Evaluation of Quantum in Tooth Remineralisation Using a Mollusc, Avian and Plant-Source of Calcium against Different Tooth Remineralising Agents

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ABSTRACT

Background: The loss of enamel is a dynamic process occasioned with periods of demineralisation and remineralisation. Hence, preventive measures against enamel dissolution and permanent damage should be a priority for oral health-care providers. It has been suggested that the beneficial health effects can be improved with calcium or calcium containing materials. There are many commercially available effective remineralising agents, however they are not economically feasible for the low economic population. Thus, there is a need for alternative sources of calcium that aid in tooth-enamel reminerasalisation.

Aims: (i) Investigated and compared invitro the potential of oyster (*Crassostrea madrasensis*) shells, domestic hen (*Gallus gallus*) eggshells and *moringa oliefera* leaves on tooth-enamel remineralisation. (ii) Comparatively evaluated invitro, the quantum of tooth remineralisation when oyster (*Crassostrea madrasensis*) shell, domestic hen (*Gallus gallus*) eggshells and *moringa oliefera* leaves on tooth enamel remineralisation were used. (iii) Compared the quantum of tooth-enamel remineralisation between oyster(*Crassostrea madrasensis*) shell, domestic hen(*Gallus gallus*) eggshells and *Moringa oliefera* leaves with CPP-ACPF (GC Tooth Mousse Plus), Bioactive glass(BioEnamel) and Fluoride enhanced Hydroxyapatite(Remin Pro)

Objectives: (i) To provide an alternative natural tooth-enamel remineralising agent that is socioeconomically viable compared to the currently available commercial counterparts. These agents been tried in the liquid state can be used in the form of paste and powder that can be, very much part of a school oral health programme especially in rural India.

(ii) Since most of the commercially available tooth remineralising agents has casein as one of the primary ingredients, it has been observed that there are numerous people allergic to casein. Hence, the alternative remineralising agents to be tried in this study can be immensely beneficial to children and adolescent individuals who have casein intolerance.

(iii) Since one of the samples to be tried out is *Moringa oliefera* (Indian drumstick tree) leaves, this calcium containing plant extract may also serve as an alternative remineralising agents especially in vegen community.

Materials and Methods: Seventy human unerupted maxillary and mandibular 3rd molars were selected for the study. Carious lesions representing preliminary stage of subsurface enamel lesion were created by placing the tooth samples in 20ml of demineralisation bath for 72 hours. The samples were divided into 7 groups having 10 samples each. The seven groups were: Group 1: (Negative Control)Demineralised enamel , Group 2 : (Positive Control) CPP-ACPF, Group 3: Bioactive glass, Group 4: Flouride enhanced hydroxyapatite, Group 5: Domestic hen eggshell solution, Group 6: Oyster shell solution, Group 7: *Moringa oliefera* extract. The samples were then kept in a vibrating waterbath for 21 days. After 21 days, the samples were then tested by Vickers microhardness test and X-ray flouresence spectroscopy.

Results: All groups were evaluated for tooth-enamel remineralisation potential. This study demonstrated that all the alternative sources of calcium are promising. However, among the various alternatives tested, domestic hen (*Gallus gallus*) egg shell (group 5), proved to be better in remineralisation potential (222.43) than the oyster shell (201.65) (group 6) and *moringa oliefera* leaves (199.42) (group 7), (Table 1). However, group 3 (Bioactive glass) exhibited maximum efficacy in remineralising tooth enamel (250.93). The p value was determined as 0.001, that implies its highy significant.

Conclusion: The alternative calcium sources (domestic hen (*Gallus gallus*) egg shell, oyster shell and *Moringa oliefera* leaves) exhibited remineralisation of tooth-enamel. Among the alternative sources, domestic hen (*Gallus gallus*) egg shell had maximum tooth-enamel remineralisation (222.43) but was not to the extent of the commercially available Bioactive glass (250.93). Nevertheless, the natural alternatives can definitely be a boon to people in developing countries of low socioeconomic groups, oral health programmes, people allergic to casein, etc.

KEY WORDS

Demineralisation, Remineralisation, tooth-enamel, CPP-ACPF, Bioactive glass, Fluoride enhanced hydroxyapatite, domestic hen (*Gallus gallus*) eggshell, oyster shell, *Moringa oliefera*, Vickers microhardness, X-ray fluorescence spectroscopy, ANOVA

INTRODUCTION

Decades of research has helped to increase our knowledge of dental caries and reduce its prevalence. However, dental caries still continues to remain as a major dental disease.[1]

'Demineralisation' and 'Remineralisation' is a balanced processes that occurs in the oral cavity wherein remineralisation is facilitated by the buffering action of saliva, permitting calcium and phosphate ions to precipitate onto the tooth and form new mineral. It is the modulation of the demineralisation-remineralisation balance is the key to prevention of dental caries.[16,17]

The conventional treatment concept for all carious teeth involves caries excavation and replacement with a restorative material. Fluoride has been recognized as the main propriety for the decline in dental caries due to its cariostatic potential. Despite its profound effect in halting caries progression, it has been met with certain limitations. Fluoride does not aid in

eliminating caries totally. Moreover, increased fluoride concentration can produce detrimental effects on the tooth.[18]

Decades of research, evolved the "minimally invasive" approach which incorporates detecting and treating these areas sooner, emphasizing on prevention rather than the traditional surgical model.[3]

Remineralising agents, help in remineralisation of the carious lesion by replenishing lost minerals like calcium, phosphate ions into the tooth structure. Several invitro and invivo studies have proven that these agents have proven to be useful in the treatment of white spot lesions, early childhood caries, dental erosion, root caries and dentin hypersensitivity.

They are delivered in the form of oral hygiene products such as chewing gum, tooth cream and even incorporated in dental restorative materials also.

The currently used are non-fluoridated agents like; Casein Phosphopeptide Amorphous Calium Phoshphate , Tricalcium Phosphate, Bioactive Glass, Xylitol, Unstabilised Calcium Phosphate with Sodium Fluoride, Arginine Bicarbonate calium carbonate complex, Calium carbonate carrier etc.[22] However, they have disadvantages of being expensive for the socio-economic population especially in third world/developing countries.

Casein PhosphopeptideAmorphous Calium Phoshphate (CPP-ACP) is derived from milk protein-casein, has exhibited anticariogenic potential invitro and insitu studies. The CPP-ACP acts as reservoir of bio-available calcium and phosphate thus facilitating remineralisation. CPP-ACP containing 0.09% fluoride is available as CPP–ACPF paste (GC Tooth Mousse Plus; GC Corporation, Tokyo, Japan). CPP–ACPF has been reported to have a greater potential for remineralisation than CPP–ACP.[4-8]

Bioactive glass (BioEnamel, Prevest Denpro, USA) has the ability to act as a biomimetic mineraliser. It enhances the remineralisation of enamel and have found to reduce sensitivity after bleaching.[16] Fluoride-enhanced-Hydroxyapatite (Remin Pro (VOCO, Germany) is yet another remineralising paste which in contrast to CPP-ACP contain calcium and phosphate in the hydroxyapatite form. In addition, fluoride and xylitol have also been included into CPP-ACP.[19] However, these chemically synthesized remineralising agents have limitations. These agents may only show superficial surface remineralisation and may have short duration of action. Also some of them tend to show gastric irritations and allergic reactions and are not economically viable to the poor-income groups. Recently, alternative sources of calcium have been researched upon. They include*; galla chinesis*, hesperidin, gum Arabic, domestic hen egg shells, etc.[20]

Molluscs, contain calcium which can be used as a good natural source. Of these, the oyster shells have exhibited the maximum calcium levels. Oyster (*Crassostrea madrasensis*) creates its own environment by secreting a shell composed or ninetyfive percent (95%) of calcium carbonate. The remainder of the shell is made up of organic material and trace amounts of manganese, iron, aluminum, sulfate and magnesium. There is a probable chance in remineralisation of tooth enamel because of the high calcium content[9].

Domestic hen (*Gallus gallus*) egg shell, has a very high percentage of bio-available calcium. When compared with other natural calcium sources, hen egg shell has low levels of toxic metals like Pb, Al, Cd and Hg.[11]

Plants, act as big reservoir for different minerals. These minerals are found to be in various parts of the plant such as in leaves, roots, barks, etc.

Moringa oliefera, (drumstick tree), contains good amount of calcium in its leaves. This can be utilised as a natural source for obtaining calcium. *Moringa oleifera* leaves are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper.[21]

However, there is limited knowledge regarding the efficacy of alternative agents when used for tooth-enamel remineralisation when compared to their commercial counterparts.Thus, the aim of this invitro study was to, comparatively evaluate the quantum of tooth-remineralisation of three natural alternative sources (domestic hen eggshell, oyster shell and *Moringa oliefera* leaves) when compared to three commercially available remineralising agents (CPP-ACPF, Bioactive glass and Fluoride enhanced Hydroxyapatite)

AIMS:

(i) Investigated and compared invitro the potential of oyster (*Crassostrea madrasensis*) shells, domestic hen(*Gallus gallus*) eggshells and *Moringa oliefera* leaves on tooth-enamel remineralisation.

(ii) Comparatively evaluated invitro, the quantum of tooth remineralisation when oyster (*Crassostrea madrasensis*) shell, domestic hen(*Gallus gallus*) eggshells and *Moringa oliefera* leaves on tooth enamel remineralisation were used.

(iii)Compared the quantum of tooth-enamel remineralisation between oyster (*Crassostrea madrasensis*) shell, domestic hen(*Gallus gallus*) eggshells and *Moringa oliefera* leaves with CPP-ACPF (GC Tooth Mousse Plus), Bioactive glass(BioEnamel) and Fluoride enhanced Hydroxyapatite(Remin Pro).

OBJECTIVES:

(i) To provide an alternative natural tooth-enamel remineralising agent that is socioeconomically viable compared to the currently available commercial counterparts. These agents been tried in the liquid state can be used in the form of paste and powder that can be, very much part of a school oral health programme especially in rural India.

(ii) Since most of the commercially available tooth remineralising agents has casein as one of the primary ingredients, it has been observed that there are numerous people allergic to casein. Hence, the alternative remineralising agents to be tried in this study can be immensely beneficial to children and adolescent individuals who have casein intolerance.

(iii)Since one of the samples to be tried out is *Moringa oliefera* (Indian drumstick tree) leaves, this calcium containing plant extract may also serve as an alternative remineralising agents especially in vegen community.

MATERIALS

(i) CPP-ACPF (GC Tooth Mousse Plus; GC Corporation, Tokyo, Japan)

(ii) Bioactiveglass (BioEnamel, Prevest Denpro; USA)

(iii) Fluoride enhanced hydroxyapatite(Remin Pro ;VOCO, Germany)

(iv) Domestic hen (*Gallus gallus*) eggshell

(v) Oyster shells (*Crassostrea madrasensis*)

(vi) *Moringa oliefera* leaves

METHODOLOGY:

Preparation of oyster shell powder. [9]

The oyster (*Crassostrea madrasensis*) shells were heated in an oven at 200 °C for 1 hour to make the shells more brittle and submitted to milling in a high-speed planetary mill with a porcelain jar and alumina balls for 15 minutes with water. The powders were heated again to 500 °C and maintained for 2 hours. To undo the clusters a new milling was performed without water for 1 minute.

Production of Eggshell Powder[10]

The eggshell powder was obtained by the process of calcination (World Property intellectual organization protocol).[23] This Calcination process was done to obtain pure powder free of pathogens and to increase the alkalinity of powder.

Eggshells were obtained from a local hatchery, the contents removed and the eggshells are cleaned in distilled water.

The eggshells were then kept in hot water bath at 100°c for 10 minutes followed by removing the membrane. They were then crushed using a sterile mortar and pestle. The crushed particles were then heated at 1200˚c in a muffle furnace and powdered to small particles.

Production of Eggshell Powder Solution and Oyster shell powder solution[11]

One gram of eggshell and oyster shells were dissolved with 20 ml of 4% acetic acid in separate test tubes. The clear fluid which was collected at the top and then transferred to beakers. The pH of the solution is tested using a pH meter.

Preparation of crude leaf extract of *Moringa oliefera* [12]

The leaves were collected and the stalk removed. The leaves were then grounded and homogenized with distilled water in an electric blender. The decoction thereafter was filtered through a sterilized cheese cloth. The clear filtrate was stored in a refrigerator at 4°C and served as the stock crude extract.

Sample preparation

Seventy human unerupted maxillary and mandibular 3rd molars that were indicated for extraction was selected for the study.

After removal of debris, calculus and soft tissue from the tooth surface, the teeth were stored in 10% formalin solution until further use. Teeth were then sectioned 1 mm below the cementoenamel junction with a slow speed diamond disc, the roots discarded and crowns used for the study.

Custom built moulds were prepared and self-cured acrylic resin is poured in them. Each tooth-crown was embedded in the resin with the buccal surface facing upward and exposed and parallel to the horizontal plane. The buccal surface was flattened and polished using 400, 800, 1000, 1200 grit abrasive paper sequentially.

A 5 mm × 5 mm window of exposed enamel was created in the middle of the sample surface by using adhesive tape and the sample was rendered resistant to acid attack by applying a uniform coat of the nail varnish around it. Once the samples were adequately dried, the

adhesive tape was removed from the tooth surface using an explorer, exhibiting a rectangular area on the enamel surface.

Demineralisation Protocol (Caries Research 2003;37:166-71)

Dental carious lesions representing preliminary stage of subsurface enamel lesion were created by placing the tooth samples in 20ml of demineralisation bath for 72 hours (CaCl2 = 2.2 Mm NaH2PO4= 2.2 Mm Lactic acid = 0.05 M, Fluoride = 0.2 ppm, solution was adjusted with 50% NaOH to a pH of 4.5). The specimens were kept in the demineralisation solution (CaCl2, NaH2PO4, Lactic acid and Fluoride) for 72 h at 37° C.

Study groups (n=70)

Group 1(n=10) (Negative Control) - Demineralised enamel suspension in artificial saliva for 21 hrs X 21 days

Group 2 (n=10) (Positive Control) - Topical application with CPP-ACPF (GC Tooth Mousse Plus), followed by suspension in artificial saliva for 21 hours X 21 days

Group 3 (n=10) — Topical application with Bioactive glass (BioEnamel), followed by suspension in artificial saliva for 21 hours X 21 days

Group 4 (n=10) — Topical application with Fluoride enhanced hydroxyapatite (Remin Pro), followed by suspension in artificial saliva for 21 hours X 21 days

Group 5 (n=10) – Suspension of the tooth samples in domestic hen (*Gallus gallus*) eggshell solution for 21 h for 21 consecutive days for remineralisation. For every 24 hours, the hen eggshell solution was freshly prepared and the samples washed twice with distilled water.

Group 6 (n=10) --- Suspension of the tooth samples in oyster (*Crassostrea madrasensis*) shell powder solution for 21 hours X 21 days. For every 24 hours, the oyster shell solution was freshly prepared and the samples washed twice with distilled water

Group 7 (n=10)— Suspension of the tooth samples in *Moringa oliefera* leaves extract for 21 hours X 21 days. For every 24 hours, the extract was freshly prepared and the samples washed twice with distilled water

The sample specimens (Groups 1- 7) were kept in vibrating water bath (REMI) containing their respective solutions.

Microhardness Testing

The surface microhardness of tooth enamel of all specimens were analysed by Vickers microhardness testing machine (Micro Vickers Hardness tester, Matsuzawa Co., Ltd, Toshima, Japan).

Atomic Analysis

Both calcium% and phosphorus% were analysed in all samples by X-ray fluorescence spectroscopy (NPP STRUCTURNAYA, DOM 5, Yekaterinburg, Russia).

Results:

The results reveal that all groups evaluated, exhibited remineraslisation of tooth-enamel. Among the alternatives tested, domestic hen (*Gallus gallus*) egg shell (Group 5), exhibited better remineralisation potential (222.43) than the oyster shells (201.65) (Group 6) and *Moringa oliefera* leaves (199.42) (Group 7) (Table 1).

However, Group 3 (Bioactive glass) exhibited maximum efficacy in remineralising of tooth- enamel. [Microhardness (250.93)(Table 1) and the Ca/p ratio (1.67)(Table 4).The p value was determined as 0.001, that implies its highy significant.

Table 1 : **Vickers Microhardness test**

The surface microhardness of all specimens was analysed by Vickers microhardness testing machine. A load of 25g applied for 5 sec and five indentations made for each specimen with a spacing of 100 microns. The average value considered as the microhardness of corresponding specimen.

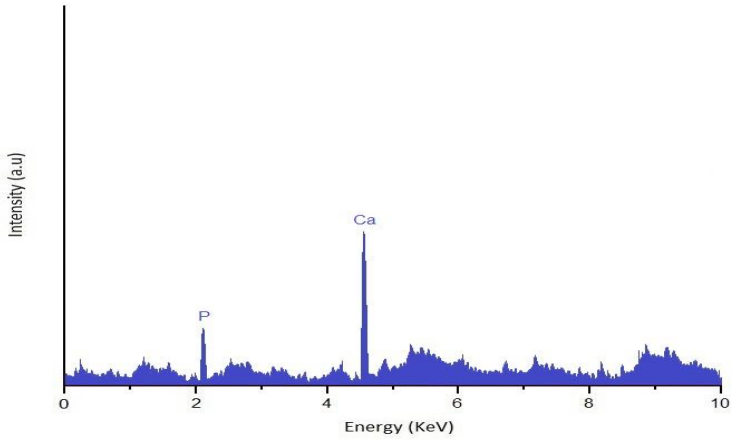
X- Ray Fluorescence Spectroscopy is based on the principle that individual atoms, when excited by an external energy source, emit X-ray photons of a characteristic energy or wavelength. The number of photons of each energy emitted from a sample are counted, the elements present may be identified and quantitated, This analysis was done in this study to obtain atomic analyses of Ca weight % and P weight %.

The Ca %, P % and Ca/P ratio are shown in Table 2 , 3 and 4 respectively.

Table 1: Vickers Microhardness test

Micro hardness	Mean	SD	SE	ANOVA	p
Control (G 1)	174.94	0.644	0.372		
CPP-ACPF (G 2)	241.78	0.042	0.024		
Bioactive glass (G 3)	250.93	1.126	0.650		
Fluoride enhanced hydroxyapatite (G 4)	234.13	0.544	0.314	4583.01	0.001
Domestic hen eggshell (G 5)	222.43	1.116	0.644		
Oyster shells (G 6)	201.65	0.264	0.152		
Moringa oliefera leaves (G 7)	199.42	0.250	0.144		
Total	217.90	25.687	5.605		

Graph 1: Data Interpretation from X-ray Fluorescence Spectroscopy



The specimen is irradiated with an X-ray source resulting the elements in the specimen to fluoresce their distinctive X-rays. The peaks (Calcium and Phosphorus) of the generated X-rays are measured using a wavelength dispersive detection device. The intensities are derived

from a number of 2D frames obtained while rotating the sample in a wide consecutive and partially overlapped scans. Then, the orientation of each of the grains is determined and the intensities are scaled and merged, so that the resulting single-crystal dataset can be used for structure solution or refinement. The values are compared with the International Centre for Diffraction Data (ICDF) to interpret the elemental composition

Table 2 : Calcium Analysis

Calcium Analysis	**Mean**	**SD**	**SE**	**ANOVA**	**p**
Control (G 1)	21.77	0.153	0.088		
CPP-ACPF (G 2)	16.82	0.108	0.062		
Bioactive glass (G 3)	16.05	0.050	0.029		
Fluoride enhanced hydroxyapatite (G 4)	19.85	0.127	0.073	2778.68	0.001
Domestic hen eggshell (G 5)	20.82	0.076	0.044		
Oyster shells (G 6)	22.87	0.104	0.060		
Moringa oliefera leaves (G 7)	25.85	0.132	0.076		
Total	20.57	3.234	0.706		

Table 3 : Phosphorus Analysis

Phosphate Analysis	**Mean**	**SD**	**SE**	**ANOVA**	**p**
Control (G 1)	16.48	0.200	0.115		
CPP-ACPF (G 2)	10.53	0.070	0.040		
Bioactive glass (G 3)	9.62	0.231	0.133		
Fluoride enhanced hydroxyapatite (G 4)	11.82	0.101	0.059	911.80	0.001
Domestic hen eggshell (G 5)	13.04	0.203	0.117		
Oyster shells (G 6)	14.40	0.180	0.104		
Moringa oliefera leaves (G 7)	17.00	0.065	0.038		
Total	13.27	2.699	0.589		

Table 4: Calcium / Phosphate Ratio

Calcium / Phosphate Ratio	**Mean**	**SD**	**SE**	**ANOVA**	**p**
Control (G 1)	1.32	0.023	0.013		
CPP-ACPF (G 2)	1.60	0.017	0.010		
Bioactive glass (G 3)	1.67	0.035	0.020		
Fluoride enhanced hydroxyapatite (G 4)	1.68	0.010	0.006	86.78	0.001
Domestic hen eggshell (G 5)	1.60	0.023	0.013		
Oyster shells (G 6)	1.59	0.026	0.015		
Moringa oliefera leaves (G 7)	1.52	0.010	0.006		
Total	1.57	0.116	0.025		

DISCUSSION

Scientific literature proposes that clinical management of tooth-enamel demineralisation should emphasize on early detection and prevention, before a restorative approach is applied.

Decades of research has lead to the advancement of technologies that can promote enamel remineralisation or down scale demineralisation thereby reinforcing and aiding oral health.
Considerable efforts have been made to limit the progression of dental carious lesions and while clinical studies remain the gold standard, standardized invitro models are the most conventional techniques in cariology research that can serve as a valuable tool for assessing anticaries efficacy of tooth remineralising agents.
In recent years, alternative remineralising agents have been researched. Recently, studies have evaluated the use of eggshells as a source for calcium oral supplement.
Studies reveal that oyster shells contain high amount of bioavailable calcium in them. The plant leaves of *Moringa oliefera* have shown to exhibit antibacterial properties.[27]
However, there is limited knowledge regarding the comparative remineralising efficacy among these alternatives and also when compared with the commercially available tooth-enamel remineralising agents. Hence, this study comparatively determined the quantum of tooth-enamel remineralisation potential of early enamel carious lesions by oyster shell solution, domestic hen eggshell solution and *Moringa oliefera* leaf extract against commercially available tooth remineralising agents (CPP-ACPF, Bioactive glass and Fluoride enhanced hydroxyapatite).
Lata S et al., reported that initial enamel lesions with intact surfaces record a low mineral content at the surface layer when compared to sound enamel thereby demonstrating a lower microhardness value at the surface than for sound enamel tissue.According to Lata et al., the demineralising solution creates a subsurface demineralisation of approximately 150 microns width with an intact surface simulating an early enamel lesion. The concentration of both calcium and phosphates in the demineralising solution was at 50% of saturation level, causing dissolution of only enamel subsurface.[11]
The addition of fluoride prevented surface demineralisation by forming fluorapatite at the surface. In this invitro study, it was observed that Bioactive glass performed better (250.93) when compared to CPP-ACPF (241.78), fluoride enhanced hydroxyapatite (234.13), domestic hen egg shell (222.43) , oyster shell (201.65) and *Moringa oliefera* leaves (199.42)(Table 1). The probable reason for this could be because of its active ingredient, calcium sodium phosphosilicate, that binds to the tooth surface in order to initiate the remineralisation process.[13]
Bioactive glass reacts with saliva inducing dissolution of calcium, phosphate and silicate ions at the glass surface and subsequent precipitation of a polycondensed silica-rich layer, that serve as a template for the formation of calcium phosphate which subsequently crystallise into hydroxycarbonate apatite.[13,14,6]
Bioactive glass, is an extensively studied biomaterial in the field of tissue engineering, bone regeneration and dentin remineralisation due to the remarkable capability of forming Hydroxycarbonate Apatite (HCA). Bioactive glass 45S5 (BAG) has been incorporated into dentifrices, desensitizing pastes and glass ionomer cements (experimentally). Although, it has been successfully proven that materials based on bioactive substance have the potential to promote remineralisation, only a limited number of studies have quantitatively monitored the remineralisation process.[6]
Reynolds EC et al., reported that CPP–ACPF has a greater potential for remineralisation than CPP–ACP [4]. In their study, CPP-ACPF was compared with the other agents. The authors said

that the CPP stabilizes the calcium and phosphate ions in dental plaque and dental enamel and keeps the calcium, phosphate ions in an amorphous, noncrystalline state that helps them to enter the tooth enamel. The CPP stabilizes ACP thereby maintaining a state of supersaturation with calcium and phosphate ions that finally reduce demineralisation and increased remineralisation.[31]

There are limited invitro studies evaluating the remineralising efficacy of fluoride enhanced hydroxyapatite pastes. Heshmat H et al., and Kamath U et al., reported no difference between CPP-ACPF and fluoride enhanced hydroxyapatite paste .The authors hypothesised that the synergistic action of the HA and fluoride, enhanced remineralisation thereby rendering the tooth more resistant to acid attacks.[19,32]

In this invitro comparative study, all the three alternatives (domestic hen eggshell, oyster shell and *Moringa oliefera* leaves) exhibited remineralisation potential. The domestic hen eggshell had remineralisation value (222.43) when compared with oyster shells (201.65) and *Moringa oliefera* leaves (199.42)(Table 1). The rationale may be due to the N-terminal sequence (Met-Ala-Val-Pro-Gln-Thr-Met-Val-Gln) of eggshell matrix proteins suggested in the increased calcium transport and considered as a potential significance of eggshell calcium when used as calcium supplements.[33,34] The increased pH of a remineralising solution is favourable, as it increases the ion activity of anions such as phosphate and hydroxyl ions in the solution. The ion activity corresponds to the concentrations of these ions in the solution. Therefore, there will be more availability of these ions for remineralisation. In addition, the basic form of phosphate anion present in hydroxyapatite is PO_4^{3-} and these anions are present in higher concentrations only at a high pH of 11-12. For remineralisation to occur, bioavailable calcium and phosphates are essential.[24,25]

Therefore, the rich bioavailability of calcium along with the high concentration of phosphates present in eggshell solution coupled with its increased pH may be responsible for remineralisation.[26]

In this study, remineralisation value of oyster shell was (201.65)(Table 1). There is a high content of calcium carbonate in oyster shells, which can be used in the formulation of medicine, in construction or as filler in polymer materials and also got remineralisation potential. Hydroxyapatite powder was synthesized using oyster shell. The prepared hydroxyapatite powders at pH 10, have exhibited improved crystallinity. The probable reason for this can be due to the presence of hydroxyapatite nanocrystalline for tooth-enamel remineralisation.[30]

Moringa Oleifera, is a plant that is very rich in nutritional elements and has been used in the treatment of many diseases. Its high content of calcium and potassium and many other natural proteins could be beneficial in presenting the required elements to remineralise a defected enamel surface[15].In this study, *Moringa oliefera* leaf extract had exhibited a value of (199.42)(Table 1). The remineralisation effect is by increasing the pH level in body fluids and therefore counteracts acidification which enhances remineralisation. *Moringa oleifera* also contains a high concentration of minerals and a large range of different amino acids. These amino acids could play a role in the regulation of mineral deposition and guidance of enamel crystals formation.[27]

Gopalakrishnan L, had investigated the role of different proteins in the remineralisation process, it compared the whey protein (45% of all amino acids) to the *moringa* protein (47%

of all amino acids) and concluded that the higher protein content of the *Moringa* leaf powders lead to better remineralisation.[27]

In addition, *Moringa oleifera* contains proanthocyanidins as evident from its compositional analysis[28]. Mikarimi, et al, demonstrated that plant-derived proanthocyanidins can increase the remineralisation of carious enamel lesions and it could be an effective natural agent for noninvasive dentistry. Its effect on the enamel is not well known but it is possible that it enhances remineralisation due to the precipitation of minerals.[29]

CONCLUSION

Within the limitation of this invitro comparative study, it is important to observe that all the alternative sources of calcium evaluated had promising results in remineralising of tooth-enamel. Among the alternative tooth-enamel remineralising agents evaluated, domestic hen (*Gallus gallus*) egg shell was superior when compared to oyster shell and *Moringa oliefera* leaves. However, among the commercially available remineralising pastes, Bioactive glass exhibited maximum tooth-enamel remineralisation (250.93), when compared to CPP-ACPF (241.73) and Fluoride enhanced nanohydroxyapatite (234.13)

Nevertheless, these remineralising alternatives may be suggested as possible agents for remineralisation of tooth-enamel, especially for a population of low socio-economic status, oral health programmes in developing countries, communities on vegan diet, those allergic to casein-paste products, etc.

However, there are other parameters the above alternative remineralising agents need to be analysed, such as; antimicrobial effect, hypersensitive reaction, stability data of the prepared powder solution, shelf life, physical changes during storage, colour, taste, clarity, specific gravity, viscosity, delivery of calcium and phosphate into the tooth-enamel subsurface, should boost the remineralising properties of saliva, should work at an acidic pH, etc. It is to be noted that remineralisation invitro may be quite variable when compared to changes occurring in the oral cavity invivo. Therefore, direct extrapolations to clinical situations must be executed discreetly only after further studies, before it can be finally proposed as alternative tooth-enamel remineralising agents.

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CONFLICT OF INTEREST :

None.

REFERENCES

1. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralisation potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in vitro pH-cycling study. J Conserv Dent. 2014;17:03-07.
2. Sivapathasundharam B, Raghu AR. Dental Caries. In: B Rajendran, B Sivapathasundharam, editor. Shafer's textbook of oral pathology, 5th ed. Elsevier publishers; 2006. Pp. 567-658

3. Madan N, Madan N, Sharma V, Pardal D, Madan N. Tooth remineralization using bio-active glass - A novel approach. J Academy Adv Dental Research. 2011;2:45-50.
4. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008;87:344-48.
5. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP–ACP and CPP–ACP with 900 ppm fluoride on eroded human enamel : An in situ study. Arch Oral Biol. 2010;55(7):541-44.
6. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization- An in vitro study using scanning electron microscope and diagnodent. Indian J Dent Res. 2011;22:77-82.
7. Shetty S, Hegde M, Bopanna T. Enamel remineralization assessment after treatment with three different remineralizing agents using surface microhardness: An in vitro study. J Conserv Dent. 2014;17:49-52.
8. Taranath A, Pai D, Chakravarthy K. The role of casein phosphopeptide-amorphous calcium phosphate products in remineralization of incipient enamel lesions and its substantivity. J Exp Integr Med. 2014;4:67-70.
9. Michele Regina Rosa Hamestera, Palova Santos Balzera, Daniela Beckerb Characterization of Calcium Carbonate Obtained from Oyster and Mussel Shells and Incorporation in Polypropylene. Materials Research. 2012; 15(2): 204-208
10. Methods of producing egg shell powder: Patents: US 20060062857 A1.
11. Bejoy Mony et al., Effect of Chicken Egg Shell Powder Solution on Early Enamel Carious Lesions: An Invitro Preliminary Study Journal of Clinical and Diagnostic Research. 2015 Mar, Vol-9(3): ZC30-ZC32
12. S. Ghasi et al. : Journal of Ethnopharmacology 69 (2000) 21–25
13. Golpayegani M, Sohrabi A, Biria M, Ansari G. Remineralization effect of topical novamin versus sodium fluoride (1.1%) on caries-like lesions in permanent teeth. J Dent. 2012;9:68-65
14. Hamba H, Nikaido T, Inoue G, Sadr A, Tagami J. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: A quantitative assessment using micro-computed tomography. J Dent. 2011;39:405-13.
15. Younis, S. H., Obeid, R. F., & Ammar, M. M. (2020). Subsurface enamel remineralization by Lyophilized *Moringa* leaf extract loaded varnish. Heliyon, 6(9), e05054
16. Basso M. Advances in remineralisation therapy. Available from: www.burkhartdental.com
17. Madan N, Madan N, Sharma V, Pardal D, Madan N. Tooth remineralization using bio-active glass - A novel approach. J Academy Adv Dental Research. 2011;2:45-50.
18. Zhang Q, Zou J, Yang Ran, Zhou X. Remineralization effects of casein phosphopeptide-amorphous on artificial early enamel lesions of primary teeth. Int J Paediatr Dent. 2011;21:374-81
19. Heshmat H, Ganjkar M, Jaberi S, Fard M. The effect of remin pro and mi paste plus on bleached enamel surface roughness. J Dent. 2014;11:131-36.
20. Nebu Philip State of the Art Enamel Remineralization Systems: The Next Frontier in Caries Management. Caries Res 2019;53:284–295
21. Raimunda Sâmia Nogueira Brilhante, et al . Research advances on the multiple uses of Moringa oleifera: A sustainable alternative for socially neglected population, Asian Pacific Journal of Tropical Medicine, Volume 10, Issue 7, 2017, Pages 621-630,
22. Dr. Sourabh Ramesh Joshi, Dr. Gowri Pendyala, Dr. Mopagar Viddyasagar, Dr. Neeta Padmawar, Dr. Asha Nara and Dr. Prajakta Joshi. Remineralizing agents in dentistry: A review. International Journal of Applied Dental Sciences 2018; 4(2): 198-199
23. WO/2004/105912: Method of producing egg shell powder

24. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2). J Clin Pediatr Dent. 2004;28:119–24.
25. González-Cabezas C. The chemistry of caries: remineralization and demineralization events with direct clinical relevance. Dent Clin North Am. 2010;54(3):469-78
26. Cochrane NJ, Reynolds EC. Calcium phosphopeptides - mechanisms of action and evidence for clinical efficacy. Adv Dent Res. 2012;24(2):41-47.
27. Gopalakrishnan L., Doriya K., Kumar D.S. Moringa oleifera: a review on nutritive importance and its medicinal application. Food Sci Hum wellness. 2016;5(2):49–56
28. Moyo B., Oyedemi S., Masika P.J., Muchenje V. Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. Meat Sci. 2012;91(4):441–447
29. Mirkarimi M., Eskandarion S., Bargrizan M., Delazar A., Kharazifard M.J. Remineralization of artificial caries in primary teeth by grape seed extract: an in vitro study. J. Dent. Res. Dent. Clin. Dent. Prospects. 2013;7(4):206–20610
30. Sawittree Rujitanapanich, Pitoon Kumpapan, Panthong Wanjanoi, Synthesis of Hydroxyapatite from Oyster Shell via Precipitation, Energy Procedia, Volume 56 2014, Pages 112-117
31. Dr. Aarushi Kataria, Dr. Naveen Nandal and Dr. Ritika Malik, Shahnaz Husain -A Successful Indian Woman Entrepreneur, International Journal of Disaster Recovery and Business ContinuityVol.11, No. 2, (2020), pp. 88–93
32. Kumar, S. (2020). Relevance of Buddhist Philosophy in Modern Management Theory. Psychology and Education, Vol. 58, no.2, pp. 2104–2111.
33. Roy, V., Shukla, P. K., Gupta, A. K., Goel, V., Shukla, P. K., & Shukla, S. (2021). Taxonomy on EEG Artifacts Removal Methods, Issues, and Healthcare Applications. Journal of Organizational and End User Computing (JOEUC), 33(1), 19-46. http://doi.org/10.4018/JOEUC.2021010102
34. Shukla Prashant Kumar, Sandhu Jasminder Kaur, Ahirwar Anamika, Ghai Deepika, Maheshwary Priti, Shukla Piyush Kumar (2021). Multiobjective Genetic Algorithm and Convolutional Neural Network Based COVID-19 Identification in Chest X-Ray Images, Mathematical Problems in Engineering, vol. 2021, Article ID 7804540, 9 pages. https://doi.org/10.1155/2021/7804540
35. Karlinsey RL, Mackey AC. Solid state preparation and dental application of an organically modified calcium phosphate. J Mater Sci. 2009; 44:34-69
36. Kamath U, Sheth H, Mullur D, Soubhagya M. The effect of remin pro on bleached enamel hardness: An in-vitro study. Indian J Dent Res. 2013;24:690-93.
37. Schaafsma A, Pakan I. Short-term effects of a chicken egg shell powder enriched dairy-based products on bone mineral density in persons with osteoporosis or osteopenia. Bratisl Lek Listy. 1999;100(12):651-56.
38. Schaafsma A, Pakan I. Effect of a chicken egg shell powder enriched dairy product on bone mineral density in persons with osteoporosis or osteopenia. Nutrition. 1999;15(2):157