

## Original Article

# Relationship of pH and the viscosity of five different iron supplements with the absorption of iron ions and enamel discoloration in the anterior primary teeth (an *in vitro* study)

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## ABSTRACT

**Background:** Various iron drops are prescribed to children to prevent iron-deficiency anemia. The current study aimed to evaluate and compare the physicochemical profile of iron drops and the effect of these medicines on the color of primary teeth.

**Materials and Methods:** On the commencement of this experimental study, the pH and viscosity of five types of iron drops were measured. For the purpose of the current study, sixty healthy anterior deciduous teeth were provided; thereafter, they were assigned to five groups and then exposed to iron drops. The color and color difference of each specimen were measured by VITA Easyshade Compact after 2 weeks. The amount of absorbed iron was determined by atomic absorption. The data were analyzed using two-way ANOVA and Tukey's test ( $P < 0.05$ ).

**Results:** As evidenced by the obtained results, all medications demonstrated acidic pH and discoloration. The viscosity values of iron drops were reported to be within the range of 2.07–33.58 cP. Based on the results of Pearson's correlation coefficient test, discoloration showed a correlation with pH and atomic absorption ( $P < 0.05$ ).

**Conclusion:** Analyzed iron drops displayed low pH and discoloration higher than 3.3 which can be easily distinguished with naked eyes. Medicine labels should include warning statements on the feasibility of dental discoloration and erosion.

**Key Words:** Deciduous tooth, iron, pH, tooth discoloration, viscosity

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## INTRODUCTION

Iron deficiency is the most widespread and prevalent nutritional deficiency that affects more than two billion people worldwide.<sup>[1]</sup> The prevalence of iron-deficiency anemia in children under 5 years of age in Iran has been reported as 18%–38%.<sup>[2]</sup> Iron deficiency in children leads to decreased work productivity, poor cognitive performance, delayed

psychomotor development, and increased morbidity due to infectious diseases.<sup>[3,4]</sup>

In the first 6 months of life, the neonates rely on the iron stores which occurred during pregnancy. Iron supplements in the form of syrups or drops are generally administered to 6–24-month-old babies.<sup>[5]</sup>

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As a national health policy to prevent iron-deficiency anemia, 6–24-month-old babies nowadays receive 15 drops of iron.

Parents typically worry about extrinsic discoloration commonly observed on deciduous teeth and the popular misconception about the association between the iron supplement and dental caries or enamel decalcification. These adverse effects of the iron supplement are reported to be the most notable reasons for the inadequate or irregular intake of iron supplementation in children.<sup>[1,6]</sup> In addition, some parents use low-dose iron in an effort to minimize tooth staining; nonetheless, low-dose iron is not effective in the prevention of anemia.<sup>[7,8]</sup>

Several studies have pointed to the protective effect of iron on enamel demineralization.<sup>[9–11]</sup> Nevertheless, in our previous study, we evaluated the pH and titratable acidity of iron supplementation and found that ferrous sulfate has acidic content and may help to increase the pH of the drop after consumption. These medicines may result in erosion of teeth.<sup>[12]</sup>

Viscosity is the resistance of a fluid to the application of shear stress; in other words, it is the friction resistance of a liquid against flowing or slipping the layers when subjected to shear stress.<sup>[13]</sup> Numerous studies have highlighted the effect of the viscosity of liquids on their absorption. It is worthy to note that oral clearance depends on the viscosity of medicine. Medicines with high levels of viscosity penetrate and stick to teeth and inner mouth surface; therefore, they remain on the tooth surface for a longer period of time.<sup>[14,15]</sup> These products may penetrate fissures and proximal areas of teeth; consequently, their harmful effects increase in direct contact with teeth.<sup>[16]</sup>

The present study aimed to investigate the effect of the viscosity of the iron supplements on the iron ion

absorption and enamel discoloration in the anterior primary teeth.

## MATERIALS AND METHODS

In this experimental study, the viscosity and pH of the five iron drops [Table 1] were initially identified. Viscosity measurements of iron supplements were carried out at the temperature of 25°C by viscometer (Cannon Capillary, USA). The pH of iron drops was evaluated using pH and mV meter (Benchtop Behineh® sat 2002).

A number of sixty anterior primary teeth were used in this *in vitro* study. All the collected teeth were extracted due to space discrepancy, mobility, or trauma. There were no developmental anomalies, enamel hypoplasia, restorations, and extrinsic or intrinsic stains in the selected teeth. The sterilization of the teeth was performed by immersion in 10% formalin for 24 h. For the preparation of specimens, the teeth were dissected at the cemento-enamel junction, and the pulpal residues in the pulp chamber were completely removed. The pulp chamber was then filled with composite resin. A label was placed on the buccal surface of prepared teeth measuring 0.5 cm × 0.5 cm. The entire area surrounding the label was coated with nail polish (Le chic, USA). The label was then removed and the glue residues were washed off with gauze and water. Tooth shades were measured at the baseline using a VITA® Easyshade® Compact (Model DEASYCHP, VITA Zahnfabrik, Bad Sackingen, Germany). The VITA Easyshade was calibrated using its calibration block, according to the manufacturer's instructions; thereafter, the color of the specimens was measured. To obtain accurate measurements, the probe tip was then inserted perpendicular at the center of each specimen and flushed into the surface of the specimens. A three-dimensional color

**Table 1: Name, type, and manufacturer of the tested iron drop**

Description	Country and company	Group label	Product name (iron drops)
30 ml iron drops, each ml contains: An ideal level of 15 mg of elemental iron in the preferred iron form of 75 mg ferrous sulfate and 12.5 mg of ascorbic acid	Vitabiotics, Britain	A	Feroglobin® drops
30 ml iron drops, each ml contain 7 mg Sucrosomial iron, the amount per serving is NRVs percent	BSK, Iran	B	Liposofer® drops
15 ml iron drops, each ml contains: 125 mg of ferrous sulfate. 7 H <sub>2</sub> O (equal to 25 mg Fe <sup>2+</sup> ) and 1.5 m of saccharin sodium (as a sweetener)	Behsa Pharmaceutical, Iran	C	Ferrous sulfate Behsa®
15 ml iron drops, each ml contains: 125 mg of ferrous sulfate. 7 H <sub>2</sub> O (equal to 25 mg Fe <sup>2+</sup> ) and 1.5 m of saccharin sodium (as sweetener)	Shahre Daru, Iran	D	Ferbolin® oral drops

NRV: Nutrient reference value; BSK: Bonyan Salamat Kasra

space containing lightness ( $L^*$ ), red-green ( $a^*$ ) and yellow-blue ( $b^*$ ) components are represented by the CIE  $L^*a^*b^*$  system.

$\Delta E(L^*a^*b^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ , where

$\Delta L^*$  is the difference between the  $L^*$  values

$\Delta a^*$  is the difference between the  $a^*$  values

$\Delta b^*$  is the difference between the  $b^*$  values

The color variation ( $\Delta E$ ) measurements were carried out three times for each sample between before immersion (T0) and 1<sup>st</sup> week (T1) ( $\Delta E$  1<sup>st</sup> week), between T1 and 2<sup>nd</sup> week (T2) ( $\Delta E$  2<sup>nd</sup> week), and between T0 and T2 ( $\Delta E$  total 2 weeks), and the average was recorded.

After the measurement of color values, the specimens of each group were immersed in 10 mL of iron drop and distilled for 3 h per day at 37°C during the test period. The samples were stored in artificial saliva during immersion periods.<sup>[17]</sup> Before the commencement of each test, all the test solutions were daily refreshed. On the completion of the immersion time, the samples were rinsed under running distilled water and air-dried. Using the same method under the same conditions and in the same manner, the color measurements were recorded after 1 and 2 weeks of experiment. Thereafter, the samples were immersed in hydrochloric acid 2M for 24 h. During this period, the samples were occasionally shaken. After the removal of teeth from the solution, the level of iron absorption in each group and the intact one were determined by atomic absorption in 248.3 nanometers longwave and resolution of 0.062 ppm.

Kolmogorov–Smirnov test was applied to test the normal distribution of the data. Intergroup mean and standard deviations were analyzed using ANOVA, and statistical significance was analyzed using Tukey's *post hoc* honestly significant difference test.  $P = 5\%$  was considered statistically significant.

## RESULTS

Table 2 depicts the mean  $\Delta E$  values of specimens at different times and media. The evaluation of mean  $\Delta E$  rate among groups revealed that color changes were clinically visible ( $\Delta E > 3$ ).

The comparison of mean T2  $\Delta E$  values among different groups indicated that the  $\Delta E$  of the Ferbolin group was significantly higher, as compared to that

**Table 2: Comparison of mean±standard deviation  $\Delta E$  value of all the groups among the five iron drops using ANOVA test**

Groups	$\Delta E$ 1 <sup>st</sup> week	$\Delta E$ 2 <sup>nd</sup> week	$\Delta E$ total 2 weeks	<i>P</i>
A	12.44±4.84	6.88±4.21	13.37±4.39	0.00
B	11.03±4.71	6.79±2.93	8.58±4.15	
C	11.36±6.00	5.22±2.60	12.43±4.51	
D	21.79±6.51	4.95±2.87	22.11±5.34	
E	7.73±4.03	5.48±4.86	9.27±4.37	

of other groups ( $P < 0.05$ ). Moreover, the lowest  $\Delta E$  values were detected in the Lipofer group. Nonetheless, no significant difference was observed among A, B, C, and E groups.

The repeated measures ANOVA was used to examine the progression of discoloration in each group suggesting significant differences across time intervals ( $P = 0.00$ ).

The values of pH and viscosity were reported to be within the ranges of 2.54–4.68 and from 2.07 to 33.58 cP, respectively. It was found that the mean values of pH and viscosity of iron drop were significantly different between iron drops ( $P = 0.00$ ). Sideral iron drops demonstrated the highest pH and the lowest viscosity in comparison to other kinds of drops. The results of Pearson's correlation coefficient test pointed to a correlation between pH and T2  $\Delta E$  values ( $P < 0.05$ ); in other words, a decreased pH increased discoloration. The results of iron absorption manifested themselves as significant differences among the groups. In addition, T2  $\Delta E$  values demonstrated a correlation with pH and atomic absorption, according to Pearson's correlation coefficient ( $P < 0.05$ ).

## DISCUSSION

Iron drop supplements are recommended for all children in Iran. Nonetheless, the discoloration of teeth after the administration of oral iron supplements reported in over half of the children who take such supplements is a matter of concern to parents.<sup>[6,18]</sup> Apart from discoloration, the physicochemical profile of these medicines is an influential factor that can affect oral health. Some agents are added to the formulation of these drops in an effort to improve their appearance, bioavailability, stability, and palatability.<sup>[15]</sup> To make these medicines more flavorful to children, acidic contents are also added which typically serve as buffering agents. These

agents preserve chemical stability, monitor tonicity, and verify physiological compatibility.<sup>[19]</sup>

As illustrated by the results, the Ferbolin group displayed a significant change in  $\Delta E$  after 2 weeks, while the color change was not significant in other groups. This can be attributed to the difference in the dosage of iron in drops. In the present study, the same volume of supplements was used; however, the iron content of the drops was not equal. Moreover, higher doses of iron were detected in Ferbolin, and reduced iron content in drops leads to less tooth color change; therefore, Lipofer drop showed the lowest discoloration.

In addition, liposomal iron (Lipofer) is a recent oral iron with high bioavailability and gastrointestinal absorption and a low incidence of side effects since it is not in direct contact with intestinal mucosa.<sup>[20]</sup> Oral liposomal iron was highly compatible and tolerated, as compared to other oral iron salts.<sup>[21]</sup> The noticeable feature of liposomal iron may be the absence of common side effects of conventional oral iron supplementation, such as discoloration of the mucous and teeth.

Numerous studies have pointed to the discoloration effect of iron on teeth.<sup>[22-24]</sup> In a current study, the color assessment was carried out using CIELAB color space. This system is used to measure color differences perceived by humans. In the present study, VITA Easyshade was applied since the reliability and accuracy values of this test were >90% as reported by Kim-Pusateri *et al.*<sup>[25]</sup> Based on the same study, an increase was observed in discoloration with the reduction of pH and increased absorption of iron. The results of the present study confirmed the results of studies conducted by Shabzندهdar who found that the absorption of iron increased in etched teeth. It can be ascribed to increased surface leading to more change in color which was most prominent in Iranian iron drop.<sup>[26]</sup>

Low endogenous pH of iron drops greatly contributes to dental erosion and could reduce the hardness of deciduous enamel.<sup>[27-29]</sup> The obtained results indicated that pH values of iron drops were within the range of 2.54–4.68. Acidic pH below 5.5 has been recognized as an indicator of dental erosion. Since primary tooth enamel is less mineralized than permanent tooth enamel, it is prone to such adverse effects as dental erosion and staining of tooth surface due to frequent usage of these low pH liquid medications.<sup>[30]</sup>

Dental erosion in primary teeth is almost three times more common, as compared to permanent teeth, and it is highly correlated with caries experience in children.<sup>[31]</sup> Xavier investigated the physicochemical characteristics of some pediatric medications and suggested the cariogenic and erosive potential of these medicines.<sup>[32]</sup> Valinoti *et al.* studied the formulations of pediatric antibiotics and revealed that these medicines, especially their frequent administration, contribute to the development of dental caries and dental erosion.<sup>[15]</sup> Pasdar reported on the reduction of hardness caused by iron and multivitamin drops.<sup>[33]</sup> Therefore, iron drop takers should be recommended to rinse their mouth immediately after iron drop ingestion and delay toothbrushing.

Martinhon *et al.* performed a study on the effect of ferrous sulfate on the reduction in demineralization of enamel blocks and the alteration of ionic composition of the formed biofilm. They demonstrated that in a cariogenic environment, ferrous sulfate significantly decreased enamel demineralization, and the percentage of surface microhardness alters in enamel blocks.<sup>[9]</sup> Peres used 10 mmol/lit concentration of ferrous sulfate in mouth rinse form and observed that exposure to ferrous sulfate after the development of erosion can reduce the dissolution of enamel and dentin structure.<sup>[34]</sup> Some studies have previously pointed to the inhibitory effect of iron on caries development;<sup>[10,11,35]</sup> however, it seems that the low pH of drops which contain iron can lead to dental erosion.

Viscosity was another factor which was investigated in the present study. The values of iron drop viscosity ranged from 2.07 to 33.58 cP. The result of Pearson's correlation coefficient revealed no correlation between viscosity and discoloration ( $P > 0.05$ ). Oral clearance rate of drops depends on their property of adherence to the enamel. Numerous studies conducted on the viscosity of drugs have indicated that liquid medications possess high viscosity. They reported that these liquid tend to retain on the tooth surface for a longer period of time and have a slow salivary clearance rate leading to enamel erosion and further adverse consequences.<sup>[15,36]</sup> This inconsistency between our results and the abovementioned findings can be attributed to the difference in the viscosity of iron drop that was low in comparison to other evaluated drugs.

The present study was conducted in laboratory conditions. It is important to take into consideration

oral environment is different from invitro conditions and it can alter the side effect of iron drops. In addition, clinical studies are necessary to determine the real extension of the iron drops problem.

## CONCLUSION

As evidenced by the results of the present study, analyzed iron drops displayed low pH and discoloration.  $\Delta E$  value in all groups was higher than 3.3 that can be easily distinguished with naked eyes. Children's medicine labels should contain warning statements on the feasibility of cause dental discoloration and erosion. The implementation of this advice lowers the risk of dental demineralization.

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## Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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