



Therapeutic Effects of Thymoquinone in a Model of Neuropathic Pain[☆]

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ABSTRACT

Background: The goal of our study was to determine the therapeutic effects of thymoquinone in a dose-dependent manner in a model of neuropathic pain following an experimentally applied spinal cord injury (SCI).

Methods: Fifty female adult Wistar albino rats weighing between 220 and 260 g were included in the study and were divided into 5 groups as follows: Group S (sham), Group C (control), Group T100 (100 mg/kg thymoquinone), Group T200 (200 mg/kg thymoquinone), and Group T400 (400 mg/kg thymoquinone). To begin the experiment, SCI was applied to all groups (with the exception of the sham group) following a mechanical and heat–cold test. Two weeks later, the mechanical and heat–cold tests were repeated, and a single normal saline dose was given to the sham and control groups, whereas 3 varying doses of thymoquinone were given to the other groups. The mechanical and heat–cold tests were repeated at 30, 60, 120, and 180 minutes after receiving thymoquinone. Finally, the animals were put to death via the removal of intracardiac blood. The levels of nitric oxide, total oxidant status, total antioxidant status, paraoxonase, malondialdehyde, tumor necrosis factor- α , and interleukin-1 β were determined in all of the blood samples.

Results: The withdrawal threshold and withdrawal latency values recorded from the mechanical and heat–cold allodynia measurements for all 3 thymoquinone groups were higher than that of the control group at all time points (ie, 30, 60, 120, and 180 minutes). There were no differences in these results between the 3 thymoquinone groups. The paraoxonase and total antioxidant status serum levels of all 3 thymoquinone groups were higher than those of the control group, whereas total oxidant status, nitric oxide, malondialdehyde, interleukin-1 β , and tumor necrosis factor- α levels were lower in the 3 thymoquinone groups than in the control group.

Conclusions: Thymoquinone is beneficial for decreasing experimental neuropathic pain following SCI. However, increasing the dose does not change the effect.

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Introduction

Neuropathic pain affects millions of people worldwide.¹ It is a chronic type of pain that worsens as it continues, and can range from mild to extreme pain levels. It can develop following illnesses of the peripheral or central nervous systems, and can also develop as a result of an injury. Despite the many experimental and animal

studies on neuropathic pain, its pathophysiologic mechanisms have not been completely elucidated.

Pain that occurs due to a spinal cord injury (SCI) can diminish quality of life and hinder successful physical rehabilitation. Neuropathic pain is difficult to relieve. Many patients have failed to benefit from current medications, and therefore there is a need to develop new medications.²

Thymoquinone is extracted from the oil of *Nigella sativa*, and in recent studies it has been reported to be immunomodulatory,³ anti-inflammatory,⁴ antitumor,⁵ antidiabetic,^{6,7} and antinociceptive.⁸ However, the effects of thymoquinone have not yet been determined for neuropathic pain following SCI.

Generally, neuropathic pain is monitored with mechanical^{9,10} and heat–cold^{8,11} allodynia tests, although it can be detected via biochemical parameters as well.⁶

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Our study was conducted to determine the dose-dependent therapeutic effects of PO thymoquinone in a model of neuropathic pain.

Methods

Animals

Following approval from our ethics committee, 50 female adult Wistar albino rats weighing between 220 and 260 g with normal motor activity were selected from the Dicle University Health Research Center. The animals were kept under standard laboratory conditions (ie, exposed to light from 12:00 pm to 12:00 am at a room temperature of 20°C–22°C) during the duration of the experiment (2 weeks). The rats were fed ad libitum. Their bladders were emptied twice a day via manual compression. During the experiment, none of the animals developed infections or paralysis of the back legs following cord trauma, and none displayed dragging behaviors.

Experimental protocol

The rats were equally divided into 5 random groups: Group S: Sham + normal saline (NS), Group C: SCI + NS, Group T100: SCI + thymoquinone 100 mg/kg/PO, Group T200: SCI + thymoquinone 200 mg/kg/PO, and Group T400: SCI + thymoquinone 400 mg/kg/PO.

All groups were subjected to mechanical and heat–cold tests before the start of the experiment. With the exception of the sham group, SCI was applied to all of the animals. Two weeks later, the mechanical and heat–cold tests were repeated, and the sham and control groups were given a single dose of NS, whereas the other groups were given the appropriate dose of thymoquinone (Sigma catalog-274666; Cas No. 490-91-5, in sterile packaging) in NS by nasogastric feeding tube. Doses of thymoquinone were chosen according to the study by Abdel-Fattah et al.⁸ The mechanical and heat–cold hypersensitivity tests were repeated at 30, 60, 120, and 180 minutes after receiving NS or thymoquinone. After all animals were anesthetized (using 80 mg/kg ketamine), they were put to death after 180 minutes by removal of intracardiac blood and perfusion of NS with 10% formaldehyde.

SCI

Anesthesia (80 mg/kg ketamine) was administered intraperitoneally to all rats in a manner that ensured the preservation of spontaneous breathing. Using interscapular distance as a reference, a 3 × 2 cm area on each animal's back was shaved, and local antiseptic (povidone-iodine) was applied. Then, lidocaine was administered as local anesthetic, which passed the cutaneous and subcutaneous tissue at the T_{5–12} levels. While under anesthesia, the rats were placed in a facedown position and were monitored with a rectal heat probe to maintain a body temperature of 37°C. The paravertebral muscle fascia were pulled apart using lateral blunt dissection until the T_{6–7} lamina could be seen. A total laminectomy was carefully applied at a single level to prevent damage to the dura. A Yaşargil aneurysm clip (model FE 721 K; Aesculap AG and Co, Tuttlingen, Germany) for standard trauma was clipped around the dura and spinal cord between T_{6–7} for 1 minute with 63 g force.¹² Then, the clip was removed and following hemostasis the incision area was closed with 3-0 silk sutures appropriate for anatomic layers. The rats were then placed in their cages.

Tests

Mechanical test

The withdrawal threshold measurement, which is the most common method used to measure mechanical allodynia, was taken with an electronic von Frey device (Model EVF3; Bioseb, Chaville, France). For the test, every rat was placed on a mesh floor inside a transparent plastic cage. The rats were kept in a silent environment at room temperature for 20 minutes, and then force (in grams) was applied to the rats through the plantar hind paw up-down method.¹³ The upper pressure limit was 15 g, and the point at which each rat pulled its paw back was recorded. This test determined the mechanical excitability threshold.

Heat (8)–cold (44) test

Flicking of the hindlimb or jumping as the withdrawal latency measurement, which is the most commonly used method to measure heat–cold allodynia using a hot–cold plate analgesia meter (Cold Hot Plate Test; Bioseb, Chaville, France), was measured. The plate can be heated to 65°C or cooled to –3°C (± 0.1°C) in ambient room temperature (20°C–25°C). Each animal was placed in a Plexiglas cylinder (165 mm diameter × 25 mm height) with a drilled cover.¹¹ A temperature of 55°C (± 1°C) was used to determine heat hypersensitivity, and a cut-off time of 60 seconds was used to prevent tissue damage. A temperature of –3°C was chosen to determine cold hypersensitivity.^{14,15}

Biochemical analysis

Blood samples were immediately centrifuged at 4000 rpm (4°C for 10 minutes), transferred into Eppendorf tubes (Relassay, Gaziantep, Turkey), and were kept at –70°C until the end of the study, which was completed in 3 months. Total antioxidant status (TAS) and total oxidant status (TOS) were measured by Erel's methods,^{16,17} whereas malondialdehyde (MDA) content was measured using a spectrophotometer, as previously described.¹⁸ Serum paraoxonase (PON) levels were measured using a spectrophotometer with a modified Eckerson method. Initial rates of paraoxon hydrolysis (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co, London, United Kingdom) were determined by measuring liberated p-nitrophenol at 405 nm (37°C).¹⁹ Plasma nitric oxide (NO; Cayman Chemicals, Ann Arbor, Michigan), tumor necrosis factor-α (TNF-α; Bender MedSystems, Vienna, Austria), and interleukin-1β (IL-1β; Bender MedSystems, Vienna, Austria) levels were determined with an enzyme-linked immunosorbent assay method.

Statistical analysis

Statistical analyses were performed using SPSS for Windows 13.0 (IBM-SPSS Inc, Armonk, New York). Biochemical values are presented as mean values (SD). Differences between groups were compared with the nonparametric Kruskal-Wallis test. A Mann-Whitney *U* test was used for binary comparisons. The Bonferroni correction was applied, and *P*s < 0.01 were considered to be statistically significant.

Results

The withdrawal threshold and withdrawal latency values recorded from the mechanical and heat–cold allodynia measurements for all 3 thymoquinone groups were higher than that of the control group at all time points (ie, 30, 60, 120, and 180 minutes) (*P* < 0.01). There were no differences in these results between the 3 thymoquinone groups (ie, T100, T200, and T400) (Figures 1–3 and Table 1).

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