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Title: Effect of *Nigella sativa* Ethanol Extract on the Nitric Oxide Content and Renal Arteriole Diameter of a Pre-eclampsia Mouse Model

Running Head: Effect of *Nigella sativa* Ethanol Extract

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ABSTRACT

Objective: The aim of this study was to investigate the effect of *N. sativa* ethanol extract on the nitric oxide (NO) level and renal arteriole diameter of a preeclampsia mouse model.

Materials and Methods: This experimental study was conducted using a post-test only control group design. Thirty BALB/c mice were divided into six groups, one negative control (normal pregnant mice), one positive control (preeclampsia model), and four groups of preeclampsia mice treated with varying doses of *N. sativa* (500 mg/BW/day, 1000 mg/BW/day, 1500 mg/BW/day, and 2000 mg/BW/day). Ethanol extract of *N. sativa* was given for five days. Data were analyzed by analysis of variance.

Results: We detected significant differences in the NO level of the preeclampsia mouse model and those that were given the ethanol extract. The animals given ethanol extract of *N. sativa* had NO levels of 85.77±4.47 µM (500 mg/BW/day), 189.04±6.01 µM (1000 mg/BW/day), 226.56±2.13 µM (1500 mg/BW/day), and 207.98±4.74 µM (2000 mg/BW/day). The mean diameter of renal arterioles showed significant differences in the treatment group of dose 1000, 1500, and 2000 mg (15.15±2.21 b µm, 16.35±2.52 b µm, and 15.76±3.03 b µm, respectively).

Conclusion: *N. sativa* ethanol extract treatment increases NO levels and enlarges the renal arteriole diameter of a preeclampsia mouse model in a dose-dependent manner.

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Keywords: Nitric oxide, renal arteriol diameter, Nigella sativa, preeclampsia

Introduction

Preeclampsia is a disorder diagnosed by an increase in blood pressure and proteinuria at twenty weeks or more of pregnancy [1]. The ischemic placenta produces soluble factors and cell debris into the bloodstream, causing systemic inflammation, together with maternal oxidative stress. This is believed to be the key factor causing endothelial dysfunction and the major symptoms leading to preeclampsia [2].

Healthy endothelial cells produce balanced amounts of endothelial derived relaxing factors (EDRFs) and endothelial-derived contracting factors (EDCFs) to support the cardiovascular system. Nitric oxide (NO) is an EDRF that induces relaxation of smooth muscles in blood vessels through guanylyl cyclase/cyclic guanosine monophosphate (sGC/cGMP). This smooth muscle relaxation causes the dilatation of blood vessels that suppresses vascular resistance and optimizes tissue perfusion, including in arteriol [3].

NO production is decreased in preeclampsia, which leads to vasoconstriction, an increase of vascular resistance, and an elevation of blood pressure [4-8]. Moreover, the vasoconstriction in renal arterioles decreases the renal blood flow.
and glomerulus filtration rate. Thus, this causes a decline in kidney performance and contributes to the increases in systemic vascular resistance and blood pressure [9, 10].

*N. sativa* (black cumin) has long been used as a medication, especially in the Middle East and India. Several studies have shown that the active compounds in *N. sativa* have anti-diabetic, anti-tumor, anti-hypercholesterolemia, anti-hypertension, anti-inflammation, and digestive protection activities [11]. In particular, thymoquinone can act as anti-inflammation agents by inhibiting NF-kB protein [12, 13]. Thymoquinone also has antioxidant activity [12, 14].

Here, we investigate the potential of *N. sativa* ethanol extract to increase NO levels as the marker of endothelial cell dysfunction. At last, it can induce the dilatation of renal arteriole in preeclampsia mice model.

**Materials and Methods**

Preeclampsia induction was conducted by injection of 0.1 ml of serum taken from severe preeclampsia patients on day 10 and 11 of gestation. Thirty mice were divided into six groups: a negative control group (normal pregnant mice), a positive control group (preeclampsia model mice), and four treatment groups.

Mice in the treatment groups were given *N. sativa* doses of either 500, 1000, 1500...
or 2000 mg/BW that previously had preeclampsia induction. Because of being an experimental animal study, no other informed consent was obtained.

The *N. sativa* seed extraction was done following published methods, with only minor modifications [15, 16]. The extract doses were based on those used by Meziti [13]. The extract was given orally from 15 days of pregnancy until 19 days of pregnancy. Following extract treatment, the NO levels in the serum of the mice were measured using a Colorimetric Griess device (RnD Systems, Minneapolis, USA). Next, the diameter of the renal artery was measured by hematoxylin-eosin staining of renal tissue.

**Statistical Analysis**

Statistical Package for the Social Sciences 16 for Windows (SPSS Inc.; Chicago, IL, USA) as statistical program application was used for data analyses. One-Way Analysis of variance (ANOVA) and Least Significance Difference (LSD) was used to the significant differences among the data for each mice groups. P values that less than 0.05 (p < 0.05) were accepted as statistically significant.

**Results**

The NO levels in the normal pregnant mice (negative control group) were significantly higher than in the preeclampsia model. The renal arteriol diameter
was also larger in the normal pregnant mice than in the preeclampsia model (Table 1).

One-Way ANOVA identified significant differences in the NO levels across the six groups (p < 0.001). The four N. sativa treated groups had higher NO levels than the preeclampsia model (85.77±4.47 µm [500 mg/BW], 189.04±6.01 µm [1000 mg/BW], 226.56±2.13 µm [1500 mg/BW] and 207.98±4.74 µm [2000 mg/BW] vs. 70.67 ± 4.86 µm [preeclampsia model]). NO levels tended to increase with increasing N. sativa dose (Fig. 1).

**Figure 1.** The effect of N. sativa ethanol extract on NO level of preeclampsia mice model

One-Way ANOVA identified significant differences in renal arteriol diameter across the six groups (p < 0.001). Moreover, the LSD test found that the renal arteriol diameter of the preeclampsia model (8.59±1.21 µm) was significantly smaller than those of mice treated with N. sativa ethanol extract (1000 mg/BW, 1500 mg/BW, and 2000 mg/BW (15.15±2.21 µm, 16.35±2.52 µm, 15.76±3.03 µm respectively). These data show that the N. sativa ethanol extract causes the renal arteriol to dilate, up to a dose of 1500 mg/BW (Fig. 2).

**Figure 2.** The effect of N. sativa ethanol extract on renal arteriol diameter of preeclampsia mice model

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Discussion

Preeclampsia induction in mice can be monitored by following the progress of key symptoms, such as increase of blood pressure, proteinuria, the increase of placental secretion (sEng), kidney abnormality, and renal arteriole vasoconstriction [17]. Angiotensin II type I receptor agonistic autoantibodies (AT1-AA) is an inducer of ROS production through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. An AT1-AA-containing serum was injected into pregnant mice [18, 19]. Moreover, this serum also contains higher TNF-α that can further induce NF-κB [20, 21]. Along with ROS, NF-κB as the transcription factor activates HIF1-α, which then leads to the increase of Soluble FMS-like tyrosine kinase 1 (sFLT 1) expression in placenta [2, 23, 24]. AT1-AA is an angiogenic factor that enters the maternal bloodstream and binds vascular endothelial growth factor (VEGF). Under these conditions, VEGF levels decrease causing endothelial cell damage due to the inability of VEGFR to bind VEGF [25]. VEGF levels are also related to the activation of endothelial NOS, also known as nitric oxide synthase (eNOS), which induces NO synthesis [26]. Thus, the reduced VEGF levels lead to a decrease in NO synthesis [27]. Furthermore, NO easily reacts with superoxide, forming peroxynitrite and reduces NO bioavailability [28, 29].

NO is an EDRF that induces the dilatation of smooth muscles in blood vessels. This activates the soluble guanylyl cyclase (sGC) enzyme, which catalyzes the
formation of cyclic guanosine 3′,5′-monophosphate (cGMP) from guanosine 5′-triphosphate (GTP) [30]. Moreover, NO/sGC/cGMP acts as an intracellular signal and leads to the activation of several effector molecules, ultimately resulting in vasodilatation and vascular resistance [31]. Thus, this can account for the lower level of NO and larger renal arteriole diameter we detected in preeclampsia mice compared to the control group [31-35].

We suspect that thymoquinone, which scavenges superoxide, is the principal active compound responsible for the changes in NO levels and renal arteriole diameter observed here [36-37]. Thymoquinone is also known to act as an anti-inflammation agent by inhibiting NF-kB in a dosage-dependent manner [13, 38, 39, 40].

Thymoquinone, as the inhibitor of NF-kB, causes a decrease in HIF1-α activation in cases of preeclampsia. Also, thymoquinone has an important role in the sFLT-1 synthesis, which leads to more VEGF to binds its receptor on endothelial cells. This increases the activation of the eNOS cascade and NO synthesis in endothelial cells.

Previous studies have shown that N. sativa extract can prevent tissue damage caused by ROS. The scavenger effect of thymoquinone and its derivates have
been investigated on several ROS and shown to have strong antioxidant activity. Thymol scavenger effect is related to singlet oxygen, while both thymoquinone and dithymoquinone have the similar activity to superoxide dismutase (SOD) [14]. Therefore, treatment of *N. sativa* prevents NO from interacting with superoxide, so that the product peroxynitrite is prevented from causing harm to the endothelial cells. Otherwise, *N. sativa* might inhibit several different targets, including calcium channels, inositol triphosphate (IP3), and intracellular Ca²⁺ release [41]. *N. sativa* induces smooth muscle relaxation by blocking voltage-operated Ca²⁺ channels (VOCC), which leads to the dilatation of blood vessels [42].

The main limitation of this study is that the result just focus on the effect of *N. sativa* ethanol extract for NO levels and renal arteriole diameters of a preeclampsia mouse model dependent of the dose that we used. Because is it *in vivo* study, we couldn’t find the real mechanism how *N. sativa* ethanol extract can increases NO levels and enlarges the renal arteriole diameter exactly. Besides we din’t test the chemical substances or chemical compound of our extracts so that we couldn’t estimate what is the chemical compound that has a significant role that involved in the mechanism exactly.

**Conclusion**

Based on the study, *N. sativa* ethanol extract treatment increases NO levels and enlarges the renal arteriole diameter of a preeclampsia mouse model in a dose-
dependent manner. Actually the NO levels and the renal arteriol diameters on preeclampsia mouse model (without *N. sativa* ethanol extract treatment) was smaller than other mouse groups.

**References**


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Table 1. The comparison of NO level and the diameter of renal arteriole between positive and negative control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Negative control (Healthy mice)</th>
<th>Positive control (preeclampsia mice)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO level (µM)</td>
<td>218.05±3.28</td>
<td>70.67±4.86</td>
<td>0.000&lt;∞</td>
</tr>
<tr>
<td>Arteriol diameter (µm)</td>
<td>20.73±4.09</td>
<td>8.59±1.21</td>
<td>0.000&lt;∞</td>
</tr>
</tbody>
</table>

Note: If p-value<α=0.05 means that the data are significantly different. If p-value>±0.05 indicates that the data are not significantly different.