Original Article

Experimental Evaluation of the Effect of Zinc Salt on Inhibition of *Streptococcus mutans*

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ABSTRACT

Statement of the Problem: The rate of antibiotic resistance in bacteria and side effects of antibiotics and oral and teeth health care products are increasing. Therefore, researchers aim at finding new alternatives to control bacteria of dental caries.

Purpose: The objective of this study was to investigate the inhibitory and bactericidal effects of different concentrations of zinc sulfate and zinc acetate solutions on the main recognized agent of dental caries, *Streptococcus mutans*.

Materials and Method: In this experimental study, different concentrations of aqueous zinc sulfate and zinc acetate solutions were prepared and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these salts for *Streptococcus mutans* were determined in compare with penicillin, chlorhexidine by micro-serial dilution method. In addition, the diameters of zone of inhibition for these salt solutions in four concentrations along with chlorhexidine, as the control, were detected by the disc diffusion method.

Results: MIC and MBC of zinc sulfate solution were higher than penicillin and chlorhexidine. There were not statistically significant differences between the MIC and MBC of zinc acetate solution, penicillin, and chlorhexidine. In 25 and 50 µg/mL concentrations, the diameters of inhibition zone for zinc sulfate were more than zinc ac

Conclusion: Zinc sulfate and zinc acetate salts with 37.19 and 31.25 µgr/mL concentration had inhibitory effect on *Streptococcus mutans* growth respectively, although, no priority in antibacterial activity of the studied zinc salts was determined in comparison with penicillin and chlorhexidine.

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Introduction

There are many kinds of bacteria in the oral cavity, which act as normal flora or pathogen. Some of these pathogens can cause dental caries or periodontal diseases. The mouthwashes and antibacterial toothpastes are prescribed for decreasing the prevalence of such diseases. [1-5]

Dental caries is the most common infectious disease in the oral cavity. The most important bacteria, responsible for dental caries, is a gram-positive facultative anaerobic *Streptococcus mutans* (*S. mutans)*, which causes decalcification of the tooth structure. [6]

Zinc with its natural concentration in saliva can be used as nutrient for oral bacteria, [7-8] although increas-

ing its concentration can show inhibitory effects on oral microorganisms. Zinc increases bacterial membrane permeability for H^+ and acidic stress. In addition, it can inhibit glycosyltransferase enzyme, limiting the primary substrate and leading to bacterial starvation. [9]

In a study, an incredible decrease in the number of *S. mutans* colonies was reported by zinc chloride mouthwash. [10] A study in 2007, reported that zinc sulfate and zinc acetate decreased the amount of oral *S. mutans*. [11] The result of the research conducted by Eisenberg *et al.* [12] showed the synergistic inhibitory and bactericidal effect of accompanying zinc ion with sanguinarine extract.

Owing to the high prevalence and incidence of dental caries, finding new methods for inhibiting *S. mutans* growth is necessary. To accomplish this, minimal side effects, besides the most potent antibacterial effects, should be considered for qualifying the new treatment methods.

This study aimed to evaluate the inhibitory effect of zinc on *S. mutans* growth, the bacteria responsible for dental caries. This might provide an opportunity for introducing new additive for dentifrices and mouthwashes in order to decrease dental caries.

Materials and Method

In order to compare the antibacterial effect of salts, we designed a study involving four test groups and a control group. Group 1 was zinc sulfate, group 2, and zinc acetate, group 3, penicillin, and group 4, chlorhexidine. The antibacterial effect of zinc sulfate and zinc acetate were evaluated in four different concentrations.

Standard strain of *S. mutans* (ATCC 35668, PTCC 1683) was obtained from Iranian Organization for Science and Technology, Tehran, Iran.

Zinc sulfate (Merck, Germany) and zinc acetate (Falcon, US) solutions were prepared in concentrations of 6.25, 12.5, 25, and 50 µg/mL. They were then sterilized by autoclave and were kept in refrigerator until used in study. Disk diffusion technique was used to determine antimicrobial effect of zinc sulfate and zinc acetate on *S. mutans*.

A bacterial suspension of *S. mutans*, cultured in blood agar for 24 hours, with 0.5 McFarland turbidity ($1.5x10^8$cfu/mL) was adjusted in normal saline. The bacterial suspension was applied with sterile cotton swab on MHA (Muller Hinton agar, Merck, Germany) with 5% blood sheep. Then, disks were prepared by putting 20 µL of each concentration of zinc sulfate or zinc acetate on sterile paper disks (PadtanTeb, Iran) with the diameter of 6mm, which were then air-dried. 10-unit penicillin disk (Mast, UK) and dried plain paper disks with 20 µL of 0.2% chlorhexidine were considered as positive controls. After that, plates were incubated at 37°C for 24 hours. Finally, inhibition zones were measured in millimeter.

To determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of zinc sulfate or zinc acetate, we employed the broth dilution method. First, 100µL of brain heart infusion broth (BHI) (Hi-Media Laboratory, India) was added to each well of 96 well micro titer plates. Then, 100µL of highest concentration of stock solutions were added to the first well and other wells were filled with lower concentrations until the last well was in order (Final concentrations of 4.8-250 µg/mL). One well contained just BHI medium as the negative control. Wells with penicillin and 0.2% chlorhexidine were also considered as positive controls and one well contained BHI and bacterial suspension as the control with bacterium. After that, micro-well plates were incubated at 37°C in 5% CO_2 incubator for 24 hours. The first well in series without sign of visible growth of bacteria was considered as MIC. MBC was determined by culturing 10µL of contents of wells that did not show sign of bacterial growth on blood agar. Plates were incubated for 24 hours at 37°C in 5% CO_2 incubator. The least concentration that inhibited the formation of *S. mutans* colony on agar was considered as MBC. The whole experiment was performed twice at intervals of one week.

Statistical analysis was performed using SPSS™ software, version 18.0 (IBM Corp., Armonk, NY, USA). Non- parametric Kruskal Wallis and Mann Whitney tests were used for comparing different groups. $p< 0.05$ was considered as statistically significant.

Results

In concentration of 6.25 µgr/mL and 12.5 µgr/mL, the inhibition zone was zero. The concentration of 25µgr/mL and 50 µgr/mL of these salts, inhibition zones were recorded.

In concentration of 50µgr/mL, the mean inhibition

zone for zinc sulfate (15.33±1.52mm) was more than that for zinc acetate (10.33±10.1mm). In concentration of 25 µgr/mL, inhibition zone of zinc sulfate (9.66± 0.57mm) was more than that for zinc acetate (6.66± 6.11mm). In comparison with chlorhexidine (16.66 mm), both zinc sulfate and acetate showed less inhibition zone.

MIC and MBC of zinc salts and control groups are presented in Table 1.

Table 1: MIC and MBC of zinc salts and control groups

Groups	MIC (µgr/mL) Mean Rank (Median)	MBC(µgr/mL) Mean Rank (Median)
Zinc sulfate	37.19(312.50)	37.19(625.00)
Zinc acetate	31.25(625.00)	31.25(1250.00)
Penicillin	20(1)	20(2)
Chlorhexidine	13(0.95)	13 (1.95)
p Value	<0.001	<0.001

As shown in table 1, MIC of zinc sulfate was more than that of penicillin and chlorhexidine and their difference was statistically significant ($p< 0.001$).Zinc sulfate showed antibacterial effect against *Streptococcus mutants* in higher concentration than that in the control group (chlorhexidine and penicillin). MIC of zinc sulfate was approximately the same as MIC of zinc acetate and there was no statistical difference between their antibacterial effect ($p= 0.867$). MIC of penicillin was more than that of chlorhexidine ($p= 0.009$). However, there was no statistically difference between MIC of zinc acetate and that of penicillin ($p= 0.136$) and chlorhexidine ($p= 0.1$). There was a statistically significant difference between the MIC of zinc sulfate and that of penicillin and chlorhexidine ($p< 0.001$). Although the inhibitory effect of zinc sulfate on *S. mutans* was reported in this study, the bactericidal effect of this salt was achieved in a higher concentration in comparison with that of the control group (penicillin and chlorhexidine). There was no statistical difference between the inhibitory effect of zinc sulfate and zinc acetate ($p= 0.807$). In contrast with zinc sulfate, zinc acetate showed no significant differences in its MIC in comparison with that in penicillin ($p= 0.136$) and chlorhexidine ($p= 0.100$). In this study MIC of penicillin was reported higher than that of chlorhexidine, ($p= 0.009$) (Table 1). Although zinc sulfate had bactericidal effect against streptococcus, its MBC was achieved in higher concentration than that of penicillin and chlorhexidine ($p< 0.001$). However, there was no statistical difference between MBC of zinc sulfate and zinc acetate ($p= 0.867$). In addition, no statistical difference was reported for MBC of zinc acetate and both penicillin ($p= 0.136$) and chlorhexidine ($p= 0.100$). The MBC of penicillin was significantly higher than that of chlorhexidine ($p= 0.009$) (Table 1).

Discussion

In this study, the inhibitory effects of different concentrations of zinc sulfate and zinc acetate on streptococcus mantas were evaluated. In addition, the authors considered two control groups of penicillin and chlorhexidine. Although these salts showed suitable inhibitory and bactericidal effects (MIC, MBC), both control groups had lower MIC and MBC for *Streptococcus mutans*.

Different researchers initiated novel studies in order to introduce new approaches for decreasing dental caries. These studies were rendered with different methods. [4, 11-12] Eislenberg *et al*. [12] reported the synergistic interaction of sanguinarine and zinc on oral streptococcus. This combination showed suitable MIC and MBC for oral streptococci. In our study, both bactericidal and inhibitory effects on *S. mutans* were registered for zinc salts.

Burguera-Pascu *et al.* [11] tested the antimicrobial effects of zinc sulfate and acetate against *S. mutans*. A significant reduction in the mean of oral *S. mutans* was recorded two hours after rinsing the zinc salts. In another study, Phan *et al.* [4] declared the inhibitory effect of zinc ion against acidogenesis of oral *Streptococcus mutans*. According to the results of this study, the bacteriostatic activity of zinc was more prominent. In the present study, there was no significant difference between bactericidal and bacteriostatic activity of zinc salts.

Another study revealed the inhibitory effects of fluoride and zinc alone or in combination on the growth of *S. mutans* colonies. These ions, in fact, prevented glucan production, an important virulence factor for streptococci. Therefore, it was inferred that zinc oral fluoride controlled cariogenicity. [13]

Nguyen *et al*. [14] reported that zinc could suppress the respiration of oral bacteria including S. *mutans* and *Fusobacterium nucleatum*. It was observed that these organisms were in close association with

developing gingivitis. Hence, zinc could decrease gingivitis by inhibiting these bacteria. Respiratory suppression of these bacteria was improved by accompaniment of sulfate and acetate with zinc ion. [14]

Nossek *et al.* [15] compared the antibacterial efficacy of zinc chloride in two concentrations (0.1% and 0.2%) with chlorhexidine 0.2%. On the other hand, chlorhexidine 0.2% had bactericidal activity. In this study, both bactericidal and bacteriostatic activity of zinc sulfate and acetate were registered. In addition, several concentrations of two types of zinc salts were examined in this study.

Watson *et al.* [16] evaluated the interaction of zinc and *S. mutans* acid production. They reported that more resorption of zinc would lead to more inhibition of acid production. [16] This study reported the significant inhibitory effect of acid production of *S. mutans* by zinc sulfate and acetate salts. Unlike the findings of this study, zinc sulfate showed more inhibitory properties against *S. mutans* than zinc acetate. [16] Having evaluated antibacterial properties of both zinc sulfate and acetate, we found no significant difference between them (p= 0.867).

In a research, the new composition of toothpaste, containing triclosan and zinc citrate and standard fluoride, was evaluated on isolated gram positive and gram-negative dental plaque bacteria. This composition showed proper inhibitory efficacy against both groups of bacteria. [1] Spencer *et al.* [17] have reported added antibacterial properties of Fuji ORTHO LC. Dobl and Nossek [10] showed the concentration-dependent antibacterial effect of zinc chloride solution. In a study, it was reported that zinc ions released shortly after zinc-based glass polyalkenoate cement synthesis, inhibited *S. mutans growth* properly. [18]

Another evaluation showed that zinc sulfate addition to glass-ionomer-based cements decreased *S. mutans* growth significantly without weakening the flexural strength and solubility of cement. [19] The results of all these studies confirm the findings of the present study. On the other hand, Giertsen *et al.* [20] could not show any *in vivo* reduction in acid formation. Bates and Navia [21] did not confirm *in vivo* effect of zinc on oral cariogenic flora. The weak effect of zinc *in vivo* may be due to factors such as dilution, chelation effects, and tooth-site.

According to these results, *in vivo* situation can affect the antibacterial properties of zinc ions because of ions dilution and chelation and oral normal cleansing mechanism. In the present study, unlike zinc sulfate, zinc acetate in lower concentration showed antibacterial effects. On the other hand, the inhibitory halo for zinc acetate was less than that for zinc sulfate. The results obtained for MIC and MBC were more valid than the results of disc diffusion because dissemination of the solution in agar was an important factor in the method of disc diffusion. Weak solubility and diffusion of zinc acetate in agar might be responsible for the lower inhibitory halo of zinc acetate.

According to the findings of this study, the mean rank of penicillin MIC was significantly more than that of chlorhexidine. Additives and alcohol ingredient of chlorhexidine mouthwash can justify this finding.

Chlorhexidine is one of the antibacterial agents, which is prescribed widely before, after surgeries, and for periodontal diseases in order to reduce the bacterial load. However, this production has some side effects such as dental staining, taste disturbance, and mucositis. [22] Regarding the side effects of routine mouthwashes and increasing rate of antibiotic resistance, although the inhibitory potency of zinc salts is lower in the control groups, using these salts in the form of mouthwash or dentifrices can be useful.

In this study, two control groups were designed and all laboratory procedures were repeated in order to decrease the undesirable errors. In addition, several concentrations of zinc salts were used. The authors suggest the use of pure chlorhexidine powder for omitting the effect of alcohol or other additives on the bacterial growth. However, the *in vivo* evaluation of zinc salts for caries prevention and anti-streptococcal activities is suggested. Evaluating the synergistic potency of zinc salts for antibacterial activation can also be beneficial.

Further studies are recommended by adding zinc compliancy to the toothpastes or mouthwashes or oral supplementary.

Conclusion

According to the findings of this study, although different concentrations of zinc sulfate and acetate inhibited streptococcus growth, compared with control gro-

ups, they did not show significant differences.

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Conflict of Interest

The authors of this manuscript certify no financial or other competing interest regarding this article.

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