

Cytotoxic Effect of Three Different Silver Diamine Fluoride: An *In-Vitro* Study

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Abstract

Aim: Dental caries that are left untreated are the major problem in pediatric public health. For decades, Silver Diamine Fluoride (SDF) has been used to arrest caries in primary and permanent teeth. Thus, the aim of the study was to assess the cytotoxic effect of three different SDF's. **Materials & Methods:** To assess the cytotoxic potential, brine shrimp was cultured and were tested by adding 5 µL, 10 µL, 20 µL and 40 µL concentrations of Kedo, Kidz-e and Fagamin to it. The number of live nauplii's present was noted after 24 hours. **Results:** As the concentration increased, the cytotoxicity of the materials increased. The Kedo group showed 7 viable nauplii after 24 hours whereas, none were observed in the other two groups. **Conclusion:** Kedo SDF showed less cytotoxic effect compared to Kidz-e and Fagamin and can be considered the best choice for arresting caries in primary and permanent teeth.

Keywords

Cytotoxicity; Nauplii; Silver diamine fluoride

Introduction

Early Childhood Caries (ECC) is the most common chronic childhood disease and its occurrence is a worldwide health problem. [1]

ECC causes pain, infection, and advanced caries will lead to the involvement of pulp and later progresses to form dental abscess. [2]

When left untreated, ECC will lead to tooth loss which can affect the dentition. More crucially, the overall health of the child will be affected in case of poor dentition. [3]

Conventional management requires surgical intervention to remove damaged tooth structure and later followed by placement of a restorative material to restore form and function.

Alternative caries management modalities such as Silver Diamine Fluoride (SDF) have been used in Japan for the past 40 years to arrest caries and minimise tooth sensitivity in primary and permanent teeth.

SDF was accepted by the US Food and Drug Administration for management of tooth hypersensitivity [4] and is said to have staining potential of skin and clothes and the need for reapplication is highly recommended. [5]

SDF 38% is a colourless liquid which has a pH of 10. It contains 24.4% to 28.8% silver and 5% to 5.9% fluoride. [6] Their exact mechanism is unknown. Its theory states that fluoride ions act mainly on the tooth structure, while silver ions like other heavy metals are antimicrobial.

SDF also combines with hydroxyapatite in an alkaline environment to form Calcium Fluoride (CaF₂) and silver phosphate.

CaF₂ provides enough fluoride to form fluorapatite which is less soluble than hydroxyapatite in an acidic environment. [7] The benefits of using SDF are to minimise caries and infection with affordable cost, their simplicity of treatment, minimal support required, non-invasive procedure and for patients who have behavioural or medical management issues. [8]

The three different SDF materials used in the present study are Fagamin, Kidz-e and Kedo-SDF. All the three materials act as a powerful cariostatic agent, caries inhibitor and remineralizer, bactericide with anti-enzymatic action, protein-coagulant and desensitizer.

It collaborates both the effects of silver and fluoride on the teeth. It can be applied in devitalized teeth, in cavity preparations for prostheses, crowns and fixed bridge cases.

Our department is passionate about child care, we have published numerous high quality articles in this domain over the past 3 years. [9-27] With this inspiration we planned to pursue research on three different SDF's. Previously our team

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has a rich experience in working on various research projects across multiple disciplines. [28-42] Now the growing trend in this area motivated us to pursue this project.

Many studies have evaluated the efficacy of SDF on caries arrest and/ or prevention [43-48] but none has compared the cytotoxic effects of three different silver diamine fluorides. Hence, the aim of the study was to assess their cytotoxicity using brine shrimp assay.

Materials and Methods

This experimental study was performed at Blue Lab, Saveetha Institute of Medical and Technical Sciences, Chennai during December 2020 to January 2021.

Brine shrimp lethality assay

Salt water preparation: Two grams of iodine free salt was weighed and dissolved in 200 ml of distilled water. 12 well ELISA plates were taken and 10-12 ml of saline was filled [Figure 1] To that 10 nauplii were slowly added to each well (5 µL, 10 µL, 20 µL, 40 µL) and three different materials were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, The ELISA plates were observed and noted for the number of live nauplii present and calculated by using the following formula.

Number of dead nauplii/Number of dead nauplii-Number of live nauplii X 100.



Figure 1: Activity of nauplii in 12 well ELISA plates using three different SDF's.

Results

Table 1 depicts the cytotoxicity of three different SDF's using brine shrimp. At 40 µL concentration, only the Kedo group showed 7 viable nauplii and no viable nauplii was observed in the other two groups. It was found that as the concentration increased, the cytotoxicity of the three materials increased [Figures 2-4, Table 1].

Table 1: Cytotoxicity of three different SDF's.

Concentration	Viable Nauplii			
	Control	Kedo	Kidz-e	Fagamin
5 µL	10	9	7	9
10 µL	10	9	7	3
20 µL	10	8	3	1
40 µL	10	7	0	0



Figure 2: Cytotoxicity of Kedo-SDF after 24 hours.

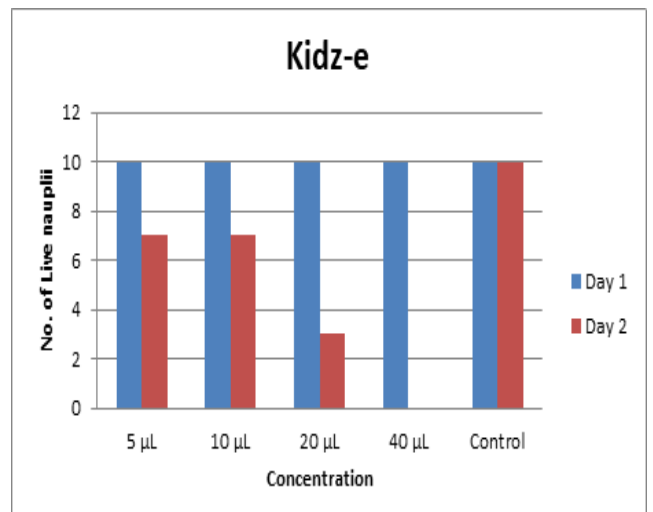


Figure 3: Cytotoxicity of Kidz-e SDF after 24 hours.

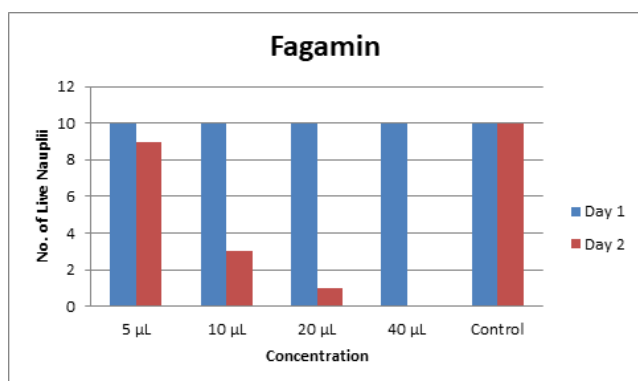


Figure 4: Cytotoxicity of Fagamin after 24 hours.

Discussion

SDF has been used as a gold standard in several researches [49,50,36] in various concentrations in primary teeth with a huge risk of active caries, [4] powerful in preventing and controlling them. [46] SDF manifests to be a cariostatic and preventive agent and its main effect on dental structures is the furtherance of calcium fluoride and silver phosphate, without any loss of calcium and phosphate ions. [51] However, side effects has been noted such as causing ulcerations and staining in oral mucosa [52] that are painful caused due to accidental contact with the solution, [53] which may disappear within 48 hours [54] and the black staining of carious tissue [55] caused due to the oxidation process of the ionic silver restrained in its formulation. Previously, chemical methods were used in the synthesis of SDF which was time efficient. But then, toxic chemicals were needed to maintain stability while synthesizing SDF chemically, which was causing a major toxicity in the environment. Keeping this into account, green technology using plant extract is rising as an eco-friendly alternative, as plant extract mediated biosynthesis is cost-effective. Possible toxicity of these products has limited the use of SDF in dental fields. We therefore undertook this study to evaluate the cytotoxicity of three different SDF's.

There was no study conducted previously comparing the cytotoxicity of SDF's. Thus, this current study confessed that at 40 µL concentration, only the Kedo group showed 7 viable nauplii and no viable nauplii was observed in the other two groups. It was found that as the concentration increased, the cytotoxicity of the three materials increased. The major death rate of nauplii was observed at 40 µL concentration in both Kidz-e and Fagamin groups. According to Sreenivasagan et al. the toxicity results showed that in lower concentrations all the nauplii survived, mild toxicity was seen in higher concentrations of silver nanoparticles. [56] Research done by Fancher et al., SDF and Fluoride Varnish (FV) can cause cell death. FV lost its cytotoxicity within 3 weeks, while SDF remained cytotoxic even after 9 weeks of rinsing. [57]

Fagamin and Kidz-e SDF both are in the state of liquid which is chemically synthesized, whereas Kedo-SDF is in gel form where SDF is added to a plant extract. This might be the reason for the low cytotoxicity observed in this *in-vitro* study. Our institution is passionate about high quality evidence based

research and has excelled in various fields. [21,58-63] We hope this study adds to this rich legacy. A limitation of this study is that its results are only *in vitro* and toxicity needs to be checked in higher living organisms. In future research, *in vivo* studies are recommended with people's acceptance values as well.

Conclusion

Kedo SDF exhibited less cytotoxic effect compared to Kidz-e and Fagamin and can be considered the best choice for arresting caries in primary and permanent teeth.

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