatively safe. Several plants have compounds of estrogenic properties, such as flavones, isoflavones and coumestan derivate. [3].

Black cumin:

(*N. sativa*) a spice that can be used as a medicinal plant and a food preservative. The seeds and the oil of *N. sativa* showed a potential treatment in traditional medicines. [4] *N. sativa* has flavinoid compounds. This plant has one clump with *N. damascene*. that to be known has a high estrogenic effect. [5]. *N. sativa* exhibited antifertility, antioxytotic activity, emmenagogum and many other activities. [5,6]

Methodology:

The general purpose of this study was to determine the effect of black cumin (*Nigella sativa*) on the LDL/HDL ratio of ovariectomized white mouse (*Rattus novergicus strain wistar*). The specific purpose were to determine the weight change, changes in levels of LDL and HDL of ovariectomized white mouse (*Rattus novergicus strain wistar*) after giving extract of black cumin (*N. sativa*).

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Black cumin seeds (*N.sativa*) were from herbal medicine shop in Malang-East Java Indonesia. Black cumin seeds were extracted by maceration procedure that produced black cumin oil. Previous research has given black cumin extract dose of 2.5 to 5 mg / kg / day to see the impact of black cumin to repair rat model of rheumatoid arthritis, and used as a dose for this study.

True experimental design with the post test only control group design was used, with 25 female mouse divided into five groups : a control of negative group, a control of positive group, and 3 treatment groups of black cumin extract at a dose of 1.25, 2.5 and 5 mg/kg/day. Extract of black cumin was done by a sonde into the mouths of mouse, six days a week for eight weeks. At the initial stage before treatment was adaptation of the mouse for 1 week then performed ovariectomy and the second adaptation for 2 weeks after ovariectomy to get hypoestrogen condition. The mouse were killed by general anathesion, and soon after that the blood was taken from the heart for examination the level of LDL and HDL in Prodia Laboratory with homegenous method.

One way ANOVA was use for statistical analysis which previously conducted normality and homogeneity test as a precondition. The test was significant if $p < 0.05$.

RESULTS AND DISCUSSION

The mean weight changes was greatest in the group P1 (51 + 8.72 g), while the mean of smallest weight changes was in group P2 (29 + 11.31 g). Mean of LDL level was highest in group P3 (13,2 + 6.8 pg/ml), and the lowest was in negative controle group (7.0 + 7.0 pg/ml). Mean of HDL level was highest in potitive controle group (52,6 + 7.96 pg/ml), and the lowest was in group P2 (45.0 + 7.14 pg/ml). The mean highest of LDL/HDL ratio was in group P3 (0.25 + 0.10), and the smallest was in negative controle group (0.15 + 0.04).

Shapiro Wilk normality test and *Levene* homogeneity test were performed to the data. Retrieved weight changes, levels of LDL and HDL levels obtained $p > 0.05$. While the ratio of LDL / HD obtained $p < 0.05$. One way ANOVA test performed on the weight changes, levels of LDL and HDL levels with results $p > 0.05$. Kruskal Walis test for the ratio of LDL / HDL was obtained value of $p > 0.05$.

Table 1: The measure of weight changes, level of LDL, level of HDL and LDL/HDL ratio

Group	N	Weight changes (g)		Level of LDL (pg/ml)		Level of HDL (pg/ml)		LDL/HDL ratio	
		Rerata	+ SD	Rerata	+ SD	Rerata	+ SD	Rerata	+ SD
Control (-)	5	31.0	14.39	7.0	3,16	46.6	10.04	0.15	0.04
Control (+)	5	35.6	11.87	12.8	4,44	52.6	7.96	0.24	0.05
P1	5	51.0	8.72	9.2	1,30	46.8	6.38	0.20	0.01
P2	5	29.0	11.31	11.2	7,60	45.0	7.14	0.24	0.13
P3	5	32.8	11.95	13.2	6,80	49.6	10.60	0.25	0.10

Tabel 5.4: Result of One way ANOVA

Variabel	F value	P value
Weight changes	2.784	0.055
Level of HDL	1.240	0.326
Level of LDL	0.612	0.659

Several epidemiological studies and clinical studies found that the ratio of LDL / HDL was an excellent predictor for the risk of CHD and effective way to monitor lipid-lowering therapies. 5-year results of a clinical trial in 4,000 males in Helsinki showed the ratio of LDL / HDL had a better prognostic value than the measurement of LDL and HDL alone [8]

PROCAM study obtained from CHD deaths increased when the ratio of LDL / HDL between 3.7 to 4.3. While other studies had found an increase of one unit the ratio of LDL / HDL caused increased risk of myocardial infarction 53-75%. [9] The ratio of LDL / HDL < 3 was a low risk, while a ratio of > 5 was a high risk. [10] NCEP recommends the ratio of LDL / HDL 2.5, and the risk of death from CVD would increase significantly if the ratio ranges from 3.3 to 3.7. [11]

Parhizkar et al, conducted a study of 40 rats in the OVX and divided into 5 groups (control group given Conjugated Equine Estrogen (CEE), Low dose group of *N.sativa* (LNS) 300 mg / kg, medium dose of NS (MNS) 600mg / kg and High dose of NS (HNS) 1200 mg / kg. The results showed *N.sativa* induce cornification and increased levels of estradiol significant at LNS group. Kusuma [12] obtained significant increase in endometrial thickness compared to the control ovariectomized mouse models. The dose of *N.sativa* used in this study was 1.25, 2.5, and 5 mg / kg BW / day. [7]

Estrogen was cardioprotective through the mechanism of direct protective effect against ischemia / reperfusion injury to the myocardium. It has been proven that there was a decrease in TNF α during an ischemic mechanism of estrogen-mediated cardiac protection [13] But the Women's Health Initiative study (WHI) can not prove that hormone replacement therapy may provide cardioprotection effects in menopausal women. [14]. Xu and WHI study showed differences in the effects of endogenous and exogenous estrogen to the cardioprotective

Until now, the effects of phytoestrogens from *N.sativa* not yet clearly understood. But there was considerable evidence that phytoestrogens can work through estrogen receptor-independent mechanism. [15] Many studies had shown that phytoestrogens bind to the estrogen receptor (ER) and showed significant estrogenic-like effect. Parkizhar, of the research mentioned *N.sativa* had estrogen-like effects.

Study of El-Dakhkhani *et al*, [16] was to determine the hypolipidemic activity of *N.sativa* in mice. In this study *N.sativa* oil given orally at a dose of 800 mg / kg for 4 weeks. The result obtained significant reduction of serum total cholesterol, LDL and triglycerides and a significant increase in serum HDL levels [16]

Tasawar *et al* [17], performed a research *N.sativa* effect on the lipid profile of patients with stable coronary artery disease. In the treatment group obtained the results of a significant reduction of the cholesterol, LDL, triglycerides, VLDL and a significant increase in HDL. The treatment group was given 500 mg / day plus a statin 10-20 mg for 180 days, while the control group was given only 10-20 mg statin. [17] Ibrahim *et al* [18] also investigated the hypolipidemic effect of *N.sativa* in 37 postmenopausal women. Given capsules containing 1 g of *N.sativa* powder after breakfast on 19 samples in the group treated for 2 months and capsules containing a placebo on 18 control samples. The results showed a significant reduction of total cholesterol, LDL and triglycerides, treatment is not obtained significant differences in levels of HDL. [18,19]

Hypolipidemic effect of NS seems not only coming from one component but was a collaboration of several substances just as thymoquinon (TQ), nigellamine, mucilage, sterols, flavonoids and polyunsaturated fatty acids (PUFAS) [20] Various mechanisms was also contribute of fiber contained in NS that can regulate cholesterol synthesis by HMG-CoA reductase, Apo-A1, Apo-B100 and LDL receptor gene [21,22,23] TQ shown to inhibit non-enzymatic lipid peroxidation in liposomes and works by binding a wide range of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radicals. [21] Other studies proved that the levels of phyto-sterols 0,33 to 0,36 % at NS that interact with some other mechanism of metabolism in the body provide a protective effect. [24]

A few things that may cause the results of this study were less significant was too small of dose. When compared with existing research, the others used a dose of 100-1000 mg of NS, while in this study only use a dose of 1.25, 2.5, and 5 mg / kg BW.

Conclusion:

Extract of black cumin (*N. sativa*) could not significant affect on LDL/HDL ratio, weight changes, LDL and HDL levels of the ovariectomized white mouse (*Rattus norvegicus wistar strain*).

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