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IN-VITRO EVALUATION OF REMINERALIZATION EFFICIENCY OF CHICKEN EGGSHELL SLURRY ON ERODED DECIDUOUS ENAMEL

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ABSTRACT

Objectives: The present study evaluated the re-mineralizing efficiency of CESP on experimentally eroded deciduous tooth enamel by Claritin syrup.

Materials and Methods: 20 extracted deciduous teeth had been selected and stored in normal saline. Each tooth was cut in a labiolingual direction into 2 halves, one half used as a control and the other used as study. Demineralization of all specimens were done by twice daily immersion in Claritin syrup for ½ an hour for 12 days to form enamel erosion. Remineralization of the study group was done by using CESP slurry twice daily application for 3min. for 10 days. Surface roughness of the specimens was measured by surface profile gauge and microhardness evaluation was done using Vickers tester. Resulted data were tabulated and ana1yzed statistically by t-test with 5% significance 1evel

Results: There was statistically significant increase in roughness values after erosion with Claritin syrup (P<0.001). however, it was significantly decreased after remineralization (*P*=0.01). There was statistically significant decreased mean value of microhardness after erosion with Claritin syrup (P<0.001). however, it was significantly increased after remineralization (*P*=0.01).

Conclusion: CESP slurry is a valuable topical treatment in deciduous teeth erosion since it decreased the roughness and increased the microhardness of the enamel surface in-vitro

KEY WORDS: Dental erosion, CESP, surface roughness, Vickers microhardness.

INTRODUCTION

Dental erosion is an acidic wearing of hard tooth tissue by chemical action of acidic dissolution that not caused by bacteria and isn't clearly related to mechanical action. [1, 2]

Erosion of dental enamel may be happened by continuous application of acidic medium either from internal or external sources [3]. From the most commonly included intrinsic sources are frequent vomiting, re-gurgitation or gastro-esophageal reflex [4]. With respect to the external sources of

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acidity, it was discovered that it likely could be because of the extreme confirmations of acidic liquids for example: carbonate containing drinks, natural fruit juices, alcoholic or vitality drink, any supplying form of vitamin C, ibuprofen and a few verbal cleanliness items, counting a few mouth washes [5].

Oral medicine is viewed as one of the external causes of dental erosion since some chronic diseases' medicines having high acidity [6]. Properly, numerous cases of tooth chemical wear have been reported as a result to oral administration of specific medications [7].

Teeth are continuously subjected to cycles of de-mineralization and re-mineralization throughout the life, but these cycles could be interrupted by excessive uptake of acidic drinks which eventually leads to disintegration of inorganic component of the teeth [8]. This chemical dissolution, in case not conveniently treated, may cause tooth sensitivity, decrease the tooth vertical dimensions, pulpal exposure and inflammation [9,10].

Some acids are contained in drug formulations as a buffering agent to save compound dependability, control constitution and as a flavoring agent to increase the agreeability to the kids. Some oral prescriptions contained citric acid which has efficient erosive effect since it has liability to bond with the calcium (Ca) in hydroxy-apatite that accelerate the hydroxy-apatite crystals disintegration rate [11]. plus, faster enamel erosion can be formed when these prescriptions are used for chronic diseases since it has at least twice daily frequent regular uptake [12].

Antihistaminic drugs are endorsed by pediatricians for quieting the side effects of occasional sensitivities, for example, runny nose; sniffling; irritated eye's secretions or nose tingling. Especially, Claritin syrup which is one of the anti-histaminic drugs which is comprehensively recommended by most pediatricians; therefore, it is exhausted by a huge prominence of children [13].

Claritin syrup has a trait of giving erosive potential because of its high acidity, since it contained citric acid, no phosphate or fluoride, and negligible amount of Ca in the chemical composition[14]. Subsequently, many reports revealed the erosive effect of Claritin syrup in dental field [15-19].

Demineralization can be re-established through utilizing calcium-phosphate compound. Advanced dental compounds containing Na F or tri $CaPo_4$ have presently been one of the foremost premier viable sources of in-organic elements essentially needed to re-mineralize enamel [20]. One of the milk derivative products is Phospho-peptide-amorphous-calcium-phosphate (CPP-ACP) which has seemed to be encouraging results both in-vivo and in-vitro in improving the enamel remineralization and treating the enamel erosion therefore, it has been effectively included in some oral and dental compounds [21]. As well, the different applications of chicken eggshell powder (CESP) were considered [22].

Chicken egg-shell is an effective origin of calcium, contains about 40% of basic calcium. It isn't just enhancing the in-vitro differentiation of chondrocyte yet additionally improving the thickness of bone mineral in female rat [23]. Moreover, CESP have improved the density of bone mineral and decreased the joint pain in post-menopausal ladies suffering from osteoporosis [24].

Numerous studies tried to evaluate the effects of various re-mineralizing agents on early enamel caries stage, however shortage of data still present about the application of CESP and its efficiency on the treatment of eroded surface of enamel caused by pediatric medications [25,26].

Withdrawal of enamel hardness is the initial sign of dental erosion. Here, a platform of the hydroxyapatite crystal still present. At that stage, the re-mineralization process by calcium, phosphorus and fluorides could be done efficiently. On the contrary, when the scaffold is completely lost, re-mineralizing agents are not significantly effective.

In the beginning of dental erosion there is a reduction in the surface hardness of the enamel however, it could be increased after re-mineralization procedures [27].

Degradation of hydroxyapatite crystals of enamel leading to mineral loss from the sub-surface layer, creating gaps among the enamel rods, and resulting in roughened enamel surface [28] The roughened surfaces cause plaque retention, which increases the demineralization rate and, possibly resulted in many oral diseases [29]. Therefore, surveying surface roughness and surface hardness of enamel is a valuable tool to confirm the efficacy of different re-mineralizing agents.

The objective of our in-vitro analysis is to investigate the re-mineralization efficiency of chicken-eggshell powder slurry on artificially induced erosive lesion in primary teeth measured by surface roughness and microhardness

Production and of Chicken Eggshell Powder (CESP)

Calcination was the process used for formation of CESP through the methodology described by World-Property-Intellectual-Organization (WO/ 105912/ 2004) [31] to get an unadulterated sterile powder with extended alkalinity. Basically (CESP) have 94-97% calcium carbonate that turned into basic calcium oxide by calcination process, which is the cause of increased alkalinity [32]. Forty chicken-eggs from a local hatchery (Tanta) have been obtained and their contents were removed, and the eggshells have been cleaned in distilled water. The eggshells then preserved in a hot water-bath at 100°c for 10 minutes to facilitate the membrane removal. Then it has been crushed by mortar and pestle. The resulted crushed powder was heat treated at 1200°C in a muffle furnace (Henan Sante Furnace Technology Co., Ltd, China) for 1 hour and finally powdered [33,34]. To form the CESP slurry, 1gm of powder was mixed with 2ml artificial saliva [35].

MATERIALS AND METHODS:

Materials

The materials used in this study are described in table (1)

TABLE (1): Materials used in the present study

Material	Description
Claritin syrup (Schering-Plough)	an anti-histaminic syrup which contains citric acid, loratadine 1 mg/ml, glycerin, flavoring agent, sodium benzoate, sugar and glycol propylene, Inactive components (corn starch, lactose monohydrate, magnesium stearate) and water. Bayer- HealthCare LLC.
Chicken-eggshell powder (CESP)	It was prepared by calcination process at Physics Department, Faculty of Science, Tanta University
Artificial saliva	It is composed of (0.843 g NaCl, 1.2g KCl, 0.051g $MgCl_2$, 20 ml of 1 % solution of tri-calcium phosphate and carboxy methylcellulose, 0.05 M of NaOH to adjust the pH to 6.8) mixed with 500 ml distilled water

Characterization of the prepared CESP:

- The chemical composition of CESP was analyzed using FTIR FT-IR spectroscopy (Perkin-Elmer-1430 Ratio Recording Infrared Spectrophotometer, USA) Then the morphology and the particle size of the powder was analyzed by Scanning Electron Microscope (SEM) (JEOL, JSM 5410, Japan.)
- To prepare CESP slurry, one gram of the prepared chicken eggshell powder was mixed with 2 ml of artificial saliva, and then the tooth was rubbed by finger with freshly mixed slurry for 3 minutes. [35]

Sample Preparation

Totally 20 freshly extracted sound deciduous anterior teeth have been collected from the pedodontics clinic, faculty of dentistry, Tanta University. The objectives of the present investigation have been explained to the children's parents and informed consents were obtained to use their extracted teeth on the research according to the guidelines on human research established by ethics committee panel of faculty of Dentistry-Tanta University.

The collected teeth were cleaned, disinfected by soaking in 5% solution of sodium hypo-chloride for 60min., and carefully examined. Any defected or cracked teeth were excluded. Then, the selected sound teeth specimens were stored in normal saline till used [36].

Roots of the selected teeth had been cut at the enamel-cementum junction using (Isomet 4000 microsaws, Buehler, USA). The crown specimens were sectioned through the bucco-lingual axis into equal two halves to utilize one of them as a control, and the others as a study. The tooth segment was embedded in Tef1on mo1d (10mm x 8mm x 2mm) with a composite resin so that their labial surface directed upwards. Si1icon-carbide papers (grades 600-1200) under running stream of water, were used to grind and polish the labia1 surfaces of the specimens to produce a highly flat surface [37].

Grouping

Group I (Control Group): one half of 20 tooth specimens were exposed to erosive syrup and left with no treatment.

Group II (Study Group): the other one half of 20 teeth specimens were exposed to the erosive syrup then re-mineralized with CESP slurry treatment.

The acidity of the anti-histaminic syrup was detected before using it as a de-mineralizing syrup by pH-electrode meter (MP 23o pH-meter, Mettler Toledo) at the Chemistry lab-Faculty of Pharmacy, Tanta University and it was found that its pH equals 3.4.

Experimenta1 methodo1ogy:

Group I (control group)

The 20 control group specimens were scheduled and examined for surface roughness and microhardness *(baseline evaluation)*.

Erosive lesion formation (36,37,38): All specimens had been soaked in Claritin syrup for ½ hour twice daily for 12 days in a shut compartment. The specimens were washed in distilled water for 5 seconds between the erosive cycles and stored in artificia1 sa1iva ti11 be used again. Artificial sa1iva was daily refreshed as well as Claritin syrup before each cycle [8]. Group I specimens were not subjected to any treatment. Following 12 days of erosion cycles, they have been examined for surface roughness and microhardness as a *(final evaluation)*.

Group II (study group)

The 20 study group specimens were scheduled, numbered and examined for surface roughness and micro-hardness as a *(baseline evaluation)* prior to application of de-mineralizing agent. The previous technique used with group I was followed.

Remineralization [39-41]: The samples were rubbed by finger with freshly mixed CESP slurry for 3 minutes twice daily. Then washed with distilled

water for 5 seconds to eliminate the excess material then kept in artificial saliva at 37 ºC for the rest of the day. This cycle was repeated for 10 days. After this time, all tooth specimens have been analyzed for roughness and micro-hardness *(re-mineralization evaluation)*.

Surface roughness evaluation [42]:

For the determination of surface roughness values, Surface Profilometer (Posi-tecter, SPG, Deflesko Co., USA) was used. The gauge is turned on and its probe is carefully positioned to the examined surface. For each specimen, five readings have been recorded and the mean value for each specimen was determined.

Surface microhardness evaluation [43]

After completing the surface roughness testing, the same specimens were used for determination of hardness values. Measuring of microhardness have been done using a microhardness indenter (ZwicRoell, west Midlands, England) as an initial assessment then following the estimated 12 days' time period of erosive cycles for the group I (control group) specimens. For group II specimens, micro-hardness measuring had been performed as an initial assessment and following the CESP re-mineralization treatment by Vickers Micro-hardness tester. Force of 100 gm have been loaded to the specimen's surface with a dwell time of 15 sec. Five indentations have been equally positioned over 1-mm diameter circle at the center of the specimens and five readings were recorded for the top surfaces of each specimen. The diagonal length of the indentations was detected, then Vickers micro-hardness number was calculated as follow: **VHN=1.854 P/d2** where:

VHN: is Vickers hardness number in Kg/mm^2,

P: is the force in Kg,

d: is the length of the diagonals in mm,

Statistical Analysis

The collected data organized, tabulated and statistically analyzed using SPSS software statistical computer package version 20. The mean and standard deviation were calculated with significance level 5%. and t-test was performed to compare between each before and after interventions.

RESULTS

Characterization of the prepared CESP

Figure (1) showed FTIR spectrum of calcined CESP. The peak at 3431 cm^{-1} is attributed to OH in calcium hydroxide that was established during absorption of H_2O by calcium oxide. The bands at 875.08 and 1428.55 cm^{-1} were related with the vibrations of the (CO_3) groups. FTIR spectra of the chicken eggshell samples calcined to 1200°C for 1 h revealed characteristic bands of the $CaCO_3$ structure at 712, 876, 1423, 1798, 2515, and 3435 cm^{-1}.

***Scanning electron microscope images (SEM)*:** the micrograph of calcined eggshell powder is shown in Figure 2. The morphology of calcined eggshell powder nearly round shaped particles was found for all the samples, with agglomeration of particles giving the shape of cloud. The powder particle size ranged from 75 nm. to 210 nm.

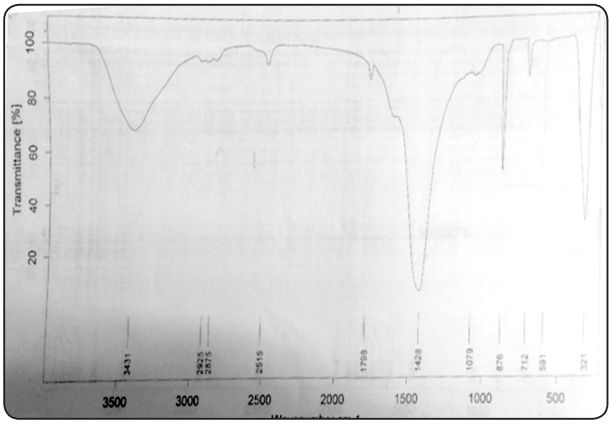

Fig. (1) FTIR spectrum of calcined chicken eggshell powder (CESP)

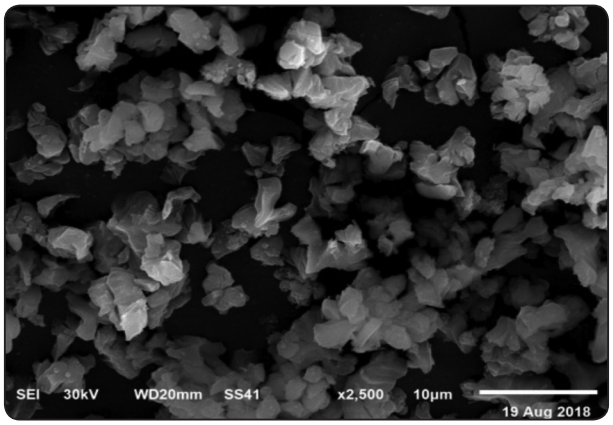

Fig. (2) SEM micrographs of calcined chicken eggshell powder (CESP)

Surface roughness

Referring to table (2), it was found that, roughness of enamel surface was increased following 12 days application of Claritin syrup. There was significantly different change ($p < 0.001$) after erosion with the antihistaminic syrup. However, after treatment with CESP slurry, there was a statistically significant decrease in enamel roughness value (P value was < 0.001).

Surface microhardness

Table (2) showed that, microhardness values were significantly decreased ($P < 0.001$) following erosive cycles with Claritin syrup. On the other hand, after treatment with CESP syrup, microhardness values were significantly increased as $P < 0.001$.

TABLE (2): Mean ±SD of surface roughness and microhardness of the tested groups

Surface roughness	1-Surface roughness at initial and following erosion with the Claritin syrup				
		Base line	**following erosion**	**T**	**P value**
	Mean ±SD	1.57±.085	2.56±0.0872	2.224	<0.001*
	2- Surface roughness after treatment with CESP slurry following erosion by Claritin syrup				
		following erosion (contro1 group)	**following re-mineralization (study group)**		
	Mean ±SD	2.56±0.0872	1.756±0.0587	18.552	<0.001*
Surface microhardness	1-Surface microhardness at initial and following erosion with Claritin syrup				
		Base line	**following erosion**		
	Mean ±SD	171±.5.33	81±3.056	16.552	<0.001*
	2- Surface microhardness following treatment with CESP slurry after erosion by Claritin syrup				
		following erosion (control group)	**following re-mineralization (study group)**		
	Mean ±SD	81±3.056	140± 6.05	3.135	<0.001*

DISCUSSION

Dental erosion is characterized by loss of the tooth surface either by extrinsic or intrinsic acidic agent [44]. This will be leading to a progressive softening of the superficial and sub-surface layer of enamel [45].

Some pediatric syrup medications have a certain acidic component that support the chemical stability, tonicity or improve their flavor, which have a role in increasing their erosive potentiality. Several types of these pediatric medicines are usually used daily in long term treatments of children [46]. As well, these children could be subjected to other sources of erosion, that will expose the tooth structure to a magnifying amount of acids [17]. Accordingly, the aim of the present investigation was to evaluate the remineralization efficiency of chicken eggshell powder (CESP) slurry on artificially induced erosion in primary teeth measured by surface roughness and microhardness.

Spectroscopic imaging is that the method of choice for monitoring and analysis of the chemical composition of the CESP because of the chemical specificity of this imaging approach [47]. Fourier Transform Infrared (FTIR) spectroscopy had been proven to be an efficiently tool in material characterization. The chemically specific information contained within the unique ''fingerprint'' region of the IR spectrum allows one to tell apart different materials within a mixture [48-50].

The medical syrup selected for the present study (Claritin) have some characteristics as high acidity as it contains citric acid - low phosphate and fluoride - and trace of calcium in the chemical composition which potentiate its erosive efficiency [15]. This was agreed with the detected acidic value = 4.2. Furthermore, Claritin syrup is the most used antihistaminic syrup in pediatric health [13,15,16,17,19].

Generally, erosion studies are conducted extra orally, to standardize many parameters [51]

Basically, there is a difference in the structure between the primary and permanent teeth. Deciduous tooth has lower minerals than permanent one therefore, progression of erosion could be faster in deciduous tooth, wherefore, it was decided to be used [52]

Saliva is playing a vital efficient role in resisting enamel erosion. This can be since; it contains a ranged salt of carbonate that can help in the neutralization of acids [53]. Therefore, within the current study, artificially prepared saliva has been chosen as a storage media among the erosive cycles.

Loss of hardened enamel by erosion is efficiently evaluated by detecting its roughness and microhardness. These two methods can accurately detect the harmful effect formed by erosive syrup [11, 54]. Therefore, these two surface evaluations were selected in the present study.

Multiple exposure of erosive medication was found to be recommended than single exposure [11,15,16] as changes will be clearly noticed following several exposures [55] therefore, it was selected to be used.

The erosion protocol of enamel has been performed by immersing all the teeth specimens in Claritin syrup for ½ hour twice daily for 12 days. The regime has been chosen to resemble the long-term use (multiple exposure) of anti-histaminic syrup as recommended by valenoti et al [16].

Surface roughness and microhardness results revealed that 12 twice daily acid exposure to Claritin syrup was efficient to form enamel de-mineralization that shown by marked increase in the roughness values and diminished micro-hardness values of the teeth specimens. This was agreed with results of Costa et al[15], Valinoti et al[16], Gaber et al[36] and Feroz et al[37]

Recently, there was a marked highlighting on the advancement of non-toxic re-mineralizing compounds [56]. Actually, CESP has a protective effect as it can act as a store of ions that prevents

enamel de-mineralization and enhances the re-mineralization process. Calcium and phosphate ions present in saliva act as building blocks for re-mineralization process and preventing the erosive de-mineralization. Also, it was found that CESP has an efficient role in the re-mineralization of the early enamel carious lesion [31]

In the present study, CESP slurry was topically applied on the demineralized tooth surface of group II twice daily for 10 days the regime that was prescribed by Darshan et al[57] and Talaat et al[40] whose regimes had showen a significant re-mineralizing effect in-vitro.

Surface roughness and micro-hardness results revealed that, topical treatment with CESP slurry significantly reduced the mean surface roughness and enhanced the micro-hardness of the de-mineralized group II tooth specimens that indicated the process of re-mineralization. The present finding is supported by those of Feroz and Moeen [25] and Feroz et al[26] who recorded enhancement on the hardness and decrease on the surface roughness following the use of CESP. Thus, it was enhancing the in-vitro re-mineralization of eroded enamel.

CONCLUSIONS

Within the limitation of the present study, the conclusions are:

1. Chicken eggshell powder slurry is an effective re-mineralized treatment against dental erosion initiated by antihistaminic syrup.
2. Claritin antihistaminic syrup had induced an extreme erosion on deciduous tooth enamel. In this way, it is very essential to the clinicians specially to be acquainted with the potential of erosion for the most used syrups.
3. Roughness and micro-hardness testing are precise and valuable strategy to evaluate the de-mineralization and re-mineralization of enamel surface in-vitro.

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