Thymoquinone in a Rat Model of Mesenteric Ischemia-Reperfusion Injury

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Abstract

Aim: The objective of this study was to assess the protective efficacy of thymoquinone at low and high doses against ischemic reperfusion (I/R) injury resulting from superior mesenteric artery occlusion in rats.

Methods: Thirty-five Wistar Albino rats were randomly divided into five groups: control, sham, two thymoquinone treatment groups (50 and 100 mg/kg), and a DMSO control. Intestinal injury was assessed using the Park/Chiu classification system. Blood samples were analyzed for liver enzymes (ALT, AST, ALP), kidney function markers (BUN), and other markers (LDH, phosphorus).

Results: DMSO and low dose thymoquinone groups showed significantly better Park and Chiu scores (p values were 0.015 and p=0.016 respectively) High-dose thymoquinone and DMSO groups had significantly higher ALT levels than the control. Sham, low-dose thymoquinone, and DMSO groups had significantly higher AST levels than the control. Both low-dose and high-dose thymoquinone groups had significantly higher BUN levels than the control.

Conclusion: Our study suggested that low-dose thymoquinone was more effective than high-dose thymoquinone in mitigating I/R-induced intestinal injury. High-dose thymoquinone appeared to have a detrimental effect on intestinal tissue, as evidenced by the lack of significant improvement in histopathological scores compared to the sham group.

***Keywords**: thymoquinone; mesenteric ischemia; reperfusion injury.*

1. Introduction

Mesenteric ischemia occurs when the visceral organs fail to receive an adequate blood supply to meet their normal metabolic demands. This condition is categorized as either acute or chronic, depending on the duration of symptoms. The most common causes of acute mesenteric ischemia (AMI) are emboli to the mesenteric arteries or acute thrombosis associated with pre-existing plaque.[1] Embolism of the superior mesenteric artery (SMA) is the most common cause of AMI.

Delayed diagnosis can lead to bowel necrosis and peritonitis, often requiring extensive intestinal resection. Intestinal tissue is highly susceptible to hypoperfusion. The SMA supplies blood to the small intestine and the proximal two-thirds of the large intestine. [2,3] Ischemic reperfusion injury is a complex pathophysiological process that occurs when blood flow is restored to ischemic tissues.

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Altered mesenteric circulation, often caused by obstruction or diminished blood flow, can lead to decreased oxygen delivery to the visceral organs, insufficient to meet their metabolic needs. [4] The initial vasodilatory response to ischemia can transition to vasoconstriction, which may persist even after blood flow is restored.[5] This early injury, primarily affecting the intestinal mucosa and submucosa, can impair the barrier function, allowing bacterial translocation.[6] Subsequently, systemic inflammatory pathways are activated, leading to worsened vasospasm, further compromising regional blood flow and causing more extensive bowel wall injury. [4,5]

Nigella sativa, or black seed, is a natural source of thymoquinone (TQ), a potent bioactive compound with a wide range of pharmacological activities. TQ has shown promise as an antimicrobial, antioxidant, anti-inflammatory, and antitumor agent, making it a subject of increasing interest in scientific research. [6,7] The antioxidant, anti-inflammatory, and anti-oxidative stress properties of thymoquinone make it a promising candidate for mitigating intestinal I/R injury.

DMSO (Dimethyl Sulfoxide) is a versatile compound with a wide range of applications, including its use as a solvent and a carrier for various medications. It has also been studied for its potential therapeutic effects on various cellular processes. DMSO is also a proper

solvent for thymoquinone. DMSO has been shown to exhibit antioxidant properties, protecting cells from oxidative stress caused by free radicals. This may contribute to its potential anti-inflammatory effects.[8] DMSO has been reported to have anti-inflammatory effects, reducing the production of inflammatory mediators such as cytokines and prostaglandins.1 This may be beneficial in conditions involving inflammation, such as arthritis.[9]

Previous research has shown that thymoquinone exhibits antioxidant and anti-inflammatory properties, which may protect against I/R injury in experimental settings.[10,11] Despite these promising findings, the optimal dose and safety profile of thymoquinone for clinical application have not been well-established. Thus, this study aimed to evaluate the protective effects of low and high doses of thymoquinone on I/R injury in a rat model of SMA occlusion.

2. Materials and Methods

2.1. Animals and Experimental protocol

Thirty-five Wistar albino rats regardless of gender difference, weighing from 200 to 250 g were used in the study. Following an overnight fast (allowing only water to drink), a midline laparotomy incision was used to access the peritoneal cavity under ketamine (Ketalar; Parke-Dawis Eczacibasi, Istanbul, Turkey), (50 mg/kg) and xylazine (Rompun; Bayer AG, Leverkusen, Germany) (10 mg/kg) anesthesia. After abdominal shaving, 10% povidone iodide was used to wipe twice with this solution and the rats were operated with sterile instruments in accordance with the rules of asepsis. Animals were anesthetized and maintained at 37°C during surgery. A midline abdominal incision was made to expose the SMA. After the surgical procedure, 10 mL of saline solution was administered intraperitoneally for hydration.

2.2. Study Group and Surgical Technique

Rats were randomly assigned to five groups of seven animals each. In the control group, the SMA was isolated without ligation. In the I/R groups, the SMA was occluded for 60 minutes using non-traumatic forceps, followed by 120 minutes of reperfusion. Treatment groups received intraperitoneal injections as follows: sham group (saline), low-dose thymoquinone group (50 mg/kg thymoquinone in DMSO), DMSO group (0.2 mL DMSO + 0.8 mL distilled water), and high-dose thymoquinone group (100 mg/kg thymoquinone in DMSO).

Abdominal incisions were closed with 3-0 polypropylene sutures. Animals were euthanized 24 hours after reperfusion under anesthesia. Tissue samples were harvested from the terminal ileum to assess intestinal injury, and blood samples were collected via cardiac puncture.

2.3. Histopathological evaluation

Terminal ileum samples were fixed in 10% formalin, processed for paraffin embedding, and sectioned at a thickness of 5 µm. Hematoxylin and eosin (H&E) staining was performed to visualize tissue morphology. After staining, the sections were dehydrated, cleared, and mounted with entellan. Slides were examined under a light microscope (Olympus BH-2) and photographed using an Olympus DP-70 digital camera.

The severity of intestinal injury was evaluated using the Park/Chiu histological scoring system (13). Scores ranged from 0 (normal mucosa) to 8 (transmural infarction), with increasing scores indicating progressive damage, including subepithelial edema, villous damage, and full-thickness tissue damage.

2.4. Biochemical Analysis

Serum levels of liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), kidney function markers blood urea nitrogen (BUN), and markers of tissue injury lactate dehydrogenase (LDH), and phosphorus were measured using standard biochemical assays.

2.5. Statistical analysis

Data were analyzed using SPSS 15.0 and SigmaStat 3.1. Continuous variables were assessed for normality using the Kolmogorov-Smirnov test (for $n \geq 30$) or the Shapiro-Wilk test (for $n < 30$). Normally distributed data were presented as mean ± standard deviation, while non-normally distributed data were presented as median. One-way ANOVA was used to compare normally distributed data between groups, followed by Bonferroni post-hoc analysis. For non-normally distributed data, the Kruskal-Wallis test was used, followed by pairwise comparisons with the Mann-Whitney U test. The Chi-square test was used to analyze categorical data. Statistical significance was set at $p < 0.05$.

3. Results

DMSO and low dose thymoquinone groups showed significantly better Park and Chiu scores (p values were 0.015 and p=0.016 respectively) when compared with the sham group. The difference was not significant between Sham and high dose thymoquinone groups (p=0.55).

When we compared the DMSO group with the both thymoquinone groups, the difference was not significant (p≥0.05). Also, the difference was not significant between the low dose and high dose thymoquinone groups (p=0.068).

Microscopic examination of the terminal ileum in the control group revealed normal intestinal tissue. Compared to the sham group, both the DMSO and low-dose thymoquinone groups showed significantly lower injury scores ($p = 0.015$ and $p = 0.016$, respectively). However, no significant difference was observed between the sham and high-dose thymoquinone groups ($p = 0.55$). Additionally, no significant differences were found between the DMSO group and either thymoquinone group ($p \geq 0.05$), or between the low-dose and high-dose thymoquinone groups ($p = 0.068$).

ALT: Median (range) ALT levels (U/L) were as follows: control group (59 [40-77]), sham group (120.5 [84-315]), low-dose thymoquinone group (138 [56-260]), DMSO group (244 [122-402]), and high-dose thymoquinone group (254.5 [99-303]). ALT level was significantly higher in high dose thymoquinone and DMSO groups when compared with the control group (*p* values were 0.03 and 0.01 respectively).

AST: Median (range) AST levels (U/L) were as follows: control group (131 [94-243]), sham group (573 [398-910]), low-dose thymoquinone group (494 [326-919]), DMSO group (1066 [503-1527]), and high-dose thymoquinone group (946.5 [495-1464]). The DMSO group had significantly higher AST levels compared to the low-dose thymoquinone group (p =0.026). Additionally, when compared to the control group, the sham, low-dose thymoquinone, and DMSO groups showed significantly elevated AST levels (p values were; p=0.001, p=0.004 and p=0.001). However, no significant difference was found between the low-dose and high-dose thymoquinone groups (p=0.133). Post-hoc analysis indicated a significant difference in AST levels between the sham and DMSO groups (p = 0.048).

BUN: Median (range) BUN levels (mg/dL) were as follows: control group (16.5 [14-18]), sham group (52.4 [19.9-102]), low-dose thymoquinone group (81.3 [16.3-143.7]), DMSO group (43.1 [18.1-106.3]), and high-dose thymoquinone group (95.4 [27.8-126]). Statistical analysis revealed a significant increase in BUN levels in both the low-dose and high-dose thymoquinone groups compared to the control group (p values were 0.023 and 0.003 respectively). However, no significant differences were found between the sham group and the thymoquinone (low and high dose groups) or DMSO groups (p values were; 0.675, 0.196 and 1,

respectively).

ALP & P: Median (range) ALP levels (U/L) were as follows: control group (53 [38-134]), sham group (88.5 [52-115]), low-dose thymoquinone group (98 [37-2769]), DMSO group (15.6 [77-203]), and high-dose thymoquinone group (129 [66-160]). Median (range) P levels (U/L) were as follows: control group (6.1 [5.3-8.5]), sham group (6.2 [5-9.3]), low-dose thymoquinone group (9 [5.8-37.6]), DMSO group (8.4 [5.2-12.6]), and high-dose thymoquinone group (9.8 [4.9-18]). No significant differences were observed in ALP ($p = 0.076$) or p levels ($p = 0.084$) among the groups.

LDH: The DMSO group exhibited significantly higher mean LDH levels (3599 ± 1221 U/L) compared to the control (1179 ± 507 U/L), sham (1604 ± 657 U/L), low-dose thymoquinone (2060 ± 989 U/L), and high-dose thymoquinone (2558 ± 1416 U/L) groups ($p < 0.05$).

4. Discussion

I/R injury is a frequently encountered event in clinical practice. While numerous studies have explored potential agents to mitigate I/R injury, an effective medical solution remains elusive. This study aimed to evaluate the protective effects of thymoquinone on I/R injury in rats and to compare the efficacy of low and high doses.

Our findings suggest that low-dose thymoquinone (50 mg/kg) is more effective in reducing I/R-induced intestinal injury than high-dose thymoquinone (100 mg/kg). While both DMSO and low-dose thymoquinone exhibited protective effects, DMSO was associated with more pronounced adverse effects on laboratory parameters.Our study contributes to the growing body of knowledge on the potential therapeutic benefits of thymoquinone in mitigating I/R injury. Additionally, clinical trials are needed to assess the safety and efficacy of thymoquinone in humans.

Ong et al. demonstrated that nanostructured lipid carrier-loaded thymoquinone exhibited reduced toxicity compared to pure thymoquinone in acute toxicity studies.[12] While both formulations (100 mg/kg) were well-tolerated in terms of mortality, they induced liver toxicity in subacute studies. [12] Similarly, our study revealed elevated liver function tests in rats treated with both 50 mg/kg and 100 mg/kg thymoquinone. However, Ong et al. also reported that both formulations at a lower dose (10 mg/kg) were well-tolerated in mice and did not induce long-term toxicity. [12]

Parlar et al. investigated the prophylactic effects of oral thymoquinone in I/R injury. In contrast, our study focused on the therapeutic effects of intraperitoneal thymoquinone administration. [10] They proposed that premedication with thymoquinone regained the disrupted contractility of the intestinal smooth muscle. In our study we administrated thymoquinone by the intraperitoneal route and this clinically reflects the therapeutic effect other than the prophylactic effect.

Histological analysis revealed normal morphology in the control group in our study. While the sham group exhibited hemorrhage and ulceration in the lamina propria, the low-dose thymoquinone group showed significant improvement in these histopathological changes. Conversely, the high-dose thymoquinone group did not demonstrate any improvement compared to the sham group. Previous studies have suggested the protective mechanisms of thymoquinone against I/R injury.

Tas et al. conducted a study to compare the protective effects of thymoquinone and melatonin against intestinal I/R injury. Their findings revealed that both agents significantly reduced oxidative stress by modulating the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, as well as decreasing the levels of lipid peroxidation marker malondialdehyde. [13] Additionally, treatment with thymoquinone and melatonin significantly decreased the number of apoptotic cells in the intestinal tissue. Aydin et al. investigated the antioxidant effects of intraperitoneal thymoquinone on intestinal I/R injury and found that it significantly reduced histopathological damage in the heart, lung, and kidney tissues, as assessed by light microscopy. [11]

Our study demonstrated that DMSO exhibited a protective effect on intestinal tissue, as evidenced by the regression of histopathological findings compared to the sham group. Low-dose thymoquinone also showed some protective effects, but the difference compared to DMSO was not statistically significant. These results suggest that the protective effects observed with low-dose thymoquinone may be partially attributed to the DMSO solvent.

In the literature, DMSO has been shown to protect tissues or even an entire organ, from ischemic damage. [14-16] DMSO captures free radicals. Wood et al. reported many known pharmacological properties of DMSO including cryoprotective and radioprotective effects, effect on serum cholesterol in experimental hypercholesteremia, and platelet aggregation antagonism[17].

Previous research has demonstrated DMSO's potential to protect tissues from ischemic damage. [14-16] Also, DMSO is known to scavenge free radicals and exhibit various pharmacological properties, including cryoprotective, radioprotective, and anti-platelet effects. [15]

DMSO is known to enhance the cellular permeability of various substances, including drugs. When mixed with DMSO, the physiological effects of the many drugs increase. The most important benefit of this effect is the potential for lower dosages requirement, which could reduce side effects and toxicity.

Intestinal I/R injury can lead to bacterial translocation and endotoxemia, resulting in damage to distant organs such as the liver and kidneys. [17-20] To assess potential systemic effects of I/R injury, we also evaluated liver and kidney function tests in our study.

Overall, neither thymoquinone nor DMSO improved laboratory parameters. However, DMSO alone appeared to exacerbate kidney function, as indicated by elevated BUN levels. High-dose thymoquinone also seemed to have a detrimental effect on kidney function. Both high-dose thymoquinone and DMSO led to increased liver enzyme levels (ALT and AST) compared to the control group, with DMSO causing a more pronounced effect on ALT levels.

4.1. Study Limitations

The number of the study population in each group was limited.

5. Conclusion

Our study suggested that low-dose thymoquinone (50 mg/kg) was more effective than high-dose thymoquinone (100 mg/kg) in mitigating I/R-induced intestinal injury. High-dose thymoquinone appeared to have a detrimental effect on intestinal tissue, as evidenced by the lack of significant improvement in histopathological scores compared to the sham group. While both DMSO and low-dose thymoquinone exhibited protective effects, DMSO was associated with more pronounced adverse effects on laboratory parameters.

Statement of ethics

This study was approved by the Ethics Committee of the Eskişehir Osmangazi University(HADYEK) (Decision no: 2015-90-480,).

Source of Finance

The authors declare that they have received no financial support for this study

Conflict of interest statement

The authors declare that they have no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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