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Evaluation of clinical and antimicrobial efficacy of propolis mouthwash in treatment of gingivitis. A randomized controlled clinical trial

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Abstract

Aim: The aim of the study was to assess and compare the clinical and antimicrobial efficacy of Propolis mouthwash (20%) with Chlorhexidine gluconate (0.2%) in the treatment of gingivitis.

Materials and methods: Sixty patients in the age group of 18-65 years, diagnosed as generalized chronic gingivitis were included for the study. Selected subjects were further divided into three groups based on randomization as Group A (Propolis mouthwash 20%), Group B (0.2% Chlorhexidine gluconate) and Group C (saline). At baseline, clinical parameters and supragingival plaque samples were collected using sterile curettes from the multiple sites in—both experimental and control groups. Respective mouthwashes were prescribed to each groups followed

by instruction. The follow up clinical parameters and supragingival plaque sample collection were collected on 15th day, further subjected to microbiological assessment for colony forming units against the most abundant supragingival plaque—microorganisms, i.e., against Streptococcus, Actinomyces, Fusobacterium and Capnocytophaga-species.

Results: Intergroup comparison of all the three groups for plaque index at follow up interval was assessed using ANOVA test and found to be highly statistically significant between the groups (F=6.812; P=0.002) whereas for gingival index at follow up, ANOVA test exhibited no statistically significant difference among all the groups at follow up visits (F=1.63; P=0.204). Intergroup comparison at follow-up for S. mutans, Actinomyces, Fusobacterium and Capnocytophaga species exhibited no statistically significant difference among all three groups.

Conclusion: The observations of this study found that Propolis mouthwash (20%) was equally effective as 0.2% Chlorhexidine in the management of gingivitis.

Clinical Significance: Propolis seems to be an efficient herbal mouthwash due to its demonstrated antimicrobial property.

Keywords: Antimicrobial, Gingivitis, Herbal, Microorganisms, Mouthwash, Propolis,

Introduction

The ultimate method for prevention of gingival diseases is maintenance by effectual level of plaque control by the individual through proper daily oral hygiene measures. It's being observed many individuals find it difficult to comply with this daily regimen due to Insufficient and inadequate brushing and flossing, deficient manipulative skills.[1]

There are several chemical plaque control agents and Chlorhexidine gluconate is considered a gold standard anti-plaque mouth wash. Though it is very effective anti-plaque agent, it does have many side effects such as tooth staining, altered taste and desquamation of oral mucosa to a lesser extent.[2]

Hence the research for a long term, ideal and a safe antiplaque and antigingivitic agent continues and has encouraged the search for other alternative agents. One such alternative agent is Propolis, a natural resinous mixture produced by honeybees from substances collected from parts of plants, buds, and exudates. Propolis is a complex of biologically active substances with major constituents being flavones, flavanones, and flavanols. It also contains a number of unidentified compounds that work together synergistically to create a balanced, nutritive substance .[3,4] Propolis has drawn the attention over a long period of time.[5]

It has been used in various formulations for dermatology, otorhino laryngology, gynecology, od ontology and veterinary medicine.[6]

Propolis has shown promising result as an anti-microbial and also found beneficial in the treatment of gingivitis and oral ulcers in pilot clinical studies[7] Propolis is also known to have antifungal,[8] antiviral,[3,4] antioxidant action [9,10] inflammation relieving and wound repair accelerating effects [11] and is used in the treatment of gingivitis, periodontal abscess, denture ulceration, stomatitis, candidal infections, dentinal hyper sensitivity, as an intracanal medicament in endo dontic procedures.[12,13]

Hence, this study was carried out to evaluate and compare the clinical efficacy and anti-microbial efficacy of Propolis mouth wash (20%) with Chlorhexidine gluconate (0.2%) in patients with gingivitis.

Materials and Methods

A randomized controlled clinical trial including 60 subjects was carried out in the Department of

Periodontics, Dayananda Sagar College of Dental Sciences, and Bangalore. The study was approved by the ethical committee and Institutional review board. All the subjects were informed about the procedures and an informed consent was obtained.

Patient diagnosed with generalized chronic gingivitis (AAP 1999 classification) but otherwise with good systemic health and willing to follow the protocols were included in the study.

Patients with adverse habits such as cigarette smoking, drug abuse, suffering from any systemic diseases, history of any dental therapy in the past two weeks, currently on antibiotic therapy, steroids or hormonal therapy, history of allergy for oral hygiene products were also excluded.

Study Design

The study design was Parallel arm RCT with total number of 60 subjects between 18 to 65 years age group. The 60 subjects were randomly divided by computerized method into three groups (20 subjects per group). Group A (Propolis group); Group B (Chlorhexidine group); Group C (Saline).

Experimental material

The experimental agent used in this study was Propolis (20%) containing mouth wash formulated at Himalaya Pharmaceuticals Limited, Bengaluru, Karnataka. The test agent and food grade alcohol were procured from Hi Tech Natural Products Limited, India. The Minimum inhibition concentration (MIC) of Propolis mouth wash was done by agar well disc diffusion method on Streptococcus, Actinomyces, and Fusobacterium and Capnocytophaga microbes. The diameter of inhibition zone was measured to the nearest whole millimeter. Among various concentration, 20% concentration of propolis was considered as it exhibited sensitivity to all above microorganisms. Cytotoxicity and cell viability was done by MTT ASSAY and 20% Propolis was found to be noncytotoxic.

Clinical Examination

A special proforma was designed to facilitate methodical recording of all the observations and information. The clinical parameters Plaque Index (Silness P and Loe, 1964) & Gingival Index (Loe H & Silness P, 1963) were recorded at baseline & 15th day follow up. Subjects were instructed to rinse with their respective mouth washes for 1 min, 10ml twice daily for two weeks. Supragingival plaque samples were also collected at baseline & 15th day (during follow up) with sterile area specific curettes from multiple sites. It was transferred immediately into a sterile vial containing 1ml of Reduced Transport Fluid and then the tubes were sealed. The vials containing samples were sent to laboratory for further process.

Microbiological Examination

The vials containing plaque samples were sent to laboratory and processed within 24 hours, quantification of microbes was recorded as colony forming units (CFU) per ml. Samples were inoculated in the enriched medium /blood agar as well as selective media, according to the culture requirements of the microorganisms. Nalidixic Acid Colistin Blood Agar was used as the selective medium for actinomyces species, which were seen as small white opaque moist colonies (Figure 1.0). Crystal violet erythromycin agar was used as selective medium for Fusobacterium nucleatum, which appeared as minute purplish mucoid colonies (Figure 1.1).

Mitis salivarius agar was used as a selective medium for Streptococcus mutans which appeared as round or spherical, raised and dark blue irregular colonies (Figure 1.2).

TBBP/Trypticase soya agar with bacitracin and polymyxin B sulphate was used as a selective medium

for Capnocytophaga which were seen as tiny brownish colonies (Figure 1.3).

Results

Intragroup comparison of clinical parameters Plaque index, Gingival index and microbial parameter such as Colony forming units of all four organisms were carried out by paired t test.

Intergroup comparison of all the three groups for plaque index at follow up interval was assessed using ANOVA test and found to be highly statistically significant between the groups (F=6.812; P=0.002) Further Post-hoc analysis showed significant difference between Group A and Group B (p=0.003); Group B and Group C groups (p=0.017) at follow-up visit whereas there was no significant difference found between Group A and Group C (p=1.00) (Table 1).

Intergroup comparison of Group A (Propolis), Group B (chlorhexidine gluconate) and Group C (Saline) for Gingival Index at baseline was done using ANOVA test and showed highly statistically significant difference between all the groups (F=4.64; P=0.014) Further Post hoc Bonferroni analysis showed significant difference between Group B and Group C (p=0.017) for GI at baseline whereas there was no significant difference found between Group A and Group B (P=1.00); Group A and Group C (P=.077) (Table 2) Intergroup comparison of the three groups for gingival index at follow up, ANOVA test exhibited no statistically significant difference among all the groups at follow up visits (F=1.63; P=0.204).

The antimicrobial efficacy of Group A (Pro polis), Group B (Chlorhexidine) and Group C (Saline) against four microorganisms (strep to coccus, Actino myces, Fuso bacterium and Capnocytophaga) was assessed based on colony forming units/ml by using culture technique.

For all the groups, the mean distribution of the pathogens was higher at baseline as compared to follow up interval.

Intragroup comparison among microorganisms

FOR Group A - (Propolis group)-Intragroup comparison among these pathogens at baseline and follow up using paired t test was found to be statistically significant difference in fusobacterium organism (p=0.037); whereas there was no statistically significant difference for S. mutans (p=0.060), Actino myces (p=0.065), Capnocytophaga (p=0.246) species. For Group B (Chlorhexidine group) -Intragroup comparison of these pathogens at baseline and follow up it was found to be highly statistically significant difference in S. mutans (p=0.08), Actino myces (p=0.013), fuso bacterium (p=0.024) whereas it was observed no statistically significant for Capnocytophaga (p=0.232) species.

For Group C (Saline group)- Intragroup comparison of these pathogens at baseline and follow up showed statistically significant in S. mutans (p=0.002), Actinomyces (p=0.023), Fuso bacterium microorganisms (p=0.05) whereas no statistically significant difference was observed for Capnocytophaga (p=0.214) species.

Intergroup comparison of Microbial Species

Intergroup comparison between Group A, Group B and Group C for S. mutans at baseline was done using Anova test and showed statistically significant difference between all the groups for S. mutans at baseline (P=0.032) whereas at follow –up no statistically significant difference among all the three was observed (p = 0.170).

Intergroup comparison of all three groups for Actino myces at baseline (p=0.960) and follow up (p= 0.366) exhibited no statistically significant difference Inter group comparison of all the three groups for Fuso bacterium at baseline (p= 0.029) was found to be

statistically significant. Post hoc analysis for Fuso bacterium at baseline showed significant difference between Group A and Group B (p=0.046) whereas there was no significant difference found between Group A and Group C(p=0.088)) and Group B and Group C (p=1.00) and at follow-up no statistically significant difference among all the three groups was observed. (p = 0.144)

Intergroup comparison of all the three groups for Capnocytophaga at baseline (p=0.234) and follow up (p=0.328) respectively. (Table no.3)

Discussion

Several individuals like to use mouthwash routinely because of the fresh feeling it gives and always prefer natural products for their oral care, since they are safer and without any side effects. Hence the research for a long term, ideal and a safe antiplaque agent continues and has also encouraged the search for other alternative agents. One such alternative agent is Propolis, which is a naturally occurring bee product consists chiefly of wax and plant extracts. It has a surprisingly wide range of beneficial properties including antimicrobial, anti-inflammatory, antioxidant, antiviral, antitumor, immune modulation. which could prove beneficial for inflammatory diseases like gingivitis. Therefore, this study was carried out to evaluate the clinical and antimicrobial efficacy of Propolis (20%) containing mouth wash in comparison to 0.2% Chlorhexidine gluconate and saline, in chronic gingivitis subjects. In this study, we evaluated the clinical parameters - plaque index (PI), gingival index (GI), at baseline and follow up i.e., on 15th day visit in both experimental and control groups. Further, antimicrobial efficacy analysis against four microorganisms i.e., streptococcus, Actinomyces, Fusobacterium and Capnocytophaga species was also done through culture technique at baseline and follow-up.

On intergroup comparison for all the three groups for plaque index (PI), highly statistically significant difference was observed which was similar to the study conducted by Tor wane et al,[14] in the mean plaque index scores. Kadav et al[15] and Murray et al[16] conducted similar studies where in their results were inconsistent with the present study. Their results reported that chlorhexidine mouthwash was more effective in inhibiting plaque than propolis, although propolis was effective in plaque inhibition in comparison with saline group. The present study reported statistically significant reduction in mean GI score in Group A (Propolis) and Group B (Chlorhexidine gluconate) from baseline to 15th day follow up; whereas there was no statistically significant reduction in mean GI score from baseline to 15 day follow up interval in Group C (Saline) on intragroup comparison.

On intergroup comparison of all the three groups for gingival index at follow up showed no statistically significant difference. Other similar studies regarding the significant clinical improvement of gingival health could not be collaborated with other authors as the detailed perusal of the available literature failed to show any such similar study. Therefore, it is not possible to compare our findings related to improvement in all the three groups with any other observed in the present study, may be attributed to its well documented antiplaque and antimicrobial action of propolis.

Several invitro studies have been carried out to assess the antimicrobial activity of propolis. A study conducted by Seoul-Hee Nam et al[17] and Nilesh Kumar et al[18], where in antimicrobial activity of propolis against various streptococcus species, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Candida

albicans and Asparagus Niger were evaluated and found that propolis has antibacterial potency. Another study conducted by Amita Coutinho[19] evaluated the effect of sub gingival irrigation with propolis extract in periodontal treatment found that there were decrease in the total viable counts of anaerobic bacteria as well as low levels of P. gingival is was observed in propolis group when compared with other groups.

The present in vivo study was assessed to evaluate the antimicrobial activity against the most predominate supra gingival plaque microorganisms Strep to coccus mutans, Actino myces Fuso bacterium and Capnocytophaga species and reduction in the colony forming units during follow up interval was observed and may be attributed to its well documented antimicrobial activity. The findings regarding the four microorganisms observed in the present study could not be collaborated with any such similar study as the detailed perusal of the available literature failed to show any such similar study.

Further Propolis is one of the promising natural products which has a significant inhibitory activity against the observed supragingival plaque microorganisms i.e., Streptococcus, Actinomyces, Fusobacterium and Capnocytophaga species and found to be non-cytotoxic on human gingival fibroblasts and is equally effective as chlorhexidine mouthwash.

However, being a short-term study with small sample size and couldn't follow the age group criteria according to WHO (World Health Organization) were the limitation of this study. The other drawbacks could be non-evaluation of substantivity of propolis in comparison with other marketed mouthwashes. The propolis mouthwash was dispensed in suspension form which requires dilution before use unlike other mouth washes available readily and this could also add to another limitation. Thus, further long-term clinical trials with larger sample size and standardized controls, are desired to validate the superiority of propolis in the treatment of gingivitis and other gingival diseases.

Conclusion

Based on the observations of this study, it was found that propolis mouth (20%) was equally effective as 0.2% Chlorhexidine gluconate in the management of gingivitis. This study also proved that propolis is one of the promising natural products which has a significant inhibitory activity against the supragingival plaque microorganisms Streptococcus, Actinomyces, Fuso bacterium and Capnocytophaga species. Hence, Propolis adds hope that it can be used safely as a mouthwash in gingivitis. Further, use of different vehicles and varied concentrations of the propolis extract to improve its substantivity are desired in long term trials to comment on its application as an oral hygiene aid adjunct to scaling in the treatment of gingival diseases.

Table 1: post hoc (Bonferroni) analysis for plaque index at baseline and follow up

	Mean Difference ± SD			P Value		
	Group A -B	Group A - C	Group B - C	Group A -B	Group A - C	Group B - C
Baseline	06875±1.82	07589±1.82	14464±1.82	291	203	002*
Follow Up	10982±1.42	01786±1.42	09196±1.42	003*	1.000	017*
*The mean difference is significant at the 0.05 level.						

Table 2: post –hoc (Bonferroni) analysis for gingival index at baseline.

	Mean Difference± SD			P Value		
	Group A -B	Group A - C	Group B - C	Group A -B	Group A - C	Group B - C
Baseline	-0.02500±1.88	0.09643±1.88	0.12143±1.88	1.000	0.077	0.017*
*The mean difference is significant at the 0.05 level.						

Table 3: Intergroup comparison of the four microorganisms at baseline and follow up

Microorganisms	Between Three Groups	Sum of Squares	Mean Square	F	P-Value
S mutans	Baseline	11424.533	5712.267	3.651	.0320*
	Follow-Up	2357.733	1178.867	1.829	.170
Actinomyces	Baseline	99.633	49.817	.041	.960
	Follow-Up	913.300	456.650	1.022	.366
Fusobacterium	Baseline	5435.200	2717.600	3.769	0.29*
	Follow-Up	390.833	195.417	2.006	.144
Capnocytophaga	Baseline	344.633	172.317	1.489	.234
	Follow-Up	136.633	68.317	1.137	.328

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Legend figure

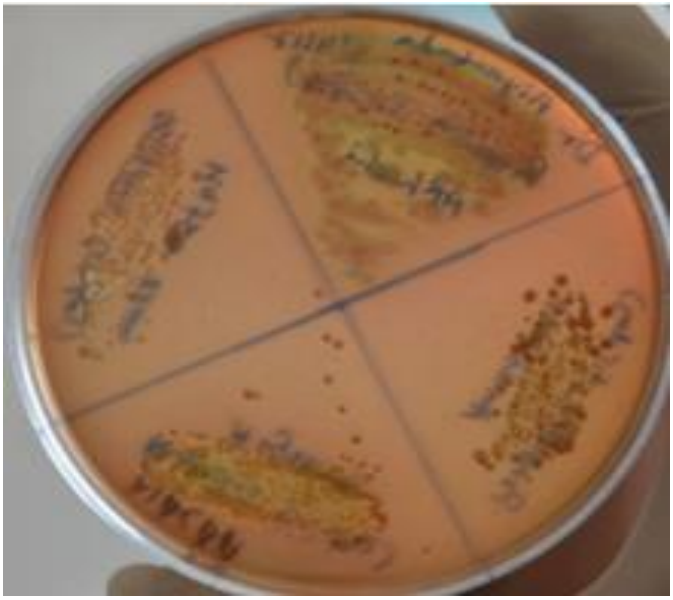

Figure 1: Growth of Actinomyces species on culture

Figure 2: Growth of F. nucleatum on culture

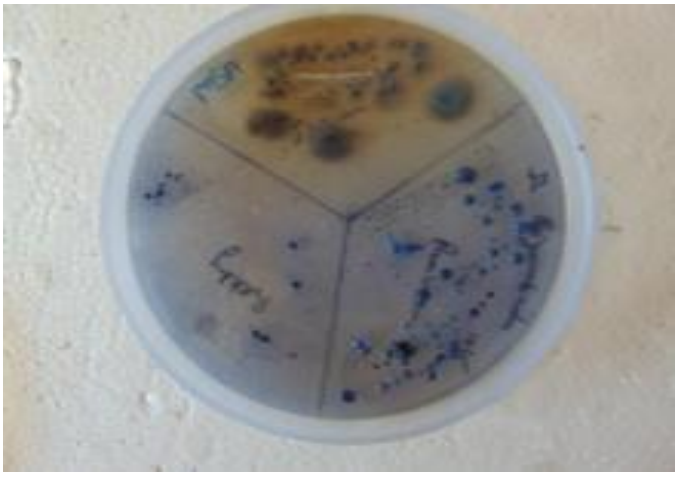

Figure 3: Growth of Streptococci on culture media

Figure 4: Growth of Capnocytophaga on culture media