

# Effects of Thymoquinone, the Major Constituent of *Nigella sativa* Seeds, on the Contractile Responses of Rat vas Deferens

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## Abstract

We have evaluated the effects of thymoquinone on smooth muscle contraction in the isolated rat epididymal vas deferens using tension recording technique. The contractile responses to norepinephrine (NE), KCl, and electrical field stimulation were recorded using an isometric transducer. Thymoquinone inhibited the contractile responses to exogenous NE (100  $\mu$ M) and KCl (80 mM) in a concentration-dependent manner. Moreover, thymoquinone reduced the amplitude of electrically-evoked contraction of vas deferens in a concentration-dependent manner. Cumulative addition concentrations of CaCl<sub>2</sub> (0.1–10 mM) to tissue bath failed to increase the amplitude of contractile responses to electrical field stimulation in the presence of thymoquinone (80  $\mu$ M). These results indicate that thymoquinone induced non-selective and concentration-dependent inhibition of contractile responses to NE, KCl, and electrical field stimulation. This action may be due to the ability of this alkaloid to interfere with the mobilization of Ca<sup>2+</sup> required for smooth muscle contraction.

**Keywords:** Thymoquinone, norepinephrine, electrical field stimulation, contraction, rat vas deferens.

## Introduction

*Nigella sativa* L. is a member of the Ranunculaceae family growing in countries bordering the Mediterranean Sea, Pakistan, India and Iran. For many centuries, *N. sativa* seeds (also called black seeds or black cumin) have been used as a food additive as well as for medicinal purposes in many countries (Jansen, 1981). This plant is one of the most exten-

sively studied, both phytochemically and pharmacologically (El-Sayed, 1998; Riaz et al., 1996; Siddiqui & Sharma, 1996; Worthen et al., 1998). Most properties of whole seeds or their extracts are mainly attributed to quinone constituents, of which thymoquinone is more abundant compound (Mahfouz et al., 1960; Filippo D'Antuono et al., 2002). Recently, a great deal of attention has been given to this pharmacologically active quinone. It has been shown that thymoquinone possesses several properties including analgesic and anti-inflammatory actions (Houghton et al., 1995; Abdel-Fattah et al., 2000), protection against chemical-induced carcinogenesis (Hassan & El-Dakhkhny, 1992; Worthen et al., 1998), the inhibition of eicosanoid generation and membrane lipid peroxidation (Houghton et al., 1995).

It has been previously shown that *N. sativa* seed extracts or essential oil inhibits the contractile responses of gastrointestinal (Aqel-Mahmood, 1993b; Gilani et al., 2001) and tracheal (Aqel-Mahmood, 1993a; Boskabadi & Shahabi, 1997; Gilani et al., 2001) smooth muscles. To our knowledge, no information is available concerned with the pharmacological effects of thymoquinone on the contractile responses of smooth muscle. The aim of the present study was to clarify whether the inhibitory effects of *N. sativa* seed extracts or essential oil on smooth muscles is related to its major active component, thymoquinone. Therefore, we investigated the effects of thymoquinone on the smooth muscle and determined its possible mechanism of action.

The isolated vas deferens has proven to be a useful preparation for a variety of pharmacological studies including characterization of the effects of novel compounds on neurotransmission in peripheral adrenergic pathway. The tissue is amenable to organ bath techniques in which the mechani-

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cal response of the smooth muscle can be recorded as a result of drug application or stimulation of the excitatory nerves of this densely innervated preparation. Thus, we studied the effects of thymoquinone on the contractile responses of the rat vas deferens induced by exogenous norepinephrine, potassium chloride, and electrical field stimulation. We further investigated the effect of thymoquinone on the electrically-induced contractile responses of vas deferens in calcium-free medium in order to clarify the role of this quinonic compound in the mobilization of extracellular  $\text{Ca}^{2+}$  required for muscular contraction. Overall, it was of interest to verify whether thymoquinone had a therapeutical potential, which could help to develop a new drug.

## Materials and methods

### Isolated rat vas deferens

Male Sprague-Dawley rats weighing 200–300 g purchased from Razi Institute (Mashhad, Iran), were used in all experiments. They were killed by a blow on the head and then vasa deferentia were dissected out. The tissues were cleaned of vascular and connective tissues while being kept in Krebs solution gassed with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Segments (2 cm in length) of the epididymal portions were then suspended vertically in 50 ml organ bath for isometric recording of mechanical activity. An initial load of 0.5 g was applied to the tissue, which was then allowed to equilibrate for at least 60 min, with the Krebs solution being changed every 15 min.

### Drugs and solutions

Norepinephrine (NE) and thymoquinone were purchased from Sigma-Aldrich Chemical Co. All other chemicals were obtained from Merck. The drugs were dissolved in distilled water. Thymoquinone was suspended in 0.8% (v/v) Tween 80. All compounds were prepared freshly each time and were applied so that the total volume of application did not exceed 0.5 ml. The composition of the Krebs solution was (mM): NaCl 118.4, KCl 4.7,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.4,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{CaCl}_2$  2.5,  $\text{NaHCO}_3$  25 and glucose 11.1. In some experiments we used calcium-free Krebs solution with the following composition (mM): NaCl 118.4, KCl 4.7,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.4,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{NaHCO}_3$  25 and glucose 11.1.

### Contractile response of vas deferens to NE

The effect of thymoquinone on the contractile response of NE was evaluated in vas deferens. The tissues were placed in a 50 ml organ bath and incubated in Krebs solution during 60 min at 37 °C, gassed with 5%  $\text{CO}_2$  in  $\text{O}_2$ . The initial muscle tension was 0.5 g, recorded with an isometric transducer coupled to a physiograph. Concentration-dependent contractions were constructed for NE alone, or in the presence of thymoquinone. Thymoquinone was added to the bath 30 min before the agonist.

### Contractile response of vas deferens to electrical field stimulation

Electrical field stimulation was repetitively delivered to the preparations by a Grass S88 stimulator via a ring electrode every 7 s with trains of 7.5 pulses, duration of 0.3 ms, and a voltage of 130 V, which produced a maximal twitch. Thymoquinone was added cumulatively to the bath to induce concentration-dependent inhibition of the evoked contractions. The responses were calculated as a percentage of the contraction of preparations in Krebs's solution evoked by electrical field stimulation.

### Contractile response of vas deferens to KCl

In this experiment, high doses of KCl (20, 40, 80 mM) were used to depolarize the tissue, which produced a typical contraction. Thymoquinone (20, 40, 80  $\mu\text{M}$ ) was added 30 min before KCl to obtain concentration-dependent inhibitory responses. The contraction of the vas deferens with KCl (80 mM), pretreated with thymoquinone was expressed as percentage of control KCl-induced contractions.

### Contractile response of vas deferens induced by $\text{CaCl}_2$ in calcium-free medium

The vasa deferentia were placed in organ bath and first preincubated during 60 min in  $\text{Ca}^{2+}$ -free Krebs's solution at 37 °C, gassed with 5%  $\text{CO}_2$  in  $\text{O}_2$ . The initial muscle tension was 0.5 g. Afterwards, cumulative doses of  $\text{CaCl}_2$  (0.1, 0.2, 0.4, 1, 2, 10 mM) were added alone or in the presence of 80  $\mu\text{M}$  thymoquinone.

### Statistical analysis

The data are expressed as mean values  $\pm$  S.E.M. and tested with analysis of variance (ANOVA) followed by the multiple comparison test of Tukey-Kramer. A *P* value less than 0.05 was considered statistically significant.

## Results

### Effect of thymoquinone on NE-induced contractile responses

Figures 1 and 2 show the contractile responses elicited by NE. The contractions were done in Krebs solution using concentrations of 10 (Fig. 1) and 100 (Fig. 2)  $\mu\text{M}$  NE. The administration of thymoquinone (10, 20  $\mu\text{M}$ ) did not modify the contractile response. However, the administration of 40, 80, and 100  $\mu\text{M}$  thymoquinone produced a significant decrease in contraction induced with low concentration of NE (10  $\mu\text{M}$ ), compared with the control ( $P < 0.001$ ). The contractile response was less inhibited when the high concentration of NE (0.1 mM) was applied, so that 40  $\mu\text{M}$  thymoquinone did not have any significant effect on the

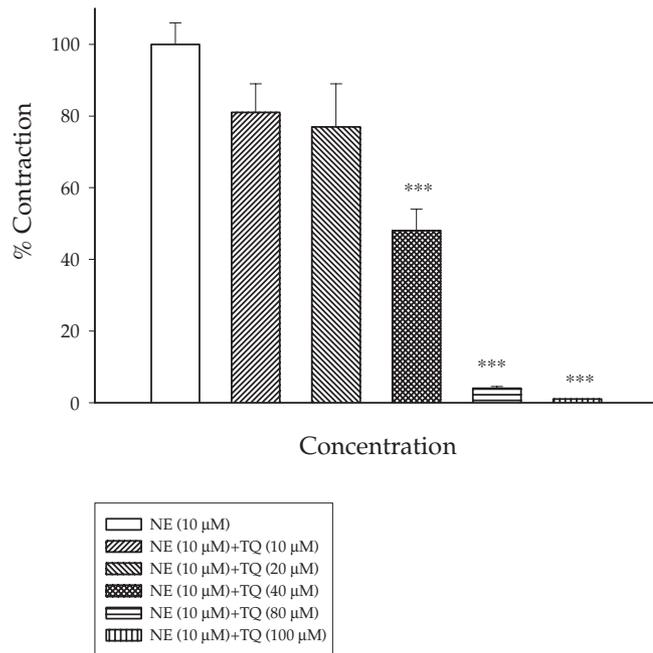


Figure 1. Effect of thymoquinone on NE (10 μM)-induced contractions in rat vas deferens. The values are given in mean ± S.E.M. for 4 experiments. \*\*\* $p < 0.001$ , as compared to control, Tukey-Kramer.

contractile response. Higher concentrations of thymoquinone (80, 100 μM) produced inhibitory effects on contraction induced by NE (0.1 mM) significantly ( $P < 0.01$ ).

### Effect of thymoquinone on the electrical field stimulation-evoked contractile responses

Electrical field stimulation caused a contraction in the vas deferens, which was significantly reduced by different concentrations of thymoquinone (Fig. 3). Incubation of the isolated vas deferens with different concentrations of thymoquinone more than 10 μM decreased the contractile response. This inhibitory effect was completely occurred when the concentration of 0.1 mM thymoquinone was applied. The inhibitory effect of thymoquinone was started after 5 min incubation and completed within 30 min. The recovery of tissue from the inhibitory effect of thymoquinone was observed 120 min after washing.

### Effect of thymoquinone on KCl-induced contractile responses

We used a high concentration of KCl (80 mM) to depolarize tissue and cause contractile response (Fig. 4). In this experiment, thymoquinone concentrations of 20–80 μM significantly blocked the contractions induced by KCl (80 mM) ( $P < 0.001$ ).

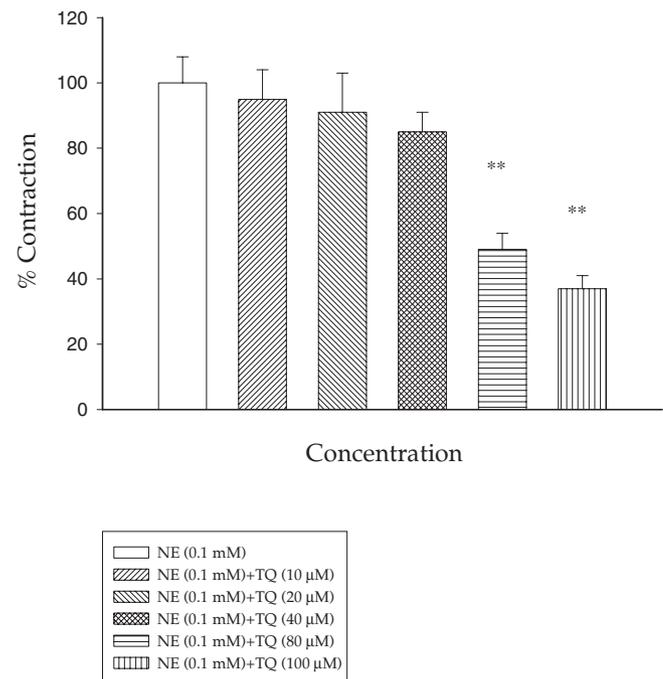


Figure 2. Effect of thymoquinone on the NE (100 μM)-induced contractions in rat vas deferens. The values are given in mean ± S.E.M. for 4 experiments. \*\* $p < 0.01$ , as compared to control, Tukey-Kramer.

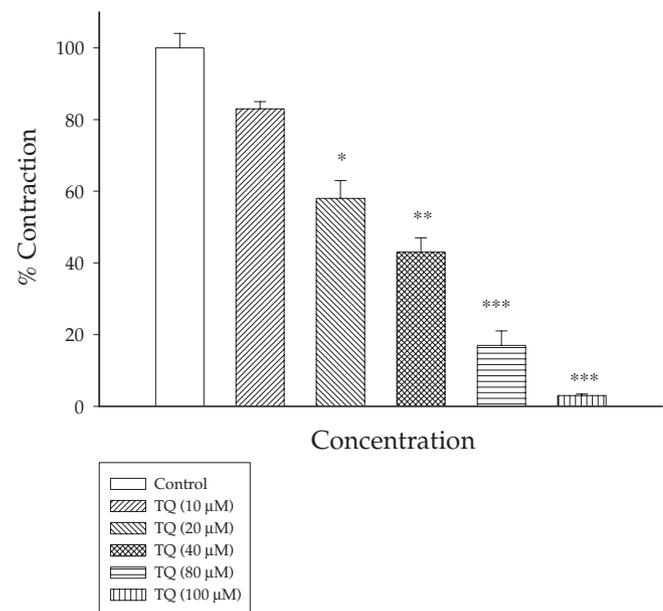


Figure 3. Decrease in contraction induced by electrical field stimulation in the rat vas deferens by thymoquinone. The values are given in mean ± S.E.M. for 4 experiments. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , as compared to control, Tukey-Kramer.

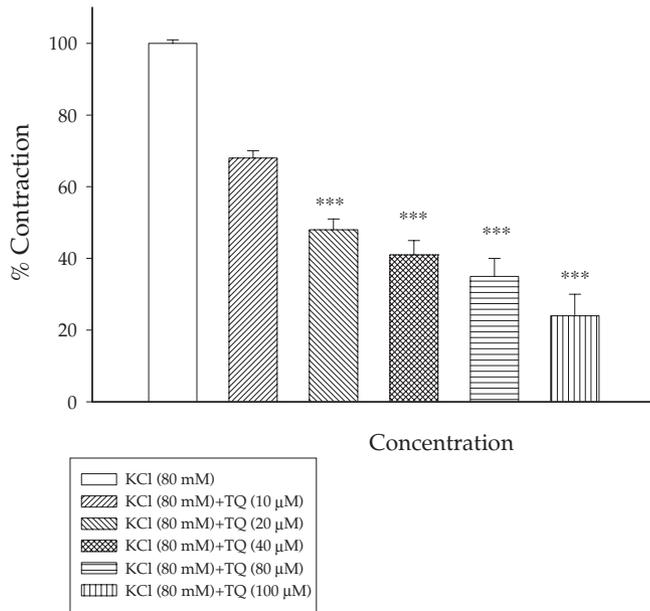


Figure 4. Effect of thymoquinone on the KCl (80 mM)-induced contraction in rat vas. The values are given in mean  $\pm$  S.E.M. for 4 experiments. \*\*\* $p < 0.001$ , as compared to control, Tukey-Kramer.

#### Effect of different concentrations of extracellular calcium on the electrically-induced contractions in calcium-free medium

After a 60 min incubation of tissue in  $\text{Ca}^{2+}$ -free Krebs solution, the contractions induced by electrical field stimulation were attenuated. In these conditions, the addition of increasing concentrations of  $\text{CaCl}_2$  (0.1 to 10 mM) to tissue bath reversed the activity of vas deferens and produced contractions in response to electrical field stimulation (Fig. 5). The maximum contractile response was obtained when 2 mM  $\text{CaCl}_2$  was applied.

#### Effect of thymoquinone on $\text{CaCl}_2$ -induced contractile responses in calcium-free medium

Figure 5 shows the inhibitory effects of thymoquinone on cumulative contractile response of  $\text{CaCl}_2$ . A significant decrease in the maximum contractile response of vas deferens with 80  $\mu\text{M}$  thymoquinone was observed, so that high doses of  $\text{CaCl}_2$  (2 and 10 mM) failed to induce contraction ( $p < 0.001$ ).

### Discussion

Contractions of the vas deferens to electrical field stimulation are mediated by a combination of noradrenaline and ATP released as cotransmitters from sympathetic nerves (Hoyle & Burnstock, 1991). In this tissue, responses to NE as well as those to KCl were significantly reduced by thymoquinone. On the basis of these data, it can be suggested that thymo-

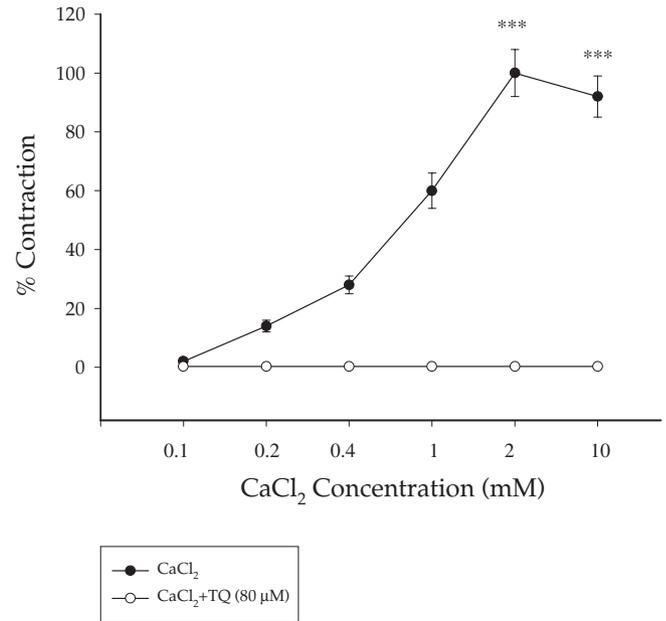


Figure 5. Effect of thymoquinone on  $\text{CaCl}_2$ -induced contractile responses in calcium-free medium. The values are given in mean  $\pm$  S.E.M. for 4 experiments. \*\*\* $p < 0.001$ , as compared to control, Tukey-Kramer.

quinone may have an antagonistic effect on adrenergic receptors of rat isolated vas deferens (a point that needs to be elucidated further). Furthermore, contractile responses induced by electrical field stimulation were significantly reduced by thymoquinone in this preparation. Considering these results, it can be suggested that the inhibitory action of thymoquinone on contractile responses of isolated rat vas deferens induced by electrical field stimulation is a postsynaptic effect.

The cytoplasmic increase of intracellular calcium concentration is one of the most common and important mechanisms within smooth muscle cells and nerve terminals, which is required for muscle contraction and exocytotic neurotransmitter release (Bolton, 1979; Spedding & Paoletti, 1992). This increase of calcium concentration could occur by an increase in influx of this ion through receptor- and/or voltage-dependent calcium channels located in the plasma membrane of the cell or can be prepared by the intracellular sources of calcium (Bolton, 1979; Spedding & Paoletti, 1992).

The calcium influx induced by depolarizing stimuli takes place via voltage-dependent calcium channels (VDCCs) (Blaustein, 1979) in the nerve endings of the smooth muscle of the rat vas deferens as well as other smooth muscles (Triggle et al., 1979; Hay & Wadsworth, 1982; Khoyi et al., 1988). In the rat vas deferens, depolarization-induced NE release is a process mediated by calcium influx through VDCCs. Probably of the N-type (Tsien et al., 1988; Lipscombe et al., 1989). In order to further study the effect of thymoquinone on mobilization of extracellular calcium, we

used a condition in electrically evoked contractions of epididymal vas deferens. As explained previously, the contractions induced by electrical field stimulation were attenuated after 60 min incubation of tissue in  $\text{Ca}^{2+}$ -free Krebs solution, but the addition of increasing concentrations of  $\text{CaCl}_2$  to the tissue bath reversed the activity of vas deferens and produced contractions in response to electrical field stimulation (Fig. 5). On the other hand, preincubation of tissue with thymoquinone significantly decreased the maximum contractile response of vas deferens, so that the high doses of  $\text{CaCl}_2$  (2, 10 mM) failed to induce contraction. In order to understand whether this effect of thymoquinone is mediated through the blockade of  $\text{Ca}^{2+}$  influx, a high dose of KCl (80 mM) was used to depolarize the tissue. However, preincubation of vas deferens preparations with thymoquinone caused a concentration-dependent inhibition of the KCl-induced contraction. The contractions induced by high KCl are dependent upon entry of  $\text{Ca}^{2+}$  into the smooth muscle cells through VDCCs (Bolton, 1979), and a substance which inhibits KCl-induced contractions is, therefore, considered to be a calcium channel blocker (Godfraind et al., 1986). Thus, inhibition of high KCl (80 mM)-induced contraction of rat vas deferens by thymoquinone may reflect restricted  $\text{Ca}^{2+}$  entry via VDCCs. Therefore, the effect of thymoquinone can be best explained by interference with the mobilization of extracellular  $\text{Ca}^{2+}$  required for muscular contraction, which will tend to impair smooth muscle cell motor activity, although, the reduction of extracellular calcium concentration may affect both smooth muscle cell contraction and the calcium-dependent part of neurotransmitter release from neurons.

There is a large body of evidence suggesting the existence of VDCC of the N-type in adrenergic nerve endings of rat vas deferens (Orallo et al., 1992). Considering this evidence, presynaptic activity of thymoquinone is possible and requires further investigation.

In conclusion, the results of present study indicate that thymoquinone induces non-selective and concentration-dependent inhibition of contractile responses induced by NE, KCl and electrical field stimulation. This action may be due to the ability of this quinonic compound to interfere with the mobilization of extracellular  $\text{Ca}^{2+}$  required for muscular contraction.

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