Comparison of Local and Commercially Available 38% SDF Products on the Inflammatory Pulp Response of Rat Molars

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Abstract

Silver diamine fluoride (SDF) is highly efficient at arresting carious lesions in both primary and permanent teeth. The penetration of SDF can trigger an inflammatory reaction in the dental pulp. The domestic development of SDF has the potential to lower expenses and expand options for dental professionals as well as medical facilities. Hence, this study aimed to compare the inflammatory reaction of rat dental pulp after applying a locally produced SDF and a commercially available product.

Thirty-two first maxillary molars of 8-week-old Wistar rats were randomly assigned to one of four groups (n = 8): group I, 38% SDF (Thai product), group II Saforide®, group III Topamine, and group IV deionized water (DI). The cavity preparation was performed and applied by SDFs in each group. Maxillary molars were obtained for histopathologic examination, and the degree of dental pulp inflammation and soft tissue organization were assessed. A statistically significant difference was considered at $p < 0.05$.

There were few or no inflammatory cells in the pulp below the pulpal wall of the cavities, and normal soft tissue organization was observed in all groups, with no significant difference among groups ($p > 0.05$). In conclusion, the application of 38% SDF (Thai product) shows no significant difference in the inflammatory reaction of dental pulp compared to the control and commercial products.

Keywords: *Dental Pulp, Inflammatory Response, Rat, Molar, Silver Diamine Fluoride*

1. Introduction

Dental caries is a disease that affects the quality of life in many dimensions. Untreated tooth decay may cause pain and discomfort, which may lead to infection and subsequently affect daily life (Oliveira, Rajendra, Veitz-Keenan, & Niederman, 2019). Traditionally, dental caries treatment is usually performed by completely removing the carious tissue and filling it with a restorative material, which creates a high risk of pulpal exposure, especially in the deep carious lesions (Innes et al., 2016). Currently, non- or minimally-invasive treatments have been proposed as an alternative therapy in the management of dental caries (Torres, Phan, Bojorquez, Garcia-Godoy, & Pinzon, 2021).

Silver diamine fluoride is a compound of ammonia and silver fluoride, which was initially expanded by Drs. Nishino and Yamaga in 1970 and is widely used as a non-invasive treatment, especially in pediatric patients with limited cooperation in dental treatment (Crystal, & Niederman, 2019). The mechanism of action

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for silver diamine fluoride is its bactericidal properties by silver ions. Silver ions can inhibit the activation of bacterial glycosyltransferase, resulting in the inhibition of biofilm formation. In addition, silver ions can inhibit the proteolysis mechanism of the enzyme collagenase, which prevents the degradation of collagen in dentin. Moreover, high concentrations of fluoride in silver diamine fluoride play a crucial role in the remineralization of tooth surfaces (Gao et al., 2021; Zhao et al., 2018). From a systematic review, it was found that silver diamine fluoride effectively inhibits and prevents dental caries in primary teeth compared to other caries control agents and no treatment group (Oliveira et al., 2019). Silver diamine fluoride at concentrations of 30% and 38% showed potential in the inhibition of dentin caries more than other concentrations (Contreras, Toro, Elías-Boneta, & Encarnación-Burgos, 2017) However, the application of silver diamine fluoride into a medium to the deep carious lesion may induce a response of dentin-pulp complex of the teeth. A systematic review by Zaeneldin, Yu, and Chu (2022) reported the direct application of silver diamine fluoride to the pulp tissue led to pulp necrosis. In contrast, the application to dentin caries did not induce an inflammatory response, and only mild inflammation was observed. This conflicting statement may arise from the different observation periods and dentitions included in this systematic review.

Currently, silver diamine fluoride is used worldwide. Topamine (DentaLife, Australia) is the first and only commercial product of 38% SDF approved by the FDA Thailand (Gao et al., 2021). If a locally produced SDF can achieve comparable or superior efficiency to existing substances on the market, it might have the potential to lower expenses and expand the variety of products available to dental professionals and hospitals. This preliminary investigation demonstrated that a locally produced SDF with a concentration of 38% had similar properties in terms of fluoride release and silver component content when compared to commercially accessible alternatives. Hence, this study aims to compare the inflammatory reaction of dental pulp to a locally produced SDF with the commercially available alternative.

2. Objectives

This study aimed to compare the inflammatory reactions of dental pulp in rat molars between a locally produced SDF and commercial products.

3. Materials and Methods

3.1 Operative procedures

The sample size was determined based on a prior study by Arifin and Zahiruddin (2017). There were 8 teeth in each group, meaning 32 teeth were needed. Hence, sixteen male Wistar rats, 8 weeks old and weighing 150–200 grams, were employed for the investigation. The procedure received approval from the Institutional Animal Care and Use Committee (IACUC) of the Chulalongkorn University Laboratory Animal Center (CULAC), Chulalongkorn University, Thailand (animal use protocol number: 2373014, approval number 2373014, date of approval: 20/06/2023). After one week of acclimatization, rats were given parental anesthesia with Zoletil (40 mg/kg) and Xylazine (2 mg/kg) intraperitoneal injections, then placed in an apparatus with an elastic orthodontic chain at the upper and lower anterior teeth to hold the jaw open. After local anesthesia with 2% mepivacaine containing epinephrine 1:100,000 (4.4 mg/kg/dose) in the first maxillary molar area, cavities were prepared by one investigator using the 3.0x magnification loupes at a working distance of 500 mm on the mesial surfaces of first maxillary molars with a slow-speed hand piece and a tungsten carbide bur (33 1/2 inverted cone bur, 0.5 mm in diameter), cooling with sterile normal saline, and drying with the paper point. The process of cavity preparation was ended when approximately one-third of the bur had been inserted into the cavity without any exposure to the pulp. The operator was blinded to the substances that were applied to the cavities.

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Thirty-two first maxillary molars were randomly assigned to one of four groups (n = 8): group I, 38% SDF (Thai product), group II, Saforide® (Toyo Seiyaku Kasei Co. Ltd., Japan), group III, Topamine (Dentalife Australia Pty. Ltd., Thailand), and group IV, DI. After isolation of the area to prevent saliva using a cotton pallet, a test substance was applied to the cavity for 3 minutes (0.5 microliters), after which the excess was removed with the paper point. After the procedure, Tramadol was injected subcutaneously as an analgesic twice daily for 2 days at a dose of 12.5 mg/kg. The rats were monitored for weight and body condition scores every 3 days after the procedure. Seven days after the procedure, the rats were euthanized with 30–70% CO_2.

3.2 Histopathologic examination

The entire maxillae were dissected and immersed in a solution of 10% buffered formalin overnight. Decalcification was performed in a solution of 10% EDTA (pH 7.4) for a period of 15 days. Decalcified maxillae were dehydrated using a series of ethanol and xylene solutions of increasing concentration and then embedded in paraffin. Sections with a thickness of 5 microns were histologically produced and stained using hematoxylin and eosin. The histological sections were examined for the extent of the inflammatory response using bright-field optical microscopy with a 40x objective lens (equivalent to 1 high-power field). The analysis was conducted by a single investigator who cross-validated the data with an oral pathologist. Histopathologic grading criteria in terms of the degree of inflammation and soft tissue organization were determined according to the Bunlunara study (Banlunara et al., 2016), as seen in Table 1.

Table 1. Histopathologic grading criteria (Banlunara et al., 2016)

Score	**Degree of inflammation: Cells were counted under a 40x objective lens (1 high-power field [HPF])**
1	Few or no inflammatory cells present in the pulp beneath the pulpal wall (1-3 cells)
2	Mild inflammation beneath the pulpal wall (4-10 cells)
3	Moderate inflammation (11-50 cells) beneath the pulpal wall and involved in coronal pulp
4	Severe inflammation or abscess formation beneath the pulpal wall
Score	**Soft tissue organization**
1	Normal or almost normal soft tissue organization beneath the pulpal wall and continued odontoblastic layer found and well-organized
2	Partial loss of soft tissue organization, discontinued or absence of odontoblastic layer beneath the pulpal wall but central part of pulp is normal. Few cells and some collagen fibers appear in the pulp tissue that is distant from the axial wall
3	Total loss of general pulp morphology and cellular organization. Some free spaces found

3.3 Statistical analysis

Statistical analysis was performed using the SPSS program for Windows, version 29.0 (SPSS, Chicago, IL). The histopathologic grading score was analyzed by the Kruskal-Wallis test and the Mann-Whitney U test. The results were analyzed and considered to possess statistically significant differences at $p < 0.05$.

4. Results and Discussion

4.1 Result

One animal died during the experiment, and one sample from the control group was exposed during the operative procedure. Therefore, there were 6 teeth in the control group and 7 teeth in the Topamine group post-treatment for histopathologic inflammation and soft tissue organization evaluation.

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After seven days, there were few or no inflammatory cells found in the pulp below the pulpal wall of cavities in all groups (Figure 1). The mean score for the degree of inflammation in all groups was 1.0 (see Table 2). There was no significant difference among groups (p = 1.0).

Normal or almost normal soft tissue organization beneath the pulpal wall and continued odontoblastic layer were found with good organization in the groups treated with 38% SDF (Figure 1A, B) and Saforide® (Figure 1C, D) with a mean score of 1.0. Meanwhile, 5 teeth (71.43%) treated with Topamine (Figure 1E, F) and 5 teeth (83.33%) treated with DI (Figure 1G, H) found normal or almost normal soft tissue organization beneath the pulpal wall but 2 teeth (28.57%) treated with Topamine and 1 tooth (16.66%) treated with DI found partial loss of soft tissue organization, discontinued odontoblastic layer beneath the pulpal wall but central part of pulp was normal. The mean scores for soft tissue organization were 1.29 and 1.17, respectively (see Table 2). However, there was no significant difference in the histopathologic soft tissue organization among groups (p = 0.203).

Table 2. Histopathologic scores for all groups in the experiment (n = 29) at 7 days post-operation

Group	Inflammation score				Mean score	Soft tissue organization			Mean score
	1	2	3	4		1	2	3	
38%SDF (n = 8)	8	-	-	-	1.0	8	-	-	1.0
Saforide® (n = 8)	8	-	-	-	1.0	8	-	-	1.0
Topamine (n = 7)	7	-	-	-	1.0	5	2	-	1.29
DI (n = 6)	6	-	-	-	1.0	5	1	-	1.17

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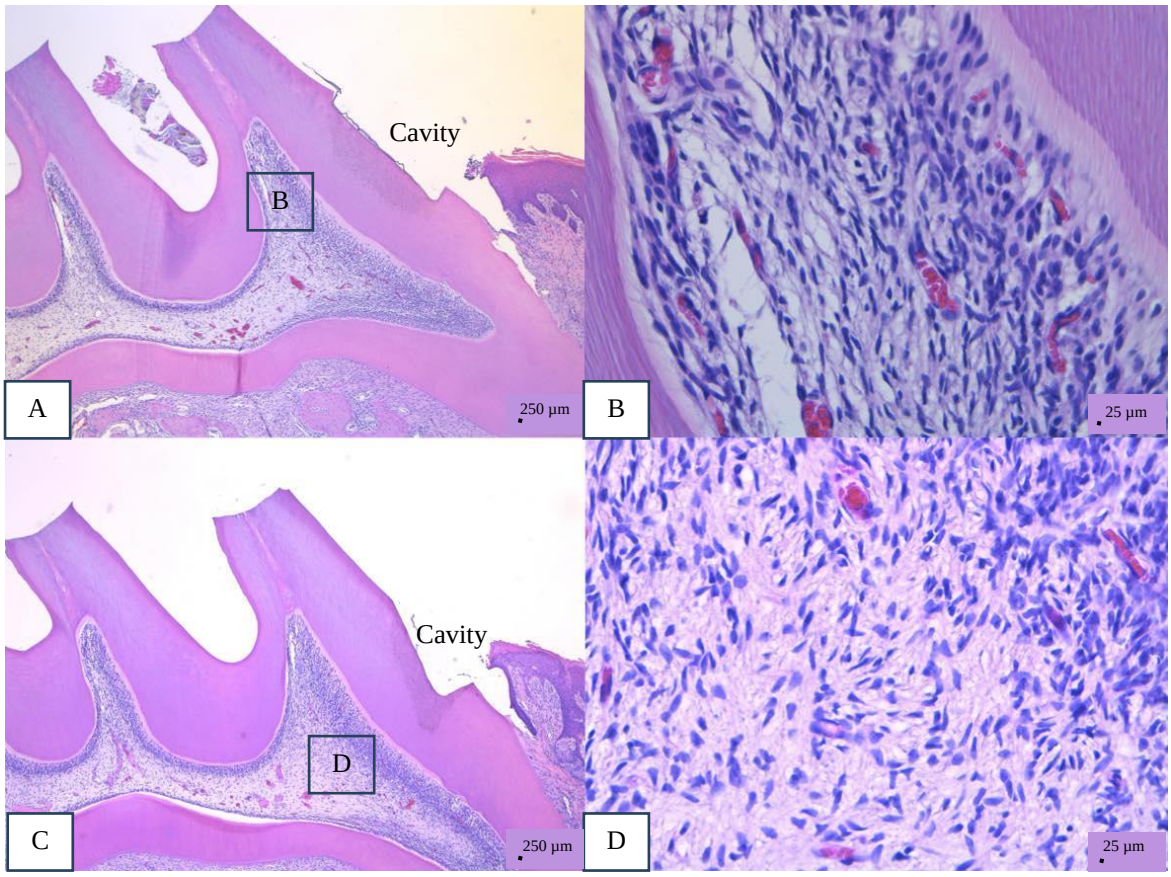

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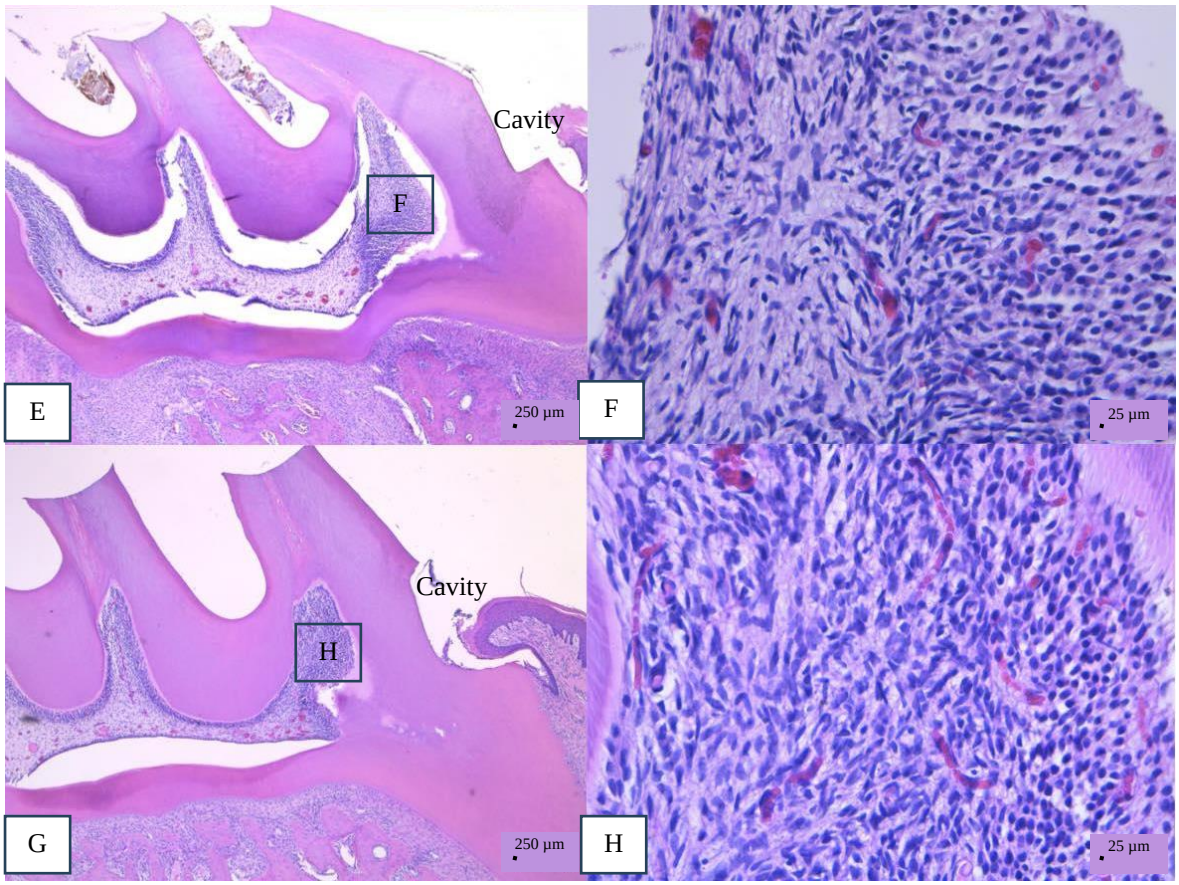

Figure 1. Depiction of the histopathology for the inflammatory response of rat dental pulp under bright-field optical microscopy with 4X and 40X objective lenses, respectively, to 38% SDF (A, B), Saforide® (C, D) Topamine (E, F) and DI (G, H)

4.2 Discussion

Based on a systematic review by Ahmed (Zaeneldin et al., 2022), it was concluded that the indirect application of SDF is biocompatible with dental pulp and has a mild inflammatory response. The current study indicated that the application of a locally produced 38% SDF compared with the commercially available products (Saforide® and Topamine) showed no difference among groups in terms of inflammatory response and soft tissue organization. There was also no difference between the control group (DI) and SDF treated group. This indicated that all SDF products are biocompatible with dental pulp. This corresponds to a previous study by Rossi, Squassi, Mandalunis, and Kaplan (2017), who studied the effect of silver diamine fluoride on

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dentin pulp complex when compared with no treatment group, which showed well-organized dental pulp with good vascularization and mild inflammatory infiltrate.

All teeth in the 38%SDF and Saforide groups exhibited normal or nearly normal soft tissue organization beneath the pulpal wall, with a continuous odontoblastic layer. However, 28.57% of the Topamine group and 16.66% of the DI group showed partial loss of soft tissue organization, with the discontinued odontoblastic layer beneath the pulpal wall, while the central part of the pulp remained normal with fewer than 250 µm of remaining dentin thickness, corresponding to a previous study by Izumi, Eida, Matsumoto, and Inoue (2007) who examined the effect of cavity preparation on the dental pulp of the upper first maxillary molar of 9-week-old Sprague-Dawley rats with remaining dentin thickness between 200-250 µm. Five days after cavity preparation without the application of any substance, partial loss of soft tissue organization was found, suggesting that the partial loss of soft tissue organization in the current investigation may have resulted from cavity preparation.

The findings of this research demonstrated that the application of a locally produced SDF did not induce an inflammatory response of the dental pulp in rat molars differently from commercially available products. This suggests that a locally produced 38% SDF solution may be a viable alternative product to use with safety and effectiveness in preventing dental caries. This research was limited only to rats. Thus, the results may not be representative of clinical use. However, further study in clinical trials should be performed.

5. Conclusion

The inflammatory reaction of dental pulp in rat molars to a locally produced SDF was comparable with commercial products. Minimal or no presence of inflammatory cells in the pulp with normal or almost normal soft tissue organization beneath the pulpal wall was found in both groups with no significant difference between them.

6. Acknowledgements

The authors express their gratitude to Associate Professor Ekarat Phattarataratip and Mr. Somchai Yordsanga for their invaluable recommendations. Thanks also to the Eighteen Company for providing the dental loupe equipment for this research. This work was supported financially by a grant from the Faculty of Dentistry, Chulalongkorn University, Thailand (DRF67020).

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