

Effect of Pomegranate Juice on Dental Plaque Microorganism (*Streptococcus Mutans*) and Its Efficacy with Listerine Mouthwash

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ABSTRACT

Dental plaque which consists of several microorganisms is considered as main etiologic factor for various oral diseases including dental caries, gingivitis and periodontitis. Different chemical antimicrobials are used as antiplaque agent which may have potential side effects. The aim of the present study was to determine the antimicrobial activity of pomegranate juice on *Streptococcus mutans* and to compare its efficacy with Listerine. The study group included 90 subjects aged 15-25 years and were divided into three groups- group I (distilled water), group II (Listerine) and group III (Pomegranate juice). Plaque sample was collected pre and post rinsing and was incubated at 37°C in TYCSB media for 48 hours and Colony Forming Unit/mg (CFU/mg) was counted. The result obtained after pomegranate juice rinse in group III showed 27.9% reduction in CFU/mg, whereas in group I and group II it was 0.3% and 9.9% respectively. The result obtained in the study clearly demonstrate that the pomegranate juice has greater antimicrobial effect against *S. mutans* than distilled water or Listerine.

Key words: Colony Forming Units, Dental plaque, Pomegranate, *S. mutans*.

INTRODUCTION

Dental plaque is a soft unmineralized bacterial deposit which forms on surfaces of teeth and dental prostheses that are not adequately cleaned.¹ Plaque can result in variety of oral diseases including dental caries, gingivitis and periodontitis. Primary preventive measure to prevent onset of oral disease is plaque control. Although mechanical plaque control methods are efficient in maintaining adequate level of oral hygiene, studies have shown that patient compliance are not adequate.² In order to overcome this, various chemico therapeutic agents have been employed. The widespread use of commercially available antimicrobial has led to advent of multi drug resistant pathogens which ultimately lead to threat to global public health.³

Herbs are nature's gift to humans. They have various bioactive components which possess enormous medicinal value with least side effects.⁴ *Punica granatum* commonly known as Pomegranate is a shrub or small tree native from Asia where several of its parts have been used as an astringent, hemostatic as well as anti-inflammatory.³ According to Ayurveda it is considered as 'A Pharmacy unto itself'.⁵ Pomegranate juice contain polyphenols, tannins, ellagic acid and

anthocyanins which are powerful antioxidants.⁶ Pomegranate preparation are used as topical application particularly for controlling oral inflammation, bacterial and fungal count in periodontal diseases and candida associated denture stomatitis.⁷

Listerine mouthwash, one of the commonly used mouthwash consist of menthol, ethanol, thymol, methyl salicylate and eucalyptol.⁸ There has been concern that the use of alcohol-containing mouthwash may increase the risk of developing oral cancer. Presence of alcohol also results in dry mouth and burning sensations.⁹

Very few studies have been carried out to evaluate effect of pomegranate juice on oral bacteria. Hence the aim of our study was to determine the effect of pomegranate juice in reducing selective dental plaque microorganism (*Streptococcus mutans*) and to compare its efficacy with commonly used mouthwash.

MATERIALS AND METHODS

Present study is a clinical trial conducted to find the effect of pomegranate juice on dental plaque microorganism.

Before conducting the study, all the subjects were informed about the nature of study and written consent was obtained. The study group consist of 90 subjects aged 15-25 years. They were divided into three groups each having 30 subject. Thorough oral prophylaxis was done and subjects were asked to refrain from oral hygiene procedure for 24 hours. Plaque sample was collected from each subject from buccal surface of maxillary central, lateral and molar teeth with the help of sterile probe. (Figure 1)



Approximately 1 mg of plaque was collected by using electronic balance in 2ml plastic test tube. (Figure 2)

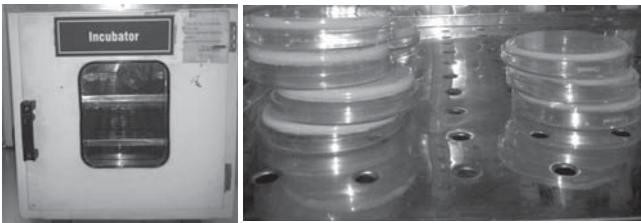


Fig 2: Incubating Plaque Sample in Tycsb Media

Subjects from group I were asked to rinse mouth with distilled water, group II with Listerine mouthwash and group III with freshly prepared Pomegranate juice without sugar for 5 minute. Second sample of plaque was collected from each subject after rinsing. Immediately after collection, samples were sent to microbiological laboratory for bacterial culture. Plaque samples from each individual before and after rinsing was inoculated in Tryptone Yeast extract cysteine with sucrose and bacitracin media (TYCSB). Inoculated plates were then incubated at 37° C for 48 hours. (Figure 3)

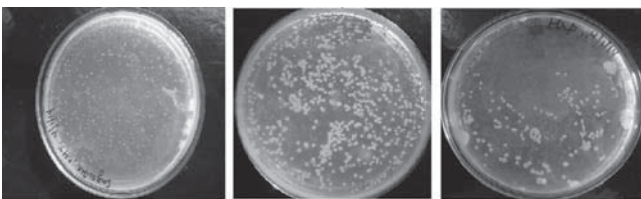


Fig 3: Colony Forming Units in Different Group

Number of Colony Forming Units (CFU) were counted using colony counter and expressed as CFU/mg dry weight of plaque.

STATISTICAL ANALYSIS

Wilcoxon’s signed rank test was used to determine the difference in number of CFU/ mg of plaque before and after rinsing. The significance level was set at P< 0.05.

RESULT

The mean number of CFU of streptococcus mutans before and after rinsing with distilled water was 99.70±4.739 and 99.40±4.695 respectively, in group II it was 100.10±4.818 and 90.20±8.189 respectively where as in group III it was 101.60±7.919 and 73.70± 8.92 respectively. (Table 1).

Table 1: Mean Value Standard Deviation and Percentage Reduction Preand Post Rinse in Different Group

Group		Mean	Standard Deviation	Percentage of reduction
GROUP I	PRE CFU/ mg	99.70	4.739	0.3%
	POST CFU/mg	99.40	4.695	
GROUP II	PRE CFU/ mg	100.10	4.818	9.9%
	POST CFU/mg	90.20	8.189	
GROUP III	PRE CFU/ mg	101.60	7.919	27.9%
	POST CFU/mg	73.70	8.920	

The mean difference of CFU before and after rinsing in group I was 0.3, group II was 9.9 and group III was 27.9. Z score and P value was calculated for each group before and after rinsing. (Table 2, Table 3)

Table 2: Z Scor and p Value Pre and Post Rinse

Group		Post Cfu/Mg-Pre Cfu/Mg
Group I	Z Score	-0.283
	P Value	0.777
Group II	Z Score	-2.807
	P Value	0.006
Group III	Z Score	-2.805
	P Value	0.005

Table 3: Z Scor and p Value Pre and Post Rinse

	Group II	Group II	Group III	I & II p value	II & III p value	I & III p value
Post Rinse	99.7±4.7	90.2±8.2	73.7±8.9	<0.001	0.006	<0.001

DISCUSSION

P. granatum most commonly known as pomegranate is a shrub native of Asia. According to Greek mythology, it was known as 'fruit of the dead', the Babylonians address it as 'agent of resurrection' and Chinese call it as 'soul concentrate' as it is homologous to human blood capable of conferring on a person longevity and immortality.¹⁰

Pomegranate is a potent anti-oxidant with anti-inflammatory and anti carcinogenic effect. The biochemical components that make it beneficial are ellagatannins, ellagic acid, punica acid, anthocyanins, flavonoids, flavonols and flavones.¹¹ According to Ross et al (2001), the anti-inflammatory effect may be attributed to its considerable immunoregulatory activity over macrophages and T and B lymphocytes.¹²

The result obtained in the present study showed pomegranate juice have significantly higher inhibitory effect against *S. mutans* compared to distilled water and Listerine. The percentage of reduction was 27.9% in group III compared to 0.3 and 9.9 in group I and group II respectively.

The antibacterial property of pomegranate has been evaluated in previous studies, which shows similar results. Trivedi and Kazmi (1979) used extracts of fruit bark to observe anti-bacterial activity of pomegranate against *Bacillus anthracis* and *Vibrio cholera*.¹³ Prashanth et al (2001) in their study stated that methanolic extract of pomegranate is active against all microorganisms tested.¹⁴ Machado et al (2003) showed similar effect against *Staphylococcus aureus* in their study.¹⁵ Study conducted by Vasconcelos et al (2006) showed that *Streptococcus sanguis* which is seen in supra-gingival plaque was sensitive to pomegranate juice. Kote et al stated that pomegranate rinse is effective against dental plaque microorganism with 23% reduction in *S. mutans* colonies.¹⁶ Subramaniam et al (2012) conducted an in vitro study and stated that hydroalcoholic extract of pomegranate showed significantly higher inhibitory effect on *S. mutans* at all concentration.⁵ Lalwani et al (2014) in their study stated that there was 52.2% reduction in CFU after rinsing with pomegranate juice.¹¹

The present study demonstrate that pomegranate juice has a significant anti-bacterial action against *S. mutans*, thus having an anticariogenic effect. Presence of various natural agents make it an efficient phyto-therapeutic agent. Pomegranate juice can be used as an alternative to various mouthwashes, thus protecting the individuals from potential side effects of commercially available mouthwashes and preventing them from developing resistance against antimicrobial agents. Thus more long term clinical trial with different concentration

of pomegranate juice and its effect on different oral microorganisms are necessary to verify its potential effects and to know a safer dose that can be taken by humans.

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