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The Comparison of the Effects of Ellagic Acid and Diclofenac Sodium on Intra-Abdominal Adhesion: An In Vivo Study in the Rat Model

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Peritoneal adhesions are seen frequently after abdominal surgery and can cause serious complications. We aimed to evaluate the effects of the oral use of diclofenac sodium and ellagic acid on formation of postoperative adhesions in rats Studies have shown that agents with anti-inflammatory properties and antioxidant substances can prevent adhesion by decreasing oxidative stress. We compared and evaluated the effects of ellagic acid that has strong antioxidant and anti-inflammatory properties and the nonsteroidal anti-inflammatory diclofenac sodium on peritoneal adhesion development in our experimental study. Laparotomy was performed with a midline incision under general anesthesia and an adhesion model was created on the antimesenteric side of the cecum in Groups I, II, and III. Group I received 85 mg/kg ellagic acid and Group II, 50 mg/kg diclofenac sodium through the nasogastric catheter while Group III received no medication. Only laparotomy was performed in Group IV. The rats were sacrificed at the end of the 14th day. Following macroscopic scoring, tissue samples were removed and subjected to biochemical and histopathologic evaluation. The degree of adhesion and the

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malondialdehyde level were decreased ($P < 0.05$), and glutathione level increased ($P < 0.05$) in Group I compared to Group II and Group III. The effects of ellagic acid on the prevention of peritoneal adhesion were found to be stronger than diclofenac sodium. This can be explained by the fact that ellagic acid is a strong antioxidant and decreases oxidative stress with anti-inflammatory and anti-angiogenic effects.

Key words: Ellagic acid – Diclofenac sodium – Intraabdominal adhesion – Rat

Peritoneal adhesions are fibrotic adhesions that are formed intra-abdominally on the visceral or peritoneal surface during the healing of peritoneal injury.[1,2] Adhesions often occur after laparotomy and are also a significant cause of postoperative morbidity.[3,4] Adhesions can be asymptomatic but can also lead to serious complications such as intestinal obstruction, perforation and fistula.[3–6]

Adhesion formation begins with mesothelial cell injury on the peritoneal membrane surface. An inflammatory process consisting of cellular elements of small venules and fibrin exudation, edema, and hyperemia begins in this region and a serous exudates develops.[5,7–9] Fibrous bands develop between fibrinous exudate and serosal surfaces, leading to formation of fibrinous bridges. Mesothelial cells produce plasminogen activator that dissolves fibrin clots, and the fibrinolytic system is engaged causing the fibrinous exudates to be rapidly resorbed. Adhesions are formed otherwise.[10–12]

DS (diclofenac sodium) is a nonsteroidal anti-inflammatory agent often used in clinical practice for postoperative analgesia and is known to have a fibrinolytic effect.[13,14] EA (ellagic acid) is a natural antioxidant reported to be present in walnuts, carrots, tomatoes, pomegranate, grape juice, grape wine, blueberries, blackberries, and strawberries in significant quantities.[15–18] EA is a phenolic acid derivative that inhibits lipid peroxidation in addition to its anti-inflammatory, antiproliferative, anti-angiogenic and anticarcinogenic effects.[16,17]

We aimed to evaluate the effects of the oral use of DS that has an anti-inflammatory effect and EA that has an antioxidant effect in addition to its anti-inflammatory effect on intra-abdominal adhesion development in comparison in the present study.

Material and Methods

This study was conducted with the permission of Kafkas University's Local Ethics Committee for Animal Experiments (KAÜ-HADYEK - 2012-31).

Animals: This study was conducted on 32 Wistar Albino female rats, 12 weeks of age, with a weight of 250 to 300 g in 4 groups with 8 animals in each group.

Group I: The EA group (85 mg/kg ellagic acid was administered orally, a daily single dose after the surgery),

Group II: The DS group (50 mg/kg diclofenac sodium was administered orally, a daily single dose after the surgery),

Group III: The control group (only an adhesion model was created but no drug was administered),

Group IV: Sham group (only laparotomy was performed).

Drug administration: Orogastric gavage was used in a daily single dose and 85 mg/kg EA (ellagic acid, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) was administered to Group I, and 50 mg/kg DS (Dikloron 50 mg, tablet, DEVA, Tekirdağ, Turkey) to Group II while no drug was administered in Group III and Group IV.

Surgical procedure: Rats were kept in the laboratory environment for a week before the experiment in all study groups and were fed with *ad libitum* standard rat chow and water. The rats were anesthetized by using intraperitoneal 10 mg/kg xylazine HCl (Rompun 2%, Bayer, Istanbul, Turkey) and 80 mg/kg ketamine HCl (Ketasol, Interhas, Turkey) combination. The ventral abdominal area was prepared for aseptic surgery and a midline incision of 3 cm was made. The cecum was revealed in Groups I, II, and III. Its anterior wall was determined and rubbed with a gauze pad held with a clamp until serous punctate hemorrhages developed. Once the adhesion model was created, the abdomen was closed in accordance with the anatomy (Fig. 1). Only laparotomy with a midline incision was performed in the Sham group (Group IV). After completion of all procedures in each group, the abdominal wall was closed in accordance with the anatomy. The animals were housed in individual cages and were postoperatively followed. A second-generation cephalosporin was adminis-

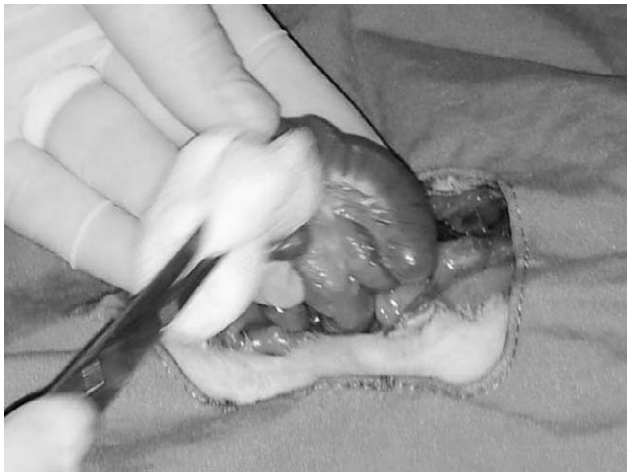

Fig. 1 Model of adhesion: The cecum was exposed and its anterior wall rubbed with a gauze held in a clamp until serosal punctate hemorrhages developed in Groups I, II, and III.

tered to the gluteal muscle for a total of 3 pre- and postoperative days.

Macroscopic evaluation: The rats were sacrificed by cervical dislocation on the 14th day under general anesthesia. A "U" incision was made in the abdomen, the abdominal wall was retracted and maximum visibility was provided. The adhesions were quantitatively evaluated and scored blindly by 2 physicians using the classification described in the literature.[18–20]

Grade 0: No adhesion

Grade 1: A single adhesive band between the organs or between the organ and abdominal wall (Fig. 2a)

Grade 2: Two adhesive bands between the organs or between the organ and abdominal wall (Fig. 2b)

Grade 3: More than two adhesive bands between the organs or between the organ and abdominal wall, or adhesions of intestinal loops with or without adhesions to the abdominal wall (Fig. 2c)

Grade 4: Viscera directly attached to the abdominal wall (Fig. 2d)

Biochemical evaluation: Tissue samples were taken from the area where the suture was placed in the cecum and malondialdehyde (MDA) and glutathione (GSH) measurements were performed spectrophotometrically according to the method reported by Beutler *et al* and Yoshioka *et al* (UV-1201, Shimadzu, Japan).[21,22]

Histopathologic evaluation: Adhesive bands were histopathologically evaluated with the affected organs in rats with adhesions. The parietal peritoneum and anterior cecum were resected and evaluated histopathologically in rats without adhesions. 5-μ-thick sections obtained from the paraffin blocks were stained with hematoxylin-eosin (HE), evaluated under a light microscope (Leica DM4000B) and recorded (Leica DFC280). Grading of lesions was performed in 10 different fields and the same magnification (HE × 100) based on inflammatory and necrotic changes as below:

(–) No lesion found
(+) Mild lesions (<10%)
(++) Moderate lesions (10–50%)
(+++) Severe lesions (<50%)

Statistics: The SPSS version 18 software (SPSS Japan Inc.) was used for statistical analysis of all data obtained from the study. Mean and standard deviations that are the central distribution criteria were calculated with statistical analysis and the differences of nominal values between the groups were evaluated using Fisher's definite chi-square

Table 1 Macroscopic, histopathologic, and biochemical results for Kruskal–Wallis H test analysis

		Groups				Process of statistical value		
Parameters		Group I (n = 6)	Group II (n = 7)	Group III (n = 6)	Group IV (n = 7)	X^2	*P* values	Differences rows
Macroscopic adhesion		13.08	18.00	19.42	4.29	16.883	0.001	1–3,2–3,3–4
Biochemical	MDA	13.42	23.57	28.00	4.00	24.577	0.000	1–2,1–3,1–4,2–4,3–4
	GSH	19.42	11.64	7.42	29.36	19.991	0.001	2–4,4–5
Histopathologic findings	Edema and hyperemia	13.57	15.29	17.83	7.00	8.653	0.034	3–4
	Inflammatory cell infiltration	8.36	18.86	19.75	7.00	17.691	0.001	1–4,2–3,1–3,2–3
	Fibrosis	11.83	14.14	18.50	10.00	7.208	0.066	-
	Adhesion	10.67	14.07	21.50	8.50	14.650	0.002	1–2,2–3
	Necrosis	10.00	11.67	21.57	10.00	17.910	0.000	1–4,3–4,4–2

Group I: EA, Group II: DS, Group III: Control, Group IV: Sham ($P < 0.05$).

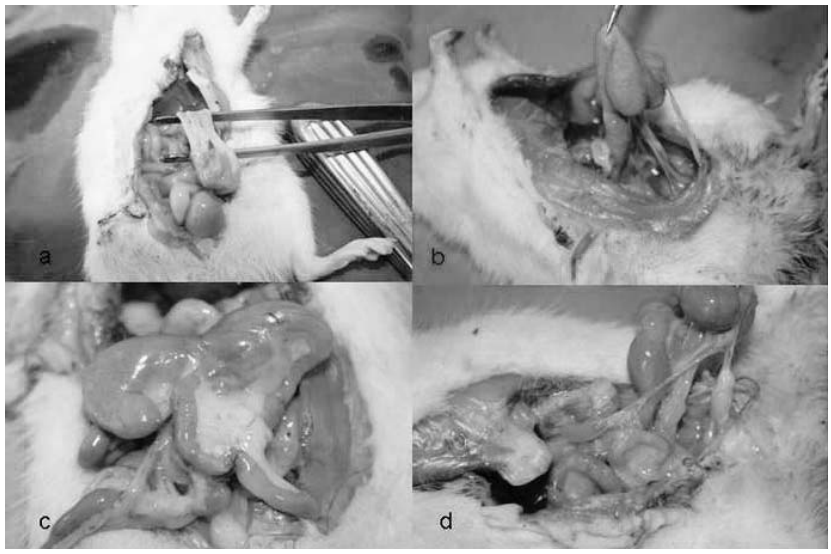

Fig. 2 Macroscopic adhesion scoring. (a): Grade 1, (b): Grade 2, (c): Grade 3, (d): Grade 4. Grade 1: A single adhesive band between the organs or between the organ and abdominal wall (a). Grade 2: Two adhesive bands between the organs or between the organ and abdominal wall (b). Grade 3: More than two adhesive bands between the organs or between the organ and abdominal wall, or adhesions of intestinal loops with or without adhesions to the abdominal wall (c). Grade 4: Viscera directly attached to the abdominal wall (d). Model of adhesion.

test in the analysis of nonparametric data. A P value <0.05 was considered significant. The significance of the difference between 2 groups regarding the parameters found statistically significant with the Kruskal-Wallis test was evaluated with the Whitney U test for the analysis of the values obtained by measurement. A P value <0.015 with Bonferroni correction was considered significant.

Results

The results obtained from the study were macroscopically, histopathologically, and biochemically categorized (Table 1).

Two animals each in Group I and Group III and 1 animal each in Group II and Group IV died in the postoperative period and these animals were not included in the evaluation. The cause of death for the rats was thought to be individual infection.

Macroscopic results

Table 1 shows the statistically significant differences between the groups according to macroscopic adhesion scoring following evaluation after relaparotomy ($P < 0.050$). Group I and Group II showed a significant difference when compared with the control group (Group III) but there was no significant difference between Group I and Group II.

There was a statistically significant difference between Group I and the other groups in terms of MDA ($P < 0.05$), but this difference was not present between Group II and Group III. However, the MDA level in Group I was lower than in Group II and Group III. There was no statistically significant difference between Group I and the other groups in terms of GSH ($P > 0.05$) but the values in Group I were higher than the others.

Histopathologic findings

Group I: Mild hyperemia and mild edematous changes were present in the vessels of the lamina propria in the tissues of 3 cases in the group where ellagic acid was administered (Fig. 3). Edema and hyperemia of the lamina propria was not seen in the other 2 cases. Cellular infiltration with sporadic neutrophil leukocytes was present. Fibrosis in the tunica serosa was observed in 1 case. No fibrosis

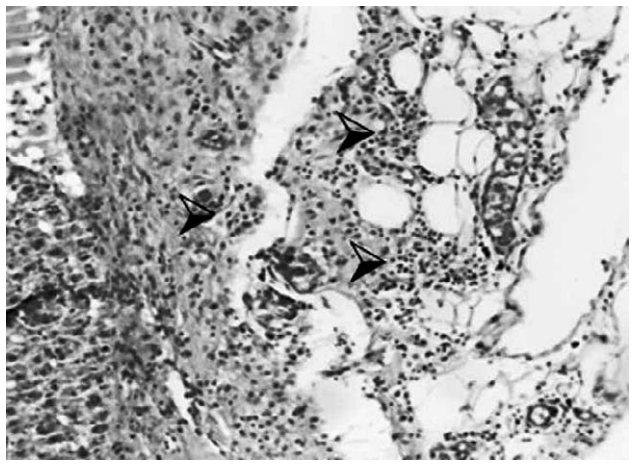

Fig. 3 Inflammatory cell in filtration in submucosa (arrowheads), H&E (× 100).

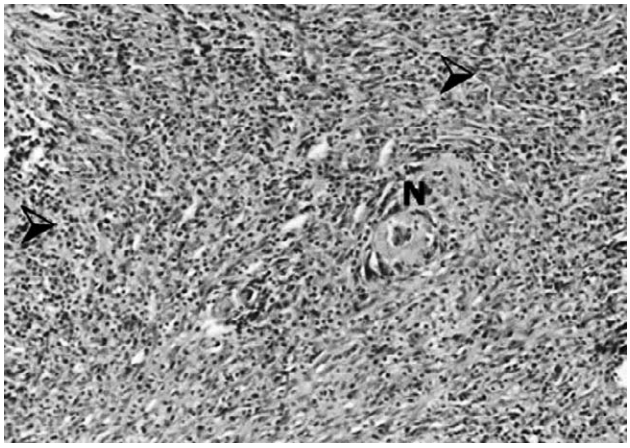

Fig. 4 Necrose (N) and inflammatory cell infiltration (arrowheads) in lamina propria, H&E,(× 100).

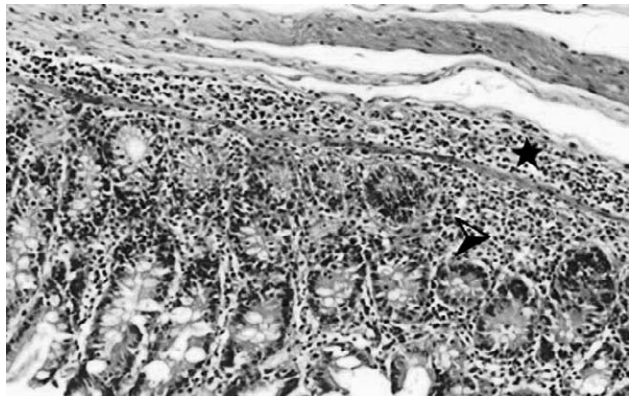

Fig. 5 Inflammatory cell infiltration in lamina propria (arrowhead) and submucosa (star), H&E (× 100).

was seen in the other cases. Adhesion of the liver to the Glisson capsule was seen in only 1 case in this group. None of the patients showed necrosis in the lamina propria.

Group II: Marked necrosis (Fig. 4) and accompanying neutrophil leukocyte infiltration, edematous changes, hyperemia, and capillarization were noticed in the lamina propria of 6 of 7 cases in the group where diclofenac sodium was administered. Degenerative changes in the muscles and mononuclear cells were observed in the lumen and around the vessels in particular in the submucosa. Mononuclear cell infiltrations were also found in the tunica muscularis and tunica serosa. Ovarium and adhesion were noticed in 1 case.

Group III:Large areas of necrosis spreading from the cecal lumen lamina epithelia to the tunica muscularis and intact or necrotic neutrophilic leukocyte infiltration (Fig. 5), edematous changes, hyperemia, and capillarization were noticed in 1 of the 6 cases in the control group. Thickening due to organized fibrosis was also observed in this layer in 4 cases. Mononuclear cell infiltration was found in the lamina propria, tunica muscularis (Fig. 6), and tunica serosa.

Group IV: The lesions detected in the patients in the sham group were usually limited to the lamina propria and submucosa. There was marked mononuclear cell infiltration in both layers. These cells were accompanied by a small number of neutrophilic leukocytes in the lamina propria in one case and by a granuloma in the submucosa in a different case (Fig. 7). Edematous changes were present in some places.

Discussion and Conclusion

Intra-abdominal adhesions after surgery is a significant cause of morbidity in the short and long term and many studies have therefore focused on their prevention.[3,4] Peritoneal adhesions occurring after abdominal surgery reduce the quality of life. Although adhesions are formed in almost all abdominal operations, there is almost no effective method to prevent them. The repair process as a result of any disruption of tissue integrity consists of inflammation, fibroblast proliferation, and then the maturation phase with connective tissue and matrix formation.[3] We aimed to investigate the effects of EA that has many effects but is mainly antioxidant and anti-inflammatory on intra-abdominal adhesion development in comparison with DS in this study.

DS is a nonsteroidal anti-inflammatory agent used in various ways as an adhesion inhibitor.[13,14] Some effects of EA have not yet been fully revealed although its antioxidant, anti-inflammatory, and anti-angiogenesis effects are known but studies on its anti-adhesive effect are limited. Furthermore, the anti-inflammatory effect of both substances is known to be through inhibition of COX-2.[13,14,23] We administered the oral form of both agents a daily single dose after the surgery and compared their effects in our study.

Positive effects of DS were reported on pleural adhesions by Lisete *et al* and after thoracic surgery by Lardinois *et al*[13,14] Although there are some studies on EA, we did not come across any that evaluated its effect on intraabdominal adhesion. There was a statistically significant difference between the groups regarding macroscopic classification in our study. Adhesion development was less

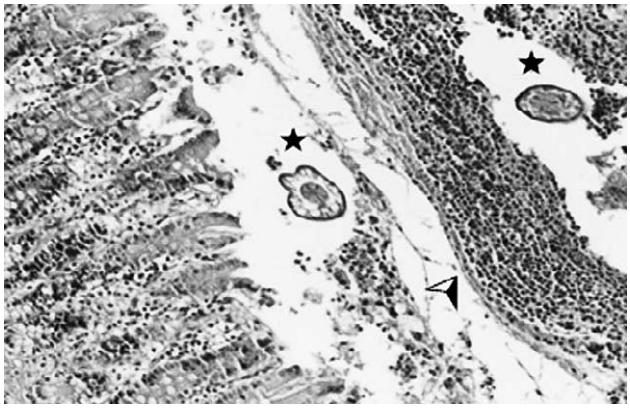

Fig. 6 Neutrophilic leukocytes (arrowhead) and larvae (stars), H&E (× 100).

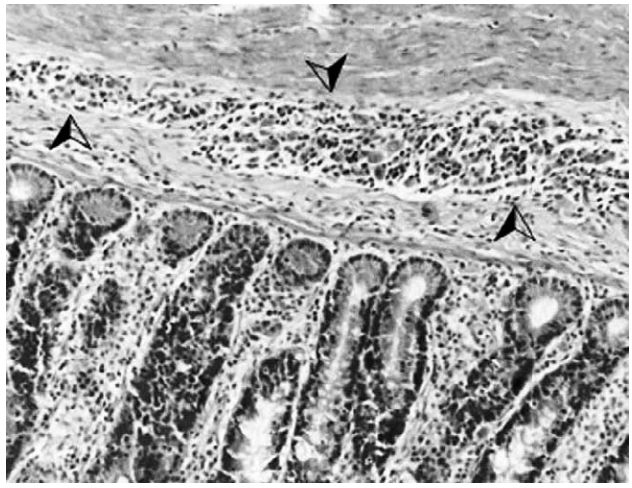

Fig. 7 Inflammatory cell infiltration in submucosa (arrowheads), H&E (× 100).

in Group I than in Group II and Group III. Various antioxidants and substances such as hyaluronic acid have been used to prevent intra-abdominal adhesions. Another study similar to ours evaluated the effect of hyaluronic acid on adhesion with macroscopic and microscopic examination. They reported that the trauma decreased to a minimum level when the tissue was covered with hyaluronic acid before the adhesion model is created and increased hyaluronic acid viscosity led to a macroscopic and microscopic decrease of intraabdominal adhesion in rats.[24]

The level of MDA, an oxidative stress parameter, has been reported to be increased in peritoneal adhesions and could be reversed to normal limits with antioxidant or anti-inflammatory agents.[3,4] A significant decrease in the MDA level with EA was reported by Böyük *et al* in rats where intestinal ischemia developed after lung trauma and by Özkaya *et al* in liver toxicity.[25,26] Similarly, the MDA levels showed a significant decrease in Group I compared to Group II and Group III in our study. A decrease was present in Group II compared to Group III but this was not as marked as in Group I. The level of GSH, an anti-oxidant in contrast to MDA, has been reported to decrease with ischemia, trauma or adhesions.[3,4,27] No statistically significant difference was found between Group I and the other groups in terms of GSH level in our study. However, the GSH level in Group I was higher than in Group II and Group III and closer to the sham group. The decrease in the MDA level and increase in the GSH level in the group where ellagic acid was administered is significant for our study. Similarly EA was reported to reduce cardiac levels of glutathione peroxidase (GPx), catalase, tumor necrosis factor alpha (TNF alpha), and malondialdehyde significantly.[17,18]

Rosillo *et al* reported that EA decreases inflammatory cell infiltration, edema, hyperemia, and necrosis in Crohn's disease and starts re-epithelialization during recovery, and linked this effect to the inhibition of cyclooxygenase (COX 2) and nitric oxide synthase (iNOS) activity.[23] Girish *et al* reported a decrease in rat liver necrosis, fat accumulation, and inflammatory reactions in rats given EA after liver toxicity was created by administering carbontetrachloride in a different study.[28]

Lisete *et al* reported that diclofenac sodium decreased leukocyte migration, edema, hyperemia, and fibrosis.[13] Lardinois *et al* reported an anti-adhesive effect through decreasing granulation tissue, collagen deposition and fibrosis.[14] We found a decrease in edema, hyperemia, inflammation, fibrosis, and adhesion in Group I as well as in Group II compared to Group III. Although a decrease was seen in Group II compared to Group III in terms of necrosis, this decrease was not as marked as in Group I. There are many products containing ellagic acid in the market but not enough information is present regarding their ingredients and side effects. Problems may arise when determining when and at what dose EA should be used. Previous studies have not clearly reported the side effects of ellagic acid.[29] Side effects need to be determined according to the dose with clinical use in future periods. The clinical use of EA can also enable the determination of the rate of prevention

for conditions such as intestinal obstruction, infertility, and chronic pain with patient follow-up and laparoscopic methods as necessary.

In conclusion, taking all our study data into account, the positive effects of the oral form of EA on the development of peritoneal adhesions are more marked than DS, a nonsteroidal anti-inflammatory drug. These positive effects could be a result of the strong antioxidant effect of EA reducing oxidative stress together with its anti-inflammatory and anti-angiogenesis effects. The clinical use of EA as for DS can be targeted with experimental studies performed by using different doses in line with our study.

References

1. Herrick EH, Mutsaers SE, Ozua P, Sulaiman H, Omer A, Boulos P *et al*. Human peritoneal adhesions are highly cellular, innervated and vascularized. *J Pathol* 2000;**192**(1):67–72
2. Hellebrekers BWJ, Trimbos-Kemper TCM, Trimbos JBMZ, Emeis JJ, Kooistra T. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. *Fertil Steril* 2000;**74**(2):203–212
3. Özler M, Ersöz N, Özerhan İH, Topal T, Öter Ş, Korkmaz A. The effect of alpha-lipoic acid in the prevention of peritoneal adhesions. *Turk J Gastroenterol* 2011;**22**(2):190–194
4. Celepli S, Kısmet K, Kaptanoğlu B, Erel S, Özer S, Celepli P *et al*. The effect of oral honey and pollen on postoperative intraabdominal adhesions. *Turk J Gastroenterol* 2011;**22**(1):65–72
5. Saed GH, Munkarah AR, Diamond MP. Cyclooxygenase-2 expressed in human fibroblasts isolated from intraperitoneal adhesions but not from normal peritoneal tissues. *Fertil Steril* 2003;**79**(6):1404–1408
6. Vrijland WW, Tseng LNL, Eijkman HJM, Hop WCJ, Jakimowicz JJ, Leguit P *et al*. Fewer intraperitoneal adhesions with use of hyaluronic acid-carboxymethylcellulose membrane. *Ann Surg* 2002;**235**(2):193–199
7. Reed KL, Fruin AB, Bishop-Bartolomei KK, Gower AC, Nicolaou M, Stucchi AF *et al*. Neurokinin-1 receptor and substance P messenger RNA levels increase during intra-abdominal adhesion formation. *J Surg Res* 2002;**108**(1):165–721
8. Golan A, Stolik O, Wexler S, Langer R, Ber A, David MP. Prostaglandins- A role in adhesion formation. *Acta Obstet Gynecol Scand* 1990;**69**(4):339–341
9. Guvenal T, Cetin A, Ozdemir H, Yanar O, Kaya T. Prevention of postoperative adhesion formation in rat uterine horn model by nimesulide: a selective COX-2 inhibitor. *Hum Reprod* 2001; **16**(8):1732–1735
10. Tito WA, Sarr MG. Intestinal obstruction. Nyhus LM (ed). Shackleford's Surgery of the Alimentary Tract. 4th ed. Philadelphia: Saunders Co, 1996:375–416
11. Dunn RC, Steinleitner AJ, Lambert H. Synergistic effect of intraperitoneally administered calcium channel blockade and recombinant tissue plasminogen activator to prevent adhesion formation in an animal model. *Am J Obstet Gynecol* 1991;**164**(5 Pt 1):1327–1330
12. Kennedy R, Costain DJ, McAlister VC, Lee TDG. Prevention of experimental postoperative peritoneal adhesions by N,O-carboxymethylchitosan. *Surgery* 1996;**120**(5):866–870
13. Lisete R, Teixeira LR, Vargas FS, Acencio M, Antonangeio L, Vaz MAC, Marchi E. Influence of antiinflammatory drugs (methylprednisolone and diclofenac sodium) on experimental pleurodesis induced by silver nitrate or talc. *Chest* 2005;**128**(6): 4041–4045
14. Lardinois D, Vogt P, Yang L, Hegyi I, Baslam M, Weder W. Non-steroidal anti-inflammatory drugs decrease the quality of pleurodesis after mechanical pleural abrasion. *Eur J Cardiothorac Surg* 2004;**25**(5):865–871
15. Mo J, Panichayupakaranant P, Kaewnopparat N, Songkro S, Reanmongkol W. Topical anti-inflammatory potential of standardized pomegranate rind extract and ellagic acid in contact dermatitis. *Phytother Res* 2013;**28**(4):629–632
16. Rogerio AP, Fontanari C, Borducchi E, Keller AC, Russo M, Soares EG *et al*. Anti-inflammatory effects of *Lafoensia pacari* and ellagic acid in a murine model of asthma. *Eur J Pharmacol* 2008;**580**(1–2):262–270
17. Lee WJ, Ou HC, Hsu WC, Chou MM, Tseng JJ, Hsu SL *et al*. Ellagic acid inhibits oxidized LDL mediated LOX-1 expression, ROS generation and inflammation in human endothelial cells. *J Vasc Surg* 2010;**52**(5):1290–1300
18. Chao PC, Hsu CC, Yin MC. Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice. *Nutr Metab* (Lond) 2009;**6**:33
19. Galili Y, Ben-Abraham R, Rabau M, Klausner J, Kluger Y. Reduction of surgery induced peritoneal adhesions by methylene blue. *Am J Surg* 1998;**175**(1):30–32
20. Hemadeh O, Chilukuri S, Bonet V, Hussein S, Chaudry IH. Prevention of peritoneal adhesions by administration of sodium carboxymethyl cellulose and oral vitamin E. *Surgery* 1993;**114**(5):907–910
21. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. *J Lab Clin Med* 1963;**61**: 882–888
22. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol* 1979;**135**(3):372–376
23. Rosillo MA, Sanchez-Hidalgo M, Cárdeno A, de la Lastra CA. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem Pharmacol* 2011;**82**(7):737–745
24. Burns JW, Skinner K, Colt J, Sheidlin A, Bronson R, Yaacobi Y *et al*. Prevention of tissue injury and postsurgical adhesions by

precoating tissues with hyaluronic acid solutions. *J Surg Res* 1995;59(6):644–652

25. Böyük A, Onder A, Kapan M, Gümüş M, Fırat U, Başaralı MK *et al*. Ellagic acid ameliorates lung injury after intestinal ischemia-reperfusion. *Pharmacogn Mag* 2011;**7**(27):224–228
26. Özkaya A, Çelik S, Yüce A, Şahin Z, Yılmaz Ö. The effects of ellagic acid on some biochemical parameters in the liver of rats against oxidative stress induced by aluminum *Kafkas Univ Vet Fak Derg* 2010;**16**(2):263–268
27. Jaeschke H. Molecular mechanisms of hepatic ischemia reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;**284**(1):G15–26
28. Girish C, Pradhan SC. Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbon-tetrachloride-induced liver toxicity in mice. *J Pharmacol Pharmacother* 2012;**3**(2):149–155
29. Corbett S, Daniel J, Drayton R, Field M, Steinhardt R, Garrett N. Evaluation of the anti-inflammatory effects of ellagic acid. *J Perianesth Nurs* 2010;**25**(4):214–220