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Analgesic effect of Anatolian propolis in mice

Farelerde Anadolu propolisinin analjezik etkisi

Kıvanç KAMBUROĞLU,[1] Tuncer ÖZEN[2]

Summary

Objectives: The aim of the present study was to assess the analgesic effect of Anatolian propolis, which is added to toothpastes as a prophylactic component for periodontal diseases.

Methods: Water-, ethanol- and acetone-extracted Anatolian propolis were prepared. The analgesic effect of the extracts was assessed using the tail-flick test in mice (n=6 per extract). Comparison among groups was made using one-way ANOVA, followed by post-hoc Scheffe test to determine significant differences among the means of the data groups. P<0.05 was accepted as indicating a significant difference.

Results: We found that water-extracted Anatolian propolis caused a significant increase, 1.61-fold (p<0.001 versus control), in the latency time using tail-flick test in mice. However, acetone-extracted and ethanol-extracted propolis led to no significant effect.

Conclusion: We proved the analgesic effect of water-extracted Anatolian propolis. Thus, propolis used in the composition of toothpastes may be beneficial in terms of its analgesic action in addition to its other favorable effects.

Key words: Analgesia; dental pain; propolis; toothpaste.

Özet

Amaç: *Bu çalışmanın amacı, periodontal hastalıklarda profilaktik bileşen olarak eklenen Anadolu propolisinin analjezik etkisinin değerlendirilmesidir.*

Gereç ve Yöntem: *Su, etanol ve asetondan ekstre edilmiş Anadolu propolisi hazırlandı. Ekstrelerin analjezik etkisi fare tail-flick testi ile değerlendirildi (n=6 her bir ekstre için). Gruplar arası karşılaştırmalar için tek yönlü ANOVA, sonrasında ise data grupları ortalamalarının arasındaki belirgin farkların saptanması amacıyla post-hoc Scheffe's testi uygulandı. P<0.05 anlamlılık sınırı olarak kabul edildi.*

Bulgular: *Sudan ekstre edilmiş Anadolu propolisi tail-flick testi ile farelerde latens sürenin belirgin şekilde 1.61 kat artışına neden oldu (p<0.001). Ancak, aseton ve etanol den ekstre edilmiş propolisin belirgin bir etkisi ortaya konamadı.*

Sonuç: *Anadolu propolisinin analjezik etkisi kanıtlandı. Propolisin diş macunlarında kullanılması diğer olumlu etkileri ile beraber analjezik açıdan da faydalı olabilir.*

Anahtar sözcükler: Analjezi; dental ağrı; propolis; diş macunu.

Introduction

Propolis, which is a resinous sticky substance that honeybees produce by mixing their own waxes with resins collected from plants, is used as a sealant and sterilant in honeybee nests.[1-4] It has been used as a folk medicine since ancient times. In modern times, it has been found to have a wide range of biological activities, such as antibacterial,[1] anti-inflammatory,[2] antioxidative,[3] hepatoprotective effects,[4] and/or tumoricidal[5] activities. Moreover, propolis may also prevent dental caries.[6,7] Because of its biological activities, propolis have been used in the composition of toothpastes.[8,9] Actually, dental use of propolis has been emphasized by several studies, and its beneficial effects, such as decreasing dentinal hypersensitivity, occluding dentinal tubules, increasing

[1]Department of Dentomaxillofacial Radiology, Ankara University, Faculty of Dentistry, Ankara;
[2]Department of Dentomaxillofacial Radiology, Dental Science Center, Gülhane Military Medical Academy, Ankara, Turkey

[1]Ankara Üniversitesi, Diş Hekimliği Fakültesi, Ağız, Diş ve Çene Radyolojisi Anabilim Dalı, Ankara;
[2]GATA, Diş Hekimliği Bilimleri Merkezi, Ağız, Diş ve Çene Radyolojisi Anabilim Dalı, Ankara

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Correspondence ***(İletişim):*** Kıvanç Kamburoğlu, DDS, MSc, PhD. Ankara Üniversitesi, Diş Hekimliği Fakültesi, Ağız, Diş ve Çene Radyolojisi Anabilim Dalı, Ankara, Turkey.
Tel: +90 - 312 - 296 56 32 **e-mail** ***(e-posta):*** dtkivo@yahoo.com

periodontal ligament cell viability of avulsed teeth, plaque-inhibiting and anti-inflammatory effects have been reported.[10-13] It is valuable to find out if propolis used in the composition of toothpastes might be beneficial in terms of its analgesic action along with its other favorable effects. De Campos et al.[14] previously demonstrated analgesic action of an ethanol extract of Brazilian propolis through acetic acid, kaolin, or zymosan models of nociception and they also reported that this extract significantly inhibited capsaicin-induced pain and reverted the hyperalgesia induced by bradykinin. Recently, Paulino et al.[15] reported that an ethanol extract Bulgarian propolis was ineffective when assessed by using hot plate and tail flick analgesia tests in mice. In a search of the literature, however, we were unable to find a published study concerning the analgesic property of Anatolian propolis.

In the present study, we sought to investigate the analgesic effect of Anatolian propolis by using tail-flick test in mice.

Materials and Methods

The propolis sample was collected during spring season in Samsun (Blacksea Region, Turkey), stored in laboratory conditions, and three different extracts were prepared as previously described.[16] Three different extracts prepared were as follows:

1. Aqueous (water) extracted propolis (AEP): Water extracted propolis was obtained by mixing 100 ml water and 30 gram propolis in ultrasonic water bath at 35 kHz for 1 h at 40°C. The extraction was then filtered through a paper filters. The extract was further concentrated to 5 ml under stream of nitrogen at 40°C.

2. Acetone extracted propolis: Thirty gram of propolis was dissolved in 100 ml acetone and evaporated in rotary evaporator. The volatile ingredients of propolis dissolved in acetone was separated and evaporated under stream of nitrogen to dryness at 40°C, and then it was dissolved with 10% dimethylsulphoxide (DMSO) in 5 ml.

3. Ethanol extracted propolis: The remaining unvolatile compounds of propolis was dissolved in ethanol 70% (100 ml) and evaporated under stream of nitrogen to dryness at 40°C, and then it was dissolved with 10% dimethylsulphoxide (DMSO) in 5 ml. The final concentration of DMSO in this solution did not exceed 1%, which had no effect per se on animal analgesia tests.

All experiments were performed at the same time every day and in the light period (10:30 - 13:00 AM). The experiments performed in this study have been carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by National Institutes of Health (USA) and the Declaration of Helsinki. Adult male albino Swiss-Webster mice (22-30 g) were subjects in our study. They were placed in a quiet and temperature- and humidity-controlled room (22 ± 2°C and 60 ± 5%, respectively) in which a 12/12 hour light-dark cycle was maintained (07 AM - PM light).

Analgesic effect was assessed using the tail-flick test, which is a thermal analgesia measurement method for rodents.[17] Tail-flick latencies were measured by a tail-flick test apparatus (Columbus, OH, Type 812). The mean of the tail-flick latencies measured in three predrug trials represented the individual baseline. The mouse tail was marked with a pen about 3 cm from the tip and the light beam was focused on this marked site. Baseline tail-flick latency for each mouse was determined and designated as the baseline latency. The intensity of light was adjusted so that baseline latencies were 2-3 s, with a cut-off time of 6 s to prevent tissue damage. Propolis or 0.9% saline (as control) were injected intradermally using a 30 gauge needle attached to a Hamilton syringe with a volume of 10 µL into the skin of the tail on the marked site. Each injection of 10 µL of the extracts contained 60 mg propolis. Test latencies were measured after drug or 0.9% saline injections. To determine whether propolis acted locally, tail-flick latencies elicited by stimulation of the tail at a site 1-2 cm more proximal from the marked site was concomitantly measured.[18] Comparison among groups were made by using One-way ANOVA, followed by post-hoc Scheffe's test to determine significant differences among the means of the data groups. $P<0.05$ was accepted as significant difference.

Results

Acetone extracted propolis (60 mg/10 µL) did not cause a change in the tail flick latency time after intradermal injection (n=6 mice). Tail-flick latency values after injection of acetone extracted propolis were similar to those of 0.9% saline at 10, 20, 30 and 60 min after intradermal injections (n=6 mice per group). Similarly, ethanol extracted propolis (60 mg/10 µL) led no significant change in the tail flick latency values after intradermal injection (n=6 mice). Tail-flick latency time of acetone extracted propolis group were similar to those of 0.9% saline at 10, 20, 30 and 60 min after injection (n=6 mice per group). However, water extracted Anatolian propolis caused a significant increase in the latency time using tail-flick test in mice, when compared with 0.9% saline group, at 10, 20, 30 and 60 min after injection ($p<0.001$) (n=6 mice per group). At 60 min, water extracted Anatolian propolis caused 1.61 fold significant increase of the latency time. Tail-flick latency values after injection of water, acetone and ethanol extracted propolis in mice are shown in Figure 1.

Discussion

In the present study, we showed the analgesic effect of Anatolian propolis. To our knowledge, this study is the first to reveal the analgesic effect of propolis using tail flick test in mice. Previously, Paulina et al.[15] reported that Bulgarian propolis was ineffective when assessed in the hot plate and tail flick test in mice. We also observed that, acetone extracted and ethanol extracted Anatolian propolis led no significant effect.

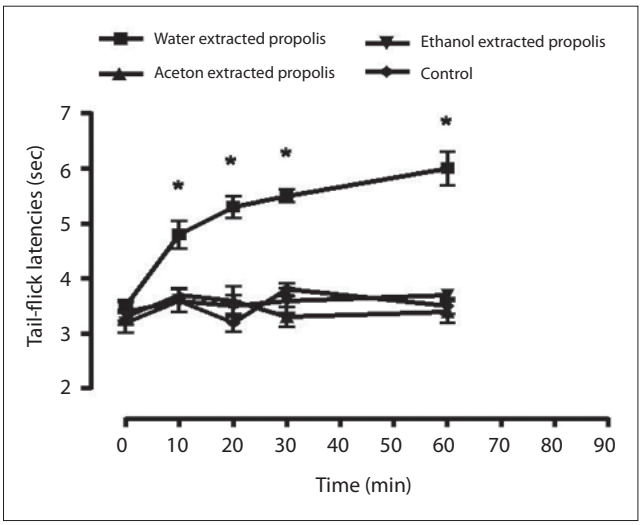

Figure 1. Tail-flick latency values (sec) after injection of water, acetone and ethanol extracted propolis in mice (n=6 per each treatment group). * $p<0.001$ versus Control.

Propolis, also known as bee glue, is a traditional remedy widely used in many countries for the management of numerous diseases, including airway disorders and cutaneo-mucosal infections, mainly those of bacterial and viral etiologies. Propolis exhibits a variety of biological activities including bactericidal, antiviral, fungicidal, anti-tumoural, anti-oxidant, and anti-inflammatory properties.[1-5]

De Campos et al.[14] previously demonstrated the anti-hyperalgesic action of an ethanol extract of propolis collected in the South of Brazil through acetic acid, kaolin, or zymosan models of nociception and also this extract significantly inhibited capsaicin-induced pain and reverted the hyperalgesia induced by bradykinin.

Chemical studies conducted with propolis extracts revealed the existence of a very complex mixture of different naturally-occurring constituents with more than 300 constituents identified to date,[1,2] such as phenolic acid, terpenes, cinnamic acid, caffeic acid, several esters, and also flavonoids. Most of these are lipophilic compounds. Since lipophilic compounds are easy to extract using ethanol, the ethanol extract of propolis (EEP) is well known and has attracted much interest. In contrast, the water extract of propolis (WEP) has been featured in few reports, even though WEP and its main constituents (including caffeoylquinic acids) have greater antioxidative effects, greater inhibitory activity against some enzymes, and greater absorbency than EEP and the constituents of EEP.[19] In the present study, Anatolian green propolis was extracted by water and it may contain more hydrophilic constituents than propolis originate from other areas. These constituents may have synergic effects, which leads propolis to have such different pharmacological activities.

In addition, propolis composition varies with the season and the geographic region; such extraordinary variability among samples from different sources leads to variation of the pharmacological properties of propolis. The biological activity of propolis is

associated mainly with phenolic compounds such as flavonoids and derivatives of hydroxycinnamic acids. In temperate zones, the main constituents are flavonoids, while in tropical zones other classes of bioactive components have been described, such as aromatic acid derivatives, specific terpenoids and prenylated p-coumaric acids and acetophenones.

Because of its wide range of biological activities, propolis have been used in the composition of toothpastes.[8,9] Previously, Mahmoud et al.[10] reported that propolis had significant effect on dentinal hypersensitivity in patients. Almas et al.[11] reported that application of propolis was effective in occluding dentinal tubules. Recently, Botushanov et al.[20] have reported that silicate toothpaste with extract from propolis shows very good plaque-cleaning, plaque-inhibiting and anti-inflammatory effect. In addition, Koo et al.[7] have also reported that mouth rinse containing propolis is efficient in reducing dental plaque accumulation. Propolis has also been shown to be an effective transport medium for the maintenance of periodontal ligament cell viability of avulsed teeth,[12] and it has recently been reported that direct pulp capping with propolis flavonoids in rats may delay dental pulp inflammation and stimulate repair of dentin.[13] We observed that water extracted Anatolian propolis caused a marked analgesic effect using tail flick test in mice whereas, acetone extracted and ethanol extracted propolis led no significant effect. Water extracted propolis is much more available than ethanol or acetone extracted propolis to be used in the composition of toothpastes. Thus, our results show that propolis used in the composition of toothpastes may be beneficial in terms of its analgesic action in addition to its other favorable effects stated above. Since seasonal and geographic differences may affect composition of propolis, further investigations are needed to clarify influence of these differences on the analgesic effect propolis.

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