World Journal of Advanced Pharmaceutical and Medical Research

Journal homepage: https://zealjournals.com/wjapmr/

ISSN: 2799-0656 (Online)

(Research Article)

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Preliminary clinical study of mucoadhesive propolis gel to gingival disorders control in patients with fixed orthodontic appliances.

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World Journal of Advanced Pharmaceutical and Medical Research, 2023, 05(02), 018–029

Publication history: Received on 15 June 2023; revised on 20 November 2023; accepted on 23 November 2023

Article DOI: https://doi.org/10.53346/wjapmr.2023.5.2.0028

Abstract

Aims: Gingival hyperplasia (GH) etiology is diverse and ranges from acute or chronic inflammatory processes and is commonly observed in patients with fixed orthodontic appliances. Propolis has been globally studied and has anti - inflammatory and antimicrobial properties, however, there are few clinical studies involving orthodontics.

Study design: This clinical study aims to obtain preliminary evidence of an oral gel containing 5% Brazilian propolis (BPG) in GH and gingivitis control on patients with fixed orthodontic appliances.

Place and Duration study: Clinic Orthodontics, Faculty of Dentistry/UFMG, Belo Horizonte, Brazil, from March to October 2013.

Methodology: Thirty patients were selected, but only 10 participated and reached the end of the study. The patients were submitted to questionnaire and a complete prophylaxis of dental structures before initiating the gel use(T0) and they were instructed to use the gel two times daily after brushing (morning and evening) during 30 days (T30). Patients were evaluated considering plaque and gingival index and photographs collection of first premolars - measuring gingiva of the clinical crown area. The SPSS version 18.0 statistical program, Shapiro Wilk normality test, presenting a normal or parametric distribution and all analyzes were performed using Student's t Test for paired samples.

Results: BPG show statistically significant reduction of GBI and GSI (GB1 p =0.207; GB2 p = 0.122), respectively. Conclusion: Considering this study model, 5% propolis gel had effective in reducing GH and gingivitis. However, a double blind and randomized clinical studies with higher number of patients should be made to give greater credibility for gel use.

Keywords: Orthodontics; Gingival Hyperplasia; Gingivitis; Clinical trials; Propolis Gel

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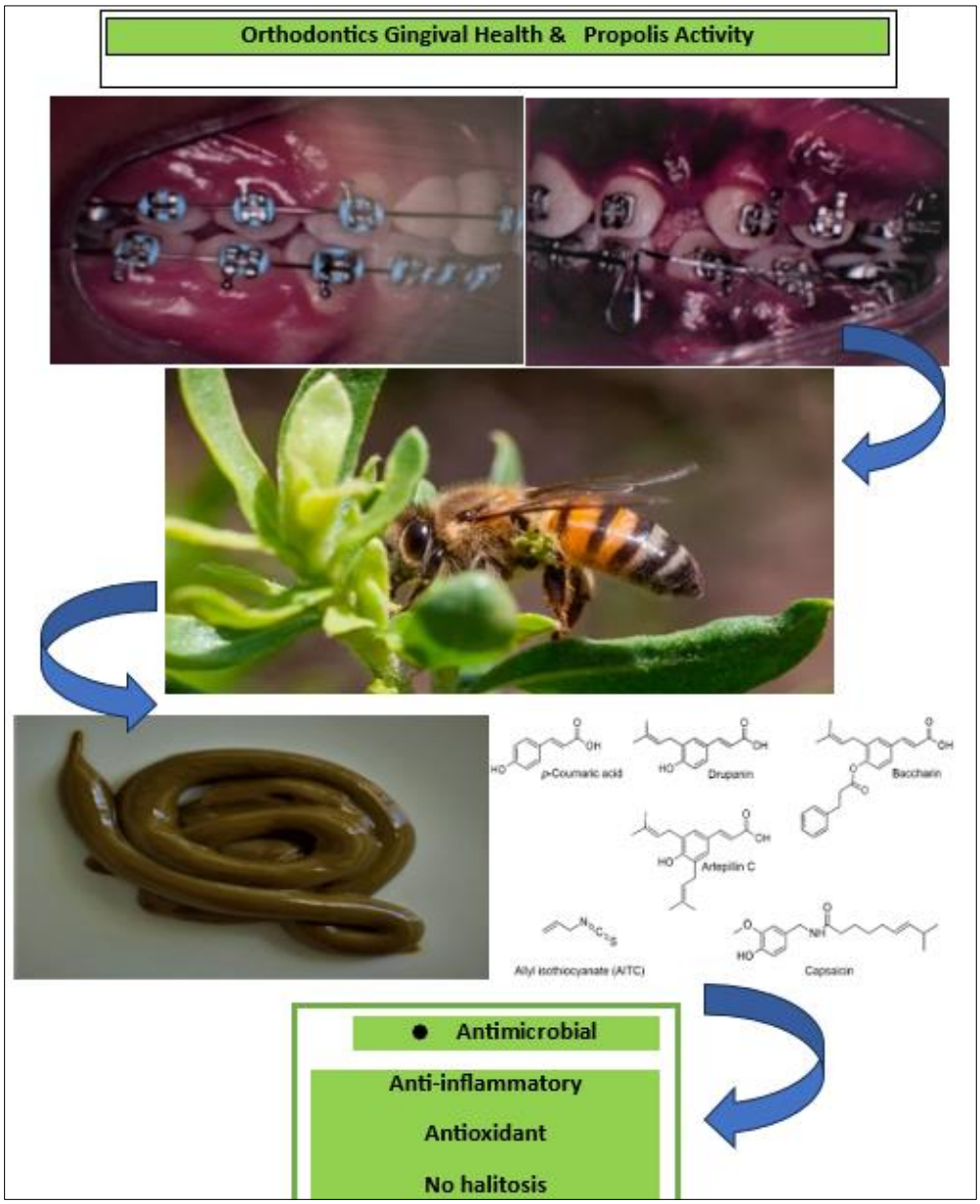
Graphical abstract



1. Introduction

Oral preventive care can inhibit disease dependent of several factors related to the patient and the professional. During orthodontic treatment, the control of dental plaque is a challenge present in clinical routine [1,2].

Fixed orthodontic appliances make a difficult for adequate oral hygiene and the patient more susceptible to the occurrence of periodontal diseases. The biofilm accumulation means a greater number of microorganisms, and increase gingival volume around the brackets. The orthodontic bands somehow alter the composition of the oral microbiota [3]. Thus, due to the difficulty and limitation in maintaining effective control of bacterial biofilm through mechanical methods, often imposed by the device itself-chemical agents are considered important adjuncts in maintaining oral health and plaque or biofilm control. Several agents are commercially available, although these chemicals can alter the oral microbiota and have undesirable side effects. For this reason, the search for alternative medicines based on natural products, such as propolis, has been the subject of research all over the world [4]. Propolis is a complex, non-toxic resin produced by *Apis mellifera* bees, whose composition varies according to the botanical origin. It performs several biological activities such as antibacterial, antifungal, healing, tumoricidal, anti-inflammatory and anesthetic [4,5]. Previous *in vitro* studies revealed the bactericidal activity of propolis extract on periodontopathogen microorganisms

[6,7, 8]. It has recently been reported that artepillin C has an inhibitory effect on the prostaglandin E2 and nitric oxide through NF-κβ modulation using the cell line of RAW 264.7 macrophages [9,10]. The anti-inflammatory activity observed in green propolis seems to be due to the presence of flavonoids and prenylated cinnamic acid. These compounds show inhibitory activity against cyclooxygenase (COX) and lipoxygenase. It is also verified that caffeic acid phenethyl ester (CAPE) has anti-inflammatory activity by inhibiting the release of arachidonic acid from the cell membrane, suppressing enzyme activities COX-1 and COX-2 [11,12,13,14]. Ethanol extracts of green propolis exhibited significant antimicrobial activity against several pathogens of the oral cavity, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum* which is the main microbiota involved in dental biofilm-related periodontal disease [15,16,17]. Currently, several products containing propolis have been developed for the use and application in the various dental specialties, considering the multiple biological properties of propolis and the resistance of microorganisms to antibiotics. [18,19, 20]. Given the increase in resistance to antibiotics by bacteria, the effects of adverse effects of some agents used in dentistry and the financial conditions prevalent in developing countries, the need to create options for prevention and treatment that are safe, effective and economical [21,22]). In this sense, the objective of this study it was verify clinical evidence of a gel containing 5% green propolis in the control gingival disorders observed in patients with fixed orthodontic appliances.

2. Materials and methods

This research was submitted and approved by the Federal University of Minas Gerais Ethics Committee, under No. 04346212.0.0000.5149 and Brazilian Platform No. 245.559.

2.1. Patients and Study Design

This research is characterized as interventional clinical study of the follow-up type, which lasted 30 days. It was conducted at the Faculty of Dentistry of the Federal University of Minas Gerais (UFMG), Belo Horizonte, from April 2011 to October 2013. The universe of this study consisted of 30 patients from Orthodontics Specialization Clinic at the UFMG, who have been undergoing orthodontic treatment for more than 6 months.

Regarding eligibility to participate in this study, we considered the following criteria [23,24].

- inclusion: be between 12 and 60 years old, in good general health and have at least 20 teeth, absence of pregnancy, non-lactating, not showing sensitivity to any component of the test product, be available for the duration of the study, present with the appliance installed for more than 6 months, agree and sign the Informed Consent Form (TCLE), present a condition of gingivitis with erythematous, edematous and probing depth of a maximum of 3 mm and index gingival ≥1.0 [25].
- Exclusion: presence of hard and soft tissue tumors and gingival growths, hyperplastic due to use or not of medication, confirmed hypersensitivity to propolis, use of antibiotic therapy up to two weeks before the start of the study.

2.2. Clinical Initial Exam

The clinical examination was performed by a single examiner, calibrated and trained, to improve the consistency of the exam. During the oral examination, special attention was paid to collecting data regarding the presence of gingivitis, gingival enlargement and the presence of alterations in the tissues of the mouth.

2.3. Index Collection

2.3.1. Gingival Bleeding Index (GBI)

The GBI was obtained considering the Gingival Index [26]. Buccal and lingual surfaces of each tooth will be quantified. The maximum score was 18 per tooth. Third molars and those teeth with cervical restorations or possessing prosthetic crowns were excluded from this analysis. The gingival total index was obtained by averaging the values observed in all areas gingival margin.

2.3.2. Gingival Severity Index (GSI)

The GSI was also evaluated [21,27]. This index measures the tooth surfaces proportion with a high gingival index (GI). The GSI indicates the proportion of counted tooth surfaces of the entire mouth with indices equal to 2 or 3 [25] or i.e., bleeding sites divided by the total number of points assessed in the entire mouth for gingivitis (number of evaluated teeth multiplied by 6.0(six).

2.3.3. Clinical analysis of gingival swelling

Only patients with Grade 2 of gingival enlargement were included. Grade 3 and Grade 4 will be indicated for orthodontic appliance removal to restore gingival normality. Patients with Grade 0 or 1 do not show significant gingival alterations to justify study inclusion. The measurement of the gingival margin was performed according Rodrigues et al. [26].

2.3.4. Image acquisition

After the clinical selection, intraoral photographs were taken, right and left sides, for the buccal surface area analysis of the upper first premolars at all times of the survey (T0 and T30), with a digital camera Canon Rebel XT (Canon USA, Inc., One Canon Plaza, Lake Success, NY, 11042, USA). All photographs were taken by the researcher, with a fixed focus of 1:1 and the mesiodistal width of the assessed tooth will be measured to establish a pattern in the angle of the photograph taken.

2.3.5. Scraping, coronal polishing and instructions

After completed the initial examination, participants were submitted to oral prophylaxis, with complete removal of plaque and calculus deposits. Participants received a guidance of the study, including printed text, soft toothbrushes, gel mouthwash containing 5% propolis and the application frequency form. Participants were instructed to apply the gel, spread it with the tongue throughout the oral cavity, twice a daily after morning and evening brushing. They were also instructed to remain with the usual habits of oral hygiene and not to use any other mouthwash during the study. To measure adherence to the application program of the gel, with each distribution of a new bottle of gel, the participant returned the already used bottle and the completed attendance form, which was inspected and initialed by the researcher. In this form, the corresponding dates and periods (morning or evening) were noted and whether used or not the oral gel.

2.4. Propolis gel

Oral gel containing 5% green Brazilian propolis (BPG) was manipulated, according to the researchers' request, by PharmaNéctar® (Belo Horizonte, Brazil), complying with the standards required by ANVISA [28] and the requirements of the ISO 9001 and GMP International. The propolis used in the manipulation of the gel is standardized, since the identification of flavonoids and other chemical constituents was carried out by means of High-Performance Liquid Chromatography and Reverse Phase (RPHPLC) (Table 1 and Figure 1).

2.5. Data Analysis

The following indicators were considered for the analysis of results: presence/absence of gingivitis GBI and GSI; presence/absence of mouth tissues alterations; surface area of maxillary first premolars; appreciation and acceptability of the product. As the sample is smaller than 50, the choice test was the Shapiro Wilk test and the data before and 30 days after the propolis use were used with significance and $p < 0.05$ values. In this way, all analyzes were performed using Student's t Test for paired samples.

Table 1 Main Chemical Components of Brazilian green propolis extract

No.	Formula	Compound	Unit (mg/g)
1	C9H8O3	p- Coumaric acid	3.56
2	C25H24O12	Dicaffeoylquinic acid	6.64
3	C15H10O7	Quercetin	1.38
4	C15H12O5	Pinobanksin	13.92
5	C15H10O6	Kaempferol	11.40
6	C14H16O4	Caffeic acid isoprenyl ester	17.47
7	C15H12O4	Pinocembrin	19.00
8	C15H10O5	Galangin	9.75
9	C16H11O6	Kaempferide	11.,60
10	C19H24O3	3,5-diisopentenyl-4-hydroxycinnamic acid*	82.96

11	C16H14O5	Sakuranetin	5.57
12	C15H10O4	Crisin	3.51
13	C16H12O7	Isorhamnetin	0.91
14	C17H14O6	Pinobanksinr-3-O-acetate	20.68
15	C9H8O2	Cinamic acid	1.55

*Artepilin C. Adapted from: [29,30].

A B

Photo Credit A/B: Prof. Dr. Vagner R. Santos

Figure 1 Physical appearance of Green Brazilian Propolis: A- Solid and powdered. B- Mucoadhesive Green Propolis Gel (BPG)

3. Results and discussion

Because it is a reduced sample with a final of 10 patients, it was decided not to make a flowchart, but to describe the entire sample. During the period available for the study, 30 individuals were selected for convenience and screening. Due to the inclusion and exclusion criteria and availability to participate in the study, 15 patients were chosen, in accordance with what was proposed by Clinical Trials and Haynes et al. [23,24], for Phase II clinical trials. However, 5 other patients dropped out during the study. The main reason for excluding patients was the inadequacy of the study model, as they did not present the minimum required plaque and gingival indices. Individuals who refused to participate during the study claimed to be afraid of the product or because they did not like the taste and smell of propolis. Four men and six women completed the study, aged between 13 and 29 years (16.9±4.6).

Tables 2 to 5 show the results obtained through the statistical tests of the Gingival Bleeding Index (GBI), the Gingival Severity Index (GSI) and Exposed Area of the Crown of Teeth (PMA) corresponding to the presence of dental plaque, gingival inflammation before and after using the propolis gel.

Table 2 Descriptive Analysis and Sample Indicators and the measurements of premolar tooth area (14) before (PMA1) and after (PMA2) use of propolis gel. Paired samples.

Indicators	**Age**	**GBI-1**	**GBI-2**	**PMA-1 (mm)**	**PMA-2 (mm)**	**GSI-1**	**GSI- 2**
Sample	10	10	10	10	10	10	10
Media	16.9	1.26	0.82	35.23	38.81	0.40	0.24
SD	4.86	0.38	0.44	7.08	6.90	0.21	0.24

Legend: GBI= Gingival Bleeding Index, PMA= Coronary surface of the premolar tooth (14), GSI= Gingival Severity Index, SD= Standard Deviation.

For a better statistical evaluation, in the case of a low number of participants, the tables show the results observed in the values of the indexes with the paired and separated samples. **Table 2** describes the values of the paired samples

with statistical difference in the values with gingival enlargement, when the gingiva invades the coronal portion, and after the use of propolis gel when there is a decrease in gingival volume and an increase in the visible surface of the tooth (PMA).

3.1. Gingival Bleeding Index (GBI)

Table 3 shown the mean GBI scores at the baseline (GBI-1) and 30-day (GBI-2) examination periods recorded with the separate and matched samples, respectively. The use of 5% propolis-based buccal gel (GBI-2) reduced gingivitis by 34,7%, which was statistically significant when comparing the 30-day score with the initial exam score ($p < 0.0016$).

Table 3 Evaluation of the Gingival Bleeding Index (GBI) in the Initial Examination before (GBI-1) and 30 days after (GBI-2) the use of propolis gel ($p<0.05$). Normality test considering paired samples. Paired Samples Test

GBI	Paired Differences	St test	Sig
	Mean ± SD		
GBI-1			
	0.4372 ± 0.469015	2.948	*0.0016
GBI-2			

Legend: GBI=Gingival Bleeding Index, St = Student t test, CI= Confidence Interval, SD=Standard Deviation, Sig= Significance (2-tailed)

The results of the indexes observed in the analysis on the severity of gingival inflammation (GSI) are shown with separate and paired samples, in Tables 4 and 5, respectively, and there was a significant difference between the indexes before and after the use of propolis gel.

Table 4 Evaluation of the Gingival Severity Index (GSI) before (GSI-1) and 30 days after (GBI-2) the use of propolis gel ($p<0.05$). Normality Test considering separate samples. Test of Normality

GSI	Kolmogorov-Smimov[a]			Shapiro-Wilk		
	Statistic	Sample	Sig	Statistic	Sample	Sig
GSI-1	0.159	10	0.200	0.94	10	
GSI-2	0.193	10	0.200	0.868	10	0.094*

Table 5. Evaluation of the Gingival Severity Index (GSI) in the Initial Examination before (GBI-1) and 30 days after (GBI-2) the use of propolis gel ($p<0.05$). Normality test considering paired samples

GSI	Paired Differences Media ± SD	St test	Sig
GSI-1			
	0.1605± 0.19838	2.558	0.031*
GSI-2			

Table 6 shows the patients' responses to the questionnaire answered in the last consultation, after 30 days of using the gel containing 5% Brazilian green propolis. The satisfaction, acceptability and indication of the product for other patients was 100%. The patients reported that there were no canker sores (7), bleeding (8) or traumatic ulcers (10) and during the use of the gel, as well as a significant improvement in the control of halitosis (9). Constant use of the gel did not interfere with either the color of the teeth or the color of the mucosa.

Table 6 Propolis gel (BPG) appreciation and acceptability by patients. Based on a questionnaire in the last consultation after 30 days of the product use.

Patient report	Patients (n=10)	Percentage (%)
Teeth and mucosa color change	No	0%
Burning	1	10%
Absence of recurrent canker sores	7	70%
No bleeding	8	80%
Traumatic Ulcers	No	0%
Improves halitosis	9	90%
Use satisfaction	10	100%
Acceptability	10	100%
Product recommendation	10	100%

This interventional clinical trial was conducted to research what would be the action of a 5% BPG on gingivitis and gingival size in patients with fixed orthodontic appliances. The 30-day treatment period was chosen because it was sufficient to verify the changes that occurred in the oral condition of the individual and also to observe the presence of adverse effects. Furthermore, this period was chosen due to the fact that the gingivitis being a chronic disease and participants remaining with their usual oral hygiene habits. As this is a phase II study, there is no determining requirement in relation to study time [31,32, 33, 34].

The difficulty of orthodontic patients in performing adequate oral hygiene makes it more susceptible to the occurrence of periodontal diseases. The means of bacterial plaque accumulation are a greater number of microorganisms, and an increase gingival volume around the brackets and close to the orthodontic bands somehow alters the composition of the oral microbiota [35]. The 5% propolis concentration was chosen considering the positive results in previous studies. In recent research [36] demonstrated in a phase II study the effectiveness of a mouthwash containing 5% of propolis with satisfactory results, in the reduction of plaque and gingivitis in the studied population. Noronha et al. [37]) in a study with oncology patients submitted to head and neck radiation, demonstrated the efficacy of 5% propolis gel in oral mucositis and candidiasis control. In both studies, the trend towards a more intact mucosa, good patient acceptance of the product and the recommendation of these patients to others. These studies served as indicators that corroborate and coincide with our results.

BPG reduced the GBI, and GSI when comparing the initial examination with the period of 30 days, the difference being statistically significant. The reduction in bleeding points was approximately 35%. Brazilian green propolis has as main bioactive component artepilin C, which in other studies showed potential anti-inflammatory activity [10,38, 39]). Propolis acts in the modulation of cytokines and inflammatory enzymes, such as prostaglandins, leukotrienes, histamines and TGF-β1 suppression [40]. Previous studies demonstrated the anti-inflammatory effect of Brazilian green propolis inhibiting proinflammatory cytokines IL-6 and TNF-α expression in MAECs [41]. Brazilian propolis have immunomodulatory effects on LPS-induced inflammation and exerts anti-inflammatory effects by inhibiting the production of IL-1α, IL-1β, IL-4, IL-6, TNF- α and MCP-1, in stimulated J774A cells [42]. Green propolis extract can also inhibit the release CXCL1/KC and CXCL2/MIP-2, inhibit peritoneal neutrophil migration, and reduce leukocyte migration and adhesion on the microcirculation [4,43]. The mechanisms of propolis activity in inhibiting the growth of microorganisms is related with some components, such as flavonoids (quercentin, galangin, pinocembrin), caffeic acid, benzoic acid and cinnamic acid, probably act on the microbial membrane or on the wall surface cell, causing structural and functional damage. Its activity against microorganisms is more related to the synergistic effect of its compounds (flavonoids, phenolic compounds and others) than for individual compounds [44].

Statistical significance did not occur, however, with the increase in the crown area of the first premolar in millimeters (mm). A trend towards an increase in the area of the crown was observed, but cannot be said statistically. This result may have occurred due to the size of the sample being small.

This study used green propolis, derived from *Baccharis dracunculifolia*, a native plant in Southeast region of Brazil, propolis type 12 [45]. The company that manipulated the BPG 5% for the research used standardized samples of crude

green propolis, which guarantees the presence of the main bioactive compounds in the oral gel [12], being of excellent quality (ISO 9001, GMP Internacional) this product is recognized by ANVISA as a topical medication. Almeida et al. [33], reported that propolis extract solution presented satisfactory antimicrobial activity and action similar to that of chlorhexidine, in addition to acting under clinical conditions, such as the presence of biofilm and gingival disease, after fifteen children using the mouthwash for fifteen consecutive days.

This work was carried out in 2013, however, we considered interesting to publish it because there are few works published in the area of orthodontics using propolis in the control of alterations caused by fixed appliances. There are various studies, but not orthodontics, used propolis in varied formulations and in different concentrations, such as gel, hydroalcoholic solution, extract and mouthwash [36, 37, 46]. In most of the afore mentioned works, the propolis was effective in regressing gingival disease and reducing plaque formation, being in agreement with the results obtained in this work [33,47]. In addition, rinsing solutions mouthwashes containing propolis are less cytotoxic to human gingival fibroblasts than chlorhexidine [44].

The positive changes reported by the individuals, such as the absence of recurrent canker scores, absence of gingival bleeding, and loss of halitosis, due to the use of BPG 5% are according to the biological properties attributed to propolis in the literature [10,48].

Evaluation of adherence to the program, access and acceptability of the product content propolis by patients were also considered in the Pereira et al. [36] and Noronha et al. [49] studies. Once acceptability, tolerance and preference are integral components of therapeutic adherence and facilitate the insertion of the product on the market.

Clinical studies often have limitations, regardless of the efforts of the researchers to do it judiciously. This study had limitations, for example: presence of unexpected reactions to the product, which resulted in the individual exclusion; difficulty in controlling participants' adherence to the study, how to contact every time the callbacks for evaluations were made; difficulty in maintain the individual's commitment. Despite the institution of a control of the use of the gel (frequency form and return of the empty vial), clinical studies have limitations with respect to the veracity of the applicability of the product by the patient, which is generally beyond the control of the researcher. It was noted that the research managed to reach some patients who used the gel in a judicious, and other patients who wrote down the use on the form, but in conversation informal said that they often forgot to use the gel. This resulted in little improvement in some patients. Despite complaining about the taste of propolis initially, patients have already observed an improvement in halitosis and no gingival bleeding, and this observation was important for the continuity of these patients in the study. Products for use on the oral mucosa should, in principle, have a pleasant taste and not interfere with the coloring of the teeth and mucosa, which could interfere with the acceptance and promotion of the product in the market. One of the very positive factors is that there are no side effects that could make the product unacceptable. In this study, the green propolis gel did not change the color of hard and soft tissues and there were no complaints about side effects that could cause interruption of treatment. This finding coincides with studies [38,49]. Despite having a small sample, this study, presented satisfactory results. He was carried out as a pilot study, with initial planning of patient follow-up by 90 days - as recommended by the ADA (American Dental Association), unfortunately this is not possible, for reasons beyond the control of the researchers, follow-up was obtained 30 days. However, a study with a larger sample is needed 90-day follow-up to confirm these results.

Abbreviations

- FO/UFMG- Faculty of Dentistry/ Federal University of Minas Gerais.
- FAPEMIG - Research Support Foundation of Minas Gerais State.
- ANVISA - Brazilian Health Surveillance Agency

4. Conclusion

Considering this study model, 5% propolis gel (BPG) had effective in reducing GH and gingivitis. However, a double blind and randomized clinical studies with higher number of patients should be made to give greater credibility for gel use.

Compliance with ethical standards

Acknowledgements

The authors acknowledge the financial support of Minas Gerais State Research Support Foundation (FAPEMIG),

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions

- AARODRIGUES- Selection and clinical care of patients, filled out the clinical questionnaire, managed the literature search.
- RTGOMES- English correction and Graphical abstract
- RCMARTINS- worked in the statistical analyses, made suggestions and corrections to the article.
- HPRETTI- Assisted the patients in diagnosing injuries, worked on correcting and analyzing the text of this article.
- VRSANTOS- Designed the study, wrote the protocol, clinical care for patients, project guidance, managed the analyses of the study, wrote and translated the article. He is the Corresponding author.

Statement of Ethical approval

The clinical work was approved by The Ethic Committee on Human Research, Federal University of Minas Gerais (COEP/UFMG): Number: CAAE 04328812-1-0000.5149.

Disclosure of Informed Consent

Informed consent to participate in the study was obtained from all participants patients.

Availability of data and materials

Data and information were obtained by completing a clinical form, anamnesis, patient history and care by health professionals, orthodontist and qualified dentists. Data were recorded and archived. Clinical files and records are in the archives of the Faculty of Dentistry of UFMG, but are not available, to secure the privacy of patients

Funding

Minas Gerais State Research Support Foundation (FAPEMIG) (17174FAPEMIG/FO/DCPCO/Mucositis and Candidiasis) - Project financing

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