Antitussive Effect of Thymoquinone, a Constituent of *Nigella Sativa* Seeds, in Guinea Pigs

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ABSTRACT

The antitussive activity of thymoquinone, a constituent of *Nigella sativa* seeds, was evaluated using the nebulized solution of citric acid 20 % in guinea pigs. Thymoquinone and codeine were injected intraperitoneally. Thymoquinone (20 and 40 mg/kg) and codeine (5 mg/kg), a prototype antitussive agent, reduced the number of cough in animals. The antitussive effect of these agents was antagonized by pretreatment with naloxone (2 mg/kg), an opioid receptor antagonist. These results suggest that thymoquinone has antitussive activity probably through opioid receptors.

Keywords: Thymoquinone, *Nigella sativa*, Antitussive, Anticough, Cough, Opioid receptors, Codeine

INTRODUCTION

The seeds of *Nigella sativa* Linn. (Ranunculaceae), commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhoea and dyslipidaemia (1). The oil and seed constituents, in particular thymoquinine, have shown potential medicinal properties in traditional medicine (2). The black cumin or *N. sativa* L. seeds have many acclaimed medicinal properties such as bronchodilatory, hypotensive, antifungal, analgesic, anti-inflammatory and immunopotentiating (3), antioxidant (4), antibacterial (5).

N. sativa seeds contain many components, but the major ones were thymoquinone (27.8%–57.0%), ρ-cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%) (1).

Thymoquinone is a pharmacologically active quinone, which possesses several properties including analgesic and anti-inflammatory actions (6-7); protective effect on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus (8) and renal ischemia-reperfusion-induced oxidative damage in rats (9), anticonvulsant (10-11), antineoplastic (12-14), and the inhibition of eicosanoids generation (7).

Thymoquinone demonstrated the anti-inflammatory effect in experimental asthma (15). The different extracts of *N. sativa* showed antitussive activity in guinea pigs (16). Thus, in this study the antitussive activity of thymoquinone was evaluated evaluated using the nebulized solution of citric acid in guinea pigs.

METHODOLOGY

Animals

Male and female guinea pigs (500-900 g) were obtained from Pasteur Institute of Iran, Tehran and maintained in animal house of School of Pharmacy,

Mashhad University of Medical Sciences. Animals were housed in a colony room with a 12/12 hour light/dark cycle at 24 ± 1 °C. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee acts.

Experimental protocol

Male and female guinea-pigs, five in each experimental group, were placed in a small Perspex box (20× 20× 40 cm) and exposed for 10 min to an aerosol of irritant agent, citric acid. The aerosol was produced by air compressed at a pressure of about 500 mmHg through a nebulizer containing 10 ml of 20% citric acid. The frequency of cough during this 10 minutes period was recorded. The extracts or agents were given intraperitoneally 30 min prior to the initiation of test (16-17).

Drugs

Codeine phosphate and naloxone were purchased from Temad Pharmaceutical Co. and Tolid Darou, respectively.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer *post-hoc* test for multiple comparisons. The p-values less than 0.05 were considered to be statistically significant.

RESULTS

As expected codeine (5 mg/kg) reduced citric acid-induced coughs (Figure 1). Thymoquinone in doses of 20 and 40 mg/kg also inhibited cough number dose dependently (P<0.001). The antitussive effects of both codeine and thymoquinone were antagonized by pretreatment with naloxone (2 mg/kg) in guinea pigs (Figures 2, 3 and 4).

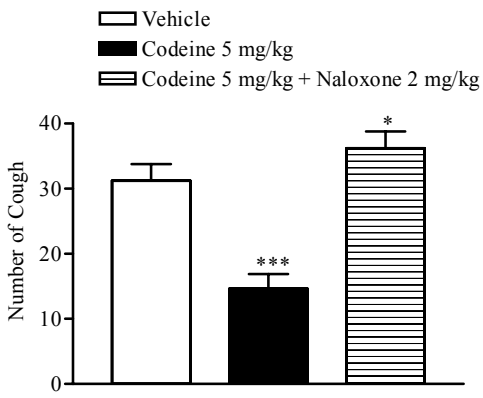

Figure 1. Antitussive effect of codeine and the pretreatment action of naloxone (2 mg/kg) in guinea pigs. Data was reported as Mean + SEM of effects of agents on 5 guinea pigs cough number, 30 min after injections.*P<0.05, ***P<0.001 *vs* Vehicle, one-way ANOVA followed by Tukey-Kramer post-hoc test.

DISCUSSION

In this study thymoquinone showed antitussive activity dose dependently which this activity was antagonized by naloxone.

The guinea pig provides a good model of the human cough reflex; this has been confirmed by a study showing the similarity in response to both citric acid and capsaicin in human and guinea pig (18). It was shown that cough produced by citric acid inhalation may be mediated, at least in part, by generation of kinins; secondary to this, a release of prostanoids also appears to participate in the response (19). Thymoquinone showed an anti-inflammatory effect during the allergic response in the lung through the inhibition of PGD2 synthesis and Th2-driven immune response (20). Thus, it is possible the anti-inflammatory effect of thymoquinone is involved in antitussive activity of this agent.

Airway acidification induces acute bronchoconstriction mainly because of the release of tachykinins after activation of sensory nerves. Pretreatment with high doses of capsaicin and with an NK2 receptor antagonist abolished and reduced,

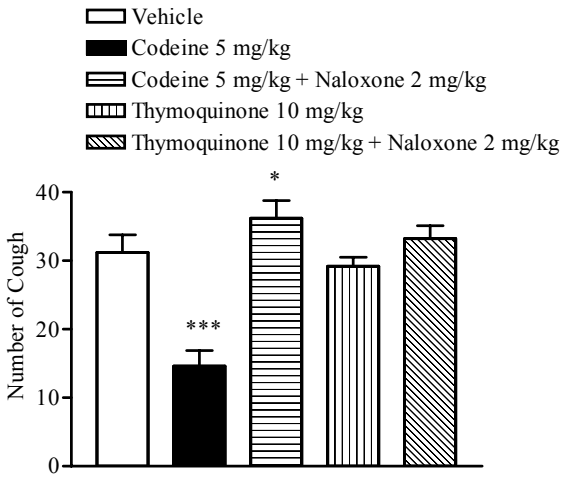

Figure 2. Antitussive effect of thymoquinone and the pretreatment action of naloxone (2 mg/kg) in guinea pigs. Data was reported as Mean + SEM of effects of thymoquinone (10 mg/kg) on 5 guinea pigs cough number, 30 min after injections. *P<0.05, ***P<0.001 *vs* Vehicle, one-way ANOVA followed by Tukey-Kramer post-hoc test.

respectively, citric acid–induced bronchoconstriction. Tachykinins and bradykinin, released by airway acidification, could also modulate citric acid–induced bronchoconstriction by their ability to subsequently release the epithelially derived bronchoprotective nitric oxide (NO). Thus, bronchoconstriction induced by citric acid inhalation in the guinea pig, mainly caused by the tachykinin NK2 receptor, is counteracted by bronchoprotective NO after activation of bradykinin B2 and tachykinin NK1 receptors in airway epithelium (21). Thymoquinone induced relaxation of precontracted tracheal preparation is probably mediated, at least in part, by inhibition of lipoxygenase products of arachidonic acid metabolism and possibly by non-selective blocking of the histamine and serotonin receptors. This relaxant effect of thymoquinone, further support the traditional use of black seeds either alone or in combination with honey to treat bronchial asthma (22). Nigellone and high concentrations of thymoquinone had a concentration-dependent inhibitory effect on the trachea when being contracted by the depolarizing effect of Ba2+. The trachea contractions induced by leukotriene-d4 were inhibited by nigellone and by thymoquinone (23). Thymoquinone possesses inhibitory effects on contractility of guinea pig isolated ileum, and that effects may be responsible for the smooth muscle relaxant activity of *N. sativa* seeds. The mechanism by which thymoquinone relaxes ileum contractility was exerted, at least in part, through an antagonistic activity on calcium channels in guinea pig ileum smooth muscle cells (24). Thymoquinone induced relaxation of precontracted tracheal preparation is probably mediated, at least in part, by inhibition of lipoxygenase products of arachidonic acid metabolism and possibly by non-selective blocking of the histamine and serotonin receptors. This relaxant effect of thymoquinone, support its effect to treat bronchial asthma (22) and may reduce cough following broncoconstriction.

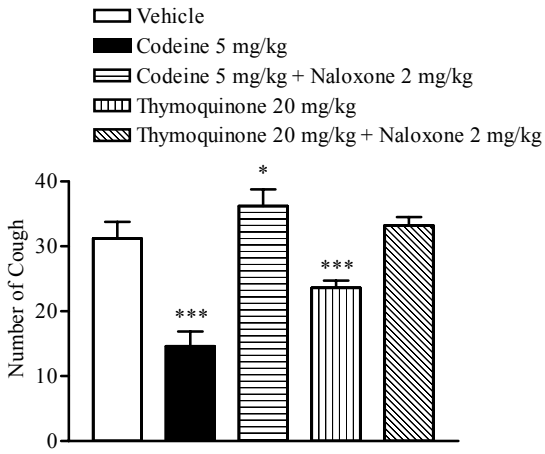

Figure 3. Antitussive effect of thymoquinone and the pretreatment action of naloxone (2 mg/kg) in guinea pigs. Data was reported as Mean + SEM of effects of thymoquinone (20 mg/kg) on 5 guinea pigs cough number, 30 min after injections. *P<0.05, ***P<0.001 *vs* Vehicle, one-way ANOVA followed by Tukey-Kramer post-hoc test.

N. sativa oil and thymoquinone produce antinociceptive effects through indirect activation of the supraspinal μ_1 and kappa-opioid receptor subtypes in mice (6). In this study, thymoquinone

and codeine antitussive activities were antagonized effectively by naloxone. This implies that the main mechanism of action of thymoquinone is mediated by opioid receptors.

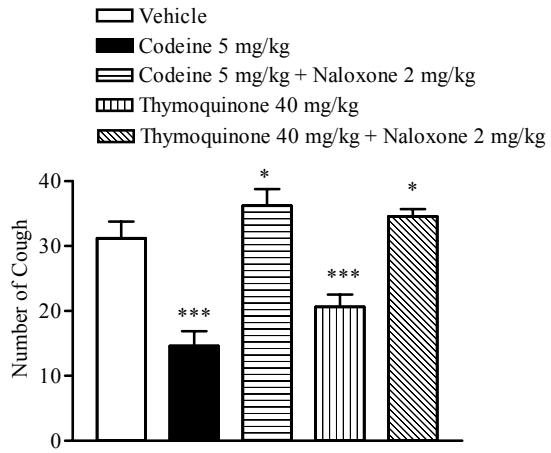

Figure 4. Antitussive effect of thymoquinone and the pretreatment action of naloxone (2 mg/kg) in guinea pigs. Data was reported as Mean + SEM of effects of thymoquinone (40 mg/kg) on 5 guinea pigs cough number, 30 min after injections. *P<0.05, ***P<0.001 *vs* Vehicle, one-way ANOVA followed by Tukey-Kramer post-hoc test.

This study showed that thymoquinone has antitussive activity and the mode of action may be done by anti-inflammatory, bronchodilatory and other effects. These effects may be mediated by opioid receptors.

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