Effect of Nano-Hydroxyapatite-Crystal-Incorporated Herbal Dentifrice in Remineralization of Incipient Caries Lesions – **A Confocal Microscopic Study**

Introduction: Dental caries is an infectious microbiological disease of the teeth that results in localized dissolution and destruction of the calcified tissues. Prevention of dental caries is considered more economical than treatment. Use of fluoridated dentifrices is considered an effective method for preventing dental caries. However, studies show that there may be risk of fluorosis following use of high fluoride dentifrices. Nano-hydroxyapatite crystals which have remineralizing potential can be combined with herbs with antimicrobial activity and used as a dentifrice without any such risks.

Aim: Compare the remineralizing effect of hydroxyapatite incorporated herbal dentifrice and conventional fluoridated dentifrice on incipient caries lesion.

Wet chemical precipitation method









XRD image



3.7 g Ca(OH)₂

1.9 ml 88% H₃PO₄

40% Miswak

Cycling for 24 hours

Nano hydroxyapatite

An aqueous solution of 0.5M calcium hydroxide was prepared by mixing 3.7 grams of Ca(OH)₂ with 100 ml of distilled water and mixed with 0.3M orthophosphoric acid (1.9 ml 88% H₃PO₄ in 100 ml distilled water) drop by drop using the funnel and beaker technique until the pH reaches 12.5. The mixture was then stirred at 300 to 350 ppm using a magnetic stirrer for 24 hours. The mixture was then centrifuged at 6000 rpm for 15 min. The precipitate obtained was then rinsed with distilled water and dried at 100°C for 7 hours. The dried precipitate was then crushed using mortar and pestle until the fine power of NHA crystals were obtained.

Fabrication of Dentifrice





50% Calcium hydroxide

Glycerol SLS

Peppermint oil Toothpaste formulation

The dentifrice formulation was prepared based on the technique suggested by Maryam Ghelichli. Herbal extractions of miswak were mixed with 40% of abrasive (calcium carbonate); then they are mixed with 20% of a humectant (glycerol and Sodium lauryl sulfate) and 2% of natural flavoring (cinnamon essence). To this formulation the formulated 50% Nano hydroxyapatite crystals was added.

Preparation of tooth sample: An enamel window of size 3mm×3mm was created using modeling wax on the buccal surface of the sample, and the rest of the surface was coated with nail varnish. The samples were placed in distilled water until further use and randomly divided into two groups. **Results**

Confocal microscopic images



I = 208.99 µm , 60.8 °



Materials and Methods



pH cycling

An early artificial caries lesion was produced in the enamel according to Ten cate and Dujisters. Each specimen was immersed in 8 ml of demineralization solution for 72 hours at 37 degrees Celsius. After artificial caries preparation, the depth of the lesion was assessed using confocal microscopy. The pHcycling models are used to simulate the dynamics of caries formation by inducing demineralization and remineralization cycles



window created using wax

cycle tooth

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

| I=278.37μm, 75.6 ⁰ | | | | |
|-------------------------------|-------|---|----------------|---------|
| | Group | Ν | Mean ± SD (µm) | p-value |







| Herbal dentifrice with hydroxyapatite crystals | 7 | 240.33 | 23.47 | |
|--|---|--------|-------|-------|
| Fluoride dentifrice | 7 | 272.75 | 29.38 | 0.043 |
| | , | 2,2.,0 | 20.00 | |

Confocal microscope image after demineralization

Group I: Test dentifrice (Herbal with Hydroxyapatite)

Group II: Fluoride dentifrice Independent 't' test Conclusion

Herbal dentifrice incorporated with hydroxyapatite had higher remineralizing potential compared to a fluoride dentifrice. References

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