

Effect of ginger extract on Mutans streptococci in comparison to chlorhexidine gluconate

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ABSTRACT

Background: The rhizome of ginger is used in cooking and for medicinal purposes such as anti-bacterial, anti-fungal, anti-inflammatory and antioxidant. The aims of the study were to test the effect of ethanolic extract of ginger on growth, adherence and acidogenicity of mutans streptococci in comparison to chlorhexidine gluconate 0.2% and de-ionized water.

Materials and methods: From saliva often volunteers (dental students 20-22 years); mutans streptococci was isolated, purified and diagnosed according to morphological characteristic and biochemical tests. Ginger was powdered and extracted, different concentrations of ginger extract were prepared. Chlorhexidine gluconate 0.2% used as a control positive; while de-ionized water was used as a control negative. In this study, in vitro and in vivo experiments were conducted. In vitro experiment, agar well technique was used to study the sensitivity of mutans streptococci to different concentrations of ginger extract and other control agents; also effect of ginger extract on the viable count of mutans streptococci, the adherence and acidogenicity of mutans streptococci were studied. In vivo experiment, the volunteers couldn't tolerate the extract.

Results: Mutans streptococci was sensitive to different concentrations of ethanolic ginger extract, but they were more sensitive to chlorhexidine gluconate than the extract. The effect of ginger extract on the viable count of mutans streptococci at concentrations (30%, 35% and 40%) showed highly significant reduction in the count of the bacteria but less than chlorhexidine effect. In the effect of the extract on the adherence of mutans streptococci, the concentrations (30%, 35%, 40%) were used and only 40% and chlorhexidine prevent the plaque formation. But in the acidogenicity of mutans streptococci procedure 35%, 40% of the extract and chlorhexidine showed effectiveness in reducing acid formation.

Conclusion: Ginger extract was effective against mutans streptococci, chlorhexidine is more effective than other agents.

Keyword: Mutans streptococci, Ginger extract, Chlorhexidine. (J Bagh Coll Dentistry 2013; 25(2):179-184).

INTRODUCTION

Ginger, the underground stem or rhizome of the plant *Zingiberofficinale* has been used to treat a wide array of ailments⁽¹⁾. It is one of the natural products having antimicrobial property against various human pathogens including oral pathogens like mutans streptococcus. Mutans streptococci have the ability to generate considerable amount of acid as a result of their metabolism of carbohydrate, to survive in acid environment so they are considered as cariogenic microorganisms^(2,3,4,5) and have a real role in dental caries, which is a disease in which the mineralized tissues of the tooth undergo progressive destruction from the surface of the tooth^(6,7), initiated through a series complex, chemical and microbial reactions associated with dental plaque biofilms that containing a number of mutans streptococci⁽⁸⁾.

Medicated oral rinse usually contains antimicrobial agents, such as chlorhexidine gluconate which is a very potent chemoprophylactic agent, it has a broad spectrum action especially against mutans streptococci group^(9,10).

But it has many side effects like staining of the teeth, altering the test of the mouth and desquamation of oral mucosa⁽¹⁰⁾.

As plant sources have been considered to be safe with fewer side effects, so that use of anti-plaque and anti-caries agents from plants has been investigated. Hence in the present study an attempt has been made to explore the antimicrobial potential of ginger (*Zingiberofficinale*) against mutans streptococcus and compare it with chlorhexidine gluconate.

MATERIALS AND METHODS

Preparation of ginger extract

The preparation of ethanolic extract of ginger carried out according to the method described by Nweze and Okafor⁽¹¹⁾, which involved the maceration of 100 gram of plant in 500 ml of absolute ethanol (ethanol 99.9%). The container was sealed with paper foil to prevent loss of volatile solvent and left at room temperature for 24 hr. At the end of this period, the contents were filtered using filter paper (No.1) into a beaker. The filtered solution then concentrated by evaporating the solvent in a hot air oven at 40°C for 24 hr. The extract powder was then weighed, kept in sterile bottles, labeled accordingly and stored in the refrigerator.

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Collection of Saliva

Stimulated saliva was collected from ten healthy looking students aged (22-24) years⁽¹²⁾.

Isolation of mutans streptococci

The collected salivary samples were homogenized by vortex mixer for 1-2 minutes, then ten-fold dilutions were performed by transferring 0.1 ml of saliva to 0.9 ml of phosphate buffer saline (pH 7.0), then from the dilution 10^{-3} of salivary samples 0.1 ml was taken and spread on the MSBA media, the plates were incubated anaerobically using a candle for 48 hr. at 37°C then incubated aerobically for 24 hr. at room temperature⁽¹³⁾, the colonies of mutans streptococci were identified on the basis of the morphology of the colonies, Gram's stain and biochemical test (The ability of mutans streptococci to ferment sugar was tested by addition of selected types of sugar (sorbitol) in a concentration of 1% in Brain Heart Infusion broth)⁽¹⁴⁾.

In vitro Experiments

- A. Sensitivity of mutans streptococci to different concentrations of ginger extract, chlorhexidine and de-ionized water. Different concentrations of ginger extract in addition to chlorhexidine (0.2%) and de-ionized water were used in this experiment. A volume of 25 ml of Mueller Hinton Agar was poured into sterile glass petridishes, left at room temperature for 24 hour. To each plate 0.1 ml of mutans streptococci inoculum was spread, left for 20 minute at room temperature then wells of equal size and depth were prepared in each plate, each well was filled with 0.2 ml of the test agents. Plates were left at room temperature for one hour then incubated anaerobically for 24 hr. at 37°C , zone of inhibition was measured by using digital vernia.
- B. Effect of ginger extract, chlorhexidine and de-ionized water on viable Count of mutans streptococci. Different concentrations of ginger extract were prepared. Brain Heart Infusion broth (pH 7.0) was distributed in test tubes by 8.9 ml in each one. One ml of the test agent was added to each tube, after that 0.1 ml of bacterial inoculum was added to both study and control tubes. From the control tube 0.1 ml was transferred to 0.9 ml of sterile phosphate buffer saline (pH 7.0) and a ten-fold dilution was performed. From dilutions 10^{-3} , 0.1ml was taken and spread in duplicate on Mitis Salivarius Bacitracin agar plates, the plates then incubated anaerobically at 37°C for 48

hour. Then colony forming unit per milliliter (CFU/ml) was counted, this value was considered as the initial count of bacteria. Study and control were incubated aerobically at 37°C for 24 hour. From each tube of the control and study 0.1 ml was transferred to 0.9 ml of phosphate buffer saline and a ten fold dilution was performed. From dilutions 10^{-3} , 0.1 ml was taken and spread in duplicate on MitisSalivarius Bacitracin agar plates, the plates then incubated anaerobically at 37°C for 24 hour. The colony-forming unit per milliliter was counted (CFU/ ml) for all the plates.

- C. Effect of Ginger Extract on adherence of mutans streptococci to tooth surface
1. Different concentrations of ginger extract were prepared. Stainless steel wire was threaded in one end in the root of previously cleaned and polished sound first premolar by using non fluoridated pumice. These all were sterilized by the autoclave.
 2. Teeth were immersed in 10 ml of the tested agents for two minutes except for control positive which was broth and bacteria without agents, the tested agents include ginger extract of concentrations (30%, 35%, 40%), CHX 0.2%, and de-ionized water.
 3. The wires and teeth were washed using sterile de-ionized water for one minute and left to dry for 5 minutes at room temperature.
 4. The teeth were immersed in 10 ml Brain Heart Infusion Broth containing 5% sucrose (pH 7.0). The study and control tubes were inoculated with 0.2 ml (2%) of bacterial isolates. All the bottles were incubated aerobically at 37°C for seven days.
- D. Effect of ginger extract on the acidogenicity of mutans streptococci
1. Different concentrations of ginger extract were prepared. Stainless steel wire was threaded in one end in the root of a previously cleaned and polished sound first premolar.
 2. Each sterilized wire and tooth inserted in a tube of 10 ml of Brain Heart Infusion Broth containing 5% sucrose at (pH 7.0), then inoculated with 2% of mutans streptococci except for the control negative which contain (5% sucrose broth with wire holding a tooth). The study and control broths were incubated at 37°C aerobically, for period of three days every 24hr. each wire holding a tooth was

transferred to a fresh 5% sucrose broth, incubated aerobically at 37°C this allowed for further accumulation of bacterial deposit.

- In the fourth day, coated teeth in addition to the control negative were immersed about 2 minutes in 10 ml solutions of ginger extract (30%, 35%, 40%), CHX 0.2% and de-ionized water. Then wires and teeth were removed and washed with sterile de-ionized water for about one minute including control negative and control positive, teeth were left to dry at room temperature for 5 minutes then placed in a fresh 5% sucrose broth at (pH 7.0) containing 1% bromocresol purple as an indicator, test tubes were incubated for seven days at 37°C. Positive reaction (acid formation) was indicated by the change in the color from purple to yellow.

Results were recorded as follows:

- (+) Yellow No effect on acid production.
- (+,-) Orange Weak effect on acid production.
- (-) Purple Effective (preventing acid production).

In vivo Experiment

The volunteers couldn't tolerate the, they removed it immediately from their mouth because of hotness, burning and numbness of the mouth (as they described).

Statistical Analysis

Data processing and analysis were carried out by using SPSS program, which provide the following:-

- Calculation and presentation of statistical parameters: mean and SD of the variables in the study.
- Student's t-test, paired t-test and analysis of variance (ANOVA) for testing the significant differences among means of different groups.
- For all the above mentioned tests, the analysis was accepted at $P < 0.05$, as the level of significance.

RESULTS

Sensitivity of mutans streptococci to different concentrations of ginger extract, chlorhexidine and de-ionized water

Diameter of inhibition zones for ethanolic extract of ginger (clear zone of no growth of mutans streptococci around each well) were found

to be increased as the concentration of the extract increased. De-ionized water showed no zone of inhibition, while CHX showed highest zones of inhibition compared to the ginger extract (Table 1).

LSD test among different concentrations of ginger extract, CHX and D.W. shows statistically, highly significant differences were found among all groups used, except between (30% and 35%) there was non significant difference (Table 2).

Effect of ginger extract, CHX and de-ionized water on viable count of mutans Streptococci

The count of mutans streptococci was recorded before the application of the tested agents, this was considered as the initial count of bacteria. After 24hr. of incubation period, the number of mutans streptococci was counted (with study agents and without). Paired t-test was used to compare between initial count of bacteria and the count after 24 hr. Statistically highly significant increase in the number of bacteria was recorded after 24 hr. (without agent) (Table 3).

ANOVA test was used to compare among count of bacteria after 24hr., ginger extract, CHX and D.W. Statistically highly significant difference was found among these groups (Table 4). LSD test among these groups was done and statistically highly significant differences were found among all of them, except between (After 24 hr.-D.W) and (CHX -40%) there were non significant differences (Table 5).

Effect of ginger extract, CHX and D.W on adherence ability of mutans streptococci

The results of this experiment showed that dental plaque was detected by using dental probe only, which accumulated on the positive controlled teeth, de-ionized water and 30%, 35% ginger extract while negative controlled teeth, teeth immersed in 40% ginger extract and in CHX showed no accumulation of dental plaque on them (Table 6).

Effect of ginger extract, CHX and D.W on acidogenicity of mutans streptococci

In this experiment, the ginger extract at 35%, 40% and CHX were effective in retardation of acid formation as were detected by the change in color from deep purple to orange, while for 30% ginger extract, de-ionized water and control +ve, the color was changed from deep purple to yellow, and the control -ve remained purple in color (Table 7).

Table 1: Inhibition zones of mutans streptococci to different agents.

Agents	Mean*	±SD	ANOVA test
Ginger extract 30%	6.9760	0.27179	F =487.725 d.f= 7 P= 0.000 HS
Ginger extract 35%	7.475	0.3206	
Ginger extract 40%	8.234	0.372	
Ginger extract 45%	9.5110	0.2210	
Ginger extract 50%	10.294	0.4699	
Ginger extract 55%	11.2710	0.7264	
CHX 0.2%	13.0400	1.19112	
D.W	0	0	

*Mean (mm).

Table 2: LSD test among different concentrations of ginger extract, CHX and D.W.

Concentration	Mean difference	P	Description
30% -35%	-0.49900	0.051	NS

*Mean (mm).

Table 3: Initial count of bacteria and the count after 24 hr. ($\times 10^4$).

	Mean*	±SD	Test
Initial count	259.40	28.737	t = -29.395 d.f= 4 P= 0.000 HS
After 24 hr. count	945.60	76.891	

*Mean (CFU/ml).

Table 4: Count of bacteria after 24 hr., with ginger extract, CHX and D.W ($\times 10^4$).

	Mean*	±SD	ANOVA test
After 24 hr. count	945.60	76.891	F = 334.548 d.f= 5 P= 0.000 HS
30%	344.40	51.252	
35%	230.40	40.104	
40%	128.60	32.921	
CHX	88.80	15.434	
D.W	965.60	54.413	

*Mean (CFU/ml).

Table 5: LSD test among the count of bacteria ($\times 10^4$) of different agents.

Concentration	Mean difference	P	Description
After 24hr.-D.W	-20.000	0.525	NS
CHX - 40%	39.800	0.212	NS

Table 6: Effect of ginger extract, CHX and D.W on adherence of mutans streptococci.

Agent	30%	35%	40%	CHX	D.W	Control -ve	Control +ve
Adherence	+	+	-	-	+	-	+

(+) Presence of plaque (Adherence).

(-) Absence of plaque (No adherence).

Table 7: Effect of ginger extract, CHX and D.W on acidogenicity of mutans streptococci.

Agent	30%	35%	40%	CHX	D.W	Control -ve	Control +ve
Acidogenicity	Yellow	Orange	Orange	Orange	Yellow	Purple	Yellow

yellow: no effect, orange: weak effect, purple: strong effect

DISCUSSION

The sensitivity of mutans streptococci to different concentrations of ethanolic extract of ginger in comparison to CHX 0.2% and de-ionized water was tested using agar diffusion technique.

Ginger ethanolic extract was able to produce an antibacterial effect by inhibiting mutans streptococci isolated from human saliva. The diameters of the inhibition zones were found to increase when the concentration of the extract increased, this may be attributed to the amount of the dissolved active constituents of the extract will be more abundant as the concentrations increase causing increased antibacterial activity of the extract. The first concentration was shown to inhibit the growth of the bacteria was 30%, although all the concentrations 30%, 35%, 40%, 45%, 50% and 55% were shown lower inhibition zone than CHX 0.2%.

The de-ionized water had zero effect on the bacteria appearing by absence of inhibition zone. In the effect of ginger extract, CHX 0.2% and de-ionized water on the viable count of mutans streptococci, the result showed that a highly significant reduction in count of these bacteria at concentrations 30%, 35% and 40% of the ginger extract was seen compared to the control after 24hr.

The antibacterial activity of ginger extract could be attributed to the chemical constituents of ginger like sesquiterpenoids with zingiberene as the main components and other components include β -sesquiphellandrene, bisabolene and farnesene⁽¹⁵⁾.

No significant difference in count of bacteria was seen for de-ionized water compared to control after 24hr., this could be explained by complete resistance of these bacteria for de-ionized water.

The ability of mutans streptococci to adhere to host surfaces is the major virulence factor, which is important in colonization of them^(16, 17, 18). It is of great importance to the development of carious lesions, and any interference with some of the mechanisms of adherence can prevent the formation of carious lesions^(19, 20). In this study, the effect of ginger extract on the adherence of mutans streptococci was tested, the results showed that the concentrations 30% and 35% of ginger extract were unable to prevent adherence of bacteria, but there was a reduction in the thickness of plaque in comparison to the control. No plaque formed on teeth immersed in 40%, this may be attributed to the inhibitory effect of these agents on growth or metabolism of these bacteria.

CHX and control negative (broth and agent without bacteria) showed no plaque formation on teeth immersed in them, while plaque was formed on control positive teeth and teeth immersed in de-ionized water.

The present study tested the effect of 30%, 35% and 40% ethanolic ginger extract, CHX and de-ionized water on acid production by mutans streptococci. The results showed change in the color of the indicator from deep purple to yellow as in 30% ginger extract, de-ionized water and control positive teeth, and this indicate a failure in the prevention or retardation of acid formation by mutans streptococci, while 35%, 40% of ginger extract, and CHX were able to change the color from deep purple to orange. Control negative remained the color purple.

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