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## Influence of Hygienic Preparations with a 3% Content of Ethanol Extract of Brazilian Propolis on the State of the Oral Cavity\*

### Wpływ preparatów higienizacyjnych zawierających 3% etanolowy ekstrakt propolisu brazylijskiego na stan jamy ustnej

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#### Abstract

**Background.** One of the most important measures to be undertaken in order to fight gingivitis and periodontitis is maintenance of proper hygiene of the oral cavity. The research to improve the content of toothpaste has continued for many years so that they should become better in terms of therapeutic abilities.

**Objectives.** The aim of this work was to determine and investigate the influence of the application of toothpaste and gel with 3% ethanol propolis extract on the state of the oral cavity.

**Material and Methods.** The research group comprised 80 adult patients divided into two subgroups: Group I, which comprised 40 patients without pathological changes within the boundaries of the periodontium, and Group II, also 40 patients endangered with the occurrence of periodontitis caused by dental plaque and lack of proper hygiene of the oral cavity. Qualification for both groups was based on an interview and analysis of clinical documentation and assessment of adequate indices such as API, OHI and SBI. The patients underwent three examinations: initial, follow-up after 7 days and after 8 weeks since the beginning of the program. Moreover, the patients were instructed about hygienic procedures of the oral cavity. Four groups (T, G, CT, CG), 20 patients each, were created from research groups I and II. They used the following preparations: T – Dental Polis DX toothpaste with propolis content, G – Dental Polis DX toothpaste without propolis content, CT – Carepolis gel with propolis content, CG – Carepolis gel without propolis content. The patients were informed about the type of hygienic preparation they were given to use (whether it contained propolis or not). Moreover, they were interviewed for their subjective evaluation of the product received.

**Results and Conclusion.** Results of the research show the effectiveness of hygienic preparations with 3% content of ethanol propolis extract in both groups of patients: without pathological changes within the boundaries of the periodontium and in the case of patients endangered with the occurrence of gingivitis caused by dental plaque (Adv Clin Exp Med 2012, 21, 1, 81–92).

**Key words:** 3% ethanol extract of propolis, dental prevention, oral cavity health, gingivitis.

#### Streszczenie

**Wprowadzenie.** Podstawowym krokiem w walce z zapaleniami dziąseł i przyzębia jest utrzymanie właściwej higieny jamy ustnej przez eliminację płytki nazębnej. Dobór właściwej metody szczotkowania, szczoteczki i środków wspomagających stanowi pierwszy etap w walce z chorobą. Od lat trwają badania nad ulepszeniem składu past do zębów i płukanek, tak by wykazywały właściwości terapeutyczne.

**Cel pracy.** Określenie wpływu pasty do zębów i żelu do czyszczenia zębów zawierających 3% etanolowy ekstrakt propolisu na stan jamy ustnej.

**Materiał i metody.** Grupa badana stanowiła 80 osób. Były to osoby pełnoletnie, zarówno kobiety jak i mężczyźni – powyżej 18. roku życia. Z grupy badanej zostały wyłonione dwie podgrupy pacjentów wydzielone ze względu

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na wstępną kwalifikację stanu przyzębia brzęznego: I grupa – bez zmian chorobowych w obrębie przyzębia brzęznego – 40 osób, II grupa – z zagrożeniem wystąpienia zapalenia dziąseł wywołanego płytką bakteryjną, brakiem dostatecznej higieny jamy ustnej – 40 osób. Pacjenci byli poddani: wstępnej kwalifikacji i badaniu wstępnemu (wizyta 1), badaniu po upływie 7 dni (wizyta 2) i 8 tygodni (wizyta 3) od rozpoczęcia programu. Przeprowadzono profesjonalny instruktaż higieny jamy ustnej. Podczas wizyt kontrolnych wyznaczono wartości wskaźników API, OHI i SBI. Z grupy badanej (I i II) wydzielono cztery podgrupy (T, G, CT, CG) liczące po 20 osób, które stosowały: T – pastę Dental Polis DX z propolisem, G – pastę Dental Polis DX bez propolisu, CT – żel z propolisem Carepolis, CG – żel bez propolisu.

**Wyniki i wnioski.** Wyniki zaprezentowanych badań, poddane analizie statystycznej, wskazują na skuteczność preparatów higienizacyjnych zawierających 3% etanolowy ekstrakt propolisu zarówno u pacjentów bez zmian chorobowych w obrębie przyzębia brzęznego, jak i u pacjentów z zagrożeniem wystąpienia zapalenia dziąseł wywołanego płytką bakteryjną (*Adv Clin Exp Med* 2012, 21, 1, 81–92).

**Słowa kluczowe:** 3% etanolowy ekstrakt propolisu, profilaktyka dentystyczna, zdrowie jamy ustnej, zapalenie dziąseł.

Gingivitis and periodontitis, apart from dental caries, are the most common diseases of the oral cavity. Bacteria existing in dental plaque are the major etiologic factor of marginal periodontitis. Moreover, there are many general and local factors modifying the onset and the course of the disease. One of the most important measures to be undertaken in order to fight gingivitis and periodontitis is the maintenance of proper hygiene of the oral cavity. Choosing an adequate method of tooth brushing, a proper toothbrush and supporting products is the first step in the fight against the disease. The research to improve the content of toothpastes and mouthwashes has continued for many years so that, besides better abrasive and polishing properties, they should become better in terms of therapeutic abilities. One of the known methods to improve them is the addition of natural ingredients (such as, ethanol extract of propolis) which are successfully used in other areas of medicine as substances that accelerate the wound healing process [1].

Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is used for small gaps (approximately 6 millimeters (0.2 in) or less), while larger spaces are usually filled with beeswax. Normally, propolis is dark brown in color, but it can be found in green, red, black and white hues, depending on the sources of resin found in the particular hive area. Honey bees are opportunists, gathering what they need from available sources, and detailed analyses show that propolis' chemical composition varies considerably from region to region, along with the vegetation [1, 2]. In northern temperate climates, for example, bees collect resins from trees such as poplars and conifers. "Typical" northern temperate propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%), waxes (30%), essential oils (10%), and pollen (5%). In neotropical regions, in addition to a large variety of trees, bees may also gather resin

from flowers in the genera *Clusia* and *Dalechampia*, which are the only known plant genera that produce floral resins to attract pollinators. *Clusia* resin contains polyprenylated benzophenones. In some areas of Chile, propolis contains viscidone, a terpene from *Baccharis* shrubs, and in Brazil, naphthoquinone epoxide has recently been isolated from red propolis, and prenylated acids such as 4-hydroxy-3,5-diprenyl cinnamic acid have been documented. An analysis of propolis from Henan, China found sinapic acid, isoferulic acid, caffeic acid and chrysin, with the first three compounds demonstrating anti-bacterial properties [1]. Extraction of propolis with the use of various solvents yielded numerous biologically active substances. Nowadays, the most common solvent used in such processes is ethyl alcohol, as it allows extraction of ingredients with high biological activity. After the ethanol is removed by evaporation, ethanol propolis extract (EPE) is obtained [3]. Propolis extracts are successfully used in the treatment of purulent dermatitis caused by bacteria and fungi, as well as in the treatment of furuncles, eczemas, trophic ulcers of the shin, decubitus ulcers and cervicitis [4]. EPE has antibacterial, antifungal, antiparasitic, anti-inflammatory and antioxidative properties, as well as being able to sweep free radicals [5–7]. European propolis differs in chemical composition from Brazilian. A difference is visible even between samples of Brazilian propolis, for example between red propolis (from northern regions of Brazil and Cuba) and green Brazilian propolis (south-eastern Brazil) [8]. Green propolis contains Artepilin C, which is present in *Baccharis dracunculifolia*. Red propolis contains high amounts of polyprenylated benzophenones. The usefulness of the of green propolis was confirmed in the treatment of oral inflammation and there was significant reduction in dental plaque formation. The Brazilian propolis used in current investigation comes from *Baccharis dracunculifolia*, known as green propolis, which has its own chemical and biological characteristics. Like other types of propolis, it

has anti-inflammatory, antioxidant, antiparasitic, and anticarcinogenic effects [8]. The antimicrobial properties of propolis have been well documented. Thus it has been shown previously that propolis is more active on Gram-positive than on Gram-negative bacteria [9]. On the other hand, *Listeria monocytogenes* is resistant to propolis, which has therefore been used to develop a selective medium for this bacterium [10]. Alcoholic extracts of propolis are active against a wide range of dermatophytes at concentrations of 0.25 to 2%, antiviral properties have also been described and the protozoa *Toxoplasma gondii* and *Trichomonas vaginalis* were killed within 24 h when incubated with 150  $\mu$ g/ml of propolis [10]. Propolis contains resins and waxes and large amounts of flavonoids which are benzo- $\gamma$ -pyrone derivatives found in all photosynthesizing cells. Flavonoids have many biological effects on animal systems but have received relatively little attention from pharmacologists [1]. Caffeic acid phenethyl ester (CAPE) extracted from propolis has also been shown to be toxic for a range of tumor-derived cell lines. A component active against *Bacillus subtilis* has been identified as 3,5,7-trihydroxyflavone (galangin). It has been suggested that the killing of *staphylococci* is the result of the combined action of several components, none of which alone are effective. Bioautograms, i.e. chromatograms overlaid with bacteria or fungi in agar media, have revealed that propolis contains more than one agent active against bacteria and *Candida albicans* [11]. In addition, the synergy between propolis and a range of antibiotics has been demonstrated in several studies [12]. Ethanol extracts of propolis have been shown to promote the regeneration of bone [13], cartilage [14] and injured dental pulp [15, 16]. Artificially induced bone tissue losses after the application of ethanol extract of propolis (EEP) showed an accelerated rate of ossification [17]. A reduction of disorders of the circulatory system was observed and inflammatory and degenerative processes are also reduced. The effects of ethanol extract of propolis on chronic inflammation were evaluated using rat adjuvant arthritis [17]. This may also be a property of their flavonoids which have been shown to be anti-inflammatory and able to stimulate the formation of collagen [18]. Aqueous solutions (0.5–1%) have been administered to human beings as aerosols for the apparently successful treatment of acute and chronic respiratory disease and have been used as eye drops [19]. A 10% alcoholic solution has been used for disinfection of hands in dental surgical practice [19]. It appears likely that the beneficial effects of propolis and honey are the result of their flavonoid content and both of these natural compounds, as well as purified flavonoids,

appear to be worthy of further assessment of their therapeutic efficacy [20–23].

The aim of this work was to determine and investigate the influence of the application of toothpaste and gel with 3% ethanol extract of propolis on the status of the oral cavity.

## Material and Methods

The purpose of the research was an assessment of the effectiveness of hygienic activities conducted with use of preparations for the maintenance of hygiene in the oral cavity which contained 3% ethanol extract of propolis. The research was conducted on patients of the Dental Clinic of the Department of Conservative Dentistry with Endodontics of the Medical University of Silesia (the Academic Center of Dentistry and Specialist Medicine in Bytom).

The research group comprised 80 adult patients, both women and men. The group was divided into two subgroups with assessment based on a preliminary evaluation of the state of their marginal periodontium: group I – without pathological changes within the marginal periodontium – 40 patients, group II – with danger of the occurrence of periodontitis caused by dental plaque and lack of proper hygiene of the oral cavity – 40 patients.

Qualification for both groups was based on an interview and analysis of clinical documentation and assessment of appropriate indices: API, OHI and SBI. The Approximal Plaque Index (API) by Lange assesses the presence of plaque in the interdental spaces. According to that index, plaque is present or absent in the approximal spaces. The Greene and Vermillion OHI index is an oral hygiene index that makes it possible to assess the debris (DI) index and calculus index (CI). In order to better visualize plaque, staining with a Plaque Indicator Kit (GC) was used. The periodontal status of patients from the research group was assessed with use of the Muhlemann-Son Sulcus Bleeding Index (SBI).

The patients underwent three examinations: a preliminary qualification and basal examination (first visit – 1), a follow-up after 7 days (second visit – 2) and an examination after 8 weeks since the beginning of the program (third visit – 3). During preliminary qualification, they were interviewed for past and current diseases, date of the last visit to the dentist, regularity of control examinations, causes of canceling dental appointments, food habits including the frequency of eating carbohydrate-rich products, frequency of tooth cleaning. The patients also received professional

training in hygienic procedures for the oral cavity which comprised: hygiene advice, training in correct tooth cleaning with a chosen method demonstrated on a model, training in quality control of tooth-brushing procedures. The state of the oral cavity of every patient was assessed during each of the follow-up visits with use of the API, OHI and SBI indices.

Four groups (T, G, CT and CG), 20 patients each, were created from research groups I and II. They used the following pastes/gels: T – Dental Polis DX toothpaste with propolis content, G – Dental Polis DX toothpaste without propolis content, CT – Carepolis gel with propolis content, CG – Carepolis gel without propolis content. The patients were informed about the type of hygienic preparation they were given to use (whether it contained propolis or not). The third visit ended with an interview about their subjective evaluation of the preparations received. The evaluation included: taste, smell, color, rheological properties – if they could obtain an optimal degree of foaming during tooth brushing, subjective feeling of the degree of tooth cleaning (tooth smoothness, sense of freshness in the oral cavity) assessed in a 5 grade scale.

Criteria of exclusion from the study: patients who did not express consent to take part in the research, juvenile patients, toothless patients, pregnant women, patients undergoing therapy for acute systemic diseases, patients with acute periodontal diseases, patients with recorded hypersensitivity to propolis or bee products.

The data obtained from every patient was treated as confidential. Each patient had their own code – initials and the last two digits of their date of birth (for example: John Doe 1960 – JD/60). The research program was approved by the Bioethics Committee of the Silesian Chamber of Medicine (resolution no. 6/2010, dated 1.03.2010).

## Statistical Method

The obtained data was analyzed statistically in two ways: quantitative and qualitative. Quantitative results showed the values of the OHI index whereas qualitative results were related to the API and SBI indices as well as an assessment of the rheological properties of the tested preparations and a subjective evaluation of the patients' impressions of the degree of comfort when using the products. Both parametric and non-parametric tests were used for statistical verification of the assumed research hypotheses. Use of the first group of tests requires verification of assumptions made for the normality and homogeneity of variance of the data, which was verified with the use of the

Shapiro-Wilk test (normality) and the Levene test (homogeneity of variance). The ANOVA method was used in order to statistically assess the dynamics of changes of the API index which occurred due to the effect of the preparations. However, due to the fact that the data was a set of repeated measurements, the Wilks' lambda test was used as a multidimensional equivalent of the F-test. As the null hypothesis of equality of means was rejected, the Tukey range test was used to compare individual means for each of the groups. The Student's t-test was used to assess the influence of the toothpaste and gel on the quantitative parameters, provided that the assumption of variance homogeneity was fulfilled. If not, a t-test with separate variance estimate (called the Cochran-Cox test) was utilized. The statistical analysis of qualitative parameters was based on non-parametric tests. More than two dependent samples were compared with use of the Friedman ANOVA test, while in the case of just two samples, the Wilcoxon signed-rank test was used. In the case of two independent samples, the Mann-Whitney U-test was applied. All tests were conducted with a significance level of  $\lambda = 0.05$  and with the use of Statistica v.8 software (SUM, Katowice, Poland).

## Results

In the case of the OHI index (Greene and Vermillion's Oral Hygiene Index) and patients of Groups I and II who used both C and T toothpastes, the value decreased so much that the median (average value) after 8 weeks was statistically different from the value during the first visit ( $p = 0.0679$ ) (Table 1). It suggests that in the case of patients who were not diagnosed with periodontal diseases but only with some tendencies due to lack of hygiene, the fact of participation in the research program and the necessity of using hygienic preparations and a toothbrush alone could have played a significant role, irrespective of the presence or absence of a propolis additive. Lack of statistical significance in the third group, who used the C and T toothpastes, suggests that the OHI index may have no analytical importance in the case of patients with a diagnosed periodontal disease. However, it may suggest that the choice of therapeutic product such as propolis may have a significant influence on elimination of hygienic negligence in the case of healthy patients or patients with minor problems (Group I, II) (Table 1). Unfortunately, those results cannot be confirmed by the results of correlative analysis of the OHI index in the case of patients who used the CT and CG gels. In their case, no statistically significant

**Table 1.** Median [min–max] for OHI index assessment of Group I and II patients who used the T and G toothpastes**Tabela 1.** Mediana [min–maks] dotycząca oceny wskaźnika OHI pacjentów grupy I i II stosujących pastę do zębów T i G

Group (Grupa)	Specimen (Preparat)	Examination (Badanie)			ANOVA Friedman (p)	Wilcoxon's signed rank test (Test kolejności par)		
		first (1) pierwsze	second (2) drugie	third (3) trzecie		comparable group pairs		
						(1):(2)	(1):(3)	(2):(3)
I	T	2 [1–2]	1 [1–2]	1 [1–1]	0.0498	0.4795	0.0679	0.4795
	G	1 [0–2]	1 [0–2]	1 [0–2]	0.6065			
	Mann-Whitney U-test (p)	0.5229	0.7494	0.6547				
	II	T	2[1–3]	2[1–3]	2[1–3]	0.0388	0.108	0.0679
	G	1[1–2]	1[1–1]	1[1–2]	0.0366	0.108	0.0679	0.4795
	Mann-Whitney U-test (p)	0.4414	0.5006	0.7728				

**Table 2.** Median [min–max] for OHI index assessment of Group I and II patients who used the CT and CG toothpastes**Tabela 2.** Mediana [min–maks] dotycząca oceny wskaźnika OHI pacjentów grupy I i II stosujących żel CT i CG

Group (Grupa)	Specimen (Preparat)	Examination (Badanie)			Friedman ANOVA (p)	Wilcoxon's signed-rank test (Test kolejności par)		
		first (1) pierwsze	second (2) drugie	third (3) trzecie		comparable group pairs		
						(1):(2)	(1):(3)	(2):(3)
I	T	1.5 [1–3]	1.5 [1–3]	1 [1–2]	0.2232			
	G	1 [0–2]	1 [0–2]	1 [0–2]	0.3679			
	Mann-Whitney U-test (p)	0.4233	0.8727	0.3367				
	II	T	3[2–3]	2[2–3]	2[2–3]	0.1146		
	G	2[1–3]	2[1–3]	2[1–3]	0.2636			
	Mann-Whitney U-test (p)	0.0621	0.0756	0.1200				

difference was observed (Table 2), despite the fact that the OHI index actually decreased in the following examinations.

The Lange API index values in the case of those groups of patients who were to start using the CT and CG gels varied from the fully correct values during the first visit. The results of the evaluation in the case of the patients from Group I yielded a range of percentage values that made it possible to qualify those patients into a range described as “quite good hygiene”, while during a follow-up visit that took place 7 days after the initial examination, their results changed the qualification to a range described as “optimal hygiene”. Moreover, during the third visit the qualification changed

back to the range described as “quite good hygiene” (Table 3, Fig. 1). Patient Group II received a qualification of “average hygiene” during the first visit and remained the same during the follow-up examinations. Further analysis showed that the patients were moderately satisfied with the properties of the CT and CG gels, which may have been the reason for an unfavorable change or a lack of anticipated improvement (Table 4).

In the case of the patients from Group I, who used the T and C toothpastes, the values of the SBI index (Muhlemann-Son's Sulcus Bleeding Index) qualified them to be included in the range described as “gingiva normal, no bleeding” and “bleeding after probing without changes in the

**Table 3.** Mean values of the API index in Group I and II patients who used the T and G toothpastes**Tabela 3.** Średnia arytmetyczna  $\pm$  odchylenie standardowe wskaźnika API u pacjentów grupy I i II stosujących pasty do zębów T i G

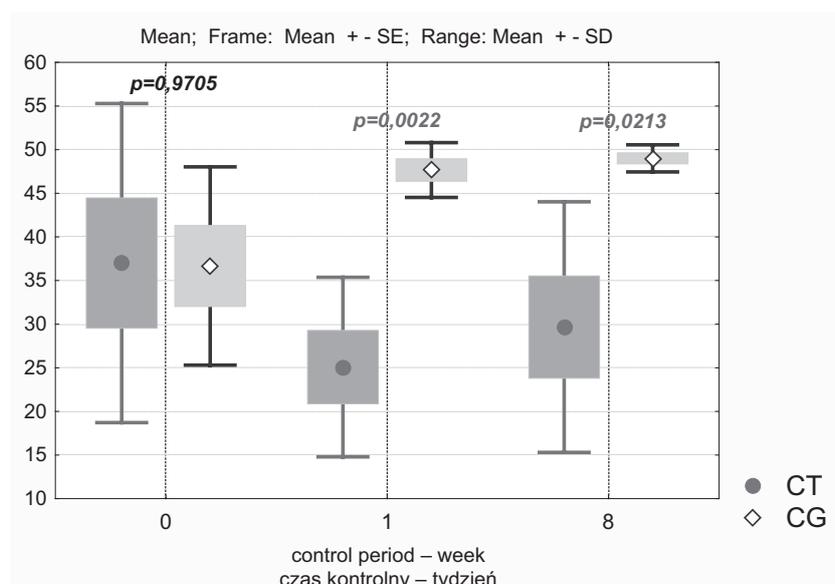
Group (Grupa)	Specimen (Preparat)	Examination (Badanie) X $\pm$ SD			ANOVA (p)	Tukey's test (Test Tukeya)		
		first (1) pierwsze	second (2) drugie	third (3) trzecie		comparable mean pairs		
						(1):(2)	(1):(3)	(2):(3)
I	T	31.0 $\pm$ 7.1	31.4 $\pm$ 15.1	21.6 $\pm$ 16.8	0.1464			
	G	29.6 $\pm$ 17.9	27.6 $\pm$ 20.7	24.9 $\pm$ 20.9	0.5280			
	t-test (p)	0.8478	0.6978	0.7518				
II	T	42.8 $\pm$ 10.8	38.6 $\pm$ 8.6	38.6 $\pm$ 14.8	0.4305			
	G	53.4 $\pm$ 14.4	42.0 $\pm$ 16.0	34.6 $\pm$ 17.7	0.1063			
	t-test (p)	0.1043	0.5833	0.6253				

a – t-test with separate variance estimate (Cohran-Cox test).

p – test probability.

a – test t z oddzielną estymacją wariancji (test Cohrana-Coxa).

p – prawdopodobieństwo testowe.

**Fig. 1.** Mean values of the API index in Group I patients who used the CT and CG toothpastes (t-test)

**Ryc. 1.** Średnia arytmetyczna  $\pm$  odchylenie standardowe wskaźnika API u pacjentów grupy pierwszej stosujących żel CT i CG (test t)

shape and color” during the initial examination. During the third examination, all patients qualified for the range described as “gingiva normal, no bleeding”. However, that tendency did not prove to be statistically significant (Table 5). In the case of the patients from Group II, who used the T and C toothpastes, the initial qualification was “bleeding after probing without changes in the shape and color” and no statistical significance was observed during the third examination (Table 5).

In the case of the patients from Group I, who used the CT and CG gels, the values of the SBI index qualified them to be included in the range described as “bleeding after probing without changes in the

shape and color” during the initial examination. During the third examination, all patients qualified for the range described as “gingiva normal, no bleeding”. In the group of patients who used the CT gel, the median of the SBI index value decreased with statistical significance in the period between the initial examination and a follow-up examination conducted after 8 weeks ( $p = 0.0431$ ) (Table 6). Use of the CG gel did not statistically change the mean values during the whole observation period. A statistical significance can be observed if the CT gel (with propolis content) is used. The longer the observation period, the better the values of the SBI index with final results that qualify to be in the range

**Table 4.** Mean values of the API index in Group I and II patients who used the CT and CG toothpastes (t-test)**Tabela 4.** Średnia arytmetyczna ± odchylenie standardowe wskaźnika API u pacjentów grupy I i II stosujących żele CT i CG (test t)

Group (Grupa)	Specimen (Preparat)	Examination (Badanie) X ± SD			ANOVA (p)	Tukey's test (Test Tukeya)		
		first (1) pierwsze	second (2) drugie	third (3) trzecie		comparable mean pairs		
I						(1):(2)	(1):(3)	(2):(3)
	CT	37.0 ± 18.3	25.1 ± 10.3	29.7 ± 14.4	0.3657			
	CG	36.7 ± 11.4	47.7 ± 3.1	49.0 ± 1.5	0.1093			
	t-test (p)	0.9705	0.0022a	0.0213a				
II	CT	55.3 ± 17.9	44.9 ± 16.2	46.6 ± 14.5	0.0631			
	CG	45.0 ± 15.4	46.9 ± 9.0	51.9 ± 15.2	0.4402			
	t-test (p)	0.2159	0.7628	0.4637				

a – t-test with separate variance estimate (Cohran-Cox test).

p – test probability.

a – test t z oddzielną estymacją wariancji (test Cohrana-Coxa).

p – prawdopodobieństwo testowe.

**Table 5.** Median [min–max] for SBI index assessment of Group I and II patients who used the T and G toothpastes, p – test probability**Tabela 5.** Mediana [min–maks] dotycząca oceny wskaźnika SBI dla pacjentów I i II grupy stosujących pastę do zębów T i G, p – prawdopodobieństwo testowe

Group (Grupa)	Specimen (Preparat)	Examination (Badanie)			Friedman ANOVA (p)	Wilcoxon's signed rank test (Test kolejności par)		
		first (1) pierwsze	second (2) drugie	third (3) trzecie		comparable group pairs		
I						(1):(2)	(1):(3)	(2):(3)
	T	0 [0–1]	1 [0–1]	0 [0–1]	0.4724			
	G	1 [0–1]	0 [0–1]	0 [0–1]	0.3679			
	Mann-Whitney U-test (p)	NS	NS	NS				
II	T	1[1–2]	1[0–1]	1[0–1]	0.2636			
	G	1[1–2]	1[1–1]	1[1–1]	0.1354			
	Mann-Whitney U-test (p)	0.6304	0.7003	0.7003				

described as “gingiva normal, no bleeding” (Table 6, Fig. 2). Patients from Group II who used the CT and CG gels were initially qualified in the range described as “bleeding after probing without changes in the shape and color”. There was no improvement or statistically significant difference during the third visit (Table 6).

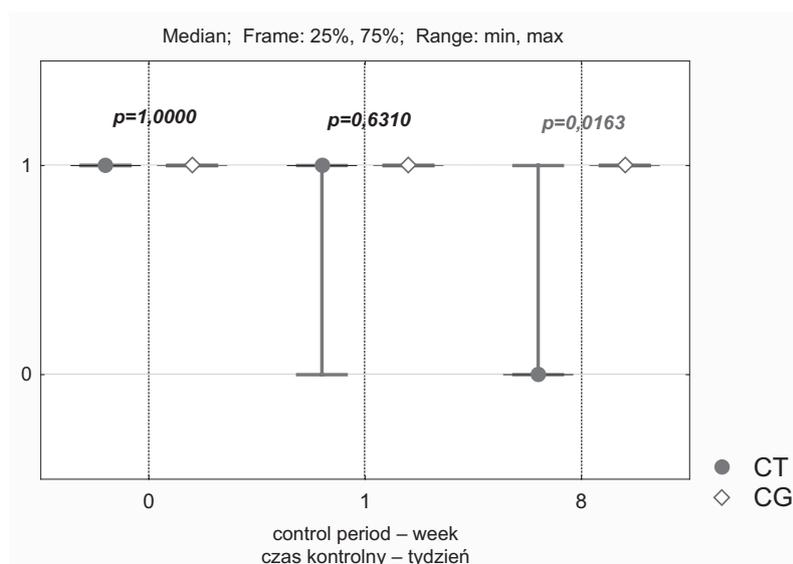
The assessment of the rheological properties of the researched preparations, as well as the patients'

impressions of the degree of comfort when using the preparations (including taste, smell, color, optimal degree of foaming during tooth brushing, subjective feeling of a degree of tooth cleaning such as tooth smoothness and sense of freshness in the oral cavity) did not prove to be statistically significant in the case of gels. However, it showed statistical significance in case of the C and T toothpastes and such parameters as taste, color and foaming

**Table 6.** Median [min–max] for SBI index assessment of Group I and II patients who used the CT and CG gels, p – test probability

**Tabela 6.** Mediana [min–maks] dotycząca oceny wskaźnika SBI dla pacjentów grupy I i II stosujących żel CT i CG, p – prawdopodobieństwo testowe

Group (Grupa)	Specimen (Preparat)	Examination (Badanie)			Friedman ANOVA (p)	Wilcoxon's signed rank test (Test kolejności par Wilkoxona)		
		first (1) pierwszy	second (2) drugi	third (3) trzeci		comparable group pairs		
I						(1):(2)	(1):(3)	(2):(3)
	T	1 [1–1]	1 [0–1]	0 [0–1]	0.0150	0.4795	0.0431	0.0679
	G	1 [1–1]	1 [1–1]	1 [1–1]	1.0000			
	Mann-Whitney U-test (p)	1.0000	0.6310	0.0163				
II	T	1[1–2]	1[1–1]	1[1–2]	0.2232			
	G	1[1–2]	1[1–1]	1[1–2]	0.2232			
	Mann-Whitney U-test (p)	0.8590	1.0000	0.9292				



**Fig. 2.** Median [min–max] for SBI index assessment of Group I patients who used the CT and CG gels

**Ryc. 2.** Mediana [min–maks] dotycząca oceny wskaźnika SBI dla pacjentów I grupy stosujących żel CT i CG

ability. The CT and CG gels did not evoke such extreme opinions in the patients and were generally better accepted (Table 7, Figs. 3 and 4).

## Discussion

Alternative or complementary medicine is a collection of concepts, means and techniques based on natural forces, nature and human powers. Some branches of complementary medicine have their origins in traditions which are several hundred years old (e.g. acupuncture). Current trends show a return to natural medicine and treatment methods, also due to the fact that patients are worried about the preventive means of

fighting many diseases and illnesses. Bee products are an example of such means that have been widely and commonly used for many years and that can improve our health as well as play an important role in the prevention of illnesses. The therapeutic potential of honey has recently been reviewed [1, 3, 14, 24–26]. Other bee products, royal jelly and propolis, have also been widely used in “folklore medicine” for centuries. As popular folk medicine, propolis is alleged to exhibit a broad spectrum of activities including antibiotic, anti-inflammatory and tumor growth arrest; some of the observed biological activities may be traced to identified chemical constituents such as caffeic acid which is antimicrobial and anti-inflammatory [2].

**Table 7.** Median [min–max] for preparation properties assessment of Group I and II patients**Tabela 7.** Mediana [min–maks] dotycząca oceny właściwości preparatów dla pacjentów I i II grupy

Property (Właściwość)	Specimen (Preparat)		Mann-Whitney U-test (p) (Test-U Manna- -Whitneya)	Specimen (Preparat)		Mann-Whitney U-test (p) (Test-U Manna- -Whitneya)
	T	G		CT	CG	
Taste (Smak)	3 [2–4]	4 [2–5]	0.0332	3 [1–5]	4 [2–5]	0.1979
Smell (Zapach)	3 [2–4]	4 [2–5]	0.1822	3 [1–5]	3 [2–5]	0.1001
Color (Kolor)	2 [1–5]	4 [2–5]	0.00001	3 [1–5]	4 [2–5]	0.2464
Foaming (Pienienie)	2 [1–4]	3 [1–5]	0.0198	3 [1–4]	4 [1–4]	0.1875
Cleaning (Oczyszczanie)	3 [1–5]	4 [1–5]	0.2336	3 [1–5]	3 [1–5]	0.9908

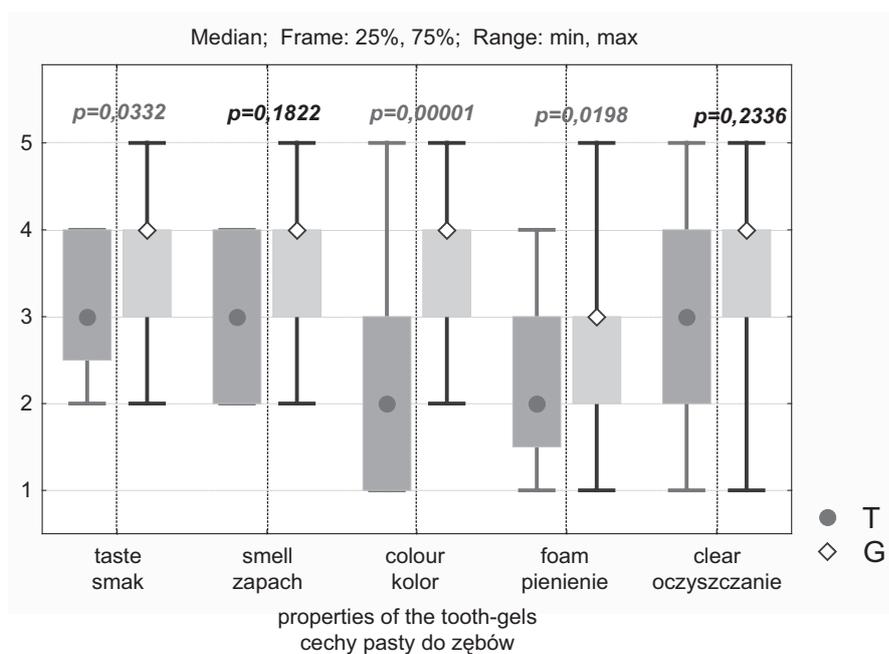
Descriptive marking scale for each property of the preparation used was limited by the following ranges: 1 – unsatisfactory, 2 – satisfactory, 3 – average, 4 – good, 5 – very good.

Skala ocen poszczególnych cech stosowanych preparatów: 1 – niedostateczne, 2 – dopuszczalne, 3 – dostateczne, 4 – dobre, 5 – bardzo dobre.

Owing to discoveries in the field of chemical composition and the confirmed usefulness of these products by both laboratory and clinical research, the term of “apitherapy” was coined [24, 25]. It is a distinguished method of treatment based on the use of products which are collected, processed or produced by bees. Such products include the following: pollen – as a product collected by bees, propolis, nectar honey, bee bread – products collected and processed by bees, royal jelly, bee venom and beeswax – products secreted by bees [25].

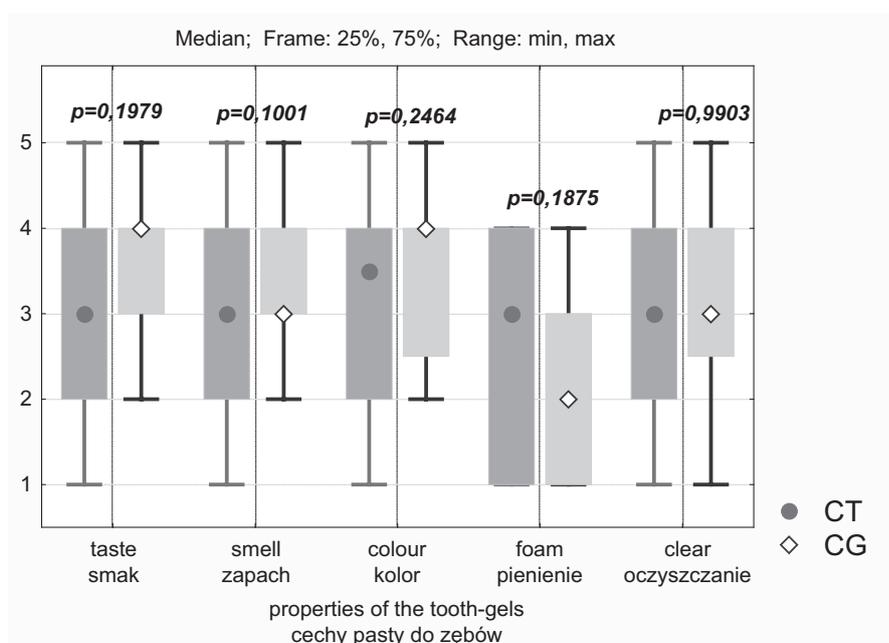
The therapeutic properties of propolis have been used in dentistry for many years, focusing on the four most well-known properties of that substance: regenerative, anti-bacterial, anesthetic and stimulation of the immune system. The regenerative features of propolis were observed by Stojko et al. in animals [19]. Soft tissue and cartilage wounds of dogs granulate quickly if the ethanol propolis extract (EPE) or a water solution of propolis are applied. Moreover, significant acceleration of cartilage and perichondrial primordia was found during histopathological and radiological examinations. Cytochemical research made it possible to notice increased mitochondrial activity of the letrozole reductase, which indicates increased cell metabolism. The therapeutic properties of propolis can be confirmed with its effectiveness in periodontal diseases, as it mitigates the course of alveolar osteitis [25]. Its anesthetic properties are used in dentin hypersensitivity, electrocoagulation of hypertro-

phic interdental papillae and minor surgical procedures. There are also reports on research tests with the use of propolis in the treatment of pulp and caries. Propolis is also used in the form of gel and rubbing ointments, a 10-percent ethanol solution for brushing and a 0.2-percent ethanol-water solution for mouthwashing used three times a day in the treatment of periodontal diseases, ulcerative gingivitis, chronic and recurrent aphthous ulcers, desquamative cheilitis and bullous and ulcerative forms of lichen planus. Treatment can be supported by chewing a 3-gram lump of propolis three times a day for half an hour, while people with dentures can sprinkle them with a powdered form. Propolis extracts have proven highly effective in soothing post-extraction pain and the treatment of alveolar osteitis. Repeated intra-alveolar application of a seton soaked with ethanol propolis extract (EPE) takes away the pain and accelerates wound healing [6, 12, 14–16]. Further research on applications of ethanol propolis extract demonstrate tests to use it in dentistry in three different forms: *in substantia*, in a 3-percent ethanol-glycerine solution and as a 3-percent ointment on an *Eucerinum anhydricum* base. EPE was rubbed into the hard tooth tissue during preparation of a cavity in order to anaesthetize it. The anesthetic effect was obtained after 3 to 4 minutes of rubbing the preparation into the walls and bottom of the cavity. An analysis of the influence of EPE in relation to the depth of the cavity demonstrated a slightly higher percentage of



**Fig. 3.** Rheological property analysis and subjective impression of patients who used the T and G tooth-pastes

**Ryc. 3.** Analiza właściwości reologicznych i odczuć pacjentów dotyczących pasty do zębów T i G



**Fig. 4.** Rheological property analysis and subjective impression of patients who used the CT and CG gels

**Ryc. 4.** Analiza właściwości reologicznych i odczuć pacjentów dotyczących żelu CT i CG



**Fig. 5.** Patient from group I, initial examination, with a color marking Plaque Indicator Kit (preparation T)

**Ryc. 5.** Pacjent z grupy I, wizyta wstępna, po wybarwieniu Plaque Indicator Kit (preparat T)



**Fig. 6.** Patient from group I, examination after week 4, with a color marking Plaque Indicator Kit (preparation T)

**Ryc. 6.** Pacjent z grupy I, wizyta po 28 dniach, po wybarwieniu Plaque Indicator Kit (preparat T)

cases in which anesthesia was complete in cases of average caries or even deep caries than in cases of surface caries. During treatment of hypersensitivity of the dentin, teeth dried and isolated from saliva were rubbed with EPE for a period of 3 to 4 minutes every 2 to 3 days. In the case of caries, the prepared cavity was washed with an EPE solution which created a thin, organic film that blocked the exit of the dentin canals after the vapor fraction disappeared. In the case of pulp treatment, not only antiseptic properties were observed, but also long-term treatment benefits could be noted. In some cases, e.g. for direct covering of the pulp, EPE *in substantia* was used instead of a ready solution, similarly to the cases of pulpotomy [16, 19].

The results of the presented research show the effectiveness of hygienic preparations with a 3%

content of ethanol propolis extract in both groups of patients: without the pathological changes of the periodontium and in the case of patients endangered with the occurrence of gingivitis caused by dental plaque (Figs 5, 6). When using propolis, particular attention should be paid to its side effects, particularly the possible occurrence of allergic reactions. Some authors claim that the frequency of allergic cases constitutes 0.25% of all propolis applications, others show this number to be as large as 0.8% of cases in which oedema of mucous membranes or conjunctivas, as well as hypotension or cardiac dysrhythmia may occur [27].

The authors have concluded that hygienic preparations with a 3% content of ethanol propolis extract efficiently support removal of dental plaque and improve the state of marginal periodontium.

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