Comparative antiplaque activity of propolis extract and chlorhexidine in vivo

Baceer A Abdullah¹
BDS, MSc (Lect)

Rafi¹ A Al-Talib¹
BDS, MSc (Lect)

Fa'iz A Al-Sultan¹
BDS, MSc (Assist Lect)

¹Department of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

ABSTRACT
This study compared the clinical efficacy of different mouthwashes [0.2% chlorhexidine gluconate (CHX), 0.5%, 1% water extract of propolis, 0.5%, 1% ethanolic extract of propolis] with distilled water in their ability to inhibit plaque accumulation.

In this double blind study, 10 (8 males and 2 females) dental students volunteers aged from 20-24 years, 1120 tooth surfaces from 280 teeth were examined. Each volunteer received a final professional tooth cleansing and was instructed to stop all mechanical tooth cleaning effort for next 5 days, where the mouthwashes used 3 times daily. Plaque system index (Silness and Löe, 1964) was obtained from teeth surfaces (buccal, lingual, mesial and distal) before and after the uses of mouthwashes.

The results of this study showed significant difference (p < 0.05) between 0.2% CHX, 0.5% and 1% water extract of propolis, 0.5% ethanolic extract of propolis and distilled water, but non significant difference (p > 0.05) between CHX and 1% ethanolic extract of propolis in their ability to inhibit plaque accumulation.

It can be concluded that the alcoholic extract of propolis may be used as adjunct to mechanical plaque control during the maintenance phase of therapy to ensure sustained low plaque level and this may meet patient approval because it is a natural substance and devoid of industrial chemical component.

Key Words: Antiplaque activity, propolis, chlorhexidine.
INTRODUCTION

Although there is an overwhelming amount of data favouring the specificity of periodontal infections, at present the elimination of plaque is still the most reliable method to prevent gingivitis and to maintain periodontal health. Self-performed plaque control measures can be laborious and difficult, since for most patients it is necessary to keep plaque at very low levels. Therefore, antibacterial products incorporated into tooth paste and mouth rinses have become important adjuncts to the traditional oral hygiene procedures.

Previous studies have shown that chlorhexidine (CHX) is an effective anti-plaque agent. Unfortunately, the toxic qualities of CHX do not reserve entirely for bacteria, a review of literature has shown CHX to be noxious to a variety of mammalian cells, including sperm, polymorphonuclear leukocytes, macrophages, skin epithelial cells, erythrocytes and gingival fibroblasts. In addition to that, CHX application directly to surgical wounds in the oral cavity can delay and alter wound healing. Also, some basic side effects were present, like discoloration of the teeth, anterior fillings and the tongue is clearly seen in CHX rinses. Some persons have complained of bitter taste and interference with their sense of taste. So the need to evaluate other antibacterial mouth rinses with minimum or no disadvantages is mandatory.

Natural products have been used for thousands of years in folk medicine for several purposes, among them propolis has attracted increased interest. It is a natural resinous material produced by honey bees and used by them to strength them, isolate and disinfect their nest. It is a sticky mass, grayish–brown in colour with slight aromatic odor, contains 50% resin and vegetative balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other substances. It possesses several biological activities such as antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant tissue regenerative, anaesthetic cytostatic.

However, the chemical composition and biological activity of propolis are highly variable depending on the geographical origin of this natural substance. Polyphenolic chiefly flavonoids are considered the primary biological active substances in propolis. Some of these flavonoids are considered antimicrobial, such as pinocembrin, galangin and sakuranetin. More recently, western researchers have investigated the antibacterial properties of this material. Propolis was active in vitro against some gram positive bacteria and tubercle bacillus. It also demonstrated limited activity against gram negative bacilli.

The aim of this study was to compare the effectiveness of propolis mouth washes (water and ethanolic extract) at different concentrations 0.5–1% with CHX 0.2% in their ability to reduce plaque accumulation and subsequent gingival inflammation.

MATERIALS AND METHODS

A- Preparation of Propolis Extracts

The raw propolis was collected from hives located in Sinjar (a small town to the North of Mosul City). The raw propolis was received in the form of hard greenish-brown lumps, chopped and extracted with water at pH 7.2 at room temperature for 5 days, then lyophilized. The watery extract (WEP) was prepared by resuspending 1 gm of the dried propolis in 100 ml of saline (1% solution) for five days, then other dilutions were prepared. The ethanolic extract (EEP) was prepared by dissolving 1 gm of lyophilized propolis in 100 ml of ethanol (95%). The solution was left to dry, then resuspended in saline at 1%, 10% ……etc.

B- Patient Selection

The patients participating in this study were dental students with at least 25 scorable teeth in good alignment with good gingival and periodontal conditions. Wisdom teeth were excluded, the volunteers having no any appliance or prosthesis, good medical history and not
taking any medication that influence the conduct of the trial.

C- Study Design

A group of 10 (8 males and 2 females) dental students volunteers aged from 20 – 24 years participated in this trial from College of Dentistry, University of Mosul. Two hundred eighty eight teeth and 1120 tooth surfaces were examined. The study was double-blind crossover design for individual subjects, each of six treatment regimens commenced on Saturday (day 1) and finished on Wednesday (day 5). These arrangements gave a washout period of at 2 days for each subject between each treatment.\(^{(20, 21)}\)

At (day 1), all volunteers received a final professional tooth cleaning, scaling and polishing with home care instruction that include three times daily tooth brushing with one dental flossing and were subsequently told to abstain all mechanical tooth cleaning effort for the next five days. They were asked to rinse, however, three times daily for one minute each time with 15 ml of distilled water mouth rinse or water extract of propolis 0.1%, 0.5% or ethanolic extract 0.5%, 1% or CHX 0.2%. On (day 5) the volunteers were exposed to a new clinical examination.

After two days wash out period, the volunteers were given professional tooth cleaning after which an additional 5 days test period was initiated. This pattern was repeated for each of the six mouthwashes.

D- Clinical Examination

All examinations were performed by one examiner. The presence of the amount of plaque was examined and scored by the use of the plaque index system (PI I).\(^{(22)}\) A plaque index was obtained from 1120 tooth surfaces (buccal, lingual, mesial and distal surfaces) of 280 teeth in 10 dental students volunteers.

E- Statistical Analysis

The statistical analysis in this study was the use of descriptive analysis (mean, standard deviation) and t–test was used to see the significant differences among the test groups at the level of 0.05.

RESULTS

Table (1) shows the plaque free surfaces before and after the use of different mouthwashes. It reveals higher plaque inhibition with CHX and ethanolic extract of propolis than other mouth rinses.

After 5 days of abstinence from all types of mechanical plaque control the mean individual PI I scores for all surfaces were 0.85 for ethanolic extract of propolis (1%), 0.97 for water extract of propolis (1%), 0.58 for CHX (positive control) and 1.24 for distilled water (negative control) as shown in Figure (1).

| Group | Pretreatment | Post Treatment |
|-------|--------------|----------------|
|       | Upper | Lower | Total | Upper | Lower | Total |
| DW    | 28    | 42    | 35    | 91    | 96    | 93.5  |
| EE (0.5%) | 30    | 37    | 35.5  | 94    | 95    | 94.5  |
| EE (1%)  | 19    | 26    | 22.5  | 69    | 73    | 71    |
| WE (0.5%) | 36    | 36    | 36    | 86    | 88    | 87    |
| WE (1%)  | 21    | 23    | 22    | 83    | 92    | 87.5  |
| CHX (0.2%) | 37    | 52    | 45.5  | 52    | 61    | 56.5  |

DW: Distilled water; EE: Ethanol extract; WE: Water extract; CHX: Chlorhexidine.

The statistical analysis (student’s test) revealed that CHX mouth rinse was more effective than water extract of propolis 0.5% and 1% and 0.5% ethanolic extract of propolis to inhibit plaque formation \((p < 0.05)\), while there is no statistical difference \((p > 0.05)\) between CHX and ethanolic extract of propolis 1% in inhibiting plaque formation as shown in Table (2).
Antiplaque activity of propolis and chlorhexidine

Figure (1): Antimicrobial effect of water extract and ethanolic extract of propolis compared with control groups

(DW: Distilled water; EE: Ethanol extract; WE: Water extract; CHX: Chlorhexidine)

Table (2): Comparative significance between different mouthwashes regarding their effect on dental plaque formation (PI I)

| Groups            | Mean ±SD | Standard Error | 95% CI | t-test | d.f | Significance (2 tailed) |
|-------------------|----------|----------------|--------|--------|-----|------------------------|
| DW-EE (1%)        | 0.23 ±0.39 | 0.13           | -0.14  | 1.7    | 4   | 0.161                  |
| DW-EE (0.5%)      | -1.4 ±0.62  | 0.28           | -0.4   | 0.63   | -0.50 | 4 | 0.64                  |
| DW-WE (1%)        | 0.12 ±0.47  | 0.21           | -0.46  | 0.7    | 0.5  | 4 | 0.58                  |
| DW-WE (0.5%)      | 0.15 ±0.21  | 9.8            | -0.12  | 0.42   | 1.5  | 4 | 0.04                  |
| DW-CHX(0.2%)      | 0.65 ±0.48  | 0.21           | 4.69   | 1.25   | 2.9  | 4 | 0.04                  |
| EE(1%)–EE(0.5%)   | 0.38 ±0.76  | 0.34           | -1.33  | 0.57   | -1.1 | 4 | 3.31                  |
| EE(1%)-WE(1%)     | -0.11 ±0.05  | 0.24           | -0.79  | 0.57   | -0.45 | 4 | 0.67                  |
| EE(1%)-WE(0.5%)   | -0.86 ±0.26  | 0.11           | -0.41  | 0.23   | -0.73 | 4 | 0.50                  |
| EE(1%)-CHX(0.2%)  | 0.41 ±0.39  | 0.17           | -3.7   | 0.89   | 2.35  | 4 | 0.07                  |
| EE(0.5%)-WE(1%)   | 0.26 ±0.38  | 0.17           | -0.21  | 0.74   | 1.55  | 4 | 0.19                  |
| EE(0.5%)-WE(0.5%) | 0.29 ±0.02  | 0.26           | -0.45  | 1.04   | 1.09  | 4 | 0.33                  |
| EE(0.5%)-CHX(0.2%)| 0.79 ±0.64  | 0.28           | -8.9   | 1.59   | 2.74  | 4 | 0.52                  |
| WE (1%)-WE (0.5%) | 2.6 ±0.41  | 0.18           | -0.48  | 0.53   | 0.14  | 4 | 0.89                  |
| WE (1%)-CHX(0.2%) | 0.52 ±0.35  | 0.15           | 8.68   | 0.96   | 3.32  | 4 | 0.02                  |
| WE (0.5%-CHX(0.2%)| 0.49 ±0.29  | 0.15           | 0.12   | 0.86   | 3.37  | 4 | 0.02                  |

DW: Distilled water; EE: Ethanol extract; WE: Water extract; CHX: Chlorhexidine

d.f: Degree of freedom; SD: Standard deviation

DISCUSSION

The polyphenolic compounds are defined as compounds having molecular weights between 500–3000 and beside giving usual phenolic reactions they possess gelatin properties such the ability to precipitate the gelatin and other proteins.23 Polyphenolic chiefly flavonoids are considered the primary active substances in propolis. Some of these flavonoids possess antimicrobial activity such as...
Antiplaque activity of propolis and chlorhexidine

...pinocembrin, galangin and sakuranetin.

The presence of many disadvantages of CHX such as tooth and anterior filling staining and bitter taste make researchers searching for new potent antiplaque agent with minimum or no side effects.

In this study CHX rinse brought about a higher effect on preventing plaque accumulation than water extract of propolis 0.5 and 0.1% (p<0.05). This result is in the vicinity of those reported by Abbas et al.\(^{21}\) regarding the effect of sanguinarine (blood root plant) and Al–Naimi\(^{24}\) regarding the effect of myrtus communis and quercus infectoria on plaque accumulation.

These findings may be attributed to the fact that both the potency and substantivity of CHX is higher than that of propolis extracts.

Although the plaque containing tooth surfaces were higher in those using ethanolic extract of propolis than those using CHX there was no statistical differences among them. This result in not in consistent with that done by Abdul–Rahman\(^{19}\) as showed that the water extract of propolis 1% was more effective in inhibiting growth of Streptococcus mutans in comparison with ethanolic extract in vitro. This may be attributed to differences in both environmental and the type of bacteria forming the dental plaque.

The results of this study showed that the ethanolic extract of propolis exert better antibacterial activity than that of water extract and this may be due to the ability of alcohol to dissolve the active ingredients of the propolis which makes the alcoholic extract of the propolis more potent than boiling water extract.

CONCLUSIONS

Propolis water extract reduce plaque accumulation but their effect is less than that of ethanolic extract and CHX. No statistical difference in PI I between CHX and ethanolic extract of propolis was found.

Therefore, the ethanolic extract of propolis may be used as adjuncts to mechanical plaque control during the maintenance phase of periodontal therapy to ensure sustained low plaque level and this may meet patients approval because it is a natural material and devoid of industrial chemical component.

REFERENCES

1. Zambon JJ. *Actinobacillus actinomycetemcomitans* in human periodontal disease. *J Clin Periodontol*. 1985; 12: 1-20.
2. Slots J, Emrich J, Genco RJ, Rosling BG. Relationships between some subgingival bacteria and periodontal pocket depth and gain loss of periodontal attachment after treatment of adult periodontitis. *J Clin Periodontol*. 1985; 12: 540-552.
3. Greenstein G, Polson A. Microscopic monitoring of pathogens associated with periodontal diseases: A review. *J Periodontol*. 1985; 56: 470-474.
4. Quirynen M, Marechal M, Van Stenenhege S. Comparative antiplaque activity of sanguinarine and chlorhexidine in man. *J Clin Periodontol*. 1990; 17: 223-227.
5. Jones CG. Chlorhexidine: It is still the gold standard. *Peridontol*. 1997; 15: 55-62.
6. Pulcher JJ, Daniel JC. The effect of chlorhexidine digluconate on human fibroblasts in vitro. *J Periodontol*. 1992; 63: 526-532.
7. Bassetti C, Kallenberg A. Influence of chlorhexidine rinsing on the healing of oral mucosa and osseous lesions. *J Clin Periodontol*. 1980; 7: 443-456.
8. Al–Talib R, Abdullah B, Al–Khateeb A. A clinical comparison of an antibacterial mouth rinses in an orthodontic patients. *J Iraqi Dent Assoc*. 2002; (under press).
9. Metzuner J, Schneidewind EM. Studies on the question of potentiating effects of propolis constituents. *Pharmaize*. 1978; 33(4): 465. (English Abstr)
10. Ghisalberti EL. Propolis: A review. *Bee World*. 1979; 60: 59-84.
11. Bankova V, Christor R, Stoey G, Popov S. Determination of phenolics from propolis by capillary gas chromatography. *J Chromatol*. 1992; 607: 150-153.
12. Grange JM, Davey RW. Antibacterial properties of propolis (bee glue). *J R Soc Med.* 1990; 83: 159-161.
13. Parky K, Koo MH, Abrey JAS, Ikegaki M, Cury JA, Rosalen PL. Antimicrobial activity of propolis on oral microorganisms. *Curr Microbiol.* 1998; 36: 3-10.
14. Villanueva VR, Bogdanovsky D, Barbier M, Gonnet M, Lavie P. Sur l'identification d'un 193,3,5,7-trihydroxy flavon (galangine) a partir de la proplis. *Ann Inst Pasteur (Paris).* 1964; 118: 84-107. Cited by: Koo H, Gomes B, Rosalen P, Anbrosano G, Park Y, Gury J. *In vitro* antimicrobial activity of propolis and Arnica montana against oral pathogen. *Arches Oral Biol.* 2000; 45: 141-148.
15. Metzner J, Bekemeier H, Paintz E, Schneidewind E. Zur antimikrobiellen wirksamkeit von propolis and propolisnhatstoffen. *Pharmazie.* 1979; 34: 79-102. Cited by: Koo H, Gomes B, Rosalen P, Anbrosano G, Park Y, Gury J. *In vitro* antimicrobial activity of propolis and Arnica montana against oral pathogen. *Arches Oral Biol.* 2000; 45: 141-148.
16. Oswa K, Yasuda H, Moruyama T, Morita H, Takeya K, Itokarra H. Isoflavonones from the heartwood of Swatzia polyphylla and their antibacterial activity against cariogenic bacteria. *Chem Pharm Bull.* 1992; 40: 2970-2974.
17. Koo H, Gomes B, Rosalen P, Anbrosano G, Park Y, Gury J. Effect of apis millefera propolis on the activities of streptococcal glucosyltransferase in solution and absorbed on to saliva coated hydroxyapatite. *Caries Res.* 2000; 34: 418-426.
18. Ibrahim FH, Hamdi ER, Osamah C. Typically applied water extract of propolis to suppress corneal neovascularization in rabbits. *Ophthal Res.* 1999; 31: 426-431.
19. Abdul–Rahman Ghy. Antimicrobial effect of propolis on *Streptococcus mutans*. Al–Rafidian Dent J. 2001; 2(Sp Iss): 299-303.
20. Hellden L, Comosci D, Hock J, Tinanoff N. Clinical study to compare the effect of stiamous fluoride and chlorhexidine mouth rinse on plaque formation. *J Clin Periodontol.* 1981; 8: 12-18.
21. Abbas DK, Thrane P, Othman SJ. Effectiveness of veadent as plaque inhibiting mouthwash. *Scand J Dent Res.* 1985; 93: 494-497.
22. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand.* 1964; 22: 121-135.
23. Bate–Smith EC, Swain T. In Comparative Biochemistry. (eds: Mason HS, Florkin AM). Vol III, Academic Press, New York. 1962; p: 460.
24. Al–Naimi WH. The effect of mouthwashes extracted from myrtus communis and querucus infectoria on dental plaque formation. MSc Thesis. College of Dentistry. University of Baghdad. 1995.

Received: 10/12/2002
Accepted for Publication: 21/1/2003