



ORIGINAL ARTICLE

The Effect of Thymoquinone, the main Constituent of *Nigella sativa*, on Endothelin level of Ovalbumin Sensitized Guinea Pigs

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ABSTRACT

Previous studies showed that thymoquinone, the main constituent of *Nigella sativa*, has bronchodilatory effects. So for determining its effect on endothelin 1 level, this study was proposed. Forty guinea pigs of both sexes were randomly divided to control (group C), animal model of asthma (group S) and sensitized groups drinking water containing 20 μ M thymoquinone (group S+LTQ) and 40 μ M thymoquinone (group S+HTQ). The mean value of endothelin in bronchoalveolar lavage and lung tissue of sensitized group was significantly higher than in group C ($p < 0.001$ to $p < 0.01$). The mean value of endothelin in bronchoalveolar lavage in pre-treated group with low and high concentrations of thymoquinone were significantly lower than S group ($p < 0.05$). The mean value of endothelin in lung tissue of S+LTQ group was significantly lower than S group ($p < 0.05$). There was not any significant difference between two pretreated groups. It confirmed thymoquinone has reducing effect on endothelin level in both bronchoalveolar lavage and lung tissue in asthmatic guinea pigs.

Key words: Thymoquinone, *Nigella sativa*, asthma, endothelin, guinea pig, bronchoalveolar lavage, lung tissue

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INTRODUCTION

Asthma is an allergic disease and its main pathophysiology is an inflammation and airway hyperresponsiveness. Many of inflammatory factors are important in development of this inflammation [1]. Many researches are being done about endothelin1 - a member of 21 amino acid peptides family, as an important inflammatory mediator [2] in pulmonary disease including asthma [1] and pulmonary fibrosis [3]. So finding some factors that reduce endothelin level can be helpful in controlling progression of disease [4]. Various medications are being used to treat asthma but nowadays the use of plants in pharmaceutical industry has been increased because medicinal plants are safer. One of these plants in traditional medicine is *Nigella sativa* [5] which grows in cold climate area. Its small black seeds contain thymoquinone and several other monotropens [6]. Its therapeutic effects have been demonstrated in both human and animal researches. Previous studies showed that it has positive effect in treatment of rat sepsis model [7] and allergic rhinitis in children [8]. Another study demonstrated that this plant has prophylactic effect on asthmatic patients [9]. In addition, its anti-inflammatory [10] and relaxant effect on guinea pig tracheal chain has been investigated [11].

As we mentioned at the beginning, increased level of endothelin is one of the important pathophysiologic mechanisms of asthma. This fact has been confirmed in another research we've done in which reducing effect of *Nigella sativa* on lung endothelin level of ovalbumin sensitized guinea pig were shown [12]. But the main constituent was not clear. For detecting the exact component of this extract on reducing the endothelin level, this study has been proposed.

MATERIALS AND METHODS

Sensitization

On the first day, 100 mg of ovalbumin (sigma-AldrichCo., Germany) was injected intraperitoneally and another 100 mg was injected subcutaneously. In 8th day, 10 mg ovalbumin was injected intraperitoneally

too. Since day 14, sensitized animals were exposed to an aerosol of 4% Ovalbumin, administered in a closed Plexiglas chamber with dimensions 20×30×20 cm, for 4 minutes daily for 18±1 days. These animals were used for experiments 1 day after the last exposure to Ovalbumin [13].

Animals and groups

Forty guinea pigs (both sexes) with weight 400 -700 grams were randomly divided to 4 groups:

- 1) Drinking water alone (group C, treated as the same as sensitized group but normal saline was used instead of ovalbumin)
- 2) Drinking water alone (group S, an animal model of asthma)
- 3) Drinking water containing 20 μM (0.0033 g/dL) thymoquinone (Aldrich Chemical Co., Germany) (group S+LTQ).
- 4) Drinking water containing 40 μM (0.0066 g /dL) thymoquinone (group S+HTQ)

Lung Lavage Endothelin measurement

One day after completion of asthma induction, animals were killed and the canola was inserted into the trachea and through which the lungs were lavaged with 5 ml of saline for 4 times (total: 20 ml). The lung lavage fluid was centrifuged at 2500×g at 4°C for 10 min. The supernatant was kept in -70 °C until the endothelin level was measured by means of special ET-1 Elisa kit (Glory Science Co., USA) [12].

Lung tissue Endothelin measurement

After finishing the lung lavage, the lungs were removed and immediately homogenized to measure ET-1 (homogenized solution: 20 mmol/L hydrochloric acid +1 mol/L acetic acid). The homogenized solution was centrifuged at 3000×g at 6°C for 5 min and the light supernatant was taken and stored at -70 °C until analyzes. Tissue ET-1 was measured using a special kit (Glory Science Co., USA) after lyophilizing with lyophilizator (Christ Aplhal 4) [12].

RESULTS AND DISCUSSION

Bronchoalveolar lavage endothelin level

The mean value of endothelin in bronchoalveolar lavage of sensitized group (36.23±2.17 ng/L) was significantly higher than in group C (18.81±1.58 ng/L, $p<0.001$). The mean value of endothelin in bronchoalveolar lavage in pre-treated group with low and high concentrations of thymoquinone was significantly lower than S group (22.82±0.83 and 24.72±1.35 ng/L respectively, $p<0.001$ for S+LTQ and $p<0.01$ for S+HTQ). However, the mean value of this parameter in these groups was still significantly higher than group C ($p<0.05$ for both). There was not any significant difference between two pretreated groups (Fig. 1).

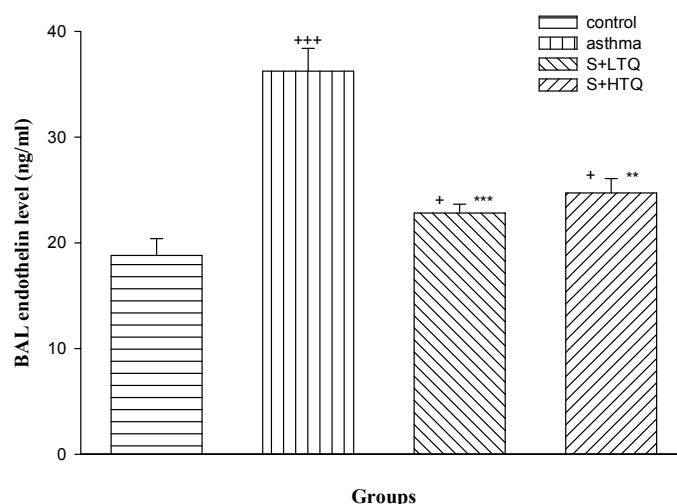


Figure 1: The mean value of BAL endothelin level (ng/L) in control (C), sensitized (S) and sensitized guinea pigs treated with low dose (S+LTQ) and high dose (S+HTQ) of thymoquinone (for each group, n=6). Statistical differences between control and different groups: +; $p<0.05$, +++; $p<0.001$. Statistical differences between S+LTQ and S+HTQ vs. sensitized group: **; $p<0.01$, ***; $p<0.001$.

Lung tissue endothelin level

The mean value of endothelin in lung tissue of sensitized group (23.96±2.79 ng/L) was significantly higher than in group C (13.08±0.98 ng/L, $p<0.01$). The mean value of lung tissue endothelin in pre-treated group with low concentration of thymoquinone was significantly lower than S group (15.81±1.79 ng/L,

$p < 0.05$). The mean value of this parameter in S+HTQ group (19.62 ± 1.37 ng/L) was still significantly higher than group C ($p < 0.01$). Again there was not any significant difference between two pretreated groups (Fig. 2).

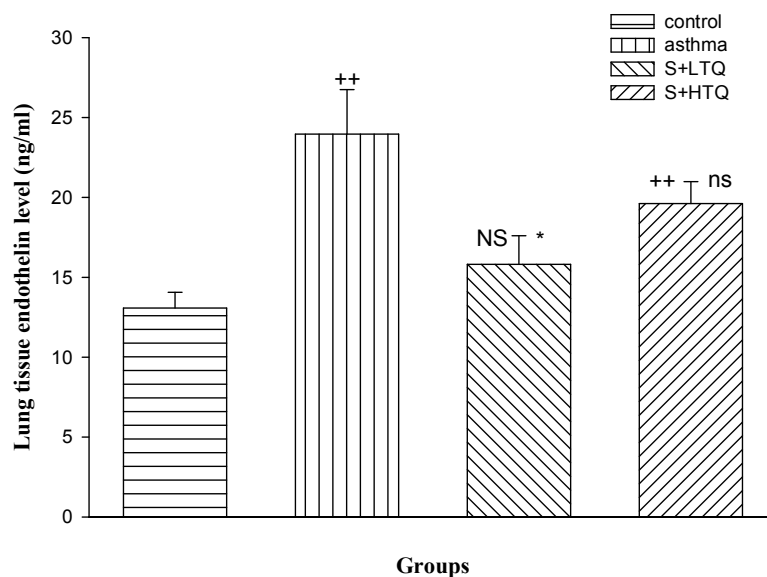


Figure 2: The mean value of lung tissue endothelin level (ng/L) in control (C), sensitized (S) and sensitized guinea pigs treated with low dose (S+LTQ) and high dose (S+HTQ) of thymoquinone (for each group, $n=6$). Statistical differences between control and different groups: NS; no significant difference, ++; $p < 0.01$. Statistical differences between S+LTQ and S+HTQ vs. sensitized group: ns; no significant difference, *: $p < 0.05$.

In the present study we investigated effect of two different concentrations of main component of *Nigella sativa* extract, thymoquinone, on both bronchoalveolar lavage and lung tissue of ovalbumin sensitized guinea pigs. Our findings showed that administration of this component has reducing effect on endothelin level in bronchoalveolar lavage and lung tissue.

As we noted above, there was significant differences in endothelin levels between ovalbumin sensitized and control group. It shows that injection and inhalation of ovalbumin caused sensitization. Endothelin measurement in bronchoalveolar lavage after treating animals with different doses of thymoquinone demonstrated that both concentrations of thymoquinone decrease endothelin level in bronchoalveolar lavage although it could not diminish it to the normal range. Lack of significant differences in endothelin levels between two treated concentrations suggested that the low dose of thymoquinone could effect as the same as higher concentration.

Moreover, the administration of low concentration of thymoquinone could lower the level of endothelin in lung tissue, however high concentration of this drug didn't have significant effect on endothelin level in comparison to sensitized group. Its cause is not clear.

These results demonstrated that thymoquinone, the main constituent of *Nigella sativa*, has reducing effect on oxidative agents like endothelin. This fact was conforming to the results of the study of Farah and his colleagues in 2005. In this study they showed the antioxidant effect of thymoquinone on cellular metabolism of A549 cells [14]. In addition, last studies showed that thymoquinone have dilatory effect on bronchial muscles by reducing bronchoconstrictors agents level [11, 15]. According to the findings of Boskabady and his colleagues in 2007, *Nigella sativa* and its components have prophylactic effect on human asthmatic attacks [9]. In our previous study we had shown that *Nigella sativa* extract could reduce the endothelin level in bronchoalveolar lavage and lung tissue which supports these new findings [12]. So thymoquinone can be useful for patients who have asthma and can lessen the severity of their disease although more studies are required.

In conclusion, thymoquinone, the main constituent of *Nigella sativa*, could prevent asthma by reduction of endothelin level in bronchoalveolar lavage and lung tissue.

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