OBJECTIVES: The antiplaque and antigingivitis effects of a gel containing 10% *Punica granatum* Linn extract were evaluated using a 21-day partial-mouth experimental model of gingivitis. Methods: 23 volunteers participated in this cross-over, double-blind study, carried out in 2 phases of 21 days each. For each period of the experiment, an acrylic toothshield was made for each volunteer to carry the test or placebo gel as well as to avoid brushing of the 4 experimental teeth (posterior teeth in the lower left quadrant). The subjects were randomly assigned to use either the placebo gel (control group) or the test gel (experimental group) and were instructed to brush the remaining teeth normally 3 times a day. On days 0 and 21, the visible plaque index (VPI) and gingival bleeding index (GBI) were recorded. Results: The results did not show statistically significant difference between control and experimental groups for either of the indices (VPI and GBI). Conclusion: The gel containing 10% *Punica granatum* Linn extract was not efficient in preventing supragingival dental plaque formation and gingivitis.

Uniterms: Dental plaque; Gingivitis; *Punica granatum* Linn.
INTRODUCTION

Gingivitis is a chronic inflammatory process limited to the gingiva and without either attachment or alveolar bone loss. It is one of the most frequent oral diseases, affecting more than 90% of the population, regardless of age, sex or race. The earliest clinical sign is the bleeding caused by a vasodilatory effect caused by an inflammatory response\(^\text{29}\). Prevention of gingivitis by daily and effective supragingival plaque control using toothbrushing and dental floss is necessary to arrest a possible progression to periodontitis\(^\text{1,11}\).

Although mechanical plaque control methods have the potential to maintain adequate levels of oral hygiene, clinical experience and population-based studies have shown that such methods are not being employed as accurately as they should by a large number of people. Therefore, several chemotherapeutic agents such as triclosan, essential oils and chlorhexidine have been developed to control bacterial plaque, aiming at improving the efficacy of daily hygiene control measures\(^\text{6,8,10,15-17,23,26}\).

The interest in plants with antibacterial and antiinflammatory activity has increased as a consequence of current problems associated with the wide-scale misuse of antibiotics that induced microbial drug resistance\(^\text{7,18}\). Natural products such as Astronium urundeuva, calendula, aloe vera, curcuma zedoaria and other herbal products have been tested with effective results\(^\text{12,19,25,29}\). Punica granatum Linn (family Punicaceae), mostly known as “pomegranate”, is a shrub or small tree native from Asia where several of its parts have been used as an astringent, haemostatic as well as for diabetes control\(^\text{1,13,24}\). In Brazil, the fruit of this tree is known as “romã” and is commonly used for treatment of throat infections, coughs and fever due to its antiinflammatory properties\(^\text{13,14}\).

In the only study available evaluating the effects of pomegranate on gingivitis, Pereira and Sampaio\(^\text{21}\) (2003) showed a significant reduction of gingival bleeding after using a dentifrice containing pomegranate extract. However, a control group was not included in that study. Therefore, the purpose of the present investigation was to assess the effects of Punica granatum Linn extract on supragingival plaque formation and development of experimental gingivitis in comparison to a control formulation.

MATERIAL AND METHODS

Subjects

Twenty-five dental students from the University of Fortaleza (UNIFOR) (12 male and 13 female aged 22 to 28 years) were enrolled in this study. All subjects had at least 20 natural teeth, among which the 4 posterior teeth in the lower left quadrant (experimental teeth). All participants, randomly screened, were informed about the nature of the study and signed an informed consent form in compliance with the guidelines of the Brazilian Health Council. Students with medical disorders, severe periodontal disease and under antimicrobial therapy, as well as smokers, pregnant women and individuals presenting a probing depth $\geq 3$ mm associated with any mandibular teeth were excluded from the trial. The protocol was approved by the University’s Ethics Committee (Report COÉTICA/Unifor).

Test and control products

The control and experimental gels were formulated and packed into tubes in a commercial drugstore. The tubes were previously coded to warrant that neither the examiner nor the volunteers knew their content, which was revealed by the pharmacist only after the study was completed. All students used both gels in alternate periods, according to a cross-over model.

Preparation of Punica granatum Linn extract

Fresh pomegranates were obtained in grocery stores and then barks and juice were separated. The barks were dried at room temperature during 5 days and then powdered. An infusion was prepared with powdered material at a ratio of 100 g powder to 1000 mL distilled water, cooled at room temperature and filtered. Thereafter, 50 g of carboxymethylcellulose was added to the infusion (1000 mL) and the mixture was kept boiling until complete dissolution to obtain the 10% gel concentration. The pomegranate extract concentration used in this work was based on the findings of previous in vitro studies\(^\text{12,20}\) that tested the gel at different concentrations and found that the 10% concentration yielded the most favorable results. A glycerin/ethanol mixture (50 mL:50 mL) was added and the solution was vigorously stirred during 15 minutes until gel formation. A very small amount of menthol (flavoring) and a conserving agent were then added. The control gel had the same formulation except for the Punica granatum Linn infusion.

Clinical design

This study was a randomized, double-blind comparison of 2 crossover groups of dental students performed in 2 experimental phases of 21 days each with a 1-month washout interval between them. A partial mouth experimental model was used\(^\text{27}\). To standardize the groups, the students were submitted to a meticulous evaluation (pre-experimental phase) to score the Visible Plaque Index (VPI) and the Gingival Bleeding Index (GBI)\(^\text{1}\) of each tooth. All teeth of each subject were polished and flossed by the examiner to eliminate plaque remnants. The importance of oral hygiene was strongly reinforced.

Toothshield fabrication

An algiln impression of the experimental teeth was taken and poured in die stone to obtain casts. On each stone cast, a 0.3-mm-thick thermoplastic mouthguard material spacer was made using a vacuum former. Upon the spacer, an individual toothshield was made of a 2-mm-thick thermoplastic mouthguard material, using the same vacuum former. The toothshield was trimmed 2 mm beyond the gingival margin to assure that gel would be in contact with the gingival margin of the experimental teeth during toothbrushing of the remaining teeth.
Thirty days after the initial phase, the volunteers were randomly assigned to 2 groups and the experimental phase began. On day 0 of both experimental periods, VPI and GBI were recorded. A personal “kit” containing a toothshield, a tube with 90 g of control or experimental gel and a commercial dentifrice with no antigingivitis properties (Sorriso, Kolynos do Brazil Ltda., Osasco, SP, Brazil) was given to all students. During each 21-day experimental period, the subjects were instructed to fill the toothshield with the gel prior to insertion in the mouth and seat it over the experimental teeth for at least 1 min. The volunteers refrained from brushing the test quadrant, while the other teeth were normally brushed three times a day using the dentifrice (same to all students). In addition to verbal instructions, the subjects were given written recommendations to follow at home. On the last day of each period (21st day), VPI and GBI indexes were recorded and the teeth were polished with pumice.

Clinical assessment

VPI and GBI indexes were recorded by the same examiner on the mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual surfaces of the experimental teeth. The gingival tissues were inspected for the presence of bleeding, recorded 10 seconds after running the tip of a WHO probe along the gingival margin (0.5-mm penetration into the sulci). The values of six sites of each tooth were recorded to obtain the VPI and GBI means. Then, VPI and GBI means for the four test teeth were calculated to determine VPI and GBI means of each volunteer. Intra-examiner agreement for VPI and GBI was calculated by repeating the measurements in 10 patients, with at least 1 hour of interval. The Kappa coefficient was used to verify the agreement between the examinations. VPI and GBI means were 0.78 and 0.85, respectively.

STATISTICAL ANALYSIS

The Mann-Whitney non-parametric test was used to evaluate statistical differences between control and experimental groups on days 0 and 21. In each group, the mean scores of VPI and GBI were compared between baseline and the end of the trial by the Wilcoxon test. Results were expressed as means and standard deviation.

**Table 1**: Plaque index (VPI) means and standard deviation on day 0 and day 21 for the control and experimental groups

|          | Control       | Experimental  |
|----------|---------------|---------------|
| Day 0    | 0.037 ± 0.085A,a | 0.034 ± 0.060A,a |
| Day 21   | 0.740 ± 0.290A,b | 0.770 ± 0.270A,b |

*means followed by the same uppercase letters on day 0 and day 21 do not differ statistically (p>0.05)
**means followed by different lowercase letters in a same column differ statistically (p<0.05)

**Table 2**: Bleeding index (GBI) means and standard deviation on day 0 and day 21 for the control and experimental groups

|          | Control       | Experimental  |
|----------|---------------|---------------|
| Day 0    | 0.003 ± 0.017A,a | 0.008 ± 0.017A,a |
| Day 21   | 0.160 ± 0.140A,b | 0.120 ± 0.090A,b |

*means followed by the same uppercase letters on day 0 and day 21 do not differ statistically (p>0.05)
**means followed by different lowercase letters in a same column differ statistically (p<0.05)

RESULTS

Twenty-three subjects completed the clinical trial. Two students were excluded from the study during the experimental phase due to third molar extraction and restorative needs. The experimental gel had good acceptance and did not show adverse effects, such as abscess, ulcerations or allergic reactions.

On day 0, in both experimental periods, minimum plaque and bleeding scores were present and tended to zero. The control and experimental groups did not show statistically significant difference to each other with respect to VPI (p=0.5385) and GBI (p=0.3392) means (p>0.05). These results indicated that both groups were well balanced at baseline (Table 1 and 2). At the 21st day, plaque (p=0.4354) and gingival bleeding (p=0.4685) were present in both groups, but the difference between them was not statistically significant (Table 1 and 2).

Comparing the means between day 0 and day 21 in each group, there was a statistically significant difference in the VPI (p=0.0000) and GBI (p=0.0001) indexes.

DISCUSSION

The inability of adult population to perform adequate mechanical toothcleaning has stimulated the search for chemotherapeutic agents added to dentifrices to improve plaque control and prevent gingivitis. Phytotherapeutic products have been investigated with these purposes and have shown satisfactory results.

The antibacterial activity of *Punica granatum* Linn has been evaluated in previous studies with good results. Trivedi and Kazmi (1979), using extracts of fruit barks have observed an antibacterial activity of pomegranate extract against *Bacillus anthracis* and *Vibrio cholerae* while Machado, et al. (2003) showed similar effect against *Staphylococcus aureus*, in agreement with Prashant and Asha (2001).

Considering that flavoring agents can promote a moderate antiplaque effect and that the contents of test and control gels were different only with respect to the presence of pomegranate extract, this agent did not have additional effect in reducing dental plaque formation. These data are not in agreement with those of a previous *in vitro*
study that showed that a bacterial strain present in supragingival plaque, namely *Streptococcus sanguis*, was sensitive to different concentrations of pomegranate extract, which demonstrated an inhibitory action similar to that of chlorhexidine. It should be highlighted, however, that *in vitro* studies do not reproduce exactly the oral conditions.

In the present study, the *Punica granatum* Linn extract gel did not avoid plaque formation during the trial, as suggested by Kakiuchi, et al. (1986) and Pereira, et al. (2001). As the gel was placed onto the toothshield in a non-diluted form, it may be speculated that gel solubilization by saliva would be necessary for its antimicrobial action to take place. The antibacterial agents present in pomegranate - the hydrolysable tannins - form complexes of high molecular weight with proteins soluble, increase bacterial lysis and moreover interfere with bacterial adherence mechanisms to tooth surfaces.

There was no significant difference between the experimental and control groups in relation to GBI at the end of the trial. These results are not consistent with those reported by Pereira and Sampaio (2003), who showed a significant reduction on gingivitis in patients using a dentifrice containing pomegranate extract. Nevertheless, it is noteworthy that a control group was not included in that study, which does not allow accessing the actual gingivitis reduction rate related exclusively to mechanical plaque control.

According to Ross, et al. (2001) the antiinflammatory effect of pomegranate may be attributed to its considerable immunoregulatory activity over macrophages and T and B lymphocyte subsets. However, in both experimental and control groups, there was a significant increase in marginal bleeding, showing that pomegranate did not avoid the development of gingivitis. The binary score used in this study only evaluate the presence or absence of bleeding; it does not allow accessing the actual gingivitis reduction rate.

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