Animal Health Prod and Hyg (2022) 11 (2) : 44 - 49

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Effects of Mucoadhesive Gel Containing Propolis on Some Biochemical and Hematologic Parameters in Rats With Experimental Periodontitis

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ABSTRACT

This study aimed to investigate the effects of propolis-containing mucoadhesive gel in experimentally induced periodontitis in rats. Propolis-containing mucoadhesive gel was prepared by using chitosan with a modified mechanical method. Thirty-five *Sprague Dawley* rats were used in the study. Rats were divided into five groups as the negative control, periodontitis + 50 mg/dL propolis, periodontitis + 100 mg/dL propolis, periodontitis + chitosan and healthy control. Experimental periodontitis was induced by placing ligatures on the inferior frontal teeth. After 11 days, the ligatures were removed, and gel applications were started. On the eighth day, blood samples were taken under anaesthesia. Haematological and biochemical analyses were performed from whole blood and serum samples. As a result of the statistical analysis, non-statistically significant decreases were determined in serum C-Reactive Protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) levels in the groups that were applied a mucoadhesive gel containing propolis. As a result, it was thought that mucoadhesive gel containing propolis might help treat periodontitis.

***Keywords**: Mucoadhesive gel, periodontitis, propolis, rat*

Deneysel Periodontitis Oluşturulan Ratlarda Propolis İçeren Mukoadesiv Jelin Bazı Biyokimyasal ve Hematolojik Parametreler Üzerine Etkileri

ÖZET

Çalışmada ratlarda deneysel oluşturulan periodontitisde propolis içeren mukoeadesiv jelin etkilerini araştırmak amaçlandı. Propolis içeren mukoadesiv jel kitosan kullanılarak modifiye mekanik bir metot ile hazırlandı. Çalışmada otuzbeş *Sprague Dawley* rat kullanıldı. Ratlar negatif kontrol, periodontitis + 50 mg/dL propolis, periodontitis + 100 mg/dL propolis, periodontitis + kitosan ve sağlıklı kontrol olmak üzere beş gruba bölündü. Deneysel periodontitis alt kesici dişe ligatür konularak oluşturuldu. Onbir gün sonra ligatürler çıkarılarak jel uygulamalarına başlandı. Sekizinci gün genel anestezi altında örnekler alındı. Tam kan ve serum örneklerinden hematolojik ve biyokimyasal analizler yapıldı. Sonuçların ististstiki analizinde propolis içeren mukoadesiv jel uygulanan gruplarda serum C-reaktif protein (CRP), interlökin-1 (IL-1), interlökin-6 (IL-6) ve tümör nekrozis faktör (TNF-α) düzeylerinde istatistiki önemde olmayan azalmalar belirlendi. Sonuç olarak propolis içeren mukoadesiv jelin periodonditis tedavisinde yararlı olabileceği düşünüldü.

***Anahtar Kelimeler:** Mukoadesiv jel, periodontitis, propolis, rat*

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Received Date: 07.10.2022 – Accepted Date: 14.11.2022

DOI: 10.53913/aduveterinary.1185773

Introduction

Propolis is a resinous natural product used for many purposes by mixing with wax after being collected from the secretions and buds of plants by honey bees (Popova et al., 2005). It has been shown in previous studies that the ethanolic extract of propolis has antiviral, antibacterial, antifungal, antiprotozoal, anti-inflammatory, anticarcinogenic, antioxidant, and local anaesthetic properties. Antibacterial activity of propolis has been demonstrated against gram-positive bacteria such as *Staphylococcus aureus (S. aureus)* (Valazquez et al., 2007) and gram-negative bacteria such as *Salmonella* (Orsi et al., 2005). It has been confirmed *in vivo* and *in vitro* that propolis inhibits the glycosyltransferase enzyme activity in *Streptococcus mutans* and *Streptococcus sobrinus* (Ikeno et al., 1991). Propolis induces the synthesis of insoluble glycans and inhibits the glycosyltransferase enzyme activity (Koru et al., 2007). When the researchers evaluated the antibacterial activity of propolis against some anaerobic oral pathogens, they reported that it was effective against *Lactobacillus acidophilus, Actinomyces naeslundii, Prevotella oralis, Prevotella melaninogenica, Porphyromonas gingivalis, Fusobacterium nucleatum* and *Veillonella parvula* due to the presence of aromatic flavonoid. Kujumgiev et al. (1999) reported the antibacterial activity of propolis against *S. aureus* and *Escherichia coli* and its antifungal activity against *Candida albicans (C. albicans)*. In addition, it has been reported that the co-administration of propolis with antibiotics increases their effectiveness 10 to 100 times and synergises with them. Propolis also has anti-inflammatory properties by inhibiting lipoxygenase enzymes and the production of prostaglandins. Its anti-inflammatory and analgesic properties are similar to aspirin, but it has fewer side effects (Poppe and Michelis, 1986). It also increases the production of interferon and antibodies.

Periodontitis is one of the comprehensive diseases in humans, so many studies have used experimental animals to understand its pathogenesis. The cause of tissue destruction in periodontitis is the immune and inflammatory response against pathogenic bacterial plaque. Interleukin 1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) stimulate the differentiation of osteoclast precursors and activate osteoclasts, inducing connective tissue destruction and bone resorption. Furthermore, IL-1β and TNF-α are well-researched markers of disease activity in the periodontium and act synergistically to cause bone resorption (Preshaw and Taylor, 2011).

A natural or synthetic polymer bonding to a biological substrate and keeping these two surfaces together for a long time by interfacial forces is defined as bioadhesion. If the physical surface is epithelial tissue or the mucus layer on the surface of a tissue, this attachment is defined as mucoadhesion. Mucoadhesion is a practical method for drug immobilisation or localisation (Boddupalli et al., 2010). Mucoadhesive drug release systems have advantages such as a long residence time of the drug in the region, localisation of the release system in a specific area, and an increase in the drug concentration gradient due to the intense contact of the drug with the mucosal surface. Chitosan is a bioadhesive polymer used in mucoadhesive formulations. Chitosan is a biologically cationic polysaccharide formed by combining a monosaccharide with a glycosidic bond. Due to its positively charged nature can bind very strongly to negatively charged materials such as cell surface and mucus. Although chitosan is used as a diluent, it is also used as a binder, lubricant or strong dispersant. The mucoadhesive properties of chitosan facilitate the local delivery of drugs and other substances in the oral cavity (Singh et al., 2011).

Therefore, this study aimed to investigate the effects of mucoadhesive gel containing ethanolic extract of propolis by experimentally constructing a periodontitis model. For this purpose, it was decided to examine the levels of proinflammatory cytokines IL-1, IL-6 and TNF and early inflammation marker CRP in rats with experimental periodontitis. It also aimed to demonstrate propolis's therapeutic efficacy in treating periodontitis, one of the gingival diseases, by observing haematological changes.

Materials and Medhods

Animals

This study was started with the approval of Adnan Menderes University Animal Experiments Local Ethics Committee dated 28/10/2020 and numbered 64583101/2020/104. *Sprague Dawley-type* male rats used in this study were obtained from Aydın Adnan Menderes University Veterinary Faculty Experimental Animal Production and Research Center. The study took 35 Sprague Dawley-type male rats with an average of 200-250 grams weight. During the experiment, the animals were made of transparent polycarbonate material of 420 x 260 x 180 mm in controlled rooms at Adnan Menderes University Veterinary Faculty Experimental Animals Unit with 40-60% humidity, optimum temperature (22°C), 12 hours of light and 12 hours of darkness. They were housed in cages made of stainless steel with cage tops. Animals were adapted to the experiment room before the study started. The water and feed were met *ad libitum*. The daily care of the rats was done from 10:00-12:00 every day.

Preparation of mucoadhesive gel formulation

A modified mechanical process was used to prepare the mucoadhesive gel containing propolis. Continuous mixing was done by adding 5% glycerol to 4% chitosan solution designed in 3% acetic acid. Propolis extract in ethanol was added to the gel at the determined doses (50mg/dL and 100mg/dL). All the formulations were stored in a screw-capped wide-mouthed beaker covered with aluminium foil in a calm and dark place (Partha et al., 2016).

Animals were divided into five groups as healthy control, negative control, chitosan, propolis 50 (50 mg/kg propolis) and propolis 100 (100 mg/kg propolis). Each group included 7 animals, and groups were kept in separate cages.10 mg/kg Xylazine (Rompun®, Bayer, Topkapı, Türkiye) and 100 mg/kg ketamine (Ketalar, Pfizer, Istanbul, Türkiye) were prepared for anaesthesia in periodontitis rat groups. 3/0 silk suture material was passed submar-

ginally to the inferior frontal teeth under anaesthesia. The knot was tied in the vestibule. On the 11th day, these ligatures were removed again under anaesthesia. The mucoadhesive gel containing 50 mg/dL and 100 mg/dL propolis was applied to the gingiva for seven days in the groups with periodontitis. The gel prepared with chitosan, used to prepare mucoadhesive gel, was applied to the rats in the chitosan group with the same method. No gel was applied to the negative control group. Seven rats were housed in different cages in the same experimental room without ligature and gel application as a healthy control group. Gel applications were made locally every day for seven days, starting when the ligature was removed. On the eighth day, intracardiac blood samples were collected under general anaesthesia.

Biochemical analysis

Serum IL-1, IL-6, TNF-α and CRP concentrations were measured using rat-specific ELISA kits (Bioassay Technology Laboratory, China) with an ELISA reader (Optic Ivymen System, Spain) according to the manufacturer's instructions.

Hematologic analysis

Haematological examinations were performed using the Abacus Junior Vet Hematology Cell Counter (Diatron MI Ltd, Hungary).

Statistic evaluation

SPSS21 (Statistical Package For Social Sciences 21SPSS INC., Chicago, IL, USA) was used to analyse the data obtained in the study. Whether the data showed normal distribution or not was evaluated with the Shapiro-Wilk test. While comparisons were made with the ANOVA test to the groups with normal distribution, the Kruskal-Wallis test was applied to the groups that did not show normal distribution. Results are shown as mean and standard deviation.

Results

When the groups were compared, no statistically significant difference in serum CRP levels could be determined. However, the CRP level of the negative control group was found to be higher than the healthy control group. Serum CRP levels were lower in rats treated with a mucoadhesive gel containing chitosan and propolis than in the negative control. Comparing the mean serum TNF-α concentrations, changes were not statistically significant. At the same time, the highest level was determined in the group with periodontitis without treatment. The level was decreased in the chitosan and 50 mg/dL propolis groups. It was lower in the 100 mg/dL propolis group than in the healthy control group. Mean serum IL-1 levels showed a slight increasing trend in all groups with no statistical significance compared to the healthy control. When the mean serum IL-6 levels were compared, no statistical difference could be found in the groups treated with healthy control negative control chitosan, 50 mg/dL and 100 mg/dL propolis.

A statistically significant difference was determined in the hemoglobin and PCT levels. In contrast, among other parameters, no statistically significant difference was observed between the healthy, negative control and chitosan and propolis-containing groups. While the hemoglobin level was higher in the negative control and chitosan gel group than in healthy rats, it was observed that it tended to decrease in the group treated with propolis-containing gels. The blood PCT level was significantly lower in rats with periodontitis that did not receive any treatment than in the healthy and treatment groups (Tables 1 and 2).

Discussion

Animal models of periodontal disease are essential in developing the scientific basis for understanding pathological processes (Graves et al., 2012). Especially rodents and rats are suitable models for experimental periodontal research (Struillou et al., 2010). The structure of the dental gingival region is similar to that observed in humans with shallow gingival sulcus and attachment epithelium to the tooth surface (Lonel et al., 2015). Some pathways in experimental periodontitis differ from human chronic periodontitis progression. Because in the model created by placing the ligature, bacterial accumulation and periodontal tissue destruction show an acute process. Despite this, it is widely used (Aral et al., 2015). Propolis antibacterial, antioxidant, antifungal, antiviral, anti-inflammatory, tissue regenerative, and wound-heal-

Table 1. Mean serum CRP, TNF-α IL-1 and IL-6 concentrations (means±standard deviation) ($\overline{X}$ ±S) in experimental periodontitis-induced and healthy control group

	Healty ($\overline{X}$ ±S)	**Nega ve Control** ($\overline{X}$ ±S)	**Chitosan** ($\overline{X}$ ±S)	**Propolis 50** ($\overline{X}$ ±S)	**Propolis 100** ($\overline{X}$ ±S)	**P**
CRP (ng/mL)	1.22±0.52	1.78±0.30	1.55±0.53	1.24±0.51	1.41±0.52	NS
TNF-α(ng/mL)	200.57±95.70	293.25±141.00	221.84±23.03	244.41±91.83	180.36±81.18	NS
IL-1 (pg/mL)	28.36±3.96	31.05±5.49	29.56±4.60	32.64±2.30	32.18±6.96	NS
IL-6 (pg/mL)	2.56±0.36	2.37±0.67	2.83±0.22	2.43±0.34	2.87±0.39	NS

NS: Non-significant

Table 2. Mean serum hematologic parameters means±standard deviation ($\bar{X}$ ±S) in experimental periodontitis-induced and healthy control group rats.

	Healty ($\bar{X}$ ±S)	Nega ve Control ($\bar{X}$ ±S)	Chitosan ($\bar{X}$ ±S)	Propolis 50 ($\bar{X}$ ±S)	Propolis 100 ($\bar{X}$ ±S)	P
WBC	9.63±3.23	11.27±3.47	8.95±3.18	9.35±1.80	9.78±2.50	NS
LYM	7.95±2.64	9.72±3.04	7.31±2.84	7.06±1.88	7.68±1.91	NS
NEU	1.34±0.66	1.37±0.55	1.33±0.50	1.92±1.57	1.63±0.87	NS
%LY	82.75±5.18	73.18±31.98	80.95±4.19	75.91±16.06	79.60±15.57	NS
%NE	13.58±3.59	12.11±2.60	15.90±5.57	20.56±16.52	16.28±5.83	NS
RBC	9.91±4.63	9.85±0.72	9.11±0.85	8.01±0.37	8.56±0.68	NS
HGB	13.56±1.39^{a}	15.25±0.48^{b}	15.85±1.00^{b}	14.30±1.21^{ab}	14.71±0.66^{ab}	*
HCT	62.17±41.17	137.16±204.76	49.72±2.89	43.83±3.72	47.33±3.52	NS
MCHC	27.13±9.66	28.53±2.40	31.83±0.72	32.68±1.98	31.14±1.70	NS
PDWc	18.08±6.03	16.20±0.55	16.20±0.67	16.20±0.50	15.90±0.69	NS
PCT	0.3±0.06^{a}	0.58±0.06^{b}	0.79±0.11^{ab}	0.76±0.13^{ab}	0.78±0.30^{ab}	*
MPW	7.04±0.16	7.30±0.49	7.05±0.64	6.98±0.43	7.07±059	NS
PDWC	33.16±0.35	32.81±2.55	33.46±2.34	32.75±1.04	32.65±1.10	NS

ab: Statistical difference between groups with different letters in the same column is significant. *: P<0.05
NS: Non-significant

ing effects allow its clinical use (Eroğlu et al., 2004). Much research has been done on propolis for the mouth, and it is widely used safely. Experiments have been done on periodontitis, gingivitis and caries in dentistry (Koo et al., 2000; Skaba et al., 2013). It has been reported that the solvent used affects the antimicrobial activity of propolis. While glycerine solutions have little inhibitory effect on bacteria, ethanol solutions create an excellent inhibitory effect against bacteria and yeasts (Castaldo and Capasso, 2002).

IL-1, IL-6 and TNF-α stimulate the differentiation of osteoclast precursors and activate osteoclasts, inducing connective tissue destruction and bone resorption (Farquharson et al., 2012; Dietrich et al., 2013). These cytokines are well-researched markers in periodontal disease activity and act synergistically to induce bone resorption (Dietrich et al., 2013; Olsen, 2015). For this reason, this study aimed to show the effect of propolis in the form of mucoadhesive gel by examining the changes in these cytokines. There was a decrease in the TNF level in the gel-applied groups, but the reductions were not statistically significant. It was observed that it was lower than the healthy control, especially in the gel group containing 100 mg of propolis.

However, IL-1 and IL-6 levels did not cause a significant change when the healthy control and experimental groups were compared. Aral et al. (2015) reported that mean plasma IL-1b levels in rats with diabetes and periodontitis increased in untreated groups and decreased in propolis treatment groups. However, the differences were not statistically significant in this study either. Nishihara et al. (2009) reported that serum TNF-α levels increased in mice with experimental diabetes and periodontitis, and then decreased on the 3rd day. Takano et al. (2010) similarly induced periodontitis in diabetic mice and reported that cytokine levels increased significantly. These differences in the studies were thought to be due to the differences in the methods of inducing periodontitis and the duration of the study.

The relationship between CRP and periodontitis has received significant attention because of the link between periodontitis and cardiovascular disease (Paraskevas et al.,2008; Bansal et al., 2014). It is common to use CRP as a marker of the relationship of periodontitis with other systemic diseases (Hajishengallis and Chavagis, 2021). The serum CRP level was higher in the negative control group, which did not receive treatment. The gel containing propolis decreased in the treatment groups, but the serum CRP levels were not statistically significant. In a meta-analysis review evaluating studies on periodontitis and CRP levels, it was reported that there is a correlation between serum CRP levels in patients with periodontitis who are not systematically healthy. It has been reported that patients with periodontitis have high CRP levels, but systemic disease causes a more severe increase in CRP. In addition, it has been reported in the literature reviews that aggressive forms of periodontitis cause a more severe increase in CRP levels. Haematological changes in

patients with periodontitis were generally recorded as high WBC, high neutrophil level, low sedimentation rate and PCV (Bothello et al., 2020). This study determined that the WBC and lymphocyte count increased in the rats with experimental periodontitis compared to the healthy control and tended to decrease in the treatment groups. Although there was no statistical difference between the changes, these results were compatible with previous studies. The decrease in PCT levels was statistically significant between this study's healthy control and untreated negative control group. The stimulus in the gingiva is characterised by leukocytes rich in inflammatory infiltrate, which can then be excreted into the systemic circulation (Ryder, 2010; Hirschfeld, 2014). Alternatively, it can stimulate the bone marrow to produce more inflammatory cells chronically through continuous local inflammation and bacterial interaction (Belkaid and Hand, 2014). In addition, periodontal bacteria can invade periodontal tissues through the ulcerated epithelium and trigger a systemic response to counter harmful effects.

Conclusion

This study showed that propolis might contribute to preventing tissue destruction and healing by reducing the systemic manifestations of inflammation caused by periodontal diseases. However, more detailed studies are needed to examine the effects of propolis applied as a mucoadhesive gel. In the new studies to be planned, different periodontitis induction methods, increasing the number of subjects and trying various dose applications will improve the data on this subject. Propolis has an important place in pharmaceuticals. Applying propolis in mucoadhesive gel form is important as a new approach. In future studies, it is predicted that propolis can be more effective in this form by examining the physicochemical properties of the gel and standardising it.

Acknowledgements

This study was summarised by the first author's Master Thesis. This study was supported by Aydın Adnan Menderes University Research Foundation (VTF-21025).

Conflict of İnterest

The authors declare that they have no conflict of interest.

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