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Original Article

BLACK CUMIN (NIGELLA SATIVA) EFFECT ON EXPRESSION OF TNF-α, IL-2, AND sFlt-1IN PREECLAMPTIC MODEL RATS

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ABSTRACT

Objective: To prove the effect of giving black cumin seed extract (Nigella sativa) to decrease levels of TNF-α, IL-2, sFlt-1, and in PE model mice.

Methods: This study is an analytical study with true experimental research design in laboratory mice (Rattus norvegicus) pregnant female has given black cumin seed extract (Nigella sativa) at a dose of 500 mg/kg/day and 2000 mg/kg/day. The treatment of all samples was carried out simultaneously and during the treatment was observed using the type of Postest Only Control Group Design. To assess whether the sample is normally distributed or not the Shapiro-Wilk test was carried out because the sample was ≤50. To assess the comparison of parameters (differences in expression of IL-2, sFlt-1, and TNF-α) between groups, the ANOVA test was used if the data were normally distributed and the Kruskal Wallis test if the data were not normally distributed.

Results: From these results, it can be seen that there are significant differences in the administration of black cumin extract on TNF-α, IL-2, andsFlt-1 expression ($p<0.001$); the administration of black cumin extract can reduce TNF-α, IL-2, and sFlt-1 expression in preeclampsia mice. The administration of 2000 mg black cumin extract can reduce TNF-α and IL-2 expression more significantly.

Conclusion: Black cumin can reduce levels of TNF-α, IL-2, sFlt-1 in models mice with preeclampsia ($p<0.001$).

Keywords: Nigella Sativa, TNF-α, IL-2, sFlt-1, Preeclampsia

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INTRODUCTION

Preeclampsia (PE) is a pregnancy disorder characterized by systemic hypertension and endothelial dysfunction [1]. PE is hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mm Hg) arising after 20 w gestation with or without proteinuria (300 mg or more, according to 24 h urine capacity) [2]. According to WHO, generally, PE complicates 2-10% of all pregnancies and this incidence is 7 times higher in developing countries (2.8% of all live births) [3]. In Indonesia, PE prevalence data are still limited, especially data at the national level. Even so, the incidence of PE is around 3-10% [4].

Besides being characterized by hypertension and proteinuria, swelling in the legs and hands is also a clinical manifestation of PE. Swelling or edema in PE is caused by increased vascular resistance as a result of endothelial dysfunction in the placenta [5, 6]. Various pro-inflammatory mediators or cytokines are thought to also play a role in the occurrence of PE and its progression, such as interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), Soluble Fms-like tyrosine kinase (sFlt-1), interleukin-6 (IL-6) and interleukin-7 (IL-7) [2, 7, 8].

Oxidative stress plays an integral part of the aging process and results from the overproduction of free radicals such as reactive oxygen species (ROS), which overwhelm the body's antioxidant defense mechanisms. Normally, antioxidants neutralize ROS and thus help to prevent overexposure from oxidative stress. However, as the body ages, antioxidant levels decline, leaving the human body susceptible to a variety of age-related pathologies, such as non-alcoholic liver cirrhosis and atherosclerotic heart disease. This decline, combined with a gradual loss of estrogen in the female reproductive system is highly associated with the various sequelae of menopause [9]. Deficiency of estrogen in menopause causes increase of free radicals and oxidative stress, which will trigger dNA apoptosis in whole cells. Lypoperoxidase level was higher in postmenopausal women compared with premenopausal (0,357±0,05 vs 0.331±0.05 mmol/l; $p<0.001$). Study by MFG Siregar concluded women in menopause experienced higher oxidative stress compared to reproductive women. This condition is similar to preeclampsia condition [10].

In recent years, researchers began to develop drugs from herbs, one of which is Nigella sativa. Nigella sativa (a plant of the ranunculaceae family) is synonymous with the critical Nigella plant and commonly referred to as Habbatussaudah, Al-Habbah Al Sawda, Habbet El-Baraka, Camoun Aswad, Schuniz and Khodria [8, 11].

Black cumin plants contain flavonoid compounds which have anti-inflammatory properties, but also have stimulant, carminative, emenagoga, galagtoga and diaphoretic properties.[8,9] In addition, black cumin also has efficacy as an antioxidant that is quite strong and can reduce blood pressure. Thymoquinone, dithymoquinone, and thymol contained in black cumin seed oil can reduce free radicals and act as an antihypertensive agent [11, 12].

The research results by Indrawan *et al.*, 2016 proved that ethanol extract of black cumin seeds was proven to reduce systolic blood pressure and proteinuria, reduce serum levels of IL-1β, IFN-ET, ET-1 in mouse PE models. Immunohistochemistry results in decreased expression of p65 in the cytoplasm and nucleus. The results of the path analysis stated that the validity value of black cumin work in reducing blood pressure and proteinuria through decreasing serum IL-1β, IFN-γ, ET-1, p65 NF-kB expression was 94.6% [13].

In a study conducted by Bambang *et al.*, 2017 it was found that black cumin in various doses can reduce proinflammatory cytokines such as TNF-α and IL6 in mice exposed by preeclampsia plasma. Doses given are 50 ppm, 100 ppm, 200 ppm, 400 ppm. The active ingredient of black cumin, thymoquinone, functions as an anti-inflammatory by inhibiting the enzymes cyclooxygenase (COX) and lipooxygenase (LOX) and inhibits transcription factors NF-kB and TNF-α [8].

MATERIALS AND METHODS

This study is an analytical study with true experimental research design in laboratory mice (Rattus norvegicus) pregnant female given black cumin seed extract (Nigella sativa) at a dose of 500 mg/kg/day and 2000 mg/kg/day. The treatment of all samples was carried out simultaneously and during the treatment was observed using the type of Postest Only Control Group Design (Notoatmodjo, 2012).

The study was conducted at the Biology Laboratory of Mathematics and Natural Sciences (MIPA) Faculty USU, starting the process of acclimatization, treatment and sampling of experimental animals. The Anatomy Pathology Laboratory of Medical Faculty USU, for an examination of the expression of IL-2, sFlt-1, and TNF-α using the Immunohistochemistry (IHC) method. The research will take place in July 2019.

The experimental animals used in this study were Rattus norvegicus laboratory mice, 10-week-old female, in a healthy/active condition. This study will only use a minimum dose (500 mg/kg/day) and a maximum dose (2000 mg/kg/day) in accordance with previous research studies. This research uses liquid black cumin extract from the brand Habbasyifa®. The injection used to make the PE rat model is LPS (lipopolysaccharide) with a daily dose of 0.5 µg/kg LPS (Escherichia coli serotype 0111: B4, Sigma-Aldrich), diluted in 2 ml of saline solution. Black cumin seed extract (Nigella sativa) is given orally through oral gavage and is prepared as needed.

Work arrangement

The number of treatment groups was 4 groups, so the total sample was 24 animals. A diagnosis of pregnancy is obtained with the presence of vaginal spermatozoa/vaginal plugs and is counted as pregnancy day 0. Group 1, negative control (normal), pregnant mice were not given any treatment in general, were given excessive food and drink (adlibitum) in their cages. Group 2, the treatment group were pregnant mice given LPS injection on the 5th day of pregnancy on the 8th day of the pregnancy the trophoblast invasion was started so that they became PE model mice but were not given black cumin seed extract (Nigella sativa). Group 3, the treatment group namely pregnant mice given LPS injection on the 5th day of pregnancy on the basis of the 8th day of the trophoblast invasion pregnancy, to become a PE model mouse and given black cumin seed extract (Nigella sativa) at a dose of 500 mg/kg BW/day for 15 d. Group 4, the treatment group was pregnant mice given LPS injection on the 5th day of pregnancy on the basis of the 8th day of the pregnancy the trophoblast invasion was started, to become a PE model mouse and given black cumin seed extract (Nigella sativa) at a dose of 2000 mg/kgBW/d for 15 d.

After that, the monitoring of systolic blood pressure carried out in mice every morning (8:00 to 10:00) was evaluated every three days. Black cumin (Nigella sativa) administration in groups 3 and 4 was given immediately after an increase in systolic blood pressure in mice. Black cumin (Nigella sativa) is given orally according to the dose of each group every day until the 15th day. On the 16th day, the four groups were terminated.

The mice then dieuthanasia using ketamine and followed by necropsy. After opening the abdominal cavity, the uterus is incised, the placenta is taken and put into a pot that already contains 10% neutral buffered formalin. Histopathological preparations were made by fixation using 10% neutral buffered formalin buffer solution and then cut and put into a specimen made of plastic. Then the dehydration process is carried out in multilevel alcohol concentration, namely 70% alcohol, 80% alcohol, 90% absolute alcohol I, absolute II for 2 h each. Then purification with xylol is then printed using paraffin so that the preparation is printed in paraffin blocks and stored in the refrigerator. The paraffin block is then thinly cut 5-6 µm thick using a microtome. The cut results are floated in warm water with a temperature of 60^0C to stretch so that the tissue does not multiply. The preparation is then removed and placed in a glass object for staining Hematoxylin and Eosin (HE). Next examined under a light microscope with 400x magnification. The parameters assessed were sFLT-1, IL-2, and TNFα expression in the placenta.

Statistical analysis

Data will be presented in the form of mean and standard deviation if the data is normally distributed. If the data is not normally distributed, the data will be presented in quartile form. Data were processed and analyzed using SPSS with a significance limit of $p<0.05$. To assess whether the sample is normally distributed or not the Shapiro-Wilk test was carried out because the sample was ≤50. To assess the comparison of parameters (differences in expression of IL-2, sFlt-1, and TNF-α) between groups, the ANOVA test was used if the data were normally distributed and the Kruskal Wallis test if the data were not normally distributed.

RESULTS

Characteristics of experimental animals

Table 1: Trial distribution group

Group	F	%
Control-(K-)	6	25.0
Control+(K+)	6	25.0
Nigella Sativa 500 (P1)	6	25.0
Nigella Sativa 2000 (P2)	6	25.0
Total	24	100.0

Systolic and diastolic blood pressure after administration of black cumin extract (Nigella sativa) in preeclampsia model mice

From the results of this study, obtained systolic and diastolic blood pressure in the negative control group, positive control group, and the treatment group. Can be seen in the negative control group blood pressure within the normal range obtained during monitoring. In the positive control group, there was an increase in systolic blood pressure during monitoring compared to the negative control group. This can be seen in fig. 1. In the group of mice given black cumin extract showed a decrease in systolic blood pressure. This indicates a change in systolic blood pressure in experimental animals.

From fig. 1 it was found that blood pressure in treatment group 1 (P1) and treatment group 2 (P2) showed a decrease in systole pressure. Systolic blood pressure decreased in mice with preeclampsia models by administering black cumin extract with a dose of 500 mg (P1) and black cumin extract with a dose of 2000 mg (P2). However, a dose of 2000 mg black cumin extract has a significantly lower systolic blood pressure than 500 mg black cumin extract.

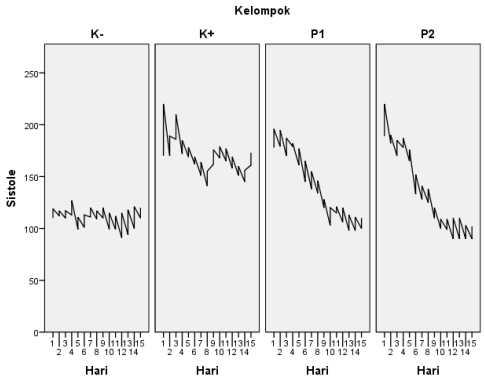

Fig. 1: Administration of black cumin extract can reduce systolic blood pressure in the case group, whereas the positive control group still shows an increase in systolic blood pressure

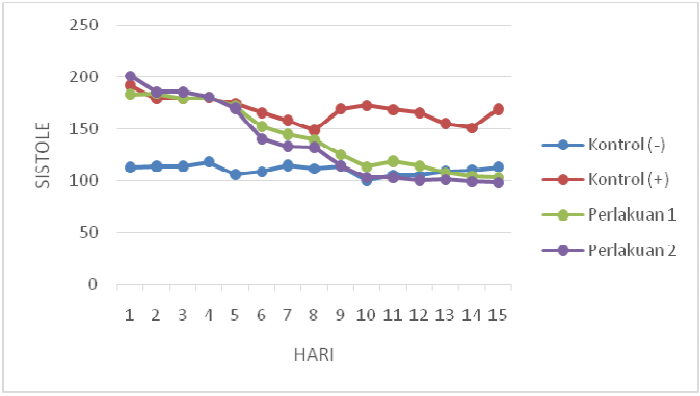

Fig. 2: Comparison of mean systole between groups

From the fig. below, it can be seen the diastole blood pressure values in the treatment group 1 (P1) at a dose of 500 mg and the treatment group 2 (P2) at a dose of 2000 mg experienced a decrease approaching diastole in the normal rat group.

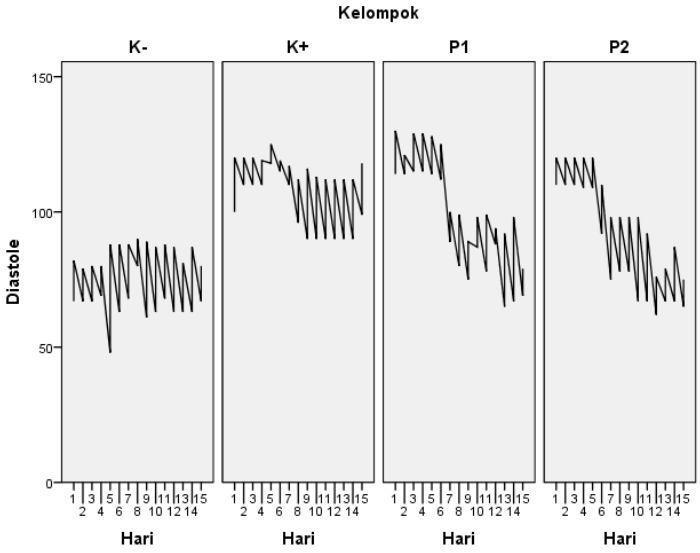

Fig. 3: The administration of black cumin extract (Nigella sativa) can reduce diastole blood pressure in the case group, approaching diastole blood pressure in the negative control group, while the negative control group still shows an increase in diastole blood pressure

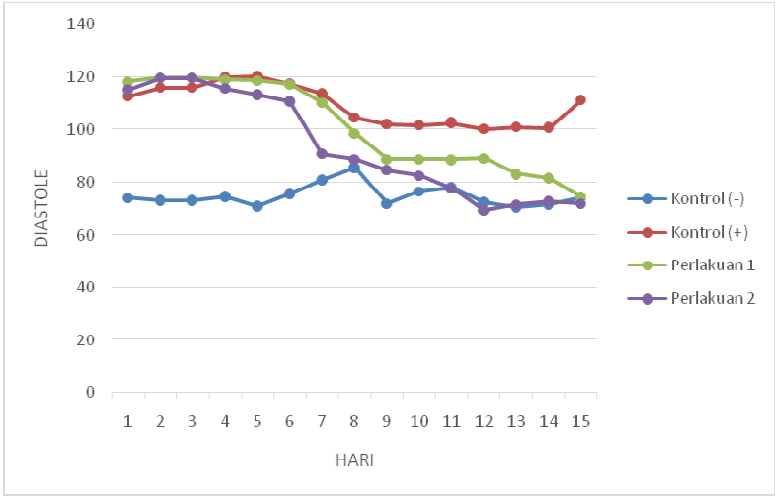

Fig. 4: Comparison of mean diastole between groups

Based on table 2. it was found that changes in blood pressure from the first day to the fifteenth day had a significant difference, where there was a significant difference in blood pressure values in the normal (K-) group and the preeclampsia (K+) group (p<0,000, CI 95%-38.59-29.10); normal mice group with mice that get 500 mg black cumin extract groups ((p<0,000, CI 95%-34.06-21.49); normal mice groups with mice that get 2000 mg black cumin extractgroups (p<0,000, 95% CI-24.85-16.22) There was a significant difference in blood pressure in the mice with LPS injectiongroup with a negative group (p<0,000, 95% CI 29.10-37.17); Significant difference between the positive control group and mice that received 500 mg black cumin extractgroup (p<0.058, CI 95%-0.19-38.59); there was a significant difference between the positive control group and micereceived 2000 mgblack cumin extract group (p<0,000, 95% CI 9.07-12.33) There was a significant difference in micegets 500 mg black cumin extract group with negative control group (p<0,000, 95% CI 21.09-34, 06) there was no significant difference in blood pressure between micegets 500 mgblack cumin extract groups with a positive control group (p = 0.58, 95% CI-12.33-0.19); there was a significant difference between mice with 500 mg black cumin extract dose groups and 2000 mg black cumin group (p<0.022, 95% CI-13.38-1.09). There was found blood pressure difference in rat received 2000 mg black cumin extractgroup with a positive control group (p<0,000, 95% CI 16.22-24.85); there was a significant difference between micegets 2000 mg dose of black cumin extract groups and positive control mice (p<0,000, 95% CI 16.22-24.85); there was a difference in blood pressure in the rat group with black cumin dose 2000 mg with negative control group (p<0,000, 95% CI-17.55-9.07); there was a difference in blood pressure in the rat group gets 2000 mg black cumin extractand the group of micegets 500 mg black cumin extract (p = 0.022, 95% CI-13.38-1.09).

Table 2: Comparison of mean values of blood pressure of mice

(I) klp	(J) klp	Mean difference (I-J)	Std. error	Sig.	95% confidence	
					Lower bound	Upper bound
K-	K+	-33.85*	1.455	.000	-38.59	-29.10
	P1	-27.77*	1.862	.000	-34.06	-21.49
	P2	-20.54*	1.296	.000	-24.85	-16.22
K+	K-	33.85*	1.455	.000	29.10	37.17
	P1	6.07	1.849	.058	-.19	38.59
	P2	13.31*	1.276	.000	9.07	12.33
P1	K-	27.77*	1.862	.000	21.49	34.06
	K+	-6.07	1.849	.058	-12.33	.19
	P2	7.24*	1.726	.022	1.09	13.38
P2	K+	20.54*	1.296	.000	16.22	24.85
	K-	-13.31*	1.276	.000	-17.55	-9.07
	P1	-7.24*	1.726	.022	-13.38	-1.09

Comparison of mean arterial pressure (MAP) in mouse group after giving black cumin extract

Based on table 3 it was found that MAP change from the first day to the fifteenth day had a significant difference, where there were significant differences in blood pressure values in normal (K-) group and the preeclampsia (K+) group (p<0.001, CI 95%-46.35-37.48); normal mice group with mice that get black cumin 500 mg groups ((p<0,000, CI 95%-33.74-23.41); normal mice group with mice that get black cumin 2000 mg groups (p<0.001, CI 95 %-25.70-17.83) There was a significant difference in blood pressure in the mice given LPS injection group with mice with administration of 500 mg black cumin groups (p<0.001, 95% CI 8.12-18.55); there was a significant difference between the positive control group and rat that received black cumin doses of 2000 mg group (p<0.001, 95% CI 16.11-24.18); there was a significant difference between the positive control group and the negative control group (p<0.001, 95% CI 23.4-33.74) There was a significant difference in rat given 500 mg black cumin extract group with a positive control group (p<0.001, 95% CI-18.55-8.12); there was a significant difference on blood pressure between micegetsblack cumin extract dose of 500 mg groups with control mice given black cumin extract dose of 2000 mg group (p<0.001, 95% CI 1.85-11.76); there was a significant difference between mice gets 500 mg black cumin extract groups and negative control mice (p<0.001, 95% CI 17.83-25.70). There was a difference in blood pressure in the rat group which administering black cumin extract at a dose of 2000 mg with a positive control group (p<0.001, CI 95%-24.18-16.11); there was a significant difference between the groups of mice with 2000 mg black cumin extract and 500 mg black cumin extract (p<0.001, 95%-24.18-16.11) CI; there was a difference in blood pressure in the rat group with black cumin dose of2000 mg with a negative control group (p<0,000, 95% CI-17.55-9.07); there was a difference in blood pressure in the rat group with black cumin dose of 2000 mg with the negative control mice group (p<0.001, 95% CI 17.83-5.70).

From the image below it can be seen the mean MAP comparison in each group of mice. Where seen MAP decrease in mice that were given black cumin extract dose of 500 mg and a dose of 2000 mg. However, a more significant decrease in MAP was seen in mice given 2000 mg black cumin extract.

Table 3: Comparison of mean arterial pressure (MAP) value in model mice

(I) klp	(J) klp	Mean difference (I-J)	Std. error	Sig.	95% confidence	
					Lower bound	Upper bound
K-	K+	-41,92	1,35	<0.0001	-46,35	-37,48
	P1	28,57	1,57	<0.0001	-33,74	-23,41
	P2	-21,76	1,18	<0.0001	-25,70	-17,83
K+	K-	41,92	1,35	<0.0001	37,48	46,35
	P1	13,34	1,57	<0.0001	8,12	18,55
	P2	20,15	1,21	<0.0001	16,11	24,18
P1	K-	28,57	1,55	<0.0001	23,4	33,74
	K+	-12,34	1,57	<0.0001	-18,55	-8,12
	P2	6,80	1,43	<0.0001	1,85	11,76
P2	K-	21,76	1,18	<0.0001	17,83	25,70
	K+	-20,15	1,21	<0.0001	-24,18	-16,11
	P1	-6,81	1,43	<0.0001	-11,76	-1,85

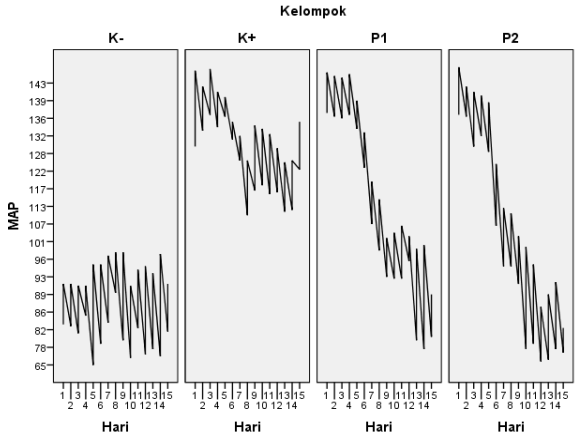

Fig. 5: Comparison of MAP between groups

Comparison of proteinuria in each study group

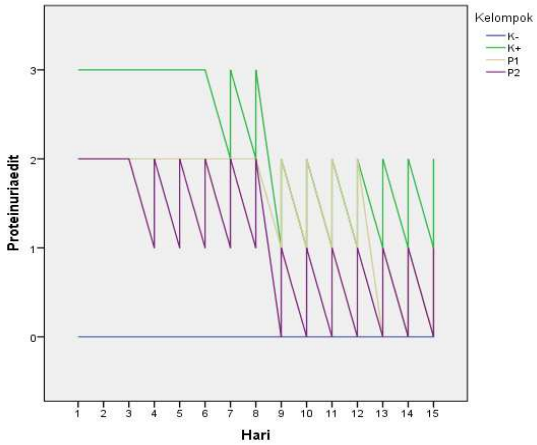

Fig. 6: Comparison of proteinuria in each group

In fig. 6, proteinuria shows a decrease per day where it appears the role of black cumin can reduce protein in the urine.

Table 4: Proteinuria in the study group from day 1 to day 15

		Proteinuria																											
		1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
		n	%	n	%	n	%	N	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
K-	-	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100
	+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K+	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	33.3	2	33.3	2	33.3	2	33.3	2	33.3	2	33.3	0	0
	+2	0	0	0	0	0	0	0	0	0	0	0	0	2	33.3	3	50	4	66.7	4	66.7	4	66.7	4	66.7	4	66.7	4	66.7	6	100
	+3	6	100	6	100	6	100	6	100	6	100	6	100	4	66.7	3	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P1	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+2	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100	6	100
	+3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P2	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	33.3	2	33.3	2	33.3	2	33.3	2	33.3	2	33.3	6	100
	+1	0	0	2	33.3	1	16.7	1	16.7	1	16.7	1	16.7	1	16.7	1	16.7	3	50	3	50	3	50	3	50	3	50	3	50	0	0
	+2	6	100	4	66.7	5	83.3	5	83.3	5	83.3	5	83.3	5	83.3	5	83.3	1	16.7	1	16.7	1	16.7	1	16.7	1	16.7	1	16.7	0	0
	+3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Comparison of TNF-α expression scores after administration of black cumin (Nigella sativa) in the study group

Based on table 5. it was found that the highest TNF-α value was found in the positive control group (K+) with a value of 7.33, while in the negative control group (K-) with a value of 0.67, in group 1 which gets 500 mg black cumin extract (P1) with a value of 1.67, and group 2 who were given black cumin 2000 mg (P2) with a value of 0.50. From these results it can be seen that there are significant differences in the administration of black cumin extract on TNF-α expression (p<0.001) and the administration of black cumin extract can reduce TNF-α expression in preeclampsia mice. The administration of 2000 mg black cumin extract can reduce TNF-α expression more significantly.

Table 5: Differences in TNF-α expression scores

TNF-α	N	Mean	SD	SE	*p value*	K-	K+	P1	P2
K-	6	0.67	0.816	0.333	<0,001		<0.001	0.311	0.864
K+	6	7.33	2.582	1.054				<0.001	<0.001
P1	6	1.67	1.862	0.76					0. 239
P2	6	0.5	0.548	0.224					

Comparison of IL-2 expression scores after administration of black cumin (Nigella sativa) in study group

Based on table 6 it was found that the highest IL-2 value was found in the positive control group (K+) with a value of 4.33, while in the negative control group (K-) with a value of 0.50, in group 1 which gets 500 mg black cumin extract (P1)) have value of 0.33, and group 2 who were given black cumin 2000 mg (P2) with a value of 0.17. From these results it can be seen that there is a significant difference in the administration of black cumin extract on IL-2 expression (p<0.001) and the administration of black cumin extract can reduce IL-2 expression in preeclampsia mice. The administration of 2000 mg black cumin extract can significantly reduce IL-2 expression.

Table 6: Differences in IL-2 expression scores

IL-2	N	Mean	SD	SE	*p value*	K-	K+	P1	P2
K-	6	0.5	0.837	0.342	<0,001		<0.001	0.838	0.684
K+	6	4.33	2.582	1.054				0.806	0.806
P1	6	0.33	0.516	0.211					0.806
P2	6	0.17	0.408	0.167					

Comparison of sFlt-1 expression scores after administration of black cumin (Nigella sativa) in study group

Based on table 7 it was found that the highest value of sFlt-1 was found in the positive control group (K+) with a value of 4.33, while in the negative control group (K-) with a value of 0.17, in group 1 which gets 500 mg black cumin extract (P1) with a value of 1.00, and group 2 who were given black cumin 2000 mg (P2) with a value of 0.17. From these results it can be seen that there are significant differences in the administration of black cumin extract on sFlt-1 expression (p<0.001) and the administration of black cumin extract can reduce sFlt-1 expression in preeclampsia mice.

Table 7: Differences in sFlt-1 expression scores

sflt1	N	Mean	SD	SE	*p value*	K-	K+	P1	P2
K-	6	0.17	0.408	0.167	<0,001		<0.001	0.904	1
K+	6	4.33	2.582	1.054				0.904	0.904
P1	6	1	1.673	0.683					0.904
P2	6	0.17	0.408	0.167					

DISCUSSION

Change in mean MAP in preeclampsia by administering black cumin extract (Nigella sativa)

Black cumin has been known to have many benefits, such as anti-inflammatory, antioxidant, and antihypertensive. Black cumin can play a role in protecting organs from damage due to oxidative stress. The content of timoquinone in black cumin acts as an anti-inflammatory by inhibiting pro-inflammatory cytokines and Nuclear Factor Kappa Beta (NF-kB) transcription factors [14]. The effect of a decrease in blood pressure associated with a decrease in cardiac lipid peroxidation products and inhibition of the activity of the angiotensin converting enzyme was seen in samples receiving N. sativa oil followed by significantly higher plasma nitric oxide levels compared to the placebo group or those receiving nicardipine. Black cumin and its active component, thymoquinone, reduce oxidative stress through calcium channel blockade and increase urine output activity that may be associated with a decrease in blood pressure [15].

Previous studies assessing the dose of N. sativa (300 mg twice daily for 4 w) with the severity of hypertension showed patients at a dose of 200 mg twice daily for 4 w and 500 mg twice daily for 6 w, respectively, showing significant systolic decrease [16]. Research conducted by Hebil *et al.* in which intravenous injection of Nigella sativa extract doses of 50, 100, 200 mg/kg showed a decrease in mean arterial pressure (MAP) (p<0.001) followed by a significant decrease in blood pressure (p<0.001) [17] The central nervous system plays an important role in the rapid control of arterial blood pressure. Thus, the observed decrease in arterial blood pressure may be caused by inhibition of the sympathetic nervous system that can control the aorta under normal conditions [18].

Changes of mean systole in preeclampsia by administration of black cumin extract (Nigella sativa)

The results of this study are in line with the study of Jarin *et al.*, 2015 in which the administration of a combination of black cumin extract (Nigella Sativa) and nicardipine showed a significant decrease in systolic blood pressure. A decrease in systolic blood pressure with Nigella sativa was also followed by a significant decrease in MDA, ACE, NADPH oxidase activity and increased activity of HO-1 in cardiac, which was also accompanied by an increase in plasma NO [19].

The antioxidant effect of Nigella Sativa is also often reported lately. Antioxidant effect of Nigela Sativa which contributes to the antihypertensive effect. Apart from its antioxidant activity, the blood pressure reduction effect of Nigella Sativa can be caused by antidiuretic, antiinflammatory, or protective effects on the kidneys [21, 22].

Endothelium has an important role in vascular muscle relaxation, where several studies have confirmed the involvement of the role of blood vessels as part of the pathophysiology of hypertension [18]. Nigella Sativa extract dose of 30 mg/ml can produce vasorelaxant effects of blood vessels. Thus, Nigella Sativa extract works the same as a blood vessel relaxant factor (Nitrite oxidant or prostacyclin) which causes vasodilation [17].

Previous studies also reported that essential oils from black cumin and thymoquinone lowered blood pressure and heart rate, both directly and indirectly through mechanisms that act as serotoninergic and muscarinic receptors. Thymol, the active component of Nigella Sativa, is known to have a role and can reduce blood pressure through work on calcium ion channels. Peixoto *et al.* reported that thymol produced a relaxing effect in aortic mice [23].

Changes of mean diastole in preeclampsia by administration of black cumin extract (Nigella sativa)

Based on research conducted by Huseini *et al.*, 2013 it was found that consumption of 5 ml of Nigella sativa every day can reduce diastolic by decreasing 12.46%. This was also done by Indrawan *et al.* in 2016, they found that a significant difference was found in endothelin 1 (ET-1) levels in mice with preeclampsia. Reduction of ET-1 by black cumin (Nigella sativa) can reduce levels of ET-1 by inhibiting the formation of peroxynitrite, which can reduce endothelial dysfunction [11].

According to the study of Sahebkar et al., 2016 daily consumption of Nigella Sativa dose of 5 ml can reduce blood pressure both systole and diastole in normal patients without any accompanying effects on the liver and kidneys. Obtained a decrease in systolic blood pressure (8.17%) and a decrease in systolic blood pressure (12.46%). Another mechanism that occurs as a blood pressure reduction effect is the diuresis effect of Nigella Sativa, from a dose of 0.6 ml/kg for 15 d can cause increase in diuresis about 16% spontaneously in hypertensive rats. The diuresis effect of Nigella Sativa compared to 5 mg/kg of furosemide which has a strong diuresis effect associated with excretion of Na, K, Cl, and urine. The results of this study mention that Nigella Sativa can reduce blood pressure through the effects of diuresis, decreased water and electrolytes which cause blood volume, cardiac output and as a main regulation of blood pressure [24].

Changes of proteinuria in preeclampsia by administration of black cumin extract (Nigella sativa)

In recent years, clinical and experimental studies of Nigella Sativa extract have shown many therapeutic effects. In addition, recent toxicological studies have shown that crude extracts of seeds and some of their active constituents (essential oils and Thymoquinone) may have a protective effect on nephrotoxicity. Data can also indicate that Thymoquinone might act as a protective agent for proteinuria and hyperlipidemia associated with nephrotic syndrome [25].

Tumor necrosis factor-α (TNF-α) expression in preeclampsia by administration of black cumin extract (Nigella sativa)

The effect of giving black cumin extract can reduce TNF-α levels along with increasing doses of black cumin in preeclampsia mice. So that the administration of black cumin extract can reduce levels of proinflammatory factors such as TNF-α. Nigella sativa has an active composition of thymoquinone that can inhibit proinflammatory cytokines such as IL-1, IL-6, and NF-kB. Thymoquinone plays a role in inhibiting inflammation through anti-inflammatory and proapoptotic pathways [16]. Oral administration of thymoquinone shows a significant reduction in the number of pro-inflammatory mediators (IL-1, IL-6, TNF-IF, IFN-□ and PGE) and also an increase in IL-10. TQ appears to suppress Fe-NTA-induced oxidative stress, hyperproliferative response and renal carcinogensis in Wistar rats.[26] A study in Iran examined the effects of Nigella sativa extract on inflammatory cytokine responses and oxidative stress status. In the study, the final TNF-α level was lower (9.42, SD: 5.72-14.83) compared to the initial TNF-α level (13.29, SD: 8.19-17.130). Significantly decreased TNF-α levels at the end of Nigella sativa supplementation when compared with placebo [17].

Interleukin-2 (IL-2) placenta expression in preeclampsia by administration of black cumin extract (Nigella sativa)

In other studies concerning the effect of black cumin administration on IL-8 levels, thymoquinone inhibits the NF-kB transcription factor by decreasing promoter activity, first by inhibiting the activity of the NF-kB signaling pathway and inhibiting cytokine and inflammatory chemokine transcription factors. Giving thymoquinone after 6 h and 24 h can reduce IL-8. There are no studies that discuss the effect of black cumin (Nigella sativa) on decreasing IL-2 levels. So it can be analogous that administration of black cumin extract (Nigella sativa) can reduce levels of IL-2 [16, 27]. Recent research shows there is a relationship between the active component of Nigella Sativa extract which can modulate the inflammatory process. Giving Nigella Sativa extract once a day for 21 d can reduce levels of pro-inflammatory mediators including tumor necrosis alpha (TNF-α) [28]. In human lymphocyte T cells, the suppressive effect of NS on IL2, IL-6, and PGE2 is not reported at this level. However, serum IL-2 suppression was found in rats treated with NS extract. The effect of IL-2 suppression from lymphocyte cells will be beneficial because it is considered as a mediator of inflammation. Also, administration of NS extracts significantly reduced PGE2 plasma levels in mice [29]. Interleukin 17A can induce pro-inflammatory cytokines, such as IL-6 [30].

sFlt-1 placenta expression in preeclampsia by giving black cumin extract (Nigella sativa)

It is known that in the condition of preeclampsia occurs increasement of sFlt-1 levels, especially in the second and third trimesters. Excessive production of sFlt-1 contributes to the pathogenesis of placental ischemia in preeclampsia. In the study of Levine *et al.*, 2014, significant levels of sFlt-1 were found in preeclampsia (±4382 pg/ml) and normal pregnancy (±1643 pg/ml). Failure from cytotrophoblast invasion will decrease the diameter of the spiral arteries followed by acute atherosclerosis which will cause endothelial damage, necrosis, and accumulation of foam cells. This will cause hypoxia and release of proinflammatory cytokines such as sFlt-1 which will cause ischemia in the placenta [31-33].

In preeclampsia also increased levels of Hypoxia Inducible Factor 1α (HIF-1 α), sFlt-1, Angiotension II type 1-Receptor Autoantibody (AT1-AA, and PlGF). This increased factor affects endothelial dysfunction, decreases Nitric Oxide (NO)) and increase Reactive Oxygen Species (ROS) and endothelin-1, this will cause changes in kidney function, increased peripheral resistance, and hypertension [34-36]. Thymoquinone content in black cumin has been known to inhibit the activity of proinflammatory cytokines that play a role in preeclampsia such as TNF-α, IL-1, IL-6, and NF-kB.[16] Thymoquinone has the effect of preventing organ damage by free radical effects by inhibiting the formation of ROS. Thymoquinone suppresses NF-κB activation Nph-κB suppression is related to activation, phosphorylation, and degradation of protein kinase B (lkBα) and inhibition of degradation and translocation from i p65. Thymoquinone inhibits p65 from DNA binding. Thymoquinone inhibits activation of NF-κB which will inhibit endothelial dysfunction in preeclampsia, this will cause a decrease in endothelial dysfunction markers such as ET-1, and allow for sFlt1. So that the administration of black cumin extract in this study was proven to reduce sFlt-1 levels in mice with preeclampsia models [37].

CONCLUSION

Black cumin can reduce levels of TNF-α, IL-2, sFlt-1 in models mice with preeclampsia ($p<0.001$). Black cumin is proven to reduce systolic blood pressure, diastolic blood pressure, and Mean Arterial Pressure.

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With the results of this study, black cumin can be recommended to be given to pregnant women who are either at risk or not, to prevent

the occurrence of preeclampsia, given the side effects that do not exist. This study is the first study to prove the anti-inflammatory effect of black cumin extract (Nigella sativa) in model mice preeclampsia against proinflammatory cytokines, TNF α. IL-2, and sFlt-1. This underlies the use of black cumin extract in cases of preeclampsia and cases of pregnancy with increased proinflammatory cytokines.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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