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Reearch Article

The Effect of Honeybee Propolis Solution as An Adjunct to Scaling and Root Planning, In Patients with Chronic Periodontitis: A Clinico-Microbiological Study.

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Abstract

Aim: This study was conducted to evaluate the clinical and microbiological effect of subgingival irrigation with honeybee propolis in patients with chronic periodontitis.

Material and Methods: A total of 20 systemically healthy subjects diagnosed with chronic periodontitis, with at least five teeth with periodontal pocket depths ranging from 4 - 6 mm were included in the study. At baseline all patients were examined to record the plaque index, gingival index, pocket probing depth, bleeding on probing, and clinical attachment levels. Two weeks later the study participants were divided into: Group I: Subgingival irrigation for two weeks with 4 ml 20% propolis hydroalcoholic solution twice a week. Group II: Scaling and root planning. Clinical and microbiological parameters were assessed after 4,6 and 8 weeks.

Results: There was no significant decrease in pocket depth at 4 weeks and 6 weeks follow up, whereas, after the irrigation procedures a significant decrease in clinical probing depth was observed at 8 weeks in group A ($p < 0.05$) when compared to group B. At week 6 and week 8, the decrease in total viable counts of anaerobic bacteria was seen in sites from group I as compared to sites from group II ($p < 0.05$). Similarly, the decrease in the proportion of sites with high levels of porphyromonas gingivalis (≤ 105 cfu/mL) was observed in sites from group I as compared to group II at 6 and week 8 weeks.

Conclusion: The results of this clinical study demonstrated the benefits provided by propolis solution and indicated that it should be considered for use as an adjunct to scaling and root planing in patients with chronic periodontitis.

Keywords: Honeybee Propolis; Root Planning; Chronic Periodontitis

Introduction

Periodontium is affected by periodontal diseases like gingivitis and periodontitis. If not controlled, periodontitis can lead to bone loss and loss of teeth. The treatment of periodontal disease is aimed primarily to reduce subgingival bacteria and eliminate the inflammation. [1] The conventional plaque control measures includes scaling and root planing, which might be associated with systemic use of antibiotics. In the last decade, the treatment has been redefined as the use of drug delivery systems in periodontal pockets is largely increased, which is advantageous for site specific drug delivery, prolonging and controlling the drug concentration. Recent advances in the field of alternative medicine have introduced various herbal products for the treatment of chronic periodontitis. Herbal extracts such as curcumin, honeybee propolis, aloevera, triphala etc. in various forms such as mouthwashes, gels, solutions have shown significant advantages over the chemical agents in the treatment of periodontal diseases.

Propolis is a natural resinous substance collected by honey bees from plant buds and bark exudates mixed with their hypopharyngeal gland secretions, beeswax, and pollen, which is sometimes called as bee glue [2].

The flavonoids in propolis have been shown to be anti-inflammatory and able to stimulate the formation of collagen [12].

Some specific effects of the aqueous extract of propolis have also been demonstrated, such as the inhibition of platelet aggregation, inhibition of prostaglandin biosynthesis *in vitro*. [4]

Few clinical studies have demonstrated subgingival irrigation with solutions containing propolis as an adjuvant in periodontal treatment [26].

Aim and Objective

The study was carried out on patients with chronic periodontitis to assess the clinical and microbiological effects of subgingival irrigation with honeybee propolis solution.

Materials and Methods

A total of 20 patients (12 females and 8 males, aged 25–57 years) diagnosed with chronic periodontitis, with at least five teeth with periodontal pocket depths 4-6mm who reported to the Department of Periodontics, Y.C.M.M. and R. D. F's Dental College, Ahmednagar India, were selected for the study. The study protocol was approved by the Ethical Committee of Y.C.M.M. and R.D F's Dental College, Ahmednagar India. Selected patients had given informed consent before start of the study.

Patients with good systemic health, chronic periodontitis, minimum of 20 natural teeth with at least one pocket per quadrant, probing depth (PD) between 4-5mm were included in the study. patients who had undergone subgingival instrumentation within 3 months, antibiotic therapy within 3 months prior to the start of the study, smokers, pregnant or nursing women, intolerance or allergy to honey products were excluded from the study.

Preparation of 20% propolis hydroalcoholic solution (study solution)

Propolis powder was obtained from 'Hitech natural products, New Delhi, India'. The propolis powder was sent to the Department of Biochemistry, Y.C.M.M. and R. D F's Dental College, Ahmednagar, India, for preparation of the study solutions. The propolis powder was mixed with 99.8% (v/v) ethanol in hermetically-sealed glass beakers at a proportion of 1 g of propolis powder to 3 ml of ethanol. Then at room temperature in darkness beakers were incubated for 1 week, with constant agitation. The resulting ethanol solutions were clarified by the process of centrifugation which was done at 7000 g for 60 s and the supernatants were collected and filtered through Whatman #4 filter paper. By evaporating to dryness under vacuum, ethanol-soluble components were collected. 20% (w/v) ethanol solution was obtained by re-dissolving extracts in pure ethanol. The final solution was stored in hermetically-sealed brown-glass vessels at room temperature. [1,5] Previous studies have proved that propolis extract can be kept stable for 6 months, maintaining its antimicrobial activity over this period [6].

Figure 1: Honey Bee Propolis Solution.

Procedure

At baseline all patients were examined in order to record the plaque index, [17] gingival index, [18] pocket probing depth, [19] bleeding upon probing,[19] and clinical attachment level [19]. Plaque was collected (sample 1) using sterile curette from the deepest portion of the pocket, and scaling and root planing of all teeth included in the study was done.

Two weeks later the study participants were divided as one of the following treatments:

Group I: Irrigation for two weeks with 4 mL 20% propolis hydroalcoholic solution two times a week. [Figure 2]

Group II: Ultrasonic scaling and root planning.

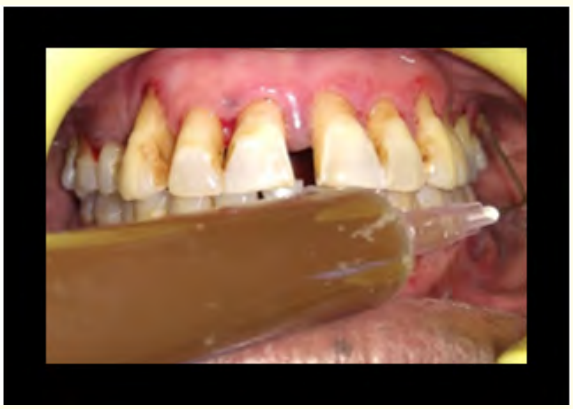


Figure 2: Subgingival Irrigation by Propolis Solution.

Subgingival plaque samples were collected after 4 weeks (sample II), 6 weeks (sample III), and 8 weeks (sample IV) after scaling and root planing procedure by the same method as performed at baseline. The samples were sent for anaerobic culturing to the Department of Microbiology, P.D.V.V.P. F's Medical College, Ahmednagar, India.

All twenty selected patients in study as well as control group were followed appointments till the end of the study.

Results

At baseline, there were no differences found between the two groups in both clinical or microbiological parameters.

The effect of treatment with subgingival irrigation with propolis solution in sites from group I was compared with clinical as well as microbiological data obtained from groups II by paired T test for equality of two population means.

When assessment of clinical parameters was done at week 4, week 6 and week 8, the reduction in the proportion of sites positive for bleeding on probing was significantly greater for group I ($P < 0.05$) than group II. There was a significant decrease in the clinical probing depth as well as sites positive for bleeding on probing even at 6 weeks, when the patients were recalled for follow-up from group I.

At 4 weeks and 6 weeks follow up there was no significant decrease in pocket depth, whereas, 8 weeks after the irrigation procedures a significant decrease in clinical probing depth was observed in group A ($P < 0.05$) when compared to group B.

At week 6 and week 8, decrease in the total viable counts of anaerobic bacteria was seen in sites from group I as compared to sites from group II ($P < 0.05$). Similarly, at week 6 and week 8 the decrease in the proportion of sites with high levels of P gingivalis (≤ 105 cfu/mL) was observed in sites from group I as compared to group II.

Discussion

The traditional therapy for periodontal disease includes scaling and root planing to disrupt the subgingival microflora [20]. Advances in technology have resulted in the introduction of a range of new methods for use in nonsurgical periodontal therapy, including machine-driven instruments, lasers, antimicrobial photodynamic therapy and local antimicrobial-delivery devices [29].

It has been shown that the local route of drug delivery can reach 100 times higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen. The reduction is observed in the total patient dose by over 400 times, hence reducing the potential problems by using systemic antibiotic drug regimens and development of antibiotic-resistant microbiota at non oral body sites [21].

In present study, one of the local drug delivery system, irrigation with honey bee propolis solution was compared between group A and group B.

It was observed that scaling and root planing effectively reduced the number of sites positive to bleeding upon probing, along with reduction in the pocket probing depth. More improvement in above parameters was recorded 4–8 weeks after the irrigation procedures in group A sites in comparison with the other sites. Studies done by Lembariti B.S., *et al* and Ludovico S., *et al* suggested that single session of scaling and root planing does not suffice in maintaining subgingival microbiota in accordance with health in patients with pocket probing depth ≥ 5 mm [24,25].

The decrease in the total feasible counts of anaerobic bacteria was observed after 2 weeks of the irrigation procedures for test group, and the reduction continued after 8 weeks of the irrigation procedures in the study.

Not only at the initial phase of the study but also after scaling and root planing procedures, the greater number of the evaluated sites presented high levels of P gingival is (>105 cfu/sample). The application of propolis extract in group A sites reduced the percentage from 80% to 50% of sites with high levels of P gingival is (>10^3 cfu/sample).

Since Wolff., *et al.* (1994) have reported that the total viable count between 10^3cfu/ml and 10^5cfu/mL are related to low numbers of periodontopathogen organisms, the presentation of microbiological parameters in terms of total viable count is of great clinical interest and the same has been expressed in the study.

Repopulation by periodontopathogen organisms usually occurs 4 - 8 weeks after scaling, [23] and it can influence the success of the therapy. There was reduction in the percentage of sites with high levels of P gingivalis, in the sites which were irrigated with propolis in group A; observation done at 2 weeks, 4 weeks, 6 weeks, and 8 weeks after propolis irrigation. The observation advocates that the effect of propolis irrigation might be perennial, affecting the repopulation process happening in the periodontal pocket.

Similarly, Morawiec., *et al.* (2015) demonstrated the antimicrobial effect of the extract of Brazilian green propolis used for maintaining hygiene after minor oral surgeries.

The use of propolis solution should be considered as complementary to scaling and root planing in patients with periodontitis as the antimicrobial and anti-inflammatory benefits are maintained even after 6 weeks of the inception of the treatment.

Some of the constituents of propolis are able to constrain cyclooxygenase and the resulting manufacture of prostaglandins. This mechanism of action is one of the many mechanisms highlighting the anti-inflammatory effect of propolis.

This complex mechanism of antimicrobial activity of propolis is caused by the coadjutant activity between phenolic and other compounds [27], chiefly by the flavonoids: pinocembrin, galangin, and pinobanksin [28].

de Freitas., *et al.* (2016) compared the treatment of periodontal disease with scaling and root planing associated with irrigation with 0.9% saline solution, chlorhexidine 0.1 and 0.5%, sodium hypochlorite and propolis extract 11% in rats. The results showed no differences between groups.

However, when Gebara., *et al.* (2003) and Coutinho (2012) evaluated clinical and microbiological parameters among patients, they found that irrigation with a hydroalcoholic solution of propolis extract 20% (w/v) as adjuvant to periodontal treatment was more effective than conventional mechanical treatment alone. These studies and present research utilized the same concentration of propolis, but they used four irrigation washes as compared to present study only one test group with irrigation by propolis solution and control group of SRP alone.

The results of this clinical study corroborate with the results of other studies and demonstrate that irrigation with a solution of propolis extract as an adjunct in periodontal treatment was more effective than SRP alone.

Since the present study has limitations in both clinical design and sample size, further randomized clinical trials using propolis must be conducted in oeder to evaluate possible clinical benefits of honey bee propolis in periodontal therapy, including trials using varying concentrations and frequencies of irrigation with honey bee propolis solution.

Conclusion

Concluding, the results of this clinical study demonstrate the benefits provided by propolis solution and indicate that it should be considered for use as an adjunct to scaling and root planing in patients with chronic periodontitis.

Figure 3: Anaerobic Gas Pack Used for Anaerobic Bacteria Culture.

Figure 4: Candle Jar Used During Anaerobic Bacteria Culture.

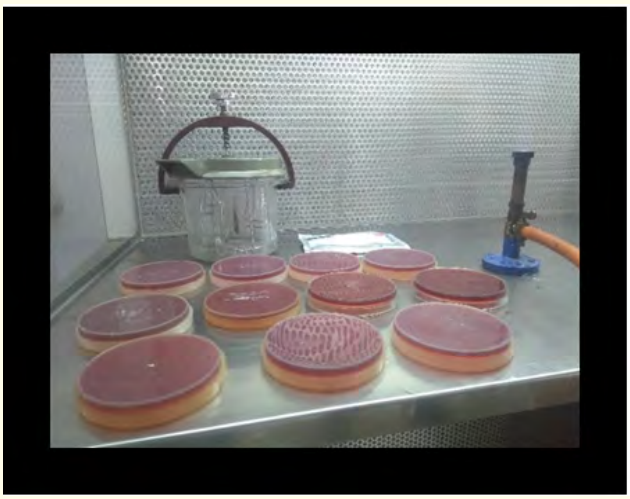


Figure 5: Blood Agar Culturing Plates.

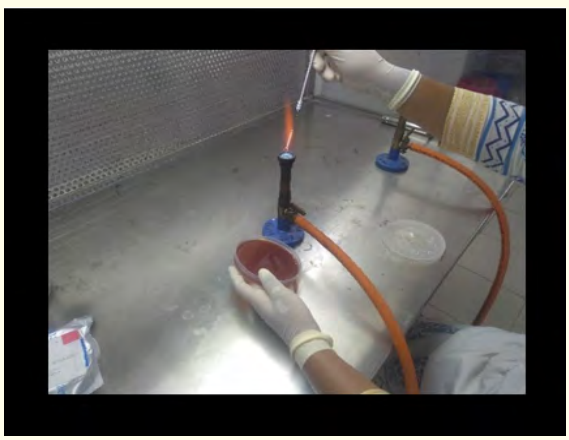


Figure 6: Streaking of Sample on Blood Agar Plate.

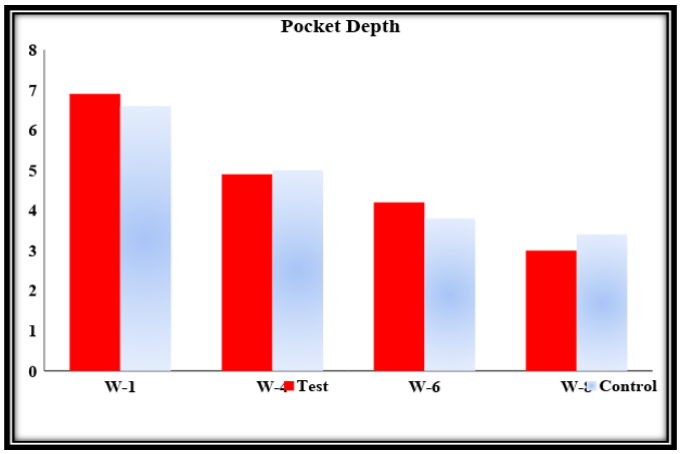
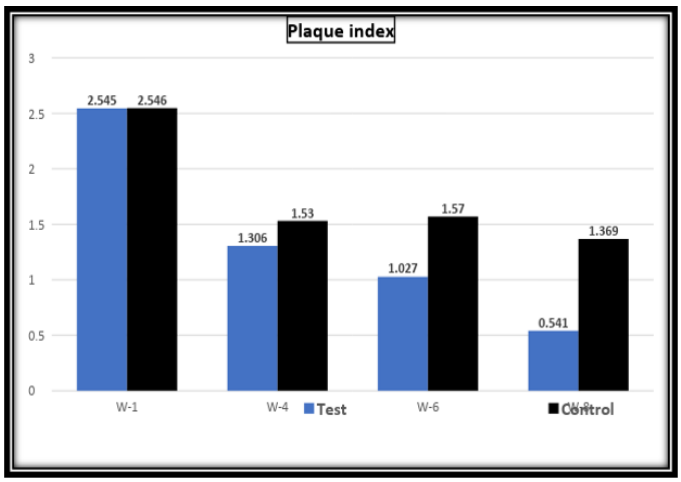


Table 1



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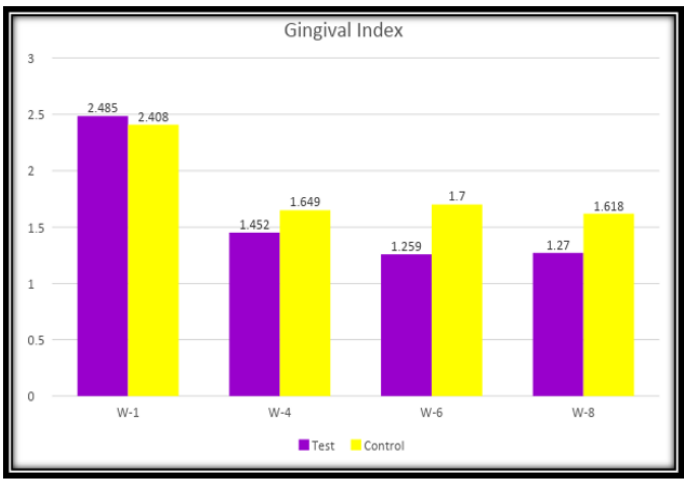

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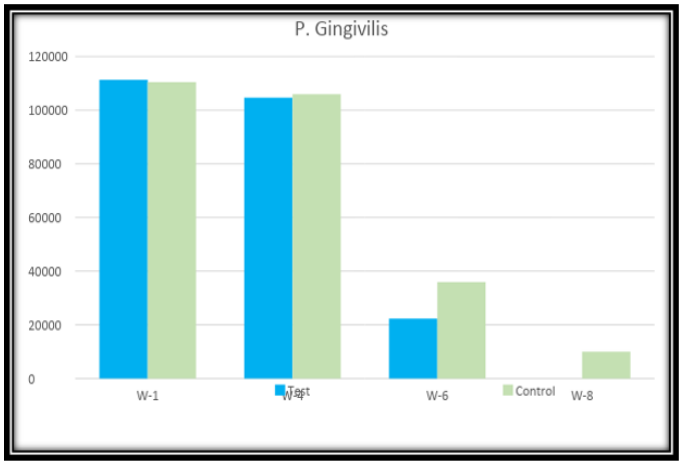

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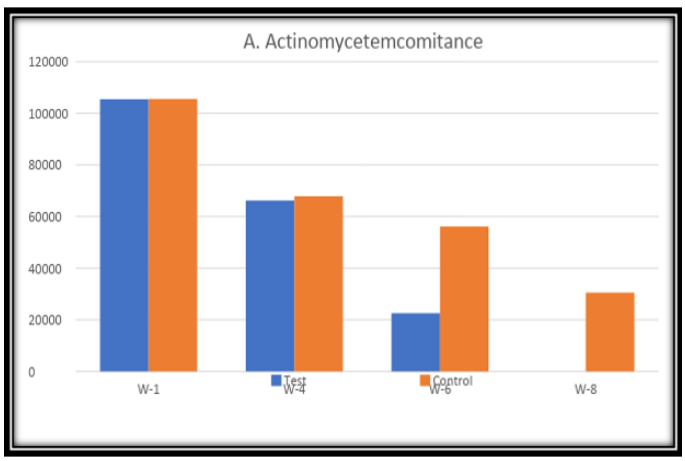

Table 5

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Volume 2 Issue 11 November 2018