

The antiplaque efficacy of propolis-based herbal toothpaste: A crossover clinical study

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Abstract

Background: In recent years, herbal products have been suggested as an economic, safe and probably effective alternative for prevention and control of various oral diseases. But still there are some products which need to be evaluated. Of lately, Propolis is one such product. To assess and compare the efficacy of herbal dentifrice containing Propolis with Miswak and Colgate total toothpastes in controlling plaque formation. **Materials and Methods:** A double blind, randomized, crossover study design was conducted among thirty healthy dental students. After oral prophylaxis all subjects were given a washout product for one week period. Subjects were then made to brush with (washout product) for 1 minute followed by 1 minute brushing with assigned test product. The baseline MGMPI plaque scores were recorded. Subjects were then refrained from oral hygiene for 24 hours, and were recalled to be re-disclosed and re-measured for plaque formation. This procedure was repeated according to crossover design after a washout period of (2 week). Statistical tests used were Kruskalwallis and Wilcoxon sign rank test. **Results:** There was a significant difference in 24 hour score between the test products evaluated. When the change from baseline to 24 hours was analyzed, the test product Propolis resulted in a consistently and significantly ($p < 0.05$) lower MGMPI mean scores than the Colgate Total and Miswak toothpastes. **Conclusion:** Propolis was found to be safe and effective in reducing plaque accumulation when compared to Miswak and Colgate total toothpaste.

Key words: Colgate, Miswak, modified gingival marginal plaque-index, plaque, Propolis

INTRODUCTION

Association of periodontal infection with organ systems such as cardiovascular system, endocrine system, reproductive system, and respiratory system makes periodontal infection a complex multiphase disease.^[1] Dental plaque is considered to be the key factor associated with both dental caries and gingival inflammation. The latter, if untreated may extend beyond the gingival margin and progress to periodontitis. This may ultimately lead

to the loss of teeth. Approaches to control dental plaque center on mechanical removal by tooth cleaning or by the use of chemicals, which prevent or reduce bacterial multiplication.^[2] Self-performed mechanical plaque removal is one of the most accepted methods of controlling plaque and gingivitis.^[3] The fact that most people experience difficulty in maintaining adequate levels of plaque control, particularly at interproximal sites, necessitates the use of chemicals for control of plaque as an adjunct to mechanical plaque control procedures.^[4] There has been a search for years for chemical agents that could supplant patient-dependent mechanical plaque control and thus reduce or prevent oral disease.^[5]

A wide range of toothpastes are commercially available and recently interest in natural products has increased. A number of controlled clinical trials have demonstrated that tooth brushing with herbal dentifrices reduces supragingival plaque and gingivitis.^[2,3,6] As a result, a number of these

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agents have been incorporated into toothpastes and mouth rinses. Miswak is one such scientifically formulated herbal toothpaste with pure extract of the Miswak plant “*Salvadore Persica*,” which has been used for centuries.^[7] Recently, several studies have reported the antibacterial effects of Miswak (chewing sticks) on cariogenic bacteria and periodontal pathogens, and inhibitory action on dental plaque formation.^[8-10]

More recently, another active agent Propolis has been used for treating different diseases and inflammatory conditions as both local and systemic applications.^[11] Propolis is available in the world markets in different forms as capsules, lozenges, tincture, and cream and recently added to the list are mouth rinses and toothpastes. Based on literature reports showing that propolis resin is a product with anti-inflammatory and bactericidal activity, several *in vitro* and some *in vivo* studies^[12-14] have been conducted in America, Australia, United Kingdom, and Europe and especially in Eastern Europe.^[15-18] Only a few studies have been conducted to assess the effects of propolis on oral health. Owing to this dearth of literature, the present study has been undertaken with an objective to assess and compare the efficacy of herbal dentifrice containing Propolis with Miswak and Colgate total toothpaste in controlling plaque formation.

MATERIALS AND METHODS

Study design, study area, and study population

A double-blind, randomized, crossover study was conducted at Pacific Dental College and Hospital, Udaipur city, Rajasthan state, India among 18-22 years old dental students.

Inclusion criteria

1. Dental students aged 18-22 years who volunteered to participate.
2. Those with a minimum of 24 natural teeth.
3. Subjects who agreed to discontinue oral hygiene measures for 24 h after the initial appointment.

Exclusion criteria

1. Those currently using any mouthwash,
2. Those on antibiotic therapy within the last 2 weeks,
3. Those having a history of hypersensitivity to any product used in the present study,
4. Those with a recent tooth extraction,
5. Those having periodontal disease,
6. Those with extensive untreated dental caries, diseases of hard and soft palate, subjects wearing orthodontic appliances or presenting with abnormal salivary flow,
7. Subjects suffering from immune compromised states such as diabetes, AIDS, hepatitis, etc.

Sample selection

A convenient sample of 30 dental students comprising of both males and females was selected.

Ethical clearance and official permission

The present study was conducted in accordance to Declaration of Helsinki. Ethical clearance was obtained from the Ethical Review Board of the Pacific Dental College and Hospital (No. PDCH/13/000234). An official permission was obtained from the principal of Pacific Dental College and Hospital, Udaipur.

Informed consent

After explaining the purpose and detailed procedure of the study, a written informed consent was obtained from all the subjects, prior to the beginning of the study.

Training and calibration

All the examinations were carried out by a single examiner. Before the commencement of the study, the examiner was standardized and calibrated for Modified Gingival Marginal Plaque-Index (MGMPI) in the Department of Public Health Dentistry by a senior faculty member to ensure uniform interpretations, understandings, and application of the codes and criteria to be observed and recorded and to ensure consistent examination. The examiner first practiced the index on a group of 10 subjects. Then the examiner applied the criteria by examining a group of 10 subjects, twice on successive days. The intra examiner reliability was assessed using Kappa statistics, which was found to be 90%.

Materials used in the study

- Forever Bright Toothgel (containing Propolis) (forever living products)
- Colgate Total (Colgate-Palmolive Company India Ltd.)
- Colgate Great Regular (Colgate-Palmolive Company India Ltd.)
- Meswak Toothpaste (Dabur India Ltd.)
- Disclosing agent (The Bombay Burmah Trading Corporation India, Ltd.)

Methodology

Before starting the study, oral prophylaxis was performed. All subjects were given a washout product, Colgate Regular Flavour toothpaste and a Colgate soft manual toothbrush, with the instructions to use only these products and to brush twice daily for the washout period (1-week). After the washout period was complete subjects reported to the Department of Public Health Dentistry and were randomly allocated to three groups of 10 participants each. Each group was randomly assigned to one of the three toothpastes (1 - Forever Bright Toothgel, 2 - Colgate Total, 3 - Meswak toothpaste). Randomization

was performed using lottery method. Then they were made to brush with Colgate Regular Toothpaste for 1-min followed by 1-min brushing with assigned test product. All products were blinded to both the subject and the examiner by way of an over-wrap. After this, all teeth were disclosed with disclosing agent. The Xu and Barnes probe^[19] was gently placed along the margin of the gingiva, and the baseline MGMPi plaque scores were recorded. Subjects were then refrained from oral hygiene for 24 h, and were recalled to be re-disclosed and re-measured for plaque formation.

The above-mentioned procedure was repeated after a washout period (2 weeks) in accordance with the crossover design, so that all three products could be tested on each subject. To ensure allocation concealment, the allocation methods were not revealed to the examiner. A statistician was not directly involved in recruiting patient generated the randomization sequence. Recruitment and assignment of patients to their groups was carried out by the trial coordinator.

Statistical analysis

Data were analyzed using SPSS version 19 software (SPSS Inc., Chicago, IL, USA). The data were found to be nonnormally distributed using Kolmogorov-Smirnov test. Kruskal-Wallis test was used for the comparison of the three toothpastes (Forever Bright Toothgel, Conventional Colgate toothpaste, Miswak toothpaste). Wilcoxon signed rank test was used for pair-wise intra (within) group of toothpastes. Confidence level and level of significance were set at 95% and 5%, respectively.

RESULTS

Table 1 shows the distribution of 30 subjects 15 males and 15 females with mean age of 19.9 ± 1.4 years.

Table 2 revealed that there was no significant difference in the mean MGMPi scores between the three groups (Propolis, Miswak, Colgate) at baseline. But when they were compared after 24 h significantly ($P = 0.01$) highest mean MGMPi score was observed in Miswak group (45.5 ± 5.0) followed by Colgate (37.4 ± 2.2). Propolis showed significantly least mean MGMPi score (35.8 ± 2.3) after 24 h. On comparing the mean difference (baseline and 24 h) of the three groups, significantly ($P = 0.01$) lowest mean difference was elicited by Propolis group.

Table 3 intragroup comparisons depicted significantly lower mean MGMPi scores among Propolis group than Colgate group and Miswak groups. Colgate group had significantly lower scores than Miswak group.

Table 1: Distribution of study subjects

Group	Number of subjects	Males	Females	Mean age \pm SD
Propolis	30	15	15	19.93 \pm 1.41
Herbal				
Colgate				

SD: Standard deviation

Table 2: Comparative assessment of the mean MGMPi scores at baseline and 24 h and their differences for each of three toothpastes

Groups	Baseline (mean \pm SD)	24 h (mean \pm SD)	P**	Difference (mean \pm SD)
Propolis	14.56 \pm 1.24	35.88 \pm 2.36	0.01	21.32 \pm 2.81
Herbal	14.65 \pm 1.28	45.51 \pm 5.08	0.01	30.86 \pm 5.47
Colgate	14.55 \pm 1.230	37.48 \pm 2.25	0.01	22.93 \pm 2.83
P*	0.88	0.01	—	0.01

*Test applied: Kruskal-Wallis, **Test applied: Wilcoxon sign rank test. Statistically significant ($P < 0.05$). SD: Standard deviation, MGMPi: Modified gingival marginal plaque-index

Table 3: MGMPi plaque scores intragroup comparisons

Group	24 h (mean rank)	P	Difference (mean rank)	P
Propolis	16.98	0.01	16.98	0.01
Herbal	44.02		44.02	
Herbal	42.45	0.01	42.45	0.01
Colgate	18.55		18.55	
Colgate	37.03	0.03	23.97	0.04
Propolis	23.97		37.03	

Statistically significant ($P < 0.05$). MGMPi: Modified gingival marginal plaque-index

DISCUSSION

The present study was conducted among 30 dental students of Udaipur to assess and compare the efficacy of herbal dentifrice containing Propolis with Miswak and Colgate Total toothpaste in controlling plaque formation.

Propolis is the generic name for the resinous product of complex composition collected by honeybees from various plant sources. Chemical analyses revealed that propolis contains more than 300 constituents among them phenolic compounds, including flavonoids as major components.^[20] Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.^[21]

In the present study, no significant difference was observed in baseline MGMPi scores between the groups which may be accounted to the oral prophylaxis which was performed before the commencement of the study. Calculus with its rough surface promotes plaque formation at a faster rate than a smooth calculus-free surface. The increased rate may result in inaccurate data and incorrect conclusion in

studies. By performing the oral prophylaxis several days prior to the plaque scoring, all calculus was removed, and while the insufficient time for calculus re-growth exists, sufficient time for plaque formation does exist.

In this study, 24 h plaque formation was assessed using the MGMP. Our results amending for the assessment of antiplaque activity showed a significant difference at 24 h after use depicting Propolis to the most effective compared to Colgate Total and Meswak. Koo *et al.*^[22] evaluated the effect of propolis mouth rinse on dental plaque accumulation and found it to be effective against plaque build up. In another study, Hidaka *et al.*^[23] showed that Propolis reduced the rate of amorphous calcium phosphate transformation into hydroxyapatite and concluded that it had a potential as an anticalculus and antiplaque agent in toothpastes and mouthwashes. Ikeno *et al.*^[24] and Hayacibara *et al.*^[25] found that the insoluble glycan synthesis and glucosyltransferase activity were inhibited by the multiple action of Propolis. This could be because of the high content of phenolic compounds, including flavonoids and dramatic compounds such as caffeic acids.^[26] Flavonoids are well-known plant compounds which have antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties.^[20] Seidel *et al.*^[27] Velazquez *et al.*^[28] Orsi *et al.*^[29] concluded that propolis was found to be very effective against Gram-positive and Gram-negative bacteria this reduction in the bacterial count could also be accounted for the reason of decline in the plaque score, as plaque consists of colonies of microorganisms.

The crossover study design employed in the present study reduced the between patient variability, because the comparison of treatment was made on the same patient that is, all the participants acted as their own control and less sample was required. As this was a double blind study, the participant's and the investigator were not aware of the treatment allocation. Furthermore, measuring the curved surface of the gingiva with a straight probe was found to be difficult, therefore, a periodontal probe was curved and contoured to reflect the curvature of the gingival margins.^[27] A few limitations of the study may be pointed out. First is that the subjects were dental students and were more likely to maintain a better oral hygiene compared to the general population. MGMP employed in the study used a 24 h plaque assessment model which could have been a drawback. Ozan *et al.*^[30] and Murray *et al.*^[31] used propolis mouth rinse and found it to be less effective on antiplaque activity. Hence, long-term clinical studies in large number of the patient population should be undertaken to evaluate the usefulness of this natural products. The actual mechanism involved behind the antimicrobial activity of propolis extracts needs to be researched. Research on microbial biofilms is proceedings on many dimensions,

with specific focus on elucidation of the genes specifically expressed by biofilm-associated organisms, assessment of different control approaches for either preventing or remediating biofilm colonization of medical devices, and development of new methods for evaluating the efficacy of these treatments.^[32]

CONCLUSION

Propolis is the most effective in reducing plaque from baseline to 24 h followed by Colgate total and Miswak toothpaste. Furthermore, long-term studies with the use of more objective measures of plaque alongside a conventional subjective index to support the findings of the present study are warranted.

REFERENCES

- Saini R, Saini S, Saini SR. Periodontitis: A risk for delivery of premature labor and low birth weight infants. *J Nat Sci Biol Med* 2011;2:50-2.
- George J, Hegde S, Rajesh KS, Kumar A. The efficacy of a herbal-based toothpaste in the control of plaque and gingivitis: A clinico-biochemical study. *Indian J Dent Res* 2009;20:480-2.
- Mullally BH, James JA, Coulter WA, Linden GJ. The efficacy of a herbal-based toothpaste on the control of plaque and gingivitis. *J Clin Periodontol* 1995;22:686-9.
- Fine DH. Chemical agents to prevent and regulate plaque development. *Periodontol* 2000 1995;8:87-107.
- Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. *J Clin Periodontol* 1988;15:488-98.
- Pannuti CM, Mattos JP, Ranoya PN, Jesus AM, Lotufo RF, Romito GA. Clinical effect of a herbal dentifrice on the control of plaque and gingivitis: A double-blind study. *Pesqui Odontol Bras* 2003;17:314-8.
- Almas K, Skaug N, Ahmad I. An *in vitro* antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses. *Int J Dent Hyg* 2005;3:18-24.
- Dabur.com. Available from: <http://www.dabur.com/Products-Health%20Care-Meswakage>. [Last cited on 2013 Oct 08].
- Sukkarwalla A, Ali SM, Lundberg P, Tanwir F. Efficacy of miswak oral pathogens. *Dent Res J (Isfahan)* 2013;10:314-20.
- Dahiya P, Kamal R, Luthra RP, Mishra R, Saini G. Miswak: A periodontist's perspective. *J Ayurveda Integr Med* 2012;3:184-7.
- Rathod S, Brahmkankar R, Kolte A. Propolis - A natural remedy. *Indian J Dent Res Rev* 2012;1:3.
- Brumfit W, Hamilton-Miller JM, Franklin I. Antibiotic activity of natural products: 1. Propolis. *Microbios* 1990;62:19-22.
- Dimov V, Ivanovska N, Manolova N, Bankova V, Niklov N, Popov S. Immunomodulatory action of propolis. Influence on anti-infectious protection and macrophage function. *Apidologie* 1991;22:155-62.
- Dobrowolski JW, Vohora SB, Sharma K, Shah SA, Naqvi SA, Dandiya PC. Antibacterial, antifungal, antiamebic, antiinflammatory and antipyretic studies on propolis bee products. *J Ethnopharmacol* 1991;35:77-82.
- Koo H, Rosalen PL, Cury JA, Park YK, Bowen WH. Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity. *Antimicrob Agents Chemother* 2002;46:1302-9.
- Ophori EA, Eriagbonye BN, Ugbodaga P. Antimicrobial activity of propolis against *Streptococcus mutans*. *Afr J Biotechnol* 2010;9:4966-9.
- Malhotra N, Rao SP, Acharya S, Vasudev B. Comparative *in vitro* evaluation of efficacy of mouthrinses against *Streptococcus mutans*, *Lactobacilli* and *Candida albicans*. *Oral Health Prev Dent* 2011;9:261-8.

18. Duailibe SA, Gonçalves AG, Ahid FJ. Effect of a propolis extract on *Streptococcus mutans* counts *in vivo*. *J Appl Oral Sci* 2007;15:420-3.
19. Xu T, Barnes VM. The development of a new dental probe and a new plaque index. *J Clin Dent* 2003;14:93-7.
20. Ansoerge S, Reinhold D, Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-beta1 production of human immune cells. *Z Naturforsch C* 2003;58:580-9.
21. Pathak P, Saraswathy, Vora A, Savai J. *In vitro* antimicrobial activity and phytochemical analysis of the leaves of *Annona muricata*. *Int J Pharm Res Dev* 2010;2:1-6.
22. Koo H, Gomes BP, Rosalen PL, Ambrosano GM, Park YK, Cury JA. *In vitro* antimicrobial activity of propolis and *Arnica montana* against oral pathogens. *Arch Oral Biol* 2000;45:141-8.
23. Hidaka S, Okamoto Y, Ishiyama K, Hashimoto K. Inhibition of the formation of oral calcium phosphate precipitates: The possible effects of certain honeybee products. *J Periodontal Res* 2008;43: 450-8.
24. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. *Caries Res* 1991;25:347-51.
25. Hayacibara MF, Koo H, Rosalen PL, Duarte S, Franco EM, Bowen WH, *et al.* *In vitro* and *in vivo* effects of isolated fractions of Brazilian propolis on caries development. *J Ethnopharmacol* 2005; 101:110-5.
26. Koru O, Toksoy F, Acikel CH, Tunca YM, Baysallar M, Uskudar Guclu A, *et al.* *In vitro* antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. *Anaerobe* 2007;13:140-5.
27. Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. *Phytother Res* 2008;22:1256-63.
28. Velazquez C, Navarro M, Acosta A, Angulo A, Dominguez Z, Robles R, *et al.* Antibacterial and free-radical scavenging activities of Sonoran propolis. *J Appl Microbiol* 2007;103:1747-56.
29. Orsi RO, Sforcin JM, Rall VL, Funari SR, Barbosa L, Fernandes JR. Susceptibility profile of *Salmonella* against the antibacterial activity of propolis produced in two regions of Brazil. *J Venom Anim Toxins Incl Trop Dis* 2005;11:109-16.
30. Ozan F, Polat ZA, Er K, Ozan U, Deger O. Effect of propolis on survival of periodontal ligament cells: New storage media for avulsed teeth. *J Endod* 2007;33:570-3.
31. Murray MC, Worthington HV, Blinkhorn AS. A study to investigate the effect of a propolis-containing mouthrinse on the inhibition of de novo plaque formation. *J Clin Periodontol* 1997;24:796-8.
32. Saini R, Saini S, Sharma S. Biofilm: A dental microbial infection. *J Nat Sci Biol Med* 2011;2:71-5.

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