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Ethanol Extract with Black Cumin (Nigella Sativa) Against sFlt-1 Level and VEGF Serum on Laboratory Mice with Preeclampsia

Deasy Irawati[1], Hidayat Suyuti[2], Titi Maharrani[3], Fitriah[1], Ani Media Harrumi[3], Suryaningsih[1], Nursalam[4]

[1]Bangkalan Midwifery Diploma Study Program, the Health Ministry Polytechnic of Surabaya, [2]Master Program in Biomedical Sciences, Brawijaya University, [3]Soetomo Midwifery Diploma Study Program, the Health Ministry Polytechnic of Surabaya, 4Faculty of Nursing, Universitas Airlangga

Abstract

Introduction: Preeclampsia is one of the complications that occur in pregnancies. This study was aimed to study the factors that affect the of giving ethanol extract with black cumin (Nigella sativa) against sFlt-1 level and VEGF serum on laboratory mice induced preeclampsia.

Method: Laboratory experimental research with posttest only control group design. This study used 30 BALB/C laboratory mice, divided into 6 groups, namely negative controls: pregnant mice injected serum from normal pregnant women, positive controls; mice modeled preeclampsia, and treatment groups 1, 2, 3 and 4 are preeclampsia mice received a dose of 500 mg, 1000 mg, 1500 mg and 2000 mg/kg weight of Nigella sativa ethanol extract for 5 days. Statistical analysis using ANOVA

Result: The mean serum sFlt-1 level in mice modeled preeclampsia and treatment group dose 500mg, 1000mg, 1500mg and 2000mg (2510.3+182.2 pg/mL, 2142.5+171.9 pg/mL, 1309+161.3 p/mL, and 1500+169.9, respectively) pg/mL) showed a significant difference ($p<0.05$) and found a decrease in serum sFlt-1 levels with increasing doses. The mean serum VEGF levels in preeclampsia mice and treatment groups were 500 mg, 1000 mg, 1500 mg and 2000 mg (50.25±2.85b pg/mL, 60.18±4.81c pg/mL respectively, 71.89±2.38d pg/mL, 66.51±1.87 e pg/mL) showed a significant difference ($p<0.05$) and found an increase in serum VEGF levels as the dose increased.

Conclusion: Giving of Black Cumin extract (Nigella sativa) decreases serum sFlt-1 levels and increases serum VEGF levels in preeclampsia mice model and the effect is dependent dose.

Keywords: *sFlt-1, VEGF, Nigella sativa, preeclampsia.*

Introduction

Preeclampsia is one of the complications that occur in pregnancies of more than 20 weeks which is characterized by an increase in systolic blood pressure greater or equal to 140 mmHg or diastolic pressure greater than or equal to 90 mmHg and the amount of proteinuria 300 mg or more than 30 mg/dL per 24 hours[1]. Preeclampsia occurs in about 3-5% of pregnant women worldwide and the number two cause of death for pregnant women. In the United States, 15% of maternal deaths are caused by preeclampsia [2,3].

The pathogenesis of preeclampsia occurs with a variety of mechanisms, but placental ischemia/hypoxia is likely to be a major factor due to disruption of trophoblast invasion[4]. Placental ischemia will stimulate excessive production of sFlt-1 or VEGFR-1. The presence of sFlt-1 as a competitor for surface VEGF receptors (Flt-1), causes VEGF cannot attach to receptors on the cell surface. This situation causes serum VEGF levels

Corresponding Author:
Nursalam
Faculty of Nursing, Universitas Airlangga
e-mail: nursalam@fkp.unair.ac.id

to decrease and induce endothelial cell damage in the glomerulus, producing a urine protein[5,6].

Black cumin has been used as a traditional medicine for thousands of years for various diseases such as asthma, coughing, bronchitis, headaches, fever, and rheumatism[7]. Seed extracts of both water and oil have the potential to be anti-tumor, antioxidant, anti-inflammatory, anti-hypertensive, anti-diabetic and anti-seizure. Thymoquinone (TQ) is the main constituent of Black Cumin essential oil[8,9]. As an antioxidant, thymoquinone synergizes with other compounds such as dithymoquinone and thymol as a free radical scavenger[10]. As TQ Anti-inflammatory inhibits activation of NFκβ. In cases of preeclampsia where placental hypoxia occurs, activation of NFκβ will affect the expression of hypoxia-inducible factor 1-α (HIF1-α)[11] which is a VEGF[12] transactivator.

By referring to the above facts because Black Cumin (Nigella Sativa) has the potential as an antioxidant and anti-inflammatory, it is necessary to conduct research on the molecular mechanism of Black Cumin extract (BC-e) on serum sFlt-1 and VEGF levels in preeclampsia mice.

Design and Method

This research is a laboratory experimental study with a posttest only control group design. This study measured serum sFlt-1 and VEGF levels in the mice model of preeclampsia after being given several doses of Nigella sativa extract. A total of 30 pregnant BALB/C mice were used in this study, divided into 6 groups: pregnant mice injected with serum of normal pregnant women were used as negative controls, preeclampsia mice were as positive controls, and 4 groups of preeclampsia mice were treated with BC-e with a dose of 500 mg, 1000 mg, 1500 mg and 2000 mg/kg of body-weight/day for 5 days. Mice model preeclampsia made by injecting serum of preeclamptic pregnant women on the 10th and 11th days of gestation each 0.1cc intraperitoneally[13.14]. The manifestation of preeclampsia in mice is obtained by finding hypertension and proteinuria on the 15th day of gestation. Maintenance of mice and modeling of preeclampsia were carried out at the Pharmacology Laboratory of the Faculty of Medicine, University of Brawijaya and got standard food and drink.

Mice are terminated at 20 weeks' gestation and blood and kidney organs are collected. Examination of sFlt-1 and VEGF levels in mice serum was measured using an ELISA kit, pg/Ml unit.

Result

The comparison test results showed a difference (p = 0.000 <) the mean serum sFlt-1 level between the negative control group (healthy mice) (579.8 ± 114.8 pg/mL) and the positive control group (preeclampsia mice) (2752.8 ± 188.7 pg/mL)) Likewise there was a significant difference (p = 0.000 <) mean serum VEGF levels between healthy groups (88.56 ± 5.58 pg/mL) with preeclampsia mice model (44.85 ± 2.15 pg/mL). As shown in table 1.

Table 1. Results of comparison of control groups

Variable	Negative Control (Healthy) Mean ± SD	Positive Control (Eclampsia) Mean ± SD	p-value
sFlt-1 serum level (pg/mL)	579.8±114.8	2752.8±188.7	0.000
VEGF serum level(pg/mL)	88.56±5.58	44.85±2.15	0.000

Based on the results of the one way ANOVA test on serum sFlt-1 level data, there were significant differences in the mean serum sFlt-1 level in the five observation sample groups (p-value<0,000). Furthermore, the Multiple Comparisons with the Least Significant Difference (LSD) showed that there was a difference between the mean serum sFlt-1 levels between the positive control group (model preeclampsia mice) (2752.8±188.7apg/mL) and the treatment group gave ethanol extract Nigella sativa doses 500mg (2510.3±182.2b pg/mL), with a dose of 1000mg (2142.5±171.9cpg/mL), with a dose of 1500mg (1309±161.3dpg/mL), and also with a dose of 2000mg (1500±169.9dpg/mL). This means that there is a treatment effect of giving 500mg, 1000mg, 1500mg and ethanol Nigella sativa extracts to serum sFlt-1 levels in preeclampsia mice.

Table 2. The influence of Nigella sativa ethanol extract on mean serum sFlt-1 levels and VEGF levels

Intervention Group	Mean serum sFlt-1 levels (pg/mL)	Mean serum VEGF (pg/mL)
Negative control	579,8	88,56
Positive control	2752,8	44,85
Preeclampsia Mice + Ethanol Extract 500 mg	2510,3	50,25
Preeclampsia Mice + Ethanol Extract 1000 mg	2142,5	60,18
Preeclampsia Mice + Ethanol Extract 1500 mg	1309,0	71,89
Preeclampsia Mice + Ethanol Extract 2000 mg	1500,0	66,51

Based on the results of the One Way ANOVA test on VEGF level data, there were significant differences in the mean VEGF levels of the five observation sample groups (p-value=0,000). Furthermore, the Multiple Comparisons with the Least Significant Difference (LSD) showed that there were significant differences in the mean VEGF levels between the positive control group (preeclampsia mice) (44.85±2.15 pg/mL) and the treatment group administered ethanol extract Nigella sativa at a dose of 500mg, 1000mg, 1500mg and 2000mg (50.25±2.85b pg/mL, 60.18±4.81c pg/mL, 71.89±2.38d pg/mL and 66.51±1.87e pg/mL). There appears to be an increase in serum VEGF levels along with an increase in the dose of ethanol extract except at doses of 2000 mg. If based on the average value of VEGF levels, the treatment group doses 1500mg show the highest value of the average VEGF level (71.89±2.38d pg/mL) compared to the group in other doses and can be considered the fastest dose in increasing VEGF levels in mice models preeclampsia.

The occurrence of a decrease in serum sFlt-1 levels and an increase in VEGF in line levels in increasing doses. The 1500mg dose seems to be the optimal dose of reducing serum sFlt-1 levels and increasing serum VEGF levels.

Discussion

This study showed that sFlt-1 serum levels in pregnant mice injected with pre-eclampsia maternal serum (2752.8±188.7 pg/mL) significantly increased compared with negative control mice. The administration of pre-eclampsia serum intraperitoneal injection with high TNF levels in pregnant mice increase blood pressure and serum sFlt-1 levels[14] which caused by an increase in angiotensin II[15].

The previous study reported that the administration of IgG injection of preeclampsia mothers increase TNF serum levels in pregnant mice. Increased levels of sFlt-1 in preeclampsia patients can reduce levels of free VEGF and PlGF in the circulation resulting in the onset of symptoms of preeclampsia[16,17]. Soluble Fms-like tyrosine kinase-1 (sFlt-1), also known as Soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) which is a soluble receptor for VEGF and PlGF[18] which acts as VEGF and PlGF against by binding and inhibiting interactions both of them against endogenous receptors[19].

This study showed the serum VEGF levels of preeclampsia mice have a significant decrease compared to control mice. The injection of serum for pregnant women PEB in pregnant mice causes pre-eclampsia-like symptoms because TNF-α found in maternal serum binds to TNF type 1 receptors (TNFR-1) mice which in turn activate the NF-κB[20] transcription factor. NF-κβ activation by TNF α may play a role in inducing HIF-1α[21] which is a transcription factor for sFLT formation in the placenta[22]. sFlt does not have a transmembrane domain and membrane[6] cytoplasmic domain, so the bond between VEGF and PlGF to sFlt-1 cannot provide a second messenger for angiogenic and has an antiangiogenic effect[5]. The presence of sFlt-1 as a competitor for surface VEGF receptors (Flt-1) causes VEGF cannot attach to receptors on the cell surface. This condition causes serum proangiogenic VEGF levels to drop by 5/6. Decreasing levels of free VEGF can also indirectly increase in blood. The low levels of free VEGF in serum could decrease in nitric oxide (NO) which cause in blood vessel vasoconstriction followed by an increase in blood pressure.

There was a significant difference in serum sFlt-1 levels in preeclampsia mice with a treatment group given a dose of BC-e dose of 500 mg, 1000 mg, 1500 mg, and 2000 mg. Antioxidant supplements to preeclampsia patients able to reduce serum sFlt-1 levels and increase serum PlGF levels. Black cumin with the main content of Thymoquinone (TQ) has the potential as

an antioxidant so that it can reduce serum sFlt-1 levels in preeclampsia mice significantly[23]. TQ is able to inhibit organ damage caused by free radicals[10]. The antioxidant effects of TQ, dithymoquinone, and thymol be able to inhibit some reactive oxygen species (ROS). TQ and dihydrothymoquinone (DHTQ) have the ability as free radical scavengers with a half inhibitory concentration (IC50) in nanomolar concentrations and micromolar[10]. All ingredients of black cumin have a strong antioxidant effect, where thymol works by quelling single oxygen production, while TQ and dithymoquinone show activities such as superoxide dismutase (SOD)[24].

There was a significant difference in the mean serum VEGF levels of preeclampsia mice with a treatment group that was given a dose of 500 mg, 1,000 mg, 1500 mg, and 2000 mg of BC-e. The effect of BC-e on increasing serum VEGF levels in preeclampsia mice is not fully understood. TQ has the ability to inhibit transcription factors, nuclear factor kappa β (NFκβ) is thought to be the cause. TQ as an inflammatory inhibitor works through anti-inflammatory and proapoptotic action[25].

TQ can inhibit the bonding of NFκβ to DNA through direct interaction with sub-unit p65. TQ will inhibit activation by IkBα kinase which in turn will inhibit degradation and phosphorylation of IκBα thereby inhibiting the activation and translocation of NFκβ from the cytoplasm to the cell nucleus[26]. Barriers to activation of NF-kβ cause decreased HIF1-α expression. In preeclampsia placenta, the inhibition of activation of HIF1-α can reduce the synthesis of sFlt anti-angiogenic factors, and ultimately increase the VEGF angiogenic factor that enters the maternal circulation.

The role of BC-e as an antioxidant is also thought to play a role in increasing VEGF levels. Antioxidant supplementation caused a significant decrease in the concentration of sFlt-1 and increased PlGF in plasma. Whereas in vitro studies showed beneficial effects of antioxidants on VEGF. BC-e has considerable antioxidant properties both in vivo and in vitro[8,24]. In its activity as an antioxidant, thymoquinone synergizes with other compounds such as dithymoquinone and thymol to capture free radicals[10].

The average increase in serum VEGF levels along with the increase in the dose of black cumin extract given and the optimal dose of NS in increasing VEGF levels in serum is 1500 mg. At a dose of 2000 mg, there is a decrease in serum VEGF levels. This is presumably because the effect of hormesis is found in the effects of the response dose[27], where at low doses black cumin ethanol extract has a beneficial effect while at high doses it has a detrimental effect.

Conclusion

Giving of Black Cumin extract (Nigella sativa) decreases serum sFlt-1 levels and increases serum VEGF levels in preeclampsia mice model and the effect is dependent dose.

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