

Protective Effect of Propolis and Bee Bread in Experimental Gastric Ulcer Model

Züleyha DOĞANYIĞIT^{1*}, Arda Kaan ÜNER², Aslı OKAN¹, Sibel SİLİCİ³

¹Yozgat Bozok University, Faculty of Medicine, Department of Histology and Embryology, 66100 Divanliyol / Yozgat, TURKEY

²Yozgat Bozok University, Faculty of Medicine student, Phase IV, 66100 Divanliyol / Yozgat, TURKEY

³Erciyes University, Seyrani Faculty of Agriculture, Department of Agricultural Biotechnology, 38100 Kayseri, TURKEY

* Corresponding author e-mail: zuleyha.doganyigit@gmail.com

Received: 11th April, 2021; accepted: 18th June, 2021; published: 15th July, 2021

A B S T R A C T

Gastric ulcer is the most common disease of the upper digestive system. The pathophysiology of the disease is associated with environmental factors and non-steroidal anti-inflammatory drugs. These factors weaken the gastric mucosal barrier and increase acid secretion, causing damage to the gastric epithelium. Although medical treatment approaches are widely used, side effects are seen. Propolis and bee bread are natural compounds obtained from the products of honeybees. Studies have shown that these compounds have antioxidant, antimicrobial and anti-inflammatory effects. In this study, it was aimed to investigate the therapeutic effects of propolis and bee bread in an animal model of gastric ulcer induced by indomethacin. 48 male Wistar rats were used in the study. Six random groups were formed as the control group, the indomethacin-treated ulcer group, the low and high-dose bee bread (perga) groups, the low and high-dose propolis groups. After injections and treatment applications, stomach tissues of animals were examined histologically and blood samples biochemically. According to the obtained findings, propolis and bee bread prevented epithelial damage in gastric ulcer induced by indomethacin and formed a histopathological structure similar to the control group ($p < 0.05$). These data indicate that propolis and bee bread have protective and therapeutic effects in the treatment of gastric ulcers.

Keywords: Bee bread, Propolis, Stomach, Ulcer, Histology

Introduction

Gastric ulcer is the most common disease of the upper digestive system. The prevalence of gastric ulcer in the western population is % 2.4 [1]. In addition, the annual incidence rates range from % 0.10 to 0.19 [2]. The

most common ulcer area in the stomach is the small curvature. Moreover, Ulcerative lesions might be seen anywhere from pylori to cardia of the gastric tissue [3]. Causes of gastric ulcer contain environmental factors

(smoking, alcohol use and microorganisms) and non-steroidal anti-inflammatory drugs (NSAID) [4]. The reduction of protective factors in the gastric physiology (prostaglandin release, gastro-mucosal barrier, and blood flow) and the increase of degenerative secretions play a role in the main pathophysiology [5]. Under normal circumstances, the gastric tissue can tolerate high concentrations of hydrochloric acid, refluxing bile salts, alcohols and nutrients at varying temperatures. This tolerance is maintained by the repair ability of the mucosal barrier. The production of prostaglandins (PG) regulates mucosal defense and increases the resistance of the stomach layer [6]. The production of bioactive PGs is provided by the enzyme cyclooxygenase (COX). In addition, NSAIDs [7], such as Aspirin and Indomethacin, prevent the production of PGs by inhibiting the production of COX. With these effects, NSAIDs have been shown to play a role in gastric ulcer development. Although treatment approaches aim to reduce acid secretion from gastric cells, side effects are commonly occur [8]. Due to these side effects, new treatment procedures based on bee products have been investigated in recent years.

Bee bread is a type of fermented pollen collected by the honeybee and stored in the honeycomb [9]. Bee bread contains approximately %24-35 carbohydrate, % 3 lipid, % 20 protein, % 3 vitamins and minerals. The bee bread contains all of the essential amino acids that the human body cannot biosynthesize, as well as proteins, vitamins such as C, B, B2, E, H, P, nicotinic acid, folic acid, pantothenic acid, sucrose, pigments, enzymes such as amylase, phosphatase, flavonoids, carotenoids and hormones [10]. The antibacterial and antioxidant properties of the fatty acid content of bee bread have been proven by scientific studies [11-13]. In addition, the positive effects of bee bread on stomach tissue have been shown in studies [14].

Propolis, a resinous substance, is prepared by honeybees to cover cracks, smooth walls in the hive and to keep humidity and temperature constant. Propolis is a natural sticky substance that honeybees collect from the resins of flowers, trees and plants and mix them with their saliva [15].

Propolis is frequently used in studies according to chemical composition and therapeutic properties [16]. The chemical content of propolis has identified more than 300 compounds [17]. Studies have shown that the observed effects of propolis may be

a result of the synergistic effect of the complex components [18]. These data show that Propolis and its extracts have many applications in the treatment of various diseases due to anti-inflammatory, antiseptic, antioxidant, antibacterial, antimycotic, antifungal, anti-ulcer,

anticancer and immunomodulatory effects. In the light of this information, effects of propolis and bee bread on gastric epithelium were investigated in the experimental gastric ulcer model in the present study.

Materials and Methods

Animals

48 Wistar albino male rats aged 8-10 weeks were used in the study. Rats were obtained from Erciyes University Experimental and Clinical Research Center (DEKAM). Ethics committee approval required for the study was obtained from Erciyes University Animal Experiments Local Ethics Committee (decision dated 15.11.2017 and numbered 17/114). Throughout the study, rats were kept in cages in groups of four, where they had unlimited access to food, on a 12-hour light and 12-hour dark cycle at 21 ± 1 °C room temperature.

Chemicals

The present study used popular type propolis collected from the vicinity of the Kayseri province in central Anatolia (Turkey). Bee bread was purchased from the Nutral Therapy Company in Kayseri (Turkey) and stored at -20°C. Using the

AOAC method, the moisture content of bee bread was determined by drying it gravimetrically at 105 °C to constant weight in a convection oven. Crude protein value was determined. A conversion factor of 6.25 was used to convert the percentage of nitrogen to the percentage of crude protein. The crude oil was extracted using a soxhlet apparatus and diethyl ether. The crude fiber content of bee bread was analyzed. Ash content of the samples was determined gravimetrically. The total carbohydrate content was calculated according to the expression: total carbohydrates = 100- (% moisture + % protein + % fat + % ash).

Experimental Groups

Control group: Saline (0.9% NaCl) 1 ml intraperitoneally (i.p.) administered group (n = 8).

END (Indomethacin) group: 1 ml volume of 25 mg/kg 1 i.p administered Indomethacin group (n = 8) [19].

High-dose Perga (bee bread) (PY) group: 1 ml volume of perga was administered 100 mg / kg by oral gavage (n = 8) [14, 20].

Low-dose Perga (PD) group: 1 ml volume of perga was administered 50 mg / kg by oral gavage (n = 8) [20].

High-dose Propolis (EPY) group: 100 mg / kg olive oil-based propolis administered by oral gavage in a volume of 1 ml (n = 8) [20, 21].

Low-dose Propolis (ELP) group: 50 mg / kg olive oil-based propolis administered by oral gavage in a volume of 1 ml (n = 8) [20].

Histological procedures

Stomach tissues were fixed with 10% formaldehyde solution to be used in histological examinations. After fixation, tissues were embedded in paraffin by applying routine further steps.

5-6 µm sections from the paraffin blocks were taken on flat slides. The prepared slides were kept in the oven for a certain period using standard histological methods. The paraffin was removed with xylene and diluted after passing through graded alcohol series. The sections were stained with

Hematoxylin-Eosin (H&E) to see the general histological structure. Gastric tissue damage scoring was made considering criteria such as surface epithelial degeneration, presence of necrotic cells, shortening of the mucosa from 50 areas. According to these criteria, 0: no damage, 1: mild damage, 2 moderate damage, 3 severe damage. The examinations were examined under the Olympus BX51 microscope.

Biochemical analysis

At the end of the experiment, blood samples taken from all groups were taken into EDTA tubes and the hematological parameters of WBC, RBC, HGB, HTC, MCV, MCH, MCHC, EOS, LYM were analyzed.

Statistical analysis

For the biochemical data obtained as a result of the research, SPSS 20.0 (Statistical Package for the Social Sciences) Software package program was used. Arithmetic Mean (\bar{x}) for descriptive statistics and standard error ($S\bar{x}$) is used to observe the difference between the groups by comparing the data with each other. The data of the variables were determined by the Shapiro-Wilk normality test, and the data of the parametric variables were analyzed by one-way ANOVA test. While Post-hoc

Tukey test is used for data showing homogeneity of variance in one-way ANOVA test; Games-Howell Post-Hoc test

was applied to the data without variance homogeneity. The level of significance was set at $\alpha = 0.05$ for all statistical tests.

Results and Discussion

Histological Results

Normal histological structure was observed in the gastric tissue of the control group. In the END group, degeneration of surface epithelial cells and shortening of the mucosa and glands were observed. A

significantly similar structure to the control was observed in the PY, PD, ELP and EPY groups (Figure 1, Table 1). Perga and propolis have been shown to provide a significant improvement over the END group at both low and high doses.

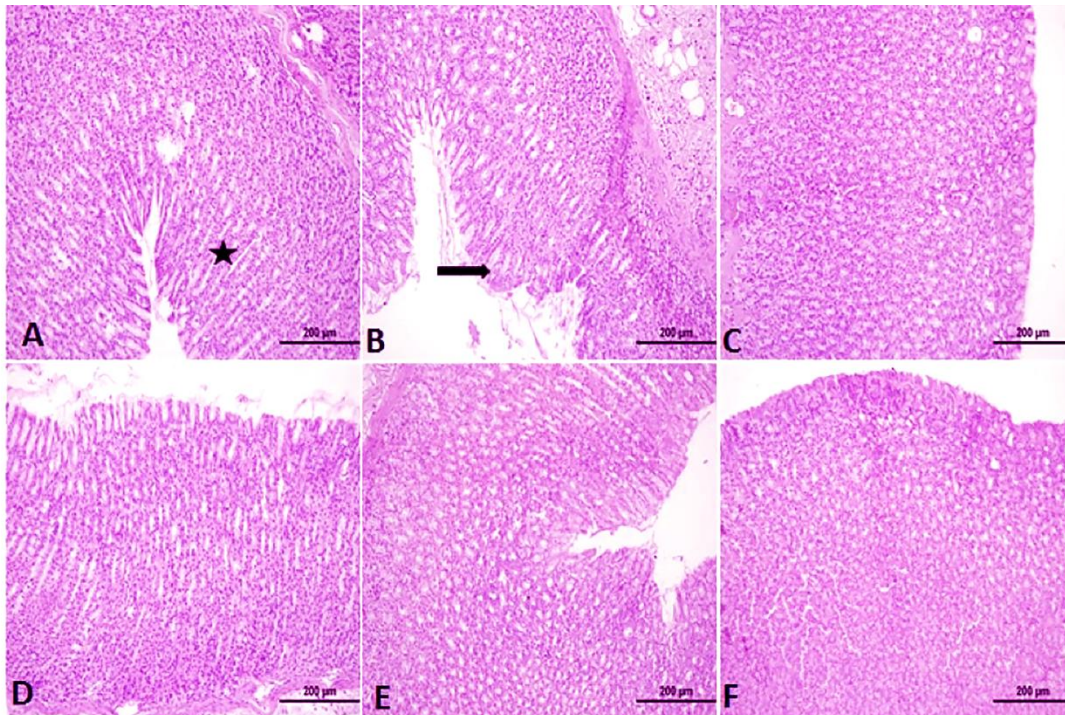


Figure 1. The normal stomach structure of the control group (A) is shown, the END group (B) showed disruption in the surface epithelium and shortening of the mucosa. PY group (C), PD group (D), ELP group (E), EPY group (F). H&E. Image magnification X200

Table 1. Histopathological evaluation results of rat stomach tissues.

Gruplar	Kontrol	END	PY	PD	ELP	EPY	p
Histopathological score	0.20±0.61 ^a	1.06±0.92 ^b	0.20±0.55 ^a	0.13±0.34 ^a	0.46±0.62 ^a	0.50±0.57 ^a	0.001

Datas are expressed as mean ± standard deviation. P <0.05 was considered significant. There is no significant difference between the groups containing the same letter (a-b).

Biochemical Results

In the biochemical analysis of blood samples, a significant decrease was detected in the number of red blood cells, hemoglobin, hematocrit, mean erythrocyte hemoglobin and lymphocyte in the indomethacin group compared to the control group. A significant decrease in lymphocyte count was observed in the perga and propolis groups compared to the

indomethacin group. The mean erythrocyte cell volume was significantly changed in the propolis groups compared to the indomethacin group. In addition, a significant decrease in eosinophil levels was found in the propolis and perga groups compared to the indomethacin group (p <0.05) (Table 2).

Table 2. Biochemical analysis results of rat blood samples.

	Groups					
	Control	END	PY	PD	ELP	EPY
WBC	9.66±3.79 ^{ab}	6.47 ±0.85 ^b	9.37 ±0.93 ^{ab}	9.65 ±1.89 ^{ab}	10.48±0.24 ^a	11.42±0.50 ^a
RBC	9.18±0.28 ^c	8.27 ±0.13 ^b	9.36 ±0.29 ^a	9.17 ±0.14 ^a	9.40 ±0.21 ^a	9.16 ±0.14 ^a
PLT	602.24±31.20	595 ±84.19	764 ±53,04	628 ±17.20	898 ±30,17	925 ±40.0
HGB	11.40±2.22 ^b	15.68 ±0.27	15.66 ±0.86	16.22 ±0.19	15.30 ±0.35	16.00 ±0.25
HCT	50.88±3.94 ^c	51.36 ±0.74	53.36 ±1.04	53.15 ±0.55	53.96 ±1.02	52.20 ±0.75
MCV	57.99±1.23 ^{ab}	60.35 ±0.55 ^a	57.06±0.99 ^{ab}	57.95±0.61 ^{ab}	57.73±0.37 ^{ab}	55.60 ±1.40 ^b
MCH	17.68±0.52 ^b	17.85 ±0.16	17.50 ±0.40	17.67 ±0.25	17.33 ±0.16	17.66 ±0.03
MCHC	30.50±0.68 ^a	29.65 ±0.27 ^b	30.66 ±0.16 ^a	30.52 ±0.33 ^a	30.0 ±0.12 ^{ab}	30.66 ±0.23 ^a

LYM	69.49±3.01 ^b	74.65 ±2.20 ^{ab}	69.63 ±1.32 ^b	81.60 ±1.99 ^a	74.79±2.94 ^{ab}	56.76 ±3.67 ^c
EOS	0.50±0.02 ^a	0.45 ±0.08 ^{ab}	0.06 ±0.03 ^b	0.57 ±0.13 ^a	0.33 ±0.03 ^{ab}	0.06 ±0.03 ^b

Datas are expressed as mean ± standard deviation. P <0.05 was considered significant. There is no significant difference between the groups containing the same letter (a-b).

Findings from the study show that propolis and bee bread have positive effects on the epithelium in the gastric ulcer model induced with indomethacin (Table 1). In histopathological examinations, the treatment groups were statistically similar with the control group (Figure 1). In addition, no significant difference was observed between the varying doses of propolis and bee bread. Accordingly, it might be speculated that bee bread and propolis application has therapeutic potential in terms of epithelial damage in the treatment of gastric ulcers.

In another study conducted in 2021, a gastric ulcer model was created in mice with intragastric Indomethacin injection. The therapeutic effects of Mexican propolis were investigated in the study. With the findings obtained, it has been reported that propolis reduces the mucosal damage in the stomach, protects the mucosal content and decreases the pro-inflammatory cytokine levels. In addition, in this study we investigated the therapeutic effect in terms of pro-inflammatory markers such as tumor necrosis factor alpha (TNF- α), interleukin

1-beta (IL-1 β) and IL-6, and the effectiveness was tested [22]. However, blood hemogram analyzes such as white blood cells, platelet, lymphocyte, and eosinophil levels highlight the strength of our study.

In another study conducted in 2020, the effects of propolis on the damage caused by *Helicobacter pylori* were investigated *in vitro*. In the results obtained, it has been shown that propolis reduces the levels of pro-inflammatory cytokines such as IL-8, IL-12, IL-1 β and TNF- α . In addition, it has been reported that propolis upregulates antioxidant enzymes [23]. These findings demonstrate that propolis has therapeutic potential for ulcers developed with *H. pylori* infection as well as medical ulcerative lesions *in vitro*. However, our study provides preliminary information for the next step as *in vivo*.

In a study conducted in 2020, an acute and chronic gastric ulcer model was created with ethanol and acetic acid in rats. The protective and therapeutic effects of green

propolis were investigated in the model. In the findings obtained, it has been shown that the application of propolis prevents gastric epithelial damage. In addition, it has been reported that it decreases the recovery time and shows an antioxidant effect by reducing oxidative stress markers [24]. The therapeutic effect against gastric ulcer may have been occurred by anti-inflammatory activity of propolis. In line with these findings, analyzing oxidative stress markers, pro-inflammatory cytokines, and blood hemogram values may be important in evaluating the therapeutic effect in future studies in terms of monitoring the efficacy of the treatment.

In a 2020 study, the gastroprotective effects of “red propolis” and an anti-inflammatory Formononetin were investigated in ethanol and indomethacin-induced gastric ulcer models. In the results obtained, reduced ulcer lesion areas have been shown that propolis applied at doses of 50, 250, 500 mg/kg. In addition, increased mucus production, antioxidant and anti-helicobacter pyloric activity were determined using propolis and formononetin combination [25]. These data suggest that the combination of propolis with anti-inflammatory agents can potentiate its therapeutic effect against gastric ulcers. In another study conducted in

2021, the protective effects of "red propolis" were investigated in gastric ulcer models induced with ethanol and hydrochloric acid. Histologically therapeutic effects at varying doses (30, 100, and 300 mg/kg) against ulcers have been shown that propolis provides. In addition, increasing levels of mucin secretion have been determined by propolis treatment. In addition, propolis has been observed to reduce oxidative stress [26]. These findings show that propolis may have different efficacy according to its local species. Furthermore, different dose administrations are important in determining the therapeutic effect. In this direction, the evaluation of different local propolis species by large dose groups in future studies may provide more robust results.

In a study, a gastric ulcer model was inducing with the application of acetic acid in rats. It has been reported that honey and pollen application to animals showed positive effects by preventing painful gastric motility [27]. Pollen known to be inhibited the lipid peroxidation, scavenges free oxygen radicals, [28, 29] and kills bacteria in bacteria studies *in vitro* [30]. However, our findings show that bee bread obtained from pollen has positive effects against gastric ulcer induced by

indomethacin by reducing epithelial damage, for the first time. Limitations of the study include the lack of evaluation of propolis and bee bread in terms of pro-inflammatory cytokines and oxidative stress-related markers. In future studies on this subject, it is important to examine these parameters in terms of evaluating the therapeutic effect.

Conclusion

In conclusion, gastric ulcer, which has a wide spectrum of etiology such as alcohol, smoking, microorganisms and NSAIDs, is a common disease that reduces the quality of life of patients. New approaches are needed due to the side effects and insufficiency of commonly used treatments. In this context, honey products propolis and bee bread, which are known to have antioxidant, anti-inflammatory, and antimicrobial effects, should be targeted in terms of gastric ulcer treatment. In addition, in further studies, evaluation of different dose groups of propolis and bee bread is necessary to determine the therapeutic window. Our findings show that the gastric epithelium damaged in gastric ulcer was significantly improved as a result of the application of propolis and bee bread. In future studies, approaches in which a wider

spectrum of doses and markers to elucidate the mechanism of the therapeutic effect are evaluated can be implemented.

Deneysel Gastrik Ülser Modelinde Propolis ve Arı Ekmeğinin Koruyucu Etkisi

Öz: Mide ülseri (gastrik ülser) üst sindirim sisteminin en yaygın hastalığıdır. Hastalığın patofizyolojisi çevresel etmenler ve non-stereoid anti-inflamatuar ilaçlarla ilişkilendirilmektedir. Bu faktörlerin mide mukozaya bariyerini zayıflatması ve asit salınımını artırması ile mide epitelinde hasar meydana gelmektedir. Medikal tedavi yaklaşımları yaygın olarak kullanılmakla beraber yan etkiler görülmektedir. Propolis ve arı ekmeği bal arılarının ürünlerinden elde edilen doğal bileşiklerdir. Yapılan çalışmalarda bu bileşiklerin antioksidan, antimikrobiyal ve anti-inflamatuar etkilere sahip olduğu gösterilmiştir. Bu çalışmada indometazin ile indüklenen gastrik ülser hayvan modelinde propolis ve arı ekmeğinin terapötik etkilerinin araştırılması amaçlanmıştır. Çalışmada 48 adet Wistar cinsi erkek ratlar kullanılmıştır. Kontrol grubu, İndometazin uygulanan ülser grubu, düşük ve yüksek doz arı ekmeği (perga) grupları, düşük ve yüksek doz propolis içeren gruplar olmak üzere 6

rastgele grup oluşturulmuştur. Enjeksiyonlar ve tedavi uygulamaları sonrasında hayvanların mide dokuları histolojik olarak ve kan örnekleri biyokimyasal olarak incelenmiştir. Elde edilen bulgulara göre propolis ve arı ekmeğinin, indometazin ile oluşturulan mide ülserinde epitel hasarını önleyerek

kontrol grubu ile benzer histopatolojik yapı oluşturduğu gözlemlendi ($p<0.05$). Bu veriler propolis ve arı ekmeğinin gastrik ülser tedavisinde umut vadeden etkilere sahip olduğunu doğrulamaktadır.

Anahtar Kelimeler: Arı ekmeği, Propolis, Mide, Ülser, Histoloji

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