

## Effect of Different Tea in Remineralization of Artificially- Induced Initial Enamel Caries of Human Teeth (Study in Vitro)

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### Key words

Green tea, black tea, Mentha picata, Ocimum asilicum, re-mineralization.

### Abstract

One of the beneficial effects of consumption different types of tea is preventing tooth decay that attributed to their mineral contents.

Purpose: to investigate the microscopical changes of different types of tea ; Camellia sinensis (black and green), Mentha spicata and Ocimum basilicum on artificially- induced initial enamel caries. Initial enamel caries-like lesions were introduced in 30 sound extracted human 1<sup>st</sup> premolar teeth using pH cycling procedure. Teeth were divided into 6 groups to test 4 types of tea ( black, green, Mentha spicata and Ocimum basilicum) and 2 control solutions (0.05% NaF and de-ionized water as positive and negative control respectively )for enamel re-mineralization, using polarized microscope. The concentration of calcium, inorganic phosphorous and fluoride ions in all tea solutions were also measured.

Results revealed that green tea produced the best enamel remineralization, while black tea and Mentha spicata were coming next and result in different mode of re-mineralization. On the other hand, teeth treated Ocimum basilicum showed mild evidence of re-mineralization. The chemical analysis indicated that both Mentha spicata and Ocimum basilicum had the highest calcium and phosphorous ions levels (higher than their fluoride concentration). Green tea had nearly equal concentration for the 3 tested ions, which were less than its counterpart black tea.

All selected tea solutions were effective in remineralizing of initial enamel caries, in the following sequence: green tea, black / Mentha spicata , then Ocimum basilicum.

### Introduction

A considerable interest has been arisen concerning the health promoting potential of tea (Camellia sinensis)<sup>(1)</sup>, which is widely consumed in the world today, 2<sup>nd</sup> only to water, as a cultural habit and part of everyday life <sup>(2)</sup>.

Tea is an aqueous extract of various dried and processed leaves <sup>(3)</sup> accordingly, there are 3 main varieties of Camellia sinensis tea; green, black and oolong <sup>(4)</sup>. They have numerous medicinal properties, mainly attributed to their antibacterial and antioxidant properties, and their bioactive chemicals, including; polyphenols, alkaloids, mineral, volatile oils <sup>(4-8)</sup>. From dental point of view, tea has preventive effect against tooth decay <sup>(7,9)</sup>. It reduces both dental plaque <sup>(10)</sup> and caries <sup>(11-12)</sup> as a

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result to its high fluoride and organic constituents<sup>(13,14)</sup> that inhibit bacterial activity<sup>(15)</sup> and re-mineralize enamel<sup>(16)</sup>. On the other hand aromatic herbal's tea like those prepared from *Mentha spicata* and *Ocimum basilicum* are used in various kind of illness<sup>(17-18)</sup>. *Mentha* is rich in essential nutrient minerals and trace elements<sup>(19)</sup>, while *Ocimum basilicum* has high antioxidant power<sup>(20)</sup> and bactericidal properties<sup>(21)</sup>. *Mentha* is used as flavor in tooth paste, mouthwash, chewing gum and confectionery<sup>(19,22)</sup>, as well as being added during brewing of different types of tea<sup>(18)</sup>. The present study aims to investigate the effects of different tea; *Camellia sinensis* (black and green), *Mentha spicata* and *Ocimum basilicum* on the remineralization of artificially demineralized enamel of extracted human teeth.

## Materials and Method

Thirty newly extracted sound human's teeth were collected (after getting the approval of the patients and the institute), cleaned and prepared to expose a circular 6mm buccal enamel window (figure-1) following Al-Obaidy's methods<sup>(23)</sup>. Initial enamel caries-like lesions were introduced following Featherstone et al<sup>(24)</sup> pH cycling procedure for 10 days. Teeth were divided equally into 6 groups. Four of them were subjected for testing solutions, named the black, green (ready prepared tea bags, both Ahmed trademark), *Mentha spicata* and *Ocimum basilicum* (locally prepared from drying clean fresh leaves). Each tea solution was prepared daily by brewing 2g crushed dried leaves in 100 ml de-ionized water for 30 second. Teeth in each group were immersed in 20ml for 4 minutes in a testing tea, then rinsed with and kept in de-ionized water at 37°C for next day. This procedure was repeated daily for 7 days<sup>(25)</sup>. The remaining 2 groups, one of them immersed by similar manner in positive control solution (0.05% NaF), the other group along the experiment immersed in negative control solution (de-ionized water). For each tooth 2 buccolingual ground sections were prepared following Gauy-Fen et al method<sup>(26)</sup>. The cutting and preparing of the samples were carried in the department of Geology in

Sulaimani University. each enamel section was fixed on glass slide by Canada baslam mounting media and examined under polarized microscope (40X magnification) for evaluating color changes in the enamel that reflect various zones of demineralization<sup>(23,25)</sup>. Calcium and inorganic phosphorous ions concentrations were assessed colorimetrically<sup>(27,28)</sup> in 100ml of above selected tea solutions. The concentration of fluoride ion was measured by ion-selective electrode (standard addition method<sup>(29)</sup>). The differences in the mean values between different solution for each tested ion separately were evaluated by t-test. P value <0.05 was considered statistically significant.

## Results

Microscopical changes of enamel sections, under polarized microscope, of all studied groups were illustrated in figure-2. Initial enamel caries-like lesions, in -ve control group, appeared as blue and red colored zones that reflect 2 areas of different demineralization (body of lesion and advance zone) (figure 2A). Such zones were totally disappeared in teeth that remineralized with fluoride ions (+ve control) (figure 2B). On the other hand, green tea produced enamel remineralization nearly similar to that observed after fluoride treatment (figure - 2C). While black tea resulted in partial remineralization, that observed in the body zone only, since small areas of red and blue colors were seen in inner layer of the carious lesion (advance zone) (figure 2D). While the *Mentha spicata* produce partial re-mineralization of inner layer and hypore-mineralization of the surface area (figure 2E). Lastly, *Ocimum basilicum* treated teeth showed mild evidence of remineralization at enamel surface only, that appeared as reduction in blue colored area (figure 2F) when compared with -ve controlled group. Chemical analysis of above solutions indicated that both *Mentha spicata* and *Ocimum basilicum* had the significantly higher calcium and phosphorous ions levels then both the green and black tea (P<0.05). While green

tea expressed significantly the lowest values for both fluoride and phosphorous ions when compared with the levels reported in both *Mentha spicata* and *Ocimum basilicum* solutions (P0.03), table-1.

## Discussion

Tea has been recommended as satisfactory drink from dental viewpoint. There have been researches on and off over 50 years on the anticariogenic properties of tea<sup>(30)</sup>. Series of well-conducted systematic studies suggested that tea extract showed several useful antimicrobial effects<sup>(31)</sup> and reported to reduce dental plaque score and caries index<sup>(9,8,12,13,14,29)</sup> as well as it reacted chemically with enamel surface<sup>(23)</sup>. The objective of selection of green and black tea in this study was based on the knowledge that enamel remineralization can be increased by tea drink<sup>(16)</sup>. Besides that, both *Mentha spicata* and *Ocimum basilicum* were proved to have variable amounts of minerals mainly calcium, phosphorous, magnesium and aluminum, as well as trace elements<sup>(19)</sup>. They are widely used as aromatic herb added to different foods and salads, and they may be mixed with black and green tea as a cocktail. Since this study, tea was intended to be prepared in a similar manner to people's habits; the home made procedure was followed, irrespective to the personal preference variation whether to drink these herbal teas highly concentrated or diluted. Beside that the effect of different temperature and adding of sugar was neglected. The initiation of caries-like lesion in the present study was conducted in ten days that demonstrated a high loss of enamel minerals. The body zone (blue in color) and advance zone (red band) were clearly identified. However, when it was compared with natural enamel carious lesion there was no surface layer, which considered as an area of active mineral re-precipitation that occur in vivo-environment<sup>(32)</sup>. Sodium fluoride re-mineralization was reported to produce transforming in the size and shape of enamel crystallite<sup>(33)</sup>. It enhanced precipitation of calcium phosphate and formation of fluorohydroxyapatite

crystals<sup>(34)</sup> accordingly, the above zones of the initial enamel caries-like lesions disappeared. Although tea plants accumulated fluoride in their leaves in level comparable to that recommended in preventive dentistry<sup>(35,36)</sup>, its concentration, according to the chemical analysis of this study, was more in black tea than in green tea. Nevertheless, in this experiment, microscopical evaluation of ground sections for the effect of green tea revealed complete re-mineralization identical to that of sodium fluoride. While in black tea treated-teeth there was only remineralization of surface area, i.e incomplete re-mineralization. This can be explained that only low level of fluoride is required to trigger the mechanism of remineralization, raising the fluoride level dose not result in greater degrees of mineralization<sup>(37)</sup> i.e only free exchangeable one can react with calcium ion. This attracts the attention to find a way to make much of fluoride in any tea in its reactable state to get the best benefit or to prevent the competition of different ions in the single solution to get reaction with the apatite crystals. Also black tea contains elements other than calcium and phosphorous such as Al, K, Mg, Mn, which may substitute calcium ion of hydroxyapatite crystals (decrease Ca/P molar ratio) and forming other crystals with various sizes and orientation of heterogenous chemical structure, which could be seen as hypomineralize area in limited quantities. Even though, in a vivo clinical trial, it was established that regular tea drinking may decrease the incidence and severity of caries<sup>(9)</sup>. Treatment of demineralized enamel surface with different concentration of black or green tea was reported to affect the microhardness in different manner. Initially black tea induced increase in enamel microhardness but further increase in its concentration significantly reduced enamel microhardness unlike the green tea, which further increase in enamel microhardness<sup>(25)</sup>. It is not well understood, which types of reactions were taken place that leads to remineralization and we suggest further chemical analysis for both green and black tea to determine the concentration of different elements

that may substitute calcium in the apatite crystals like Mg and Na and the way they result in rebuilding of these crystals. On the other hand, there was a remarkable enamel remineralization in *Mentha spicata* treated teeth especially in the deeper zone. Unfortunately, there was no previous published literature to compare our results with. However, this encourages the cultural habit of adding *Mentha* during brewing of tea to get better remineralization in both surface and deep zones of the carious lesion. Yet this needs further investigation since there is a chance to have competition of different minerals during

the process of remineralization as stated above. Lastly, enamel re-mineralization produced by *Ocimum basilicum* was minimum. Conclusion and suggestion: all selected tea solutions were effective in remineralizing of initial enamel caries. But before concluding which type of tea is the best for tooth remineralization, the results of this study need to be confirmed since different tea concentrations or aromatics or sweeteners additives may change these results.

Table (1):- Fluoride, calcium and phosphorous ions concentrations in all tested tea solutions

Tea solutions	Ca <sup>++</sup> (mg/100ml)		P <sup>+</sup> (mg/100ml)		F <sup>-</sup> (mg/l)	
	mean	sd	mean	sd	mean	sd
Black tea	0.88	1.35	1.98	0.66	1.29	0.08
Green tea	1.0	0.77	1.02	0.15	0.73	0.079
<i>Mentha spicata</i>	10.08	2.5	2.48	0.55	1.24	0.084
<i>Ocimum basilicum</i>	15.78	1.09	2.32	0.2	1.19	0.075



Fig.(1):- A window on the buccal surface of maxillary first premolar.

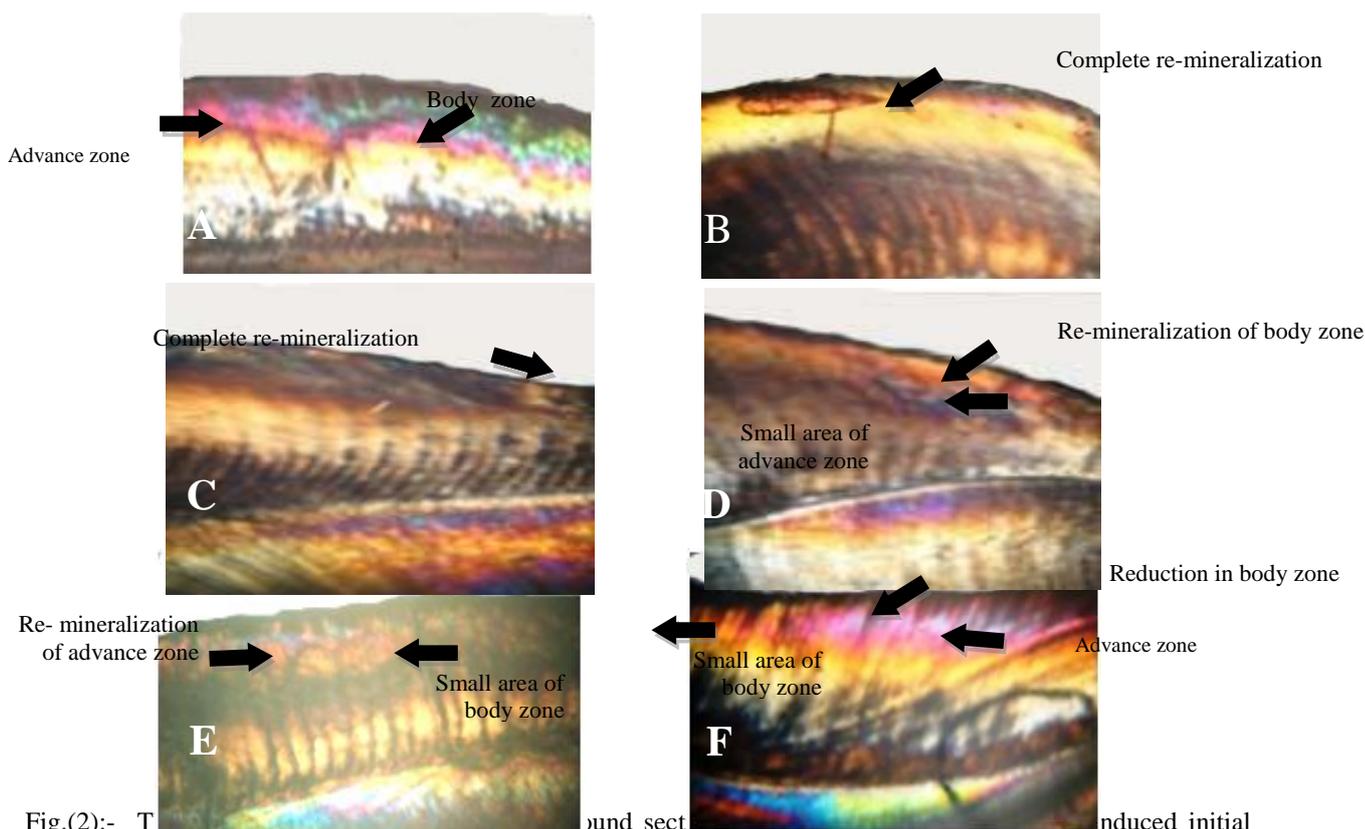


Fig.(2):- Transverse section of enamel induced initial enamel caries, and after treatment with; (B) fluoride, (C) green tea, (D) black tea, (E) *Mentha spicata*, and (F) *Ocimum basilicum*, (polarized light microscope, X40).

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