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ORIGINAL ARTICLE

The Effects of Thymoquinone (*Kalonji*) on Abdominal Adhesion in Experimental Abdominal Adhesive Model

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Abstract

The aim of this study was to investigate the effects of thymoquinone on abdominal adhesion development in an experimental abdominal adhesion model. Forty-five female Wistar albino rats weighing 260–280 g were used in the study. The rats were randomized into 3 even groups. Control, serum physiological (SP), and thymoquinone (TQ) groups were formed. In the experimental abdominal adhesion model, caecum serosa was brushed until the petechial hemorrhages were seen. No additional procedures were performed in the control group except surgery protocol. SP was injected over the caecum in the SP group. In the thymoquinone group, the abdomen was closed after the application of prepared thymoquinone solution on the caecum surface. On the 21st day, tissue samples from sacrificed rats were examined macroscopically and microscopically, and statistically evaluated. There was a statistically significant difference between the control group and the thymoquinone group in the microscopic evaluation ($p = 0.006$). However, there was no statistically significant difference between SP group and control group, and between thymoquinone group and SP group. Macroscopic evaluation revealed a statistically significant difference between the thymoquinone group and the control group, and between the thymoquinone group and the SP group ($p = 0.009$, $p = 0.027$, respectively). In the microscopic and macroscopic evaluation, it was observed that thymoquinone had a decreasing effect on postoperative adhesions. We think that this effect of thymoquinone is due to its anti-inflammatory and antioxidant properties. However, the available data are not sufficient for this effect to be used in medical treatment. Further studies are needed in the future.

Keywords Thymoquinone · Abdominal adhesion · Anti-inflammatory · Antioxidant

Introduction

The most common reason for intraabdominal adhesions is previous intraabdominal surgical procedures. Abdominal adhesion may develop in more than 90% of patients undergoing intraabdominal surgery [1, 2]. Adhesions are considered a defensive system of the body, which helps to regain vascularity on the defected and ischemic surfaces and maintain the vitality of the tissue in this region. However, adhesions often cause pain, intestinal obstruction, infertility, and even death [3, 4].

Nigella sativa (NS) is a plant of the family Ranunculaceae. *Nigella sativa* is commonly known as Black seed. It is commonly used condiment in vegetables and pickles in India. It is called *kalonji* in India. It may have anti-cancer activity also [5]. The seeds of the NS plant are consumed as food and spices. The fats and compounds in the NS seed, in particular the thymoquinone (TQ), show potential medicinal properties. Its antioxidant and anti-inflammatory properties have been shown in studies [6]. The anti-inflammatory effect of thymoquinone in inflammatory component diseases such as allergic ensafalomyelitis, arthritis, and colitis has been shown. TQ is referred to as a potent inhibitor of thromboxane B2 and leukotriene B4. TQ inhibits arachidonic acid metabolism in

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peritoneal leukocytes by cyclooxygenase (COX) and 5-lipoxygenase [6–8] pathways. We think that the potent inhibitory effect of thymoquinone on thromboxane B2 and leukotriene B4, on the metabolism of arachidonic acid in cyclooxygenase and lipoxygenase pathways and its existing anti-inflammatory, antioxidant, antibacterial properties, helps to reduce intraabdominal adhesions.

Material and Method

Thirty to 70% humidity, room temperature, and 12 h light, 12 h dark environment were provided for all rats included in the study and they were taken care of for 1 week before the experiment. All rats were fed "ad libitum" with standard laboratory food and water. The ethics committee approved the Kobay Experimental Animals Laboratory Company Animal Ethical Committee ınstructions. It was supported by Kırıkkale University Scientific Research Projects Department (Project no: 2013/51).

Forty-five female Wistar albino rats weighing 260–280 g were used in the study. Rats were divided into three groups as control, SP, and thymoquinone groups. Intraperitoneal ketamine (Ketalar®, 500 mg 10 ml vial Pfizer; 90 mg/kg) and xylazine (Rompun®, Bayer, Leverkusen, Germany; 10 mg/kg) were administered to all rats and the abdomen was opened with midline incision following general anesthesia. Caecum was exposed, and its serosa was brushed until petechial hemorrhages were seen. The abdomen was closed without additional treatment in the control group. SP group was applied 1 cc of SP after the surgical procedure and the abdomen was closed. In the thymoquinone group, 10 mg/kg thymoquinone (Sigma Aldrich Co., St. Louis, MO, USA) solution was prepared in 1 cc SP, applied to caecum surface, and the abdomen was closed.

Twenty-one days postoperatively, the rats were nursed according to their natural nutrition and shelter conditions. On the 21st day, tissue samples obtained from sacrificed rats were examined macroscopically and microscopically (Figs. 1 and 2). In the macroscopic evaluation, a modified diamond scale was used [9].

Histopathological Evaluation

After adhesion scoring; in rats with adhesion, adhesive band was resected with the affected organs while in the absence of adhesion, the anterior of the cecum was resected with the parietal peritoneum, and the histopathological examination of samples was performed in Kırıkkale University Faculty of Medicine Pathology Department laboratory. Pathological samples were fixed in vessels, containing 10% buffered formol.

The samples which were followed by classical laboratory method were buried in paraffin blocks. Sections of 5 μm thickness were taken onto the slides. It was stained with hematoxylin-eosin dye and examined by light microscopy. The investigating pathologist evaluated the specimens blindly. After histopathological examination, the samples were evaluated according to the microscopic classification defined by Zuhlke [10].

Statistical Analysis

Statistical analysis was performed using the SPSS ver. 23 (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean ± standard error. Both macroscopic and microscopic adhesion classification grades were presented in numbers (i.e., grade 0 = 0, grade 1 = 1, grade 2 = 2, grade 3 = 3, and grade 4 = 4). Due to the limited number of rats in each group, nonparametric methods were used for statistical analysis. For intergroup analysis, the Kruskal-Wallis variance analysis was used to analyze significant differences among the groups. A p value of less than 0.05 was considered statistically significant.

Findings

The results of the macroscopic evaluation are shown in Table 1.

The results of the microscopic evaluation are shown in Table 2.

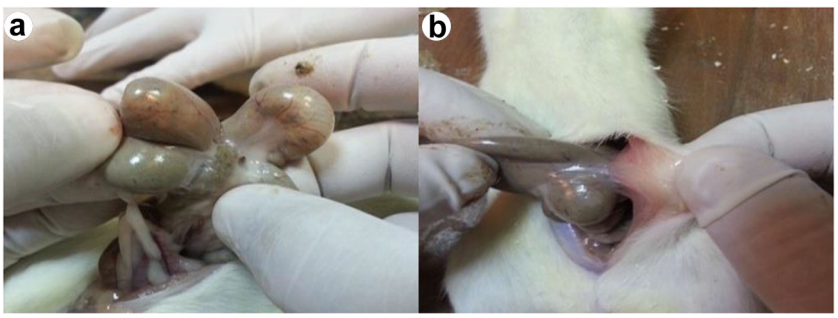

Fig. 1 **a** Control group intraabdominal adhesion. **b** Thymoquinone group intraabdominal adhesion

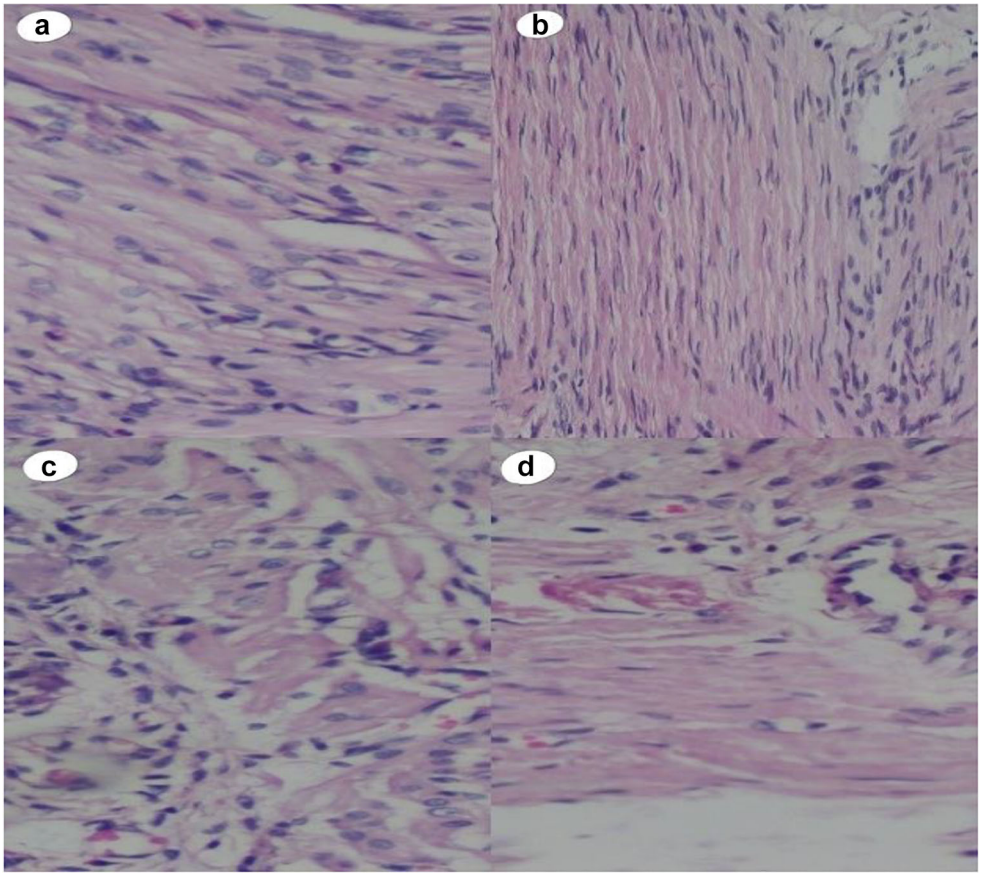

Fig. 2 **a** Zuhlke, microscopic adhesion classification grade 1. **b** Zuhlke, microscopic adhesion classification grade 2. **c** Zuhlke, microscopic adhesion classification grade 3. **d** Zuhlke, microscopic adhesion classification grade 4

The mean macroscopic grade was 1.71 ± 0.72 in the thymoquinone group, the mean macroscopic grade was 2.69 ± 0.94 in the control group, and the mean macroscopic grade was 2.47 ± 0.91 in the SP group. A statistically significant difference was found between the three groups in terms of macroscopic grade ($p = 0.018$) (Table 1).

The groups were evaluated bilaterally among themselves. Macroscopic evaluation revealed a statistically significant difference between the thymoquinone group and the control group, and between the thymoquinone group and the SP group ($p = 0.009$, $p = 0.027$, respectively). However, there was no statistically significant difference found between the SP group and the control group ($p = 0.544$).

In microscopic evaluation, the mean microscopic grade was found 1.71 ± 0.82 in the thymoquinone group, 2.85 ± 0.98 in the control group, and 2.33 ± 0.97 in the SP group. There was a statistically significant difference between the three groups in terms of microscopic grade ($p = 0.017$) (Table 2).

The groups were evaluated bilaterally among themselves. There was a statistically significant difference found between the control group and the thymoquinone group in the microscopic evaluation ($p = 0.006$). However, there was no statistically significant difference between SP group and control group, and between thymoquinone group and SP group ($p = 0.179$, $p = 0.085$, respectively).

Table 1 Distribution of groups according to macroscopic grade

Macroscopic	Thymoquinone *N*, %	Control *N*, %	SP *N*, %
Grade 1	6 (42.9%)	1 (7.7%)	2 (13.3%)
Grade 2	6 (42.9%)	5 (38.5%)	6 (40%)
Grade 3	2 (14.3%)	4 (30.8%)	5 (33.3%)
Grade 4	–	3 (23.1%)	2 (13.3%)
Total	14 (mean grade 1.71 ± 0.72)	13 (mean grade 2.69 ± 0.94)	15 (mean grade 2.47 ± 0.91)

Table 2 Distribution of groups according tomicroscopicgrade

Microscopic	Thymoquinone *N*, %	Control *N*, %	SP *N*, %
Grade 1	7 (50%)	1 (7.7%)	3 (20%)
Grade 2	4 (28.6%)	4 (30.8%)	6 (40%)
Grade 3	3 (21.4%)	4 (30.8%)	4 (26.7%)
Grade 4	–	4 (30.8%)	2 (13.3%)
Total	14 (mean grade 1.71 ± 0.82	13 (mean grade 2.85 ± 0.98	15 (mean grade 2.33 ± 0.97)

Discussion

Postoperative intraabdominal adhesions and resulting reoperations are an important cause of long-term morbidity and mortality. There are a large number of studies in the literature aimed at preventing adhesions. In spite of the improvements in surgical techniques and technological opportunities, products that could reduce adhesion formation in clinical use could not be developed.

Inflammation characterized by extravasation of serum and cellular elements against trauma, ischemia, infection, and foreign bodies is the first response to peritoneal injury [11]. Therefore, if it is planned to prevent adhesions, control of mediators in the inflammatory-anti-inflammatory process should be targeted [12].

Some authors thought that reducing fibrin levels would be effective in reducing AA formation. Heparin was thought to be effective both by reducing accumulation of fibrin in the environment and by reducing the amount of released exudate fibrin. However, it has caused bleeding when it should be used in high doses [13].

In some studies, substances aiming to reduce the contact of the tissues that may be adherent are included. For example, amniotic membrane [14], synthetic barriers (surgicel, oxidized cellulose) [15, 16] can be given. Although most of the substances used in these studies have decreased intraabdominal adhesions, these products were not effective enough to be used clinically.

Many substances with anti-inflammatory properties have been used to reduce AA formation. Among these, steroids are used to reduce intraabdominal adhesions as well-known anti-inflammatory agents. As they may be useful in theory, the antiadhesive effects of corticosteroids are still controversial, since they also have fibroblast growth-stimulating- and intrinsic fibrinolytic activity-reducing effects [17, 18]. Again, the anti-inflammatory effects of ankaferd and self-renewal, as well as inflammation-reducing properties of mesenchymal stem cells on intraabdominal adhesions, have been investigated and it has been demonstrated that these reduced adhesion formation [19, 20].

Anti-inflammatory and antioxidant properties of thymoquinone isolated from seed of *Nigella sativa* plant used as food and spice in daily life were emphasized [21, 22]. In our study, we found that TQ decreases AA formation due to increased inflammation.

Conclusion

When the results of the study are examined, it is seen that abdominal adhesions are lower in the thymoquinone group compared with the other two groups both macroscopically and microscopically. We think that this effect of thymoquinone is due to its anti-inflammatory and antioxidant properties. To demonstrate the potential beneficial effects of thymoquinone, it should be supported by further experimental and clinical studies.

Compliance with Ethical Standards

Ethics Committee Form Description Local Animal Experimental Ethics committee of Kobay Experimental Animals Laboratory Company met in 30 November 2012 Friday in the presidency of Veterinarian A. Begüm Buğdaycı Açıkkol. The project entitled "The Effects of Thymoquınone on Abdomınal Adhesıon in Experımental Abdomınal Adhesıve Model' which is conducted by Professor Oral Saygun in Kırıkkale University Faculty of Medicine, Department of General Surgery is investigated and found suitable for ethical principles in Kobay Experimental Animals Laboratory Company Animal Ethical Committee ınstructions.

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

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