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Effect of Sucrosomial® Iron and Iron Drop Diluted with Natural Fruit Juice on Microhardness of Primary Enamel

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A B S T R A C T

Objectives: Considering the high prevalence of consumption of iron drops, and the resultant reduction in microhardness of primary enamel, this in vitro study aimed to assess the effects of Sucrosomial® iron and iron drop diluted with natural fruit juice on microhardness of primary enamel.

Materials and Methods: This in vitro, experimental study evaluated 45 extracted sound primary anterior teeth, that were randomly assigned to three groups (n=15) of Sideral, Irofant, and Irofant + natural apple juice. The titratable acidity and pH of solutions were measured. After measuring the baseline microhardness by a Vickers hardness tester, the teeth in the three groups were exposed to the respective iron drop solutions at 37°C for 5 minutes. They were then rinsed with distilled water, and their secondary microhardness was measured. Data were analyzed using the dependent Student t-test, ANOVA, and ANCOVA (alpha=0.05).

Results: Irofant had the lowest pH and the highest titratable acidity among the tested solutions. A reduction in enamel microhardness occurred in all groups after exposure to iron drops (P=0.0001). The reduction in microhardness was significantly greater in Irofant group compared with Irofant + natural apple juice (P=0.0001). Also, the reduction in microhardness was significantly greater in Irofant + natural apple juice compared with Sideral iron drop group (P=0.0001).

Conclusion: Sideral iron drop with Sucrosomial iron has minimal adverse effect on microhardness of primary enamel. Also, dilution of iron drops with natural apple juice can be suggested as an effective strategy to decrease their adverse effects on microhardness of primary enamel.

Keywords: Hardness; Dental Enamel; Tooth, Deciduous; Sucrosomial Iron

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INTRODUCTION

Iron deficiency anemia is a common form of anemia during childhood [1]. Iron deficiency decreases the physical, mental and behavioral abilities of children [2]. Iron supplementation by using iron drops is one suggested strategy to treat iron deficiency. The commonly prescribed iron drops contain citrate, and have high acidity, which can cause erosion, decrease enamel strength, and enhance the development of dental caries [3]. Scanning electron microscopic examinations have indicated that exposure of tooth structure to an acidic environment increases the absorption of iron and changes the structure of primary enamel [4]. Changes in the structure of primary enamel following iron drop consumption have created some concerns for many dental clinicians. Also, dark discoloration of primary teeth following iron consumption often leads to discontinuation of use of this necessary supplement by some parents [5]. One suggested strategy by the clinicians to decrease the side effects of iron

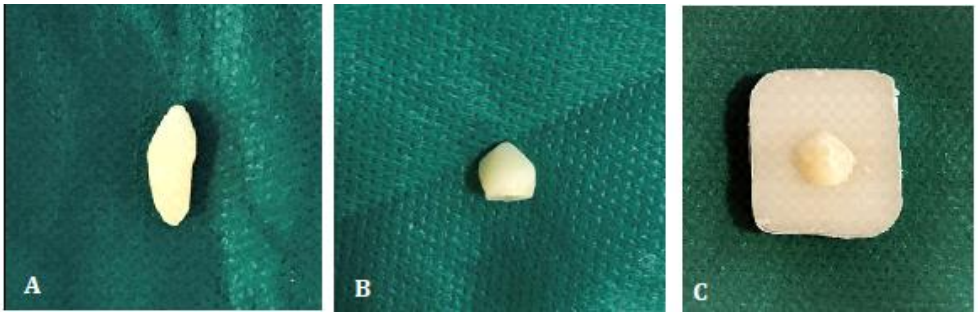

Fig. 1. Preparation of samples: (A) tooth selection, (B) cutting the crown at the cementoenamel junction, (C) mounting the tooth in acrylic resin

drops is to dilute them with fruit juice, which often leads to their better acceptance by children as well [6,7]. Pediatricians recommend daily use of apple juice by infants starting at 6 months of age [7].

A new iron drop was recently marketed with the brand name of Sideral (Junia Phrarma, Italy), the manufacturer of which claims that it has Sucrosomial® iron, which prevents the exposure of tooth structure to iron and subsequently prevents the side effects such as tooth discoloration [8]. Some previous studies have demonstrated that Irofant iron supplement (Kharazmi Co., Iran) [9] decreases the microhardness of primary enamel [4,10,11]. Since no previous study has evaluated the effect of dilution of iron drops with fruit juice or the effect of Sideral iron supplement on microhardness of primary enamel, this study aimed to compare the microhardness of primary enamel following exposure to Irofant, its mixture with natural apple juice, and Sideral iron drop by measuring the Vickers hardness number (VHN).

MATERIALS AND METHODS

This in vitro, experimental study evaluated extracted sound human primary anterior teeth (A, B and C) with no caries, cracks, fracture, restoration, or coronal hypoplasia [3,12,13] (Fig. 1A). The teeth had been extracted for purposes not related to this study. The study was approved by the ethics committee of Islamic Azad University (IR.IAU.DENTAL.REC.1398.015).

The sample size was calculated to be 15 in each group according to previous studies [3,10–14] and also based on a pilot study with 3 samples in each group using one-way ANOVA feature of PASS 15 software assuming alpha=0.05, beta=0.2, effect size of 0.4, and standard deviation of 25 for microhardness assessment.

The teeth had been extracted within the past 3 months [3,4,10]. The teeth were stored in 0.9% saline (0.9% sodium chloride; Shiraz Serum Co., Iran), at room temperature [3,4,10]. In this study, the microhardness of primary enamel was measured before and after exposure to iron drops using a Vickers hardness tester (Buehler, USA). The study was carried out in four phases as follows:

Phase I: Measuring the pH and titratable acidity of the solutions:

First, the important parameters related to erosion caused by iron drops including the titratable acidity and the pH of solutions were measured. The pH of both iron drops, including Sideral (Junia Phrarma, Italy) and Irofant (Kharazmi, Iran), was measured by a digital pH-meter (WTW, Germany); while, the Maguire method was used to determine the titratable acidity [15].

Phase II: Sample preparation and measuring the baseline microhardness:

To prepare the samples, the tooth crowns were cut at the cementoenamel junction (Fig. 1B). The contents of the pulp chamber were completely removed [11,13]. Next, the samples were mounted in acrylic resin such that their lingual surface was in the acrylic and their labial surface faced up, and remained exposed [10] (Fig. 1C). A sticker measuring 4 x 4 mm was applied on the labial surface of tooth crowns, and the area surrounding this window was coated with colorless nail varnish [3,10].

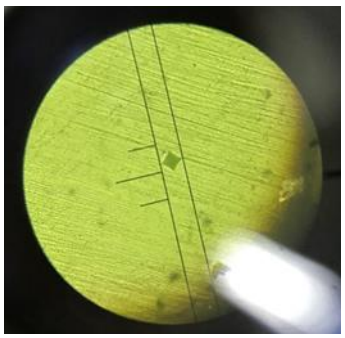

Fig. 2. Indentation caused by load application under a microscope

The sticker was then removed. By doing so, the testing area was standardized in all teeth. To achieve a smooth enamel surface, the labial surface of the teeth was slightly polished with 800, 2000, and 5000-grit silicon carbide abrasive papers (Lijian; China) under running water [3,10,11,14].

Polishing of the labial surface of each sample with each grit of silicon carbide abrasive papers was performed with 90-degree rotation relative to the previous grit, in order to obtain a smooth surface without any scratch for measurement of microhardness. To measure the microhardness of specimens, a Vickers hardness tester (Wilson Hardness Tukon 1202, Buehler, USA) was used. For this purpose, 50 g load was applied for 10 seconds by the device [10]. To measure the baseline microhardness of the samples, a flat and smooth part of the labial surface was selected for load application.

A conical indenter with a square-shaped cross-section applied load to the surface to create a square-shaped indentation with two equal diameters (Fig. 2). Finally, by rotating the head of the tester, the two diameters of the indentation were measured under a microscope by its ruler feature (D1 and D2) to calculate the mean diameter (D). The VHN was calculated using the formula below:

$$HV = \frac{2\mathrm{p}\sin(\frac{\theta}{2})}{D^2}$$

Where D is the mean diameter of the indentation (mm), P is the applied load (kgf), and θ is the angle between the opposite surfaces of the diamond indenter (θ is 136° in Vickers indenter).

To measure the microhardness of each sample, the VHN was measured at three points on each surface, and the mean of the three values was calculated and reported as the VHN of each specimen [10]. Teeth with a VHN between 239 and 478 (normal range) were selected for the study, and those with a VHN out of this range were excluded and replaced [10]. After measuring the baseline VHN, the final 45 samples were randomly divided into three groups of 15. Random allocation of the samples to the groups was performed using a table of random numbers in PASS software.

Phase III: Exposure to iron drops:

The samples were then exposed to iron drops (Table 1) as follows:

Table 1. Characteristics of iron supplements evaluated in this study [8,9]

Supplement	Ingredients	Iron ions	Company	Country
Irofant	Ferrous sulfate heptahydrate, anhydrous citric acid, sodium saccharin, aserola flavor, sorbitol, ethanol, sucrose, water, sodium metabisulfite, ethyl butyrate.	25 mg/mL	Kharazmi Co.,	Iran
Sideral	Stick ingredients: Sucrosomial iron (iron pyrophosphate, sucrose esters of fatty acids, sunflower lecithin spray-dried on rice flour and tricalcium phosphate. pregelatinised rice starch), maltodextrins. Gluten free. Bottle ingredients: Water, sucrose, maltodextrins, flavor; preservatives: potassium sorbate and sodium benzoate; thickening agent: xantan gum; acidity regulator: citric acid, emulsifier: sucrose esters of fatty acids. Gluten free.	7 mg/mL	JuniaPharmaSrl Co.,	Italy

Group 1: Each sample in this group was exposed to 15 drops of Sideral iron drop.
Group 2: Each sample in this group was exposed to 15 drops of Irofant and 15 drops of natural apple juice. To prepare the apple juice, red apples were peeled and juiced. Apple juice was filtered 2 to 3 times to become completely clear.
Group 3: Each sample in this group was exposed to 15 drops of Irofant.
The samples in each group were exposed to iron drops at 37°C for 5 minutes in a shaker incubator (IKA, Roentgen, Germany) [3,10].

Phase IV: Secondary microhardness measurement:

After 5 minutes, the samples were removed from the solution and rinsed with distilled water [3,10,13]. The microhardness of all samples was measured again by the Vickers hardness tester in a blind manner. The change in microhardness of each sample was then calculated by subtracting the baseline and secondary microhardness values. Data were analyzed using the Fisher's exact test, dependent Student t-test, one-way ANOVA, and ANCOVA. The significance level was set at 5% (P<0.05).

RESULTS

The Shapiro-Wilk test was applied to assess the normal distribution of microhardness data. The results showed that the data were normally distributed (P>0.05). The Fisher's exact test and one-way ANOVA revealed no significant difference in the mean baseline (primary) microhardness of the three groups (P=0.067). However, the mean secondary microhardness (after exposure to iron drops) of the three groups was significantly different (P=0.002, Table 2). The dependent Student t-test revealed a significant difference between the baseline and secondary microhardness of each group (P=0.0001, Table 2). ANCOVA was used to analyze the effect of type of solution on secondary microhardness after adjusting for the baseline microhardness. The results showed that type of solution had a significant effect on secondary microhardness (P=0.0001). Pairwise comparisons of the groups were then performed using the Bonferroni test, which revealed that:

- The secondary microhardness of Sideral group was significantly higher than that of Irofant + apple juice group (P=0.0001).
- The secondary microhardness of Sideral group was significantly higher than that of Irofant group (P=0.0001).
- The secondary microhardness of Irofant + apple juice group was significantly higher than that of Irofant group (P=0.0001).

The pH and titratable acidity [measured by the Maguire technique (15)] of the iron drop solutions consumed in this study are also listed in Table 3.

DISCUSSION

The current results showed that in all groups, iron drop decreased the microhardness of primary enamel. Also, Irofant iron drop caused maximum reduction in microhardness (P=0.0001). Sales-Peres et al. [16] evaluated the effect of an iron-containing mouthwash on erosion of enamel and dentin, and concluded that the iron-containing mouthwash significantly decreased the microhardness of enamel. Pasdar et al. [10] evaluated the effect of several iron drops and multivitamins on microhardness of primary enamel and reported that Irofant iron drop caused maximum reduction in enamel microhardness,

Table 2. Comparison of baseline (primary) and secondary microhardness of the groups

Solution	Baseline microhardness	Secondary microhardness	Within-group comparison
Sideral	311.93±40.60	285.74±39.91	T=8.74 P=0.0001
Irofant+apple juice	292.85±38.62	239.28±38.85	t=13.72 P=0.0001
Irofant	326.35±35.36	240.09±33.98	t=15.82 P=0.0001

Table 3. Titratable acidity and pH of Sideral and Irofant iron drops and combination of Irofant and apple juice

Solution	Mean pH	Titratable acidity
Sideral	4.33	0±0.325
Irofant+apple juice	3.167	0.85±0.112
Irofant	1.903	1.54±0.026
Apple juice	4.284	---

which was in agreement with our findings. Tabari et al. [3] reported an increase in enamel microhardness following the application of silicon oil and nano-hydroxyapatite powder on the teeth exposed to several types of iron drops; however, they also added that exposure to Irofant significantly decreased the enamel microhardness. In contrast to our study, Eskandarian et al. [11] evaluated the effect of three types of iron drops on microhardness of primary enamel under an artificial cariogenic challenge and reported that iron drops had no significant effect on demineralization of tooth structure. In their study, the teeth were polished with abrasive papers after immersion in cariogenic solution containing iron drops, and then underwent micro-hardness measurement. This process can eliminate the most superficial enamel layer exposed to iron drops, and can lead to different results.

In the present study, enamel microhardness decreased by 26% after exposure to Irofant iron drop. The erosive effect of compounds depends on their titratable acidity and the amount of H+ ions (pH) [15,17-19]. However, iron has an inhibitory effect on tooth demineralization [20-24]. The titratable acidity of Irofant iron drop is 1.54, and its pH is around 1.98, which is much lower than the critical pH for enamel solubility. It appears that the formulation of iron in Irofant iron drop and the citric acid added to this product increase its titratable acidity and decrease its pH, resulting in higher erosive potential. Citric acid is often added to iron supplement formulations to increase iron absorption and improve their taste [6,15]. The amount of citrate in Irofant iron drop is 498 ppm [10]. Citric acid can bond to calcium present in hydroxyapatite and increase the enamel solubility as such [15,17-19]. Hekmatfar et al. [12] reported an association between the pH and titratable acidity with iron uptake by the tooth structure in use of five iron supplements. Also, they showed that all iron drops were relatively acidic, and Irofant iron drop had a lower pH than the remaining four iron drops. The acidity of iron drops can cause dental erosion. Thus, acidic iron drops can decrease the enamel microhardness and have the potential to cause dental erosion.

Sideral iron drop was also evaluated in the present study. The results showed a reduction in enamel microhardness after exposure to Sideral iron drop by 8%. However, this reduction was minimum among the three groups (P=0.0001). To date, no previous study has evaluated the microhardness of primary enamel exposed to Sideral. Iron supplements contain iron often in the form of ferrous or ferric, and cause adverse gastrointestinal effects due to poor absorption of iron and the unabsorbed ions. Sideral iron drop contains Sucrosomial iron, and thus, it does not cause the gastrointestinal problems associated with supplemental iron intake [25]. In Sucrosomial iron formulation, the ferric pyrophosphate is protected by a phospholipid membrane similar to the structure of intestinal cells. Thus, iron is easily absorbed by the intestinal lining. By doing so, the absorption of iron is enhanced with minimal gastrointestinal complications, and tooth staining in children is also prevented [25]. Moreover, the pH of Sideral iron drop is 4.33 and its titratable acidity is 0.85; thus, it can be considered a weak acid. Although Sideral contains citric acid according to the information provided by the manufacturer, it appears that the resultant structural changes in the enamel are minimal following its consumption due to the presence of milk proteins and tricalcium phosphate in its composition. The present results showed that dilution of Irofant iron drop with natural apple juice decreased

enamel microhardness by 18%; however, this reduction was significantly smaller than that caused by pure Irofant iron drop (P=0.0001). To the best of the authors' knowledge, no previous study has investigated the effect of diluted iron drops on enamel microhardness. Clinicians often recommend the consumption of iron along with fruit juice, particularly orange juice, to enhance iron absorption [6,7]. Since there is no difference in iron absorption between the use of iron drops with apple juice or orange juice [26], and the fact that daily consumption of apple juice is recommended for infants starting at 6 months of age [7], we used natural apple juice with a pH of 4.28 to dilute the iron drop. It appears that natural apple juice increases the pH and decreases the titratable acidity of Irofant iron drop, resulting in smaller reduction in enamel microhardness. It should be noted that artificial fruit juices are not suitable for dilution of iron drops due to their acidic nature [27].

One strength of the present study was assessment of enamel microhardness following exposure to Sideral iron drop and a combination of Irofant iron drop and natural apple juice for the first time. Also, the pH and titratable acidity of the solutions were measured in this study as important factors affecting the enamel microhardness.

One limitation of this study was the fact that the results cannot be well generalized to the clinical setting. In this study, similar to previous studies [3,10], each tooth was exposed to 15 drops for 5 minutes. However, it is not known whether exposure to iron drops for 5 minutes can well simulate the situation in the clinical setting. Moreover, in use of supplements with high titratable acidity, stimulation of saliva flow and the buffering capacity of the saliva should also be taken into account due to the presence of additional citric acid in their composition. Further studies are required to assess whether long-term use of such supplements can increase the risk of dental erosion.

CONCLUSION

The current results showed that Irofant iron drop significantly decreased the enamel microhardness. Sideral iron drop with its Sucrosomial iron had minimal adverse effect on primary enamel microhardness, and can be a good alternative to Irofant iron drop. Also, iron drops can be diluted with natural apple juice to decrease their adverse effects on primary enamel microhardness.

CONFLICT OF INTEREST STATEMENT

None declared.

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