Journal of Pharmaceutical Research International

32(20): 13-19, 2020; Article no.JPRI.59845
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Effect of Nano-hydroxyapatite Crystal Incorporated Herbal Dentifrice on Remineralization of Incipient Caries Lesion- A Pilot Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS and DA managed the analyses of the study and write up of the article. Authors IMA and RPK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/JPRI/2020/v32i2030724
Editor(s):
(1) Dr. Q. Ping Dou, Wayne State University, Detroit.
Reviewers:
(1) Tania Regina Riul, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brazil.
(2) Dakshita, Kothiwal Dental College and Research Centre, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59845

Original Research Article

Received 02 June 2020
Accepted 07 August 2020
Published 26 August 2020

ABSTRACT

Fluoridated dentifrices are considered as an effective method for preventing dental caries. Nano-hydroxyapatite crystals which have remineralizing potential can be combined with herbs with antimicrobial activity and used as a dentifrice without any risks like cytotoxicity and adverse effects. Nano-hydroxyapatite crystals are formulated by wet chemical precipitation method and tested using X-ray diffractometer. An herbal dentifrice is formulated from extract of *Salvadora persica* to which Nano-hydroxyapatite crystals are added. A 3x3mm wide window has been created in 14 extracted first premolars which are then processed through a pH cycling for 7 days. The depth of the lesion is assessed using a confocal microscope. The difference in mean depth of the lesion between test dentifrice (Herbal dentifrice with Hydroxyapatite) (240.33.± 23.47) and fluoride dentifrice (272.75±29.38) was found to be statistically significant. Herbal dentifrice with Hydroxyapatite group

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mean depth of the lesion was 763.13+66.18 before and 240.33+23.48 after the remineralization cycle. In the Fluoride dentifrice group the mean depth of the lesion is 763.14±66.18 before and 272.55+29.38 after the pH cycle. Herbal dentifrice incorporated with hydroxyapatite had higher remineralizing potential compared to a fluoride dentifrice.

Keywords: Dental caries; fluorides; herbal medicine; hydroxyapatites; in vitro techniques; primary prevention; toothpastes.

1. INTRODUCTION

Oral cavity is a battlefield of continuous demineralization and remineralization [1]. Enamel is the highest mineralized tissue of the body which is mainly composed of 96% inorganic material, 4% being organic material and water. However, demineralization of enamel is a common phenomenon. The ratio of demineralization and remineralization determines the hardness of the tooth structure [1,2]. The increased demineralization leads to formation of incipient lesion which initially appears as whitish spots in the enamel and progresses to dental caries [3].

The prevention and biomimetic treatment of early caries lesions, particularly in individuals at high risk for developing caries, which is a prevalent chronic oral disease worldwide, are on-going challenges for the dental research and public health communities [4]. Also the prevention of carious lesions is cost effective compared to restoration. Proper brushing may aid in maintenance of good oral hygiene thereby reducing the incidence of carious lesions [5].

Several studies have proven that dental caries in its early stage of formation can be remineralized, and this remineralization can be facilitated by such agents as fluoride, delivered via either mouth rinse or dentifrice [6]. However, extensive use of fluoride mainly in the form of a dentifrice has contributed to a rising incidence of dental fluorosis [7]. Hence, there is a need for an alternative caries remineralizing and preventive agent as effective as fluoride. In recent times, there has been renewed interest in naturally occurring products [8,9]. Several studies have shown that miswak (*Salvadora persica)* has significant antimicrobial activity against both aerobic and anaerobic bacteria [10,11]. However, the remineralizing capacity of the herb is questionable. Hydroxyapatite is an important source of calcium and phosphate, very important for the remineralization of demineralized enamel areas [12,13].

Incorporation of hydroxyapatite crystals with *Salvadora persica* may have a synergistic effect against dental caries with both antimicrobial efficacy and remineralization capacity. According to the epidemiological triad for the dental caries causes, there is a requirement to evaluate the host factors such as oral health knowledge, oral hygiene practices, dental visits, and eating habits among the school going children.We have successfully completed numerous epidemiological studies [14–20,21] and in vitro studies [22,23,24,25] for the betterment of our community [26,27,28,29,30,31] Hence a study was planned to compare the remineralizing effect of hydroxyapatite incorporated herbal dentifrice and conventional fluoridated dentifrice on artificially created enamel lesions.

2. MATERIALS AND METHODS

An in-vitro experimental study was conducted in the Department of Public Health Dentistry. The sample size was estimated to be 7 per group based on the study done by Roza Haghgoo et al., using G power software version 3.1 with a power of 95%. Ethical committee approval was obtained prior to the start of the study. Extracted sound human maxillary premolar with enamel defects, dental fluorosis, fracture or micro cracks (under magnification of 4X), wasting diseases and caries or restorations were excluded from the study.

2.1 Pre-study Procedures

Teeth were collected and stored in a 10% formalin solution until further use. The buccal surface of each tooth was coated with acid resistant nail varnish leaving a window of 3×3 mm. This was done to limit the area of demineralization and remineralization only in the window area.

2.2 Preparation of Solution

The buffered de-/re-mineralizing solutions were prepared using analytical grade chemicals and deionized water.

2.3 Preparation of Toothpaste

Nano-hydroxyapatite crystals are formulated by wet chemical precipitation method and tested using X-ray diffractometer (Fig.1).

Herbal extractions of miswak, were mixed with 10-50% of abrasive (like silica, calcium carbonate, and sodium hydrogen carbonate), 50% Nano hydroxyapatite crystal and Salvadora powder); then they are mixed with 10-30% of a humectant (glycerol, Sodium lauryl sulphate), and ultimately 1-3% of natural flavoring (peppermint oil) (Fig. 2).

2.4 pH Cycling

The pH- cycling models are used to stimulate the dynamics of caries formation by inducing demineralization and remineralization cycles (Fig. 3).

2.5 Depth of the Lesion Assessed after pH Cycling

The treated enamel blocks to be then analyzed using confocal microscope. These values are noted, tabulated and compared with sound and demineralized zones of the same specimen. This is to be done for two groups (Fig. 4).

2.6 Statistical Analysis

Normality of the data was assessed using Shapiro Wilks and Kolmogorov Smirnov test. Data to be entered in Microsoft excel spreadsheet and analyzed using SPSS software (version 21). Independent 't' test was used to compare between the groups. Mean imputation was done to replace the lesion depth on demineralization.

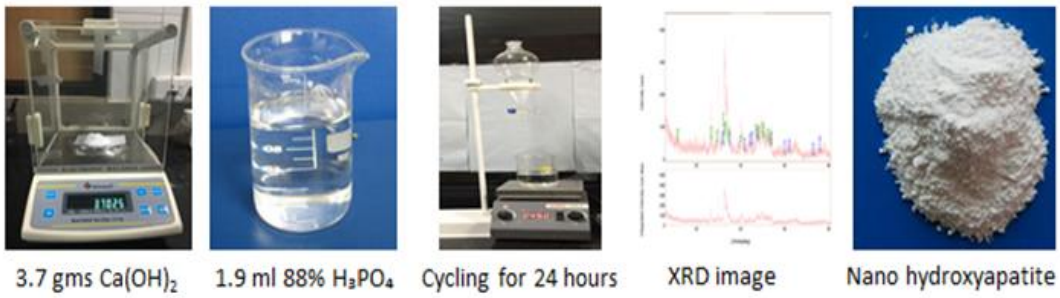

Fig. 1. Formulation of Nano hydroxyapatite crystal using wet chemical precipitation method and XRD image was used to assess the quality of the crystal obtained

Fig. 2. Depicts the Formulation of dentifrice and pictures of the Chemicals / raw materials

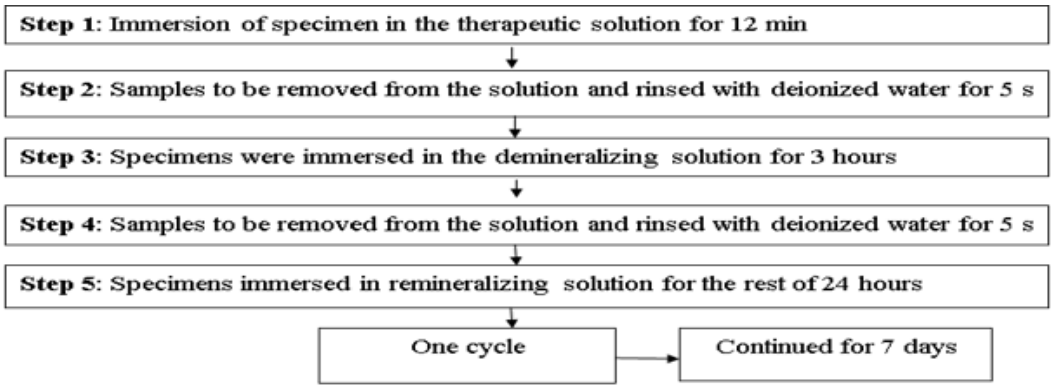

Fig. 3. The pH- cycling models

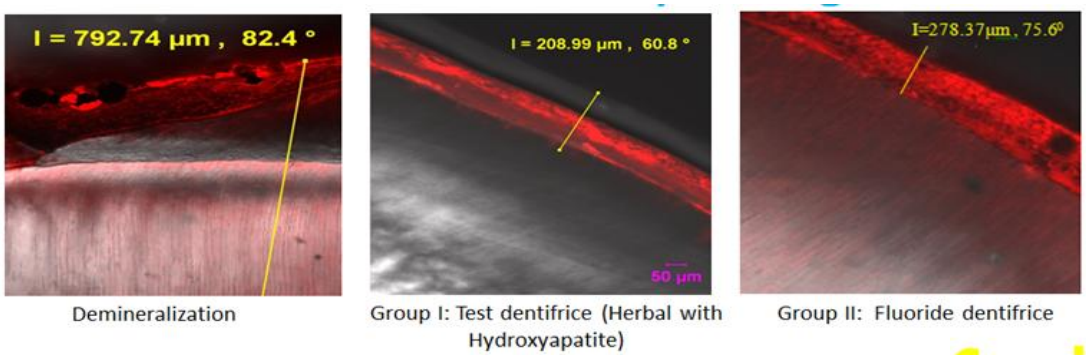

Fig. 4. Depicts the confocal microscopic image of demineralization and remineralization among different groups

3. RESULTS AND DISCUSSION

Dental caries is one of the most prevalent chronic diseases of people worldwide; individuals are susceptible to this disease throughout their lifetime [13,32]. Affected teeth cannot be saved always and may have to be extracted when extensively damaged. Brushing is an important in-house method of maintaining oral hygiene which helps in preventing dental caries. Often this mechanical plaque control method of brushing is associated with chemical plaque control of using toothpaste [33].

Fluoride is an important ingredient in dentifrices. Nearly 80 years on and fluoride remains one of dentistry's key strategies for the prevention of dental caries [33,34]. However, Fluoride-containing toothpaste can be toxic if swallowed in large amounts [35]. Hydroxyapatite is an important source of calcium and phosphate, which helps in remineralization of demineralized enamel areas [33,34]. *Salvadora persica* has strong antimicrobial effects against cariogenic microorganisms [36]. Formulating a dentifrice with nano-hydroxyapatite crystals (n-HA) and *Salvadora persica* as active ingredients may have a synergistic effect. Herbal dentifrice with Hydroxyapatite showed a statistically significant difference in lesion depth before and after demineralization and remineralization cycle. The mean depth of the lesion was 763.13±66.18

Table 1. Comparison of depth of the lesion between two different groups

Group	N	Mean ± SD		F value	p-value
Herbal dentifrice with hydroxyapatite crystals	7	240.33	23.47	0.939	**0.043***
Fluoride dentifrice	7	272.75	29.38		

Table 2. Comparison of depth of the lesion in herbal dentifrice with hydroxyapatite crystals before and after remineralization

Parameter	N	Mean ± SD		p-value
Decalcification	7	763.14	66.18	**0.001***
Re-calcification	7	240.33	23.48	

Table 3. Comparison of depth of the lesion in fluoride dentifrice before and after remineralization

Parameter	N	Mean ± SD		p-value
Decalcification	7	763.14	66.18	**0.001***
Re-calcification	7	272.55	29.38	

before and 240.33±23.48 after the remineralization cycle (Table 2). In the Fluoride dentifrice group the mean depth of the lesion is 763.14±66.18 before and 272.55±29.38 after the pH cycle. The difference was found to be significant statistically (Table 3). Thus, herbal dentifrice with Hydroxyapatite (240.33.± 23.47) had better remineralizing effect than fluoride dentifrice (272.75±29.38). Both the dentifrices exhibited remineralizing effects following the pH cycle. However, the results of this study could not be compared due to lack of similar studies.

Limitations of this study may be that the tooth may have greater susceptibility than others due to the age of donors and exposure to environmental factors like fluoride and the amount of demineralization in the cycle is much greater than in the oral environment. Hence, no in-vitro study can replace an in vivo study.

Further in vivo-studies have to be done to evaluate the combined anticariogenic and antimicrobial effectiveness of the *Salvadorapersica* and nano-hydroxyapatite crystals.

4. CONCLUSION

A herbal dentifrice with *Salvadora persica* and nano hydroxyapatite crystals as an active ingredient was formulated and remineralizing effectiveness was assessed using confocal microscopy. Both fluoride dentifrice and herbal dentifrice with hydroxyl apatite have remineralizing effectiveness. However, the newly formulated herbal dentifrice with nano hydroxyapatite crystal was found to be more effective in remineralizing the artificial caries lesion.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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