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Role of intraperitoneal propolis treatment in preventing postoperative peritoneal adhesions

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ABSTRACT

This study aimed to evaluate the anti-inflammatory and anti-oxidant properties of propolis and its effect on the fibrinolytic system in postoperative adhesions.

The rats were randomly divided into 3 groups of 10, and a modified cecal abrasion model was created. Group 1 was the sham group. Group 2 received 0.4 mL of ethanol (96%), while Group 3 was administered 0.4 mL of propolis (900 mg/kg) and ethanol (96%) solution intraperitoneally. The adhesions were evaluated macroscopically and microscopically.

The adhesion in the propolis group was significantly higher according to the scores of Nair (ethanol–propolis $P > 0.002$ and sham–propolis, $P > 0.023$) and Zühlke (ethanol–propolis $P > 0.033$ and sham–propolis $P > 0.024$). Propolis administration was significantly associated with increased fibrosis ($P > 0.01$) and vascular proliferation ($P > 0.01$).

The increase in peritoneal adhesion in the propolis group might have been due to propolis being retained in the abdomen longer than normal and the higher concentration of the solvent.

Key words: Anti-inflammatory, anti-oxidant, peritoneal adhesion, propolis

INTRODUCTION

Intra-abdominal adhesions are a dynamic, fibroproliferative, and inflammatory defense mechanism created by the peritoneum against damage (1). Fibrous adhesions occur 10 days after peritoneal injury and reach maximum levels in 2–3 weeks. The most important natural protection mechanism preventing the formation of adhesions is the fibrinolytic system. The balance between plasminogen activators and inhibitors is crucial in determining the formation of normal healing or adhesion formation. Therefore, plasminogen activator inhibitor-1 (PAI-1) is considered to be the most important factor in the development of adhesions, and a high concentration of PAI-1 is found in the peritoneal tissue of patients with accompanies large adhesions (2). Postoperative adhesions constitute an important problem for surgeons in terms of reoperations (3). Propolis is a honey bee product containing plant tissue resin (4). Mouse and rabbit studies have shown that the hydroalcoholic solution of propolis has an anti-inflammatory effect after its topical application, injection, or oral administration (5). This study aimed to evaluate the anti-inflammatory and anti-oxidant properties of propolis and its effect on the fibrinolytic system in postoperative adhesions.

MATERIAL AND METHODS

This study was conducted experimentally at Baskent University Laboratory of Experimental Animals. Prior to the study, the ethical committee approval (project number DA 13/58) was obtained. Thirty male young Sprague–Dawley rats (aged 6–9 months) weighing between 284 and 405 g (mean 342 g) were used in the study. The rats were kept at room temperature at 21–23°C during the study, provided standard food and water, and monitored for 21 days. Before the surgery, fasting was not performed to prevent stress factors in the animals. All surgical

procedures were performed in a clean environment. Anesthesia and analgesia were provided using 7 mg/kg intraperitoneal (IP) xylazine and 50 mg/kg IP ketamine. After anesthesia, the abdominal skin was shaved, and the surgical field was cleaned using 10% povidone–iodine solution. After the rats were covered with sterile drapes, a 3-cm midline incision was made. Using the modified cecal abrasion model (6), an abrasion was created by rubbing a gauze sponge 60 times on a 1-cm^2 area on the cecum wall. Subsequently, the 1-cm^2 area was excised in the parietal peritoneum adjacent to the cecum. Powder-free gloves were used during the procedure. Extreme care was taken to avoid perforation and excessive bleeding. After the intestines were replaced into the abdomen, the midline was closed with continuous sutures using 5/0 monocryl.

Study groups

Propolis originating from the Rize–Artvin region was obtained from Hacettepe University to be used in the study. Ethanol was chosen as the solvent because it is known as one of the best solvents of propolis. A 96% solution was used due to the better solubility of ethanol at higher concentrations. Three experimental groups, each consisting of 10 rats, were formed. Group 1 was the sham group, in which the abdomen was closed without applying any solution. After the same abrasion procedure, Group 2 was administered 0.4 mL of IP 96% ethanol solution while Group 3 received 0.4 mL of the prepared solution mixture (900 mg/kg propolis and 96% ethanol) intraperitoneally, and the abdomen was closed. The rats were sacrificed on the 21st day after the procedure, and the thorax was opened to collect approximately 10 mL of intracardiac blood. The abdomen was opened using an inverted-U incision, and intra-abdominal adhesions were graded macroscopically using the Nair adhesion score scale (7).

The bands and the tissues attached to the bands were fixed with 10% formaldehyde solution and embedded in paraffin blocks. Then, the sections were cut and stained with hematoxylin–eosin (H&E) and trichrome. Adhesions were microscopically classified using Zühlke scoring (8). In addition to the Zühlke classification, all rats were microscopically examined for vascular proliferation (mild, moderate, and severe), inflammation (mild and moderate), fibrosis (mild, moderate, and severe), and foreign body reaction (present and absent). Tissue plasminogen activator (TPA), PAI-1, prostaglandin E2 (PGE2), α2-macroglobulin, α1-antitrypsin, malondialdehyde (MDA), and glutathione (GSH) concentrations in the blood samples were examined for the evaluation of the fibrinolytic and oxidative systems.

Statistical analysis

The Kruskal–Wallis test was used to test the significance of the differences in the mean values of three or more groups for the data that did not show normal distribution. The groups that caused significant differences were determined using the Bonferroni–Dunn test, which allowed comparing the mean rank scores of groups. The chi-square test was used to compare the qualitative categorical variables between the groups. The hypothesis was that a statistically significant difference existed between at least two study groups. The values were compared using a P value of 0.05, in which $P < \alpha$ was considered to indicate statistical significance while $P > \alpha$ was not statistically significant.

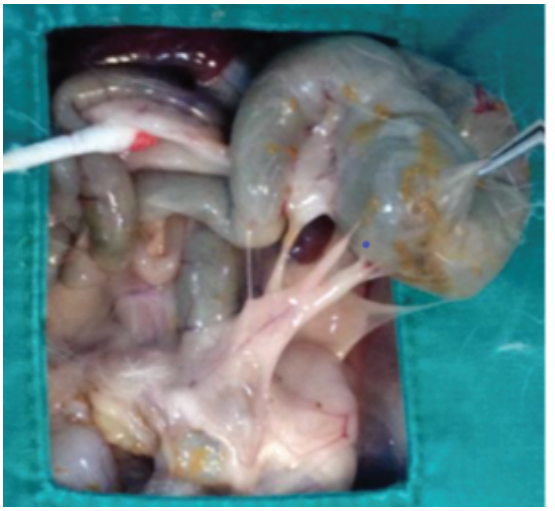
RESULTS

The results of macroscopic and microscopic evaluations are given in Table 1. More macroscopic adhesions were observed in the propolis group, with a statistically significant difference ($P = 0.002 < 0.05$). The paired comparisons of the Nair adhesion scores revealed statistically significant differences between the ethanol and propolis groups ($P = 0.002 < 0.05$) and between the sham and propolis groups ($P = 0.023 < 0.05$).

Adhesion was observed more in the propolis group ($P = 0.011 < 0.05$). The paired comparisons of the microscopic Zühlke adhesion scores showed that the differences between the ethanol and propolis groups ($P = 0.033 < 0.05$) and those between the sham and propolis groups ($P = 0.024 < 0.05$) were statistically significant. In the propolis group, more fibrosis ($P = 0.001 < 0.05$), vascular proliferation ($P = 0.010 < 0.05$), and foreign body reaction ($P = 0.007 < 0.05$) were observed.

The intra-abdominal photographs of the rats in the propolis group are presented in Figure 1, in which propolis appears yellow on the organs inside the abdomen. According to the Nair scale, six rats had grade 3 adhesions and four had grade 4 adhesions.

Grade 3: Multiple bands.



Grade 4: Adhesion to the abdominal wall.

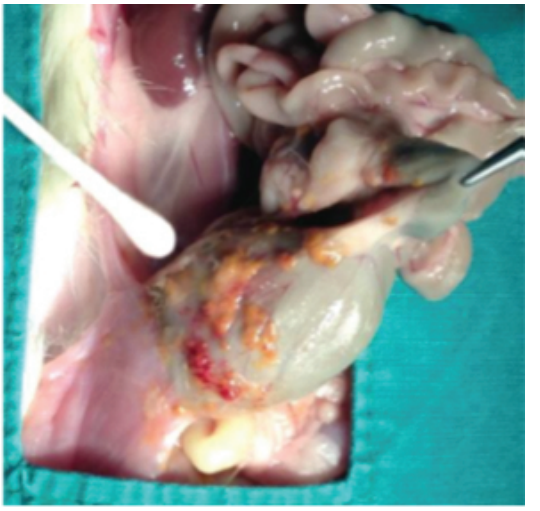


Figure 1 Macroscopic appearance of the abdomen of the rats administered propolis.

The fibrinolytic and oxidative system parameters were evaluated during biochemical analysis, and the results are given in Table 2. According to the data obtained, the PAI-1 values ranged from 19.0 pg/mL to 21.1 pg/mL in the sham group, 18.3 pg/mL to 22.3 pg/mL in the propolis group, and 18.0 pg/mL to 25.4 pg/mL in the ethanol group. The PAI-1 variable was not statistically significantly different between the three groups ($P = 0.082 > 0.05$). The TPA values varied between 1.31 ng/mL and 5.18 ng/mL in the sham group, 1.30 ng/mL and 3.45 ng/mL in the propolis group, and 1.08 ng/mL and 7.66 ng/mL in the ethanol group. The TPA levels in the sham group were statistically significantly higher ($P = 0.029 < 0.05$).

Table 2 Macroscopic and microscopic evaluation of the groups

Group		Sham ($n = 10$)	Ethanol ($n = 10$)	Propolis ($n = 10$)	P value
Nair classification	0		2		
	1	1	4		
(grade)	2	6	1		
	3	3	2	6	
	4		1	4	($P = 0.002 < 0.05$)
Zühlke classification	1	2			
(grade)	2	5	8	2	
	3	3	2	6	
	4			2	($P = 0.011 < 0.05$)
Fibrosis	Mild	6	7		
	Moderate	3	3	4	
	Severe	1		6	($P = 0.001 < 0.05$)
Inflammation	Mild	5	7	6	
	Moderate	5	3	4	($P = 0.668 > 0.05$)
Vascular proliferation	Mild	2	4		
	Moderate	6	6	5	
	Severe	2		5	($P = 0.010 < 0.05$)
Foreign body reaction	Present	4	2	9	
	Absent	6	8	1	($P = 0.007 < 0.05$)

Table 2 Comparison of the biochemical parameters of the groups

Group	Sham (n = 10) (mean rank)	Ethanol (n = 10) (mean rank)	Propolis (n = 10) (mean rank)	P value
PAI-1	18.45	10.45	17.6	0.082 > 0.05
TPA	20	10.65	15.85	0.029 < 0.05
α1-Antitrypsin	12.3	21.75	12.45	0.023 < 0.05
α2-Macroglobulin	10.3	24.1	12.1	0.001 < 0.05
PGE2	14.7	16.15	15.65	0.932 > 0.05
GSH	20.8	14.8	10.9	0.040 < 0.05
MDA	13.45	15.35	17.7	0.554 > 0.05

GSH: glutathione, MDA: malondialdehyde, PAI-1: plasminogen activator inhibitor-1, PGE2: prostaglandin E2, TPA:tissue plasminogen activator

The ranges of the α1-antitrypsin values were determined as 3.01–7.62 mg/mL for the sham group, 1.35–7.97 mg/mL for the propolis group, and 3.82–8.95 mg/mL for the ethanol group (P = 0.023 < 0.05). The α12-macroglobulin values were found to range from 0.51 µg/mL to 4.68 µg/mL in the sham group, 0.49 µg/mL to 10.47 µg/mL in the propolis group, and 4.08 µg/mL to 20.60 µg/mL in the ethanol group; α12-macroglobulin level was significantly higher in the ethanol group (P = 0.001 < 0.05).

The PGE2 values varied between 0.21 ng/mL and 2.15 ng/mL in the sham group, 0.32 ng/mL and 1.86 ng/mL in the propolis group, and 0.44 ng/mL and 3.01 ng/mL in the ethanol group. The PGE2 variable did not show a statistically significant difference between the groups (P = 0.932 – 0.05).

The GSH value, an oxidative stress parameter, was determined as 1.10–5.57 µmol/g in the sham group, 1.15–3.49 µmol/g in the propolis group, and 1.38–3.97 µmol/g in the ethanol group. The GSH levels were higher in the sham group (P = 0.040 < 0.05). Finally, the range of MDA value was 4.5–14.8 nmol/g, 4.7–18.4 nmol/g, and 4.3–18.1 nmol/g for the sham, propolis, and ethanol groups, respectively, with no statistically significant difference between the groups (P = 0.554 > 0.05).

DISCUSSION

The present study was valuable due to the limited number of published articles on intraperitoneal propolis administration to prevent peritoneal adhesions. A study conducted in Brazil reported that that propolis contained various components through which it exhibited anti-inflammatory and anti-oxidant properties and effects on the fibrinolytic system and wound healing; also, ethanol was the best solvent for propolis (9). In the present study, more adhesions were seen in the propolis group according to the macroscopic Nair adhesion scale and microscopic Zühlke adhesion scale, and the results were statistically significant. The microscopic examination also revealed that the propolis group had more vascular proliferation, fibrosis, and foreign body reaction compared with the other groups at a statistically significant level. However, the microscopic evaluation of inflammation showed no significant difference between the three groups. Murtaza et al. reported that the administration of intraperitoneal caffeic acid phenethyl ester reduced inflammation and fibrosis in a chronic colitis model (10). In another study performed on 40 rats, Aysan et al. applied honey to the abrasion area and left 5 mL of honey in the peritoneal cavity in one group of rats. In contrast, in the control group, they washed the area with intra-abdominal saline after abrasion and left 5 mL of saline in the peritoneal cavity. The authors sacrificed the rats on the tenth day and macroscopically observed less adhesion in the honey group; the difference between the two groups was statistically significant. They attributed the reducing effect of honey on adhesion to its efficacy in accelerating wound healing and acting as a barrier due to its high density resulting in its slow

absorption from the peritoneal cavity (11). Mirzoeva *et al.,* who treated rats with a propolis-enriched diet for 3 weeks and compared them with the group fed a standard diet, reported that the inflammatory biochemical values were high in the propolis group and suggested that this paradox was adapted to macrophages and triggered the inflammatory process against the long-term anti-inflammatory response. In the same study, the inflammation-reducing effect of propolis components was shown to be dose dependent (12). Sforcin found that the short-term administration of propolis provided better results for the immune system (13). In another study, Askari et al. reported that the effects of oral propolis were dose dependent and increased adhesion through anti-inflammatory and anti-oxidant effects in parallel to the increase in dose (14). In the present study, it was considered that despite the anti-inflammatory effect of propolis, its presence in the peritoneal cavity for 3 weeks caused the adaptation of the inflammatory system and re-stimulated inflammation, and therefore similar rates of inflammation were observed in all groups. In addition, during the sacrifice process, yellowish particles were macroscopically found on the organs in the abdomen, and adhesions were higher in these areas in the propolis group. The function of propolis as a mechanical barrier was not observed as previously reported for the honey application; on the contrary, propolis increased foreign body reaction, thus resulting in a higher level of vascular changes and fibrosis. This was explained by the prolonged presence and high amount of intraperitoneal propolis.

A peritoneal injury activates the inflammatory system and leads to an increase in the level of PGE2 in the peritoneal fluid and inhibition of plasminogen activator activity (PAA). PGE2 triggers the genesis of adhesions, and the inhibition of PAA results in decreased fibrin degradation. The balance between plasmin activators and inhibitors determines adhesion formation (2). The present study evaluated the inhibitory and activating factors affecting adhesion and examined inflammation through different pathways. No statistically significant difference was observed between the three groups in terms of the PGE2 values. Mirzoeva et al. showed that 80 mg/kg propolis (dissolved in less than 1% ethanol) in the form of intraperitoneal lavage reduced the PGE2 level, but the PGE2 level did not change in a separate group of rats fed propolis orally by 0.2% of their weight (12). Considering these findings, it was concluded that the PGE2 levels were affected by the administration of propolis dose-dependently in the present study. The TPA levels were statistically significantly higher in the sham group, but no statistically significant difference was found in PAI-1 levels between the three groups. Cardenas *et al.* reported that increased TPA levels correlated with decreased PAI-1 levels (15).

The present study found that the parameters of the pro-fibrinolytic and anti-fibrinolytic system were not correlated according to the macroscopic and microscopic analyses. The co-existence of high PAI-1 and TPA levels in the propolis group was attributed to the formation of fibrin above the fibrin degradation capacity of peritoneal plasma. The levels of plasma inhibitors α1-antitrypsin and α2-macroglobulin were statistically significantly higher in the ethanol group. Despite the lack of a significant difference in adhesion formation between the sham and ethanol groups in macroscopic and microscopic examinations, the presence of high levels of plasmin inhibitors was explained by the fact that when ethanol was administered together with propolis as a solvent, the inflammatory effect of the former was suppressed by the anti-inflammatory effect of the latter. Concerning the remaining parameters, the GSH levels were statistically significantly higher in the sham group, while the MDA values did not differ statistically significantly between the three groups. In their study on 40 rats, Celepli et al. showed that the oral application of honey and pollen reduced the MDA levels, simultaneously increased the GSH levels, and decreased fibrosis, inflammation, and adhesion (16). In the present study, the lowest GSH level and the highest MDA level were found in the propolis group. The anti-oxidant effect of propolis was suppressed by the immune system due to adaptation and the process changed in an inflammatory direction.

In conclusion, the IP administration of propolis solution (900 mg/kg propolis and 96% ethanol) increased macroscopic and microscopic adhesions, as well as fibrosis, vascular proliferation, and foreign body reaction. The effect of propolis on increasing intraperitoneal adhesions contrary to previous reports

was considered to be due to its longer presence in the peritoneal cavity than normal, the high amount of propolis, and the high concentration of its solvent. It is recommended IP administration of propolis by providing shorter-term contact in the form of peritoneal lavage, reducing the concentration of the solvent, and reconsidering a lower dose adjustment for propolis.

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