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Influence of Propolis Extract on Bleaching Effectiveness and Enamel Physical Properties

Utjecaj ekstrakta propolisa na učinkovitost izbjeljivanja i fizikalna svojstva cakline

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Abstract

Objectives: This study evaluated the effects of propolis extract on dental enamel following tooth bleaching with 35% hydrogen peroxide (HP). **Materials and Methods:** Sixty bovine enamel specimens were divided into six groups: Artificial Saliva (Control); Whiteness HP35% (HP35%); Whiteness HP35% + potassium nitrate desensitizer (HP35%+KF); Whiteness HP35% + potassium nitrate desensitizer + propolis extract (HP35%+KF+P); Whiteness HP35% + neutral fluoride + propolis extract (HP35%+F+P); and Whiteness HP35% + propolis extract (HP35%+P). The in-office bleaching protocol consisted of three sessions (3 × 15 minutes). Color change (ΔE^* , ΔE_{00}^* , ΔWID), surface roughness (Ra), and microhardness (KHN) were evaluated at baseline (T_1) and after 24 hours (T_2). Color parameters were analyzed using generalized linear models ($p \leq 0.05$), and Ra and KHN were assessed using a generalized linear mixed model for repeated measures ($p \leq 0.05$). **Results:** All HP-treated groups showed significantly greater color change compared to the control group. Surface roughness increased after bleaching; however, groups treated with desensitizers, fluoride, and/or propolis showed lower Ra values at T_2 compared to baseline. For microhardness, only the HP35%+KF+P group maintained KHN values comparable to the control group after bleaching. **Conclusion:** Propolis extract did not compromise the whitening efficacy or negatively affect enamel surface roughness or microhardness following treatment with high-concentration hydrogen peroxide.

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Introduction

Tooth bleaching is one of the most sought-after treatments in dentistry by individuals seeking a more esthetically pleasing smile (1). The bleaching mechanism involves a redox reaction of hydrogen peroxide (HP), which dissociates into low-molecular-weight free radicals. These radicals diffuse through dental tissues and react with organic pigment macromolecules, leading to the breakdown of double bonds (2). As a result, light is more efficiently reflected by the dental structure, thus producing a brighter appearance (3).

There are two main types of bleaching procedures available: in-office bleaching and supervised at-home bleaching (4, 5). A common side effect of these treatments is hypersensitivity during or after the sessions (6). This is associated with

Uvod

Izbjeljivanje zuba jedan je od najtraženijih tretmana u stomatologiji među pojedincima koji traže estetski ljepši osmijeh (1). Mehanizam izbjeljivanja uključuje redoks-reakciju vodikova peroksida (HP) koji disocira na slobodne radikale niske molekularne težine. Ti radikali difundiraju kroz zubna tkiva i reagiraju s makromolekulama organskoga pigmenta, što rezultira razgradnjom dvostrukih veza (2). Kao rezultat toga, svjetlost se učinkovitije reflektira od zubne strukture stvarajući svjetliji izgled (3).

Dvije su glavne vrste postupaka izbjeljivanja: izbjeljivanje u ordinaciji i izbjeljivanje kod kuće pod nadzorom (4, 5). Uobičajena nuspojava tih tretmana jest preosjetljivost tijekom sesija ili poslije njih (6). To je povezano s koncentracijom slo-

the concentration of free radicals in the bleaching gel, which can reach the dental pulp through dentinal tubules (7). This process may trigger an inflammatory response, the release of chemical mediators, and changes in local microcirculation, ultimately resulting in hypersensitivity. The condition typically manifests as acute and transient pain, especially within the first 24 hours following in-office bleaching with high-concentration agents (8, 9).

Although the pain and discomfort caused by bleaching are temporary, their intensity can cause some patients to discontinue the treatment (7). Therefore, implementing preventive measures to minimize side effects is essential (10). The most common approach involves applying topical remineralizing agents containing glutaraldehyde, potassium nitrate, calcium-based compounds, or neutral fluoride (10, 11). Various protocols have been proposed to reduce hypersensitivity before and after bleaching, including the use of anti-inflammatory agents, corticosteroids, analgesics, and natural extracts to improve patient comfort and satisfaction (12).

Among natural products, propolis extract has gained increasing attention in dentistry due to its anti-inflammatory, analgesic, healing, and anesthetic properties, which has been supported by several studies (12, 13, 15). Flavonoids present in propolis are known to inhibit free radical formation (17, 18) and are considered the main components responsible for its anti-inflammatory effects. Specifically, caffeic acid phenethyl ester (CAPE)—a flavonoid found in propolis which modulates inflammatory responses (13, 17).

Propolis is a natural resinous material produced by bees that has been widely used since ancient times (18). Its mechanism of action involves the deposition of resinous substances that occlude the dentinal tubules (14, 15), thereby reducing the hypersensitivity resulting from bleaching treatment. Propolis has antibacterial, antiviral, and anti-inflammatory properties (17). Due to its anti-inflammatory properties, it could be a product used after high concentration whitening treatments, since one of the most prevalent adverse effects of this procedure is exacerbated pain (8).

However, no studies to date have investigated the use of propolis extract following in-office tooth bleaching or its effect on color stability. Since propolis has a dark coloration, its application may potentially interfere with bleaching efficacy. Therefore, the objective of this study was to analyze the effects of propolis extract on dental enamel and compare them with those of commercial remineralizing agents after high-concentration (35%) in-office bleaching. The null hypotheses tested were: (1) the application of propolis does not interfere with whitening efficacy, and (2) the protocols do not affect the properties of enamel surface in terms of microhardness and surface roughness following high-concentration bleaching.

Materials and Methods

Preparation of Specimens

Bovine anterior teeth were extracted and stored in a 0.1% thymol solution (Proderma, Piracicaba, São Paulo, Brazil). Debris was removed using a scalpel blade, and the crowns

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Materijali i metode

Priprema uzoraka

Prednji goveđi zubi izvađeni su i pohranjeni u 0,1-postotnoj otopini timola (Proderma, Piracicaba, São Paulo, Brazil). Ostaci su uklonjeni skalpelom, a krune su odvojene od

were separated from the roots using a double-faced diamond disc (KG Sorensen 7020, SP, Brazil) in a low-speed micromotor under constant irrigation. Prophylaxis was performed using rubber cups (American Burrs, Brazil) with a pumice and water paste (2:1). Mesiodistal and incisocervical cuts were made using a metallographic cutter (Isomet 1000, Buehler, Illinois, USA) equipped with a precision diamond disk (4" × 0.12 × 1/2).

Enamel-dentin specimens measuring 4 × 4 mm with a height of 3 mm were obtained. Dentin surfaces were prepared using #600 grit silicon carbide sandpaper, while enamel surfaces were progressively polished with #600, #1200, #2000, and #4000 grit silicon carbide sandpapers under constant irrigation (Arotec, Cotia, SP, Brazil). Final polishing was performed using felt discs and decreasing-grit diamond pastes (1/2 μm and 1/4 μm). Specimens were cleaned in an ultrasonic cleaner (Marconi, Piracicaba, São Paulo, Brazil) for 10 min to remove residues (19).

Tooth Staining Protocol

Specimens were immersed in a black tea solution (1.6 g black tea; Leão Junior S.A., Curitiba, PR, Brazil) prepared in 100 mL distilled water, boiled for 3 min, and steeped for 5 min. The solution was stored at 37 °C and replaced every 24 h for 6 days. After staining, residues were removed with #4000 grit silicon carbide sandpaper, and specimens were stored in artificial saliva (Ca 1.5 mmol/L; P 0.9 mmol/L; 150 KCl mmol/L; 0.1 mol/L Tris buffer, pH 7.0) at 37°C (±1°C) for 14 days. Saliva was replaced daily (Serra et al., 1992). Initial color measurements were performed using a spectrophotometer to exclude outliers, and specimens were randomized into groups (19).

Group Division

After specimen preparation and initial blocking for color and microhardness (to exclude samples with greater standard deviations), the specimens were randomized. The sample size calculation was performed using www.openepi.com, with a significance level of 5% and statistical power of 80%, thus ensuring proper group allocation according to the bleaching protocol and products (Table 1): Artificial Saliva (Con-

korijena dijamantnim diskom s dvama licima (KG Sorensen 7020, SP, Brazil) u mikromotoru male brzine, uz stalno ispiranje. Profilaksa je provedena gumenim čašicama (American Burrs, Brazil) s pastom od plovuće i vode (2 : 1). Meziodistalni i incizocervikalni rezovi učinjeni su metalografskim rezačem (Isomet 1000, Buehler, Illinois, SAD) opremljenim preciznim dijamantnim diskom (4" × 0,12 × 1/2).

Dobiveni su uzorci cakline i dentina dimenzija 4 × 4 mm i visine 3 mm. Dentinske površine pripremljene su brusnim papirom od silicij-karbida granulacije #600, a površine cakline postupno su polirane brusnim papirom od silicij-karbida granulacije #600, #1200, #2000 i #4000 uz stalno ispiranje (Arotec, Cotia, SP, Brazil). Završno poliranje obavljeno je filcanim diskovima i dijamantnim pastama smanjene granulacije (1/2 μm i 1/4 μm). Uzorci su očišćeni u ultrazvučnom čistaču (Marconi, Piracicaba, São Paulo, Brazil) tijekom 10 minuta kako bi se uklonili ostatci (19).

Protokol bojenja zuba

Uzorci su uronjeni u otopinu crnoga čaja (1,6 g crnoga čaja; Leão Junior S.A., Curitiba, PR, Brazil) pripremljenu u 100 mL destilirane vode, kuhani 3 minute i natapani 5 minuta. Otopina je pohranjena na 37 °C i mijenjana svaka 24 sata tijekom 6 dana. Nakon bojenja ostatci su uklonjeni brusnim papirom od silicij-karbida granulacije #4000, a uzorci su pohranjeni u umjetnoj slini (Ca 1,5 mmol/L; P 0,9 mmol/L; 150 KCl mmol/L; 0,1 mol/L Tris pufer, pH 7,0) na 37 °C (± 1 °C) tijekom 14 dana. Slina je mijenjana svakodnevno (Serra i sur., 1992.). Početna mjerenja boje provedena su spektrofotometrom da bi se isključile odstupajuće vrijednosti, a uzorci su nasumično podijeljeni u skupine (19).

Podjela u skupine

Nakon pripreme uzorka i početnog uklapanja u blokove za boju i mikrotvrdoću (da bi se isključili uzorci s većim standardnim devijacijama), uzorci su randomizirani. Izračun veličine uzorka obavljen je s pomoću www.openepi.com, s razinom značajnosti od 5 % i statističkom snagom od 80 %, osiguravajući pravilnu raspodjelu skupina prema protokolu izbjeljivanja i proizvodima (tablica 1.): Umjetna slina (kon-

Table 1 Description of the products used and their composition
Tablica 1. Opis korištenih proizvoda i njihov sastav

Products • Proizvod (Sigla)	Specification • Specifikacija	Composition • Sastav
Artificial Saliva (Control) • Umjetna slina (kontrola)	Artificial saliva • Umjetna slina	Ca 1.5 mmol/L; P 0.9 mmol/L; 150 KCl mmol/L; 0.1mol/L TRIS buffer solution
Whiteness HP 35% (HP 35%)	Whiteness HP 35% Batch • Serija: 071123	H2O2-30–35 wt%, mixture of pigments, thickener • H2O2-30 – 35 tež. %, smjesa pigmenata, zgušnjivač
KF 0,2% (KF)	Desensitizer • Desenzibilizator KF (FGM, Brazil) Batch • Serija: 130923	Potassium Nitrate: 5%, Sodium Fluoride: 0.2%, Deionized Water, Glycerin, Neutralizing Agents, Thickener • Kalijev nitrat: 5%, natrijev fluorid: 0,2 %, deionizirana voda, glicerol, neutralizirajuća sredstva, zgušnjivač
Propolis Extract • Ekstrakt propolisa (P)	Propolis Extract • Ekstrakt propolisa (Apis Flora, Brazil) Batch • Serija: 003000524	Alcohol-free Propolis Extract and Water • Ekstrakt propolisa i voda bez alkohola
Gel Neutral Fluoride (F)	Neutral fluoride (Maquira, Brazil) Batch • Serija: 459823	Sodium Fluoride 2%, Deionized Water, Glycerin, Thickener • Natrijev fluorid 2 %, deionizirana voda, glicerol, zgušnjivač

trol); Whiteness HP35% (HP35%); Whiteness HP35% + KF desensitizer (HP35%+ KF); Whiteness HP35% + KF desensitizer + Propolis Extract (HP 35%+ KF+P); Whiteness HP35% + Neutral Fluoride + Propolis Extract (HP 35%+ F+P); and Whiteness HP35% + Propolis Extract (HP35% + P).

Bleaching Treatment

Specimens were bleached with 35% hydrogen peroxide gel (Whiteness HP, FGM, Joinville, SC, Brazil) following the manufacturer's instructions. The gel was applied in three 15-min intervals per session over three sessions at 7-day intervals. Gel amounts were measured using an analytical balance (Shimadzu, Japan). After each application, the gel was removed with cotton swabs, and specimens were rinsed with distilled water (4, 23).

Application of remineralization agents

After each bleaching session, the designated remineralization agent was applied for 15 min following the manufacturer's instructions (FGM, Joinville, SC, Brazil). Specimens were rinsed with distilled water and stored in artificial saliva until the next session (20, 21).

Color Analysis

Color evaluation was conducted with the specimens positioned in a Teflon device inside a light chamber (GTI, Newburg, NY, USA) to standardize the light conditions during the assessments, using a "daylight" setting. A spectrophotometer (Konica Minolta CM-700d) was calibrated before each test, and color measurements were obtained using ΔE , ΔE_{00} , and ΔWID (Final – Initial) standards (19). Color was assessed with a spectrophotometer (Konica Minolta CM-700d), calibrated before each test. Measurements were obtained using a Teflon device placed inside a standardized light chamber (GTI, Newburg, NY, USA) under daylight conditions. Parameters included ΔE , ΔE_{00} , and ΔWID (Final – Initial) (19).

Microhardness Analysis

Microhardness was assessed initially and 24 h after treatments using a microhardness tester (Shimadzu HMV-2000, Tokyo, Japan) with a Knoop diamond indenter. A 50-g load was applied for 5 s across five vertical columns per specimen, and mean values were calculated (19).

Surface Roughness Analysis

Surface roughness was analyzed using a surface profile measuring device (Mitutoyo Surfitec SJ-410, São Paulo, Brazil) with the specimens mounted on an acrylic planing device. Each specimen was marked on the edges to define a reference starting point for measurements. Surface roughness was measured three times at different angles (180°, 130°, and 90°) relative to this mark to ensure consistent assessment of the same area before and after treatment. The following parameters were used: a cutoff length of 0.25 mm, static load of 5 N, distance of 3 mm, and speed of 0.5 mm/s (21).

trola); Bjelina HP 35 % (HP 35 %); Bjelina HP 35 % + KF desenzibilizator (HP 35 % + KF); Bjelina HP 35% + KF desenzibilizator + ekstrakt propolisa (HP 35 % + KF + P); Bjelina HP35 % + neutralni fluorid + ekstrakt propolisa (HP 35 % + F + P); Bjelina HP 35 % + ekstrakt propolisa (HP 35 % + P).

Tretman izbjeljivanja

Uzorci su izbjeljeni gelom s 35-postotnim vodikovim peroksidom (Whiteness HP, FGM, Joinville, SC, Brazil) prema uputama proizvođača. Gel je apliciran u trima intervalima od 15 minuta po sesiji tijekom triju sesija u intervalima od 7 dana. Količine gela mjerene su analitičkom vagom (Shimadzu, Japan). Nakon svake primjene gel je uklonjen vatom, a uzorci su isprani destiliranom vodom (4, 23).

Primjena remineralizacijskih sredstava

Nakon svakog izbjeljivanja nanese se određeno sredstvo za remineralizaciju tijekom 15 minuta prema uputama proizvođača (FGM, Joinville, SC, Brazil). Uzorci su isprani destiliranom vodom i pohranjeni u umjetnoj slini do sljedećeg izbjeljivanja (20, 21).

Analiza boje

Procjena boje provedena je na uzorcima postavljenima u teflonski uređaj unutar svjetlosne komore (GTI, Newburg, NY, SAD) kako bi se standardizirali uvjeti osvjetljenja tijekom procjena, koristeći se postavkom „dnevno svjetlo”. Spektrofotometar (Konica Minolta CM-700d) kalibriran je prije svakog ispitivanja, a mjerenja boje dobivena su korištenjem standarda ΔE , ΔE_{00} i ΔWID (konačno – početno) (19). Boja je procijenjena spektrofotometrom (Konica Minolta CM-700d) kalibriranim prije svakog ispitivanja. Mjerenja su dobivena korištenjem teflonskog uređaja postavljenog unutar standardizirane svjetlosne komore (GTI, Newburg, NY, SAD) pod uvjetima dnevnog svjetla. Parametri su uključivali ΔE , ΔE_{00} i ΔWID (konačno – početno) (19).

Analiza mikrotvrdoće

Mikrotvrdoća je procijenjena inicijalno i 24 sata nakon tretmana s pomoću testera mikrotvrdoće (Shimadzu HMV-2000, Tokio, Japan) s Knoopovim dijamantnim utiskivačem. Opterećenje od 50 g primijenjeno je tijekom 5 sekunda preko pet vertikalnih stupaca po uzorku, a izračunate su srednje vrijednosti (19).

Analiza hrapavosti površine

Hrapavost površine analizirana je uređajem za mjerenje profila površine (Mitutoyo Surfitec SJ-410, São Paulo, Brazil) s uzorcima postavljenima na akrilni uređaj za blanjanje. Svaki uzorak označen je na rubovima kako bi se definirala referentna početna točka za mjerenja. Hrapavost površine mjerena je tri puta pod različitim kutovima (180°, 130° i 90°) u odnosu na tu oznaku da bi se osigurala dosljedna procjena istog područja prije i poslije obrade. Korišteni su sljedeći parametri: duljina odreza od 0,25 mm, statičko opterećenje od 5 N, udaljenost od 3 mm i brzina od 0,5 mm/s (21).

Statistical Analysis

Descriptive and exploratory statistical analyses were performed on the results. Generalized linear models were used to analyze the effects of the bleaching agent on ΔE^*ab , ΔE_{00} , ΔWID , microhardness, and roughness, as the assumptions of the ANOVA method were not met. Generalized linear models allowed the analysis of variables with error distributions that differed from the normal distribution. All analyses were conducted using R software at a significance level of 5%.

Results

As presented in Table 2, the color parameters, ΔE , ΔE_{00} , and ΔWID , exhibited comparable values among the groups subjected to bleaching treatments. Furthermore, all bleached groups demonstrated significantly greater color change compared to the control group maintained in artificial saliva (ΔE : $p = 0.0006$; ΔE_{00} : $p = 0.0003$; ΔWID : $p = 0.0002$).

As shown in Table 3, the groups treated with desensitizing agents, fluoride, and propolis extract exhibited a reduction in surface roughness (Ra) values compared to the baseline measurement (T1). The HP 35% + KF + P group maintained surface microhardness 24 hours after the high-concentration bleaching protocol, with no statistically significant difference in comparison to the control group. In contrast, other experimental groups experienced significant decreases in surface microhardness ($p = 0.0452$).

Discussion

The use of propolis after high-concentration bleaching showed no statistically significant difference in bleaching efficacy at 24 h post-treatment, thus indicating that the first

Statistička analiza

Na rezultatima su provedene deskriptivne i eksplorativne statističke analize. Generalizirani linearni modeli korišteni su za analizu učinaka sredstva za izbjeljivanje na ΔE^*ab , ΔE_{00} , ΔWID , mikrotvrdoću i hrapavost, s obzirom na to da pretpostavke metode ANOVA nisu bile ispunjene. Generalizirani linearni modeli omogućili su analizu varijabli s distribucijama pogrešaka koje su se razlikovale od normalne distribucije. Sve analize obavljene su s pomoću R softvera na razini značajnosti od 5 %.

Rezultati

Kao što je prikazano u tablici 2., parametri boje – ΔE , ΔE_{00} i ΔWID – pokazali su usporedive vrijednosti među skupinama podvrgnutima tretmanima izbjeljivanja. Nadalje, sve izbijeljene skupine pokazale su značajno veću promjenu boje u usporedbi s kontrolnom skupinom održavanom u umjetnoj slini (ΔE : $p = 0,0006$; ΔE_{00} : $p = 0,0003$; ΔWID : $p = 0,0002$).

Kao što je prikazano u tablici 3., skupine tretirane desenzibilizirajućim sredstvima, fluoridom i ekstraktom propolisa pokazale su smanjenje vrijednosti hrapavosti površine (Ra) u usporedbi s početnim mjerenjem (T1). Skupina HP 35 % + KF + P održala je mikrotvrdoću površine 24 sata nakon protokola izbjeljivanja visokom koncentracijom, bez statistički značajne razlike u usporedbi s kontrolnom skupinom. Suprotno tomu, u ostalim eksperimentalnim skupinama zabilježeno je značajno smanjenje mikrotvrdoće površine ($p = 0,0452$).

Rasprava

Korištenje propolisa nakon izbjeljivanja visokom koncentracijom nije pokazalo statistički značajnu razliku u učinkovitosti izbjeljivanja 24 sata poslije tretmana, što upućuje na

Table 2 Mean (standard deviation) of ΔE and ΔE_{00} as a function of desensitizers and median (minimum; maximum) of ΔWID by desensitizer and time.

Tablica 2. Srednja vrijednost (standardna devijacija) ΔE i ΔE_{00} kao funkcija desenzibilizatora i vremena medijana (minimum; maximum) of ΔWID by desensitizer and time.

Group • Skupina	ΔE	ΔE ₀₀		ΔWID		
	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)	Time • Vrijeme		p-value	
			Baseline • Početna vrijednost	After 24h • Poslije 24 h		
			Median (minimum; maximum)	Median (minimum; maximum)		
Control • Kontrola	3.16 (2.57) b	2.50 (2.08) b	9.62 (-11.78; 18.00) Aa	9.25 (3.11; 14.21) Ab	0.9594	
HP 35%	8.47 (4.51) a	6.48 (3.48) a	10.43 (-11.24; 23.68) Ba	20.70 (14.58; 24.25) Aa	0.0093	
HP 35% + KF	7.80 (4.09) a	6.19 (3.02) a	13.55 (-0.21; 22.82) Ba	20.21 (8.72; 27.09) Aa	0.0051	
HP 35% + KF + P	8.87 (3.20) a	6.75 (2.46) a	11.39 (1.97; 18.67) Ba	21.35 (15.83; 24.71) Aa	0.0069	
HP 35% + F + P	8.86 (3.22) a	6.94 (2.54) a	13.54 (1.27; 17.45) Ba	21.82 (19.06; 24.65) Aa	0.0051	
HP 35% + P	8.38 (2.84) a	6.43 (2.17) a	9.93 (1.23; 19.11) Ba	20.80 (17.85; 23.98) Aa	0.0051	
p-valor • p-vrijednost			0.8617		0.0002	

ΔE : $p=0.0006$. Different letters indicate statistically significant differences ($p \leq 0.05$). ΔE_{00} : $p=0.0003$. Different letters indicate statistically significant differences ($p \leq 0.05$). ΔWID : Different letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences ($p \leq 0.05$). • ΔE : $p = 0.0006$. Različita slova označavaju statistički značajne razlike ($p \leq 0,05$). ΔE_{00} : $p = 0.0003$. Različita slova označavaju statistički značajne razlike ($p \leq 0,05$). ΔWID : Različita slova (velika slova vodoravno i mala slova okomito) označavaju statistički značajne razlike ($p \leq 0,05$).

Table 3 Mean (standard deviation) of Roughness (Ra) and Microhardness (KHN) by desensitizer and time.**Tablica 3.** Srednja vrijednost (standardna devijacija) hrapavosti (Ra) i mikrotvrdoće (KHN) desenzibilizatora i vremena.

Desensitizer • Desenzibilizator	Roughness (Ra) • Hrapavost (Ra)		Microhardness (KHN) • Mikrotvrdoća (KHN)	
	Time • Vrijeme		Time • Vrijeme	
	Baseline • Početna vrijednost	After 24h • Nakon 24 h	Baseline • Početna vrijednost	After 24h • Nakon 24 h
	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)	Mean (standard deviation) • Srednja vrijednost (standardna devijacija)
Control • Kontrola	0.052 (0.033) Aa	0.047 (0.012) Ac	370.08 (29.54) Aa	333.92 (48.14) Aab
HP 35%	0.046 (0.010) Ba	0.061 (0.016) Ab	370.24 (30.08) Aa	309.77 (58.79) Babc
HP 35% + KF	0.042 (0.007) Ba	0.079 (0.026) Aa	371.36 (32.10) Aa	274.75 (44.31) Bc
HP 35% + KF + P	0.041 (0.005) Ba	0.063 (0.019) Aab	369.58 (26.82) Aa	330.75 (55.11) Aab
HP 35% + F + P	0.045 (0.012) Ba	0.070 (0.013) Aab	369.96 (27.51) Aa	290.08 (59.27) Bbc
HP 35% + P	0.044 (0.006) Ba	0.063 (0.020) Aab	370.29 (28.23) Aa	341.73 (41.68) Ba

Roughness: p (group) = 0.2087; p (time) < 0.0001; p (interaction) = 0.0683. Different letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences ($p \leq 0.05$). **Microhardness:** p (group) = 0.0849; p (time) < 0.0001; p (interaction) = 0.0452. Different letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences ($p \leq 0.05$). **Hrapavost:** p (grupa) = 0.2087; p (vrijeme) < 0.0001; p (interakcija) = 0.0683. Različita slova (velika slova vodoravno i mala slova okomito) označavaju statistički značajne razlike ($p \leq 0.05$). **Mikrotvrdoća:** p (grupa) = 0.0849; p (vrijeme) < 0.0001; p (interakcija) = 0.0452. Različita slova (velika slova vodoravno i mala slova okomito) označavaju statistički značajne razlike ($p \leq 0.05$).

null hypothesis was not rejected. Propolis extract, a dark-colored product, negatively affects color difference after three bleaching sessions. However, the present study demonstrated an effect similar to that of standard bleaching protocols. The second null hypothesis was rejected because of significant differences observed in the surface roughness and microhardness after the treatment. Tooth bleaching with 35%–38% HP can alter enamel morphology, reduce microhardness, and cause loss of hard-tissue volume (8).

In this study, the effects of propolis extract on dental enamel after bleaching sessions were evaluated and compared with those of other products, such as potassium nitrate and fluoride gels, which are currently used both individually and in combination as desensitizers. Produced by bees, propolis is a natural resinous substance that has been utilized for its therapeutic properties since ancient times (18). Its action mechanism includes the deposition of resin-like compounds that block dentinal tubules, which helps alleviate hypersensitivity caused by bleaching procedures (14, 15). Due to its anti-inflammatory properties, it could be a product used after high concentration whitening treatments, as one of the most prevalent adverse effects of this procedure is exacerbated pain (8); however, there were no studies in the literature regarding color impairment after tooth whitening.

Considering the use of HP 35% and the experimental method with propolis extract, no studies have shown similar results, as this extract had not yet been evaluated following bleaching treatments. The results showed that the bleaching efficacy of propolis was similar to that of other groups treated with 35% HP, HP35%+KF, and HP35%+F ($p > 0.05$). The mechanism of action of HP 35% involves a redox reaction that penetrates the enamel and dentin, releasing free radicals that oxidize dark pigments. This process increases dentin permeability and exposes dentinal tubules, leading to temporary hypersensitivity. Desensitizing agents such as potassium nitrate and sodium fluoride mitigate this effect by acting as tubular blockers. A previous study (20, 21) demonstrated

to da prva nulta hipoteza nije odbačena. Ekstrakt propolisa – proizvod tamne boje – može negativno utjecati na razliku u boji nakon triju sesija izbjeljivanja. Međutim, autori ove studije pokazali su učinak sličan onomu kod standardnih protokola izbjeljivanja. Druga nulta hipoteza odbačena je zbog značajnih razlika uočenih u hrapavosti površine i mikrotvrdoći nakon tretmana. Izbjeljivanje zuba s 35 % do 38 % HP-a može promijeniti morfologiju cakline, smanjiti mikrotvrdoću i prouzročiti gubitak volumena tvrdog tkiva (8).

U ovoj studiji procijenjeni su učinci ekstrakta propolisa na zubnu caklinu nakon sesija izbjeljivanja i uspoređeni su s učincima drugih proizvoda, poput kalijeva nitrata i fluoridnih gelova koji se trenutačno koriste pojedinačno i u kombinaciji kao desenzibilizatori. Propolis, koji proizvode pčele, prirodna je smolasta tvar koja se zbog svojih terapijskih svojstava koristi od davnina (18). Njegov mehanizam djelovanja uključuje taloženje spojeva sličnih smoli koji blokiraju dentinske tubule, što pomaže ublažiti preosjetljivost prouzročenu postupcima izbjeljivanja (14, 15). Zbog svojih protuupalnih svojstava mogao bi se koristiti nakon tretmana izbjeljivanja visokom koncentracijom, zato što je jedan od najčešćih štetnih učinaka toga postupka pojačana bol (8); međutim, u literaturi nije bilo studija o promjeni boje nakon izbjeljivanja zuba.

S obzirom na upotrebu HP-a od 35 % i eksperimentalnu metodu s ekstraktom propolisa, ni u jednoj studiji nisu dobiveni slični rezultati, s obzirom na to da taj ekstrakt još nije procijenjen nakon tretmana izbjeljivanja. Rezultati su pokazali da je učinkovitost izbjeljivanja propolisom bila slična onoj u drugim skupinama tretiranim 35-postotnim HP-om, HP-om 35 % + KF i HP-om 35 % + F ($p > 0.05$). Mehanizam djelovanja HP-a 35 % uključuje redoks-reakciju koja prodire u caklinu i dentin oslobađajući slobodne radikale koji oksidiraju tamne pigmente. Taj proces povećava propusnost dentina i izlaže dentinske tubule, što rezultira privremenom preosjetljivošću. Sredstva za desenzibilizaciju poput kalijeva nitrata i natrijeva fluorida, djelujući kao tubularni

that these agents, when combined with bleaching gels, reduce sensitivity and prevent changes to enamel surface morphology caused by this therapy. Notably, they do not reduce the bleaching efficacy of HP 35% (11).

The intense coloration of propolis extract did not compromise the outcome of tooth whitening. This may be attributed to the fact that the oxidation process induced by HP 35% occurs in the deeper layers of enamel and dentin, while the application of propolis is superficial and performed in an aqueous medium. Furthermore, the use of propolis extract did not alter the surface microhardness (KHN) of dental enamel after high-concentration bleaching. Following whitening therapy, surface microhardness was reduced in all groups, except for HP35%+KF+P, which maintained values comparable to the control group. Although no previous studies have evaluated this combination, the result may be attributed to a synergistic effect of potassium nitrate and propolis.

Potassium nitrate is well known for its ability to reduce dentin hypersensitivity, primarily by forming a transient protective layer on the enamel surface. Meanwhile, propolis is rich in flavonoids and other phenolic compounds with anti-inflammatory, antioxidant, and antimicrobial properties, which may act synergistically to reduce oxidative stress and promote the formation of a superficial protective layer on the enamel surface. These combined effects likely contributed to limiting the initial mineral loss caused by hydrogen peroxide, thereby helping to preserve microhardness values similar to those observed in the control group.

Although the HP 35% + KF + P group exhibited microhardness values comparable to the control group, this finding should be interpreted cautiously due to potential high data variability and limited sample size. Therefore, further studies with larger sample sizes are needed to confirm the protective effect of this protocol. In addition, all groups subjected to bleaching treatment showed a significant increase in surface roughness ($p < 0.05$), which aligns with previous findings demonstrating that HP 35% temporarily alters enamel surface properties (4, 8, 9, 24).

Propolis extract contains flavonoids and phenolic compounds with antimicrobial and anti-inflammatory effects that may indirectly influence enamel demineralization and remineralization processes (25). However, since it does not release calcium or phosphate ions essential for hydroxyapatite formation, it is not classified as a conventional remineralizing agent (22). Nevertheless, no significant differences in enamel roughness were observed between the groups, indicating that propolis does not adversely affect this parameter compared with other remineralizing agents.

The evaluation was performed 24 h after the last bleaching session, a timeframe intentionally selected to isolate the immediate effects of the applied agents before the onset of natural enamel remineralization by saliva. This approach enabled a clearer understanding of the short-term impact of propolis extract on color, surface roughness, and microhardness. Although longer follow-up periods might provide additional insights, the current findings demonstrate that propolis does not compromise bleaching efficacy or alter enamel surface integrity in the early phase following high-concen-

trators, ublažavaju taj učinak. U prethodnoj studiji (20, 21) istaknuto je da ta sredstva, kada se kombiniraju s gelovima za izbjeljivanje, smanjuju osjetljivost i sprječavaju promjene u morfologiji površine cakline prouzročene ovom terapijom. Važno je napomenuti da ne smanjuju učinkovitost izbjeljivanja HP-om 35 % (11).

Intenzivna obojenost ekstrakta propolisa nije ugrozila ishod izbjeljivanja zuba. To se može pripisati činjenici da se proces oksidacije izazvan HP-a 35 % događa u dubljim slojevima cakline i dentina, a nanošenje propolisa površinsko je i provodi se u vodenom mediju. Nadalje, upotreba ekstrakta propolisa nije promijenila površinsku mikrotvrdoću (KHN) zubne cakline nakon izbjeljivanja visokom koncentracijom. Poslije terapije izbjeljivanja površinska mikrotvrdoća smanjena je u svim skupinama, osim kod HP-a 35 % + KF + P, koja je održala vrijednosti usporedive s kontrolnom skupinom. Iako ni jednoj dosadašnjoj studiji nije procijenjena ova kombinacija, rezultat se može pripisati sinergističkom učinku kalijeva nitrata i propolisa.

Kalijev nitrat dobro je poznat zbog svojega svojstva smanjenja preosjetljivosti dentina, ponajprije stvaranjem prolaznoga zaštitnog sloja na površini cakline. U međuvremenu, propolis je bogat flavonoidima i drugim fenolnim spojevima s protuupalnim, antioksidacijskim i antimikrobnim svojstvima koji mogu sinergijski djelovati kako bi smanjili oksidacijski stres i potaknuli stvaranje površinskoga zaštitnog sloja na površini cakline. Ti kombinirani učinci vjerojatno su pridonijeli ograničavanju početnog gubitka minerala prouzročena vodikovim peroksidom, čime su pomogli u očuvanju vrijednosti mikrotvrdoće sličnih onima uočenim u kontrolnoj skupini.

Iako je skupina HP 35 % + KF + P pokazala vrijednosti mikrotvrdoće usporedive s kontrolnom skupinom, ovaj nalaz treba tumačiti oprezno zbog potencijalno visoke varijabilnosti podataka i ograničene veličine uzorka. Zato su potrebna daljnja istraživanja s većim uzorcima da bi se potvrdio zaštitni učinak ovog protokola. Uz to, sve skupine podvrgnute tretmanu izbjeljivanja pokazale su značajno povećanje hrapavosti površine ($p < 0,05$), što je u skladu s dosadašnjim nalazima koji pokazuju da HP 35 % privremeno mijenja svojstva površine cakline (4, 8, 9, 24).

Ekstrakt propolisa sadržava flavonoide i fenolne spojeve s antimikrobnim i protuupalnim učincima koji mogu neizravno utjecati na procese demineralizacije i remineralizacije cakline (25). No budući da ne oslobađa kalcijeve ili fosfatne ione bitne za stvaranje hidroksiapatita, ne klasificira se kao konvencionalno remineralizirajuće sredstvo (22). Ipak, nisu uočene značajne razlike u hrapavosti cakline između skupina, što upućuje na to da propolis ne utječe negativno na taj parametar u usporedbi s drugim remineralizirajućim sredstvima.

Evaluacija je provedena 24 sata poslije posljednjeg izbjeljivanja, a vremenski okvir namjerno je odabran kako bi se izolirali neposredni učinci primijenjenih sredstava prije početka prirodne remineralizacije cakline slinom. Taj pristup omogućio je jasnije razumijevanje kratkoročnog utjecaja ekstrakta propolisa na boju, hrapavost površine i mikrotvrdoću. Iako bi dulja razdoblja praćenja mogla pružiti dodatne uvide,

tration bleaching. Further clinical studies are needed to confirm its effectiveness against dentin hypersensitivity following bleaching treatments.

Conclusion

Propolis extract did not reduce tooth whitening effectiveness nor adversely affect enamel surface roughness or microhardness after high-concentration hydrogen peroxide treatment. The group treated with HP35% combined with potassium nitrate and propolis exhibited microhardness values similar to the control group. However, these findings should be interpreted with caution due to a limited sample size, and further studies with larger samples are needed to confirm the potential benefits of propolis in this context.

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trenutačni nalazi pokazuju da propolis ne ugrožava učinkovitost izbjeljivanja, niti mijenja integritet površine cakline u ranoj fazi nakon izbjeljivanja visokom koncentracijom. Potrebna su daljnja klinička istraživanja da bi se potvrdila njegova učinkovitost kad je riječ o preosjetljivosti dentina nakon tretmana izbjeljivanja.

Zaključak

Ekstrakt propolisa nije smanjio učinkovitost izbjeljivanja zuba, niti je negativno utjecao na hrapavost ili mikrotvrdoću površine cakline nakon tretmana visokokonzentriranim vodikovim peroksidom. Skupina tretirana s HP-om 35 % u kombinaciji s kalijevim nitratom i propolisom pokazala je vrijednosti mikrotvrdoće slične kontrolnoj skupini. Međutim, ove nalaze treba tumačiti s oprezom zbog ograničene veličine uzorka pa su potrebna daljnja istraživanja s većim uzorcima kako bi se potvrdile potencijalne koristi propolisa u ovom kontekstu.

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Sažetak

Svrha rada: U ovoj studiji procijenjen je učinak ekstrakta propolisa na zubnu caklinu nakon izbjeljivanja zuba 35-postotnim vodikovim peroksidom (HP). **Materijali i metode:** Šezdeset uzoraka govede cakline podijeljeno je u šest skupina: Umjetna slina (kontrola); Whiteness HP 35 % (HP 35 %); Whiteness HP 35 % + desenzibilizator kalijeva nitrata (HP 35 % + KF); Whiteness HP 35 % + desenzibilizator kalijeva nitrata + ekstrakt propolisa (HP 35 % + KF + P); Whiteness HP 35 % + neutralni fluorid + ekstrakt propolisa (HP 35 % + F + P) i Bjelina HP 35 % + ekstrakt propolisa (HP 35 % + P). Protokol izbjeljivanja u ordinaciji sastojao se od triju sesija (3 × 15 minuta). Promjena boje (ΔE^* , ΔE_{00} , ΔWID), hrapavost površine (Ra) i mikrotvrdoća (KHN) procijenjene su na početku (T_1) i poslije 24 sata (T_2). Parametri boje analizirani su s pomoću generaliziranih linearnih modela ($p \leq 0,05$), a Ra i KHN procijenjeni su s pomoću generaliziranog linearnog miješanog modela za ponovljena mjerenja ($p \leq 0,05$). **Rezultati:** Sve skupine tretirane HP-om pokazale su značajno veću promjenu boje u usporedbi s kontrolnom skupinom. Hrapavost površine povećala se nakon izbjeljivanja; međutim, skupine tretirane desenzibilizatorima, fluoridom i/ili propolisom pokazale su niže Ra vrijednosti pri T_2 u usporedbi s početnim vrijednostima. Kad je riječ o mikrotvrdoći, samo je skupina HP 35 % + KF + P održala KHN vrijednosti usporedive s kontrolnom skupinom nakon izbjeljivanja. **Zaključak:** Ekstrakt propolisa nije ugrozio učinkovitost izbjeljivanja, niti je negativno utjecao na hrapavost ili mikrotvrdoću površine cakline nakon tretmana visokokonzentriranim vodikovim peroksidom.

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