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The effect of oral honey and pollen on postoperative intraabdominal adhesions

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Background/aims: *We evaluated the effect of oral usage of honey and pollen, either separately or together, on postoperative intraabdominal adhesions.* ***Methods:*** *Forty rats were randomly separated into 4 groups of 10 rats each. Abrasion was performed on the cecum, and a patch of peritoneum located opposite to the cecal abrasion was completely dissected. Group 1 rats received no treatment; Group 2 rats received 4 g/kg/day honey; Group 3 rats received 4 g/kg/day pollen; and Group 4 rats received 4 g/kg/day honey and pollen mixed in equal amounts, in addition to the standard feeding for postoperative 21 days. All the rats were sacrificed on the 21st day. Following the adhesion scoring, tissue specimens of the peritoneum and bowel were subjected to histopathological investigation. The tissue and blood specimens were also taken for biochemical analysis to investigate the antioxidant capacity.* ***Results:*** *Adhesion scores were significantly different between the control and other groups. No dense adhesion was observed in the treatment groups. Tissue malondialdehyde levels were significantly different between the control and honey and honey+pollen groups. Superoxide dismutase and glutathione-peroxidase levels were significantly different between the control and other groups. Catalase levels were different between the control and honey groups. Plasma antioxidant levels were different between the control and other groups. The pathological scores for fibrosis and inflammation were significantly different between the control and other groups.* ***Conclusions:*** *Honey and pollen were found to be effective in preventing postoperative intraabdominal adhesions, and these effects were thought to be a result of their antiinflammatory and antioxidant properties.*

Key words: Intraabdominal adhesions, honey, pollen, oxidative stress

Oral bal ve polenin postoperatif intraabdominal adezyonlar üzerine etkisi

Amaç: *Bal ve polenin oral yolla ve tek tek ya da birlikte kullanımının postoperatif intraabdominal adezyonlar üzerine etkisi değerlendirilmiştir.* ***Yöntem:*** *Kırk adet rat, rastlantısal olarak her biri onar adet rat içeren dört gruba ayrıldı. Çekum üzerinde abrazyon oluşturuldu ve bu çekal abrazyon bölgesinin karşısındaki periton parçası eksize edildi. Birinci grup herhangi bir tedavi almadı. Postoperatif 21 gün boyunca; standart beslenmeye ek olarak ikinci gruba 4 g/kg/gün bal, üçüncü gruba 4 g/kg/gün polen ve dördüncü gruba 4 g/kg/gün eşit miktarda karıştırılmış bal ve polen verildi. Tüm ratlar 21. gün sakrifiye edildi. Adezyon skorlamasını takiben; histopatolojik değerlendirme için periton ve barsaktan doku parçaları, ayrıca antioksidan kapasitenin araştırılması amacıyla biyokimyasal analiz için doku ve kan örnekleri alındı.* ***Bulgular:*** *Adezyon skorları, kontrol grubu ile diğer gruplar arasında belirgin olarak farklıydı. Tedavi gruplarında hiçbir ratta yoğun adezyon gözlenmedi. Doku malondialdehit seviyeleri kontrol grubu ile bal ve bal+polen grubu arasında belirgin olarak farklıydı. Süperoksit dismutaz ve glutatyon-peroksidaz düzeyleri kontrol grubu ile diğer gruplar arasında belirgin olarak farklıydı. Katalaz düzeyleri ise kontrol ve bal grupları arasında farklıydı. Plazma antioksidan düzeyleri de kontrol grubu ile diğer gruplar arasında farklıydı. Fibrozis ve inflamasyon yönünden patolojik skorlar kontrol ile diğer gruplar arasında belirgin olarak farklıydı.* ***Sonuç:*** *Bal ve polenin intraabdominal adezyonları önlemede etkili olduğu ve bu etkilerinin bu maddelerin antioksidan ve antiinflamatuvar etkilerine bağlı olduğu sonucuna varıldı.*

Anahtar kelimeler: İntraabdominal yapışıklıklar, bal, polen, oksidatif stres

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INTRODUCTION

Peritoneal adhesions can be defined as abnormal fibrous bands in the abdominal cavity formed between organs or tissues, or both, that are normally separated. Postsurgical adhesions severely affect the quality of life of millions of people worldwide, causing small bowel obstruction, difficult reoperative surgery, chronic abdominal and pelvic pain, and female infertility (1). Although some surgical adjuvants have been developed and evaluated for the purpose of decreasing postsurgical adhesion formation, an ideal agent for use in adhesion prevention in surgical practice has not yet been identified.

Honey is a super-saturated sugar solution produced by honey bees from the nectar of plants. It has a long tradition of use in wound healing and has been referred to extensively in the medical literature since ancient times (2). Honey has bactericidal, bacteriostatic, antifungal, antiviral, scolicidal, antioxidant, antitumoral, and anti-inflammatory effects (3-6).

Pollen is a product collected from many species of plants visited by bees. Pollen and pollen products have been shown to have several beneficial applications for use in humans. It has been successfully used for the treatment of some cases of benign prostatitis and for oral desensitization of children with pollen allergy (7). It has been reported that pollen has free radical scavenging activity (8).

According to the antibacterial, antioxidant and antiinflammatory properties of honey and the antioxidant and antiinflammatory properties of pollen, we planned to use oral honey and pollen to determine their effects on experimental postoperative intraabdominal adhesions and oxidative stress.

MATERIALS AND METHODS

Forty Wistar-Albino male rats, weighing 250 ± 25 g, were housed individually in wire cages under constant temperature (21 ± 2°C) with a 12–hour (h) light-dark cycle. The animals were allowed free access to water and standard rat chow. Twelve hours before anesthesia, animals were deprived of food, but had free access to water 2 h before anesthesia. No enteral or parenteral antibiotics were administered during the study. The procedures in this experimental study were performed in accordance with the National Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Ankara Research and Training Hospital.

Study groups and surgical procedure

Rats were randomly divided into four groups of 10 animals each. All animals were anesthetized by intramuscular injection of 30 mg/kg ketamine hydrochloride (Ketalar®, Parke-Davis, Istanbul) and 5 mg/kg xylazine (Rompun®, Bayer, Istanbul). Liposomal povidone-iodine hydrogel (Repithel®) was purchased from Mundipharma GmbH (Limburg, Germany).

The abdomen was shaved and prepared with povidone-iodine. Under sterile conditions, a midline laparotomy was performed. The cecum was abraded with a sterile gauze until subserosal hemorrhage had developed. A 1x1 cm patch of peritoneum located opposite to the cecal abrasion was completely dissected.

In Group 1 (control group), adhesion induction was performed and no treatment was given. In Group 2 (honey group), after adhesion induction, 4 g/kg/day honey was given by nasogastric route. In Group 3 (pollen group), adhesion induction was performed and 4 g/kg/day pollen was given by nasogastric route. In Group 4 (honey+pollen group), 4 g/kg/day honey and pollen mixed in equal amounts were given by nasogastric route. Animals were allowed access to food and water after the operation. All operations were performed by the same surgeon. Rats were sacrificed on the postoperative 21st day. Adhesions were classified by a surgeon who was unaware of the groups, according to a scoring system based on the evaluation of the appearance, extent and strength of the adhesions (Table 1).

Table 1. Adhesion scoring system

Score	Extent	Appearance	Strength
0	No	No	No
1	< 25%	Filmy, avascular	Separated easily
2	25-50%	Dense, avascular	Separated by traction
3	50-75%	Dense, capillary vascularization	Sharp dissection needed for separation
4	> 75%	Dense, vascular	Sharp dissection needed for separation

**Adhesion score is equal to total amount of each part of adhesion. Possible highest score is 11.*

Peritoneal and intestinal tissue samples were taken for histopathological evaluation. Additionally, blood and intestinal samples were taken for antioxidant activity analyses.

Evaluation of oxidative stress

Postmortem liver samples were taken and kept on an ice bath until homogenization. The tissues were homogenized in serum physiologic solution (20 wt/vol), then centrifuged at 4,000 g for 15 minutes (min), and upper clear supernatants were used in the assays. All the procedures were performed at 4°C throughout the experiments. Protein level of the clear supernatants was studied by Lowry's method. Malondialdehyde (MDA) levels (nmol/mg) and glutathione-peroxidase (GSH-Px) (mIU/mg) enzyme activities were measured in the supernatants. MDA levels were measured by thiobarbituric acid reactive substances method. After the samples were preincubated with fish oil and xanthine–oxidase system at room temperature for 1 h, MDA level was determined. GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm. Plasma levels of MDA and GSH were determined by the same method (17-19).

Histopathological evaluation

The histopathological analyses were carried out in the Pathology Department of Ankara University School of Medicine. Histopathological examination was performed by using light microscopic analyses. The samples obtained from the abraded cecal tissue and the adjacent peritoneal tissue were fixed in 10% neutral buffered formalin solution for 2 days (d). Tissues were washed in running water, and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96% and 100%). After dehydration, specimens were placed into xylene to obtain transparency and embedded in paraffin. Embedded tissues were cut into 5 µm-thick sections and were stained with hematoxylin and eosin and trichrome. Histopathologic examinations were performed by a pathologist blinded to the study groups. The samples stained with hematoxylin and eosin were examined for inflammation, and the presence of fibrosis was evaluated in the hematoxylin/eosin- and trichrome-stained samples using a semi-quantitative scoring system (Tables 2 and 3) (20,21).

Statistical analyses

Data analysis was performed using the SPSS 15.0 package program. Data were presented as mean ± standard deviation. The differences among the groups were evaluated by one-way ANOVA or Kruskal-Wallis variance analysis, where appropriate. When analysis of variance showed a significant difference, the post-hoc multiple comparison test was applied to demonstrate the differences, which were considered statistically significant at $p<0.05$.

RESULTS

No rats died during the study. All rats were sacrificed by high-dose diethyl ether inhalation on the postoperative 21st day.

Adhesion scores

The mean adhesion scores are summarized in Table 4. There was a significant difference between the control group and the honey, pollen, and honey+pollen groups (p values 0.025, 0.035, and 0.025, respectively). The differences between the honey, pollen and honey+pollen groups were not statistically significant ($p>0.05$). Dense adhesion was not seen in any of the treatment groups.

Oxidative stress

The tissue levels of MDA, superoxide dismutase (SOD), GSH-Px, and catalase (CAT) levels are

Table 2. Inflammation scoring system

SCORE	Degree of Inflammation
0	No inflammation
1	Giant cells, lymphocytes, and plasma cells
2	Giant cells, plasma cells, eosinophils, and neutrophils
3	Inflammatory cell infiltration and microabscess formation

Table 3. Fibrosis scoring system

Score	Degree of Fibrosis
0	No Fibrosis
1	Mild
2	Moderate
3	Severe

Table 4. Mean adhesion scores of the groups

Groups	Mean adhesion scores	*p* values
Control	6.00 ± 2.92	
Honey	2.00 ± 1.72	0.025*
Pollen	2.40 ± 1.31	0.035*
Honey+Pollen	2.40 ± 1.51	0.025*

** Significantly different from control group*

summarized in Table 5. According to the MDA levels, there was a significant difference between the control and honey and honey+pollen groups (p=0.013 for honey group, p=0.033 for honey+pollen group), but the difference between the control and pollen groups was not significant (p>0.05). SOD and GSH-Px levels were significantly different between the control and other groups (for SOD levels, p= 0.007-honey, 0.003-pollen, 0.004-honey+pollen; for GSH-Px levels, p= 0.001, 0.004, 0.001, respectively). When we compared the CAT levels, the difference between the control and honey groups was significant (p=0.033), but the difference was not significant between the control and other groups (pollen and honey+pollen) (p>0.05).

The mean plasma MDA and GSH-Px levels of the groups are given in Table 6. There was a significant difference between the control and other groups according to MDA and GSH-Px plasma levels (for MDA, p=0.003, 0.011, and 0.002; for GSH-Px, p=0.009, p=0.001, and 0.028, respectively, for honey, pollen, and honey+pollen groups).

Histopathological results

The histological images of the pathological scores are shown in Figures 1, 2 and 3.

The pathological scores for fibrosis and inflammation are summarized in Tables 7 and 8, and mean pathological scores of the groups are given in Table 9. The differences between the control and honey (p=0.012 for fibrosis; 0.048 for inflammation), pollen (p=0.023 for fibrosis and inflammation), and honey+pollen (p=0.042 for fibrosis; 0.007 for inflammation) groups were statistically significant. There was no significant difference between the honey, pollen and honey+pollen groups (p>0.05).

DISCUSSION

Postoperative adhesions form after trauma to the peritoneal cavity and are a result of the biochemi-

Table 5. The tissue antioxidant levels of the groups

Groups	MDA (nmol/ml)	SOD (U/ml)	GSH-Px (mIU/ml)	CAT (IU/ml)
Control (n=10)	0.586 ± 0.252	25.30 ± 8.93	8.60 ± 1.41	4.57 ± 1.06
Honey (n=10)	0.329 ± 0.181*	48.45 ± 22.62*	34.46 ± 6.78 *	5.94 ± 1.50*
Pollen (n=10)	0.528 ± 0.314	45.50 ± 10.62*	22.28 ± 10.12*	5.89 ± 1.57
Honey+Pollen (n=10)	0.336 ± 0.184*	46.86 ± 19.36*	22.82 ± 10.42*	5.58 ± 1.52

** Significantly different from control group*

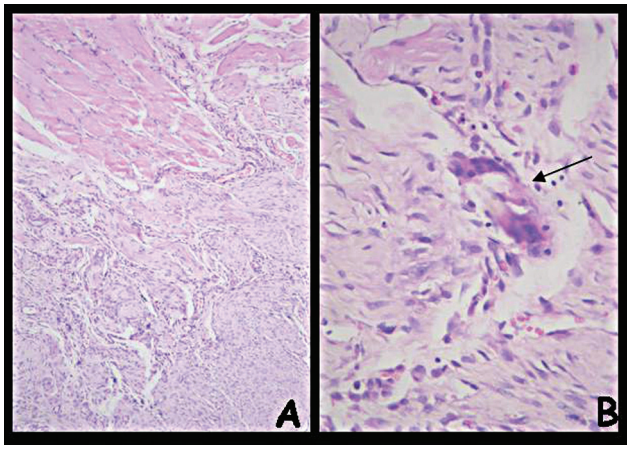

Figure 1. Inflammation Score 1: Mild serosal inflammation (hematoxylin and eosin staining). **A.** Multinuclear giant cells and a small number of lymphocytes and plasma cells with formation of fibrosis are seen (x100). **B.** Phagocytosis of a foreign body by a histiocytic giant cell is seen (marked with arrows) (x400).

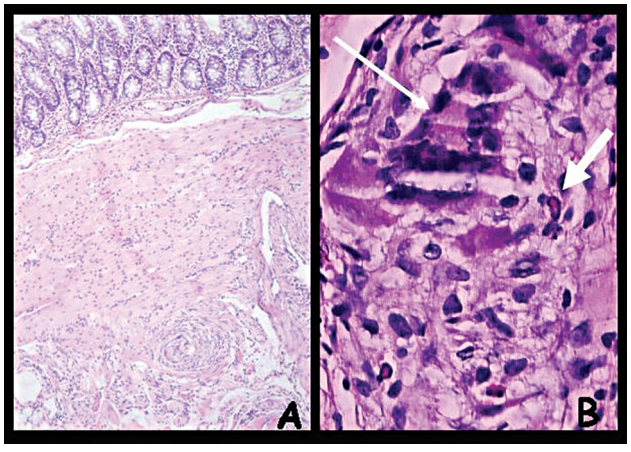

Figure 2. Inflammation Score 2: Moderate inflammation (hematoxylin and eosin staining). **A.** The inflammation focus near the serosal surface of the colon is seen (x100). **B.** Inflammatory cells including multinuclear giant cells (marked with long arrow), leukocytes (marked with short arrow), lymphocytes, and plasma cells are seen (x1000).

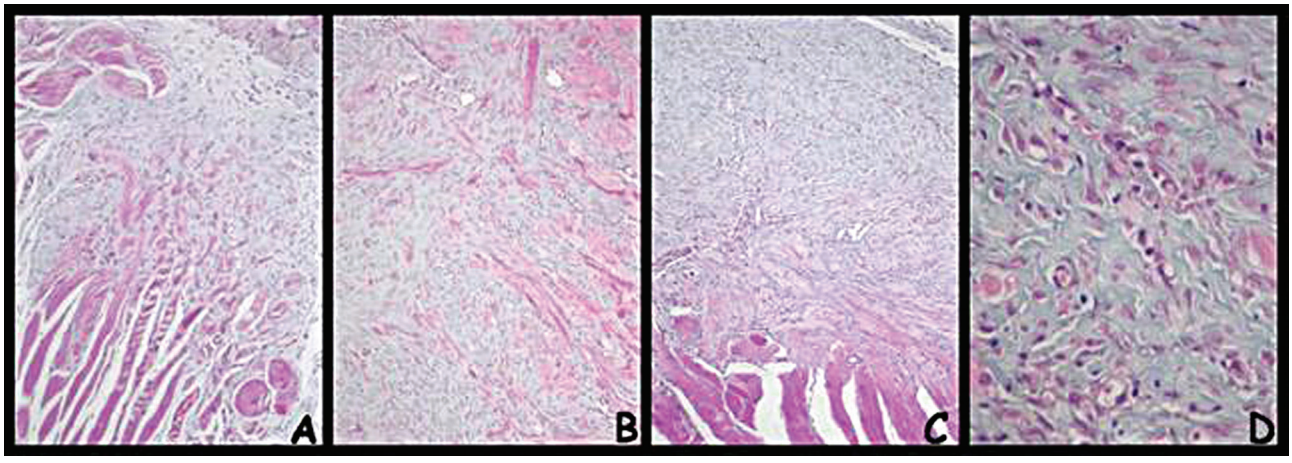

Figure 3. Fibrosis (green), residual muscle fibrils (red). **A.** Mild fibrosis-fibrosis score 1 (x100). **B.** Moderate fibrosis-fibrosis score 2 (x100). **C, D.** Severe fibrosis-fibrosis score 3 (x100, x400) (Trichrome staining).

Table 6. Plasma MDA and GSH-Px levels of the groups

Groups	MDA (nmol/mL)	GSH-Px (mIU/mL)
Control (n=10)	2.7829 ± 1.2141	112.810 ± 24.5302
Honey (n=10)	1.2981 ± 0.8154*	142.560 ± 17.5344*
Pollen (n=10)	1.4116 ± 0.7862*	169.9200 ± 29.2585*
Honey+Pollen (n=10)	1.3106 ± 0.9378*	146.400 ± 48.9114*

** Significantly different from control group*

Table 7. Pathological scores for fibrosis

	Score 0 (n)	Score 1 (n)	Score 2 (n)	Score 3 (n)
Control (n=10)	1	3	3	3
Honey (n=10)	5	4	1	0
Pollen (n=10)	5	3	1	0
Honey+Pollen (n=10)	5	3	1	1

Table 8. Pathological scores for inflammation

	Score 0 (n)	Score 1 (n)	Score 2 (n)	Score 3 (n)
Control (n=10)	1	3	3	3
Honey (n=10)	3	5	2	0
Pollen (n=10)	5	3	2	0
Honey+Pollen (n=10)	6	3	1	0

Table 9. Mean pathological scores of the groups

	Fibrosis	Inflammation
Control (n=10)	1.80 ± 0.32	1.80 ± 0.32
Honey (n=10)	0.60 ± 0.06*	0.50 ± 0.07*
Pollen (n=10)	0.80 ± 0.08*	0.70 ± 0.08*
Honey+Pollen (n=10)	0.70 ± 0.07*	0.70 ± 0.07*

** Significantly different from control group*

cal and cellular response that occurs in an attempt to repair the peritoneum. Adhesions are the leading cause of small intestinal obstruction after abdominal surgery and can be the source of significant morbidity, in some cases leading to mortality. Most of the adhesions are acquired as a result of peritoneal injury, the most common cause of which is abdomino-pelvic surgery. Less commonly, adhesions may form as the result of inflammatory conditions, intraperitoneal infection or abdominal trauma (1).

The biological processes that result in either uncomplicated repair or the development of adhesions include migration, proliferation and/or differentiation of several cell types, including inflammatory and immune cells, mesothelial cells and fibroblasts. Substances produced locally by these cells regulate tissue remodelling and angiogenesis, as well as synthesis and deposition of the extracellular matrix, which are central to the development of adhesions (14).

The goal of adhesion prevention is to abolish or reduce the incidence, severity, extent, and consequences of adhesions while retaining normal healing and preventing infection. Over the years, several strategies to prevent postoperative adhesion formation have been proposed, based on what has been learned about the underlying pathophysiology. Unfortunately, although numerous different strategies have been evaluated, few have been successful, and some have even been deleterious. To this day, there are no means of completely preventing postoperative adhesion formation. Since careful surgery does not eliminate or prevent adhesion

formation completely, there are some surgical adjuvants that have been developed and evaluated for the purpose of decreasing postsurgical adhesion formation, such as nonsteroidal antiinflammatory drugs, corticosteroids, streptokinase, dextran, heparin, oxidized regenerated cellulose, polytetrafluoroethylene, hyaluronan, and carboxymethylcellulose (1).

Many studies have tried to explain the underlying mechanisms in adhesion formation. The results of these studies have shown that reactive oxygen species and inflammatory reactions play important roles in adhesion formation. Raa et al. (15) demonstrated that reactive oxygen species had an important role in the complex pathophysiology of postoperative adhesion formation and scavenging reactive oxygen species *in vivo*. Roy et al. (16) demonstrated the colocalization of inflammatory cells and their derivative reactive oxygen species in human peritoneal tissue. They suggested that adhesions were not nonfunctional scar tissue, but that they were highly cellular, vascularized and dynamic structures containing inflammatory cells, oxidants and angiogenic factors.

Honey has a long tradition of use for wound healing and has been referred to extensively in the medical literature since ancient times. Honey has bactericidal, bacteriostatic, antifungal, antiviral, scolicidal, antioxidant, antitumoral, and anti-inflammatory effects (3-6). The antiinflammatory action of honey has been investigated, but no definite mechanism has been identified (2).

The antioxidant properties of honey have been well documented in recent studies (17,18). Schramm et al. (17) found that phenolic antioxidants from processed honey were bioavailable, and they increased the antioxidant activity of plasma. Gheldof et al. (18) also showed that the *in vivo* serum antioxidant capacity increased significantly in humans following consumption of buckwheat honey. These studies showed that the antioxidant effect of honey was not only local, but also had a systemic effect.

The adhesion preventive effect of honey, at least in part, might be due to its antiinflammatory activity. It provides glucose supply for leukocytes, modulates the activation state of immunocompetent cells, affects the proliferative activity of human B and T lymphocytes and the activity of phagocytes, and stimulates human myeloid cell lines (19,20).

In an experimental study, Bothin et al. (21) showed that bacterial flora play a significant role in adhesion formation. The antimicrobial effects of honey have been well documented in many studies (3-5). This effect of honey may play an important role in postoperative adhesion prevention.

Aysan et al. (22) and Yuzbasioglu et al. (23) found that intraperitoneal honey administration reduced postoperative peritoneal adhesions. We evaluated colonic anastomotic healing and postoperative intraabdominal adhesion formation in an experimental study and found that oral administration of honey significantly reduced adhesion formation. We concluded that this effect of honey might be attributable to its antioxidant and antiinflammatory effects (24). In the present study, we found that honey and pollen had positive effects on preventing postoperative intraabdominal adhesions, as shown previously for honey in the abovementioned studies.

Pollen is a product collected from many of species of plants visited by bees. In general, compared to many standard human foods, pollen is rich in protein and low in fat, and possesses a wealth of minerals and vitamins. Pollen and pollen products have been shown to have several beneficial applications for human use. It has been successfully used for the treatment of some cases of benign prostatitis and for oral desensitization of children with pollen allergy (7,25,26).

It has been reported that both pollen and propolis extracts and their respective isolated compounds have free radical scavenging activity (27). This property of pollen seems to be important in the prevention of various diseases such as cancer, cardiovascular diseases and diabetes, among others. Lee et al. (28) found that pine pollen, as a kind of Chinese traditional medicine, was a potential antioxidant and beneficial in inflammatory conditions.

In the present study, we evaluated postoperative adhesion formation and found a significant difference between the control group and the honey, pollen and honey+pollen groups. The differences between the honey, pollen and honey+pollen groups were not statistically significant. Dense adhesion was not seen in any of the treatment groups.

For evaluating the underlying mechanism of this adhesion preventive effect of honey and pollen, we determined tissue levels of MDA, SOD, GSH-Px, and CAT, and blood levels of MDA and GSH-Px. We also evaluated the antiinflammatory effects of

honey and pollen by histopathological examination. According to tissue MDA levels, there was a significant difference between the control and the honey and honey+pollen groups, but the difference between the control and pollen groups was not significant. SOD and GSH-Px levels were significantly different between the control and other groups. When we compared the CAT levels, the difference between the control and honey groups was significant, but the difference was not significant between the control and other groups. We found a significant difference between the control and other groups according to MDA and GSH-Px plasma levels. These results indicated that the antioxidant effects of honey and pollen (not only local, but also systemic) might have a role in the adhesion preventive effects of these substances. Some oxidative stress parameters, such as tissue MDA and CAT levels, were not different between the control and pollen groups. We suggest that these inefficacious results might be due to inadequate doses of pollen.

The pathological scores for fibrosis and inflammation were significantly different between the control and other groups. There was no significant difference between the honey, pollen and honey+pollen groups. These results highlight that the antiinflammatory effects of honey and pollen might have a role in the adhesion preventive effects of these substances.

In conclusion, the administration of oral honey and pollen reduced postoperative adhesions significantly. Although further studies are needed for evaluation of the exact mechanism, we concluded that the adhesion preventive effect of honey and pollen might be due to the antiinflammatory, antioxidant and partly antimicrobial effects of these substances. According to the data obtained from this study, pollen was less effective than honey, but these results might be attributable to an inadequate dose of pollen as used in this study. Since there was no standard dose for pollen in the literature, ideal doses of pollen should be evaluated in further studies.

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