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ADJUNCTIVE EFFECT OF PROPOLIS GEL TO NON-SURGICAL STAGE II PERIODONTITIS THERAPY: A CLINICAL AND IMMUNOLOGICAL STUDY

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KEYWORDS

Periodontitis, non-surgical, propolis gel, (IL1, IL6).

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ABSTRACT

Introduction: Periodontitis affects more than 1/2 of adults over the age of 30. Scaling and root planning is a non-surgical, gold standard that determines important objectives to control bacterial infections and reduce inflammation related to periodontal plaque. In recent years, natural products have become increasingly popular; Propolis is a natural material produced by bees and effective in treating periodontal disease. **Aim:** This study was designed to evaluate the effect of propolis gel as an adjunctive non-surgical treatment on improving clinical parameters (PI, GI) and reducing pro-inflammatory mediator (IL1, IL6) levels in stage II periodontitis. **Methodology:** Sixty patients with stage II periodontitis will be selected from the out- patient clinic of Oral Medicine and Periodontology Department, Faculty ofDentistry, Suez Canal University. Moreover, it will be divided randomly into two equal groups. Control group: will receive scaling and root planning only. Test Group: will receive scaling and root planning and propolis gel adjunctive to non-surgical therapy. At baseline, 1 month and 3 months and for each group clinical parameters (PI, GI) assessed and proinflammatory mediators (IL1, IL6). **Results** in the intergroup comparison of IL1 and IL6 levels at different time intervals, the propolis group showed a statistically significant decrease in IL1 and IL6 levels compared to the SRP. The mean IL-1 was lower in the study group than in the control group after 1 month and 3 months. (p-value). In the intergroup comparison of clinical parameters (Gingival index, plaque index) at different time intervals there is no statistically significant difference. **Conclusion:** Subgingival delivery of propolis gel showed enhanced results as an adjunct to SRP in patients with stage II periodontitis as assessed by clinical and biochemical parameters.

INTRODUCTION

Periodontitis is an inflammatory disease of the tissues that support teeth caused by certain microorganisms. Over time, it destroys the periodontal ligament and alveolar bone, forming pockets and bone loss. In chronic periodontal disease, biologically active substances in bacterial plaque trigger an inflammatory response in the gingival soft tissues and periodontium [(1).]

The primary key of periodontal treatment is to remove pathogenic bacteria, correct reversible risk factors and prevent recolonization to

avoid disease recurrence. The standard non-surgical treatment for periodontal disease is scaling and root planning (SRP) [2].

Antibiotics and other antimicrobial medicines are frequently used in clinical settings as adjuvants to treat periodontitis. Recently, natural remedies have drawn a lot of interest. Numerous [3] studies have demonstrated the abundance of physiologically active substances found in herbal products, essential oils, and purified phytochemicals.in developing nations, almost 80% of people still receive their medical treatment through traditional means. The use of traditional botanicals to treat periodontal diseases has been the subject of immune reports [4].

Natural products, as one application of complementary and alternative medical therapies (CAM), provide a natural and cost-effective intervention to alter the course of many chronic diseases and may aid in regenerating various living tissues [5].

One of these natural products is propolis, a non-toxic resin produced by bees that has antibacterial, antifungal, anti-inflammatory, antioxidant, and antitumor properties, which have attracted the attention of medical and dental researchers. Honey bees collect propolis, a complex natural resinous material, from plant, bud, and bark exudates, mixing it with their hypopharyngeal gland secretion, beeswax, and pollen. Propolis chemical composition varies according to its source [6].

The various and wide-ranging impacts of propolis on oral health have resulted in its application in treating periodontal diseases. Using propolis extracts for subgingival irrigation during periodontal therapy has demonstrated superior outcomes compared to root planing and scaling. Additionally, propolis extracts are advantageous for periodontal diseases when applied in gingival pockets. Research focusing on histological and morphological aspects revealed that regular use of propolis helps prevent additional bone loss in periodontal conditions in rats [7].

Also found that using propolis extracts in the lab had antimicrobial effects not only against bacteria that cause gingival disease (Capnocytophaga gingivalis, Prevotella intermedia, Fusobacterium nucleatum, and Porphyromonas gingivalis), but also against bacteria that cause upper respiratory infections (Staphylococcus aureus, Escherichia coli, and candida albicans [8].

By examining the impact of propolis gel as an adjuvant to SRP on enhancing clinical parameters and lowering pro-inflammatory mediator levels in stage II periodontitis, which reflects the clinical outcomes, this study aims to help clarify this issue.

MATERIALS AND METHODS

The study was conducted on sixty patients with stage II periodontitis with grade B attending the periodontology clinic, Faculty of Dentistry Suez Canal University. Ethical consideration regarding patient well-being and confidentiality were undertaken and informed written consents were signed by the patients before commencing the study explaining all clinical examinations, procedures, and follow-up after approval of the Research Ethics Committee (REC) (approval no. 428/2021), faculty of dentistry, Suez Canal University.

Sample size calculation

The sample size for this study was calculated according to Charan and Biswas (2013) using the following equation:

$$N = \frac{(Z\alpha)^2 * (S)^2}{(d)^2}$$

N = Total sample size.

Z_α = Is Standard normal variate and is it equal 1.96 at P< 0.05.

SD= Standard deviation of variables.

d=Absolute error and precision.

Zα	SD	d
1.96	7.90	2

Total sample size N=

$$\frac{(1.96)^2 x (7.90)^2}{(2)^2} = 59.39 \approx 60 \text{ samples.}$$

The total sample size calculations revealed that a sample size should be **60** samples.

Groups	Descriptive	No. of samples
Group I	Control group	**30**
Group II	Study groups	**30**
Total samples		**60**

propolis was purchased form Emtnan. Egypt. ELISA Kit was imported from Sigma-Aldrich®, USA. Carbopol 940 polymer purchased form Al-Gomhoria® Company for chemicals, Egypt.

Preparation of propolis gel formulation

Propolis extract (PE) was made using the maceration procedure and included a propolis-to-ethanol ratio of 1/15 (weight to weight). The sample was allowed to macerate for 72 hours at room temperature while remaining in the dark. After filtering through the Whatman No. 4 filter paper, the filtrate was then evaporated at 50 degrees using a rotary evaporator (automatic 24/7 evaporation, Heidolph, Ger(4many). To create the propolis mucoadhesive gel, Carbopol 940 polymer was employed. The amount of concentrated extract in gel base is (4% W/V) and the ratio of propolis to polymer is 1:1[9].

Preoperative procedures:

The recorded clinical parameters are:

Occlusal stent was fabricated for each patient. A mouth impression was made using irreversible hydrocolloid material (alginate), A stent was then fabricated in the area of interest, covering the occlusal surface of the tooth being treated and the occlusal surfaces of One mesial and one distal stent were extended to cover the coronal third of the teeth involved. Grooves were placed on the occlusal stent using a periodontal probe before the treatment to compare tissue changes.

For each group, the clinical parameters (plaque index, gingival index were recorded at baseline before SRP, and then again at one month and three months.

Patients grouping

All patients were given oral hygiene instructions including plaque control measures and were instructed not to use any type of chemical plaque control. included thirty patients were treated with nonsurgical therapy (scaling and root planning) only. The second group (the tested group) included thirty patients with scaling and root planning and application of propolis gel inside the pocket after complete isolation, using a blunt syringe [9]. Finally, the clinical parameters (plaque index, gingival index were recorded for both groups immediately before the treatment (at baseline) and after 1 month and 3 months.

Gingival crevicular fluid (GCF) sampling

After recording the clinical parameters, the area was isolated with cotton rolls, and gingival crevicular fluid was collected by paper point size (30) from the pocket and kept in place for 30 seconds. Then, the levels of (IL1, IL6) were measured using human ELISA kit (Abcam, UK) according to manufacturer instructions. Briefly, 50 μL of standards or samples were added to appropriate wells. Then, a 50 μL antibody cocktail (capture and detector antibodies) was added to all wells and preserved at room temperature it will be investigated by ELISA technique to assess the level of (IL1, IL6). After taking the needed amounts from Gingival crevicular fluid samples by paper point size (30) for (IL1, IL6) measurements.

Statistical analysis

All results were collected, tabulated and statistically analyzed using statistics software (SPSS version 26, IBM, USA).

P-value was considered significant when it was ≤0.05 and highly significant when it was ≤ 0.001.

RESULTS

Interleukin- 1 (IL-1)

There was a statistically significant difference between the control and study groups regarding IL-1 after 1 month or 3 months. Between zero and one month, there is a significant difference p= (0.02) and a highly significant difference between 1 month and three months p= (0.005).by Friedman's test. (Fig. 1, table 1)

Table (1) *Comparison between the control and study groups regarding the value of IL1 measured at baseline , one month and 3 months.*

Time	IL-1		p-value
	Control Group (N=30)	Study group (N=30)	
Baseline (Mean ±SD)	4.95 ± 1.14	4.46± 1.10	**0.15ns**
1 month (Mean ±SD)	4.92 ± 1.22	3.85 ± 1.19 #	**0.02***
3 months (Mean ±SD)	4.8 ± 1.3	3.29 ± 1.18 #	**0.005***
p-value	**<0.241**	**<0.001***	

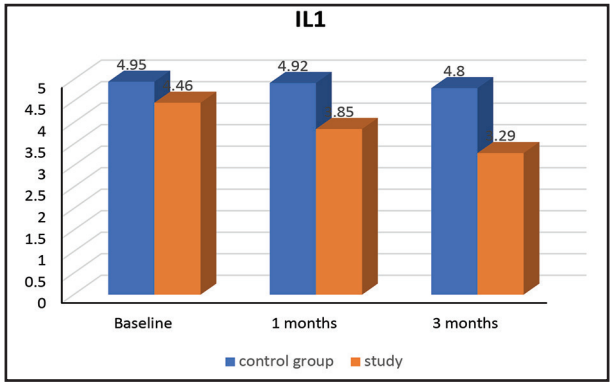

Fig. (1) Shows different intervals of IL-1 for both groups.

Interleukin -6 (IL-6)

There was a statistically significant difference between the control and study group regarding IL-6 either after 1 month or 3 months, as the mean IL-6 was lower in the study group than the control group after 1 month and 3 months. (P-value <0.05). Between baseline and 1 month, there is a significant difference P= (0.01), and 1 month and three months a highly significant difference P= (0.001). (Fig. 2, table 2)

Table (2) *Comparison between the control and study groups regarding IL-6 measured at baseline, 1 month, and 3 months.*

Time	IL-6		p-value
	Control Group (N=30)	Study group (N=30)	
Baseline (Mean ±SD)	5 ± 0.09	4.66± 1.09	**0.35ns**
1 month (Mean ±SD)	4.9 ± 1	4 ± 0.95 #	**0.01***
3 months (Mean ±SD)	4.79 ± 0.87	3.44 ± 0.87 #	**0.001***
p-value	**<0.052***	**<0.001***	

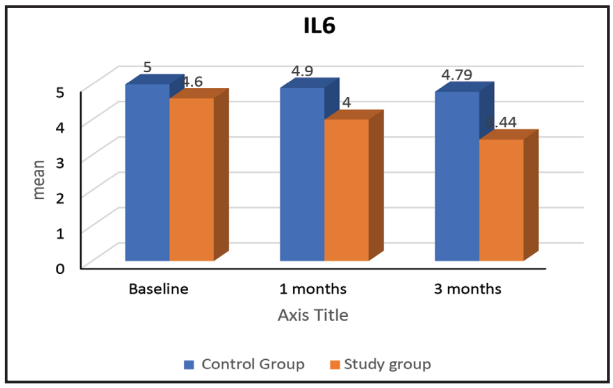

Fig. (2) Shows the bar chart showing the mean IL-6 for the study and control groups.

Clinical assessment values:

1. Plaque index:

The mean and standard deviation (SD) for plaque index for the control and study groups were presented in Table (3) and Figures (3).

A- Intragroup comparisons:

The plaque index (PI) in the control group recorded an average of 2.7± 0.45, 1.86±0.51, and 1.66±0.48 at 0, 1, and 3 months, respectively, which showed highly significant differences within the control group (intragroup difference) as revealed by Friedman's test. (Table 3, Figure 3)

PI in the study group recorded an average of 2.5±0.51, 1.87±0.80, and 1.5±0.51 at 0, 1, and 3 months, respectively, which showed a high significant difference within the study group (intragroup difference) as revealed by Friedman's test.

Table (3) *Comparison between the control and study groups regarding plaque index measured at baseline, 1 month, and 3 months.*

Time	Plaque index		p-value
	Control Group(N=30)	Study group (N=30)	
Baseline (Mean ±SD)	2.7± 0.45	2.5±0.51	**0.53ns**
1 month (Mean ±SD)	1.86±0.51 #	1.87±0.80 #	**0.59ns**
3 months (Mean ±SD)	1.66±0.48 #	1.5±0.51 #	**0.53ns**
p-value	**<0.001***	**<0.001***	

**; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$), # significant difference ($p \leq 0.05$) with baseline.*

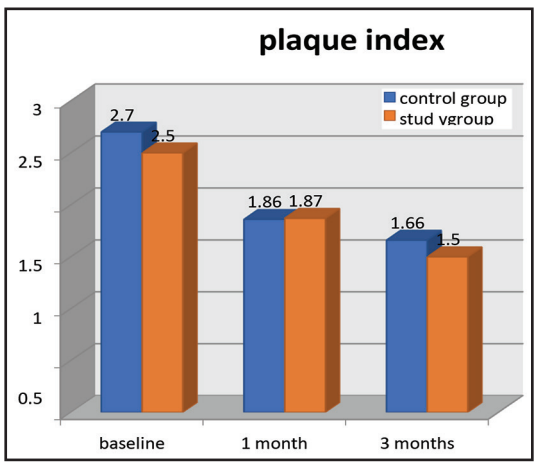

Fig. (3) Bar chart showing the mean plaque index for the study and control groups.

B-Intergroup comparisons:

As shown in Table (3) and Figure (3), there was no statistically significant difference between the control and study groups regarding plaque index at baseline, 1 month, or 3 months.

2- Gingival index:

The mean and standard deviation (SD) for the gingival index for control and study groups were presented in Table (4) and Figures (4)

A-Intragroup comparisons:

The data of the gingival index (GI) in the control group are collected in Table (4), and figures (4) recorded an average of 1.93 ± 0.25 at baseline, 1.73 ±1.02 within 1 month, and 1.26 ±0.45 within 3 months, which showed a markedly significant difference within the control group (intragroup difference) as revealed by Friedman's test.

As shown in Table (4), the gingival index (GI) in the study group recorded an average of 1.85± 0.53, 1.57±0.64, and 0.92±0.47 at 0, 1, and 3 months, respectively, which showed highly significant differences within the study group as revealed by Friedman's test.

B- Intergroup comparisons:

There was no statistically significant difference between the control and study groups regarding gingival index at baseline, 1 month, or 3 months.

Table (4) *Comparison between the control and study groups regarding gingival index measured at baseline, 1 month, and 3 months.*

Time	Gingival index		p-value
	Control Group (N=30)	Study group ((N=30)	
Baseline (Mean ±SD)	1.93 ± 0.25	1.85± 0.53	**0.53ns**
1 month (Mean ±SD)	1.73 ±1.02	1.57 ±0.64	**0.776ns**
3 months (Mean ±SD)	1.26 ±0.45	0.92 ±0.47 #	**0.174ns**
p-value	**0.001***	**<0.001***	

**; significant (p ≤ 0.05) ns; non-significant (p>0.05),# significant difference (p ≤ 0.05) with baseline.*

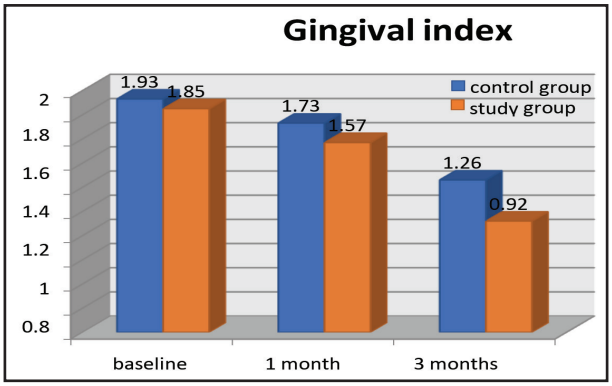

Fig. (4) Shows a bar chart showing the mean gingival index for the study and control group.

DISCUSSION

Periodontitis is a chronic inflammatory of the tissue of infection origin that, if improperly treated, can destroy the periodontal tissue and ultimately tooth loss [10].

Recent research shows that oral health and disease are characterized by complex microbiological interactions between bacterial origins and environmental influences, systemic factors, host

genetic factors, drug treatments, and the host immune system [11].

Mechanical cleaning is the biofilm and reducing the bacteria. However, mechanical instrumentation may sometimes insufficient to control the disease due to tissue-invasive pathogens or other tooth-related anatomic factors [12].

Various non-chemical alternative products, such as lasers, topical and systemic antibiotics like azithromycin, and chemicals such as hyaluronic acid, antiseptics, and anti-inflammatory agents with positive effects on the immune system, are reported to be beneficial. Due to the fewer side effects of medicinal herbs such as green tea, Cordia Verbenaceae (a native plant of the Brazilian coasts), Mikania levigate, and aloe vera and propolis, in combination with SRP, can be beneficial in periodontitis treatment [13].

Propolis is a potent antioxidant and is used in the management of different systemic conditions [14]. It has been used extensively in medicine for centuries, with antibacterial, antiviral, antifungal, antitumor, and immunomodulatory effects [14].

The present study was designed as a controlled clinical trial to study the effect of propolis gel as an adjunctive to non-surgical treatment on improving clinical parameters and reducing pro-inflammatory mediators (IL1, IL6) levels in stage II periodontitis.

Patients with stage II periodontitis were selected because those cases are usually responsive to non-surgical periodontal therapy [15].

The participants were divided into two groups. Group I was treated with SRP only, and group II was treated with SRP combined with local delivery of propolis gel.

The primary clinical outcome measures were changed in gingival index, plaque index, and Inflammatory mediators (IL1, IL6) from GCF sample.

The present study showed that mean PI & GI scores were improved in both groups however, there was no statistically significant difference in the two groups at 1 and 3 months compared to the baseline. This could be attributed to the proper, meticulous local debridement consisting of scaling and root planning at the sites and propolis marked anti-inflammatory and antimicrobial effects.

The present findings were consistent with a study by **Seth *et al.,*** [16] who concluded both PI and GI significant improvement with adjuvant therapy from the initiation of treatment to the 90-day time point; while between-groups; differences there were not statistically significant.

These results were in parallel with the study by **Sukmawati *et al.,*** [17] interleukin-1β concentration was measured using an ELISA assay kit. IL-1β was analyzed statistically, and there were significant differences between the reduction values. On curettage + 10% propolis group (Group A) and curettage + 1% tetracycline group (Group B) with ($P< 0.05$).

These results were in parallel with the study by **Park *et al* [18],** on immunological parameters are illustrated. Crevicular IL-6 showed a significant reduction in the test group (which received propolis as irrigation with SRPP) between baseline and eight weeks ($p = 0.006$).

In contrast, the present study was inconsistent with **Ö Ebrem *et al*** [19]. As a result of the statistical analysis, non-statistically significant decreases were determined in IL-1, IL-6, and TNF-α levels in the groups that were applied a mucoadhesive gel containing propolis in experimentally induced.

However, Still, more improvements were seen in test group sites in the level inflammatory mediators, based on the present study findings, propolis gel demonstrated promising outcomes in decreasing the level of proinflammatory mediators (IL1, IL6).

CONCLUSION

In comparison to the control group, the propolis gel-treated group showed better immunological outcomes. more decrease in level of proinflammatory mediators (IL1, IL6) in study group.

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