Efficacy of Propolis as an Adjunct to Scaling and Root planing in patients with chronic periodontitis - placebo controlled study

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Abstract: Introduction: Propolis is one of the few natural remedies that have maintained its popularity over a long period of time. It's a natural antibiotic is a resinous substance that honey bees collect from tree buds, sap flow, shrubs and other botanic sources. Propolis is a subject of recent research in dentistry, since there is some evidence that propolis may actively protect against oral disease due to its antimicrobial, anti-inflammatory and host modulatory properties. The present research was undertaken to test the efficacy of propolis as an adjunct to SRP on clinical Parameters and cytokine levels in patients with chronic periodontitis. Methodology: 15 patients diagnosed with chronic generalized periodontitis were included in the study. Two quadrants with maximum number of sites with PPD measuring ≥ 6mm were selected from each patient. The clinical parameters like MGI, BOP, PPD, CAL were recorded for the selected quadrants in all teeth excluding third molars. 3µlitre of GCF were collected from a site with deepest probing pocket in each quadrant. Then the patients were subjected for full mouth SRP .Then each quadrant is randomly assigned for group-I and group-II. In test group, the quadrant will be subjected for Irrigation with 3mL of 30% Propolis solution per tooth on 0, 7, 15 & 21st day. In placebo group, the quadrant will be subjected for Irrigation with 3mL of distilled water per tooth on 0, 7th, 15th and 21st day. In both the groups clinical parameters and GCF collection was repeated after two months and three months. Results: Three months following nonsurgical therapy and sub-gingival irrigation of 30% propolis four times (once in every week), there was significant improvement in clinical parameters and immunological parameters i.e. IL-1β, IL-4. Conclusion: Sub gingival irrigation of 30% propolis as an adjunct to SRP is beneficial in reducing the periodontal inflammation and arresting the periodontal tissue destruction when followed up for 3 months. [Annaji S NJIRM 2017; 8(4):17-22]

Key words: Propolis, GCF, Inteleukin -1β, Interleukin - 4, Periodontal inflammation, Chronic periodontitis.

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Introduction: Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both.[1] Periodontal disease is a ubiquitous infection in humans displaying the classic hallmarks of the inflammatory response. Late sequel of periodontal diseases is the loss of alveolar bone, mobile teeth leading to a demolished periodontium. Therefore, earlier detection and treatment leads to improved outcomes for patients.[2]

The main transgressors in periodontal diseases are gram negative bacteria that elicit a host response, which cause an aftermath of osseous and soft tissue destruction. The host response plays the role of a second fiddle through mediators like proteinases, cytokines and prostaglandins causing severe tissue loss.[2] Cytokines are the linchpins in mediating numerous biological activities including proliferation, development, differentiation, homeostasis, regeneration, repair and inflammation.

Endothelial cells are also capable of releasing IL-1β, [3, 4, 5] in response to LPS and endotoxins. Furthermore, IL-Iβ can initiate its own gene expression in a variety of cells. Periodontal ligament and gingival fibroblasts challenged with IL-1β in vitro release PGE2 in a dose dependent manner and secrete collagenase and matrix metalloproteinase. IL-Iβ causes the levels of cyclooxygetiase-2 (COX-2) mRNA to escalate and may increase the stability of COX-2 in vivo. The higher tissue COX levels induced by IL-1β may in part explain the higher levels of arachidonic acid metabolite PGE2 consistently observed in the gingival crevicular fluid of patients with periodontitis. [6]

Interleukin-4 (IL-4) is a key player for the growth and proliferation of B lymphocytes.[7] It is secreted mainly by T-helper 2 cells and also by macrophages, monocytes, mast cells, basophils and non-immune cells such as fibroblasts and endothelial cells.IL-4 attributes it's name" T-cell derived B cell stimulatory factor" because of its stimulatory effect on B lymphocytes. It was found that IL-4 could induce apoptosis of macrophages. In periodontitis sites where IL-4 levels were diminished, there was

persistent agglomeration of activated macrophages, leading to carnage of the periodontium.[8] It has also been hypothesized that localized absence of IL-4 at the sites of gingival inflammation plays a crucial role in the progression of gingivitis to periodontitis.[9] Therefore IL-4 has an intrinsic role in the regulation of the immune inflammatory response.

Propolis is a resinous substance obtained from honey bees by the mixing of their hypo pharyngeal glands secretions with the digested product of resins that honey bees collect from tree buds, sap flow, shrubs and other botanic sources. It has antibiotic properties.[3] The pharmacologically active molecules in the Propolis are flavonoids and phenolic acids and their esters. Propolis has anti-fungal 4 action against Candidaalbicans[10,11] and anti-bacterial action against a range of oral micro organisms and viruses. It may be as effective as acyclovir against herpes simplex virus. It also has shown to have immune-modulatory activities.[12] In dentistry, Propolis has been used in dentifrices and as mouth rinse[13,14] possesses anti-microbial activity against streptococcus mutants present in the oral cavity. Subgingival irrigation with Propolis extract as an adjunct to periodontal treatment may also be more effective than scaling and root planing alone in reducing periodontal bacterial colonies.[15]

According to the literature lot of studies have been done to find out the antimicrobial effects of propolis in the dentistry but the action of Propolis on cytokines in GCF has never been studied, therefore the present study is undertaken to study the effect of propolis on periodontal tissue inflammation, destruction and healing by monitoring the interleukins IL-1β and IL- 4.

Methods: Source of Data: Fifteen patients who visited the Department of Periodontics, Coorg institute of Dental Sciences, Virajpet, who were diagnosed with generalized chronic periodontitis, ages ranging from 30-55 years were selected for the study and followed up to 3 months upon taking clearance from Institutional Ethical committee and consents from the patient.

Method of Collection of Data: Two quadrants from each patient with maximum number of sites with periodontal pocket depth measuring ≥ 6mm were selected.

The base line measurements for clinical parameters like Plaque Index (PI), Modified Gingival Index (MGI), BOP, and PPD, CAL will be recorded for the selected quadrants for all teeth excluding third molars. 3µlitre of GCF will be collected from a site with deepest probing pocket in each quadrant which was repeated after 2 months and 3 months post-operatively. All the patients will be subjected for full mouth SRP .Then each quadrant is randomly assigned for group-I and group-II. All participants were explained the need and objective of the study. Only those subjects who gave consent for the study were included in the study.

• PROPOLIS (GROUP I): In test group, the quadrant will be subjected for Irrigation with 3mL of 30% Propolis solution per tooth on 0,7th,15th and 21st day.

• PLACEBO (GROUP II): In placebo group, the quadrant will be subjected for Irrigation with 3mL of distilled water per tooth on 0, 7th, 15th and 21st day.

In both the groups clinical parameters and GCF collection will be repeated at two months and three months.

Inclusion Criteria:

- Patients diagnosed with chronic periodontitis and having good systemic health.
- Minimum six teeth in each quadrant.
- Minimum one tooth with pocket depth ≥ 6mm.

Exclusion Criteria: Systemic illness (diabetes mellitus, cancer, human immune deficiency syndrome, bone metabolic diseases, or disorders that compromise wound healing, radiation, or immunosuppressive therapy)

- Smoking.
- Pregnancy or lactation.
- Systemic antibiotics with in previous six months.
- Chronic use of NSAIDs.
- Periodontal therapy within a year.

Observations and Results: The study population consisted of 15 subjects, age ranging 35 – 53 years with 8 males and 7 females. This is a split mouth study design where two quadrants were selected and assigned randomly for one of the interventions in patients who are diagnosed with chronic periodontitis. Upon subjecting the patients for one of the interventions, the subjects were followed up for 3 months. The clinical parameters like PI, MGI, BOP, PPD

and CAL were measured before the intervention (baseline) and repeated at 2 months and 3 months post operatively.

The mean and standard deviation (S.D) were calculated for all the clinical parameters at each intervals and subjected for statistical analysis by keeping p – value < 0.05 as statistically significant. For intra-group comparison of variable "student paired t – test" was used and for comparison between the group "repeated test of ANNOVA" was used.

The mean scores of PI and MGI showed improvement after 2 months and 3 months in both the groups. There was a slight better improvement in propolis group compared to placebo group at both the intervals indicating an added benefit of propolis as an adjunct to Scaling and Root planing in controlling plaque and gingival inflammation.(Table -1)

The mean value of Pocket probing depth (PPD) in Propolis group at baseline was 5.867±0.192 which gradually decreased to 5.200±0.107 and 4.8±0.089 at two and three months respectively with p value < 0.05 which is statistically highly significant. In the placebo group the PPD at baseline 5.867±0.192 which gradually decreased to 5.200±0.107 and 5.067±0.089 after two and three months respectively with p value < 0.05 which was highly statistically significant when compared from baseline to 2 months and 3 months. (Table –1).

Similarly, the mean value of Clinical attachment level (CAL) in propolis group at baseline is 9.133±0.192 which gradually decreased to 8.667±0.126 and 8.267±0.159 at two and three months respectively with p value < 0.05 which is statistically highly significant. In the placebo group the CAL at base line 9.133±0.192 which gradually decreased to 8.667±0.126 and 8.467±0.159 after two and three months respectively, the difference in mean is statically significant with p – value 0.014 and 0.012 respectively. (Table – 1).

The GCF samples were collected from deepest periodontal pocket from each quadrant for analysis of Interleukin – 1β and Interleukin – 4 at baseline, 2 months and 3 months post-operatively using ELISA kits.

The mean±S.D of Interleukin -1β in propolis group at baseline is 57.347±0.794 which decreased to 47.355±0.751 and 47.005±0.622 at two and three months respectively. When compared the means between baseline to 2 months and then to 3 months post- operatively, the reduction is statistically highly significant with p – value 0.001. (Table – 2), and when compared between two months and three months p-value is 0.003. In the placebo group the Interleukin -1β at baseline is 55.88±0.794 which gradually decreased to 50.955±0.751 and 48.472±0.662 after two and three months respectively. When compared between the intervals, the difference in mean is statistically highly significant with p – value 0.001. (Table – 2).

Similarly, an anti- inflammatory bio-marker like Interleukin – 4 was also considered in this study to check the efficacy of SRP and propolis. The mean and S.D of IL-4 levels in propolis group was 48.755±0.819 at baseline, which increased to 54.663±0.946 at two months after intervention which further increased to 56.519±0.918 at three months post-operatively. When compared the means at different intervals, the difference is statistically highly significant with p – value 0.001. (Table – 2).

In placebo group the mean and S.D at baseline is 47.826±0.819 which increased to 52.933±0.946 and 56.119±0.918 at two months and three months respectively. When compared the means at different intervals, statistically highly significant difference in means is noticed with p – values 0.001. (Table – 2).

When intergroup comparison is done at different intervals by using repeated measures of ANOVA Test. Statistically highly significant difference in means are seen with GI and IL - 1β levels with p – value 0.001. All other clinical parameters showed statistically non-significant differences with p – value > 0.05. Except with IL – 4 levels where P – value was 0.05 (Table – 3)

Discussion: Lipopolysaccharide is a key microbial stimulus that initiates the host response at periodontal disease sites and triggers monocytes to release inflammatory mediators,[16, 17] the levels of monocytic inflammatory mediators in GCF may be ideal markers of disease activity at a particular site. In the present study, we examined IL-1β, IL – 4 which is considered to be the cytokine that are powerful mediators of inflammation. Interleukin- 1β promotes development of an inflammatory response, amplifies inflammation and modulates a lot of immunological

processes. An increasing body of evidence indicates that all of these IL-1β-dependent mechanisms may contribute to the inflammation to destruction of bone and periodontal attachment loss, which are characteristic features of periodontal disease. Many studies have reported that GCF IL-1β levels are significantly elevated in all forms of periodontitis.[18, 19] Also, An increasing amount of data indicate that assessment of certain humoral factors, including IL-1β, IL-4, IL-8 and MMP-8, in gingival crevicular fluid might provide a good diagnostic tool to monitor the course of periodontitis.[20,21,22] It is also suggested that the measurement of these mediators in gingival crevicular fluid could be helpful to estimate the effect of periodontal treatment. When compared with the healthy control subjects, patients with chronic periodontitis demonstrated significantly higher levels of IL-1β.[23,24] Our observations are in line with previous studies where authors noticed a correlation between the levels of IL-1β and periodontal pocket depth.[25] We also found that scaling and root planing significantly reduced the GCF levels of IL-1β. The effect of nonsurgical therapy on IL-1β levels has been studied previously, and it has been shown that scaling and root planing resulted in reduction of gingival crevicular fluid IL-1β. Gamonal *et al* stated that there was a weak correlation between clinical parameters and the level of IL-1β 2 months after scaling and root planing.[26]

IL-4 a 20kDa product of the cells was originally described as a B-cell growth factor. It has shown to suppress the abilities of LPS involved monocyte or macrophage to secrete PGE_2, IL-1β, TNF-α which are shown to be bone resorbing mediators. In addition, IL-4 enhances production of IL-1ra in human monocytes. Recent studies have demonstrated an absence of IL-4 producing T-cell at sites of periodontal inflammation. Therefore, it was hypothesized that the localized absence of IL-4 at the site of gingival inflammation plays a fundamental role in the progression from gingivitis to periodontitis. Recent study using rheumatoid arthritis model in animals have also found that IL- 4 injected directly into the lesion prevents the tissue destruction observed in control sites.

According to a controlled clinical trail the propolis as local delivery antimicrobial agent was effective against periodontal pathogenic bacteria, fungus, yeasts and healing wound in the mouth.[27] In this study, the effect of propolis irrigation as an adjunct to SRP was compared with a placebo (distilled water) in the same patient in two different quadrants, (Split Mouth Design) of 15 patients diagnosed with chronic periodontitis.

The clinical parameters considered were PI, GI, BOP, PPD and CAL. Similarly, pro-inflammatory (IL-1β) cytokine and an anti- inflammatory (IL-4) cytokines were also considered to check the inflammatory changes in the underlying Periodontal tissues. For cytokine analysis, 5µl of GCF was collected from deepest probing pocket site in each quadrant (propolis and placebo sites) then subjected for ELISA to check the levels of interleukins in picograms(pg). Both propolis and placebo groups showed improvement in clinical parameters from baseline to two months and three months. When inter group comparison was carried out, the differences in means were statistically not significant except in relation to gingival index (GI) and Interleukin-1β (IL-1β) (Table – 3), Where propolis as an adjunct to SRP showed a significant improvement in GI scores which was further reflected in decrease in pro-inflammatory cytokine levels (IL -1β).

Similarly, the sites with advanced periodontal destruction i.e. PPD 5.867±0.192 have showed mean IL-4 concentration of 48.755±0.819pg at baseline which was in contrast to a study by Kabashima*et al* who reported no detectable IL -4 in GCF from severe inflammation sites, but was detected after periodontal treatment[28]. The results of the present study also showed increased levels of IL -4 after periodontal treatment. The increase in concentration of IL -4 after intervention was high in propolis group with a statistically significant difference (p value -0.001) indicating an added benefit of propolis as an adjunct to SRP in arresting the periodontal destruction and shifting the disease process towards healing.

Numerous studies have reported beneficial outcomes of scaling and root planing treatment in both clinical and microbial parameters. Our observations showed that three months following nonsurgical therapy and sub-gingival irrigation of 30% propolis four times (once in every week), there was significant improvement in gingival inflammation (GI). These findings are contradictory with the result obtained by Kooh*et al* and Dodwad *et al* where propolis containing rinse was marginally better than negative control.[29,30] This may be due to differences in study designs opted.

However, the gingival crevicular fluid level of markers of inflammation, i.e. IL-1β, was still elevated. Thus, these findings might suggest that short-term nonsurgical therapy resulted in an improvement in clinical signs of inflammation but that the inflammatory and destruction processes within periodontal tissues were not entirely eliminated. Therefore, further investigations with 6 to 12 months follow up are necessary to appreciate the preliminary results of this study.

As there were no previous studies which tested the efficacy of propolis on Interleukin levels in GCF, direct comparison was not possible. Future studies considering higher concentration of propolis and longer duration of application in larger sample size must be carried out to further confirm the preliminary results of this study.

Future Prospects:-

- Propolis as host modulating agent.
- To validate whether these changes were mainly due to antibacterial or anti-inflammatory properties of propolis.

Conclusion: Within the limitations of this study it can be concluded that 30% propolis sub gingival irrigation as an adjunct to SRP is beneficial in reducing the periodontal inflammation and arresting the periodontal tissue destruction when followed up for 3 months.

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Conflict of interest: None

Funding: None

Cite this Article as: Annaji S,Philip J, Jagadish P, Radhika B, Shahid S. Efficacy of Propolis as an Adjunct to Scaling and Root planing in patients with chronic periodontitis. Natl J Integr Res Med 2017; 8(4):17-22