The antibacterial effects of ellagic acid on Mutans Streptococci in comparison to Chlorhexidine

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ABSTRACT

Background: Ellagic acid is a nutraceutical contains a naturally occurring poly-phenol compound that has strong anti-oxidant and anti-carcinogenic properties. According to previous researches ellagic acid has anti-viral, anti-bacterial. Mutans streptococci are the most cariogenic microorganism responsible for initiation of dental caries. Ellagic acid is used in treatment of several diseases besides its effect on periodontal disease.

Materials and Methods: This study was conducted to test the effects of Pomegranate extracts (ellagic acid) on growth, adherence effects and acidogenicity of Mutants Streptococci.

Results: The results showed that all concentration of ellagic acid were effective in inhibition of Mutans Streptococci, The inhibition increased with increasing the concentration of ellagic acid, starting from 0.625 to 10 mg/ml. However the concentration of ellagic acid 5, 10 mg/ml showed stronger effects than chlorhexidine 0.2% and deionized water.

Conclusion: The effects of both 5, 10 mg/ml ellagic acid were tested on the viability counts of salivary Mutans Streptococci in vitro. The results showed that highly significant differences (P<0.001) were reported between 5, 10 mg/ml of ellagic acid when compared to control in the reduction of viable counts of Mutans Streptococci in vitro, on the other hand, the effective concentrations of ellagic acid 5 and 10 mg/ml were able to inhibit the adherence of mutants streptococci to teeth surface and to retard the process of acid production of mutans streptococci in vitro.

INTRODUCTION

The mouth is the mirror of health as the oral cavity is the inhabitant of thousands of microorganisms, Candida, bacteria and viruses. Neglecting may lead to prevalent diseases which are dental caries and periodontal diseases.

Mouth rinses are the simplest vehicles for anti plaque agents, the most common being a water alcohol mixture to which flavour, non-ionic surfactant and humectants are added to improve cosmetic properties, most anti plaque agents are compatible with this vehicle. Prevention mouth rinses have been clinically effective in reducing the plaque, inflammation and caries characteristic of an oral cavity with pathogenic balance of micro biota, and prevent oral ulceration.

Recently, there has been considerable interest in nutraceutical industry in preventive medicine in the quest for natural anti oxidant from plant material.

Ellagic acid is a naturally occurring poly phenolic constituent found in different fruits such as pomegranates that has strong antioxidant properties and potent anti carcinogen.

This study is the first Iraq was able to be found connecting effect of pomegranate extract (ellagic acid) on mutans streptococci. Results of this study showed that antibacterial effect of ellagic acid may inhibit the growth and adherence of these bacteria. However there is still a lack of information regarding the effect of ellagic acid on Mutans Streptococci and other cariogenic bacteria.

Testing the antimicrobial effect of Ellagic acid in comparison to Chlorhexidine gluconate 0.2% in this study may open the door for using it routinely in dental practice for prevention of oral disease. The aims of study include the following:

1- Preparation of Ellagic acid from pomegranate extract and compare it with the standard Ellagic acid
2- To test the antibacterial effects of Ellagic acid on the viable counts, adherence, and acidogenicity of salivary mutants streptococci in vitro.
3- To test the effects of Ellagic acid mouth rinse on the viable counts of salivary streptococci and mutants streptococci and on salivary flow rates and pH in vivo.
4- To carry out the above 2&3 tests in comparison to chlorhexidine 0.2% and de-ionized water.

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MATERIALS AND METHODS

The present study involved four in vitro and one in vivo experiment, concerning the effects of pomegranate extract (ellagic acid) on the viable counts, adherence, and acidogenicity of Mutans Streptococci. It was conducted in laboratory of Ministry of Science and Technology (MOST), Chemistry research Centre, during the periods from October 2008 to May 2009.

Culture Media used to test the sensitivities of Mutans Streptococci to different concentrations of ellagic acid invitro according to Agar well diffusion technique. Ten recently extracted sound first premolar teeth were collected until ready for use, then the teeth cleaned and used in experiments to test the adherence and acidogenicity of Mutans Streptococci after immersed the teeth at different concentrations of ellagic acid and Chlorhexidine 0.2%.

Stimulated salivary samples were collected from 18 volunteers before and after rinsing, they are male person and their ages between 32-43 years.

RESULTS

Identification of Mutans streptococci by the following:
1. Colony morphology
2. Microscopic examination
3. Biochemical tests

Extraction of ellagic acid from Iraqi pomegranate and identification

A hundred grams of white flesh pomegranate was group and mixed using mechanical mixer to obtain soft slurry. A mixture of 200ml triethylamine and 20% aqueous ethanol in a ratio 1.9 (V/V) was added to the formed slurry and mixed for 1hour at 25°C the resulting mixture was allowed to stand for 24 hours and then filtered by vacuum. High performance liquid chromatography (HPLC) used to determine the ellagic acid content and the compare the prepared ellagic acid with as show in the figure: 1

Table 1: Zone of inhibition in millimeter (mean and standard deviation SD) of ellagic acid, chlorhexidine, and deionized water.

<table>
<thead>
<tr>
<th>Deionized Water</th>
<th>0.2% Chlorhexidine</th>
<th>Ellagic acid</th>
<th>Concentration mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD Mean</td>
<td>SD Mean</td>
<td>SD Mean</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.939</td>
<td>16.85</td>
</tr>
<tr>
<td></td>
<td>0.52</td>
<td>6</td>
<td>8.52</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Statistical test between 0.2% chlorhexidine and each concentration of ellagic acid.

<table>
<thead>
<tr>
<th>Concentration of ellagic acid mg/ml</th>
<th>t-test</th>
<th>P-value</th>
<th>descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.625</td>
<td>0.891</td>
<td>0.062</td>
<td>NS</td>
</tr>
<tr>
<td>1.25</td>
<td>6.84</td>
<td>0.021</td>
<td>S</td>
</tr>
<tr>
<td>2.50</td>
<td>7.49</td>
<td>0.0011</td>
<td>HS</td>
</tr>
<tr>
<td>5.0</td>
<td>7.013</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>10.0</td>
<td>6.85</td>
<td>0.0019</td>
<td>HS</td>
</tr>
</tbody>
</table>

P < 0.001 high significant, p < 0.05 significant. NS non significant D.F = 8

Figure 2: The effect of different agents EA 5mg/ml, CHX and DW on viability count of MS X 10^3 in vivo

Figure 3: Salivary pH three mouth washes, Washes, CHX, DW and EA at five time intervals.
DISCUSSION

Sensitivities of MS to different concentrations of Ellagic acid in vitro (agar well diffusion)

Sensitivities of MS to different concentrations of Ellagic acid by agar well diffusion method had been tested in this study. The results showed that ellagic acid were able to inhibit the growth of mutans streptococci, this finding were in coincidence with (5,8).

The diameter of zones of inhibition of MS were increased as the concentration of ellagic acid increased and when reach 5mg/ml, 10mg/ml the zones of inhibition were much higher than0.2% CHX with highly significant differences (p<0.001), this finding may be explained by the fact that, the ellagic acid that had antibacterial effects against MS and inhibit its growth.

The effects of Ellagic acid on the viable counts of MS in vitro

In this experiment, the viable counts of MS after using different concentrations of ellagic acid (0.65-10mg/ml) were tested in vitro. The results showed that, highly significant reduction (p<0.001) in the viable counts of MS in 5mg/ml and 10mg/ml compared to the control. On the other hand, ellagic acid 5mg/ml and 10mg/ml showed more effective in reduction of the viable counts of MS than of 0.2% CHX in vitro and in vivo on the plaque bacteria in generals shown in table 1 and 2.

The effects of Ellagic acid on the adherence of MS in vitro

An important cariogenic determinant of MS is the ability of adherence on the tooth surface. (9,10) In this experiment, 5mg/ml and10mg/ml had been tested, it have the ability to interfere with adherence of MS in vitro .The result showed that the dental plaque could be eliminated and the effects of ellagic acid on caries prevention could be truly reflected of MS in presence of sucrose but prevent adherence of plaque formed by MS to a glass slide in vitro.

The effects of Ellagic acid on the acidogenicity of MS in vitro.

Acidogenicity is an important cariogenic potential of these bacteria, the fermentation of carbohydrates results in acid production, this acid may lead to demineralization of enamel surface and initiation of carious lesion (11,12). Ellagic acid0.625mg/ml 1.25 mg/ml 2.5mg/ml,5mg/ml,10mg/ml, were tested it had the ability to interfere with acid production of MS in addition to CHX and de-ionized water.

The effects of ellagic acid on salivary counts of streptococci and mutants streptococci in vivo

Aqueous extract of ellagic acid 5 mg/ml, 10mg/ml were tested for its effects on salivary streptococci and mutants streptococci colony forming unit counts among group of volunteers in comparison to deionized water and CHX. Bacterial counts were estimated at different time intervals including before rinsing as a base line,1 min after rinsing , after 30,60 ,and 120 minutes. The results of this study indicate that 5, 10 mg/ ml had highly significant antimicrobial activity against streptococci and mutants streptococci as it can reduce the viable count of the bacteria EA has high ability of reduction the salivary counts of the two types of bacteria as shown in figure 2.

Effects of ellagic acid on salivary flow rates (ml/min)

The three mouth rinses produce immediate increase in salivary flow rates which continue to increase after half an hour then began to drop down slowly until it approximate the base line after two hours. The possible explanation is that any mechanical stimulation in form of mouth rinses can increase the salivary flow rates.

The effects of Chlorhexidine lead to slightly increase in salivary flow rates than ellagic acid and de-ionized water after half an hour from rinsing as shown in figure 4.

Effects of ellagic acid on salivary pH

The three mouth rinses produce immediate increase in salivary pH which continue to increase after half an hour, then began to drop down slowly until it approximate the base line after more than two hours, ellagic acid stimulated the salivary pH to increase more than CHX and de-ionized water and cause increase in pH after rinsing with ellagic acid after half an hour compared with time before rinsing, since ellagic contain six hydroxyl groups in its main structure as shown in figure 3.
REFERENCES