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Original Article

Effects of garlic extract on salivary pH: a clinical study

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Abstract The medicinal role of garlic (*Allium sativum)*, has been recognized since ancient times. Antimicrobial activity is one of the various medicinal properties associated with garlic. This property makes garlic a potential anticariogenic agent in protecting against *Streptococcus mutans*, which is acidogenic, aciduric and cariogenic in the oral cavity. The salivary pH is therefore a reflection of caries activity, being inversely proportional. The hypothesis of this study was that the use of garlic incorporated mouthwashes may be an effective strategy in resisting decrease in the salivary pH and thus exhibit cariostatic effect. The objective of the present study was to elucidate the effects of garlic, as a mouthwash, on the salivary pH as against a commercially popular mouthwash, chlorhexidine, and a combination of the two, in caries active and caries resistant individuals. The study subjects were grouped into four groups: garlic group, chlorhexidine group, combination group (garlic combined with chlorhexidine) and control group (water rinse) and these were further subdivided into caries active and caries resistant subgroups. The results show that the best benefits were obtained from the combination mouthwash in the caries active subgroup. Individually, the garlic and chlorhexidine mouthwashes were also effective in preventing substantial drops in salivary pH, post-glucose mouth rinse, in both subgroups. Interestingly, the pH values obtained after rinsing just with water fared second best among the four groups and being very close to the combination mouthwash, confirming the benefits of plain water in accordance with the principles of "hydrotherapy".

Keywords: Chlorhexidine, dental caries, garlic, hydrotherapy, salivary pH.

Introduction

Garlic (*Allium sativum*) has always been known to play an important role in the field of medicine throughout the history of mankind. Garlic is of central Asian origin, but widely recognized and used as a valuable spice and remedy for various ailments (Ankri and Mirelman, 1999; Harris *et al.*, 2001*;* Shokrzadeh and Ebadi, 2006). In Germany, for instance, the sale of garlic preparations competes with sales of leading drugs (Tripathi, 2009).

Chemical analyses of garlic cloves have shown the presence of sulphur-containing compounds. The beneficial antimicrobial effects of garlic are attributed to these sulphur based compounds, the significant ones being allicin and the products of its breakdown namely diallyl sulphide (DAS) and diallyl disulfides (DADS) (Fig. 1) (Bakri and Douglas, 2005; Masaadeh *et al*., 2006; Tripathi, 2009; Chen *et al*., 2009). The reactive allicin molecules so produced have a very short half-life, as they react with many of the surrounding proteins, including the allinase enzyme, making it into a quasi-suicidal enzyme (Ankri and Mirelman, 1999). The biological activity of allicin extracted from fresh garlic is ascribed to (a) its function as an antioxidant, (b) its ability to attach the sulphur (SH) groups in enzymes, proteins and modify their activities, thereby inhibiting the sulfhydryl enzymes and (c) its ability to rapidly penetrate into cells through the cell membranes (Ankri and Mirelman, 1999).

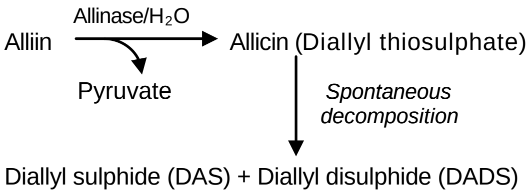

Fig. 1 Generation of allicin from a garlic clove.

The sulphur compounds are known to have an inhibitory effect on *Streptococcus mutans (S. mutans)* harboured in the dental plaque, whose acidogenicity leads to demineralization of the teeth and dental caries (Masaadeh *et al*., 2006). This antimicrobial action could be applied in the prevention of dental caries. *In vitro* studies have shown that garlic extract has an inhibitory effect on periodontopathic and cariogenic bacteria (Masaadeh *et al*., 2006; Chen *et al*., 2009).

Thus the extracts of garlic may be used to break the chain of caries aetiology, by affecting the host factors and the microbial flora. Garlic extracts stimulate the flow of saliva and facilitates the clearance of the substrates from the mouth (Groppo *et al*., 2007). It reduces the *S. mutans* counts, their acid production and prevents the demineralisation of tooth structure (Masaadeh *et al*., 2006; Chen *et al*., 2009).

Based on these facts a clinical study was done to evaluate the effects of garlic, introduced as water based mouthwash, on the salivary pH. The effects were compared with a popularly recommended chlorhexidine mouthwash and water rinse acting as control. A study was also done, incorporating the garlic into the chlorhexidine mouthwash to evaluate whether garlic effects were in synchrony with chlorhexidine.

Materials and methods

Four types of mouthwash were prepared for the present study, namely, garlic, chlorhexidine, combination (mixed garlic and chlorhexidine) and control (water purified by reverse osmosis process).

The garlic mouthwash was prepared by diluting 1000 ppm allicin liquid (Allicin International, U.K.) to 100 ppm (Groppo *et al.*, 2007). Adding allicin to water extends its half life to about 30-40 days. The addition of water stabilizes the allicin, and being water soluble, allows allicin to be absorbed into the water, which would become highly antibiotic, and leading itself to many creative applications (Ankri and Mirelman, 1999; Bakri and Douglas, 2005).

The chlorhexidine rinse was prepared by diluting a commercially available chlorhexidine (50 ppm) mouth wash in 1:1 ratio with distilled water (Harris *et al.*, 2001).

The combination mouthwash was prepared with the garlic and chlorhexidine solutions being equally mixed in a 1:1 ratio. Both the solutions are known to possess antibacterial properties and hence were mixed in equal proportions to assess whether they exhibited “synchronous” benefits.

Glucose (10%) rinse was prepared by mixing 10gm of glucose in 100ml of distilled water (Shafer *et al*., 1983). The glucose rinse was used as the common substrate for all the groups acting as a component of the cariogenic challenge.

The subjects selected for the study were in the age group of 18 to 40 years (31.6 +/- 4.6; n=80). Subjects who did not have caries were included in the caries resistant group. Subjects having a minimal of three active carious lesions were included in the caries active group. Each of the group had an equal number of caries active and caries resistant subjects.

These subjects were categorized into four groups of 20 subjects each, namely, garlic group, chlorhexidine group, combination group and control group.

All the subjects underwent oral prophylaxis before the collection of the saliva. Unstimulated whole saliva was collected from each subject and the pH was recorded using an electronic pH meter (Labin Services, Bangalore, India). The pH of this saliva was considered as baseline pH. The subjects were given glucose (10%) rinse following which the salivary pH was recorded at 5th, 10th, 15th and 20th minute to determine the salivary response to the cariogenic challenge. After an hour, the subjects were given a second glucose (10%) rinse followed by each respective mouthwash (10 ml; for 1 minute) as applicable (Shafer *et al*., 1983). The pH was recorded as before. The percentage drop in the pH was calculated, taking the baseline as 100%. The pH curve (pH versus time in minutes) was obtained for each group after rinsing with the different mouth wash solutions. The mean ± standard deviation of the recorded pH values and percentage drop in the pH before and after the use of the various mouthwashes were calculated for comparison. The pH of the rinsing solutions was measured. The data analysis was done using the general linear model and the repeated measure ANOVA. In comparison of the various groups, $p \leq 0.05$ was considered to be significant.

Results

Garlic group

In the caries resistant group, the base line pH was 6.6 +/- 0.42 (100%). On exposure to a 10% glucose rinse, the pH fell to 6.14+/- 0.54 (92.9%) at the 5th minute, and gradually increased to 6.27+/-0.38 (94.87%) at the end of 20 minutes. Subsequent to mouthwash, the pH fell to 6.11+/- 0.40 (92.44%) at 5th minute and had reached 6.37 +/- 0.37 (91.84%) by the end of 20 minutes (Fig. 2a).

In the caries active group, the baseline pH was 6.62+/-0.39 (100%). After the glucose rinse, the pH of saliva fell to 6.21+/- 0.71 (93.83%) at the 5th minute, and gradually rose to 6.34+/-0.38 (95.8%) at the end of 20 minutes. Subsequent to garlic mouthwash, the pH fell to 5.94+/- 0.54 (89.75%) at 5th minute and had risen to 6.39 +/- 0.32 (96.55%) at the end of 20 minutes (Fig 2b).

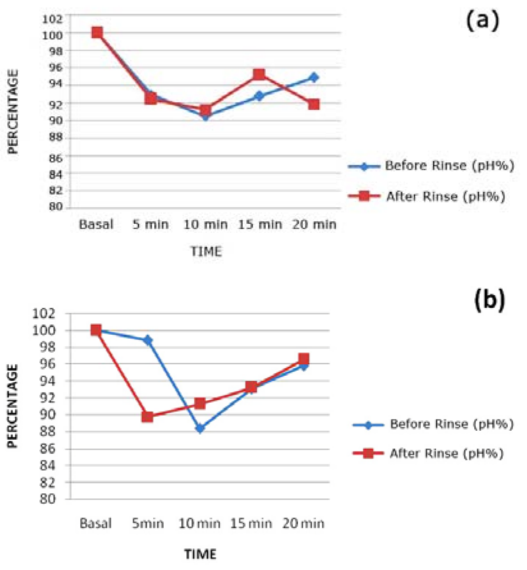

Fig. 2 pH variation curves for garlic group (a) caries resistant subgroup (b) caries active subgroup (pH=mean percentage; n=20).

Chlorhexidine mouthwash group

In the caries resistant group, the baseline pH was 6.33+/- 0.28 (100%). On use of glucose rinse, the pH dropped to 5.86+/-0.23 (92.59%) at the 5th minute and reached 6.3 +/- 0.33 (99.54%) by 20th minute. Following the use of chlorhexidine mouthwash, the pH dropped to 6.28+/- 0.47 (99.22%) at the 5th minute and reached 6.31 +/- 0.46 (99.54%) at the end of 20 minutes (Fig. 3a).

In caries active group, the baseline pH was 6.44 +/- 0.54 (100%), wherein the pH dropped to 5.63 +/- 0.44 (87.43%) at 5th minute and reached 6.01 +/- 0.54 (93.34%) at end of 20 minutes on rinsing with glucose rinse. On the use of mouthwash, the pH dropped to 5.85 +/- 0.39 (90.85%) at the 5th minute and reached 6.21 +/- 0.36 (96.6%) at the end of 20 minutes (Fig. 3b).

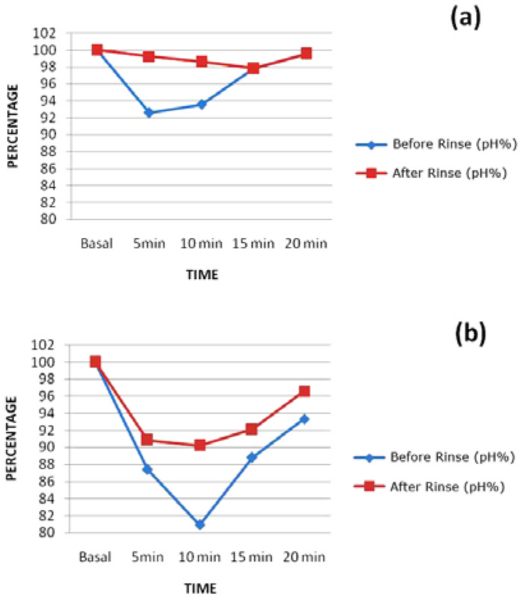

Fig. 3 pH variation curves for chlorhexidine group (a) caries resistant subgroup (b) caries active subgroup (pH = mean percentage ; n=20).

Combination (garlic and chlorhexidine) mouthwash group

In caries resistant group, the baseline pH was found to be 6.38 +/- 0.57 (100%). On rinsing with glucose solution, the pH dropped to 5.91+/- 0.43 (92.61%) at 5th minute and gradually rose to 6.23 +/-0.36 (97.62%) at the end of 20 minutes. Subsequent to the mouthwash, the pH dropped to 5.94 +/- 0.49 (93.08%) at 5 minutes and reached 6.47 +/- 0.22 (101.38%) at 20 minutes (Fig. 4a).

In the caries active group, the baseline pH was 6.25 +/- 0.13 (100%) and on rinsing with glucose, the pH had dropped to 5.66 +/- 0.27 (90.56%) by 5th minute and rose to 6.22 +/- 0.28 (99.52%) at the end of 20 minutes. Subsequent to mouthwash, the pH dropped to 6.1 +/- 0.43 (97.6%) at 5th minute and at the end of 20 minutes, it was 6.33 +/- 0.24 (101.28%) (Fig. 4b).

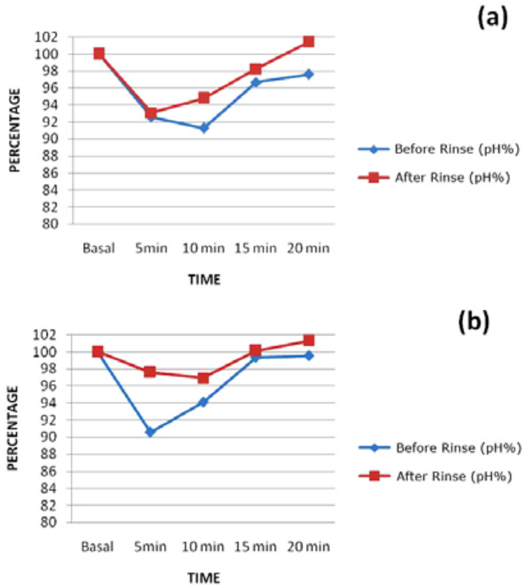

Fig. 4 pH variation curves for combination group (a) caries resistant subgroup (b) caries active subgroup (pH = mean percentage ; n=20).

Control group

In caries resistant group, the basal pH was 6.41+/- 0.09 (100%). On rinsing with glucose the pH fell to 6.17+/- 0.28 (96.25%) at 5th minute and rose to 6.32+/- 0.22 (98.59%) at the end of 20 minutes. Subsequent to the use of water rinse, the pH fell to 6.17+/- 0.13 (96.25%) at 5th minute and rose to 6.34+/- 0.15 (98.9%) at the end of 20 minutes (Fig. 5a).

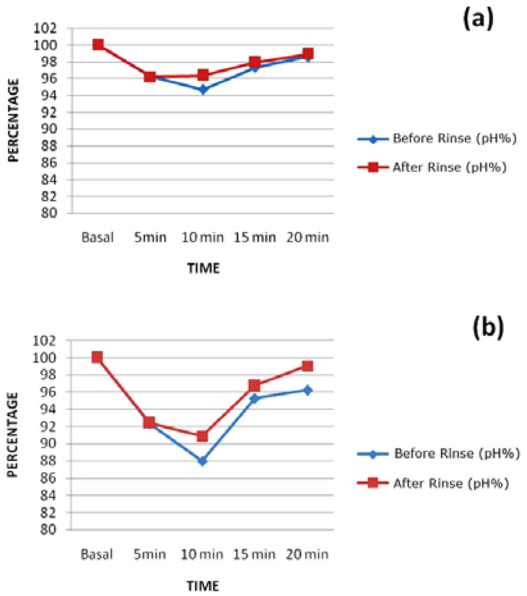

Fig. 5 pH variation curves for control group (a) caries resistant subgroup (b) caries active subgroup (pH = mean percentage ; n=20).

In the caries active group, the baseline pH was 6.44+/- 0.46 (100%). On rinsing with glucose, the pH fell to 5.95 +/- 0.37 (92.34%) at 5th minute and reached 6.2 +/- 0.42 (96.22%) at the end of 20 minutes. On rinsing with water, the pH dropped to 5.97 +/- 0.48 (92.42%) at 5th minute and by end of 20 minutes had reached 6.39+/- 0.47 (96.22%) (Fig. 5b).

The pH values (mean percentage for n=20) of the caries active subgroups of all the four groups i.e., garlic, chlorhexidine, combination and water control, at various time points of 0, 5, 10, 15, and 20 minutes were plotted as shown in Fig. 6. The drop in pH of saliva was maximum in the chlorhexidine group (5.80 +/- 0.32) followed by the garlic group (5.95+/-0.42) while the combination (6.03+/-0.30) and control groups (6.17 +/- 0.13) had the least decrease in pH.

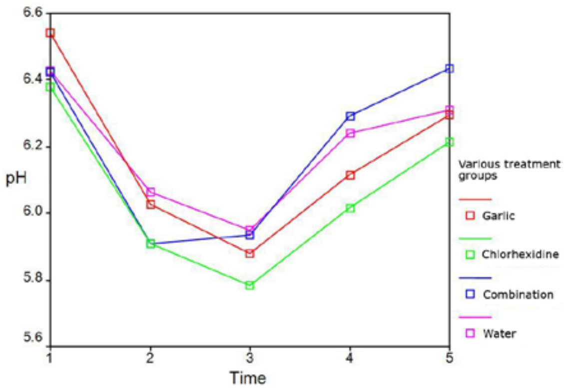

Fig. 6 pH curves for caries active subgroups of the garlic, chlorhexidine, combination and control groups (pH values= Mean pH for n=20 each).

Comparison of the results of glucose rinse followed by the group specific mouthwash showed no statistically significant difference between the 4 groups (i.e. garlic, chlorhexidine, combination and water) in this study.

In the caries resistant group, chlorhexidine mouthwash, alone, was able to reduce the pH fall significantly (p=0.005) (i.e., a significant difference in the pH curve could be seen before and with use of chlorhexidine mouth wash). However, in the caries active group, the combination mouthwash was able to prevent the fall of the salivary pH significantly following the exposure to glucose rinse (p=0.032).

When each mouthwash group was considered on the whole without distinction into caries active and caries negative groups, only the chlorhexidine group maintained a significant resistance to decrease in the pH (p=0.010). The pH of the rinse solutions were acidic in nature as compared to water which was used as rinse in the control group (Table 1).

Table 1 pH of mouth rinses used in the present study

Solution	pH
Garlic mouth rinse	5.6
Chlorhexidine mouth rinse	5.77
Combination mouth rinse	5.5
Water rinse	7.2

Discussion

Mouthwashes are widely used as an adjunct to mechanical oral hygiene procedures for their analgesic, anti-inflammatory, antimicrobial activity and anti-caries activity (Masaadeh *et al*., 2006; Tamaki *et al*., 2006; Ribeiro *et al.,* 2007; Chen *et al*., 2009; Farah *et al.*, 2009). Several adverse effects have been attributed to the use of mouthwashes currently available in the market such as taste alteration, unpleasant taste, increased risk of caries due to fermentation and alcohol content, and discoloration of teeth (Farah *et al.*, 2009; Lee and Schmitz, 2009).

Among the patients who participated in the present study, 28% remarked on the unpleasant taste of the chlorhexidine mouth rinse and in contrast 25% preferred the taste of garlic mouth rinse, which they had previously used. Majority of the patients were however 'neutral' in their preference to the taste of the various mouthwashes (Table 2).

Table 2 Patients response towards the various mouthwashes

Group (n=20 each)	Preference	Neutral	Dislike
Garlic	25 %	60 %	15 %
Chlorhexidine	20 %	52 %	28 %
Combination	15 %	73 %	12 %
Water	-	100 %	-

Many studies on chlorhexidine have shown that the reduction in oral micro flora on usage of the mouthwash is transient and dose related. The bacterial counts rapidly return to baseline levels within a week to a month depending on the dosage and term of use (Grönroos *et al*., 1995; Mangundjaja *et al.*, 2001; Tamaki *et al.*, 2006; Ribeiro *et al.,* 2007; Hassan *et al*., 2008).

Due to these factors, there is always a quest for new and improved products, emphasis being placed on natural/nature identical products. Garlic based products are the focus of such research with the recent development and availability of purified extracts for use (Lenander-Lumikari and Loimaranta, 2000; Bakri and Douglas, 2005; Masaadeh *et al*., 2006; Groppo *et al*., 2007; Chen *et al*., 2009; Lee and Schmitz, 2009). Garlic is also proven to have antioxidant, anti-atherogenic, hypolipidemic, anti-microbial, antithrombotic and anti-hypertensive activity. The garlic extract and its purified component, allicin, have the antimicrobial activity which is of particular interest, especially the inhibitory activity on *S. mutans* (Ankri and Mirelman, 1999; Harris *et al.*, 2001; Staba *et al*., 2001; Cutler and Wilson, 2004; Bakri and Douglas, 2005; Shokrzadeh and Ebadi, 2006; Masaadeh *et al*., 2006; Groppo *et al*., 2007; Fujisawa *et al*., 2008; Chen *et al*., 2009).

Masaadeh *et al.* (2006) found that different concentrations of garlic extracts inhibited several micro-organisms including those associated with caries. A clinical study showed that 250mg/ml concentration of garlic extract was inhibitory to *S. mutans* (Groppo *et al*., 2007). The Minimum Inhibitory Concentration (MIC) for *S. mutans,* OMZ 175, was found to be 8.0mg/ml and the Minimum Bactericidal Concentration (MBC) was 8.0mg/ml whereas the MIC and MBC for patient isolate *S. mutans* was 32mg/ml and 128mg/ml respectively (Grönroos *et al*., 1995).

In the present study, glucose solution (10%) was used for rinsing, to act as a suitable substrate for activation of the oral microflora. The objective was to simulate the cariogenic challenge presented to the oral environment on consumption of a carbohydrate containing foodstuff (Shafer *et al*., 1983). As expected, only in the chlorhexidine mouth rinse user, caries active group did the pH fall below the critical pH, i.e., 5.45, whereas no such decrease in the pH was seen in any of the caries resistant groups.

In the caries active group, subsequent to the use of chlorhexidine mouthwash, the pH fell to 5.81 (90.23%) in the 10^{th} minute, yet remained above the critical pH level. Subsequent to mouthwash, in caries resistant group, the time taken for the pH drop was prolonged to 15 minutes. In the caries active group, the chlorhexidine mouthwash was effective in reducing the pH fall and the endpoint pH was greater than without intervention.

In the garlic caries active group, the pH fell to 5.86 (88.39%) after the glucose rinse. But subsequent to the use of mouthwash, the pH remained above 5.94 (89.75%). Thus the fall in pH was reduced and the endpoint pH was greater on the use of garlic mouthwash despite the exposure to a glucose rinse. Studies have shown that exposure to garlic increases acid production by *S. mutans,* but in the long term, inhibit its growth. In short term period, within 24 hours, this acid production is countered by salivation in response to the spicy flavour of garlic and we hypothesize this may also be because of its inhibitory effect on *S. mutans* (Chen *et al*., 2009). The spicy flavour stimulates salivary flow. Stimulated saliva contains greater concentration of bicarbonate ions in addition to other substances. This increases the buffering capacity of saliva and its ability to clear the acid and substrates from the plaque thus improving the pH of saliva and increasing resistance to caries (Newbrun, 1989; Lenander-Lumikari and Loimaranta, 2000; Fejerskov and Kidd, 2008; Chen *et al*., 2009). However more long term studies (with a minimum duration of six months to one year), preferably on garlic incorporated mouthwashes are required to determine its sustenance.

Moreover, there are reports of oral mucosal burn patients as an allergic reaction to garlic (Eming *et al.,* 1999; Rao *et al.*, 2009). Therefore, a thorough history has to be taken to rule out allergies. The characteristic flavour of garlic is not favoured by some patients, but other patients found it to be more palatable than the chlorhexidine mouthwash.

A combination of chlorhexidine and garlic presented the best results. The pH drop was significantly reduced and end point pH was increased after mouthwash usage. These improvements were more substantial in the caries active compared to the caries resistant groups (Fig. 6).

Interestingly, in the control group, water rinsing showed effectiveness close to that of combination group in reducing the drop in pH following glucose mouth rinse. This was more effective in the caries active group than the caries resistant group. This fact goes a long way to reiterate that water by itself can have astounding effects in modifying the salivary pH, and thus can be used as daily routine regimen with the advantage of its feasibility and non side effects. It is noteworthy that G.V. Black (1914) was aware that the dental decay can be perfectly controlled by the use of tooth brush and plain water by the patient (Fejerskov and Kidd, 2008). The therapeutic modality of hydrotherapy considers disease processes to be pH mediated. The principle is to balance the pH of the body fluids like tissue fluids and saliva by regulating the intake of water (DeBruyne *et al.,* 2008; Stookey, 2008).

In the present study, the best results were obtained with the combination mouthwash of garlic and chlorhexidine, which probably produced a synergistic effect. However, the side effects of chlorhexidine still have to be considered.

Conclusion

The present study reveals that garlic is effective in minimising the fall of salivary pH after a cariogenic challenge, in a similar way to that of chlorhexidine. But the best results were obtained when they were both combined and used, making them synergistic in their actions. Thus the natural extracts of garlic have an anticariogenic property related to its antimicrobial effect and the characteristic flavour, thus having the effects on salivary pH. The characteristic flavour is significant as it induces salivation and salivary clearance. The result also shows that water rinse effectively prevents significant pH drops after carbohydrate consumption and thus is a simple and effective measure in preventing demineralisation and caries. This is in accordance with many reported studies. Thus the use of garlic incorporated combination mouthwashes or water rinsing may be a step forward in prevention of fall of salivary pH, thereby rendering more resistance against development of new carious lesions.

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