Comparative antiplaque activity of propolis extract and chlorhexidine *in vivo*

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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.

الخلاصة

تضمنت هذه الدراسة السريرية مقارنة فعالية الغسولات الفموية (كلورهيكسيدين كلوكونيت ٠.٢%، مستخلص العكبر المائي ٠.٥% و ١% ومستخلص العكبر الكحولي ٠.٥% و ١%) مع الماء المقطر في تقليل تكوين الصفيحة الجرثومية .

شملت هذه الدراسة ١٠ متطوعين (٨ ذكور و ٢ إناث) من طلبة كلية طب الأسنان/جامعة الموصل تراوحت أعمارهم ما بين ٢٠–٢٤ سنة وتم فحص ٢٨٠ سن و١١٢٠ سطح سني.

تم إزالة الصفيحة الجرثومية المتكونة على سطوح الأسنان من قبل المختص مع إعطاء إرشادات وتعليمات بخصوص العناية بالفم ، ثم طلب من المتطوع بالتوقف عن كافة الفعاليات التي تساهم برفع ومنع تكون الصفيحة الجرثومية لمدة خمسة أيام مع استعمال الغسولات الفموية الخاضعة للدراسة خلال هذه الفترة ثلاث مرات يومياً.

استعمل مؤشر الصفيحة الجرثومية حسب (Silnes and Löe, 1964) لقياس تراكم الصفيحة الجرثومية على سطوح الأسنان الداخلة في الدراسة قبل وبعد استعمال الغسولات الفموية.

أظهرت نتائج هذه الدراسة اختلافا معنويا بين كلورهيكسيدين كلوكونيت ٠.٢%، مع المستخلص المائي لمادة العكبر ٠.٥ و ١%، المستخلص الكحولي لمادة العكبر ٠.٥% والماء المقطر، كما بينت هذه الدراسة عدم وجود اختلاف معنوي بين كلورهيكسيدين كلوكونيت ٠.٢% مع المستخلص الكحولي لمادة العكبر ١% في قابليتها على منع تكون الصفيحة الجرثومية.

يمكن الاستنتاج من هذه الدراسة حول إمكانية استخدام الغرغرة المستخلصة كحوليا من مادة العكبر كعامل مساعد إلى جانب استخدام الفعاليات الميكانيكية الأخرى مثل الفرشاة ومعجون الأسنان للسيطرة على تكون الصفيحة الجرثومية وإبقائها في المستوى الأدنى وهذا يتوافق مع تقبل المريض لهذه المادة لكونها طبيعية وخالية من المكونات الكيميائية الصناعية.

INTRODUCTION

Although there is an overwhelming amount of data of favouring the specificity of periodontal infections,[1–3] 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.[4]

Previous studies have shown that chlorhexidine (CHX) is an effective antiplaque agent.[5] Unfortunately, the toxic qualities of CHX do not reserved 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.[6] In addition to that, CHX application directly to surgical wounds in the oral cavity can delay and alter wound healing.[7] 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.[8]

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.[9] It possesses several biological activities such as antibacterial, antiviral, antifungal, antiinflammatory, antioxidant tissue regenerative, anaesthetic cytostatic.[10–12]

However, the chemical composition and biological activity of propolis are highly variable depending on the geographical origin of this natural substance.[13] Polyphenolic chiefly flavonoids are considered the primary biological active substances in propolis.[12] Some of these flavonoids are considered antimicrobial, such as pinocembrin, galangin and sakuranetin.[14–16]

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.[17]

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.[18]

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.[19]

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 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 once 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 (Pl 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 Pl 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).

Table (1): The percentage of plaque containing surfaces before and after mouth rinses usage

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).

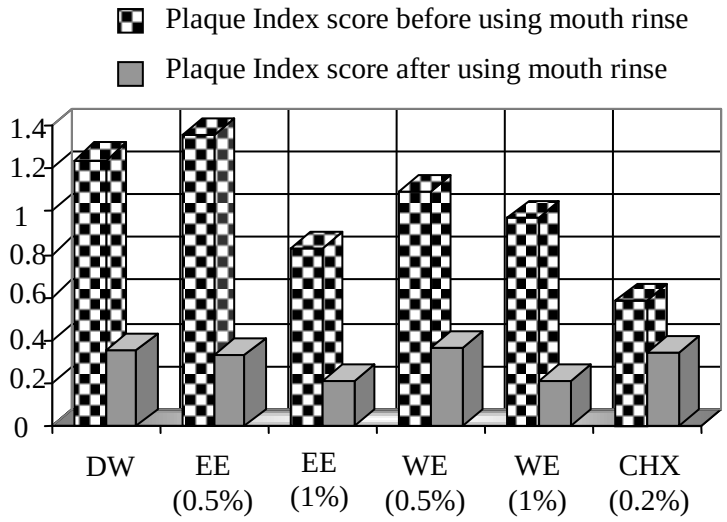

Figure (1): Antimicrobial effect of water extract and ethanolic extract of propolis compared with control groups

(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 (Pl I)

Groups	Mean	±SD	Standard Error Mean	95% CI		t-test	d.f	Significance (2 tailed)
				Upper	Lower			
DW-EE (1%)	0.23	0.39	0.13	-0.14	0.62	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	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

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 querucus infectoria on plaque accumulation.

These findings may be attributed to the fact that both the potency and substaintivity 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 Pl 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.

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