

Antimicrobial effect of propolis on *Streptococcus mutans*

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ABSTRACT

We choose two types of propolis extracts; watery and ethanolic; to estimate their activity against the most cariogenic microorganism; *Streptococcus mutans*, and evaluate this activity *in vitro* compared with chlorohexidine and tetracycline as negative controls, using the turbidity method. Both watery extract of propolis -WEP- and ethanolic extract of propolis -EEP- show good inhibitory effect with significant variance compared with chlorohexidine, which also had better effect than tetracycline against *Streptococcus mutans*. The WEP at (1%) was the best antimicrobial solution in this study.

Key Words: Propolis, antimicrobial agent, *Streptococcus mutans*.

الخلاصة

مادة العكبر ينتجها النحل ويستخدمها في لصق خلاياه لتقيته الكثير من الاصابات الجرثومية والفطرية. تم استخدام نوعين من المستخلصات هما المستخلص المائي والكحولي لمادة العكبر كمادة مضادة لجرثومة المكورات السبحية الطافرة المسبب الرئيس لتسوس الأسنان، وكان المستخلص المائي (١%) ذو فعالية مضادة للجرثوم المعزول من حالات نخر الأسنان وبفارق معنوي عالي عن مادة الكلور هيكسيدين، وكان تأثير المستخلص الكحولي جيداً عند (١٠%).

INTRODUCTION

When bees started to gather in hives some (50) million years ago; they had a problem; the same problem that no doctor likes to face: "how do you avoid bacterial infection when (50,000) bodies are crawling over one another night and day?" ⁽¹⁾.

Certain trees such as poplar, willow, horse chestnut and birch; exude a sap which has antibiotic qualities to protect that tree from infection.

Bees gathered this sap, metabolized it and took it back to the hive, they put it all over the hive so every bee could brush against it and become immunized ⁽²⁾.

Propolis is a natural resinous product which is a sticky mass, greenish-brown in color with a slight aromatic odor, contains (50%) resin and vegetative balsam, (30%) wax, (10%) oils, (5%) pollen and (5%) other substances.

The ethanolic extracts of propolis -EEP- exhibit a range of pharmacological activities, such as antimicrobial, antiinflammatory, anesthetic, and cytostatic properties ⁽³⁾. Polyphenolic, are considered the primary biological active substances in propolis ⁽⁴⁾.

However, the chemical composition and biological activity of propolis are highly variable depending on the geographical origin of this natural substance ⁽⁵⁾.

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The microorganisms play an essential role in the pathogenesis of dental caries and consequently provide a prime target for the prevention of caries by antibiotics or vaccines.

Streptococcus mutans is the most cariogenic microorganism, responsible for caries through their production of insoluble glucans and various fermentation activities⁽⁶⁾.

Our aim in this study is to evaluate the antimicrobial activity of the watery and ethanolic extracts of propolis against the *Streptococcus mutans in vitro*.

MATERIAL 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⁽⁷⁾.

The watery extract (WEP) was prepared by resuspending (1) gm of the dried propolis in (100) ml of saline (1% solution); then other dilutions prepared.

The ethanolic extract (EEP) was prepared by dissolving (1) gm of the lyophilized propolis in (100) ml of ethanol (95%), the solution was leaved to dry, then resuspended in saline at (1%), (10%), etc⁽⁸⁾.

B. Antimicrobial study

The antimicrobial activities of WEP and EEP against *Streptococcus mutans*; (18) hours cultures of the bacteria, which isolated from dental plaque specimen at the College of Dentistry used for this aim.

To each (4) ml of Brain Heart Infusion broth BHI (oxoid), we add (0.1) ml of the (18) hours *Streptococcus mutans* growth; and (0.1) ml of the examined solution (0.1% and 1% of WEP, 1% and 10% of EEP; 0.25% of chlorohexidine and 5% of tetracycline); chlorohexine and tetracycline were used as control treatments, where both were antibacterial agents used in dental clinics.

Cultures were incubated at (37) °C for (48) hours at least three replicates of each treatment were inoculated as well as the normal or untreated microorganism culture.

The turbidity or the absorbance of the cultures were determined by the turbidity method⁽⁹⁾, using spectrophotometer at (550) nm^(10,11).

C. Statistical analysis

The results (absorbance values) were tested for their significance by Duncan test at ($p < 0.05$) probability.

RESULTS AND DISCUSSION

Table (1) shows the mean values of the optical density or absorbance by the spectrophotometer, analyzed statistically and the data from the examined WEP and EEP solutions were compared with the same treatments by chlorohexidine and tetracycline, we found that chlorohexidine was better than tetracycline as anti-*Streptococcus mutans* agent.

Table (1): The analysis of absorbance values of different treatments against *Streptococcus mutans*

Treatment	Mean Absorbance	\pm Standard Deviation	Duncan's Groupings
Control + ve (<i>Streptococcus mutans</i> alone)	1.400	± 0.100	A*
Chlorohexidine	0.613	± 0.015	C
Tetracycline	0.920	± 0.100	B
WEP 1%	0.113	± 0.057	E
WEP 0.1%	0.633	± 0.030	C
EEP 1%	0.483	± 0.156	CD
EEP 10%	0.346	± 0.155	D

* Different letters mean significant differences.

The WEP at (1%) was the best treatment among the four solutions used (1% and 0.1% of WEP, 1% and 10% of EEP) and statistically better than chlorohexidine and tetracycline, also we found good antimicrobial activity for the (10%) EEP, where the (0.1%) WEP and (1%) EEP had the same effect as chlorohexidine (figure 1).

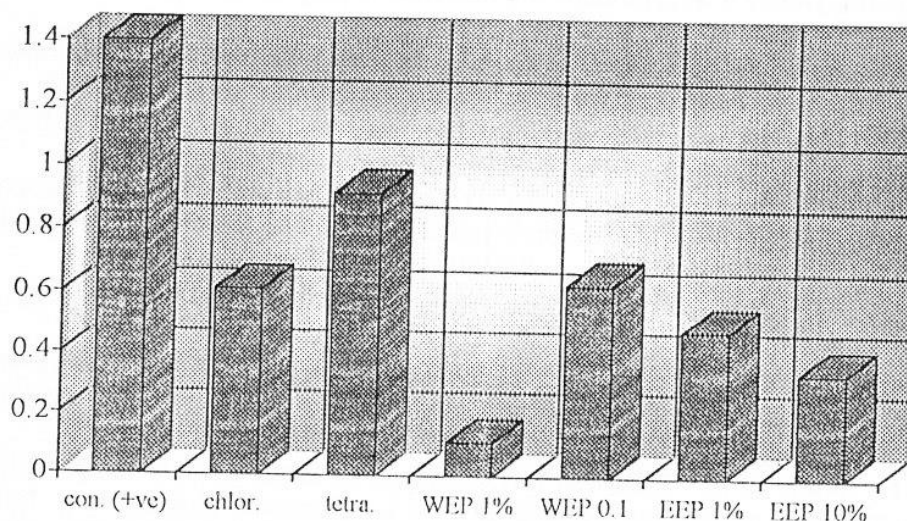


Figure (1): Antimicrobial effect of WEP and EEP compared with control treatments

A number of studies were done to evaluate the anti-inflammatory effect of propolis⁽¹²⁾ allergic effect⁽¹³⁾, antibacterial activity^(1, 14) and anti caries effect on Laboratory animals⁽¹⁵⁾.

The majority of these researches used the ethanolic extract of propolis (EEP) which gave a good inhibitory or anti fungal or anti inflammatory action ; also they found that the effect of EEP was mainly against the glucosyltransferase enzymes produced by *Streptococcus mutans*^(5, 11, 15).

Some of the flavonoids in propolis were considered to be antimicrobial agents, also pinocembrin and galangin⁽⁵⁾.

We suggest the (1%) solution of watery extract WEP as the simplest and the most effective antimicrobial suspension for the clinical use in dental clinics, also we can say that propolis; could be a promising antiplaque / anticaries agent.

We suggest further studies to compare the different types of propolis in Iraq according to the geographic area, also to find the exact chemical composition in the water extract in order to use this solution in dentistry for irrigation in dental operation or as a mouth rinse by the patient himself.

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