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Using Iron Supplements for Prevention of Dental Caries: An Experimental Study

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Background: Dental carries are the most widespread disease among humans, caused by the bacteria growing in the dental plaque. *Streptococcus mutans* is known as the main bacteria-inducing dental carries.
Objectives: The aim of the present study was to investigate the effect of some commercially available iron supplements on the growth of this bacteria and dental carries.
Materials and Methods: The antimicrobial effects of six different types of iron supplements were assessed using the well-diffusion method. Furthermore, the effects of these supplements on the beginning and progression of the process of dental carries were investigated. The SPSS software package version 20 was used for statistical tests.
Results: At the concentration of 100%, the highest and the lowest inhibitory effect were for Ferrokids-drops (32 mm) and Irovit (26 mm), respectively. At a concentration of 50%, Vitane (26 mm) supplement and Irovit supplement (21 mm) had the highest and lowest effectiveness, respectively. Moreover, at a concentration of 10%, the highest and the lowest inhibitory effect were for Vitane and Irovit, respectively. The positive control group had the highest number of decayed teeth, while the group that received Vitane iron supplements was the one with the lowest number of decayed teeth. There were no decayed teeth in the negative control group.
Conclusions: In spite of what the parents believe, iron supplements not only do not cause dental carries but also they have preventive and inhibitory effects on them. Furthermore, it is highly recommended that supplements containing both iron and zinc elements should be preferred over supplements containing only iron elements.

Keywords: Dental Caries; Iron; Dental Plaque; *Streptococcus Mutans*

1. Background

Dental carries are the most prevalent chronic infectious disease in human beings that stem from the bacteria colonized in dental plaques (1). *Streptococcus* mutans has been recognized as the primary cause of this disease. In certain circumstances, these bacteria can break down sugar and other carbohydrates in foods and drinks and then transform them into lactic acid and other organic acids. These products are acidic in nature and can solve the tooth enamel by dropping the pH of the tooth surface below the critical point (lower than 5.5). Glycosyltransferase (GTF) enzymes of S. mutans play a key role in the adhesion of the bacteria to the tooth surface and biofilm formation and consequently tooth decay (2, 3).

Dental carries can be prevented by avoiding sugary foods and using preventive techniques including training, fluoride therapy and sealant therapy (4). Another approach for reducing tooth decay that is caused by sucrose is to use proper mineral supplements such as iron supplements. This issue has been studied by several studies and it was indicated that adding iron to the diet reduces the rate of tooth decay. On the other hand, iron deficiency is the most widespread type of malnutrition in developing countries. In order to solve this problem effectively, iron supplements are prescribed for children under five years old (5, 6).

Moreover, in countries where iron deficiency anemia is common, there is also a high prevalence of dental caries, however these two phenomena are not necessarily directly interrelated. Many in vivo and in vitro studies, carried out in this field, support the anti-caries properties of iron, however, the antibacterial mechanism of iron and its role in controlling tooth decay is still a controversial debate among researchers (4, 7).

The mechanism by which iron inhibits the *S. mutans* growth is by reducing acidogenicity of *S. mutans* and interfering with the metabolism of sucrose, by which the

amounts of extracellular polysaccharides are reduced (8). Furthermore, it has been demonstrated that iron suppresses the activities of glycoprotein transferase enzymes that are produced by the bacteria. Nevertheless, some studies have shown that iron at a concentration of 70 μgr/mL has no effect on both the acidogenicity of dental biofilm and reduction of the amount of extracellular polysaccharides in the biofilm that were exposed to 10% to sucrose (9, 10), however, more studies are needed in this field.

Dental carries must be regarded as a major health problem among Iranian children. Overall, 7.3% of Iranian children aged between two and six have anemia (11). The use of iron supplements may help prevent iron deficiency among these children. Iron supplements are usually available in the form of drops and syrups for children. In addition to the unpleasant taste of some of these supplements, one of the major obstacles in their use is their discoloring effect on the teeth. Interestingly, this discoloring effect is wrongly assumed by the parents as a sign of tooth decay, which is why they tend to limit the use of these food supplements for their children (3, 12). According to our literature review, there are no studies from Iran that have assessed the effects of iron supplements on the activities of *S. mutans* bacteria.

2. Objectives

The aim of the present study was to investigate the effect of some commercially available iron supplements on the growth of *S. mutans* and dental carries.

3. Materials and Methods

3.1. Evaluation of the Antimicrobial Effects of Iron Supplement Drops

In order to evaluate the antimicrobial effects of the supplements, standard strains of *S. mutans* (ATCC 35668) were used. The brain-heart infusion (BHI) medium (Merk) was used to culture the primary strains, and the well diffusion method was applied to measure and compare the antimicrobial effects of iron supplements. Pits with a diameter of 5 mm were created on the surface of Mueller Hinton agar plates (Merk) and each of them was filled with 30 μL of supplements at various concentrations of 10%, 50%, and 100%. The bacteria that were cultured in the BHI medium for eight hours at a concentration of 0.5 McFarland (1.5 × 108 cfu/mL) were transported on Mueller Hinton agar plates. These plates were placed in the incubator at 35°C for 18 hours. Penicillin (Mast) and sterile saline were used for the positive and negative groups, respectively (13).

3.2. Dental Blocks

One-hundred and twenty-eight primary teeth, extracted due to a lack of space or trauma, were selected for this study. All of these teeth were examined to find anomalies such as caries, developmental disorders, enamel fractures, discoloration, and pathological changes in the internal or external pulp; if the tooth had one of these anomalies it was excluded from the study. All of the final samples were stored in distilled water at room temperature until use in the experiments. In order to prepare the samples, the crown and the root of the tooth were cut off from the Cement-Enamel junction (CEJ) region. Next, the residue in their pulp was fully evacuated, and their chamber was carefully cleaned and each pulp was filled by composite. In the next step, all the surfaces of the teeth were sealed by nail polish except a window with dimensions of 0.4 × 0.4 centimeters, which was labeled by a piece of paper. Before starting the experiments, this piece of paper was removed and the residue of the adhesives was washed with water and gas (14). Figure 1 shows these teeth and their windows.

3.3. Evaluation of the Effects of Iron Supplements on Block Tooth Decay

The decay causing environment was composed of BHI medium, sucrose, and a freshly cultured (18 - 24 hours) *S. mutans*. A ten-well micro plate was allocated to each sample, followed by the addition of the aforementioned drug complexes alongside the BHI medium to the micro plates (15). The drug samples used in this study were as follows; supplemental iron drops, Feriron, Vitane, Ferrokids-Drops, Ferbolin, Irovit, and Ferrodrop. A brief description about these supplements is provided in Table 1.

Figure 1. Preparing the Teeth Using a Window on the Buccal Surface

Based on the compounds and the environment in which the samples were placed, eight groups with 16 dental blocks were prepared, as follows; groups 1 - 6: 5 mm of the artificial decay causing environment + 0.5 mL from each supplement with a concentration of 10%. The positive control group: 5 mL of the artificial decay causing environment without supplements. The negative control group: 5 mL of the artificial decay causing environment without *S. mutans* bacteria. All groups were incubated under microaerophilic conditions at 37°C for 60 days in a candle jar. Every 48 hours, the growth mediums were replaced and tooth samples were transferred to the fresh medium. The beginning of the dental caries process (decalcification) was determined by the caries diagnostic device (coxo, china) and its progress (dental pitting) was evaluated using the finger touching method (16). Decalcification and pitting processes were evaluated on days 15, 30, 45 and 60, and the results were recorded.

3.4. Statistics Analysis

Chi-square test was used to assess the relationship between the groups with iron supplements on days of 15, 30, 45 and 60, with regards to incidence of tooth decay. The SPSS software package version 20 was utilized to carry out the statistical tests. All statistical analysis was performed at significant level of 0.05.

4. Results

At a concentration of 100%, the highest inhibitory zone was for the Ferrokids-drop supplement (32 mm), and at concentrations of 10% and 50% Vitane had the highest inhibitory zones of 26 and 22 mm, respectively. In the case of Irovit supplement, for three concentrations of 100%, 50%, and 10%, the diameter of inhibitory zones were of 23, 21 and 10 mm, respectively. These results are shown in Table 2.

The assessment of the number of decayed tooth in each group after 60 days indicated the following results (as described before, each group composed of sixteen teeth); the highest number of decayed teeth belonged to the positive control group with 11 decayed teeth. As mentioned previously, in this group no supplement was used. In the group that received the Fer-iron supplement, there were seven decayed teeth. In the two groups that received Ferrodrop and Ferrodrop supplement there were six decayed teeth. In the groups that received Ferrokids-drop and Irovit supplement there were five decayed teeth, and the group with Vitane had the lowest number of decayed teeth. Furthermore, there was no decayed tooth in the negative control group (Table 3). In addition, there was no significant difference between the number of decayed teeth on days 15, 30, and 45.

Table 1. The Ingredients of the Drug Samples in the Present Study

The Commercial Name of the Iron Supplements (Manufacturer)	The Amount of Iron Content (Gram Per mL)	Type of Iron Supplement
Ferbolin (Shahre Darou)	25	Ferrous sulfate
Ferrokids-drops (Eurho Vital)	16	Ferrous gluconate
Ferrodrop (Donyaye Behdasht)	25	Ferrous sulfate
Vitane oral drops (VITANE)	17.14	Ferrous gluconate
Fer-iron (Darou Pakhsh)	25	Ferrous sulfate
Irovit (VITANE)	15	Ferrous gluconate

Table 2. The Antibiotic Inhibition Zones (mm)

Supplements	Concentration 100%	Concentration 50%	Concentration 10%
Vitane	29	26	22
Irovit	23	21	10
Ferrokids-Drop	32	24	11
Fer-iron	27	23	15
Ferrodrop	31	25	13
Ferbolin	30	25	13

Table 3. The Number of Decayed Teeth After Sixty Days for Each Group [a]

	Negative Control	Positive Control	Ferbolin	Ferrodrop	Fer-Iron	Ferrokids-Drop	Irovit	Vitane
Undecayed teeth	16 (100)	5 (31/25)	10 (62/5)	10 (62/5)	9 (56/2)	11 (68/7)	11 (68/7)	13 (81/2)
Decayed teeth	0 (0%)	11 (68/7)	6 (37/5)	6 (37/5)	7 (43/7)	5 (31/2)	5 (31/2)	3 (18/7)
Total	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)

[a] The values are presented as No. (%).

5. Discussion

Dental carries, as a common health problem all around the world, are a bacterial infectious disease in which dental mineral tissues are dissolved and decomposed (17). Nowadays, it has been accepted as a fact that some species of *streptococcus* such as *S. mutans* play a key role in the formation of plaque and, consequently, dental caries. If the concentration of *S. mutans* increases above 50% of the total amount of bacteria, the decay process will begin. As a result, it can be inferred that controlling the concentration of *S. mutans* lowers the critical point (50%) and can be an effective strategy for preventing dental caries. Accordingly, the aim of the present study was to examine the inhibitory effects of some commercially available iron complements on *S. mutans* growth using the well diffusion method (18, 19). Three different concentrations of iron supplements were applied in the present study. As it was expected, the 100% concentration of iron supplement had the highest effectiveness in controlling the growth of the bacteria. Moreover, Ferrokids-drop iron supplement had the highest effectiveness at the 100% concentration while Vitane was the most effective at the concentrations of 50% and 10%. These results are consistent with the results obtained by previous studies (20, 21).

Vitane iron supplement contains zinc in addition to iron, therefore it can be deduced that zinc and iron have synergistic effects in controlling the growth of *S. mutans*. The same results were observed in other studies, such as researches by Al-Shalan et al. and Martinhon et al. (13, 22). In the study carried out by Thakib et al. the highest inhibitory effect was observed at the concentration of 50%, while in the study carried out by Martinhon et al. the concentration of 100% was the most effective (22). According to most studies performed in this field, it can be concluded that iron acts as an inhibitory agent in *S. mutans* growth. Because of the difference between the compounds of commercial iron supplements produced and consumed in various countries, the effective concentration reported by previous studies are different from each other.

Hernandez-sierra et al. demonstrated that zinc can suppress the growth of *Streptococcus* species (23). Bates et al. and Harrap et al. also obtained the same results (24, 25). Furthermore, in the present study, it was indicated that Vitane iron supplement has the highest inhibitory effect on *S. mutans* growth due to its zinc contents.

It has been shown by a high volume of studies that there is a significant relationship between iron deficiency and dental caries (4, 13). An iron supplement is an effective way for preventing such deficiencies. Dental discoloration is one of the main obstacles in using these supplements. Use of oral iron compounds that are commonly available in the form of ferrous sulfate are normally avoided by children due to their bad taste and discoloring effect on tooth surfaces (21, 26).

Another aim of the present study was to assess the beginning of the dental carries process in the presence of an artificial decay-causing environment containing *S. mutans* bacteria and iron supplement drops. The findings of these sets of experiments indicated that iron supplements could suppress the growth of bacteria effectively and hence prevent dental caries, as well. While a difference was observed between the various types of supplements used in this study and their dental carries inhibition compared with the control group, yet this difference was not statistically significant ($P > 0.05$). These findings are similar to the findings reported by Al-Shalan et al. and Eshghi et al. from Iran and the results obtained by Martinhon et al. and Alves et al. from other countries (1, 4, 22, 27). Moreover, Vitane had the highest inhibitory effect on dental carries prevention, which is because of the zinc element that is included in this particular type of iron supplement.

According to the results of the present study, it can be concluded that despite what parents believe, iron supplements not only do not cause dental carries but also have preventive and inhibitory effects. One of the main problems in using iron supplements is black discoloration of teeth after use. In order to prevent this problem, it was recommended that teeth and mouth should be washed with abundant water immediately after administration of supplements. As shown in the present study, supplements containing both iron and zinc have the highest inhibitory effect on the growth of *S. mutans*; accordingly it is highly recommended that this type of supplement should be preferred over supplements containing only the iron element.

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Mohammad Esmaeilzadeh, Farzad Mojarad, Zakieh Donyavi, and Rasoul Yousefi Mashouf: advisors in dental and microbiology issues and contribution in writing the paper. Negar Khezri Sarijeh: doing dental experiments and contribution in writing the paper.

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