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Effect of Propolis on Healing in Experimental Colon Anastomosis in Rats

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ABSTRACT

Introduction: Propolis is the generic name for the resinous substance collected by honeybees, which is known to have antioxidant, anti-inflammatory, apoptosis-inducible effects. Anastomotic dehiscence after colorectal surgery is an important cause of morbidity and mortality. We aimed to assess the effect of propolis on healing in an experimental colon anastomosis in rats.

Methods: Forty adult male Wistar albino rats were randomly assigned into 5 treatment groups with 8 rats in each: Group I, anastomosis+no treatment; Group II, anastomosis+oral propolis (600 mg/kg/d); Group III, anastomosis+oral ethyl alcohol (1 cc/d); Group IV, anastomosis+rectal propolis (600 mg/kg/d); Group V, anastomosis+rectal ethyl alcohol (1 cc/d). The bursting pressures, hydroxiproline levels and histopathological changes in each group were measured.

Results: When bursting pressures were compared between groups, we observed that they were increased in the groups treated with propolis in contrast to all other groups. Hydroxiproline levels in the propolis groups were also significantly increased in contrast to the other groups. There was also a statistically significant difference in histopathological changes between the treatment types. When propolis administration methods were compared, we did not observe a statistically significant difference.

Conclusion: Propolis has a significantly favourable effect on healing in experimental colon anastomosis, independent from the method of administration.

Keywords: anastomosis; colon; healing; propolis

INTRODUCTION

Propolis is the generic name for the resinous substance collected by honeybees from various plant sources, and which has been used in folk medicines in many regions of the world. Besides their well-known antioxidant activity,[1,2] propolis also inhibits certain enzyme activities such as lipooxygenase, cyclooxygenase, gluthathione-S transferase, xanthine oxidase and matrix metalloproteinases (MMPs).[3–6] Some propolis ingredients have been shown to have antitumour,[7,8] anti-inflammatory[5,9] and apoptosis-inducible effects as well as antimetastatic effects in colon adenocancer.[10] Previous studies[11] have shown that propolis had an anti-inflammatory effect at a dose of 300–600 mg/kg but its effect became toxic at a dose of 2000–7000 mg/kg.

Anastomotic dehiscence after colorectal surgery is an important cause of morbidity and mortality.[12,13] Anastomotic healing and the nutritional, metabolic, infectious and technical factors that may

influence the strength of the anastomosis in the colon have been investigated extensively in many clinical and experimental studies.[13–16] There have been significant improvements in techniques, and adverse effects of different systemic and local factors have been documented.[13–16] Yet, anastomotic leakage following colon anastomosis is still a serious clinical complication increasing mortality and morbidity.

MMPs constitute a family of enzymes that are structurally related neutral proteinases. They can degrade extracellular matrix proteins, particularly the collagen metabolism, which is assumed to be an important factor influencing the outcome of intestinal anastomotic healing.[17]

In the present study we aimed to determine the effect of propolis on healing in an experimental colon anastomosis. We measured the bursting pressures, hydroxiproline (HP) levels and histopathological changes in order to determine the effect.

MATERIALS AND METHODS

Forty adult male Wistar albino rats, weighing between 260 and 290 g were used. The animals were acclimatised for 1 wk to our laboratory conditions prior to experimental manipulation. They had free access to standard laboratory food and water. The protocol of this study and animal experimental procedures were approved by the ethical committee of Mustafa Kemal University's veterinary school.

Preparing 5% Propolis Extract

A 5% propolis tincture was prepared by mixing 1900 ml 70% ethyl alcohol and 100 g propolis as described by Krell.[18] The preparations were kept in a container, sealed at the top and shaken twice daily for 1 wk. The mixture was filtered and kept in a clean, dark bottle at 4°C until it was used. Chemical analysis was performed on the extract and results have confirmed that propolis extract from the Hatay region (from where the propolis was derived) had high concentrations of the aromatic acids such as benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglcine.[19]

All of the animals were fasted overnight before surgery. Anaesthesia was administered with an intraperitoneal injection of 10 mg/kg xylazine (Rompun®; Bayer, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar®; Parke-Davis, Istanbul, Turkey). The surgical procedures were performed using clean and sterile instruments. Ten per cent povidone-iodine solution was used for the disinfection of the skin. After placing sterile drapes, a 3-cm midline incision was made. Upon entering the abdominal cavity, the sigmoid colon was identified and the colon divided exactly 3 cm from the peritoneal reflection while preserving the vascular arcades. Approximately 1 cm of colonic segment was resected. This resected specimen was preserved at −20°C for later determination of the HP concentration in normal colon tissue and was assessed as the intact colon group. The free ends of the colon inside the abdomen were anastomosed with a single layer of interrupted inverting 6/0 polypropylene sutures (Prolene®; Ethicon, Edinburgh, Scotland) placed 1 mm apart. The fascia and skin layers were closed separately with running 4/0 silk sutures (Mersilk®; Ethicon, Edinburgh,

Scotland). To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously during the operation. All operative procedures were performed by the same investigator. Each animal was given free access to food and water the morning after the procedure. Because propolis was dissolved in ethyl alcohol, it was also administered in ethyl alcohol. Therefore, to ensure that the results obtained were not a consequence of ethyl alcohol administration, all other groups were also administered ethyl alcohol.

The animals were randomly assigned into 5 groups: Group I, anastomosis+no treatment (n=8); Group II, anastomosis+oral propolis (600 mg/kg/d; n=8); Group III, anastomosis+oral ethyl alcohol (1cc/d; n=8); GroupIV, anastomosis+rectal propolis (600 mg/kg/d; n=8); Group V, anastomosis+rectal ethyl alcohol (1 cc/d; n=8). An intact colon group was included to reflect the normal ranges of the rats used in the study. The results collected from this group were used to compare HP measurements between groups. Rats were killed by intracardiac puncture during anaesthesia at the seventh postoperative day. Researchers were all blinded to the randomisation of the study.

To assess the mechanical strength of the anastomosis, 1 end of the excised segment was sealed with a suture. The free end was then catheterised with a polyurethane tube (2-mm outer diameter), and a stay suture was tied circumferentially, incorporating both tissue and tube to prevent air leakage. The external end of the tube was connected to an infusion pump and a mercury manometer by way of a Y-shaped adapter. The colon segment was then placed in a saline-filled container, and air was pumped into the tube at a rate of 5 ml/min. The blood pressure reading was taken at the instant the pressure decreased suddenly (caused by bursting of the anastomosis), or when bubbles were seen. This was recorded as the 'bursting pressure'.

After measuring bursting pressures, a 5-mm-wide ring of tissue, including the anastomosis, was removed. Half of this removed tissue was wrapped in aluminium foil and preserved at −20°C for later measurement of HP levels at the anastomosis site. The other half was stored in 10% formaldehyde for later assessment of histopathological features. When brought to room temperature, samples' dry weights were recorded and, successively, the amount of HP was determined following the methods used by Bergman et al.[20] Absorbance was read using a Shimadzu spectrophotometer (UV-120−02; Kyoto, Japan), and the collagen concentration was expressed as micrograms of HP per grams tissue of dry weight.

For histopathological assessment, the tissues that were fixed in 10% formaldehyde were stained with haematoxylin and eosin and were evaluated at x20 to x200 magnifications under the light microscope. The healing parameters and inflammatory changes, granulocytic cell infiltration, mononuclear cell infiltration, fibroblastic cell infiltration, necrosis, exudate, and capillary formation were assessed semiquantitatively by assigning a score of 0 to 3 to each tissue specimen. Presence or absence of peritonitis was recorded as this had been previously observed by Nursal et al.[13]

Statistical Analysis

The results are expressed as the mean±standard error of the mean. Differences among the groups were evaluated using 1-way analysis of variance, and multiple comparisons between the groups were performed with a post-hoc test (Tukey's HSD test). Differences were considered statistically significant when $P<0.05$. Data were analysed by statistical software (SPSS for Windows 11.5; SPSS, Chicago, Ill, USA).

RESULTS

When bursting pressures were compared between groups (Table 1), we observed that bursting pressures were increased in the propolis groups in contrast to all other groups. This increase was not significant between the propolis groups (Groups II and IV) and the intact colon group, but was significant between the propolis groups and the oral ethyl alcohol (Group III) and rectal ethyl alcohol (Group V) groups ($P<0.001$ and $P<0.001$, respectively). We also observed a statistically significant difference between the no treatment group (Group I) and the rectal ethyl alcohol group (Group V) ($P<0.001$ and $P=0.025$, respectively).

When HP levels were compared between groups (Table 2), the highest levels were observed in the intact colon group. The difference between the intact colon group and the others were statistically significant ($P<0.001$, $P=0.018$, $P<0.001$, $P=0.001$ and $P<0.001$, for Groups I–V, respectively). HP levels in the oral propolis group (Group II) were significantly increased in contrast to the no treatment group (Group I; $P=0.004$), the oral ethyl alcohol group (Group III; $P<0.001$) and the rectal ethyl alcohol group (Group V; $P<0.001$). When the rectal propolis group (Group IV) was compared with

Table 1. Bursting pressure measurements of the anastomosis.

	Group I	Group II	Group III	Group IV	Group V
Bursting pressure, mmHg	141.8±16.6	158.8±14.4	128.8±10.6	157.6±10.8	122.2±12.3

Data expressed as mean±standard error of the mean. The mean pressure was significantly higher in the propolis groups.
Group I=anastomosis+no treatment; Group II=anastomosis+oral propolis (600 mg/kg/d);
Group III=anastomosis+oral ethyl alcohol (1 cc/d); Group IV=anastomosis+rectal propolis (600 mg/kg/d);
Group V=anastomosis+rectal ethyl alcohol (1 cc/d).

Table 2. Hydroxiproline measurements for all groups.

	Intact colon	Group I	Group II	Group III	Group IV	Group V
Tissue, μg/g	3.656±1.039	1.551±274	2.677±724	1.159±140	2.333±441	1.201±326

Data expressed as mean±standard error of the mean. The mean pressure was significantly higher in the propolis groups.
Group I=anastomosis+no treatment; Group II=anastomosis+oral propolis (600 mg/kg/d);
Group III=anastomosis+oral ethyl alcohol (1 cc/d); Group IV=anastomosis+rectal propolis (600 mg/kg/d); Group V=anastomosis+rectal ethyl alcohol (1 cc/d).

Table 3. Mean values collected from histopathological assessment.

	Group I	Group II	Group III	Group IV	Group V
Granulocytic cell infiltration	2.62±0.52	1.50±0.54	2.63±0.52	1.63±0.52	2.75±0.46
Mononuclear cell infiltration	1.13±0.35	2.38±0.52	1.38±0.52	2.38±0.52	1.88±0.64
Fibroblastic cell infiltration	1.13±0.35	2.25±0.71	1.63±0.52	2.25±0.46	1.25±0.46
Epithelisation	0.50±0.54	2.50±0.54	1.25±0.46	2.25±0.46	1.13±0.35
Necrosis	1.38±0.52	0.25±0.46	1.25±0.46	0.25±0.46	2.25±0.46
Exudate	1.63±0.52	0.25±0.46	1.38±0.52	0.25±0.46	2.50±0.54
Capillary formation	1.50±0.54	2.25±0.71	1.63±0.52	2.25±0.46	1.50±0.54
Microscopic peritonitis	0.50±0.54	0.13±0.35	0.75±0.46	0.25±0.46	0.88±0.35

Data expressed as mean±standard error of the mean. Assessment was based on a scoring system from 0 to 3: '0' indicates no inflammation; '1' indicates mild inflammation; '2' indicates moderate inflammation; '3' indicates severe inflammation. There was significant difference between propolis groups and other groups (P<0.05). Group I=anastomosis+no treatment; Group II=anastomosis+oral propolis (600 mg/kg/d); Group III=anastomosis+oral ethyl alcohol (1 cc/d); Group IV=anastomosis+rectal propolis (600 mg/kg/d); Group V=anastomosis+rectal ethyl alcohol (1 cc/d).

the other groups, levels were significantly higher in contrast to Group I, Group III (P=0.003) and Group V (P=0.004). The levels did not show an important difference between Groups II and IV.

When studying histopathological changes, mononuclear cell infiltration, fibroblastic cell infiltration, epithelisation and high capillary formation were considered favourable changes, whereas granulocyte infiltration, necrosis, exudate formation and microscopic peritonitis were considered unfavourable changes.

As seen in Table 3, when mean histopathological changes were compared between groups, there was a statistically significant difference between the propolis groups (Group II and IV) and all other groups (P<0.05). But when propolis groups were compared with each other, we did not observe a statistically significant difference in the results. There was no statistically significant difference between the ethyl alcohol and anastomosis groups.

DISCUSSION

In recent years the biological and pharmacological properties of propolis have received great attention in the scientific community.[1,10,17,19] This is mainly stemmed from the discovery that propolis could exhibit a broad spectrum of biological activities, e.g. antiviral, anti-inflammatory, anticarcinogenic activity, hepatoprotective activity,[1] and anti-oxygen stress activities. The composition of propolis depends on the vegetation at the site of collection. Up to now, more than 180 compounds, mainly polyphenols, have been identified as constituents of propolis.[11] The major polyphenols are flavonoids, accompanied by phenolic acids and esters, phenolic aldehydes and ketones. Other compounds

in propolis are volatile oils and aromatic acids (5%–10%), waxes (30%–40%), resins, balms and pollen grains, which are a rich source of essential elements such as magnesium, nickel, calcium, iron and zinc.[21]

Anastomotic dehiscence after colorectal surgery is an important cause of morbidity and mortality.[12,13] Investigating wound healing and attempting to improve its outcome necessitates process quantification. Parameters for anastomotic repair and adhesion formation are mechanical, biochemical or histological. Anastomosis healing and the nutritional, metabolic, infectious and technical factors that may influence the strength of the anastomosis in the colon have been investigated extensively in many clinical and experimental studies.[13–16] There have been significant improvements in technique and adverse effects of different systemic and local factors have been documented.[13–16] Yet, anastomotic leakage following colon anastomosis is still a serious clinical complication increasing mortality and morbidity.

Anastomotic healing is affected by the degree of primary inflammatory response; the rate of mucosal re-epithelisation; the amount, strength and maturation rate of new collagen; and collagenolysis in the initial 3 d of the postanastomotic period. Strength of an anastomosis is based on the collagen fibres and their maturation at the submucosa.[14,22] When an anastomosis is constructed in the gastrointestinal tract, an inflammation occurs as a response to traumatic injury and foreign material such as sutures.[23,24] This inflammation is a normal constituent of wound healing. However, if it is exaggerated, wound healing is delayed because of increased collagenolysis. This delay causes the impediment in anastomotic healing in the presence of intra-abdominal infection.[23–25] The anastomotic area is already inflamed, and the endothelium in the peri-anastomotic area is already activated during the early phase of wound healing.[24,26] As a result of the pro-inflammatory and chemo-attractant properties of the anastomosis, activated circulating polymorphonuclear leucocytes secondary to sepsis-induced injury may accumulate easily in the peri-anastomotic area, increase the inflammatory reaction, and delay healing. Together with the proteolytic enzymes, oxygen free radicals derived from activated polymorphonuclear leucocytes and circulating xanthine oxidase may increase collagenolysis in the peri-anastomotic area, which delays wound healing.[27]

MMPs constitute a family of enzymes that are structurally related neutral proteinases. MMPs can degrade extracellular matrix proteins, particularly the collagen metabolism, which is assumed to be an important factor influencing the outcome of intestinal anastomotic healing. Experimental studies have shown that diminished postoperative integrity of colon anastomosis is due to degradation of existent collagen in the narrow zone around the sutures and it is known that this collagenolysis is mediated by at least 2 types of MMPs.[28] It has also been shown that significantly higher expressions of MMP occurred in the bowel wall of the leakage groups in studies.[29] Propolis has been suggested to inhibit MMPs in experimental studies.[17]

In this study we aimed to investigate the effect of propolis on healing in an ex-

perimental colon anastomosis. In order to achieve our aim we measured the bursting pressures, HP levels and histopathological changes in different treatment groups. In order to assess the potentially different effects we constituted 2 different groups of propolis. At the end of the study we found that propolis showed an anti-inflammatory effect in experimental colon anastomosis. This could be attributed to its previously suggested inhibitory effect on MMPs, although as MMPs were not measured in this study, this cannot be confirmed here. The effect of propolis on bursting pressures and HP levels were favourable in our study but the mechanism of this favourable effect is not certain. Future studies should be performed to show the effect of potential mechanisms such as antibacterial, antioxidant activity, inhibition of certain enzyme activities such as lipooxygenase, cyclooxygenase, gluthathione-S transferase and xanthine oxidase. In the present study oral propolis and rectal propolis had similar effects but the study groups were not large enough to conclude on the potentially different effects of alternative methods of administration. This could be another consideration for future studies.

In conclusion we found that propolis, independent of method of administration, had significantly favourable effects on healing in experimental colon anastomosis.

REFERENCES

1. Nagaoka T, Banskota AH, Tezuka Y, Saiki I, Kadota S. Selective antiproliferative activity of caffeic acid phenethyl ester analogues on highly liver-metastatic murine colon 26-L5 carcinoma cell line. *Bioorg Med Chem.* 2002;10:3351–3359.

2. Vieira O, Laranjinha J, Madeira V, Almeida L. Cholesteryl ester hydroperoxide formation in myoglobin-catalyzed low density lipoprotein oxidation: concerted antioxidant activity of caffeic and p-coumaric acids with ascorbate. *Biochem Pharmacol.* 1998;55:333–340.

3. Koshihara Y, Neichi T, Murota S, Lao A, Fujimoto Y, Tatsuno T. Caffeic acid is a selective inhibitor for leukotriene biosynthesis. *Biochim Biophys Acta.* 1984;792:92–97.

4. Michaluart P, Masferrer JL, Carothers AM, et al. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. *Cancer Res.* 1999;59:2347–2352.

5. Chan WS, Wen PC, Chiang HC. Structure-activity relationship of caffeic acid analogues on xanthine oxidase inhibition. *Anticancer Res.* 1995;15:703–707.

6. Ploemen JH, van Ommen B, de Haan A, Schefferlie JG, van Bladeren PJ. In vitro and in vivo reversible and irreversible inhibition of rat glutathione S-transferase isoenzymes by caffeic acid and its 2-S-glutathionyl conjugate. *Food Chem Toxicol.* 1993;31:475–482.

7. Tanaka T, Kojima T, Kawamori T, et al. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. *Carcinogenesis.* 1993;14:1321–1325.

8. Frenkel K, Wei H, Bhimani R, et al. Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Res.* 1993;53:1255–1261.

9. Fernandez MA, Saenz MT, Garcia MD. Anti-inflammatory activity in rats and mice of phenolic acids isolated from Scrophularia frutescens. *J Pharm Pharmacol.* 1998;50:1183–1186.

10. Liao HF, Chen YY, Liu JJ, et al. Inhibitory effect of caffeic acid phenethyl ester on angiogenesis, tumor invasion, and metastasis. *J Agric Food Chem.* 2003;51:7907–7912.

11. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem Toxicol.* 1998;36:347–363.

12. Waninger J, Kauffmann GW, Shah IA, Farthmann EH. Influence of the distance between interrupted sutures and the tension of sutures on the healing of experimental colonic anastomoses. *Am J Surg.* 1992;163:319–323.

13. Nursal TZ, Anarat R, Bircan S, Yildirim S, Tarim A, Haberal M. The effect of tissue adhesive, octyl-cyanoacrylate, on the healing of experimental high-risk and normal colonic anastomoses. *Am J Surg.* 2004;187:28–32.

14. Sakallioglu AE, Yagmurlu A, Dindar H, Hasirci N, Renda N, Deveci MS. Sustained local application of low-dose epidermal growth factor on steroid-inhibited colonic wound healing. *J Pediatr Surg.* 2004;39:591–595.

15. Hendriks JM, Hubens G, Wuyts FL, Vermeulen P, Hubens A, Eyskens E. Experimental study of intraperitoneal suramin on the healing of colonic anastomosis. *Br J Surg.* 1999;86:1171–1175.

16. Turkcapar AG, Demirer S, Sengul N, et al. The adverse effects of octreotide on the healing of colonic anastomosis. *Surg Today.* 1998;28:279–284.

17. Jin UH, Chung TW, Kang SK, et al. Caffeic acid phenyl ester in propolis is a strong inhibitor of matrix metalloproteinase-9 and invasion inhibitor: isolation and identification. *Clin Chim Acta.* 2005;362:57–64.

18 Krell R. *Value-Added Products From Beekeeping. FAO Agricultural Services Bulletin No. 124.* Food and Agriculture Organization of the United Nations: Rome; 1996.

19. Sahinler N, Kaftanoglu O. Natural product propolis: chemical composition. *Nat Prod Res.* 2005;19:183–188.

20. Bergman I, Loxly R. Two impaired and simplified methods for the spectro-photometric determination of hydroxyproline. *Ann Chem.* 1963;35:1961–1965.

21. Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. *Fitoterapia.* 2002;73(suppl 1):S1–S6.

22. Brasken P, Lehto M, Renvall S. Changes in the connective tissue composition of the submucosal layer of colonic anastomosis. *Acta Chir Scand.* 1989;155:413–419.

23. Kologlu M, Yorganci K, Renda N, Sayek I. Effect of local and remote ischemiareperfusion injury on healing of colonic anastomoses. *Surgery.* 2000;128:99–104.

24. Thornton FJ, Barbul A. Healing in the gastrointestinal tract. *Surg Clin North Am.* 1997;77:549–573.

25. Kologlu M, Sayek I, Kologlu LB, Onat D. Effect of persistently elevated intraabdominal pressure on healing of colonic anastomoses. *Am J Surg.* 1999;178:293–297.

26. Graham MF, Blowquist P, Zederfeldt B. The alimentary canal. In: Cohen IK, Deigelman RF, Lindblad WJ, eds. *Wound Healing: Biochemical and Clinical Aspects.* Philadelphia: WB Saunders; 1992:433–449.

27. Demirogullari B, Sonmez K, Turkyilmaz Z, et al. Comparison of consequent small bowel anastomoses after transient ischemia: an experimental study in rats. *J Pediatr Surg.* 1998;33:91–93.

28. Agren MS, Andersen TL, Mirastschijski U, et al. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. *Surgery. 2006*;140:72–82.

29. Stumpf M, Klinge U, Wilms A, et al. Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. *Surgery.* 2005;137:229–234