

Evaluation of Anti-gingivitis efficacy of *Punica Granatum* mouthwash and 0.2% chlorhexidine gluconate mouthwash through a 4 day Randomized Controlled Trial**Deepak Ranjan Dalai¹, Pradeep Tangade², Himanshu Punia³, Saraswati Ghosh⁴, Nisha Singh⁵, Yogesh Garg¹**

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ABSTRACT:

Aim: To evaluate the anti-gingivitis efficacy of *Punica Granatum* mouthwash through a 4-day randomized controlled trial and compare it with 0.2% chlorhexidine and distilled water.

Materials and Methods: A double blinded, randomized and parallel designed clinical trial was conducted involving 45 volunteered dental students. The student subjects were divided into 3 groups (15 students in each group). At Baseline, thorough professional prophylaxis and polishing was done. Each volunteer had to rinse 10 ml of their respective mouthwash i.e. test mouthwash (*Punica Granatum* mouthwash, Group A), control mouthwash (.2% chlorhexidine, Group B) and placebo (distilled water, Group C), twice daily for 1 minute for 4 consecutive days. Then Gingival Index scores were evaluated at 5th day by the same investigator, and the differences were compared statistically by ANOVA and Student's 't'-test.

Results: On 5th Day of the study, it was found that there was significant reduction in GI scores in *Punica Granatum* mouthwash group and Chlorhexidine Gluconate group, and no significant reduction was seen in Distilled water group. Increase in Gingival health and reduction of GI score was more in *Punica Granatum* group than other two groups.

Conclusion: *Punica Granatum* can be used as an effective mouthwash and can be used as an alternative to chlorhexidine mouthrinse as it has similar properties without the side-effects of the latter.

Keywords: Chlorhexidine Gluconate, Gingival Index, Hydroalcoholic extract, Plaque, *Punica Granatum*

INTRODUCTION

Chemical plaque inhibitors play a crucial role in controlling plaque.¹Antiseptics that are provided as chemical plaque inhibitor can be delivered as mouthwash, irrigator, spray, chewing gum or varnish.^{2,3}Among the various delivery methods, mouthwash is the second most accepted method for delivery of anti-microbial agent.⁴

Among the various plants like *Astroniumurundeuva*, *Calendula*, *Aloe vera*, used for herbal products *Punica Granatum*, is the one that has been recently tested and its various parts have proved effective as herbal medicine. *Punica Granatum*, commonly known as "pomegranate" is a shrub that belongs to family Punicaceae. Its several parts

have been tested as astringent, haemostatic, and in diabetes disorders.⁵

Chlorhexidine gluconate is an organically cationic bis-guanide and is the most widely accepted broad spectrum antiseptic mouthwash that is equally effective against gram positive, gram negative bacteria and other microorganisms like yeast, fungi and virus. Chlorhexidine gluconate alters bacterial cell membrane, thereby showing its anti-bacterial property.⁶ Since been three decades dental professionals have been using chlorhexidine gluconate and thus is considered as the primary agent for maintaining gingival health. It has used as positive control, for testing anti-gingivitis efficacy of various agents.⁷ However, long term use of this agent leads to numerous local side effects like brownish discoloration of teeth, restorative materials and tongue; mucosal ulcerations, alteration of taste, paraesthesia, parotid swelling, and increased supra-gingival calculus formation etc.⁸

Keeping the faith of people for natural products and the side effects of Chlorhexidine gluconate in mind, the present study was conducted to compare the anti-plaque efficacy of *Punica Granatum* mouthwash (designated as Group 1), 0.2% chlorhexidine gluconate mouthwash (designated as Group 2) with distilled water (designated as Group 3) through a 4 day plaque accumulation survey.

MATERIALS AND METHODS

This study was a triple-blinded, parallel designed randomized controlled clinical trial conducted in Department of Public Health Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, India. It included a total of 45 dental students enrolled to B.D.S. course, who were in 3rd year and 4th year and had moderate gingivitis. Of the total subjects 22 were males and 23 were females who participated in the study. The mean age of the subjects was 21.46 ± 0.2712 , and the age range of the subjects was 18 to 23 years. Before the

conduct of the study, Institutional Ethical clearance was obtained. Informed consent from the participants was taken from all the participants prior to the study. Based on previous literature⁹ on testing of anti-gingivitis efficacy of various agents, it was found that 45 participants (15 in each group) would be sufficient to determine a difference between the Gingival Index scores of the 3 mouthwashes.

The armamentarium used in the study included diagnostic instruments, Periodontal Probe, 0.2% chlorhexidine gluconate mouthwash, pomegranate mouthwash, distilled water, sterile dispensing dark colored bottles, measuring cups.

Chlorhexidine Gluconate (0.2%) of pharmaceutical grade was purchased from the local market of Moradabad, Uttar Pradesh.

Fruits i.e. *Punica granatum* were collected from the city. The entire process of extract preparation was done in the Dept. of Pharmacy, Teerthanker Mahaveer University, Moradabad, U.P. Pomegranate mouthwash was prepared from Hydro-Alcoholic Extract (HAE) of *Punica Granatum*. The Hydroalcoholic extract was prepared from 6-7 whole fruits that were cut into small pieces mixed with a mixture of ethanol and distilled water at a ratio of 1:1, v/v. The material thus obtained was filtered and evaporated at 60°C till one-third of its original volume was obtained. Distilled water was used to restore the volume. 1ml sample was evaporated in the oven, in order to get insoluble residues /ml, which were further used to adjust the concentration of mouthwash. The final hydroalcoholic extract concentration ranged from 50-75 mg/ml.

Punica granatum mouthwash preparation and color matching (Figure 1) of all the three testing agents were done in the Dept of Pharmacy, Teerthanker Mahaveer University, Moradabad, U.P.

Index used for recording plaque was Gingival Index given by Silness and Loe (1963).



Figure 1: Three interventions after colour matching

Inclusion criteria

Systemically healthy subjects with moderate gingivitis, who provided informed consent, were included in the study.

Exclusion criteria

Subjects with antimicrobial therapy, with habit of smoking, or consumption of any other form of tobacco were excluded from the study.

Study design

A total of 45 dental students from 3rd and 4th year B.D.S. course with moderate gingivitis from Teerthanker Mahaveer Dental College and Research Centre were enrolled in the study according to inclusion and exclusion criteria. At first, baseline recording (GI scores) were done to determine the gingival health of the subjects. All the recordings were made by a single examiner and colored photographs were taken. By Simple Random Sampling (Computerised Lottery Method¹⁸) the subjects were allocated to 3 different (15 subjects each). After randomization of subjects, professional scaling and polishing was provided to all the subjects. The subjects were asked to refrain from regular oral hygiene practices like tooth brushing, dental flossing and use of any mouthwash other than the therapeutic dose of prescribed respective mouthwash. Group A subjects were instructed to rinse with 10 ml of *Punica Granatum* Mouthwash twice daily for 1 minute. Group B subjects were instructed to rinse with 10 ml of 0.2% chlorhexidine gluconate mouthwash

twice daily for 1 minute, which is the standard therapeutic regimen for chlorhexidine mouthwash. Group C subjects were instructed to rinse with 10 ml of Distilled water twice daily for 1 minute. The trial was conducted for the period of 4 days and the participants were provided with a schedule time table, so that they don't skip their rinse. The subjects were provided with same type of toothbrush and toothpaste and were instructed to follow a common standardized brushing technique. On the 5th Day, Gingival index scores were recorded by the same examiner who was unaware of the mouthwash used by the subjects. Post-rinsing GI scores of groups A, B, and C were then statistically compared by ANOVA and student's 't'-tests. Subjects were asked for any side effects upon use of mouthwash by subjective and clinical examination. None of the subjects had developed any side effect. After the trial period, subjects were asked to return to their habitual oral hygiene practices.

RESULTS

A total of 45 subjects participated in the study with the mean age of 21.46 ± 0.2712 . There were no dropouts in the study.

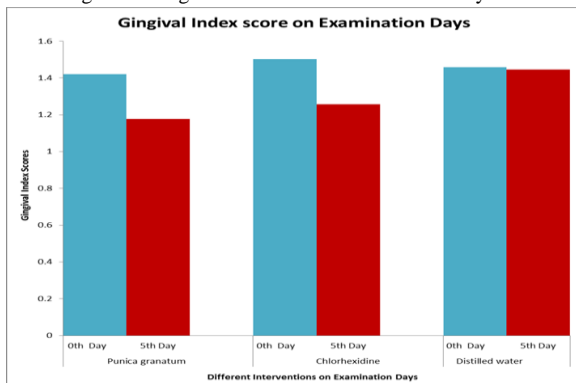
On recording of the Gingival Index on the 5th day, it was observed that there was a significant reduction in Gingival Index scores ($p < 0.05$) in Group A (*Punica Granatum*) and Group B (Chlorhexidine Gluconate). But no significant reduction was seen in Group C (Distilled Water). The mean decrease in Group A was from 1.421 ± 0.1524 on 0th Day to 1.177 ± 0.9862 on 5th day, which was significant with % reduction of 17.17%. The mean decrease in Group B was from 1.502 ± 0.1720 on 0th Day to 1.258 ± 0.1127 on 5th day, which showed a significant reduction of 16.25% which was less in comparison to *Punica Granatum* group. The mean decrease in Group C was from 1.458 ± 0.1690 on 0th Day to 1.447 ± 0.1367 on 5th day, with a % reduction of 0.75% (Table 1, Figure 2).

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Group	Time	Mean ± SD %	Reduction (%)	P value
Punica Granatum (A)	0 th Day	1.421 ± 0.1524	0	<0.05
	5 th Day	1.177 ± 0.9862	17.17	
Chlorhexidine (B)	0 th Day	1.502 ± 0.1720	0	<0.05
	5 th Day	1.258 ± 0.1127	16.25	
Distilled water (C)	0 th Day	1.458 ± 0.1690	0	>0.05
	5 th Day	1.447 ± 0.1367	0.75	

Table 1: Gingival index scores of Punica Granatum, chlorhexidine gluconate and distilled water at baseline and 5th Day.

Figure 2: Gingival Index scores on 0th and 5th days



DISCUSSION

Punica Granatum is a shrub of Asian nativity and has various parts that are put to use in herbal medicine. Over the decades, very few studies have been conducted to show the clinical efficacy of *Punica Granatum* on oral conditions. Present study showed that pomegranate mouthwash led to significant reduction in gingivitis.

Analysis of Gingival Index scores showed that *Punica Granatum* mouthwash was more effective in reducing gingival score than chlorhexidine gluconate and distilled water. Similar results were obtained by the study conducted by Ahuja S et al.⁹ and Hafajee et

al.¹⁰ The result of the present study are contradictory to the findings of the study done by Salgado et al.¹¹ on 10% *Punicagranatum* gel. It was found that the *Punica Granatum* gel was not effective in preventing gingivitis and reducing gingival Index score than chlorhexidine gluconate. Gel solubilisation would be necessary to reduce gingivitis. In the present study, direct interaction of *Punica Granatum* mouthwash with saliva led to positive results. The presence study showed an increase in gingival health which is in accordance to the findings by Batista ALA et al.¹² In another study it was proved that pomegranate hydrolysable tannins play role in inhibiting the synthesis of several pro-inflammatory mediators, that provides anti-inflammatory property to *Punica granatum*.¹³ Herbal products are safer to use as antiseptic mouthwash than chlorhexidine gluconate mouthwash. In the present study, no side effect of *Punica Granatum* mouthwash was found on subjective and clinical examination. Similar results were obtained from the study conducted on herbal mouthwash and chlorhexidine mouthwash by Parwani SR et al.¹⁴

In the quest for finding a better alternative to Chlorhexidine gluconate mouthwash in terms of side effects, various herbal products have been tried with fruitful results. The anti-gingivitis efficacy of *Punicagranatum* has been successfully tested in the present study.

A 4- day period may be insufficient to evaluate the anti-gingivitis property of Punica Granatum mouthwash, which is a limitation of the study. So, more of extensive research should be conducted for long-term period to better identify the beneficial properties and determine its wider applicability.

CONCLUSION

In the present study it was found that *Punica Granatum* mouthwash showed more reduction in gingival index scores without any side effects as compared to 0.2 % chlorhexidine gluconate mouthwash. As Punica Granatum is

cost-effective as compared to the cost of 0.2% chlorhexidine gluconate mouthwash, in low socio-economic status population, presently tested mouthwash can be a better adjunct to 0.2% chlorhexidine gluconate mouthwash. *Punicagranatum* is a newly researched herbal product used in field of dentistry. So, more clinical and microbiological studies on a long-term period are required to better understand the advantages and disadvantages of the product.

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