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Antibacterial Effect of Local Propolis Extracts and Pastes Against Enterococcus Faecalis

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الخلاصة

الأهداف:ان الهدف من هذه الدراسة هو البحث عن نشاط المستخلص الايثانولي(30%)(40%) والمستخلص المائي لعكبر النحل ضد بكتريا السبحياتالبرازية و كذلك البحث في نشاط المعجون التحريبي المحضر باستخدام المستخلص الايثانولي(30%) و المعجون التحريبي المحضر باستخدام المستخلص المائي للعكبرواجراء المقارنة مع نشاط ماءات الكالسيوم المستخدم كعلاج داخل قناة الجذر. **المواد وطرائق العمل** :تم قياس نشاط المستخلص الايثانولي والمائي للعكبر باستخدام طريقة الكدورة وذلك بقياس كمية الضوء الممتص بواسطة الخلايا البكتيرية باستخدام جهاز المطياف،وتم قياس نشاط المعجون التحريبي ومقارنته مع ماءات الكالسيوم داخل الاسنان المقلوعة بطريقة عد المستعمرات البكتيرية. **النتائج**:اثبتت النتائج ان جميع المستخلصات والمعاجين المحضرة باستخدام العكبر لها تأثيرامضادا لبكتريا السبحياتالبرازية. **الاستنتاجات**:للعكبر المستخدم في البحث تأثير مضاد لبكتريا السبحياتالبرازية سواء كان على شكل مستخلص او ضمن تركيب المعجون التحريبي.

ABSTRACT

AIMS:The aim of this study was to determine the antibacterial effect of ethanolic and aqueous extracts and pastes of propolis against *Enterococcus faecalis* and compare it with that of calcium hydroxide (intracanal medicament) (in vivo and ex vivo). **MATERIALS AND METHODS**:The antibacterial effect of the 30%,40% ethanolic and aqueous extract of propolis as well as of calcium hydroxide were detected by the turbidity method. The turbidity of the bacterial cultures was measured by light absorption in spectrophotometer and the antibacterial effect of propolis pastes prepared from ethanolic extract as well as that prepared from aqueous extract was examined on teeth and compared with that of calcium hydroxide by colony counting method. The result were analyzed by ANOVA test and Post Hoc tests. **RESULTS**:All propolis extracts and pastes were effective against *Enterococcus faecalis*. **CONCLUSIONS**: The propolis has antibacterial effect against *Enterococcus faecalis* in its extract and paste forms.

Key words: Propolis, Intracanal medicaments, *Enterococcus faecalis*.

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INTRODUCTION

Bacteria are the main causative agent in the development of pulpal and periapical inflammation.[1] *Enterococcus faecalis*is gram positive facultative anaerobic bacterium commonly recovered from previously root-filled teeth with persistantperiapical lesion.[2]Its ability to invade dentinal tubules is considered within the factors that enable this bacterium to be a persisting endodontic pathogen.[3]The major reduction of bacteria in the root canal is achieved mechanically by endodontic files and chemically by irrigation with several substances.[4] To optimize the disinfection of the root canal system, the use of intracanal medicament is needed.[5] Calcium hydroxide is one of the most commonly used intracanal medicament.[6] The buffering action of dentine neutralizes the action of calcium hydroxide at deeper layer of dentinal tubules resulting in the survival of microorganism.[7] Therefore, research for new substance is necessary and propolis may be one of the choices.[8] Propolis or bee glue, as commonly named, is a natural resinous mixture produced by honey bees from substances collected from parts of plant, buds and exudated.[9] In

general, propolis composed of 50% resin and vegetable balsam,30% wax,10% essential and aromatic oils,5%other substances including organic debris.[10] Propolis has been used in folk medicine since it has many biological properties such as antimicrobial, anti-inflammatory ,antioxidant activities.[11] Biological activities of propolis are directly related to its chemical composition .[12]The most important pharmacologically active components in propolis are flavonoids, phenolics and aromatics.[13] In recent studies, propolis and some of its cinnamic and flavonoid components were found to be traducing cytoplasmic membrane of bacteria and inhibits bacterial mobility, which may contribute to the antimicribial action .[14]

Aims of the study

1. Evaluate the antibacterial effect of propolis based paste as intra-canal medicaments against *E. faecalis*.
2. Compare the antibacterial effect of propolis based paste with that of calcium hydroxide paste.
3. Determine the antibacterial effect of ethanolic and aqueous extract of propolis against *E. faecalis*.

MATERIALS AND METHODS

Ethanolic extract of propolis

Propolis sample was obtained from Al-Quba town in Mosul city, Propolis sample was cut into small pieces and extracted by dissolving 30 gm of propolis in 70%ethanol and completing the volume to 100 ml of 70%ethanol to obtain 30%ethanolic extract ,and 40 gm of propolis was dissolved in 70%ethanol and completing the volume to 100 ml of 70%ethanol to obtain 40%ethanolic extract of propolis, then these samples were kept in dark bottle at room temperature and after seven days with intervals shaking, extracts were filtered with filter papers and ready to use for this study.[2]

Aqueous extract of propolis

Fifty grams of propolis sample(that was cut into small pieces)was added to 100 ml of distilled water and left to boil for 60 minutes then down to room temperature ,extracts were filtered and ready to use for this study.[15]

Preparation of experimental pastes

Ethanolic extract propolis paste was prepared by mixing the following (12.5 gm of 30%ethanolic extract of propolis , 22 gm calcium hydroxide powder , 7 gm propylene glycol and 10 gm distilled water).The mixture transferred into homogenizer for good mixing after that put on the vortex for make it more homogenous .The paste incubated at 37^{o}C for 24 hrs. The same steps were followed in the preparation of aqueous extract propolis paste but adding aqueous extract of propolis instead of ethanolic extract of propolis.[16]

Bacterial strain

Enterococcus faecalis cultures used in this study was acourtesy from previous researcher.[17] The cultures were refreshed in 4ml Brain heart infusion broth(BHI broth) incubated at 37^{o} C for 24 hrs , a loopfull from the BHI broth was streaked on petri dish of *Enterococcus faecalis* medium(HiMedia Lab. Pvt.Ltd.) , and Incubated for 24hrs at 37^{o}C;then fifteen colonies from this agar taken and inoculated in 4ml BHI broth incubated at 37^{o}C for 4 hrs .

Determination of antimicrobial effect of propolis extracts

Turbidity method was used to determine the antimicrobial effect of the tested material,In summary the method was dependent on measuring the amount of absorded light passing through solution,light absorption is assumed to be abscent when transmitted through a clear solution ,however when the solution loss its

clearity and become turbid as microbes grow in it,the light absorption will be changed. Light absorption was measured using spectrophotometer at wave length of 540 nanometer.[5] This method was achieved by using a series of test tubes containing an equal amount of sterilized BHI broth(2ml),they were divided according to the tested materials .So as for each tested material one tube contained broth and (0.1)ml of the tested material and three tubes contained (0.1)ml of tested material and (0.1)ml of 24 hours bacterial

growth suspension and another three tubes contained only (0.1)ml of bacterial suspension.Then these test tubes were incubated at 37^0 C for 24 hrs.The turbidity of each test tube was measured using a spectrophotometer at 540 nanometer .[18]

The antibacterial effect of propolis pastes

Preparation of the samples

Fifty extracted human permanent single rooted teeth with mature apices werecollected, the root surface were cleaned with a curette, stored in distilled water to prevent dehydration. All the teeth were decoronated with a safe-sided diamond-disk, and root length was standardized to 13 mm. All root canals were prepared by protaper rotary system to a length 1mm from the apical foramen(working length 12mm). During instrumentation ,each root canal was irrigated with 2%NaOCl solution using diposable syringe between each file to prevent canal blockage.After instrumentation each root canal was irrigated with 17%EDTA followed by 5.25% NaOCl to remove inorganic and organic debris.The apical foramen was sealed with epoxy resin to prevent bacterial leakage and the external root surface was coated with two layers of nail varnish .The roots for each experimental group were embedded in silicone impression material to the level of cervical border of the root.After complete setting of the impression material .the embedded roots were covered with aluminum foil,and then adapted in stainless steel box which also covered with aluminum foil and placed in the autoclave at 121^0C for 15 min for sterilization.[19]

Inoculation of root canals with E.faecalis

The root canals were dried with sterilized paper points and injected with 0.1 ml bacterial suspension of bacterial colonies was injected into each root canal and incubated at 37^0C for 3 days.[20]

Disinfection of samples

The application of intracanal medicaments (ethanolicpropolis paste, aqueous extract propolis paste and calcium hydroxide) was done by the use of sterilized absorbent endodontic paper points. Fifteen paper points were saturated with each medicament to length of 12mm(the woking length)and then inserted into the root canals, the samples were sealed with aluminum foil. Then each group was subdivided into three subgroups of five samples and incubated for different experimental time of 1,3 and 7 days.with noted that five samples left without any medicaments and considered as control group.

Microbiological sampling from treated root canal

After each type of treatment ,sample was taken from each root canal using sterilized K-type file size 45 which was inserted inside the root canal to full working length.then the file is rotated 360 in clock wise direction for engagement of dentin. The sample was transferred immediately to tube containing 1ml BHI broth. The solution was shaked for 20 second, then was cultured on agar plate, culturing was done by spreading using swab on Enterococcus agar and incubated at 37^0C for 24 hrs.[21] After 24hrs of incubation ,the number of bacterial colonies were counted, then multiplied by the dilution factor.

The following statistical methods were used to analyze and assess the results via

SPSS V.11.5 for Windows:

1. Descriptive statistics include , mean , standard deviation, standard error.
2. Analysis of variance (ANOVA) was used to find the effect of different variables followed by Duncan test to find the best treatment.

RESULTS

All the tested materials (propolis extracts and calcium hydroxide) show antibacterial effect against *Enteroccocus-faecalis* with no significant difference between 30%ethanolic extract and calcium hydroxide after 24 hrs. A s shown in Figure (1) and Table (1). The three pastes show significant effect against *Enterococcus faecalis*, with no significant difference

between them during different experimental time. As shown in Figure(2), Table (2), Table (3),and Table (4).

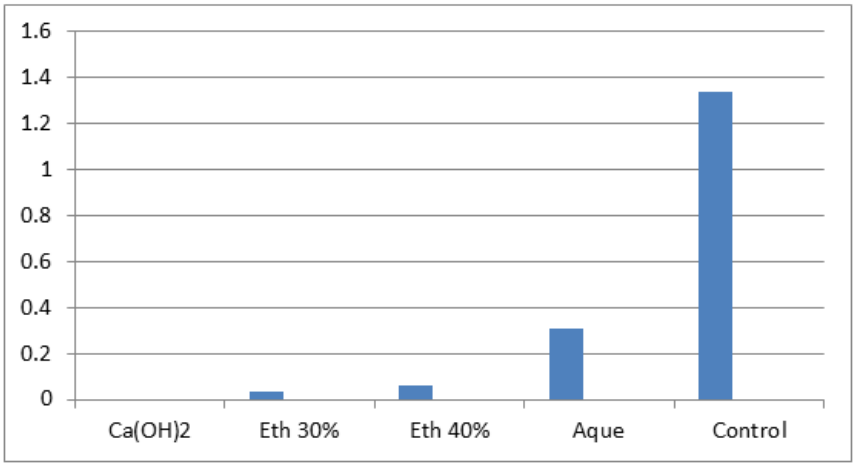

Figure (1): Histogram illustrating the antimicrobial effect of propolis extracts.
*Ca(OH)2 =Calcium hydroxide paste
*Eth 30% =30% ethanolic extract of propolis
*Eth 40% =40% ethanolic extract of propolis
*Aque =Aqueous extract of propolis

Table (1):Comparison between the antibacterial effect of propolis extracts and calcium hydroxide

	N	Mean ±Std.Deviation	Std.Error	Duncan*
Cont	3	1.33700 ± .009644	.005568	D
30%EEP	3	.03400 ± 00.33464	.002000	A
40%EEP	3	.05800 ±.007211	.004163	B
AEP	3	.30733± .040266	.023247	C
$Ca(OH)_2$	3	.00000±.00000	.00000	A
Total	15	.34727± .524695	.135476	

*Different letters mean signifigant differences

Table (2):Antibacterial effect of ethanolic extract propolis pastes

	N	Mean ±Std.Deviation	Std.Error	Duncan*
Cont	5	6780.00 ± 1848.513	826.680	B
7days	5	160.00 ±114.018	50.990	A
3days	5	320.00 ± 376.829	168.523	A
24 hrs	5	980.00 ± 683.374	305.614	A

*Different letters mean signifigant differences

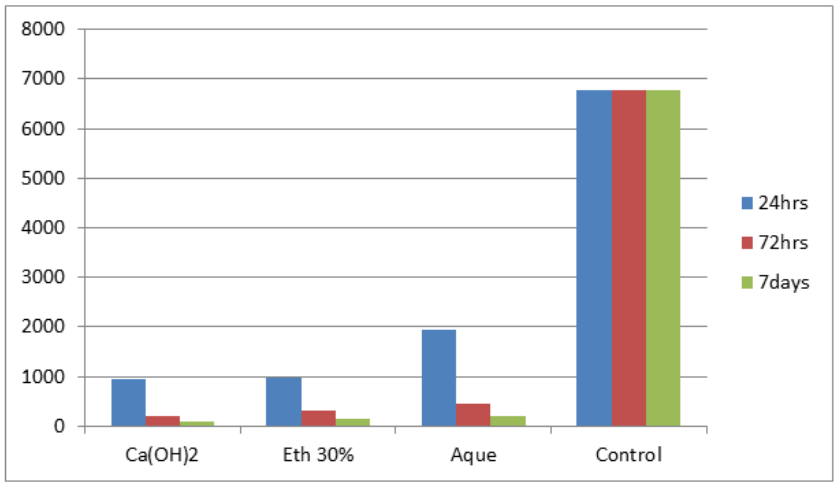

Figure (2): Histogram illustrating the antibacterial effect of propolis pastes
*Ca(OH)2 =Calcium hydroxide paste
*Eth 30% =30% ethanolic extract propolis paste
*Aque =Aqueous extract propolis paste

Table (3):Antibacterial effect of aqueous extract propolis paste.

	N	Mean ±Std.Deviation	Std.Error	Duncan*
Cont	5	6780.00 ± 1848.513	826.680	C
7days	5	200.00 ± 100.00	44.721	A
3days	5	440.00 ± 207.364	92.736	A
24 hrs	5	1940.00 ± 630.872	282.135	B

*Different letters mean signifigant differences

Table (4):Antibacterial effect of calcium hydroxide paste.

	N	Mean ±Std.Deviation	Std.Error	Duncan*
Cont	5	6780.00±1848.513	826.680	B
7days	5	100.00±70.711	31.623	A
3days	5	200.00±200.000	89.443	A
24 hrs	5	940.00±250.998	112.250	A
Total	20	2005.00±2975.421	665.324	

*Different letters mean signifigant differences

DISCUSSION

The in vitro antibacterial activity of local propolis (ethanolic and water extracts) was evaluated against most resistant bacteria isolated from failed endodontic case.[12] The facultative anaerobic *Enterococcus faecalis* are frequently found in case of failure of endodontic treatment and has shown resistant to calcium hydroxide(the most common used intracanal medicament)in the deeper layer of dentinal tubules due to the buffering action of dentin that neutralize the action of calcium hydroxide.[5] The resinous hive product has been used as a remedy for treatment of many diseases in folk medicine since ancient time, many studies showed the inhibitory effect of propolis

extract on some important virulence factors of many microorganism.[14]

Oncag et al(2006)observed that propolis has good in vitro antibacterial activity against *Enterococcus faecalis* in the root canal of extracted teeth,suggesting that it could be used as intracanl medicament.[22] Rammani and Mathew (2012) showed that propolis when used as intracanal medicament was effective against E.faecalis .[23] In another earlier study , Kujumgiev et al. (1999) showed that 10% propolis was effective against all tested microormanisms including E.faecalis.[24] Kustarci et al.(2011) showed that all propolis extracts were showed antimicrobial effect against all tested microorganism including E.faecalis.[25] Bolla et al.(2012) showed that propolis was effective against E.faecalis.[26] All these results are in an agreement with our findings. the finding of Kousedghi et al.(2012) suggest that propolis could be mixed with calcium hydroxide as anantibacterial agent and used as an intracanal dressing material.[27]

CONCLUSION

As conclusion both ethanolic and aqueous extract of local propolis show good antibacterial activity against *Enterococcus faecalis,*which enable the to be chosen as intracanal medicament.And both ethanolic and aqueous extracts propolis paste show significant antibacterial effect with no significant difference with that of calcium hydroxide.

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