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Effect of Chinese Green Tea on Enamel Surface Characteristics in an in-vitro Erosion Model

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ABSTRACT

Objectives: Erosive tooth wear is a common worldwide problem. It manifests due to consumption of high caloric and low pH acidic drinks such as carbonated soft drinks and fruit juices, which cause irreversible damage to dental hard tissues. The aim of this study was to evaluate the effect of Chinese green tea on surface characteristics of eroded enamel in an in-vitro erosion model.

Materials and methods: Twenty sound extracted human premolars were selected. Micro-hardness and surface roughness were measured before conducting the experimental test to serve as raw data (control), after immersion in Coca -Cola with a pH of 2.8 for 1 hour, 3 times a day for 3 days and then after immersion in green Chinese tea solution for 1 hour 3 times a day for 3 days. The surface micro-hardness was measured using Vicker's diamond under a load of 50 grams for 15 seconds while the surface roughness was measured using Taly-surf**.**

Results: The data were analysed using paired t-test. The micro-hardness measurements obtained before green tea treatment (Mean=102.46, SD=24.82) were significantly lower than those which were obtained after green tea treatment (Mean=158.9, SD=41.44), p=0.0001. The roughness measurements obtained before green tea treatment (Mean=7.34, SD=1.76) were significantly higher than those which were obtained after green tea treatment (Mean=5.14, SD=1.8), p=0.0001.

Conclusion: Chinese green tea positively affected the surface characteristics of eroded enamel.

KEYWORDS

Chinese green tea, Coca-cola, Dental erosion, Micro- hardness, Roughness.

INTRODUCTION

Erosive tooth wear is a common problem affecting adults and children worldwide. Dental erosion is a chemical process by which the

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dental hard tissue is damaged without bacterial involvement[1]. The dental erosion prevalence has been investigated by many epidemiological studies performed in both developed and developing countries. Those studies showed large different prevalence between age groups[2], countries, and location[3-7].

Dental erosion is caused by sustained direct contact with acidic substances having pH reaching the threshold of 5.5. These are either intrinsic acids (e.g., regurgitation gastro oesophageal reflux and vomiting) or extrinsic acids (e.g., acidic beverages and medications). The position of the tooth and the salivary mineralization and properties might take part as biological factors of erosion, as low salivary flow is considered a high risk for erosion and the salivary pellicle formation is known to reduce the erosion development and progression.

Soft drinks, including carbonated drinks, fruit juices and sport drinks (e.g., Citrus fruit Juices, Pepsi, beer) have very high acidity (low pH). Also, many solid and semisolid foods are also acidic in nature (e.g., oranges, pineapples, plums)[8]. Factors such as method and frequency of intake of acidic drinks as well as proximity of tooth brushing after intake may influence susceptibility to erosion[9]. A previous study compared the effect of three different type of carbonated soft drink (Pepsi, sprite and Coca-Cola) on the release of calcium from bovine tooth enamel using an atomic absorption spectrophotometer. The result showed that prolonged consumption of soft drink could lead to severe enamel loss[10].

Vitamin C tablets, aspirin or hydrochloric acid supplements which are acidic in nature can also cause erosion when chewed or held in the mouth before swallowing due to the constant contact between tooth surfaces and the medication[11]. Environmental and occupational factors in selected populations may contribute to dental erosion, for example swimmers who work out regularly in pools with high acidity or workers in industrial factories with continuous exposure to acidic vapours[12]. One study investigated the prevalence of dental erosion among 132 Young Regular Swimmers, found out dental erosion in 25% of the 12-17 year-olds, and in 50% of 18-25 years-olds[13].

GERD (Gastroesophageal reflux disease) is a common condition, estimated to affect 7% of the adult population on a daily basis and 36% at least one time a month. In this condition gastric contents pass involuntarily into the oesophagus and can escape up into the mouth[14]. Gastric acids, mainly hydrochloric acid, with pH level (1.0-3.0) will lead to dental erosion when it is regurgitated into the oesophagus and mouth and become in contact with teeth. However, the patient with Gastro-oesophageal reflux disease may not be aware of the condition until dental changes appear[15].

Vomiting may be spontaneous as a result of medical condition as cyclic vomiting syndrome in children, peptic ulcer, gastritis, vomiting that occur with pregnancy or self-induced by the patient itself as in anorexia and bulimia nervosa[16]. One study assessed dental erosion in sixty six individuals (63 females and three males, mean age 27.7 years) were or had been experiencing self- inducing vomiting. Dental erosions were found in 46 individuals (69.7%), 19 had enamel lesions only, while 27 had both enamel and dentine lesions[17].

Demineralization of dentin by erosive process results in exposure of an outer layer of fully demineralized organic matrix until sound inner dentin is reached[18]. The removal of minerals from dentin makes the degradation of the dentin matrix more easily, i.e., the dentin matrix cannot be degraded unless it is demineralized[19]. One of proteases enzymes that can chemically degrade the organic matrix of dentin is matrix metalloproteinases (MMPs); it is present in dentin and saliva. MMPs especially MMPs 2, 8, and 9 are found to be responsible for hydrolysing the components of the extracellular matrix (ECM) and degradation of the collagen matrix of dentin during erosive processes[20]. When the pH drops in the presence of intrinsic or extrinsic acids MMPs get activated. In addition, realizing of phosphorylated proteins during demineralization of dentin organic matrix can reactivate the inhibited host MMPs, thus enhancing the degrading activity[21].

In this sense, the presence of MMPs on eroded dentin would be likely to increase erosion progression, which could be prevented by the use of MMP inhibitors. One of the MMP inhibitor that employed in both vivo and vitro in order to prevent degradation of collagen in dentin was chlorhexidine[22].

Green tea is a leading beverage in the Far East for thousands of years before it became a popular hot and cold beverage of great consumption around the whole world. Many of the studies suggested that consumption of green tea is associated with risk reduction of several pathologies and a growing number of evidence mentioning a beneficial role of green tea in the oral health. Green tea defences and protects dental surface and cells from malignant transformation and against bacterial induced dental caries. Green tea polyphenols, especially epigallocatechin-3-gallate (EGCG), another MMPs inhibitor were found to have inhibitory activity against MMPs[23].

One systemic review investigated the relationship between green tea and dental erosion. They selected only eight articles from 2000 to 2012, seven articles were laboratory studies and only one article was cross-sectional observational study. Based on the studies' results, it was concluded that sugar-free black or green tea have no erosive effect on teeth while ice tea, fruity, floral and sugary tea have erosive effects. They also found out that green tea compared with other types of tea (fruity tea, citrus tea, black tea and floral tea) has the least erosive effect on teeth. The anti-erosion effect of sugar-free black or green tea could be explained by their high pH value, the pH of black tea and green tea is about 5.7 and 6.3, while fruity tea with high citric acid content have low pH around 2,98 to 3.95 and pH of ice tea is about 3.00. They also found that modification of black and green tea by adding calcium, phosphate, fluoride ions could enhance the anti-erosion effect[24].

Another study was evaluating the effect of green tea on eroded dentin in compression with distilled water and 0.2% chlorhexidine. Surface hardness loss (% SHL), wear and surface roughness analysis were measured. The results of the study showed no significant difference between chlorhexidine and green tea group, however there was a significant difference between green tea and distilled water group[25].

Another study analyzed the protective effect of tooth pastes containing MMP inhibitors as chlorhexidine and green tea on dentin erosion in vitro. They found that toothpastes containing MMP inhibitors were as effective as those based on NaF in preventing dentine erosion and abrasion[26].

However, there is still a need for more clinical and biological studies to support guidelines for green tea intake as part of prevention and treatment of specific oral pathologies. Therefore, the objective of this study was to evaluate the effect of Chinese green tea on surface characteristics (micro-hardness and roughness) of eroded enamel in an in-vitro erosion model

MATERIALS AND METHODS

1. Sample preparation:

Twenty sound human premolars extracted for orthodontic reasons from orthodontics' clinics in Jeddah, Saudi Araba were selected; the selected teeth were free from any defects such as cracks, dental caries or enamel hypoplasia and were not having any restorations. Thoroughly the selected teeth were cleaned from organic debris and disinfected using sodium hypochlorite, after that the teeth were stored in saline in a divided sealed container. Each tooth was coded with a number from one to twenty. Micro-hardness and roughness value of each tooth were measured before conducting the experimental test to serve as raw data (control).

2. Green tea preparation:

Ten grams of sealed and dry raw Chinese green tea leaves (Fuan Ming Hung Tea Co) were poured into 100 mL of boiled water. The green tea leaves were steep in the water for 5 minutes after that it was filtered with strainer and cooled off for 10 minutes at room temperature.

3. Experimental procedure:

The teeth samples were subjected to cyclic procedures of erosion and treatment repeated over a three-day period for each.

First, in a coded sealed container each specimen was immersed in 10 ml of Coca cola (The Coca-Cola Bottling Company of Saudi Arabia (CCBCSA), pH 2.3 for 1 hour at room temperature three times a day for three days. After each erosive cycle, the samples were rinsed with deionized water and stored in artificial saliva (Glandosane, Boots Pharmacy, London) for 8 hours. Then after the erosive cycle was finished, the surface microhardness of the enamel of each tooth was measured using Vicker's diamond micro-hardness tester (Microhardness, Zwick Roell Indentec ZHV, Germany). Also, the surface roughness of the enamel of each tooth was measured by using Taly-surf® (from Taylor Hobson Precision, Inc.). The mean values of both micro-hardness and surface roughness were calculated for each sample.

After that, in the coded sealed container each specimen was immersed in 10 ml of the prepared green tea for one hour at room temperature three times a day for three days. After each treatment cycle, the samples were rinsed with deionized water and stored in artificial saliva for 8 hours. Then after the treatment cycle was finished the micro-hardness values and surface roughness of each tooth were measured using the same procedures that mentioned before.

The average micro-hardness values and surface roughness before and after treatment were calculated and compared to each other.

3.1: Measurement of surface micro-hardness:

Micro-hardness of the teeth specimens in enamel were measured using Vicker's diamond, as shown in Figure 1. Using micro-hardness tester (Microhardness, Zwick Roell Indentec ZHV, Germany), each enamel specimen was pressed with loads of 50 grams (0.4903 N) for 15 seconds. An average of three readings for each test condition was recorded as the HV and HRC value of a specimen. Mean hardness values were then calculated for each of the enamel surfaces. Figure 2, shows the images of the micro-hardness indentation of enamel surface measured by Vicker's diamond. More details can be found in the following publication[27].

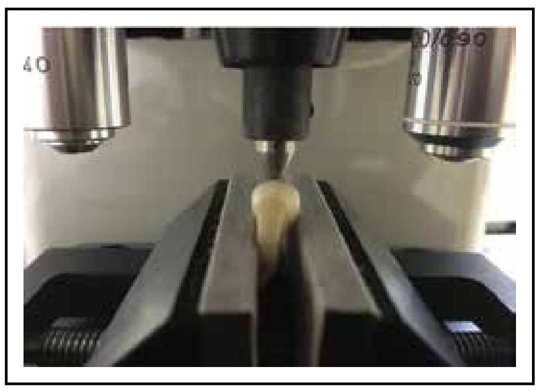

Fig. (1) Micro-hardness test-rig setup

3.2: Measurement of surface roughness:

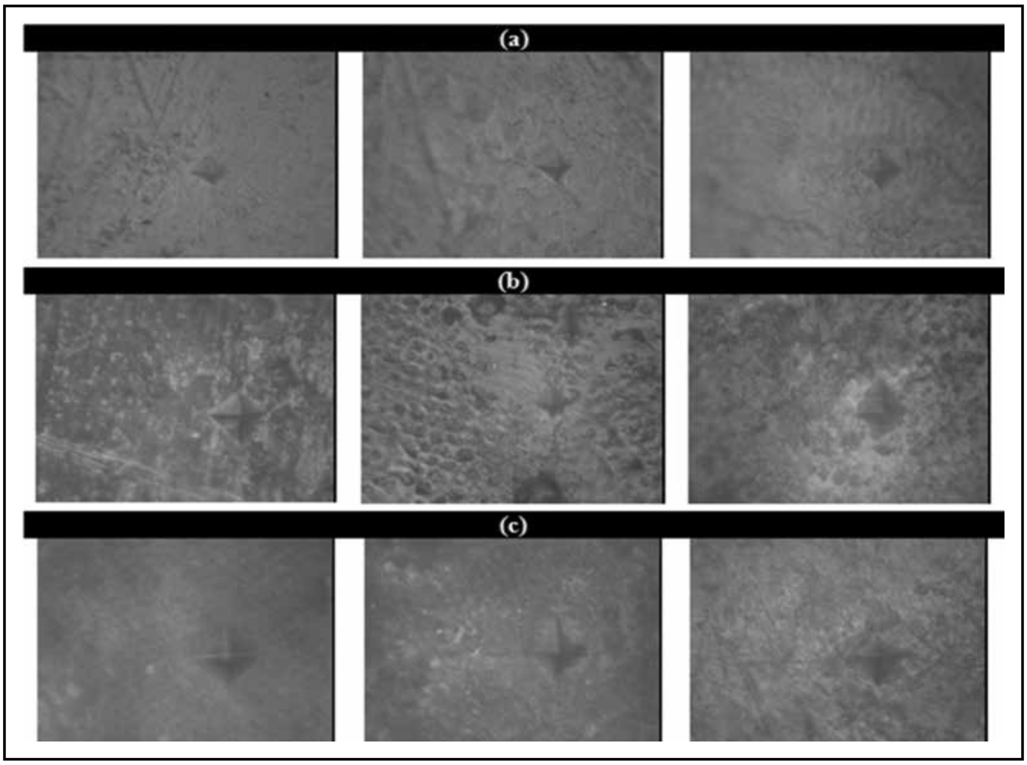

Fig. (2) Images of the micro-hardness indentation of enamel surface measured by vicker's diamond; (a) Control, (b) eroded by Coca Cola, (c) treated by Green Tea

The surface profile of the teeth specimens were quantitatively analysed in order to determine the statistical standard parameter of average roughness, R_a, using Taly-surf® (from Taylor Hobson Precision, Inc.). A nominal 2 μm stylus was used with a normal load of 0.7 mN and selectable traverse speed down to 0.5 mm s^{-1} which conforms to British Standards. Every test condition was repeated at least three times at different "*new*" locations on a rod bar surface in order to ensure the repeatability and reproducibility of the results. The "*new*" location was at least ±200μm from the previous one (Figure 3). More details see the following publications [28,29,30].

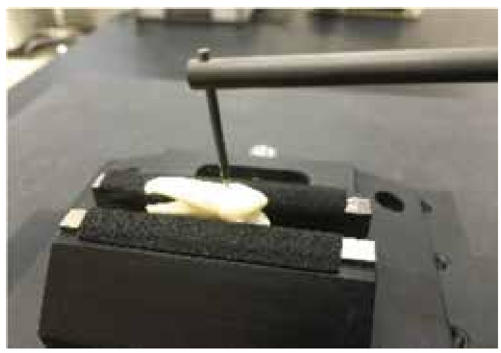

Fig. (3) Surface roughness test-rig setup

4. Statistical analysis

Analysis of the data was performed with the Statistical Package for Social Sciences (SPSS21 for Windows 8, SPSS Inc.). First, normality tests were used to determine if the data had been drawn from normal distribution; the main tests used for the assessment of normality were Kolmogorov-Smirnov[a], Shapiro-Wilk. Secondary, Paired samples T.test was used in statistical evaluation of means of micro-hardness and roughness after consumption of soft drink (Coca-Cola) and after treatment by green tea.

RESULTS

Tables 1 and 2 show the p-values of both micro-hardness and roughness data were higher than (0.05), which indicate the normal distribution of both data according to Kolmogorov-Smirnov[a], Shapiro-Wilk tests.

According to all normality tests and graphs "normal Q-Q plot" (Figures 4, 5) that have been used in the study, we can assume that the data of micro-hardness and roughness after consumption of soft drink (Coca-Cola) and after treatment by green tea were normally distributed. Based on those results, the test that has been chosen for statistical analysis of normally distributed data was Paired samples T.test as shown in tables 3 and 4.

Table (1) *Test for normality of micro-hardness values (HV)*

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
COLA HV	.163	20	.170	.951	20	.386
G.TEA HV	.100	20	.200*	.976	20	.869

**. This is a lower bound of the true significance* *a. Lilliefors Significance Correction*

Table (2) *Test for normality of roughness values (R_a)*

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
COLA Ra	.163	20	.200	.929	20	.151
G.TEA Ra	.163	20	.200*	.964	20	.622

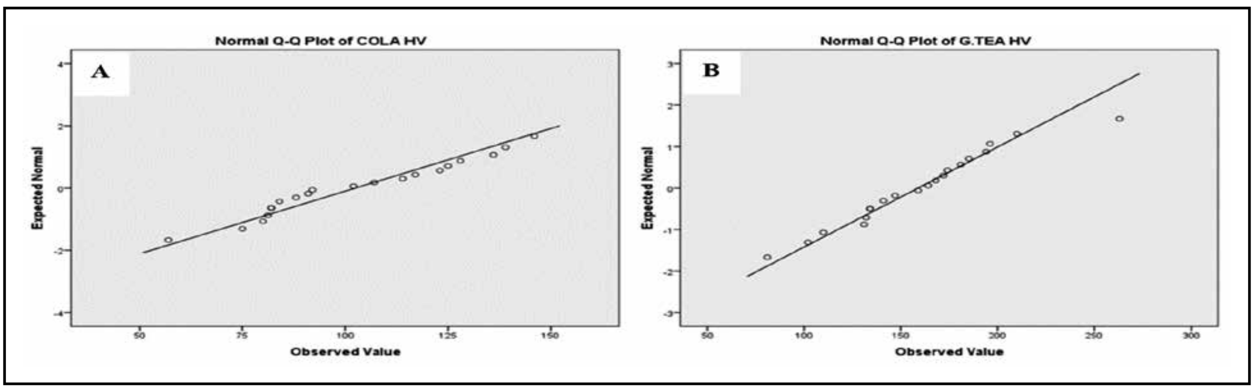

Fig. (4) Normal Q-Q plot for micro-hardness values: A. Coca Cola hardness values, B. Green tea hardness values

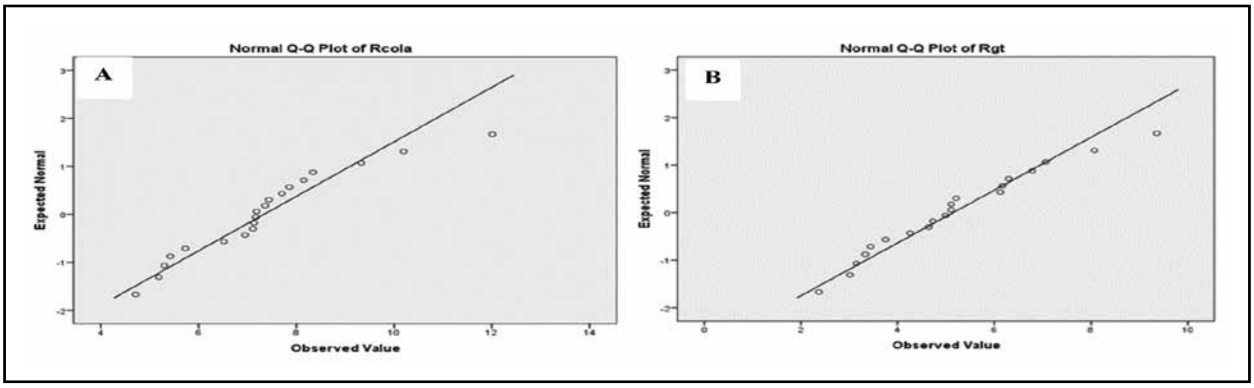

Fig. (5) Normal Q-Q plot for roughness values: A. Coca Cola roughness values, B. Green tea roughness values

Table 3 shows the Micro-hardness measurements obtained before and after treatment of eroded teeth by green tea using dependent samples (paired t-test). This revealed that scores obtained before green tea treatment (Mean=102.46, SD=24.82) were significantly lower than those which were obtained after green tea treatment (Mean=158.9, SD=41.44) (t (-7) =19, p=0.0001.

Table 4 shows the roughness measurements obtained before and after treatment of eroded teeth by green tea using dependent samples (paired t-test). This revealed that scores obtained before green tea treatment (Mean=7.34, SD=1.76) were significantly higher than those which were obtained after green tea treatment (Mean=5.14, SD=1.8) (t (-8) =19, p=0.0001.

Tables 5 and 6 show the mean and standard deviation of micro-hardness and surface roughness values obtained before and after treatment of eroded teeth by green tea. The percentage of reduction in surface micro-hardness after immersion of the tooth in Coca-Cola was 76%, while the percentage of surface roughness has increased to 16% from the original surface roughness. The percentage of improvement of surface micro-hardness after immersion of eroded tooth in green tea was increased to 55%. While the percentage of reduction in the surface roughness of eroded tooth was 30%.

Table (3) *Paired samples test for Micro-hardness values (HV)*

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	COLA HV G.TEA HV	56.4400	36.20640	8.09600	-73.38512	-39.49488	-6.971	19	.0001*

*: *Significant at* $P \leq 0.05$

Table (4) *Paired samples test for roughness values (Ra)*

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	COLA R_a G.TEA R_a	2.1970	1.20749	.27000	1.63189	2.76213	8.137	19	.0001*

*: *Significant at* $P \leq 0.05$

Table (5) *Mean and Standard deviation of Micro-hardness values (HV)*

	Raw Data (Control)	Coca-Cola	Green Tea
Mean	317.4	102.5	158.9
SD	30.85	24.20	40.39

Table (6) *Mean and Standard deviation of roughness values (Ra)*

	Raw Data (Control)	Coca-Cola	Green Tea
Mean	6.4	7.3	5.1
SD	1.77	1.72	1.76

DISCUSSION

This study confirmed the expected adverse softening effect of acidic soft drink (Coca-Cola) on the tooth enamel surface, which causes the decrease on surface micro-hardness and increases surface roughness of the enamel after the 3 days immersion cycle of teeth. The percentage of reduction in surface micro-hardness after immersion of the tooth in Coca-Cola was 76%, while the percentage of surface roughness has increased to 16% from the original surface roughness.

The present study also confirmed that green tea has no erosive effect on enamel, this finding agrees with the result of Jaâfoura et al.[24], which stated that sugar-free black or green tea have no erosive effect on teeth while ice tea, fruity, floral and sugary tea have erosive effects. On the contrary, it protects the enamel from further demineralization and surface loss by its ability to increase surface micro-hardness and decreases surface roughness of enamel. The percentage of improvement of surface micro-hardness after immersion of eroded tooth in green tea was increased to 55%. While the percentage of reduction in the surface roughness of eroded tooth was 30%.

This improvement on enamel surface is a strong evidence that green tea reduces the wear of enamel and enhances their surface quality, which agree with Kato et al.[31] result. They also found that the dentin wear was reduced significantly when 10 volunteers rinsed with green tea for 1 minutes between each erosive (5 min, cola drink) and abrasive challenge (30s, tooth brushing).

The findings in the study also agree with the results of Mirkarimi and Toomaria[21], who investigated the effect of green tea on eroded dentin by measuring micro-hardness before and after immersion of the tooth in green tea. They found that the mean ± SD microhardness value (Kg force/mm^2) after immersing teeth in coke was 46.5±2.79 and after immersion of the eroded teeth in green tea solution, the microhardness value increased to 54.51± 4.46.

The enamel surface roughness results in the present study confirm the results of Moraes et al.[25] study, which observed the dentin roughness after immersing the disk of coronary dentin in citric acid for 60 second, the mean and standard division of roughness was 0.25 (0.04). While after immersion of the coronary dentin on green tea for 5 minutes, the mean and standard division of roughness decreased to 0.20 (0.04).

The observed results in the study can be explained by the presence of Matrix metalloproteinases (MMPs) in enamel and dentin. The MMPs belong to a larger family of proteases known as the metzincin superfamily which is responsible for the breakdown of extracellular matrix including fibrillar and non-fibrillar collagens in normal physiological processes. Before mineralization of the teeth, MMPs may take part in the organization of enamel and dentin organic matrix and control the proteoglycan turn-over and remodelling of enamel and dentin and therefore regulate mineralization. MMPs especially MMPs 20, also known as enamel metalloproteinase, degrades amelogenin which is the major protein component of the enamel matrix that organizes the enamel rods during tooth development and regulates the initiation and growth of hydroxyapatite crystals during the mineralization of enamel[32].

When the pH drops in the presence of intrinsic or extrinsic acids as coca cola which was used in this study the MMPs get activated. When they get activated they start to hydrolyse the components of the extracellular matrix (ECM) and degrade the collagen matrix during the erosive processes of enamel. Individuals with high concentration of MMPs have a higher susceptibility to dental erosion, in this sense; the presence of MMPs on eroded enamel would likely o increase erosion progression, which could be prevented by the use of MMP inhibitors[23].

One of MMPs inhibitors that were used in this study in order to prevent enamel erosion was green tea. Green tea has the ability of protecting the eroded enamel by increasing the surface micro-hardness and decreasing the surface roughness of eroded enamel. The results can be explained in two ways either green tea is having the ability to form protective layer on enamel surface that prevent further erosion. Another probable explanation might be the presence of green tea polyphenols, especially epigallocatechin-3-gallate (EGCG) (7380 mg per 100 g of green tea), which acted as potent inhibitors of matrix metalloproteinase activities (MMPs). Epigallocatechin-3-gallate (EGCG) seems to exhibit a hydrogen bonding and hydrophobic interaction with collagenases, which is responsible for the change in the secondary structure of collagenases and consequently for their inhibition, therefore, it could reduce the degradation and breakdown of extracellular matrix in enamel thus decreases the enamel erosion[8].

However, there are some limitations in this study which are: it must be acknowledged that the protocol employed in the present study does not suggest that the effect of green tea in protection of eroded enamel by increase the micro-hardness and decrease the surface roughness is due to its inhibitory action on MMP activity, as we did not test this directly. Furthermore, the erosion cycles of the tooth was performed under in vitro condition, which lacks the natural protective effects of the oral environment like saliva and their buffering capacity and their protective minerals component like fluoride and calcium which aid in re-mineralization process after demineralization by extrinsic acid. Although in the study we used artificial saliva to stimulate the oral environment but it was not fair enough to produce the protective effect of the natural saliva.

CONCLUSION

According to the findings of the present study, Chinese green tea positively affected the surface characteristics of eroded enamel.

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