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RESEARCH ARTICLE

Effectiveness of a Standardized Propolis Extract in Non-Surgical Periodontal Therapy

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Abstract

We determined the polyphenol content in a defined volume of chemically characterized and standardized propolis sample to evaluate its effectiveness in patients with chronic periodontitis. After having determined the polyphenol content of a given volume of propolis extract and characterized the molecular profile, 150 patients were enrolled, randomly divided into three groups and subjected to three different treatments, Scaling and Root planing (SRP) associated with propolis, SRP with 1% chlorhexidine gel and SRP only. Before the treatments, Full Mouth Plaque Score (FMPS), Pocket Depth at Probing (PPD), Full Mouth Bleeding Score (FMBS), Impaired furcations according to the Hamp Classification, Mobility, Gingival recession and Clinical Attack Level (CAL) were evaluated. A significant reduction of all the studied variables was observed in the three examined groups even if in the group treated with propolis, after 6 weeks, the reduction, and therefore the improvement, was higher than SRP treatment alone and in the presence of 1% chlorhexidine gel. Finally, there were no significant differences in the reduction of PPD and CAL between the groups treated with chlorhexidine and the group treated with SRP alone. Compared to the other two groups, Subjects treated with 10.4 mg propolis showed a significant improvement in all four variables. This study shows that propolis could be used as a natural adjuvant in the treatment of periodontal disease.

Keywords: Propolis; Periodontitis; Polyphenols; Non-surgical Therapy

Introduction

Periodontitis is an oral disease characterized by loss of connective tissue and alveolar bone, development of periodontal pocket and related gingival bleeding [1]. Generally, dental plaque bacteria initiate periodontitis, but abnormal host defenses to bacterial pathogens may play important roles in its progression [1, 2]. As periodontitis is induced by dental biofilm (polymicrobial community) characterized by an inflammatory response mediated by the host's immune system that determines the loss of periodontal attachment [1], it can no longer be considered a simple bacterial infection but must be interpreted as a complex disease with multifactorial etiology [2]. Socransky et al. divided the microorganisms located in subgingival zones into five complexes [3]. One of them, the so-called "red complex", which includes *Tannerella forsythensis*, *Porphyromonas gingivalis* and *Treponema denticola*, is strongly associated with a greater depth of periodontal and/or peri-implant pockets and increased bleeding on probing.

Alveolar bone resorption causes the most serious, irreversible and in most cases chronic damages associated with periodontitis. Consequently, osteogenic and/or antiresorptive compounds are generally considered useful in the treatment of periodontitis [4, 5]. For example, bisphosphonates, supplementation with zinc, chitosan and metformin are employed in the field of dentistry due to their bioactive properties [4, 5]. Treatment of periodontal disease is also directed towards the removal of the subgingival microflora. Root debridment, scaling and root planing (SRP) are the most widely used therapeutic approach [6, 7]. However, complete mechanical removal in deep periodontal pockets is difficult to accomplish. When exclusively applied, It cannot eliminate the pathogenic microflora due to the localization of the bacteria in soft tissues or in areas not accessible to periodontal instruments [8]. Due to the complex ecosystem within the subgingival pocket, the combined use of mechanical instrumental therapy with antimicrobial agents has been proposed to reduce the need for surgical treatment of the periodontal pockets [9]. Local antimicrobial therapy has the advantage of providing good effective drug concentrations at the site of infection with minimal and low risk of bacterial resistance [8, 10]. Consequently, the clinical use of antibiotics and other antimicrobial agents, as adjuvants for the treatment of periodontitis, has been extensively studied [11-13]. There are currently several antimicrobials on the market, but the need to identify products without the side effects of synthetic drugs [14] is driving research towards natural remedies [15].

Particular attention has been paid to propolis [16-20] for its antibacterial [21], anti-inflammatory and antioxidant [22] activities. Propolis is a resinous substance collected by bees from the buds and bark of plants, in particular in Europe from poplar and birch. It is a substance of purely vegetable origin, even if the bees, after harvesting, process it by adding wax, pollen and enzymes produced by their own body, and use it in the construction, adaptation and protection of hives [16]. The composition of propolis is very complex and it is closely linked to its vegetable origin depending on the phytogeographic characteristics of the collection site and the season of collection [23-25]. Furthermore, the extraction methods and the different solvents used (ethanol, methanol and water) can vary the concentration of the components and influence the properties of propolis producing finished products with different chemical composition and bioactivity. It should be emphasized that propolis used for nutraceutical, medicinal and cosmetic purposes must not contain wood or metal fragments along with bee residues, parasites or harmful substances attributable to the environment and beekeeping practice, such as heavy metals and acaricides [26]. Propolis contains more than 300 different biocompounds that can be divide into main large groups [27-29] constituted of resins (~45-55%), wax and fatty acids (~25-35%), essential oils and volatile substances (~10%), and pollen (~5%). Particular mention deserves the group of flavonoids which are contained in large quantities in the resin of propolis (up to 20% of weight) [30]. They are endowed with a high chemical reactivity, anti-free radical action, anti-inflammatory, antithrombotic, vaso- and gastro-protective, and immunological activity. The bee modifies the structure of flavonoids originally present in plants by removing the sugars contained in the organic compounds thanks to the enzymes produced by their salivary glands [31]. The synergy of the action of the various components of propolis focus the attention not on a single compound but on the properties of its phytocomplexes.

Many studies have demonstrated the multiple biological activities of propolis [32-38] and highlighted its properties in the dental field [15, 39-41]. In fact, the antimicrobial activity of propolis against various periodontal pathogens has been largely demonstrated, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* and *Prevotella interme-*

dia [42, 43]. Moreover, propolis samples were observed to have a broad spectrum of antimicrobial activity against vancomycin- and methicillin-resistant *Streptococcus aureus* [21] and *Enterococcus faecium* [43]. The antimicrobial properties of propolis against oral pathogens are attributable to the flavonone pinocembrin the flavonol galangin and the phenethyl ester caffeic acid (CAPE) with the mechanism of action probably based on the inhibition of bacterial RNA-polymerase [42]. Finally, the antibacterial properties of propolis on periodontopathogenic bacteria have been confirmed by other authors [11, 42, 44, 45].

The aim of the study is to evaluate the efficacy of a standardized sample of propolis associated with the Scaling and Root Planing (SRP) treatment in comparison with an antiseptic of proven efficacy, 1% chlorhexidine gel, and with only SRP treatment, in patients with chronic periodontitis, by evaluating the clinical parameters. Due to the considerable variability of the different types of propolis used in the various studies available in the scientific literature, a propolis extract with standardized polyphenolic composition and content was used in this research.

Materials and Methods

Propolis sample

Propolis cannot be used as raw material but it is necessary to use particular extraction methods to purify the raw propolis from unwanted and inert material and to preserve active components such as polyphenolic compounds [46]. Many extraction methods can be found in the literature using different solvents such as water, ethanol, methanol, hexane, acetone and chloroform [47, 48]. However, the most common technique is ethanol extraction able to produce finished products with a low concentration of wax and a high content of bioactive compounds [49]. The Multi Dynamic Extraction (M.E.D.®) is a patented procedure [50] characterized by a variable alcohol content extraction capable of purifying all polyphenolic compounds having different solubility from the raw propolis. Consequently, M.E.D.® extracts are pure in inactive resins and rich in polyphenols. In particular, the brown propolis extracts are characterized by the presence of a biologically active polyphenolic complex identified in six main polyphenols (M.E.D.® fingerprint, galangin, chrysin, pinocembrin, apigenin, pinobanksin and quercetin) having a relative concentration always greater than 25% w/w [21]. In this work we used a hydroalcoholic extract (Sarandrea Marco & Co.) containing Propolis M.E.D.® obtained as previously described.

The composition and characterization of the polyphenols of the propolis sample used in the study were determined by HPLC (Jasco) interfaced with UV and MS detectors. To date, HPLC equipped with MS detector is the most suitable and reliable analytical tool for the identification and quantification of the active propolis biomolecules [47]. Propolis phenolic acids and flavonoids were separated using a 250 x 4.6 mm Discovery-C18 column (from Sigma-Aldrich). The chromatographic separation was carried out by means of 0.5% acetic acid and acetonitrile with a gradient from 0 min to 90 minutes from 50% of A/50% of B up to 100% B at a flow of 1.0 ml/min. The UV detector was set at 260 nm. The Agilent 1100 Series VL (Agilent Technologies, Inc.) mass spectrometer was further used online with HPLC. The electrospray interface was set in negative ionization mode with capillary voltage at 3,500 V and a temperature source of 350°C with full scan 200-2200 Da. 10 µl of sample was injected at a standardized concentration expressed as total content of polyphenols evaluated by a spectrophotometric assay according to Folin-Ciocalteau assay by using a calibration curve constructed with pure galangin.

Patients of the study

150 patients were recruited, 83 women and 67 men, aged between 25 and 76, in care at the "Fra Orsenigo" Odontostomatology Center (San Pietro Hospital, Rome, Italy). Outpatient services including the visit are part of the normal follow-up of patient management.

Patients aged 35-80 years diagnosed with chronic generalized periodontitis [51] having at least 20 teeth (excluding 3rd molars) and at least 1 element with depth of pocket at probing (PPD) > 5 mm and bleeding on positive probing (excluding 3rd molars) in

at least 2 quadrants were included into the study. Chronic generalized periodontitis was diagnosed by a first objective examination to verify the actual presence of periodontal disease with a study of the location of the periodontal bag, its depth, width and bone involvement. Then a periodontal chart is performed (including probes) followed by a complete intraoral x-ray and photos.

Subjects were excluded in the case of periodontal treatment in the last six months or presence of systemic diseases capable to influence therapy (diabetes mellitus, neoplasms, bone metabolism disorders, disorders that impair healing, radiation, immunosuppressive therapies, anticoagulant therapies). Moreover, possible patients were excluded in case of assumption of antibiotics in the last six months or anti-inflammatory drugs in the last three months, or in the presence of pregnancy or contraceptive hormone intake, psychological or physical limitations that may limit home oral hygiene, allergy to propolis or bee products, smoking (to avoid alcohol-smoking association) and lack of consent to the study.

Study Design

This was a single-center, controlled, randomized, double-blind clinical study carried out in accordance with the ethical standards established in the Declaration of Helsinki. The informed consent was obtained from all participants prior to their enrollment. The present study and related experimental protocol were approved by the ethic committee of the "Fra Orsenigo" Odontostomatology Center of San Pietro Hospital, Rome, Italy.

Three treatments were identified, one for each group of patients: 1) treatment A was SRP with ultrasound and standard Gracey curette and propolis; 2) treatment B was SRP with ultrasound and standard Gracey curette and chlorhexidine and 3) treatment C was SRP with ultrasound and standard Gracey curette.

Clinicians participating in the study operated independently of each other respecting a chronological sequence.

The first visit was carried out by a single examiner, during which medical history, consent and ERSE were collected. The following parameters were then recorded on a special periodontal pocket chart: full mouth plaque score (FMPS), pocket depth at probing (PPD), full mouth bleeding score (FMBS), clinical attachment level (CAL) by evaluating 6 points per tooth, impaired furcation according to Hamp classification, mobility and gingival recession. A CPC15 periodontal probe was used for the evaluation.

The treatment was carried out by two operators. All patients underwent oral hygiene instructions and motivation. The techniques and aids were recommended considering the gingival biotype, the presence of gingival recessions and/or abrasion of the enamel. The standard of oral hygiene was checked at each visit and further instructions were given if necessary. For the entire duration of the study, the use of other oral antimicrobial solutions was not allowed.

After evaluating only the periodontal pocket chart, the patient was then subjected to 1) supragingival ablation with EMS ultrasound insert A, and 2) root planing with Gracey Mini curette No: 3/4.7/8.11/12.13/14 (anesthesia at the patient's discretion) and EMS periodontal sonic inserts. According to the group, propolis sample or chlorhexidine was applied to periodontal pockets with PPD > 5 mm.

After polishing, the patient rinsed his mouth with plenty of water to remove traces of saliva and blood for about 20 sec. After washing the dental surfaces with the air/water jet spray, a dose of 0.2 ml of extract of propolis or 0.2 ml of chlorhexidine was applied, with the use of a disposable syringe, in the periodontal pockets. The product was applied starting from the bottom of the furcations for a usual time of 30 sec. At the end of the exposure time, the excess product was removed in the treated areas rinsing with physiological solution.

Re-evaluation was performed at 6 weeks. The examining clinician, without having access to the baseline data and the type of treatment, collected again all the values above reported.

Statistical analysis

Statistical analysis was conducted by using the SPSS statistical package. Descriptive analysis (frequency distribution) and t-test for categorical variables were performed.

Results

Sarandrea propolis shows the presence of many biologically active polyphenols formed of phenolic acids and bioflavonoids characterized by HPLC-MS (Figure 1A) and HPLC-UV (Figure 1B). Moreover, it has a biologically active polyphenolic complex composed of six main polyphenols formed of apigenin, chrysin, galangin, pinobanksin, pinocembrin and quercetin with a relative concentration of 27.8%, typical of MED Propolis [50]. 10.4 mg of total polyphenols are contained in the propolis dose, 0.2 ml used in this study, applied to each pocket.

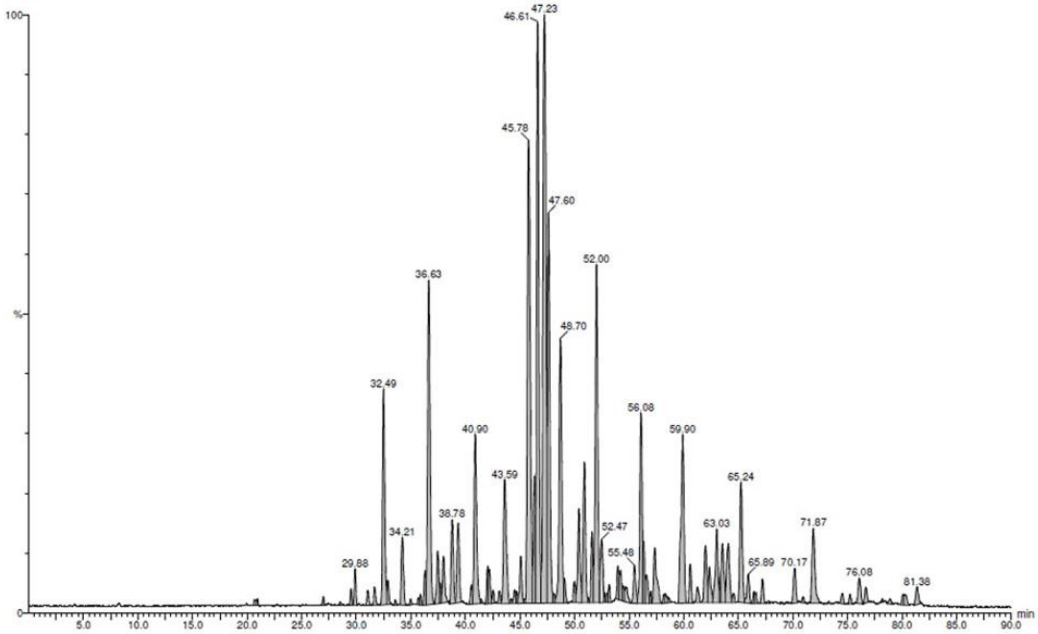


Figure 1A: HPLC profile of Sarandrea MED Propolis polyphenol species detected by single quadrupole mass detector

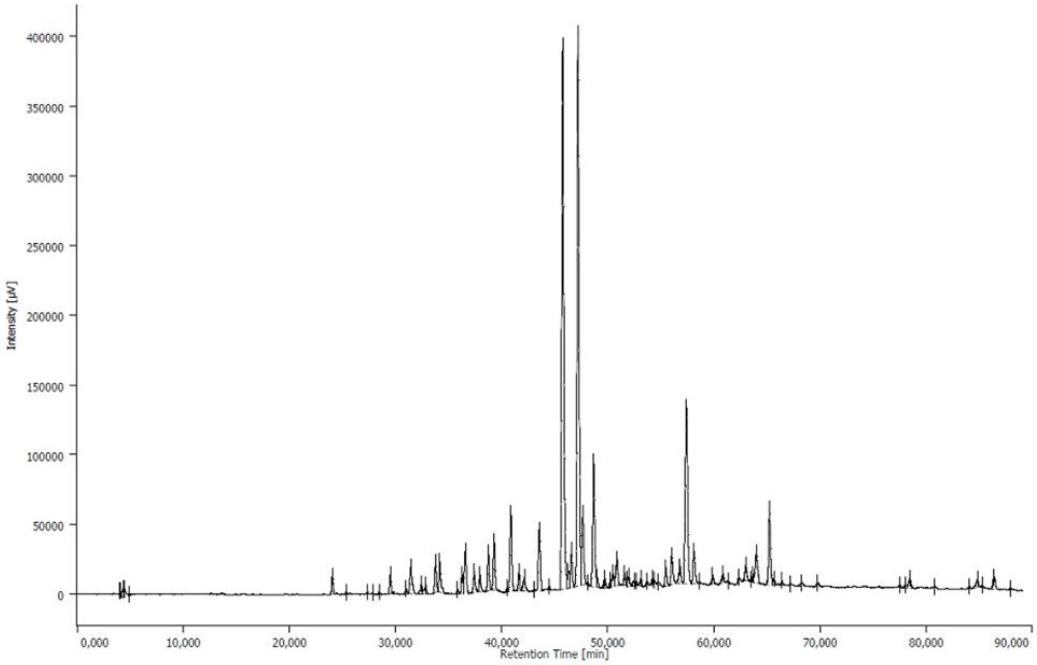


Figure 1B: HPLC chromatogram of Sarandrea MED Propolis polyphenol species obtained by UV detector

Three groups of fifty patients were used. Group A consisted of subjects subjected to SRP and propolis treatment, group B was related to SRP and chlorhexidine treatment and group C was submitted just to SRP treatment. Table 1 reports the characteristics of the participants to the three groups showing no significant differences. Moreover, no local allergic reactions, pain, swelling or other side effects were observed during the study.

	Group A	**Group B**	**Group C**
Women	32	26	25
Men	18	24	25
Middle age	56.25 ± 10.60	56.35 ± 12.23	55.55 ± 12.40
Former smokers	27	14	22
Stress	6.2 ± 2.01	6.8 ± 1.67	5.8 ± 1.80
Familiarity	20	20	12

Table 1: Characteristics of the groups under investigation

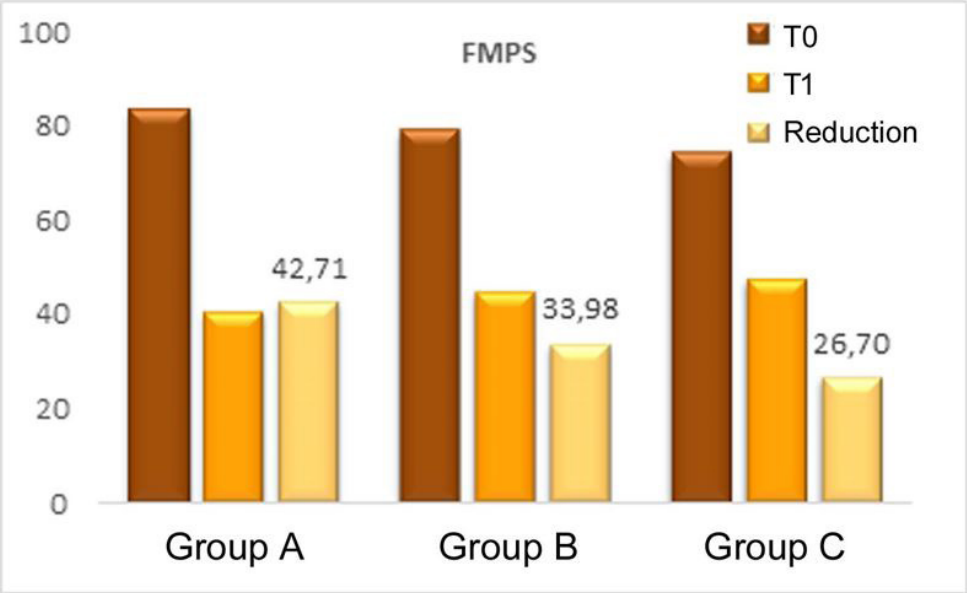
For each group, the values of FMPS, FMBS, PPD and CAL were measured before (time 0) and after treatment (time 1). The averages of the clinical parameters collected at T0 (Table 2) show that in the three groups the values of the FMPS and FMBS are well above the threshold values, respectively 20% and 10% [52], defined as success criteria and stability [53]. The presence of plaque represents a heavy risk factor, bacteria being the most important etiological factor of periodontal disease, while the presence of bleeding after standardized probing indicates the presence of gingival inflammation. Table 2 also reports the values of the investigated parameters after six weeks of treatment.

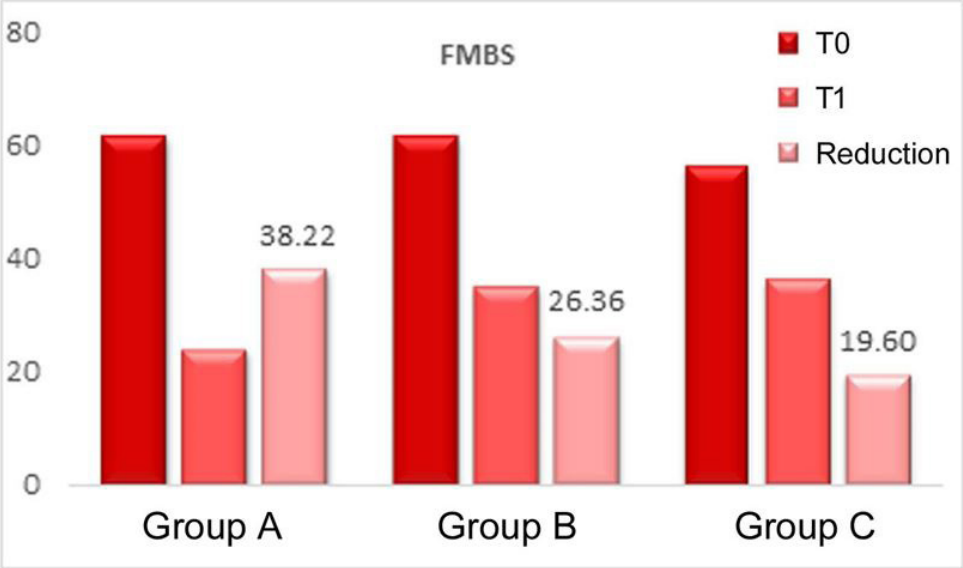
	Time 0			**Time 1**			**% of differences between T1 and T0**		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
FMPS	83.28	79.00	74.28	40.57	45.02	47.58	-51.3	-43.0	-35.9
FMBS	61.58	61.52	56.12	23.80	35.16	36.52	-61.4	-42.8	-34.9
PPD	4.03	3.86	3.76	3.08	3.13	3.11	-23.6	-18.9	-17.3
CAL	4.71	4.50	4.51	3.71	3.75	3.85	-21.2	-16.7	-14.6

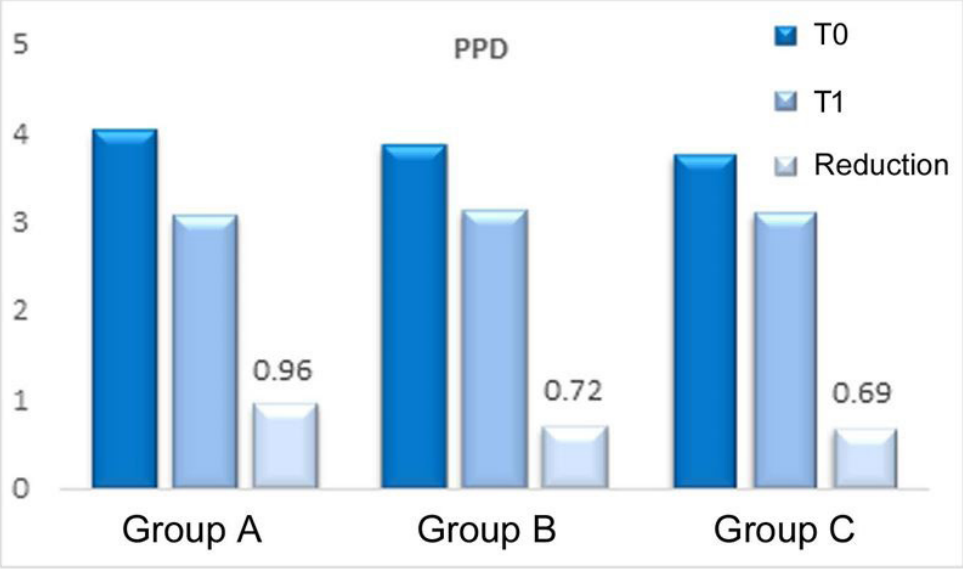
Table 2: Averages of the variables at T0 and T1

The values of the variables of the three groups at the initial time were found homogeneous with averages not significantly different. On the contrary, the differences between the values observed after six weeks of treatment compared to T0 were observed to be highly effective and significant, greater than $p<0.00001$ (not shown).

To verify the differences related to the efficacy of the various treatments, the differences of the means of Table 2 were tested through the analysis of variance to underline the differences in the averages between the different pairs of treatments and their ordering with respect to effectiveness. Based on the statistical evaluations, treatment A was found significantly higher than the other two while treatment B was significantly higher than C but only for the first two variables, FMPS and FMBS and not for PPD and CAL (Table 2 and Figure 2).


FMPS
T0
T1
Reduction
100
80
60
40
20
0
42,71
33,98
26,70
Group A
Group B
Group C


FMBS
T0
T1
Reduction
80
60
40
20
0
38.22
26.36
19.60
Group A
Group B
Group C


PPD
T0
T1
Reduction
5
4
3
2
1
0
0.96
0.72
0.69
Group A
Group B
Group C

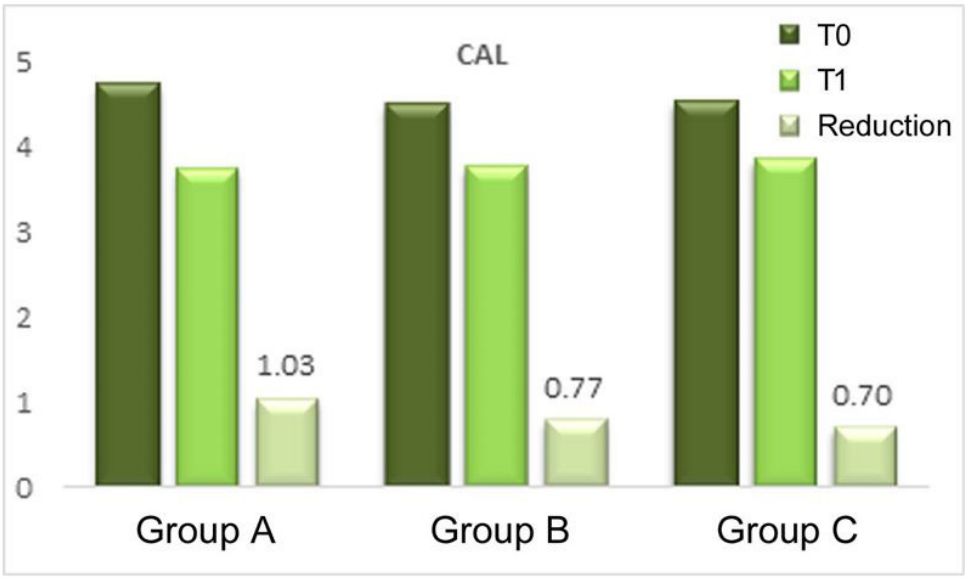


Figure 2: Progression of the variables and their absolute differences for the three tested groups

Figure 3A reports the clinical appearance of the tissues before the treatment with non-surgical periodontal therapy showing the presence of soft and mineralized deposits. Moreover, the clinical aspect of the tissues under reevaluation after 6 weeks of treatment A, consisting of SRP with ultrasound and standard Gracey curette and propolis, is illustrated in Figure 3B.

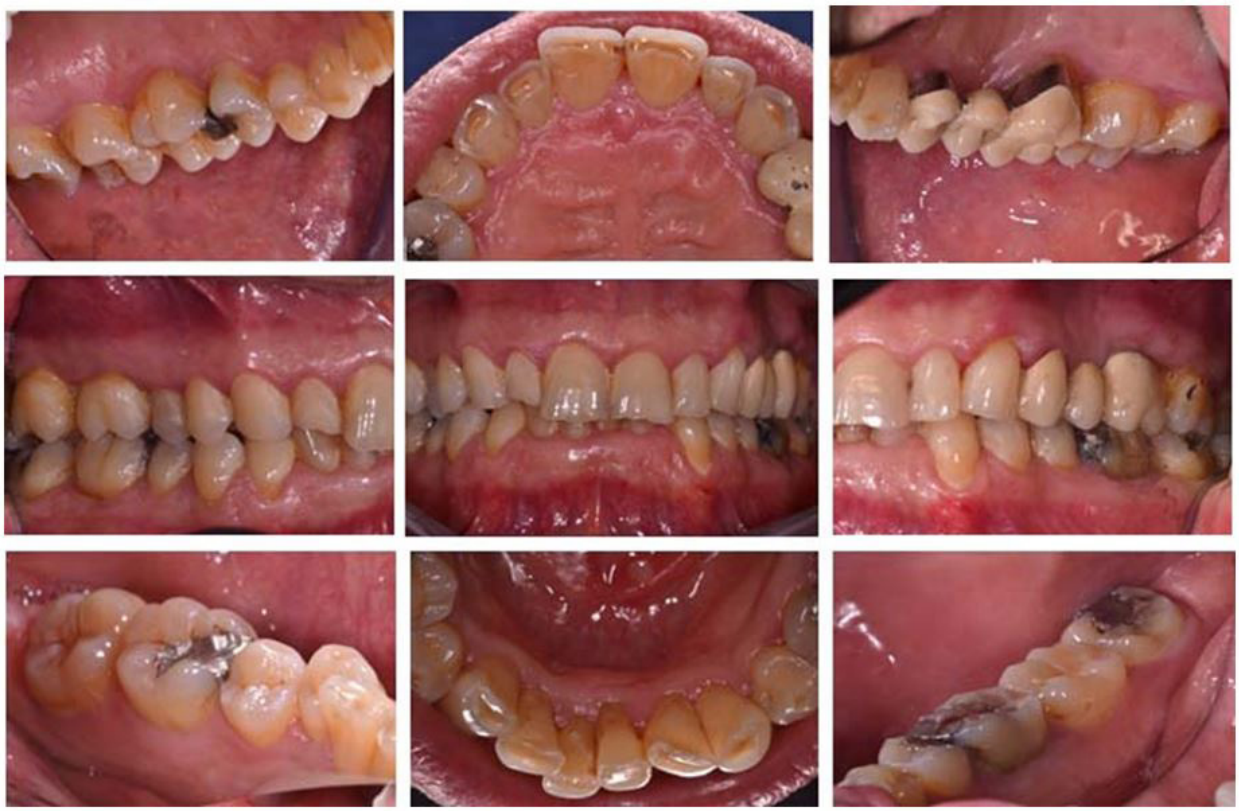


Figure 3A: Tissues before the treatment with non-surgical periodontal therapy

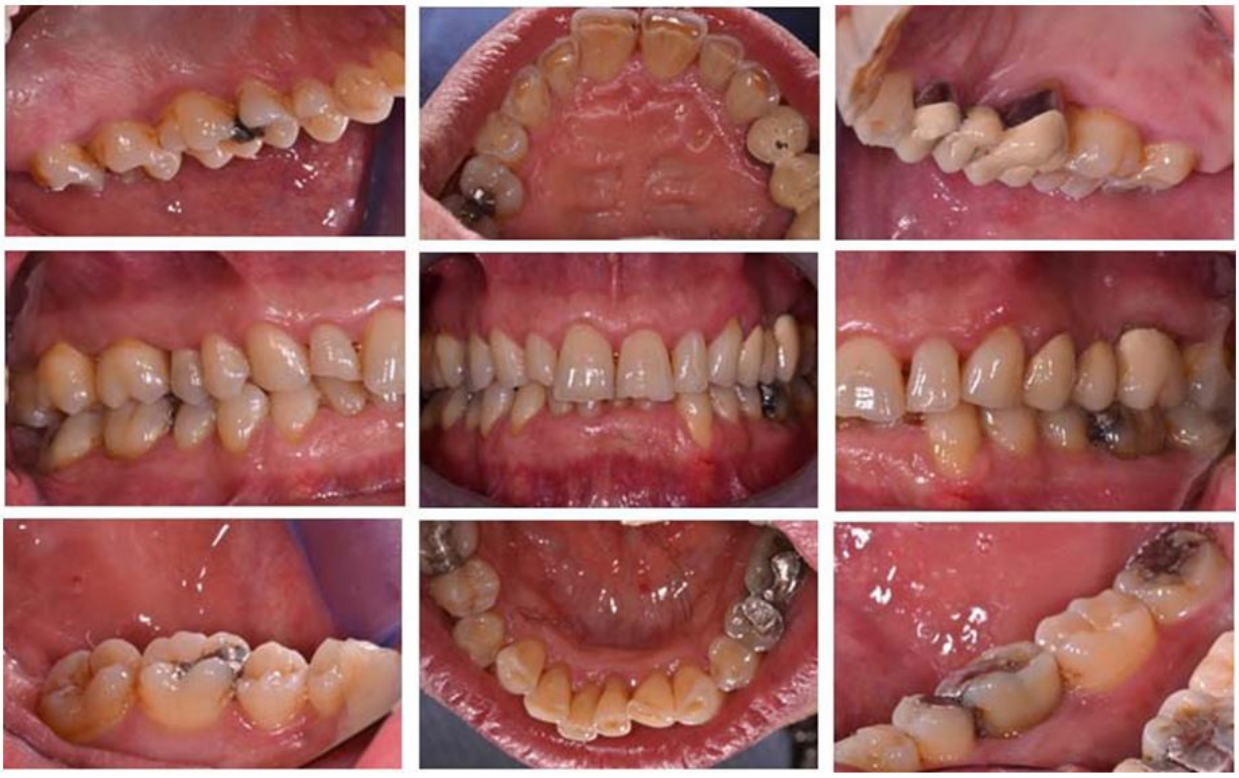


Figure 3B: Clinical aspect of the tissues after 6 weeks of treatment A, SRP with ultrasound, standard Gracey curette and propolis

Discussion

Periodontitis is an extremely widespread disease, representing the sixth disease in the world by incidence. It affects 45-50% of the population and within this percentage, 10-15% has a severe form. However, only 25% Italians are aware of being affected and know the consequences that it entails (https://www.sidp.it/media-download/ta2lvlg.pdf?v=19052020225823). Propolis is a natural and non-toxic hive product, and its content in flavonoids and polyphenols with antimicrobial, anti-inflammatory and antioxidant activity could prevent the progression of the disease. Based on this largely known capacity, the present study aimed to demonstrate the effectiveness of propolis in the non-surgical treatment of periodontitis. A significant reduction of all the considered variables was observed in the three examined groups even if in the group treated with propolis, after 6 weeks, the reduction, and therefore the improvement, was higher than SRP treatment alone and in the presence of 1% chlorhexidine gel. In fact, compared to the other two groups, Subjects treated with propolis showed a significant improvement in all four variables. On the other hand, there were no significant differences in the reduction of PPD and CAL between the groups treated with chlorhexidine and the group treated with SRP alone.

The results of the present study are consistent with some of the previous studies [54]. These effects are probably related to the anti-inflammatory and antibacterial effects of propolis, specifically on orange-complex bacteria [55, 56]. The reduction of PPD, bleeding and plaque indices in the treatment with propolis were also highlighted in the study by Cutler et al, which showed how the application of propolis also reduces interleukin-1 beta (IL-1beta) levels up to 7 days and prostaglandin-E2 (PGE) up to 14 days [57]. The effectiveness of propolis in the treatment of periodontal disease is also confirmed by recent reviews with meta-analysis. In a study of 2021 [58], randomized trials and non-randomized clinical studies were systematically reviewed and 224 studies were detected. The main conclusions of this analysis was that propolis is safe and can improve the periodontal disease treatment reducing probing pocket depth compared with treatment with a placebo. Another review [59] confirmed that all analyzed studies related to the use of propolis have shown a potentially safe antimicrobial agent in dentistry. A further review focused the attention of polyphenols in oral health able to strengthen the dental enamel, to decrease the development of dental plaque formation, to inhibit the progression of dental caries and development of dental pathogens and to show anti-inflammatory properties [60].

Conclusion

Our study demonstrated the significant reduction of FMPS, FMBS, PPD and CAL in the group affected by periodontitis treated with Scaling Root Planing (SRP) and a standardized and chemically characterized propolis sample containing 10.4 mg of total polyphenols compared to groups treated with SRP only and SRP with Chlorhexidine. It is desirable that propolis due to its characteristics, convenience, easy availability and therapeutic properties becomes an alternative treatment option for periodontitis during non-surgical periodontal therapy. The use of standardized and characterized propolis extracts also ensure the correlation between polyphenol dose and activity and its effectiveness.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

SC and BM were responsible of the clinical treatment and evaluation. GM was a supervisor of the clinical study. CA and ZV were external consultants. GF and VN performed the propolis analysis. VN wrote the paper. All authors revised the paper and approved the final manuscript.

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