Antimicrobial activity of chitosan in combination with silver diamine fluoride against *Streptococcus mutans* - An in-vitro study

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**Article History:**
Received on: 17 Jul 2020
Revised on: 19 Aug 2020
Accepted on: 20 Aug 2020

**Keywords:**
Silver diamine fluoride, Chitosan, Antimicrobial, Streptococcus Mutans

**ABSTRACT**

Silver diamine fluoride (SDF) is a recently introduced caries arresting agent. Some of its side effects include staining of teeth and cytotoxicity. Chitosan has an anti-cariogenic and plaque-reducing effect. Thus, considering the side-effects of SDF and the antibacterial efficacy of chitosan, the present study was done to evaluate the antibacterial effectiveness of SDF and chitosan in combination against *Streptococcus mutans*. 60 sterile Petri dishes were sub-divided into five groups of 12 Petri dishes each: Group 1: 38% SDF, Group 2: 2% chitosan, Group 3: 3% chitosan, Group 4: 38% SDF + 2% chitosan, Group 5: 38% SDF + 3% chitosan. Three different volumes: 30 μl, 60 μl, 90 μl were used. The agar well diffusion method was used to evaluate the antibacterial activity. The highest zone of inhibition was found in Group 1: 38% SDF at 90 μl and the lowest was found in group 3: Chitosan 3% at 30 μl. Group 1-38% SDF showed higher antibacterial activity amongst all the other four groups under study. And a comparable antibacterial effect between group 1: 38% SDF at 30 μl and Group 5: 3% chitosan and 38% SDF at 30 μl. SDF at 38% showed higher antibacterial activity against *S.mutans* and a comparable antibacterial effect between group 1: 38% SDF at 30 μl and Group 5: 3% chitosan and 38% SDF at 30 μl. Thus, considering the side effects of SDF, further studies are required in this aspect to test the cytotoxicity of SDF and chitosan in combination.

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ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11i4.3255

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**INTRODUCTION**

A sucrose-induced ecological change in the bacterial composition towards the cariogenic process leads to dental caries. Thus, caries can be called as a biofilm induced, saliva mediated acid demineralisation of enamel and dentin of teeth (AAPD). These cariogenic conditions favour the growth of specific conditional pathogens which compete efficiently with the commensal microflora for the tooth surface resulting in dental caries. (Marsh, 2010). The oral microflora mainly involved in caries is *Streptococcus mutans* (Loesche, 1986). It is a gram-positive, facultatively anaerobic bacteria. Generally, it persists as a normal member of the oral bacterial community, while in the presence of fermentable carbohydrates, these acid-producing microorganisms dissolve the tooth structure causing damage (Caufield and Griffen, 2000; Marsh, 2003).

Chitin is found abundantly in nature, and it is a polysaccharide of animal origin characterised by a fibrous structure. This forms the basis of the main constituent, which includes the outer skeleton of insects and crustaceans like shrimps, crabs and lobster (Kumar et al., 2005). The resultant product after reduction of acetylated amine group to 40-35% is a
co-polymer referred to as chitosan (Goy et al., 2009). Chitosan is a weak base insoluble in water, but soluble in dilute aqueous acidic solutions. The solubility of chitosan is mainly dependant on its biological origin, molecular weight and degree of acetylation (Shepherd et al., 1997).

Chitin and chitosan have a broad spectrum of antimicrobial activity, and several investigations had been done testing its antimicrobial property against a wide range of target organisms such as algae, bacteria, yeasts and fungi in experiments involving in vivo and in vitro interactions such as chitosan in different forms (Goy et al., 2009). Thus, it is widely used in several areas such as healthcare, pharmaceuticals, agriculture, food production, tissue engineering and cosmetics (Erpaçal et al., 2019).

Low molecular weight chitosan had been reported to prevent the adsorption of Streptococcus mutans to hydroxyapatite (Kim and Shin, 2013).

Silver diamine fluoride, referred to as SDF, is a recently introduced material which has multiple beneficial effects such as inhibition of demineralisation, conservation of collagen from degradation, increasing microhardness of dentine post-application and inhibiting the active growth of cariogenic bacteria (Mei et al., 2013). Despite the merits as mentioned and benefits of SDF, one of the side-effects associated with its usage is the staining of the tooth surface and restorative materials due to the presence of residual silver ions (Thomas et al., 2019).

Hence, considering the side-effects of Silver diamine fluoride usage and the antibacterial efficacy of chitosan against Streptococcus mutans, this present study was done to evaluate and compare the antibacterial efficacy of SDF and chitosan when used in combination and individually against Streptococcus mutans.

MATERIALS AND METHODS

This study was undertaken in the Microbiology laboratory of Saveetha Dental College, Chennai. Approval for the study design, protocol and methodology was obtained from the Institutional Research Committee Committee of Saveetha Dental College and Hospitals. The standard strain of S. mutans NCTC10449 was used to test the antimicrobial activity of the groups under study. Freshly prepared chitosan was procured from ‘The Himalayan Chemicals Pvt Ltd.’ in the concentrations of 2% and 3%. Whereas, for Silver diamine fluoride, commercially available 38% SDF (Fagamin) was used.

Sixty sterile Petri dishes of 90 mm were taken and were divided into five groups of 12 Petri dishes each. The groups are: Group 1: 38% SDF, Group 2: 2% chitosan, Group 3: 3% chitosan, Group 4: 38% SDF + 2% chitosan, Group 5: 38% SDF + 3% chitosan. For combining SDF and chitosan, a 1:1 ratio was used, i.e., 100 µl of 2/3% chitosan was mixed with 100 µl of silver diamine fluoride using a Vortex mixer. Three different volumes: 30 µl, 60 µl, 90 µl were used in the study and compared. Each group with 12 Petri dishes was divided, the subgroup of 4 plates each according to the three volumes being tested.

The agar well diffusion method was used to evaluate the antibacterial activity of the five groups under study. The fresh bacterial suspension was dispensed on the surface of Mueller Hinton agar plates and the respective study agent according to the assigned group-chitosan or SDF or its combination was incorporated into the wells, and the plates were incubated at 37% C for 24 hours. The zone of inhibition was recorded in each plate and noted.

RESULTS

The zone of inhibition in the Petri dishes of all the five groups Figure 1 was measured in mm and noted. Comparison of the antimicrobial activity of the five groups under study against Streptococcus mutans in terms of mean zone of inhibition is given in Table 1. The highest zone of inhibition was found in Group 1: 38% SDF used alone at 90 µl and the lowest zone of inhibition is located in group 3: Chitosan 3% used alone at 30 µl. Group 1- 38% SDF used alone showed higher antibacterial activity against all the other four groups under study. There was a comparable antibacterial effect between group 1: 38% SDF at 30 µl and Group 5: 3% chitosan and 38% SDF at 30 µl. [Table 1, Graph 1]

DISCUSSION

Dental caries has a multifactorial origin. It was noted that an average of 27% of toddlers between 18 and 36 months of age presented at least one deciduous tooth with caries (Saúde, 2004). Thus it is crucial to prevent and control the spread of dental caries as early as possible. Therefore, within this context, it is vital to use agents that control the progression of lesions while waiting for the treatment to be eventually done or for the adaptation of the children towards new hygiene and nourishing habits (Ditterich, 2006).

Silver diamine fluoride proves to be a cariostatic and preventive agent and its main effect on dental structures is the promotion of calcium fluoride and silver phosphate, without any loss of calcium.
Groups (a-e): (a) Group 1: 38% SDF, (b) Group 2: 2% chitosan, (c) Group 3: 3% chitosan, (d) Group 4: 38% SDF + 2% chitosan, (e) Group 5: 38% SDF + 3% chitosan

Figure 1: Zone of inhibition noted in all five groups under study

Table 1: Zone of Inhibition of Streptococcus mutans of the five groups measured in mm

| Study group                  | Zone of inhibition in mm (Mean) |
|------------------------------|--------------------------------|
|                              | 30μL   | 60μL   | 90μL   |
| Group 1: 38% SDF             | 24.36  | 38.55  | 40.22  |
| Group 2: Chitosan 2%         | 11.25  | 12.12  | 14.15  |
| Group 3: Chitosan 3%         | 11.14  | 13.21  | 14.24  |
| Group 4: 38% SDF + Chitosan 2% | 21.34  | 21.25  | 25.44  |
| Group 5: 38% SDF + Chitosan 3% | 23.23  | 26.18  | 31.32  |

Graph 1: Antimicrobial activity in terms of the mean zone of inhibition of Streptococcus mutans in the five groups represented in mm
and phosphate ions (Triches et al., 2010). Silver diamine fluoride is an easy-to-apply colourless liquid, and thus no technical devices are required for its administration. It is available in 10%, 12%, 30% and 38% concentrations (Ditterich, 2006). According to the in vitro study by Montandon, Speranca, silver diamine fluoride has antibacterial activity against S. mutans and higher the concentration used, higher were the effects. Thus in this present study, 38% SDF was used (Montandon and Speranca, 2000). However, cytotoxicity of SDF raises concerns regarding its use. It was observed that SDF remained cytotoxic even after nine weeks of rinsing. The sdf was found to have toxic effects on human gingival fibroblasts and may cause long term effects (Fancher, 2019).

Chitosan proves to be the second most common biopolymer found in nature after cellulose. Chitosan is produced by the deacetylation of chitin, which is a skeletal material of arthropods, and also occurs in the cell walls of fungi (Erpaçal et al., 2019). In a study, chewing gums containing chitosan were tested, and antimicrobial activity was checked. The study results showed that chitosan serves to be effective in controlling the number of cariogenic bacteria where tooth brushing is difficult (Hayashi et al., 2007). Water-soluble chitosan is found to directly inhibit the growth of typical cariogenic bacteria, Streptococcus mutans, at pH 6.5 without causing demineralisation of the tooth surface. And when used as a mouthwash, it exhibits an antibacterial and plaque-reducing effect (Fujiwara et al., 2004). The cytotoxic effects of an experimental biomaterial prepared with chitosan were evaluated in one study and found that there are no cytotoxic effects of chitosan on the cultured stem cells of an extracted milk tooth. The fact that there was no reported cytotoxicity of using such material has widened the area of use of the material (Subhi, 2018). Another in vitro study evaluated the cytotoxicity of chitosan derivatives on cells and found low cytotoxicity in the test results proving that they could be used as good biomaterials (Wei et al., 2019).

No previous study was done comparing the antibacterial activity of chitosan and silver diamine fluoride against Streptococcus mutans. Thus this current study revealed that Silver diamine fluoride at 38% in all three volumes showed the highest antibacterial activity compared to chitosan used alone and the combination of SDF and chitosan against Streptococcus mutans. However, the antibacterial activity of silver diamine fluoride at 30 μl was comparable to that of the combination of 3% chitosan and 38 SDF at 30 μl in terms of mean zone of inhibition of Streptococcus mutans. The cytotoxic and discolouration aspect of Silver diamine fluoride and the antibacterial effects of chitosan, further studies are required in this aspect to test the cytotoxicity of the materials and its combination and consider it for future evaluations.

CONCLUSION

Within the limits of the current in-vitro study, it may be concluded that 38% of SDF possessed higher antimicrobial activity against chitosan and the various combinations. However, there was comparable antibacterial efficacy against S. mutans when 3% chitosan and 38% SDF were used in combination at 30 μl. Thus considering the biocompatibility and antibacterial effects of chitosan and its combination with SDF, further studies are required in this aspect to investigate the cytotoxic effects of these materials to confirm the conclusions of the current study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that there is no funding support for this study.

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