Original Article

Comparison of Icodextrin with Thymoquinone: A New Hope for Postoperative Adhesions

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Abstract

Objective: The purpose of this study was to compare the effects of thymoquinone and icodextrin in rats within the framework of an experimental adhesion model. **Materials and Methods:** Rats were separated into three groups: (1) a control group consisting of rats that had 2 ml of isotonic solution administered intraperitoneally, (2) an ICO group administered with 2 ml of 4% icodextrin, and (3) a TQ group administered thymoquinone (10 mg/kg), all following cecal abrasion. The three groups underwent a reoperation on the 7th postoperative day. Hydroxyproline levels were analyzed in the resected adhesive tissues, and histopathological investigations were conducted. Blood samples were collected for biochemical analyses. **Results:** Fewer postoperative adhesions were observed in the ICO and TQ groups compared with the control group. A comparison of the TQ and ICO groups revealed lowers levels of postoperative adhesions in the TQ group. Compared with the control group, malondialdehyde, 8-OH-deoxyguanosine/deoxyguanosine (8-OHdG/10dG), Coenzyme Q10 (CoQ10), and CoenzymeQ10/reduced CoenzymeQ10 (CoQ10/CoQ10H) values were found to be lower in the TQ and ICO groups. When the TQ and ICO groups were compared with respect to their biochemical parameters, the results for all of the four parameters were found to be statistically significantly lower in the TQ group ($P < 0.000$). The levels of hydroxyproline in the control, ICO, and TQ groups were found to be (mean ± standard deviation) 502.25 ± 90.39 μg/g, 342.13 ± 66.61 μg/g, and 287.88 ± 49.59 μg/g, respectively. **Conclusions:** A comparison of the antiadhesive effects of thymoquinone and icodextrin revealed thymoquinone to be more effective. These results indicate that thymoquinone is an efficient and strong antiadhesive molecule.

Keywords: *Icodextrin, postoperative adhesions, rat, thymoquinone*

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Introduction

Peritoneal adhesions that develop following abdominal surgery have been a big problem since the beginning of modern surgical practice.[1] The development of peritoneal adhesion is, in fact, the result of the normal wound healing process. Peritoneal adhesions are caused and initiated by damage to mesothelial cells on serosal surfaces. This is followed by the formation of a soft fibrin gel matrix within 72 h by the serosanguinous exudate secreted from the subserosal connective tissue. Normally, this fibrin gel matrix is degraded by the fibrinolytic activities of mesothelial cells and then eliminated. Adhesions thus form when the fibrin gel matrix is not eliminated, for whatever reason. These causes include a decrease in fibrinolytic activity or the formation of fibrin gel matrix in quantities too large to be eliminated through fibrinolytic activity.[2] Formation of peritoneal adhesions may lead to clinical problems, such as intestinal obstruction, chronic abdominal pain, infertility, and chronic pelvic pain. Peritoneal adhesions

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increase the likelihood of organ injury when a second abdominal surgery is required and thus tend to increase morbidity and mortality.[3]

Although the most common cause of adhesions is surgery, ischemia, hemorrhage, trauma, infection, malignancy, intra-abdominal foreign body, and a long-term peritoneal dialysis may also result in the formation of adhesions.[4] Adhesions lead to patients having various health problems, while also resulting in a significant burden on health systems. The annual cost of adhesion-related problems is estimated to exceed $1 billion in the United States alone.[5] Many agents have been tested in experimental and clinical studies with the intention of preventing intra-abdominal adhesions. The agent icodextrin is a molecule known to prevent adhesions.[5] Nevertheless, a number of studies have reported that results relating to icodextrin are somewhat controversial.[5,6]

The antioxidant, anti-inflammatory, and anticancer effects of thymoquinone, which is the active form of Nigella sativa (seed extract), have been investigated since the 1960s both within the frame of *in vitro* and *in vivo* studies. Its antioxidant and anti-inflammatory effects have been observed in various disease models, such as encephalomyelitis, diabetes, asthma, and carcinogenesis.[7]

Thymoquinone is a molecule that has been investigated in a multitude of studies over the years. However, there are only a limited number of studies evaluating its antiadhesive effects. More importantly, there are no studies in the literature comparing the antiadhesive effect of thymoquinone with an agent such as icodextrin.

Materials and Methods

Animals

Since there was the possibility of death due to anesthesia or surgery, the study included three groups, each consisting of 10 rats. The study was conducted with thirty 4-month-old female Wistar albino rats, the weights of which ranged from 250 to 300 g. Following surgery, the rats were divided into the following three groups (n = 8):

- Control group: The group administered with 2 ml of isotonic solution after cecal abrasion
- ICO group: The group administered with 2 ml of 4% icodextrin following cecal abrasion
- TQ group: The group administered with thymoquinone dissolved in 2 ml of isotonic solution following cecal abrasion.

Surgical procedures

Mechanical and antibacterial bowel preparations were not done. After an 8-h fasting period, the rats were anesthetized by administering ketamine hydrochloride intramuscularly, at a dose of 75 mg/kg (Ketalar, Eczacıbaşı, İstanbul, Turkey). Abdominal entry was performed by means of an abdominal midline incision. The cecum was located in the abdomen, and abrasion was applied using a soft-bristle toothbrush until abraded hemorrhagic areas were formed over the cecum. Following abrasion, the rats in Group 1 were intraperitoneally administered 0.9% NaCl solution, while those in Group 2 were intraperitoneally administered 2 ml of 4% icodextrin solution, and those in Group 3 were intraperitoneally administered 10 mg/kg thymoquinone (code: 274666 Sigma-Aldrich) dissolved in 2 ml of 0.9% NaCl. Following this procedure, the abdominal muscle layer and skin were sutured separately using 3-0 silk (3-0 silk, Dogsan) sutures.

On the 7th postoperative day, an incision was made under anesthesia. The intra-abdominal adhesions were graded according to the Mazuji's adhesion scale [Table 1], without opening them. To determine tissue hydroxyproline and inflammation levels, the cecum was excised together with its overlaying peritoneal adhesion, if present. Following collection of blood samples from all three groups for assaying biochemical parameters, in order to research the systemic toxic effects of thymoquinone, the rats' liver, kidneys, and brain tissues were extracted for examination.

Histopathological analyses

Tissues removed by necropsy for histopathological analyses were fixed in 10% formalin solution for 48 h and then washed under flowing tap water for 10 h. Following routine tissue treatment with alcohol and xylol series, collected tissue samples were embedded into paraffin blocks. Sections of 4 μm thickness were obtained from each block, and the preparations were placed on slides for histopathological examination. These preparations were stained with hematoxylin and eosin, and also with Masson's trichrome, to evaluate the fibrous tissues in adhesions more accurately. The preparations were then evaluated under a light microscope (Leica DM 1000, Germany).

Determination of hydroxyproline level

We used the method developed by Hutson *et al*. to the analysis of hydroxyproline. Sample rat intestinals were stored at −80°C and wet weight of each sample was recorded. The tissue samples were homogenized in 1 ml of 6N HCI with a mechanical homogenizer. Then, 200 μl of homogenate was placed in a clean glass test tube and 3.8 ml 6N HCI was added. 100 ml of 2 mM sarcosine standard in water was added to each tube, after which the tubes were 2.1. Materials tightly were capped and placed in a 110°C heating block for 18 h. The hydrolysates were

allowed to cool to room temperature and neutralized with 4 ml of 6M NaOH. Each sample was brought to a pH of 9.56 ± 1.0 with 6M NaOH. Aliquots of 900 ml of this solution were removed for the subsequent derivatization process. Derivatization procedure was the same as described by Hutson *et al*.[8]

Determination of Biochemical Parameters

After sacrificing rats in all groups, 2 ml of intracardiac blood was collected. From the collected blood, malondialdehyde (MDA), 8-OH-deoxyguanosine/deoxyguanosine (8-OHdG/10dG), and Coenzyme Q10 (CoQ10) and CoenzymeQ10/reduced CoenzymeQ10 (CoQ10/CoQ10H) parameters were examined using high-performance liquid chromatography method.

Statistical analysis

The groups exhibited normal distribution and were therefore compared using the parametric one-way ANOVA test and the *post hoc* honest significant difference test. Results were expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (IBM Corp., Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.,) statistical software.

Results

Due to anesthesia-related complications, two rats from each group died before the operation. No postoperative complications developed in any of the three groups.

Macroscopic results

In macroscopic evaluations, 6 (80%) of the animals in the control group (first group) were determined to have increased connective tissue in both the peritoneum and cecum that was thick, tight, and not easily separable and was accompanied by extensive (cecum, small intestine, and stomach) vascular adhesion (++++). When an attempt was made to separate this adhesion, severe tissue injury was identified, and the tissues could not be separated. In two of the other rats, severe peritonitis was identified, and the adhesion was easily separable (+++). Five (70%) of the animals in the abrasion + icodextrin group (second group) had pieces of adhesions in different regions of both peritoneum and cecum; adhesions were of medium density and easily separable. Very thin adhesions comprising separate pieces (+) were observed in three of the other rats; these adhesions were thin and easily separable from their site of attachment (++). Four animals in the abrasion + thymoquinone group (third group) had adhesions with very thin connective tissue both in the peritoneum and cecum; this adhesion

Table 1: Modified from the Mazuji Adhesion Scale

Grade	Description of Grade	Severity
Grade 0	No adhesion	-
Grade 1	Very thin adhesion consisting of separate pieces	+
Grade 2	Easily separable, medium-density adhesion consisting of separate pieces	++
Grade 3	Easily separable yet dense and complete adhesion	+++
Grade 4	Not easily separable, very dense, complete, and extensive adhesion	++++

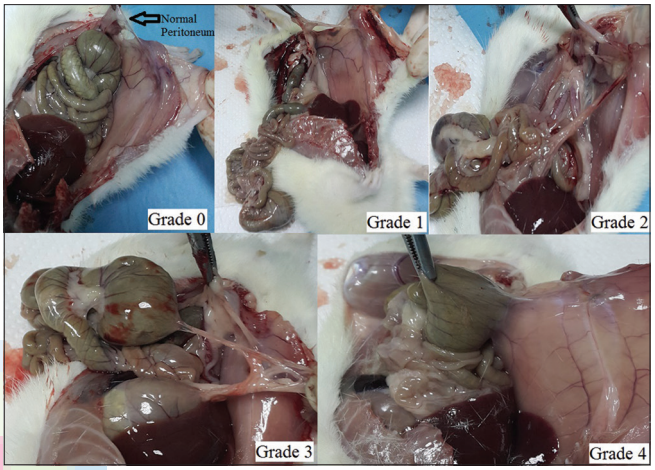

Figure 1: Macroscopic appearance

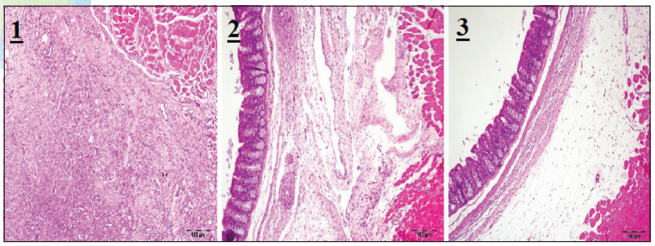

Figure 2: Group 1: Severe adhesion between peritoneum and cecum, revascularization, hyperemia in vessels, and mononuclear cell infiltration; Group 2: loose adhesion between peritoneum and cecum, revascularization, and mononuclear cell infiltration; Group 3: loose adhesion between peritoneum and cecum, mild mononuclear cell infiltration (H and E, Bar: 100 μm)

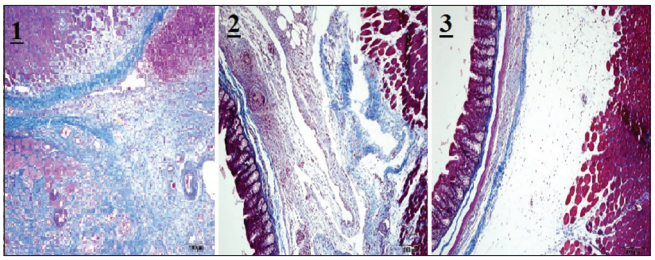

Figure 3: Group 1 severe adhesion between peritoneum and cecum, proliferation of tight connective tissue; Group 2: loose adhesion between peritoneum and cecum, connective tissue proliferation and Group 3: very loose fibrous tissue between peritoneum and cecum. Masson's trichrome, Bar: 100 μm

comprised separate pieces that were not connected with one another. No adhesion was identified in the four other rats; however, thin fibrous bands were observed in the cecum and peritoneum, possibly associated with mild hyperemia [Figure 1].

Microscopic results

No pathological signs were identified in any of the three groups during microscopic examinations of the liver, kidney, and brain tissues. On examination of the peritoneal and intestinal tissues, 6 (80%) of the animals in the control group (first group) were found to have an increase in tight connective tissue, a thickening of the serosa, microabscesses, infiltration of mononuclear cells, hyperemia, and numerous revascularizations, in both the peritoneum and cecum Figure 2. Severe peritonitis was identified in the other two animals, while the inflammatory processes were slightly milder (++). Five (70%) of the animals in the abrasion + icodextrin group (second group) were found to have mild fibrous tissue proliferation and infiltration of lymphoplasmacytic cells (++) at the site of adhesion, both in the peritoneum and cecal serosa. In three of the other animals, the fibrous band was quite thin, and a mild lymphoplasmacytic cell infiltration (+) was observed. Four animals in the abrasion + thymoquinone group (third group) were determined to have a proliferation of very thin connective tissue and mild lymphoplasmacytic cell infiltration (+) at the site of adhesion, both in the peritoneum and cecum. Adhesions could not be detected in the four other animals, while mild fibrous tissue, hyperemic vessels, and an extremely small number of plasma cells were observed at the site of abrasion in these animals [Table 2].

To evaluate the fibrous tissue reaction, the tissues were stained with Masson's trichrome stain, and scoring was performed according to the system shown in Table 3.

[Table 3] Eight (80%) of the animals in the abrasion group (first group) were determined to have an increase in tight connective tissue,[3] both in the peritoneum and cecum. Seven (70%) of the animals in the abrasion + icodextrin group (second group) were found to have developed mild fibrous tissue between the peritoneum and cecal serosa.[2] Five of the animals in the abrasion + thymoquinone group (third group) were determined to have extremely mild fibrous tissue proliferation between the peritoneum and cecal serosa [Figures 2 and 3].[1,2]

To summarize, the absence of any pathological signs in all groups during the examination of the liver, kidney, and brain tissues revealed that the applications of thymoquinone and icodextrin did not have a systemic toxic effect. The severe inflammatory processes observed

Table 2: Microscopic scoring of the adhesion severity

Grade	Definition	Severity
Grade 0	No inflammation	-
Grade 1	Mild inflammation; lymphocytic and plasmacytic infiltration	+
Grade 2	Moderate inflammation; infiltration of plasma cells, eosinophils, and leukocytes and mild fibrous tissue proliferation	++
Grade 3	Severe ınflammation; fibrous tissue proliferation, micro abscesses, revascularization	+++

Table 3: Microscopic scoring of the severity of fibrosis

Group	Definition
1. Score when fibrosis formation is absent	0
2. In case of extremely mild activity	1
3. In case of marked connective tissue proliferation	2
4. In case connective tissue proliferation is severe and has gained maturity	3

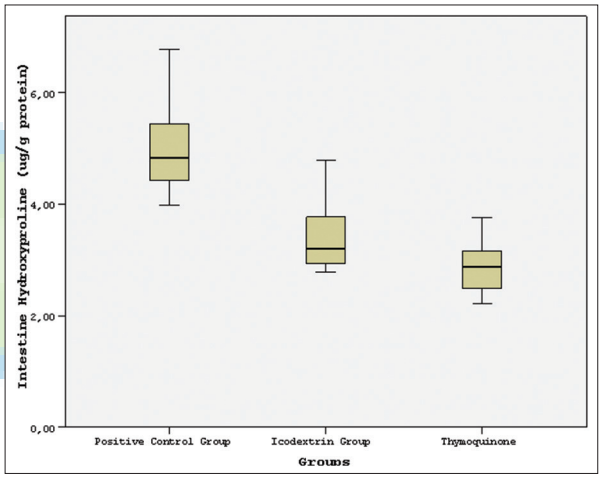

Figure 4: Comparison of hydroxyproline among groups

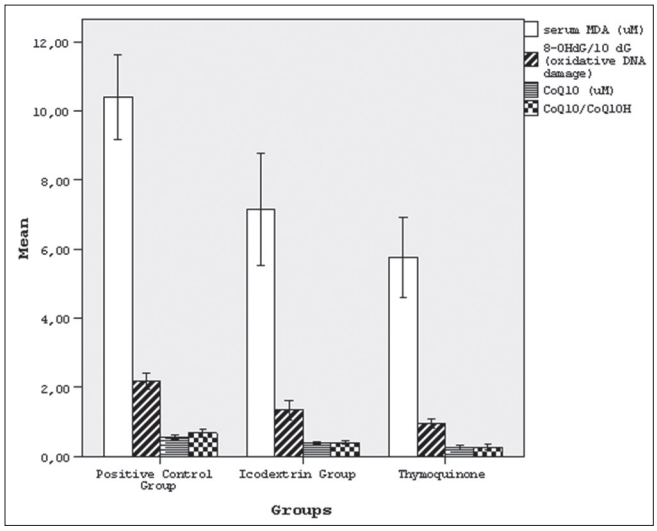

Figure 5: Comparison of biochemical results with respect to groups

in the first group decreased very markedly in the groups treated with icodextrin and thymoquinone. The group receiving thymoquinone was determined to be better, in this respect, than the group receiving icodextrin, although this difference was not significant.

Results of hydroxyproline and biochemical findings

Hydroxyproline levels in the ICO and TQ groups were lower compared with the control group, and the difference between them was statistically significant (ICO, $P < 0.001$ and TQ, $P < 0.000$). Comparison of the ICO and TQ groups revealed hydroxyproline levels to be lower in the TQ group, although this difference between the two groups was not statistically significant ($P < 0.297$) [Figure 4].

Biochemical results

Results for MDA: Mean values in the ICO group (7.1444 ± 1.61) and TQ group (5.7495 ± 1.16) were lower than the value for the control group (10.3936 ± 1.22) ($P < 0.000$). A comparison of the ICO and TQ groups revealed that mean levels in the TQ group were lower, although the difference between the two groups was not statistically significant ($P < 0.122$).

Results for 8-OHdG/10dG: Mean values in ICO group (1.3387 ± 0.277) and TQ group (0.9517 ± 0.14) were lower compared with the control group (2.1675 ± 0.236) ($P < 0.000$). A comparison of the ICO and TQ groups revealed that the mean value of the TQ group was lower, and that this difference was statistically significant ($P < 0.007$).

Results for CoQ10 (uM): Mean values in ICO group (0.5570 ± 0.064) and TQ group (0.2520 ± 0.063) were lower compared with the control group (0.5570 ± 0.064) ($P < 0.000$). A comparison of the ICO and TQ groups revealed that the mean value in the TQ group was lower, and that this difference was statistically significant ($P < 0.000$).

Results for CoQ10/CoQ10H: Mean values in ICO group (0.4002 ± 0.048) and TQ group (0.2631 ± 0.083) were lower compared with the control group (0.6791 ± 0.117) ($P < 0.000$). A comparison of the ICO and TQ groups revealed that the mean value in the TQ group was lower, and that this difference was statistically significant ($P < 0.000$) [Figure 5].

Discussion

To date, various methods and agents have been tested to prevent adhesions from developing in the peritoneum after abdominopelvic procedures. The common objective of all these studies was to block one of the steps leading to the formation of adhesions.[1] Solid or liquid agents that could form a barrier between the damaged surfaces, fibrinolytic agents, and many agents with anti-inflammatory effects have been tested to this end. Nonetheless, an agent that is fully effective in preventing postoperative abdominal adhesions, while also having minimal side effects, has not yet been discovered.[8] Many solid and liquid barriers have been tested in clinical and experimental investigations revealed in the literature. The main solutions used as liquid barriers are crystalloid, dextran, hyaluronic acid and icodextrin solutions. Crystalloid solutions are rapidly absorbed, while solutions with dextran are associated with serious side effects such as transient ascites, edema, and peritonitis; consequently, these agents are not used to prevent adhesions. Currently, the most commonly used liquid barriers are hyaluronic acid and icodextrin.[9] An important advantage of these agents is their longer presence in the abdominal cavity without being absorbed.[9]

Since icodextrin is a large molecule, and can remain in the abdomen for long periods of time without being absorbed, it maintains and isolates damaged serosal surfaces, reducing the formation of adhesions. Icodextrin is a large-molecular-weight glucose polymer that is degraded to maltose and glucose by a-1.4-bound amylase and maltase. Amylase is widely distributed in the body; however, its level of activity is lower in the peritoneal cavity. Consequently, icodextrin placed in the abdominal cavity remains in the abdomen for up to 5 days and reaches the systemic circulation by being slowly absorbed through the lymphatic system.[10] Various studies argue for the antiadhesive effect of icodextrin, although some of these studies and their results are somewhat controversial.[6,7]

Solid barriers are nonabsorbable and bioabsorbable films, gels, or solid membranes. Prospective randomized studies also have demonstrated that bioresorbable membranes, including hyaluronic acid and carboxymethyl cellulose, decrease the incidence and grade of postoperative adhesions.[11] However, it is important to bear in mind that they may also lead to marked impairments in anastomoses, and that their use must be avoided in patients who have undergone intestinal anastomosis.[11,12] Agents that prevent adhesion, other than the ones that form a barrier, generally act by inhibiting one of the steps of adhesion formation, or by increasing fibrinolytic activity. In this context, various agents such as nonsteroidal anti-inflammatory drugs, corticosteroids, calcium channel blockers, antagonists of histamine, antibiotics, fibrinolytic drugs, antioxidants, and vitamins have been tested for this purpose.[9,13-18] TQ is a phytochemical with strong antioxidant properties. In a study by Umar *et al*., it was shown that TQ increases

the activities of antioxidant enzymes glutathione, catalase (CAT), and superoxide dismutase. Furthermore, Umar *et al.* also determined that it suppresses the increases in nitric oxide and myeloperoxidase levels.[19]

Houghton *et al.* described that TQ exerts its anti-inflammatory effect by preventing the production of eicosanoids, such as thromboxane B2 and leukotriene B4.[20] TQ also has an immunomodulatory effect. Studies have shown that it suppresses the production of tumor necrosis factor-alpha and interleukin-6 (IL-6), and decreases the secretion of cytokines, such as IL-1 beta and IL-8 in mixed lymphocyte cultures, as well as their blood and tissue concentrations. TQ decreases tissue damage and edema, mainly through these effects.[21,22]

There are only a few studies related to the antiadhesive effect of thymoquinone. These studies report that thymoquinone has the effect of decreasing intraabdominal adhesion.[23,24]

In our study, macroscopically detected and classified postoperative adhesions were markedly less common in the ICO and TQ groups compared with the control group. A similar result has been histopathologically detected as well, with a markedly decreased formation of connective tissue being identified in the ICO and TQ groups.

Hydroxyproline is produced intracellularly during the course of collagen synthesis. Hydroxyproline level is an important indicator of collagen formation and thus of the severity of adhesion formation. The formation of adhesions and tissue hydroxyproline levels are linearly correlated. In the proliferative phase of wound healing (i.e., between the 5th and 14th days), collagen production increases, leading to higher levels of hydroxyproline in the tissues. Increased collagen production is not a desired situation for antiadhesive effect.[25,26] In the study by Bozdag *et al.*, it was observed that the intraperitoneal application of TQ decreases the hydroxyproline levels, causing intra-abdominal adhesion formation to a lesser extent.[24] In our study, hydroxyproline levels in the ICO and TQ groups were, in agreement with the above-mentioned study, found to be comparatively lower. The lowest level of hydroxyproline was observed in the TQ group; however, this difference was not statistically significant ($P < 0.297$).

Hydroxyproline levels in the liver and kidneys were also evaluated in our study and were found to be statistically significantly lower in the ICO and TQ groups compared with the control group ($P < 0.297$). Furthermore, no pathological signs were observed in any of the groups where the liver, kidney, and brain tissues were examined, indicating that the applications of thymoquinone and icodextrin did not exert a systemic toxic effect.

MDA, an end product of lipid peroxidation, is used to indicate the level of oxidative damage.[27] Plasma and tissue MDA levels are measured as indicators of free radicals.[28] Gotloib *et al*. reported that peritoneal fibrosis and sclerosis caused by oxidative stress are identified with peritoneal adhesions, wrapping of intestinal loops, and the existence of a fibrous tissue layer in an animal PD model.[29] In our study, the mean MDA levels in the ICO and TQ groups were lower compared with the control group, and these differences were statistically significant. A comparison of the ICO and TQ groups revealed that the MDA levels in the TQ group were lower. In light of these results, it may be concluded that thymoquinone protects the tissues from oxidative damage by decreasing lipid peroxidation.[30]

All alterations in the molecular integrity of genetic material, caused by the effects of endogenous or exogenous factors, are defined as DNA damage. DNA damage may occur for reasons such as oxidative stress, ischemia–reperfusion injury, and deficiency of Vitamin B12.

8-Hydroxy-2'-deoxyguanosine/deoxyguanosine (8-OHdG/10dG) is a marker that indicates oxidative DNA damage.[31] Morishita *et al*. reported that oxidative DNA damage is related with peritoneal inflammation, fibrosis, revascularization, and sclerosis.[32] The findings of this study show that peritoneal damage is correlated with the 8-OHdG level. In our study, the value of the 8-OHdG/10dG ratio was found to be significantly lower in the TQ group. These results demonstrate that DNA damage caused by oxidative damage was markedly reduced, especially by thymoquinone.[32]

CoQ10 (ubiquinone) is a vitamin-like compound that acts as a coenzyme in key enzymatic reactions during energy production in cells. It exists in nearly every type of cell and is fat soluble. CoQ10 acts as an electron transporter of the respiratory chain in mitochondria. CoQ10 prevents the initiation of lipid peroxidation and damage to biomolecules by interacting with oxygen-derived radicals and singlet oxygen.[33] It acts with free radicals as an intermediary product and is exposed/subject to electron reduction reactions. Free radicals, which are not stable, attain stability by gaining one electron from ubiquinone. Coenzyme Q is an important antioxidant when it gains this characteristic.[34] The CoQ10/CoQ10H (ubiquinol) ratio is an important marker of oxidative stress,[35] and in our study, this ratio was found to be lower in the ICO and TQ groups compared with the control group. It was determined to be statistically lower in the TQ group compared with both of the other two groups, and this result showed that thymoquinone leads to a lower release of oxygen

radicals with its antioxidant effect, thus protecting tissues from oxidative damage.

Conclusions

Icodextrin was found to be effective in decreasing postoperative adhesions. However, thymoquinone appears to be more effective than icodextrin. If further studies were to be performed, we believe that thymoquinone – a molecule with strong antioxidant and anti-inflammatory effects – would very likely become an agent used by surgeons to decrease the occurrence of postoperative adhesions.

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Conflicts of interest

There are no conflicts of interest.

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