Original Article

Cukurova Anestezi ve Cerrahi Bilimler Dergisi

The Analgesic Effect of Thymoquinone in the Reserpine-Induced Experimental Fibromyalgia Model

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Abstract

Aim: Fibromyalgia is a chronic pain syndrome characterized by widespread pain and sensory hypersensitivity, for which effective and well-tolerated treatments remain limited. This study aimed to evaluate the analgesic effect of thymoquinone (TQ) in a reserpine-induced experimental fibromyalgia model in rats. **Methods:** Male Wistar rats were randomly allocated into three groups (n = 6 per group): control, reserpine, and reserpine + thymoquinone. Fibromyalgia-like pain was induced by subcutaneous administration of reserpine (1 mg/kg) once daily for three consecutive days. Thymoquinone (100 mg/kg) was administered orally four days after the final reserpine injection. Mechanical allodynia and thermal hyperalgesia were assessed using the von Frey filament test and the hot-plate test, respectively, at baseline and at 30, 60, 120, and 180 minutes following treatment. **Results:** Baseline mechanical and thermal nociceptive thresholds did not differ significantly among groups. Thymoquinone treatment resulted in a significant increase in mechanical withdrawal thresholds compared with both control and reserpine-only groups at 60, 120, and 180 minutes ($p < 0.01$), indicating a marked attenuation of reserpine-induced mechanical allodynia. No statistically significant differences were observed between groups in hot-plate latency times at any time point. **Conclusions:** Thymoquinone exerts a significant analgesic effect on mechanical allodynia in a reserpine-induced fibromyalgia model, suggesting its potential as a novel therapeutic agent for fibromyalgia-associated pain. Further studies are warranted to clarify its mechanism of action and modality-specific analgesic profile.

Keywords: Fibromyalgia; Thymoquinone; Reserpine; Mechanical allodynia; Experimental pain model

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1. Introduction

Fibromyalgia (FM) is a chronic pain disorder characterized by widespread musculoskeletal pain, fatigue, sleep disturbances, and cognitive impairments. Its prevalence is estimated to range from 2% to 8% globally, with a higher incidence among women[1]. The pathophysiology of FM remains incompletely understood, but evidence suggests a complex interplay of central sensitization, neuroinflammation, and dysregulation of neurotransmitter systems, including serotonin and catecholamines[2]. Experimental models, such as the reserpine-induced fibromyalgia model, have been developed to mimic these features in rodents. Reserpine, a monoamine-depleting agent, induces persistent pain,

fatigue, and depressive-like behaviors by depleting biogenic amines such as serotonin, norepinephrine, and dopamine, thereby replicating key aspects of FM symptomatology[3].

Current therapeutic options for FM, including analgesics, antidepressants, and anticonvulsants, provide limited efficacy and are often accompanied by undesirable side effects[2]. Thymoquinone (TQ), a bioactive compound derived from the seeds of Nigella sativa (commonly known as black seed), has garnered attention for its multifaceted pharmacological properties. TQ exhibits potent anti-inflammatory, antioxidant, and analgesic effects, which have been demonstrated in various preclinical models of pain and inflammation[4,5]. These effects are thought to be mediated through the modulation of pro-inflammatory cytokines, inhibition of oxidative stress, and interaction with pain signaling pathways, including the opioid and nitric oxide systems[4].

Despite its promising therapeutic potential, the efficacy of TQ in the context of fibromyalgia has not been extensively investigated. The present study aimed to assess the analgesic effect of thymoquinone in the reserpine-induced experimental fibromyalgia model, with the goal of elucidating its potential as a novel therapeutic agent for FM.

2. Materials and Methods

2.1. Animals

The experiments were conducted on male Wistar rats aged 10–12 weeks (weighing 200–220 g) obtained from the Experimental Medicine Research and Application Center of Selçuk University. Animals were housed in acrylic cages with free access to water and food (6 rats per cage) and randomly assigned to experimental groups. The experimenter was blinded to treatments. All procedures were carried out in accordance with the Ethical Standards for Experimental Pain Research in Animals[6] and approved by the local Ethics Committee (Selçuk University Experimental Medicine Research and Application Center Ethics Committee, Decision No: 2023/62).

2.2. Drugs

All drugs were obtained from Sigma-Aldrich (St. Louis, MO). Reserpine was dissolved in acetic acid and diluted with saline to a final concentration of 0.5% acetic acid, and administered subcutaneously (1 mg/kg). Thymoquinone was dissolved in corn oil and administered orally at a dose of 100 mg/kg.

2.3. Reserpine-Induced Pain Model

The experimental reserpine-induced pain model was established as described by Nagakura et al.[3]. Reserpine (1 mg/kg, s.c.) was administered using a 23-G needle on the loose skin over the neck once every 24 hours for three consecutive days. Control groups received three subcutaneous injections of 0.5% acetic acid in saline (1 mL/kg). The effect of thymoquinone was assessed four days after the final reserpine injection. Behavioral assessments were performed at 30, 60, 120, and 180 minutes following thymoquinone administration.

2.4. Experimental Groups

Animals were randomly divided into three groups (n = 6 per group): Group I (Control): rats received subcutaneous 0.5% acetic acid in saline and oral corn oil; Group II (Reserpine): rats received reserpine (1 mg/kg, s.c.) for three days followed by oral corn oil; Group III (Reserpine + Thymoquinone): rats received reserpine followed by oral thymoquinone (100 mg/kg). All behavioral tests were conducted by an experimenter blinded to group assignments.

2.5. Von Frey Filament Test

The paw withdrawal response to tactile stimuli was assessed using von Frey filaments (Bioseb, BIO-VF-M). Animals were acclimatized for one hour in acrylic test cages

with a perforated metal floor. Filaments were applied to the center of the plantar surface of the right hind paw using the up-down method[7].

2.6. Hot-Plate Test

The hot-plate test was conducted according to previously reported methods[8,9]. The animal was placed on a metal plate maintained at 55 ± 1°C, and the latency of nociceptive responses (licking, twitching, or jumping of the hind paw) was measured[10]. A cut-off time of 45 seconds was established to prevent tissue damage.

2.7. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 26.0. Data distribution was assessed using the Shapiro–Wilk test. Since the data did not meet the assumption of normality, non-parametric tests were employed. Descriptive statistics were presented as median values with interquartile ranges (median [Q1–Q3]). Comparisons among the three groups were conducted using the Kruskal–Wallis test followed by post hoc pairwise comparisons with Bonferroni correction. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Von Frey Filament Test

Baseline mechanical sensitivity (t_0) did not differ significantly between groups ($p = 0.931$). Significant differences were observed at subsequent time points: at 60 minutes (t_{60}), the median withdrawal threshold was significantly higher in the TQ-treated group compared to both control and reserpine-only groups ($p = 0.0003$); at 120 minutes (t_{120}), the difference remained statistically significant ($p = 0.0037$); at 180 minutes (t_{180}), a persistent significant difference was noted ($p = 0.0005$) (Figure 1). These findings indicate that thymoquinone treatment resulted in a significant reduction in mechanical allodynia induced by reserpine administration.

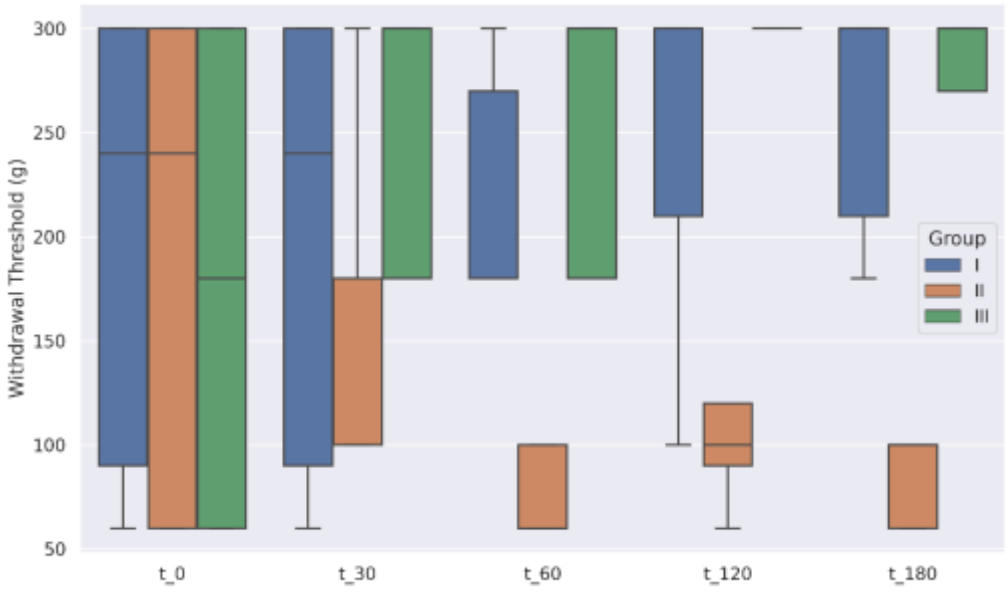

Figure 1. Mechanical withdrawal threshold (g) measured by the von Frey filament test at different time points in Groups I, II, and III

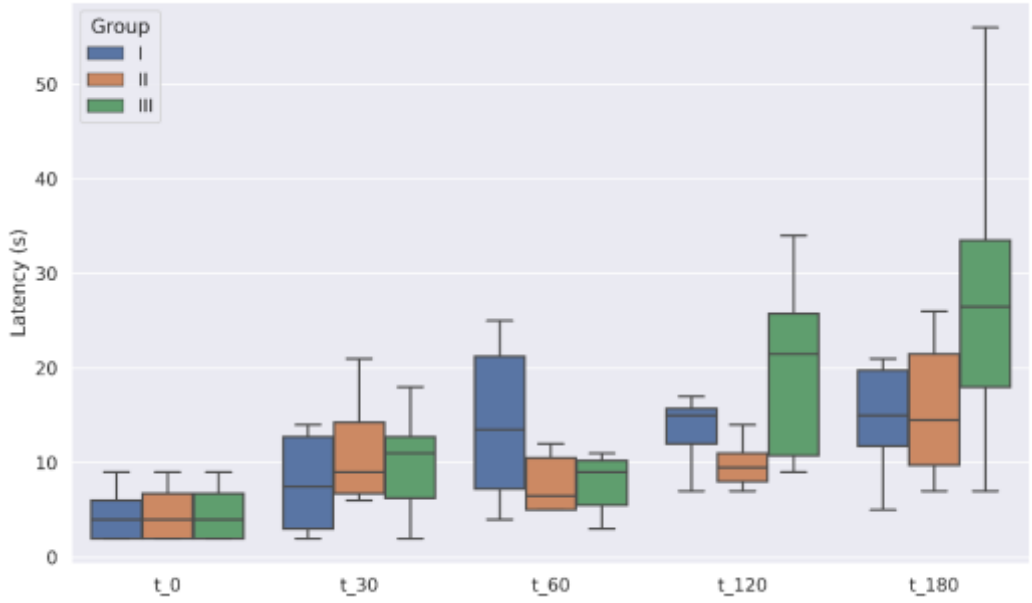

Figure 2. Hot-plate test latency times (s) at different time points in Groups I, II, and III.

3.2. Hot-Plate Test

There were no statistically significant differences between groups at any time point in the hot-plate test: t_0: $p = 0.987$; t_{30}: $p = 0.776$; t_{60}: $p = 0.314$; t_{120}: $p = 0.089$; t_{180}: $p = 0.375$ (Figure 2). Although the TQ-treated group exhibited numerically higher latency times at 120 and 180 minutes, these differences did not reach statistical significance.

4. Discussion

The findings of this study demonstrate that thymoquinone (TQ), a bioactive compound derived from Nigella sativa, exerts a significant analgesic effect in a reserpine-induced fibromyalgia (FM) rat model, particularly in attenuating mechanical allodynia. This is consistent with a growing body of evidence suggesting the therapeutic potential of TQ in modulating pain and inflammation through neurochemical and antioxidant pathways.

Samad et al. demonstrated that TQ reverses neurobehavioral alterations in reserpine-treated rats by enhancing antioxidant enzyme activities such as superoxide dismutase and glutathione peroxidase, and reducing lipid peroxidation—key markers of oxidative stress implicated in FM pathology[11]. This biochemical stabilization complements our behavioral findings, where a sustained increase in withdrawal threshold was observed in the von Frey test.

Anaeigoudari provided a broader context by reviewing the antidepressant and antinociceptive effects of Nigella sativa and its constituent TQ, asserting that TQ interacts with multiple pathways including opioid receptors, nitric oxide signaling, and cytokine inhibition, which could underlie its broad-spectrum efficacy in pain models[12].

Although TQ demonstrated a clear benefit in the von Frey test, the hot-plate test results were not statistically significant. This might reflect the specificity of TQ's analgesic action toward mechanical hypersensitivity rather than thermal pain. These nuances are also observed in the work of Aboutaleb et al., who compared myricitrin and TQ in reserpine FM models and noted differential responses between mechanical and thermal pain modalities, linked to divergent molecular signaling routes such as SIRT1/NF-κB pathways[13].

Broader reviews of animal models for FM have raised concerns about the predictive validity of the reserpine model for clinical FM[14]. However, its utility in replicating monoaminergic dysregulation and chronic pain behaviors remains valuable for early-stage pharmacological screening.

Taken together, these results suggest that TQ has significant potential as a candidate for FM therapy, primarily through mechanisms involving monoamine restoration, anti-inflammatory modulation, and antioxidant defense enhancement. Nevertheless, its limited efficacy in thermal nociceptive paradigms highlights the need for further exploration into multimodal pain modulation.

5. Conclusion

This study highlights the significant analgesic potential of thymoquinone (TQ) in alleviating mechanical allodynia in a reserpine-induced fibromyalgia (FM) model, supporting its role as a promising candidate for FM treatment. TQ's effects are likely mediated by its ability to restore monoamine levels, reduce oxidative stress, and modulate inflammatory signaling. However, the lack of significant impact on thermal hyperalgesia underscores the need for further research to better understand its modality-specific analgesic profile. Future studies should focus on elucidating the precise molecular mechanisms underlying TQ's action, including chronic dosing regimens, long-term safety profiles, female animal models, and comparative studies against existing pharmacotherapies such as duloxetine or pregabalin.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by M.S., B.G.Y., S.D., and M.S.U. The first draft of the manuscript was written by M.S. and all authors commented on previous versions. M.S. and M.S.U. supervised the whole process and critically reviewed the drafting process. All authors read and approved the final manuscript.

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Ethical Approval: All procedures were carried out in accordance with the Ethical Standards for Experimental Pain Research in Animals and approved by the Selçuk University Experimental Medicine Research and Application Center Ethics Committee (Decision No: 2023/62).

Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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