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Remineralization of Dentine Caries Using *Moringa Oleifera* Based Nano-Silver Fluoride: A Single-Blinded, Randomized, Active-Controlled Clinical Trial

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Abstract

Introduction: Dental caries is one of the most common chronic childhood diseases, and many decayed teeth remain untreated in underdeveloped and developing countries. This clinical trial aimed to evaluate the remineralization effect of *Moringa oleifera*-based nano-silver fluoride (NSF) on deciduous dentin caries **Materials and Method**: This study was a randomized, single-blinded clinical trial. A total of 138 teeth with carious lesions belonging to 83 children were selected and randomly assigned into three groups. Clinical evaluation was performed at 1 and 3 months after intervention. **Results**: The percentage of arrested caries within the groups were 86%, 42%, and 37% for NSF, MI varnish (GC, Japan), and FluoroDose varnish groups (Centrix Inc, USA), respectively. Results of 1 and 3 months follow-up period were same. Statistically significant difference was found between study groups ($P < 0.001$). **Conclusion**: The present study showed that *M. oleifera*-based NSF could be used for arresting dental caries when applied directly to the carious lesion.

Keywords: clinical trial, dental caries, *Moringa oleifera*, nano-silver fluoride, MI varnish (5% NaF + 2% CPP-ACP, GC, Japan), FluoroDose varnish (5% NaF, Centrix Inc, USA)

Introduction

Dental caries is one of the most common chronic childhood diseases, and many decayed teeth remain untreated in the underdeveloped and developing countries.[1]

Numerous nonrestorative methods had obtained more attention as there was high evidence that the development of caries could be slowed down or halted. Examples of these methods are: brushing teeth with fluoridated paste and the usage of varnish with fluoride, silver diamine fluoride liquid, chlorhexidine, xylitol, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[2]

Nano-silver fluoride is a new formulation of silver nanoparticle and fluoride that has antimicrobial and preventive properties. Unlike silver diamine fluoride it does not cause any dark discoloration to tooth surface and it is an excellent anticaries agent.[3]

The aim of this study is to evaluate the remineralization effect of *Moringa oleifera*-based nano-silver fluoride (NSF) on deciduous dentin caries [Text S1].[4]

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Materials and Method

The study protocol was registered in the clinicaltrials.gov (NCT04930458) and approved by ethical committee of University of Baghdad (approval number: 306321).

The study design was a randomized, single-blind trial (the participants were blind to the type of treatment). Parents/guardians were fully informed regarding the study design, objectives, and probable advantages, side effects, and the need for the follow-up. Written informed consent was obtained.

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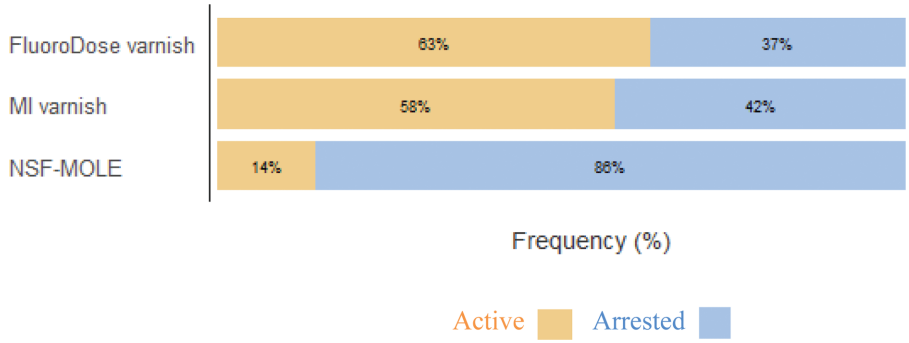

Figure 1: Percentage of arrested and active caries among intervention group (NSF-MOLE) and active control groups (MI varnish and FluoroDose varnish).

Inclusion criteria

Children between ages 5 and 10 years who had at least one carious lesion with dentin exposed based on the ICDAS II (International Caries Detection and Assessment System –Code 5: dentin cavity easily visible with the naked eye where the surface of cavity feels soft or leathery on gentle probing).[5]

Exclusion criteria

Sensitivity to remineralizing agents, pulp exposure, or abscess/fistula.

Green synthesis of NSF using *Moringa oleifera* leaf extracts

Fresh *M. oleifera* plant material was collected and separated from the stems, washed with distilled water, and air-dried to remove residual debris.[6] Extract was prepared by using 5 g of fresh leaf which was transferred to 250 mL beaker and 100 mL of deionized water was added to the leaf. The mixture was boiled for 15 minutes. The extract was cooled at room temperature and then filtered using filter paper number 001 (Thomas Baker Pvt Ltd, India) to obtain clear extract, which was used for the synthesis of silver nanoparticles.[6]

From the plant extract, 10 mL was added to 100 mL of 1 mM $AgNO_3$ (Thomas Baker Pvt Ltd, India). The mixture was stirred and heated using hotplate magnetic stirrer (Labinco, the Netherlands) for 20 minutes at 70°C. The color of aqueous solution was changed from colorless to yellowish-brown color, which was an indication to the silver nanoparticles formation.[7] At the end of the reaction sodium fluoride (NaF; Riedel-de Haën, Germany; 10.104 ppm) was added to improve the stability and the cariostatic efficacy of the solution and the stirring was continued overnight. The final NSF solution was stored in a dark container until further use.[8]

Sample size calculation

The sample size calculation was based on previous studies.[1,9] Using G*power software version 3.1.9.7 (www.gpower.hhu.de/) the sample size was estimated to be a minimum of 35 lesions for each group (by which each tooth with carious lesion was considered as a single unit). Extra sample was taken for all the groups to compensate the possible drop out for any reason. Total 186 children between 5 and 10 years old ages were examined for the present study but only 83 of them were enrolled as they met the inclusion criteria. They were divided randomly, using a randomization website [https://www.graphpad.com/quickcalcs/randomize2/] into three groups as explained by CONSORT flow diagram [Figure S1].[10]

(1) Intervention group: NSF using *M. oleifera* leaf extracts (NSF-MOLE; 0.25 μg/mL F + 60 μg/mL Ag).
(2) Active control group A: MI varnish (5% NaF + 2% CPP-ACP; GC, Japan).
(3) Active control group B: FluoroDose varnish (5% NaF; Centrix Inc, USA).

Intervention

The affected tooth surface was gently cleaned and then dried with cotton and gauze. For NSF-MOLE group, the gingival tissue of the targeted tooth was protected with petroleum jelly (Cerkamed, Stalowa Wola, Poland) and isolation was carried out using cotton rolls. Fine microbrush was dipped into the agent (each tooth received one drops of NSF, equivalent to 10 mg). The NSF-MOLE was left in contact with the tooth surface for 2 minutes, and the child was instructed not to eat or drink anything for at least 45 minutes.[1]

For MI varnish group, the varnish was applied in a thin layer using a disposable brush. The parents were instructed that the child should avoid eating hard foods and prevent from brushing teeth for 4 hours.

For FluoroDose varnish group, thin film of the varnish was applied on to the treated tooth using disposable brush. The varnish was let dry for approximately 10 seconds. The parents were instructed that the child should remain on a soft food diet for 2 hours, and not to brush for a minimum of 4 to 6 hours.

After 1 and 3 months, the treated teeth were examined with WHO probe according to the criteria of active/arrest of ICDAS II.[11]

Statistical analysis

The statistical analysis was performed using SPSS 22 (SPSS Inc, Chicago, IL, USA). Comparison between the groups was made using chi-square test.

Results

Eighty-three children (with 138 carious lesion) were enrolled into the study (with only one surface carious lesion by tooth; mean age: 7.091 ± 1.61 years, male: 57.83%) [Figure S1].[10] Results of 1 and 3 months follow-up period were same. Statistically significant difference was found between the study groups ($P < 0.001$) [Figure 1]. Subgroup analyses showed statistically nonsignificant deference between maxillary and mandibular tooth ($P < 0.05$) and first and second deciduous tooth ($P < 0.05$).

Discussion

Untreated dental caries compromises the general health, social well-being, and educational opportunities of children from low-income countries due to the lack of financial resources and insufficient access to basic oral care. To handle untreated dental caries the Arresting Caries Treatment program had been applied.[12]

In this study, a biological approach using *M. oleifera* leaf extract was used in production of silver nanoparticles instead of chemical synthesis used in other studies.[1,9,13] This green synthesis method is considered as eco-friendly, simple, one step (biogenic) approach as studies have shown that *M. oleifera* leaf extract can act as reducing and capping agent for production of silver nanoparticle instead of the hazardous chemicals used in the chemical synthesis method.[6,14,15]

In this study, the inclusion criteria and the outcome measurements were based on the ICDAS II, which had been proven to be reliable, valid, and reproducible in assessing primary caries activity according to different studies.[16]

The present clinical trial was the first trial to evaluate the effectiveness of NSF-MOLE to arrest dentine caries in primary molars and comparing it with other agents for a follow-up period of 1 and 3 months. This study showed that NSF-MOLE was effective in arresting the carious lesion (85.71%) and this result was comparable to the results of other clinical trials.[1,3,10,15] This result was in agreement with that of a meta-analysis by Gao *et al*.,[17] in which the overall proportion of arrested dentine caries was 65.9% (95% CI: 41.2%–90.7%) following the usage of silver diamine fluoride. Also, a systematic review by Contreras *et al*.[18] showed that "silver diamine fluoride, at concentrations of 30% and 38%, is more effective than other preventive management strategies for arresting dentinal caries in the primary dentition."

NSF-MOLE group showed a higher clinical and statistical efficacy in arrestment of carious lesion than MI varnish group after 1 and 3 months of follow-up period. The percent of caries arrestment in the MI varnish group and in sodium fluoride varnish group was 43.59% and 37.14%, respectively.

The result of the current study showed that there was no significant difference between MI varnish group and sodium fluoride varnish group and this result means that the incorporation of CPP-ACP in the fluoride varnish had no effect on its cariostatic features. This finding was consistent with that of Shahmoradi *et al*.'s[19] findings, they concluding that "adding calcium and phosphate compositions does not appear to enhance or inhibit the performance of fluoride varnishes."

While, it was not in consistent with the results of an in vitro study conducted by Varma *et al*.,[20] who evaluated the remineralization potential of Clinpro XT varnish (USA) containing tricalcium phosphate and MI varnish and concluding that MI varnish containing CPP-ACP had the highest release of fluoride as compared to the Clinpro fluoride-releasing varnish. The in vitro study of Salman *et al*. showed that MI varnish had a higher effect in reducing caries lesion depth than NaF varnish with more mineral depositions. It was also observed that specimens treated with MI varnish had a significantly greater percent of calcium/phosphate ratio.[21]

Fluoride may cause neurotoxicity in laboratory animals, including effects on learning and memory.[22] Meta-analysis of 27 studies published over 22 years performed by Choi *et al*.[23] suggested an inverse association between high fluoride exposure and children's intelligence and the result supports the possibility of adverse effects of fluoride exposures on children's neurodevelopment.

Limitations of the study

The limitations of this study included a relatively short follow-up period of 3 months. Additionally, the investigator could not be blinded during the application process because of the different materials used. After completing the trial, children with the remaining active carious lesion in all groups were referred for treatment.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. dos Santos Jr VE, Vasconcelos Filho A, Targino AG, *et al.* A new "silver-bullet" to treat caries in children – nano silver fluoride: a randomised clinical trial. J Dent 2014;42:945–51.
2. Lo E, Duangthip D. Non-restorative approaches for managing cavitated dentin carious lesions. In: Pediatric Restorative Dentistry. Cham: Springer; 2019. pp. 141–60.
3. Nagireddy VR, Reddy D, Kondamadugu S, Puppala N, Mareddy A, Chris A. Nanosilver fluoride – a paradigm shift for arrest in dental caries in primary teeth of schoolchildren: a randomized controlled clinical trial. Int J Clin Pediatr Dent 2019;12:484–90.
4. Jawad D. Supplementary text. figshare. Figure. 2022. https://doi.org/10.6084/m9.figshare.19753975.v1.
5. Gugnani N, Pandit IK, Srivastava N, Gupta M, Sharma M. International Caries Detection And Assessment System (ICDAS): a new concept. Int J Clin Pediatr Dent 2011;4:93.

6. Moodley JS, Krishna SB, Pillay K, Govender P. Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential. Adv Nat Sci: Nanosci Nanotechnol 2018;9:015011.
7. Bindhu MR, Umadevi M, Esmail GA, Al-Dhabi NA, Arasu MV. Green synthesis and characterization of silver nanoparticles from Moringa oleifera flower and assessment of antimicrobial and sensing properties. J Photochem Photobiol B: Biol 2020;205:111836.
8. Akyildiz M, Sönmez IS. Comparison of remineralising potential of nano silver fluoride, silver diamine fluoride and sodium fluoride varnish on artificial caries: an in vitro study. Oral Health Prev Dent 2019;17:469–77.
9. Tirupathi S, Nirmala SV, Rajasekhar S, Nuvvula S. Comparative cariostatic efficacy of a novel Nano-silver fluoride varnish with 38% silver diamine fluoride varnish a double-blind randomized clinical trial. J Clin Exp Dent 2019;11:e105–12.
10. Jawad D. Supplementary figure 1.jpg. figshare. Figure. 2022. https://doi.org/10.6084/m9.figshare.19753921.v1.
11. Dikmen B. ICDAS II criteria (International Caries Detection and Assessment System). J Istanb Univ Fac Dent 2015;49:63–72.
12. Yee R, Holmgren C, Mulder J, Lama D, Walker D, van Palenstein Helderman W. Efficacy of silver diamine fluoride for arresting caries treatment. J Dent Res 2009;88;644–7.
13. Butrón-Téllez Girón C, Mariel-Cárdenas J, Pierdant-Pérez M, Hernández-Sierra JF, Morales-Sánchez JE, Ruiz F. Effectiveness of a combined silver nanoparticles/fluoride varnish in dental remineralization in children: in vivo study. Superf y Vacío 2017;30:21–4.
14. Das S, Parida UK, Bindhani BK. Green biosynthesis of silver nanoparticles using Moringa oleifera L. leaf. Int J Nanotechnol Appl 2013;3:51–62.
15. Al-Nerabieah Z, Arrag EA, Comisi JC, Rajab A. Effectiveness of a novel nano-silver fluoride with green tea extract compared with silver diamine fluoride: a randomized, controlled, non-inferiority trial. Int J Dent Oral Sci 2020;7:753–61.
16. Ekstrand KR, Luna LE, Promisiero L, *et al.* The reliability and accuracy of two methods for proximal caries detection and depth on directly visible proximal surfaces: an in vitro study. Caries Res 2011;45:93–9.
17. Gao SS, Zhang S, Mei ML, Lo EC, Chu CH. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health 2016;16:1–9.
18. Contreras V, Toro MJ, Elías-Boneta AR, Encarnación-Burgos MA. Effectiveness of silver diamine fluoride in caries prevention and arrest: a systematic literature review. Gen Dent 2017;65:22.
19. Shahmoradi M, Hunter N, Swain M. Efficacy of fluoride varnishes with added calcium phosphate in the protection of the structural and mechanical properties of enamel. BioMed Res Int 2017; 2017:pp 1–7.
20. Varma V, Hegde KS, Bhat SS, Sargod SS, Rao HA. Comparative evaluation of remineralization potential of two varnishes containing CPP–ACP and tricalcium phosphate: an in vitro study. Int J Clin Pediatr Dent 2019;12:233–6.
21. Salman NR, ElTekeya M, Bakry N, Omar SS, El Tantawi M. Comparison of remineralization by fluoride varnishes with and without casein phosphopeptide amorphous calcium phosphate in primary teeth. Acta Odontol Scand 2019;77:9–14.
22. Chioca LR, Raupp IM, Da Cunha C, Losso EM, Andreatini R. Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. Eur J Pharmacol 2008;579:196–201.
23. Choi AL, Sun G, Zhang Y, Grandjean P. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. Environ Health Perspect 2012;120:1362–8.