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REMINERALIZATION AND ANTIBACTERIAL EFFICACY OF DIFFERENT CONCENTRATIONS OF AQUEOUS STEVIA EXTRACT AND GREEN TEA SOLUTIONS IN COMPARISON WITH FLUORIDE-BASED MOUTHWASH ON INITIAL ENAMEL CARIOUS LESION- AN INVITRO STUDY

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ABSTRACT

Aim: This in vitro study evaluated and compared the remineralizing potential of different herbal extracts aqueous solutions versus fluoride mouthwash by assessing the enamel surface microhardness and antimicrobial susceptibility.

Materials and methods: Sixty extracted premolars were used in this study and equally distributed into six groups of 10 teeth each. Group A: teeth treated with 0.5% stevia aqueous solution; Group B: teeth treated 5% stevia aqueous solution; Group C: teeth treated with 0.5% green tea aqueous solution; Group D: teeth treated with 5% green tea aqueous solution; Group E: teeth treated with Fluoride mouthwash as a positive control group; negative control group: teeth not subjected to any treatment and stored in artificial saliva. The teeth of each group were subjected to microhardness assessment at baseline, after 48 hours demineralization, and after 7 days remineralization phase. The antibacterial activities of herbal extracts and fluoride against S. mutants and Lactobacillus were quantitatively measured by an antimicrobial susceptibility test.

Results: After 7 days of treatment, the highest mean value was recorded in group D (282.69 Kgf/mm^2) with the least mean value recorded in the control group (168.66 Kgf/mm^2). The difference between groups was statistically significant (p= 0.001). The 5% green tea extract showed the highest mean value of inhibition zone (10.6 mm) against S. mutants' while fluoride showed the highest mean value of inhibition zone (14.6 mm) against the Lactobacillus.

Conclusion: An aqueous solution of 5 % green tea is an effective remineralizing agent with antimicrobial activity against S. mutans.

KEYWORDS: enamel remineralization, green tea, stevia, fluoride, initial caries.

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INTRODUCTION

Dental caries caused by a dynamic process that develops when demineralization exceeds remineralization. Its multifactorial disease influenced by the pathogenic factor [1]. It is a gradual process where noninvasive intervention can arrest it in the early stages [2]. Early diagnosis of incipient carious lesions has resulted in a new transaction in preventive dentistry in the form of remineralization[3]. Remineralization can be defined as "the transport and deposition of mineral elements, mainly calcium and phosphate, into the caries lesion that have been lost due to demineralization of the tooth tissue" [4].

The initial caries lesion can be defined as "a primary lesion which has not reached the stage of an established lesion with cavitation" [5]. It is therefore could be treated by ultra-conservative or minimal intervention dentistry [5]. Fluoride-containing dentifrices and mouthwashes have been widely used and investigated to prevent demineralization of dental enamel or to achieve the remineralization effect, as well as the antimicrobial effect against S.mutans. Antimicrobial agents can reduce dental plaque and limit the creation of new plaque, which is the cause of tooth caries and periodontal disease. There is an increasing demand for herbal-based remedies in both developing and developed countries as a result of the growing awareness towards the natural products due to their availability, affordable prices and the least side effects [6]. Herbal extracts can be used as an antibacterial agent that is safe and effective in reducing pathogenic bacteria [7]. These useful types of plants can be a reliable alternative of many medicines [8].

Dietary control is one of the most significant aspects of dental caries prevention. However, dental habits are difficult to break, especially when they are tied to the eating of carbohydrates. The most commonly consumed fermentable carbohydrates, sucrose, is linked to a high rate of dental caries. The oral bacteria in dental plaque quickly digest sucrose, resulting in the release of acids. In the dynamic caries process, these acids are responsible for the demineralization of the dental tissues [9].

Stevia is extracted from the Stevia Rebaudiana Bertoni plant. It contains a natural sweetener called steviosdeo (a molecule of complex sugar). Stevia, as an added sweetener, has been investigated recently and was shown to be noncariogenic through its antibacterial effect on microorganisms associated with the production of tooth decay, low acidogenic potential and antiplaque effect [10].

Green tea is one of the most herbals used in many researches due to their high content of polyphenols (catechins) like epigallocatechin gallate (EGCG) that exhibits profound inhibitory effect on both collagenase and elastase. It also possesses antibacterial effects as a result of the presence of bioactive compounds like as polyphenols, minerals, and volatile oil. Its remineralizing effect is aided by high fluoride concentration [11].

Therefore, the purpose of this study is to assess and compare the efficacy of stevia and green tea aqueous solutions with different concentrations on enamel remineralization as well as their antibacterial activity. The null hypothesis was that the application of the herbal extract solutions did not have any effect on remineralization potential of artificially demineralized enamel with no antibacterial activity.

MATERIALS AND METHODS

Materials

Remineralizing solutions used

a) A commercially available green tea brand; (Lipton tea ≈ 6.5 ppm fluoride, imported and packed in Egypt by Unilever Mashreq Co.)[12, 13].

b) Refined Stevia rebaudiana extract powder (from the Agriculture Research Center, Giza, Egypt).

c) Amine fluoride 0.125 gm mouthwash (Ezaflour mouthwash, Multipharma Co., Egypt).

Methods

Study design

Sixty extracted premolars were used in this in vitro study. Premolars had been equally distributed into five experimental groups, each of 10 teeth, according to the remineralizing agents used, and one Negative Control Group. Group A (n=10): teeth were treated with 0.5% stevia aqueous solution; Group B (n=10): teeth were treated 5% stevia aqueous solution; Group C (n=10): teeth were treated with 0.5% green tea aqueous solution; Group D (n=10): teeth were treated with 5% green tea aqueous solution; Group E (n=10): teeth were treated with Fluoride mouthwash (Ezaflour) as appositive control; negative control group (n=10): teeth not subjected to any treatment and stored in artificial saliva. All teeth (experimental and negative control groups) were subjected to demineralization protocol for 48 hours. Teeth of each group were subjected to microhardness assessment at baseline, after 48 hours demineralization, and after 7 days remineralization phase. The antibacterial activities of the herbal extracts and fluoride against S. mutants and Lactobacillus were quantitatively measured by antimicrobial susceptibility test**.**

Sample size calculation

To Evaluate the remineralizing effect of Aqueous Stevia extracts and green tea in Comparison with Fluoride-based Mouthwash on Initial Enamel Carious Lesion, ANOVA test or an equivalent non-parametric test was used for comparison between 6 experimental and control groups. According to Rajab et al (2018) [14], surface micro-hardness varied from 158.9±40.4 in experimental group, to 317.4±102.5 in control. Based on Rajab et al (2018) [14] and Using G power statistical power Analysis program (version 3.1.9.4) for sample size determination [15], A total sample size (n=60); equally divided to 10 in each group) will be sufficient to detect a large effect size (f) =0.49, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.

Preparation of 0.5%, 5% Stevia aqueous extract

The powder obtained was weighed up to 0.5g, 5g and then mixed with 100 ml of sterile distilled water in a sterile glass flask for 5 minutes. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filter paper and kept in an airtight container [16].

Preparation of 0.5%, 5% green tea aqueous extract

The powder obtained thus was weighed up to 0.5 g, 5g and then mixed with 100 ml of sterile distilled water in a sterile glass flask for 5 minutes. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filter paper and kept in an airtight container[17].

Sample preparation

Sixty sound extracted premolars for orthodontic and surgical reasons were used in this study. Consent was obtained from patients (ranged from 18 to 25 years old) with the approval of using their teeth before extraction according to the guide of the research ethics committee. The study proposal was reviewed and approved by the Research Ethics Committees (REC) of the Faculty of Dentistry, Cairo University, Egypt on 29/3/2022. With approval number 29.3.22. Teeth with restorations, enamel cracks, caries, erosion, developmental defects, or white spot lesions were not included [17].

Disinfection of the selected premolars was done using a solution of 5.25% sodium hypochlorite solution for 1 hour. Decoronation of all selected molars was carried out by sectioning the roots 2 mm cervical to the cementoenamel junction using a water-cooled diamond saw (Isomet® 5000 Linear Precision Saw; Buehler Ltd., Lake Bluff, USA) [18].

The crowns were scraped with a hand scaler and washed under running tap water to remove

any residual tissues and debris; then polished with fluoride free pumice paste. An acid-resistant varnish coating was applied on all teeth surfaces except a window of 2 mm X 2 mm on the middle third of the buccal and lingual surfaces [17, 19].

Using a water-cooled diamond saw each tooth was sectioned into 2 halves mesio-distally. Custom-made plastic molds were prepared with the dimension of 3 mm height and 20 mm diameter poured with cold cure acrylic resin (Acrostone dental factory, Egypt). The buccal and lingual halves of each tooth were fixed using superglue on the custom-made acrylic resin block; so that the buccal and lingual surfaces were available for treatment to be treated(17). For easy identification, each acrylic disc with the glued sample was numerically coded at its base using a waterproof permanent marker. Each group of samples was put in a separate glass container containing 10 ml of artificial saliva at 37°C in the CO2 incubator (D180-P air jacket CO2 incubator, Mira lab, Cairo, Egypt).

Artificial Non-cavitated Initial Enamel Lesion Formation

Artificial caries lesion has been produced by immersing the teeth in a demineralizing solution (10 mL for each specimen) for 48 hours. Then, they were rinsed with distilled water and stored in artificial saliva to simulate the oral cavity conditions. Every 12 hours, the demineralizing solution was renewed to prevent depletion of solution. The demineralizing solution composed of 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide (KOH) to maintain a pH of 4.4 (17).

Enamel Surface treatment with the remineralizing solutions

The samples in the experimental groups (A, B, C, D, E) were immersed in 20 ml of the corresponding solutions for 5 minutes at 37°C: 0.5% and 5% stevia, 0.5% and 5% green tea, fluoride mouth wash, 3 times a day for 7 days. Finally, the samples were rinsed carefully with distilled water. After drying the samples with clean absorbent, each group was returned to its container containing artificial saliva (20 ml) in the incubator.

Teeth of the control group were stored in the artificial saliva for 7 days the experimental time and not subjected to any type of treatment. The artificial saliva was changed once daily to avoid the risk of its saturation hence interfering with the treatment process [26].

Artificial saliva

Artificial saliva was prepared by mixing 500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to maintain a pH of 6.8 [20].

Enamel Surface Microhardness Assessment (HV)

Enamel surface microhardness was measured at baseline of sound untreated enamel, after 48 hours demineralization and after 7 days of the remineralization. The surface microhardness of the enamel specimens was measured with Digital Display Vickers Microhardness Tester equipped with a Vickers diamond indenter and a 20X objective lens. For 10 seconds a load of 50g was applied to the surface of the specimens. Three indentations were evenly placed on the surface of each specimen and not closer than 0.5 mm to the adjacent indentations. The diagonals lengths of the indentations were measured by a built-in scaled microscope and Vickers values were converted into microhardness values. Microhardness value was obtained using the following equation: HV=1.854 P/d2; Where HV is Vickers hardness in Kgf /mm2, P is the load in Kgf and d is the length of the diagonals in mm.

Antimicrobial Susceptibility Test

The Susceptibility Tests were performed according to NCCLS recommendations (National Committee for clinical laboratory Standards, 1993).

Screening tests regarding the inhibition zone were carried out by the well diffusion method (Hindler et al., 1994). The inoculum suspension was prepared from colonies grown overnight on an agar plate, and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). The compounds were dissolved in dimethyl sulfoxide (DMSO) with different concentrations (10,5,2.5 mg/ml). the inhibition zone was measured around each well after 24h at 37C°. Controls using DMSO were adequately done [21].

Statistical analysis

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 18 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows. Numerical data were summarized using mean, standard deviation and confidence interval. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups with respect to normally distributed numeric variables were compared by one-way analysis of variance (ANOVA) test, followed by Tukey's post hoc test when ANOVA revealed a significant difference. The percent change was calculated by the formula: *(value after-value before) / value before X100*

All *p*-values are two-sided. The significance level was set at $p \leq 0.05$.

RESULTS

Enamel Surface microhardness results

At baseline and after demineralization, all groups showed decrease in enamel surface microhardness with statistically significant difference between groups ($p < .01$). Tukey's post hoc test revealed no significant difference between control group, group E, group A and group B groups. There was no difference between green tea subgroups.

After remineralization, all groups showed decrease in microhardness values except Group C (237.79 Kgf/mm^2) and Group D (282.69 Kgf/mm^2), with the greatest decrease recorded in the control group (168.66 Kgf/mm^2). The difference between groups was statistically significant ($p < .01$), Tukey's post hoc test revealed nonsignificant difference between Group C, E, A and B Stevia groups Table (1).

Overall (from baseline to remineralization), the only group that showed a percent increase was group D (3.36%). All other groups showed a percent decrease, with the greatest percent decrease recorded in control group (-50%), followed by group E (-44.05%), then group A (-26.85%), then group B (-25.26 %); with the least decrease recorded in group C (-16.26%). The difference between groups was statistically significant ($p < .01$) Tukey's post hoc test revealed that group E and control group were not significantly different. Moreover, both group A, B and C were not significantly different Table (2).

Antibacterial activity results

The antibacterial activities of herbal extracts and fluoride against S. mutants and Lactobacillus were quantitatively measured, considering inhibition zones of more than 6 mm as a positive outcome. In both bacterial cultures, stevia didn't produce inhibition zones against S. mutants and Lactobacillus. In streptococcus mutants' culture, the 5% green tea extract showed the highest mean value of inhibition zone (10.6 mm) followed by the 0.5% green tea and fluoride (9.8 mm). Regarding the Lactobacillus culture the fluoride showed the highest mean value of inhibition zone (14.6 mm) followed by 5% green tea extract (12 mm) and the lowest value recorded for the 0.5% green tea (8.2 mm) table (3) figure (1).

TABLE (1) Descriptive statistics and comparison of recorded mean value in different groups (ANOVA test)

		Mean Kgf/m^{m}2	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Maxi	F	P
					Lower Bound	Upper Bound				
Baseline	Group A	327.45 [a]	6.89	2.18	322.52	332.38	316.33	336.40	8.98	<.001*
	Group B	334.69[a]	11.59	5.06	316.73	339.62	305.67	347.33		
	Group C	282.17 [b]	46.77	14.79	248.71	315.63	221.33	344.97		
	Group D	275.43[b]	39.72	12.56	247.02	303.84	236.33	331.00		
	Group E	328.17 [a]	16.00	3.67	326.39	342.98	323.67	352.83		
	Control group	336.08 [a]	29.50	9.33	314.98	357.18	307.67	386.93		
After Demin-eralization	Group A	279.97 [a]	10.30	3.26	272.61	287.34	264.67	294.27	9.972	<.001*
	Group B	267.95 [a]	31.22	9.87	245.61	290.29	209.00	290.07		
	Group C	212.83[b]	49.28	15.59	177.57	248.09	150.00	290.90		
	Group D	201.70[b]	47.78	15.11	167.52	235.89	149.67	266.33		
	Group E	270.38 [a]	18.57	5.87	257.10	283.66	239.67	286.20		
	Control group	274.61[a]	31.90	10.09	251.79	297.43	231.67	311.10		
After remin-eralization	Group A	238.87 [a,b]	72.72	23.00	186.85	290.89	101.33	281.00	5.051	<.001*
	Group B	250.92[a,b]	57.62	18.22	209.70	292.15	185.90	345.43		
	Group C	237.79 [a,b]	71.05	22.47	186.96	288.61	104.87	291.43		
	Group D	282.69 [a]	26.92	8.51	263.44	301.95	252.40	328.30		
	Group E	183.45 [a, b]	74.75	23.64	129.98	236.92	99.90	304.83		
	Control group	168.66[c]	44.36	14.03	136.92	200.39	100.00	210.40		

Significance level p≤0.05, *significant

Tukey's post hoc test: within the same comparison (observation time), means sharing the same superscript letter are nonsignificant

TABLE (2) Descriptive statistics and comparison of percent change in different groups at each interval (ANOVA test)

		Mean %	Std. Dev	Median	95% Confidence Interval for Mean		Min	Max	F	P
					Lower Bound	Upper Bound				
After demin	Group A	-14.52[c]	1.80	-14.6	-15.80	-13.23	-16.44	-12.34	2.875	.023*
	Groip B	-19.96[b]	8.93	-18.17	-26.35	-13.57	-35.79	-10.59		
	Group C	-24.38[b]	12.71	-17.19	-33.47	-15.29	-44.73	-12.75		
	Group D	-27.30[a]	10.09	-26.83	-34.52	-20.08	-39.57	-14.28		
	Group E	-17.47[b]	6.40	-15.59	-22.05	-12.89	-28.80	-10.72		
	Control group	-18.08[b]	8.99	-19.06	-24.51	-11.65	-31.46	-5.34		

After remin	Group A	-14.63[b]	26.23	-4.48	-33.40	4.13	-63.46	6.05	10.689	.000*
	Group B	-5.75[b]	20.50	-5.88	-20.42	8.91	-32.90	21.91		
	Group C	15.21[a]	41.41	24.06	-14.42	44.83	-42.99	73.31		
	Group D	49.75[a]	47.15	49.23	16.02	83.48	.08	119.35		
	Group E	-32.10[c]	26.37	-37.05	-50.96	-13.23	-64.53	7.59		
	Control group	-38.16[c]	15.55	-33.24	-49.28	-27.03	-66.14	-25.22		
Overall (from baseline to after remin)	Group A	-26.85[b]	22.68	-18.43	-43.08	-10.63	-69.45	-11.28	9.658	.000*
	Group B	-25.26[b]	15.45	-30.53	-36.31	-14.22	-43.50	-1.88		
	Group C	-16.26[b]	23.60	-15.15	-33.14	.63	-52.80	15.04		
	Group D	5.36[a]	22.17	12.29	-10.50	21.23	-23.75	32.56		
	Group E	-44.05[c]	22.70	-55.16	-60.28	-27.81	-68.33	-9.19		
	Control group	-50.00[c]	12.06	-46.35	-58.63	-41.37	-67.95	-37.13		

Significance level p≤0.05, *significant

Tukey's post hoc test: within the same comparison (interval), means sharing the same superscript letter are nonsignificant

TABLE (3): Mean and standard deviation (SD) values of inhibition zone of different groups

Variables	**Inhibition Zone**			
	Streptococcus Mutants		**Lactobacillus**	
	Mean (mm)	**SD**	**Mean (mm)**	**SD**
0.5% stevia	0	0	0	0
5% stevia	0	0	0	0
0.5% green tea	9.8[b]	0.4472	8.2[c]	0.4472
5% green tea	10.6[a]	0.5477	12[b]	0.7071
Fluoride	9.8[b]	0.8367	14.6[a]	0.5477
p value	< .001*		<.001*	
f value	479.466		1138.7	

Significance level p≤0.05, *significant

Fig. (1) Inhibition zone of different groups

DISCUSSION

Dental caries is one of the common, chronic, multifactorial disease that affect the hard tooth structure wide world. Based on the World Health Organization (WHO) reports, dental caries, showed less worries in many parts of the industrialized world, but still occupies a lot of concern as an important public health problem in many developing countries. The statistics suggest that dental caries affect 60-90% of school going children in developing countries [22].

Therefore, a great improvement in dental caries treatment may be achieved by applying caries preventive strategies, non-invasive caries management techniques, and paying attention to high-risk groups that are represented by patients with low economic-social status in many countries [23]. Fluoride was the first antibacterial and remineralizing material used to prevent dental caries and periodontal diseases. The use of fluorides in different forms showed favor results in combination with other professional care techniques [22, 24, 25]. Fluoride has been proved to promote the formation of apatite and enhance the remineralization of teeth even in very low concentration of less than 0.5 ppm in saliva [17]. Many studies recorded higher enamel microhardness results for teeth exposed to fluoride in its organic form as (amine fluoride) than inorganic fluoride (sodium fluoride) [26]. Despite the presence of fluoride as a normal mineral found naturally in water, but fluoride toothpaste contains a higher concentration than fluoridated water does that is not allowed to be swallowed. Although fluoride helps prevent tooth decay by keeping tooth enamel strong and inhibiting the growth of bacteria in plaque, but when it is in the stomach, it can cause irritation, leading to nausea, vomiting, and diarrhea. Also a high-fluoride strategy cannot be followed to avoid the potential for fluorosis as an adverse effects due to overexposure to fluoride [3][27].

Accordingly, our study was focused on the using Green tea and Stevia for their effect of remineralization on initial enamel caries using microhardness test and their antibacterial efficiency by the Antimicrobial Susceptibility Test**.**

Stevia extracts with different concentration have been used to prove their mineralizing and antibacterial effect on enamel surface. Stevia is a plant called Stevia rebaudiana Bertoni, comes from the border of Paraguay and Brazil that contains a chemical substance called sativoside (a molecule of complex sugar) and was used as a natural sweetener for drinks and in pharmaceutical properties. Stevia is composed of reducing sugars (4.5%), moisture (10.73%), fiber (5.3%), proteins (13.68%), fat (6.13%) and carbohydrates (63%) [28]. Stevia proved to have anticariogenic effect against S.mutans, S.sorbinus, L.acidophilus, and C.albicans. Also, it has anti plaque effect by reducing biofilm formation and considered as healing agent at periodontium level [3, 9, 10, 23]. Stevia prevents dental caries by reducing the acid production and inhibiting bacterial adhesion on tooth surface. The findings of many studies have shown that the leaves of stevia contain compounds such as diterpenoid steviol-glycosides, sesquiterpenes, bis-nor-diterpene, sterols, and flavonoids which have many systemic therapeutic properties [8, 16].

Green tea as a cheap and available herbal in markets containing polyphenolic compounds attracted our interest to compare it with stevia. The polyphenols present in other daily intakes of the human diet such as coffee, cereals, and fruit. Some of the biological benefits of polyphenols are antioxidant, anticancer, and anti-inflammatory effects [25]. Some in vitro studies done on green tea extracts suggested an activity against several metabolic activities of mutans streptococci, resulting in a decrease in growth and virulence. Tea is known to have catechins like epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin, epicatechin. Isolated green tea catechins such as

EGCG have already been shown to be inhibitors of collagenase, elastase and insoluble glucan catalyzed by glucosyltransferase from mutans streptococci. Also, it showed reduction of mutans streptococci adherence to glass [25][29]. Other studies have also demonstrated the inhibition of salivary amylase activity that may contribute significantly to reducing the cariogenicity of starch-containing foods by extracts of a commercial tea [17]. Green tea contains high fluoride content [17,30] and also high pH value which is about 6.3 [14] that may be the reason of being a remineralizing agent.

All enamel specimens (experimental, positive and negative control groups) were subjected to demineralization phase for 48 hours. The demineralizing solution used in this study of pH 4.4 to simulate the pH drop that happens in the oral cavity; as it can cause enamel dissolution and demineralization [17]. Artificial saliva was used as a storage medium for all experimental and negative control teeth to simulate the remineralizing capacity of human saliva [31], as it contains inorganic electrolytes (calcium, phosphorus, and fluoride) that are important participants in the remineralization process [32]. All specimens were subjected to enamel surface microhardness test (Vickers Microhardness Tester) at baseline, after demineralization and after 7 days of remineralization with different concentrations of stevia and green tea, fluoride mouthwash (positive control) as well as the specimens stored in the artificial saliva without any treatment (negative control). Measuring the surface microhardness is a simple, rapid, and nondestructive method for assessing the mineralizing effect of the different solutions [11].

Regarding the surface microhardness results at baseline and after demineralization, results showed a significant decrease in the microhardness value of demineralized enamel surfaces, indicating loss of minerals, in all groups with nonsignificant difference between them. These results proved the demineralizing effect of the demineralizing solution used in our study [17].

Green tea with both concentrations confirmed the highest remineralizing effect on initial demineralized enamel which were in agreement with Aidaros et al and Talaat with no harmful effect on enamel [14]. The results was also supported by S. Amal et al study who concluded that green tea was the highest remineralizing efficacy than 0.05% sodium fluoride [33]. This may be referred to the highest fluoride percentage in the green tea used in this study. This finding is consistent with Malinowaska et al [36] who reported that the fluoride content in green tea infusion after 5 minutes of brewing was 0.59-1.83 mg/L in green tea infusion [34].

The 5% stevia group showed better microhardness values than the 0.5% stevia group. This was in contrast with Kishta-Derani at al who concluded that 5% stevia recorded the greatest microhardness loss [35]. This could be referred to the cariogenic diet used that mimic the three meals.

Regarding the microhardness results from baseline to remineralization, the only group that showed a percent increase was group D while all other groups showed a percent decrease. This could be attributed to the action of Proanthocynidine-based components in green tea which has an extremely high affinity to bind to Proline-rich proteins such as collagen, forming a proline-PA complex. Although traditionally mature dental enamel is considered to be free of collagen, but Type I collagen is found in enamel [11].

It may also referred to the arginine presents in green tea [36] that was proved to have a remineralizing effect through the production of ammonia from arginine metabolism. Ammonia production via arginine deaminase metabolism pathway contributes to pH homeostasis [37]. Other trace elements such as calcium, zinc, sodium, phosphorus and fluorine [36] that could enhance hydroxyapatite and fluorapatite crystals formation.

Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as theobromine [36] that is effective in remineralization of enamel lesions [38]. The synergetic effect between fluoride and some components such as tannin, catechin, caffeine and tocopherol that is found in green tea could prevent the calcium ions from being released in acidic solutions [39]. These results in agreement with Yu et al [47] who demonstrated that tannin and catechins rather than fluoride contribute to the tea anti-cariogenic effect.

Stevia with both concentrations caused decrease in surface enamel microhardness denoting for demineralization process. Giacaman et al found that the 8% stevia, did induce enamel demineralization compared to the negative control (0.9% NaCl) [40]. Despite the fact the stevia is effectively anti-cariogenic against Streptococcus mutans in agreeable with many studies [41-44], it is still retaining some enamel demineralization potential [28].

By contrast, Demirez et al [45] who evaluated the Ca, P, and Ca/P ration of teeth using the EDX analysis, stevia group showed better results than the distilled water group. This could be referred to the different concentration of plant extract or the time of study which was 5 days. The enamel caries depth of Acetone, Ethanol, Methanol and Aqueous stevia extracts 20% was studied and concluded that the acetone and ethanol ones, were less depth than conventional sweeteners such as glucose and fructose [8].

Amine fluoride mouth wash treated group (group E) showed less microhardness results in comparison to other herbal groups. It may be noted for the kind of the invitro study that lack to the oral soft tissues which serve as reservoirs for fluoride [46]. In an in vivo environment, fluoride may be retained on a large surface area of soft tissue as the tongue, that may increase the availability of the active agents and impact remineralization in a different way than what happened in ours study.

Regarding the antibacterial activities of herbal extracts and fluoride against S. mutants and Lactobacillus were quantitatively measured. Stevia didn't produce inhibition zones against S. mutants and Lactobacillus. This was inconsistent with many previous studies [47, 48, 23] that proved the antibacterial effect of stevia that could be due to synergic action of adding stevia with other active ingredients such as chitosan, fluoride, ginger, green tea and pomegranate.

The 5% green tea extract showed the highest mean value of inhibition zone in streptococcus mutants' culture (10.6 mm) followed by the 0.5% green tea and fluoride (9.8 mm). Amine fluoride showed the highest mean value of inhibition zone (14.6 mm) followed by 5% green tea extract (12 mm) and the lowest value recorded for the 0.5% green tea in the Lactobacillus culture. The presence of Catechins (the sub group of flavonoids) in green tea possess strong bactericidal as well as antibacterial activity [30]. Green tea catechins maintain the salivary pH at a normal range, which is not a favorable condition for cariogenic bacteria to grow [49]. In previous studies green tea as mouth rinses was an effective alternatives to NaF mouth rinse and toothpaste [49] [50][51]. These flavonoids have the ability to bind and precipitate macromolecules such as bacterial enzymes that affects the metabolic activity of bacteria [47, 52].

CONCLUSION

Within the limitation of this study it was concluded that:

- An aqueous solution of 5 % green tea is an effective remineralizing agent on initial enamel carious lesion with antimicrobial activity against S. mutans.
- An aqueous solution of 5 % green tea could be a non-expensive available alternative to amine fluoride mouthwash.

- Stevia has neither remineralizing effect on initial enamel carious lesion nor antibacterial activity against S. mutans and lactobacillus.

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