Evaluation of Natural Products for Maintaining Gingival Condition and Preventing Caries during Orthodontic Treatment: Part II: Raisins

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Abstract: The purposes of this study were to investigate: (1) the effect of chewing raisins on the plaque pH in orthodontic patients. (2) The effect of chewing raisins on the bacterial count in dental plaque. (3) The *in vitro* effect of raisins on growth of plaque bacteria. Twenty 12- to 18-year-olds, orthodontic patients participated in this randomized controlled study. Raisins were tested against sucrose and sorbitol as positive and negative controls. The pH of saliva was measured with digital pH meter prior to (baseline) and 2, 5, 10, 20, and 30 minutes after chewing raisins or rinsing with a control solution. Plaque samples were obtained from five sites using a sterile periodontal probe. *S. mutans*, lactobacilli and *P. gingivalis* were isolated and counted. The bacteria were incubated in nutrient media at 37^{o}C for 24 hours. The antibacterial activity of raisins was tested against commonly used antibiotics using the sensitivity test by disc diffusion method. The results showed significant differences in the minimum pH in raisins and sucrose groups when compared to sorbitol ($P \leq .05$ and $P \leq .01$ respectively). Raisins showed less pH drop than sucrose ($P \leq .05$). The pH in raisins group showed rapid recovery and did not reach the critical value for decalcification (5.5). Bacterial counting showed significant reduction in the number of studied microorganisms after chewing raisins. Raisins showed significant growth inhibition of all studied strains which was comparable or even more than antibiotics tested. The results of the present study support the beneficial oral effects of raisins, but would recommend further clinical researches before the use of raisins as an alternative to the traditional remedies for prevention of dental caries and gingivitis during orthodontic treatment.
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1. Introduction

Fixed orthodontic appliances often complicate tooth brushing even in motivated patients.[1] Increased plaque mass has been reported in orthodontic patients and has been linked to enamel demineralization (white spots), gingivitis and periodontitis.[1-5] When plaque is not removed, the prolonged cariogenic challenge (pH below 5.5) leads to enamel demineralization and tooth decay.[1-5] Mechanical plaque removal remains the primary and most widely accepted means of maintaining good oral hygiene. [4-7] Increased levels of mutans streptococci, lactobacilli and other periodontopathic bacteria were detected in the oral cavity after bonding orthodontic attachments.[3,8,9] Chemotherapeutic agents such as chlorhexidine and fluoride preparations have been successfully used for controlling plaque and gingivitis during orthodontic treatment.[10-16] However; most of these chemotherapeutic agents have undesirable side effects which make it inappropriate for long-term use. Recently, there was a growing trend toward the use of natural food products as an alternative in maintaining the oral and gingival health. Toothpaste and oral rinse containing sanguinaria (extract from the rhizomes of the bloodroot plant) has been shown to provide a better long-term option.[17] Cranberry mouth rinse has been shown to reduce enamel demineralization and gingival inflammation during orthodontic treatment.[18] Recently, raisins have been a focus of controversy. Raisins were considered by many investigators as acidogenic [49–51] and have been shown to have cariogenic potential in laboratory rats [22]. It was believed that the sticky nature of raisins make it more cariogenic because they are difficult to clear off the tooth surfaces [23]. However, studies have shown that sticky foods are not necessarily all retentive and no correlation has been found between stickiness and retention of foods on teeth.[24] Among the foods evaluated, raisins were almost completely cleared from tooth surfaces 5 min after chewing and swallowing. [24] Raisins consist of 60% sugars, mainly glucose and fructose, while no sucrose is detected [25]. It is well known that, sucrose, the main dietary sugar, serves as a substrate for the synthesis of adherent glucans in human dental plaque.[26] Raisins contain polyphenols and flavonoids compounds which have potent antimicrobial properties. [27] Although various

in vitro studies have been performed to investigate the mode of actions of these phytochemicals and their effects on bodily functions, much less attention has been paid to their effects on oral health and disease prevention. The effect of raisin-containing cereals on plaque pH in young children has been investigated.[28] The results showed that consumption of raisins did not reduce plaque pH below 6 over the 30-minute testing period. [28] Another study, demonstrated that raisins fractions had growth inhibitory activity against P. gingivalis and F. nucleatum, inhibited in vitro adherence but did not suppress acid formation by *S. mutans*. [29] The aims of the present study were to investigate: (1) The effect of chewing raisins on the plaque pH in orthodontic patients. (2) The effect of chewing raisins on the bacterial count in dental plaque. (3) The *in vitro* effect of raisins on growth of plaque bacteria.

2.Materials and Methods

Twenty orthodontic female patients, ranging in age from 12 to 18 years, participated in this randomized controlled study. All patients were under active treatment with fixed orthodontic appliances at the Orthodontic department, Faculty of Dental Medicine (Boy), Al-Azhar University, Cairo, Egypt. Informed consents were obtained for each patient prior to the start of the study. Exclusion criteria included subjects on antibiotic therapy 2 weeks before the test session, subjects with xerostomia and those with allergy to any of the test products. The study comprised three parts:

Part I (Plaque pH Measurements):

The endogenous pH of the raisins ethanol extracts (Thompson seedless raisins, California, USA) and control solutions were measured using a digital pH meter (Orion model 230A, Thermo Scientific Inc., Tokyo, Japan). The electrode was calibrated before measurement using standard buffers of pH 4.0 and 7.0. The pH was read after allowing the reading to stabilize for 30 seconds. Measurements were repeated three times and the means were recorded.

Plaque collecting (sampling) method: [30-32] All subjects were required to refrain from brushing their teeth or using any oral hygiene aid for 24 hours and to abstain from any food or drink (except water) for at least 2 hours before each test session. These criteria conformed to the guidelines of the Plaque Acidity Working Group of the Food, Nutrition, and Dental Health Committee of the American Dental Association.[33] The patients were given visits once a week. At each visit, baseline plaque samples were collected with a spoon excavator from all accessible surfaces of upper central incisors, buccal surfaces of upper first molars and premolars, lingual surfaces of lower molars and incisors. The subjects were asked to swallow immediately before plaque collection to minimize salivary contamination, and during sample collection, care was taken to avoid contamination with blood or saliva. Then the patients were asked to chew and ingest 10 gm of raisins (Thompson seedless raisins, California, USA) in 2 min or rinse with 15 ml of sucrose or sorbitol (10% positive and negative control solutions) for 1 min. Post consumption plaque samples were collected at 2, 5, 10, 20, and 30 minutes and pH was estimated in the same manner. Only one material was tested at each visit in a randomized order, with at least a 7 days interval between each test day to avoid any carryover effect. The plaque samples were mixed with 20 µl of distilled water and the pH was measured with a micro-combination electrode (Orion model 9802BN, Thermo Scientific Inc., Tokyo, Japan) in conjunction with a portable pH meter (Orion model 230A, Thermo Scientific Inc., Tokyo, Japan). Calibration of the system was carried out before each test. In between each reading, the electrode was cleaned with a stream of distilled water and placed in a standard solution of pH 7.0.

Part II (Bacterial Counting):

The base line and 30 min plaque samples were collected in 2 ml of sterile thioglycollate broth transport media in screw capped vials and immediately transferred to the laboratory (The regional Center for Myology and Biotechnology, Culture and Sensitivity Unit, Al-Azhar University, Cairo, Egypt). Isolation and identification of Streptococcus Mutans, Lactobacillus Acidophilus and prophyromonas Gingivalis were done according to acknowledged protocol.[34]Bacterial counting was done by using standard pour plate method. [35]

Part III Bacterial Sensitivity Test:

The antimicrobial activity of raisins was studied using the sensitivity test by disc diffusion method.[36] Antibiotic discs {Penicillin (25µg), Oxytetracycline (10µg), chloramphenicol (30µg) and Cefaclor (30µg)} 5 mm diameters were placed in the center of the agar plates. The discs of raisins were prepared by mixing raisins with ethanol in a mixer until yield a paste consistency, then shaped to discs 5 mm diameter and allowed a time to dry and evaporate ethanol. The disc of each preparation was placed equidistant on each of the streaked nutrient agar plates and incubated for 24 hours at 37°C. The diameter of the inhibition zone including the diameter of the disc was measured in mm with a divider.

Statistical Analysis:

The data were collected, tabulated and then analyzed using ANOVA and Tukey's post hoc by using SPSS 17 software. *P*-value of less than 0.05 was considered statistically significant.

3. Results

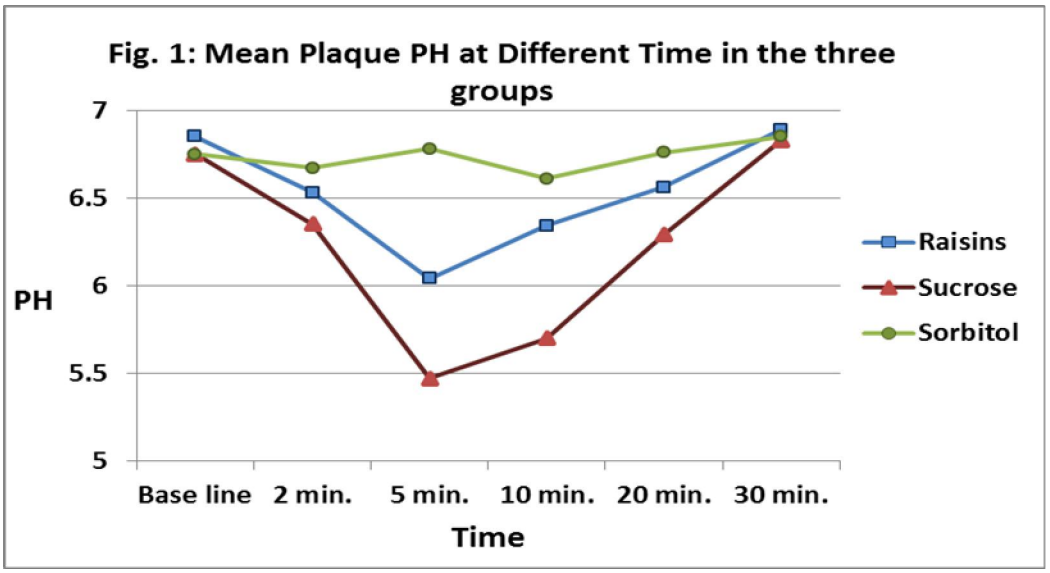
Part I (Plaque pH Measurements):

Means (X) and Standard Deviations (SD), one way analysis of variance (ANOVA) and Tukey's HSD test for plaque PH in different groups are presented in table 1and fig.1. At 5 and 10 minutes, there were significant drop in plaque pH in raisins and sucrose groups when compared to sorbitol ($P \leq .05$ and $P \leq .01$ respectively). At the same test periods, the pH values in sucrose group were significantly lower than in raisins group ($P \leq .05$). The maximum pH drop in both groups occurred at 5 and 10 min.; however the pH in raisins group showed rapid recovery at 20 min. and did not fall below the critical value for decalcification (5.5). On the other hand, the pH in sucrose group fell below the critical value and showed slow recovery at 30 min. The pH curve for sorbitol was almost straight line with no significant drop over time during the test period.

Table 1: Means (X) and Standard Deviations (SD), one way analysis of variance (ANOVA) and Tukey's HSD test for plaque pH in different groups

Groups	PH												ANOVA	
	Base line		2 min.		5 min.		10 min.		20 min.		30 min.		F	Sig.
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD		
Raisins	6.85^{a}	(.51)	6.53^{a}	(.49)	6.04^{b}	(.21)	6.34^{b}	(.18)	6.56^{a}	(.19)	6.89^{a}	(.25)		
Sucrose	6.75^{a}	(.44)	6.35^{b}	(.33)	5.47^{c}	(.27)	5.70^{c}	(.27)	6.29^{b}	(.18)	6.83^{a}	(.27)	17.440	.000***
Sorbitol	6.75^{a}	(.42)	6.67^{a}	(.44)	6.78^{a}	(.48)	6.61^{a}	(.46)	6.76^{a}	(.52)	6.85^{a}	(.52)		

Means with similar letters are not significantly different
(a – b) and (b – c) are significant at $P \leq 0.05$, (a – c) are significant at $P \leq 0.01$, *** is significant at $P \leq 0.001$



Part II (Bacterial Counting):

Means and Standard Deviations (SD) and paired samples t-test for bacterial count before and after chewing raisins are presented in table 2 and fig.2. There was significant reduction in the number of *Streptococcus mutans*, lactobacilli and *Prophyromonas gingivalis* after chewing honey ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.01$ respectively).

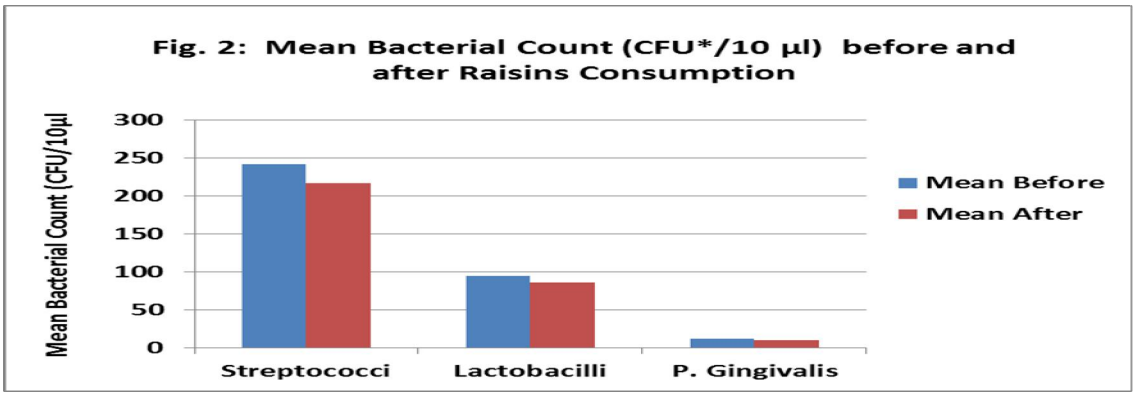
Part III Bacterial Sensitivity Test:

Means and standard Deviations (SD) of Inhibition Zones (mm) for raisins and tested Antibiotics and Tukey HSD test for comparison the effect of raisins on bacterial growth of different strains are presented in table 3 and fig. 3, 4. Raisins showed significant growth inhibition of streptococcus mutans when compared with Cefaclor ($P \leq 0.05$) but no significant differences were found with other antibiotics. Inhibition zones (mm) for *P. gingivalis* were not significantly different with raisins or any of the tested antibiotics. Inhibition zones (mm) for lactobacilli were significantly larger with raisins than chloramphenicol and Cefaclor ($P \leq 0.001$ and $P \leq 0.05$ respectively) but no significant differences were found among raisins, penicillin and Oxytetracycline.

Table 2: Means and Standard Deviations (SD) and paired samples t-test for bacterial count (CFU*/10 µl) before and after chewing raisins.

		Mean (CFU*)	±SD	t – test				
				Mean Difference	±SD	Std. Error	T - value	Sig.
Streptococci	**Before**	242.4	138.72	25.20000	26.36833	8.33840	3.022	.014**
	After	217.2	116.0					
Lactobacilli	**Before**	94.6	22.8	8.40000	6.71979	2.12498	3.953	.003***
	After	86.2	17.2					
P. Gingivalis	**Before**	12.0	16.0	1.80000	1.68655	.53333	3.375	.008***
	After	10.2	14.6					

* CFU = Colony Forming Unit, ** $P \leq 0.05$, *** $P \leq 0.01$



* CFU = Colony Forming Unit

Table 3: Means and standard Deviations (SD) of Inhibition Zones (mm) for Raisins and Different Antibiotics and Tukey HSD test for comparison the effect of Raisins on bacterial growth of different strains

		Mean	±S. D	Tukey HSD test		
				Mean Difference	±Std. Error	Sig.
Streptococci mutans	**Raisins**	20.9	3.5			
	Penicillin	18.2	3.5	2.70000	1.37073	.373NS
	Oxytetracycline	20.4	3.1	.50000	1.37073	.999NS
	Chloramphenicol	19.2	2.0	1.70000	1.37073	.815NS
	Cefaclor	16.4	2.9	4.50000	1.37073	.021*
P.Gingivalis	**Raisins**	17.9	3.5			
	Penicillin	16.8	3.4	1.10000	1.26139	.952NS
	Oxytetracycline	19.3	2.4	-1.40000	1.26139	.875NS
	Chloramphenicol	18.3	2.1	-.40000	1.26139	1.000NS
	Cefaclor	15.6	2.6	2.30000	1.26139	.460NS
Lactobacilli	**Raisins**	23.5	3.2			
	Penicillin	21.4	3.1	2.10000	1.25536	.555NS
	Oxytetracycline	21.5	2.8	2.00000	1.25536	.607NS
	Chloramphenicol	15.2	2.0	8.30000	1.25536	.000***
	Cefaclor	19.2	2.6	4.30000	1.25536	.014*

NS = Non Significant, * $P \leq 0.05$, *** $P \leq 0.001$

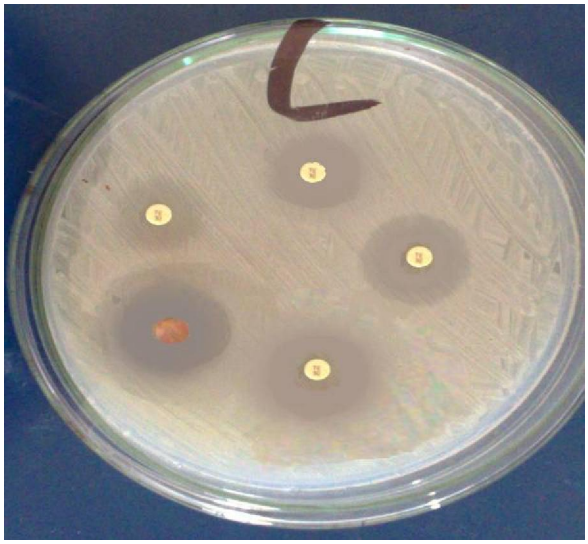
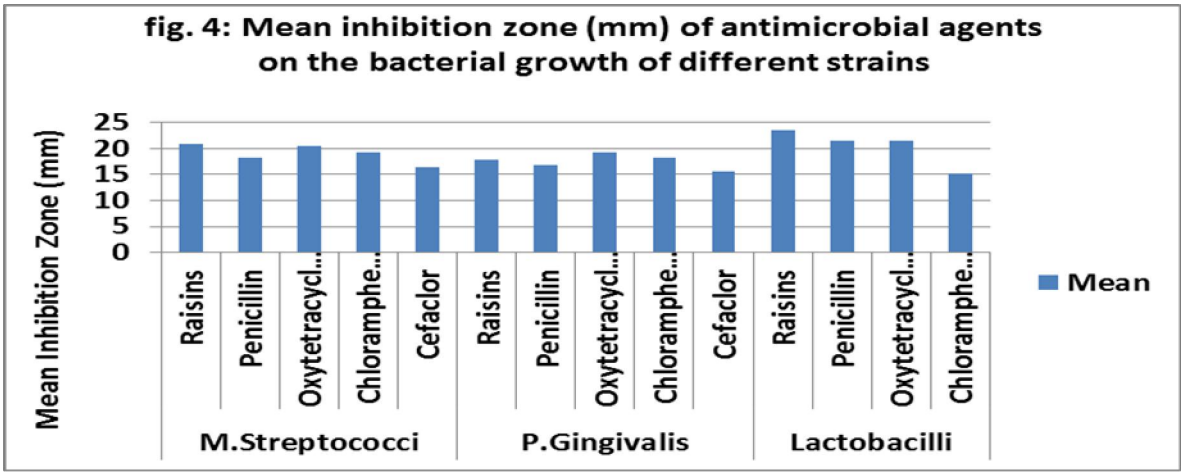


Fig. 3: Inhibition zones of raisins versus tested antibiotics



4. Discussion

Enamel demineralization and gingival inflammation, the plaque mediated microbial diseases, are considered as potential risks during orthodontic treatment. Dental plaque has been implicated as the prime etiologic factor in dental caries, gingivitis, and periodontal disease. [37–39] When compared to sorbitol, raisins causes pH drop much less than sucrose ($P \leq .05$ and $P \leq .01$ respectively). The pH values in sucrose group were significantly lower than in raisins group ($P \leq .05$). The pH in raisins group showed rapid recovery at 20 min. and did not decline below the critical value for decalcification (5.5). These findings are in agreement with previous studies on the acidogenicity of dental plaque.[28, 40-42] The effect of raisins and raisin-containing bran cereal on in vivo plaque acidogenicity was examined in young children. [28] It was found that raisins did not reduce the plaque pH below pH 6 over the 30-min test period. Compared with commercial bran flakes or raisin bran cereal, a lower plaque pH drop was noted in children who consumed a raisin and bran flake mixture when no sugar was added ($P \leq 0.05$). [28]

Raisins contain polyphenols, flavonoids, and high levels of iron that may benefit human health. However, their oral health benefits are less well understood. In the present study, raisins significantly reduce the number of streptococcus mutans, lactobacilli and prophyromonas gingivalis ($P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.01$ respectively). The sensitivity test has indicated that raisins significantly inhibit growth of streptococcus mutans, P. gingivalis and lactobacilli. These findings are in agreement with conclusions made in other studies that reported antimicrobial activity from raisin products.[29, 43-46] Several triterpenoid compounds isolated from Thompson seedless raisins were found to inhibit bacteria associated with dental caries and periodontal disease. [28]

Antimicrobial compounds from raisins were isolated, identified and investigated for their ability to inhibit growth of *Streptococcus mutans*, *Porphyromonas gingivalis,* and *Fusobacterium nucleatum*.[29] Their ability to inhibit biofilm formation by *S. mutans* in the presence of sucrose was also determined. Both chloroform and ethyl acetate fractions demonstrated growth inhibitory activity against *P. gingivalis* and *F. nucleatum*. Subsequent purification of these extracts afforded 5-hydroxymethyl furaldehyde and a large quantity of a triterpenoid, oleanolic acid. The latter exerted preferential growth inhibitory activity against *P. gingivalis* and inhibited *in vitro* adherence of *S. mutans* biofilm. Earlier *in vitro* studies have shown that oleanolic acid inhibited insoluble glucan synthesis of *mutans streptococci* in the oral cavity.[43-46]

Conclusions

1. Raisins reduced the plaque pH but not to the critical value for demineralization.
2. Raisins reduced *Streptococcus mutans*, *P. gingivalis* and lactobacilli count in plaque.
3. Raisins inhibited the growth of theses strains *in vitro*.
4. Although the *in vitro* tests does not actually represent the oral environment, the findings of the present work suggesting that raisins has a potential as a promising natural agent for maintaining oral health during orthodontic treatment.
5. Further studies to evaluate the long-term effect of raisin consumption on plaque microflora and acidogenicity are necessary.

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