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Original Article

In vitro evaluation of antibacterial effect of combination of honey and Aloe vera extract against *Enterococcus faecalis* and *Escherichia coli*

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ABSTRACT

Antimicrobial agents are used as intra-canal drugs or canal cleaners for complete dental root canal cleaning**.** This new study attempts to compare the antibacterial effect of 2% chlorhexidine and combination of honey and Aloe vera extract (H-Av mixture) against *Enterococcus faecalis* (*E.faecalis*) and *Escherichia coli* (*E.coli*) which are the most important pathogens isolated from infectious root canals. *Enterococcus faecalis* (ATCC® 29212™) and *Escherichia coli* (ATCC® 25922™) were cultured in the tryptic soy broth medium. Dilution of H-Av mixture by the method of macrodilution to determine Minimum Inhibitory Concentration was investigated. In this paper agar well diffusion and colony count methods were also used to ensure the accuracy of the results. The results were statistically analyzed by student's t-test. The significant level established at 5% ($P<0.05$). The results of the methods of macrodilution, colony count and Agar well diffusion confirm the inhibitory effect of H-Av mixture on *E.coli* and *E. fecalis*. Statistically, there was no significant difference between the antibacterial effect of 2% chlorhexidine and 25% H-Av mixture ($P>0.05$) Increasing drug resistance to antimicrobial compounds needs to study of new drugs against pathogens. H-Av mixture with benefits such as availability, good taste and easier use than chlorhexidine, and fewer side effects can be a good option for intracanal irrigation after clinical trials.

INTRODUCTION

Antimicrobial drugs are used for various purposes in dentistry. One of these goals is during root canal treatment. An infected root canal has a complex morphology that mechanical tools are not sufficient to completely disinfect the canal (1). Antimicrobial agents are used as intra-canal drugs or canal cleaners along with mechanical tools for complete canal cleaning. Sodium hypochlorite and 2% chlorhexidine are common detergents used for this purpose. However, their use has many side effects. Sodium hypochlorite can cause tissue toxicity, emphysema, and allergic reactions, as well as unpleasant odors and tastes (2). Chlorhexidine also changes tooth color, loss of taste, burning sensation and dry mouth (3).

In order to solve these problems, extensive research has been done to investigate the antimicrobial properties of natural agents. Aloe vera is a plant that has cosmetic properties and is a member of the Liliaceae family. Aloe vera gel contains 98% water and the rest contains antioxidants, minerals, flavonoids, amino acids and vitamins. The amount of these compounds differs depending on the species and conditions of plant growth (4). Its treatment range includes burn relief, as a laxative and immune system stimulant (5,6). Another property of Aloe vera is its antimicrobial potential against various microorganisms such as *Staphylococcus aureus* and *Mycobacterium tuberculosis* (7,8).

Honey is another example of natural agents used to treat various diseases. Research has shown that honey is effective against almost all infectious agents, and is also used to improve wound healing (9). The combination of honey and Aloe vera extract (H-Av mixture) in the form of syrup is available to strengthen the immune system and increase the body's resistance to viral diseases (10). Since the antibacterial effect of honey and Aloe vera have been shown in studies, there is not any study on the combined effect of honey and Aloe vera extract in this regard. The aim of this study was to investigate the antibacterial properties of H-Av mixture on *Enterococcus faecalis (E. Faecalis) and Escherichia coli (E. coli)*, which are the most important endodontic pathogens isolated from infectious root canals.

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MATERIALS AND METHODS

Ethical Considerations

This in-*vitro* experimental Study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethical code: IR.TBZMED.VCR.REC.1398.142).

Bacterial Strains Preparation

Enterococcus faecalis (ATCC® 29212™) and *Escherichia coli* (ATCC® 25922™) microorganisms were prepared from research organization for science and technology. The vials of these lyophilized microbes, which are in the form of compressed powders, cut under sterile conditions. The bacteria were cultured in test tubes containing tryptic soy broth medium (Ibresco,IR).

H-Av Mixture Preparation

H-Av mixture available in Hakim Honey Company, which according to the contents of the product, it contained 50% honey and 70% Aloe vera.

Antibacterial Susceptibility Testing

Macrodilution method

The macrodilution method, based on the Clinical and Laboratory Standard Institute (CLSI) protocol, was used to compare the effect of H-Av mixture and 2% chlorhexidine on bacteria and determining the Minimum Inhibitory Concentration (MIC). This method was investigated in 4 different concentrations (10%, 15%, 20% and 25%) of H-Av mixture on both pathogens, and for each species, two positive and negative control tubes were used as control groups (12 tubes in total). The positive control tube does not contain H-Av mixture and has a studied species and 2% chlorhexidine. The negative control tube also contains H-Av mixture and does not contain any microbes. The tubes were placed in incubator at 35˚C for 48 h. After this time, we checked the transparency and turbidity of each and cultured the microbes in each tube to ensure growth or non-growth. About 10 ml of the solution of each tube is cultured inside the plates containing blood agar medium. The growth rate of each species was assessed after 48 hours at 35˚C in incubator. Counting of colonies showed the effect of the H-Av mixture on pathogenic factors.

Agar Well Diffusion Method

The agar well diffusion was done in petri dishes with a diameter of 90 mm comprising Hilton agar molar (BBL 211438 Becton Dickinson, Sparks, MD, USA) to a depth of 4 mm for bacteria. Then a well (4 mm in deep and 6 mm in diameter) was cut in the midpoint of the agar. Sterile pipette was used to place 500 µl of 25% H-Av solution in each well. The plates were then incubated for 48 hours at 35 ° C. After incubation, the diameter of the growth inhibition zones was measured in mm using electronic calipers. Each experiment was repeated five times and the mean and standard deviation were calculated.

Statistical Analysis

The results were statistically analyzed by student's t-test. The software used in this study was SPSS.17 and P value less than 0.05 was considered significant.

RESULTS

The results of macrodilution methods were evaluated using colony counting that are presented in Table 1. The greatest effect of H-Av mixture was observed on E.fecalis, where this bacterium had no growth at 250 mg/ml (20%) and 300 mg/ml (25%) concentrations of H-Av mixture. *E.coli* also showed no growth at 300 mg/ml of this mixture. In agar well diffusion test (see Table 2), the diameter of inhibitory zone of H-Av mixture in plates of *E.fecalis* and *E.coli* were measured at 32 and 25 mm, respectively, indicating the extraordinary effect of this compound on the two species. Statistically, there was no significant difference between the antibacterial effect of 2% chlorhexidine and the H-Av mixture ($p>0.05$).

DISCUSSION

In this study, the antibacterial properties of H-Av mixture in the laboratory were investigated. The results showed that the H-Av mixture had a good inhibitory effect on the growth of *E.faecalis* and *E.coli*. This study is important because of the upward trend to use natural products in dentistry. The increase of these studies is due to the fact that plant extracts are safe for the body's health and are widely available. Both microorganisms that were examined in this study are bacteria that are abundant in periapical lesions. *E.faecalis* is a microorganism that may contaminate the root canals and may be present in the root canals failure than in primary infection cases (11). *E.coli* is present in infective root canals and is a standard organism used in antibacterial testing (12).

Studies have shown that aqueous and ethanolic extracts of Aloe vera are effective in inhibiting the growth of microorganisms (13-15). The internal mucosal mass of Aloe vera contains anthraquinone, which can be a factor in the antimicrobial properties of this plant (16). Agari et al. showed that extracts from internal jelly and leaves of Aloe vera have a good antimicrobial effect on clinical bacteria (13). However, in a study conducted by Ehsani et al., Aloe vera gel showed a weak antibacterial effect on *E.fecalis* in disc diffusion and microdilution tests (17).

Honey has antimicrobial properties due to its osmotic properties. This property inhibits bacterial growth by removing water from the bacterial cell. Honey contains the lysozyme, which is known to be an antibacterial agent. The flavanoid antibacterial agent, low pH, hydrogen peroxide concentration and phytochemical nature are other factors that cause the antimicrobial properties of honey (18,19). Wilkinson et al. studied the effect of 13 types of honey on *E.coli* bacteria and showed that honey has bacteriocidal and bacteriostatic properties on gram-negative and gram-positive bacteria (20). In another study that tested the effect of three types of honey on *E. coli*, it was shown that honey has

Table 1. Mean values of bacterial counts (cfu/ml)

Bacteria	Groups					
	H-Av mixture 100mg/ml (10%)	H-Av mixture 200 mg/ml (15%)	H-Av mixture 250 mg/ml (20%)	H-Av mixture 300 mg/ml (25%)	Positive Control (2%Chlorhexidine)	Negative Control group
E. coli	>100.000	>100.000	80.000±10.000	0	0	0
E.fecalis	50.000±10.000	18.000±2000	0	0	0	0

Table 2. The average of the diameter of inhibitory zone of bacteria in agar well diffusion test

Solutions \ Bacteria	E. coli	E.fecalis
25% H-Av mixture	25 mm	32 mm
2%Chlorhexidine	27 mm	29 mm
p-value	0.23	0.18

more antimicrobial properties than gentamicin (21). Widespread MICs from various honeys against the equal class of microorganisms have been stated, indicating differences in the antibacterial effects of different honeys. This difference can be owing to differences in the amount of growth of pathogens, the technique of testing and the origin of microorganisms. Another notable point is the difference in the regions. In study of mercan et al. that antibiotic activity of various honey samples in Turkey were investigated; honey from the Izmir region had the greatest effect on *E.coli* and *Staphylococcus aureus*; and honey produced from mugla had the greatest effect on *Candida albicans* (22). Tan et al. showed that honey is prepared from different sources, the antibacterial effects of which differ according to its origin and processing (23). There may also be a fact that the type of honey produced by bees depends on the plants and flowers that grow in different logics and different seasons, which makes a difference in the antibacterial effects of honey.

The results of colony count showed that H-Av mixture at a concentration of 25% completely inhibited the growth of the studied bacteria. In studies examining the antibacterial effect of Aloe vera and chlorhexidine against *E.faecalis*, chlorhexidine has shown better results than aloe vera (24-26). In the agar well diffusion test, H-Av mixture at a concentration of 25% did not show significant difference in the diameter of inhibitory zone compared to 2% chlorhexidine. In this method, the antibacterial solution is placed directly in front of the bacteria. This method resembles to the Disk-diffusion method and has the advantages of simplicity and low cost, with the difference that in the agar well diffusion method, more and more suitable space can be provided for the studied material. On the other hand, some disks are very expensive or some of them are very difficult to produce in the country (27).

Increasing drug resistance to antimicrobial compounds necessitates the study of new drugs against pathogens. H-Av mixture with benefits such as availability, good taste and easier use than chlorhexidine, and fewer side effects can be a good option as an antibacterial drug for intracanal irrigation. In future studies, it is recommended to evaluate the H-Av mixture as a cleanser or intracanal drug in the biofilm model before its clinical use.

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