



Color Change of Primary Teeth Following Using 4 Types of Iron Supplements Available in the Iranian Pharmacopeia

Soudeh Tayebi¹, Mohammad Esmailzade², Loghman Soufi Rezai³, Farnoush Fotovat^{4*}, Roya Najafi Vosogh⁵, Niloufar Faregh⁶

Abstract

Background: Iron supplementation plays an important role in the growth and development of children. However, iron causes persistent discoloration of primary teeth, which creates some concerns for the parents. This study aimed to assess the color change of the primary teeth following the use of four types of iron supplements available in the Iranian pharmacopeia.

Methods: In this in vitro experimental study, 60 primary incisors (120 tooth surfaces) with intact crowns were collected and randomly divided into 5 groups (1 control and 4 experimental groups). The color of the teeth was then measured at baseline (time 0) and 24, 48, 72, and 96 hours after the immersion in solutions containing 250 mL of artificial saliva in the control group and artificial saliva plus iron supplements containing 100 mg of iron in the experimental groups using the Vita Easy Shade Compact. Finally, the data were analyzed using the ANOVA test and pairwise comparisons were made using Tukey's and least significant difference tests via SPSS, version 23.

Results: The primary teeth showed a significant color change after 24 and 48 hours of immersion in the solutions ($P < 0.05$) but no significant change was noted after 72 and 96 hours of immersion ($P > 0.05$).

Conclusions: In general, the color change of the primary teeth was not significantly different following exposure to the four iron supplements. Eventually, the Iranian and foreign-made iron supplements caused a similar color change in the teeth.

*Correspondence to

Farnoush Fotovat, Assistant Professor, Department of Prosthodontics, Hamadan University of Medical Sciences, Hamadan, Iran
Tel:09188185838, Email: ffovotat67@yahoo.com

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Background

Adequate nutrition plays a major role in physical growth and development. In addition, nutritional requirements may vary depending on body size, physical activity, and health status. Iron is considered as an important element that is required by the human body (1) and is found in all viable tissues including the enamel, dentin, and dental pulp (2-4). Children also need iron for growth and development, as well as the synthesis of red blood cells. The highest amount of iron in the human body is found in hemoglobin present in red blood cells, which has the important task of carrying oxygen from the lungs to the tissues (4-6).

Further, iron supplements in Iran are often in the form of iron drops. However, tooth discoloration is a common complication of iron drop consumption (3,7-9). Edible iron compounds available in the Iranian market are

Highlights

- ▶ Iranian and foreign made supplements have similar effect on teeth color.

mainly in the form of ferrous sulfate, which has a bad taste and causes the black discoloration of the primary teeth (4,10) and is unpleasant for the children and parents (11,12).

It is believed that the extrinsic staining of the teeth or dental plaque, which is highly prevalent in children taking iron supplements, has a metallic origin (13). Iron uptake is less by the sound surfaces of the primary teeth following the use of iron drops, and therefore, less discoloration occurs on such surfaces. However, by an increase in the surface energy of the teeth due to the development of white spot lesions or caries on enamel surfaces, the amount of iron

¹Assistant Professor, Department of Pediatrics, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran

²Pedodontist, Tehran, Iran

³Associated Professor, Department of Operative and Aesthetic Dentistry, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran

⁴Assistant Professor, Department of Prosthodontics, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran

⁵PhD Candidate, Department of Biostatistics, Faculty of Health, Hamadan University of Medical Sciences, Hamadan, Iran

⁶Dentist, Hamadan, Iran

uptake and the magnitude of discoloration would increase accordingly (14).

On the other hand, the iron inhibits the progression of dental caries since it increases enamel resistance. Furthermore, iron salts decrease acid production by oral bacteria, as well as the count of *Streptococcus mutans* in the dental biofilm (13,15).

The staining of the primary teeth is regarded as a common concern of parents since it can decrease social communications and the self-esteem of the children due to the dark, unaesthetic appearance of the teeth. Such discolorations can also be mistaken for caries. Similarly, the lack of knowledge about such discolorations is a common cause of frequent dental visits of parents to pediatric dentists (2,7,14,16,17).

The three main characteristics of the color include the hue, chroma, and value (18). Several methods are available for the assessment of the degree of tooth discoloration following staining. In the present study, the Vita Easy Shade Compact (DEASYCUBU, Germany) was used for this purpose (2).

In addition, 4 commonly used types of iron supplements available in the Iranian pharmacopeia were evaluated, the details of which are provided in Table 1.

Iron drops are routinely prescribed for children by pediatric physicians. However, the resultant discoloration of the primary teeth is of great concern for the parents and leads to their frequent dental visits. In some cases, the parents discontinue iron drop consumption by their children since they believe that it causes dental caries while it is critical for the growth and development of their children. Thus, to find an iron supplement with the minimal discoloration of the primary teeth, this study aimed to compare the color change of the primary teeth following utilizing four commonly used types of iron drops that are available in the Iranian pharmacopeia.

Materials and Methods

In this in vitro, experimental study, 4 iron supplements were evaluated, including Ferbolin (ShahrDarou, Sharyar, Iran), Ferrodrops (DonyayeBehdasht, Sharyar, Iran), Irovit (Vitane, Germany), and Ferroglobin (Vitabiotics, UK). Moreover, 60 extracted sound primary incisors (120 surfaces) were collected from different pediatric dental clinics in Hamadan and stored in artificial saliva. The

teeth were cleaned by the pumice paste (Rotec GmbH and Company, KG, Germany) and prophylactic brush and sectioned mesiodistally. All teeth were then mounted in acrylic resin and the entire tooth surface was coated with nail varnish except for a square-shaped window at the middle third of the crown measuring 2x2 mm. The color of the teeth was measured using the Vita Easy Shade Compact (DEASYCUBU, USA)

The sample size was calculated to be 24 teeth in each group (a total of 120 teeth) according to a previous study by Shabzندهدار et al (12) and assuming type one error of 5% and the power of 90%.

Next, the teeth were randomly divided into 5 groups (1 control and 4 experimental groups). The control samples were immersed in 250 mL of the artificial saliva. Additionally, the samples in the experimental groups were immersed in solutions containing 250 mL of artificial saliva and iron supplements containing 100 mg of the pure iron (6.6 mL, 4 mL, 4 mL, and 6.6 mL of Irovit, Ferbolin, Ferrodrops, and Ferroglobin, respectively). The teeth were removed from the solutions after 24, 48, 72, and 96 hours (2) and rinsed with artificial saliva. The tooth color was measured using the Vita Easy Shade Compact (DEASYCUBU, USA). In addition, the fresh solutions were prepared and the teeth were immersed in fresh solutions. The color of teeth was measured again at the designated time points, followed by determining the amount of the color change (ΔE) using the following formula (1).

$$\Delta E = \left[(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2 \right]^{1/2}$$

The color of the teeth was determined by measuring a^* , L^* , and b^* parameters in CIE Lab color space. The L^* , a^* , and b^* indicate darkness/lightness, redness (+)/greenness (-), and yellowness (+)/blueness (-), respectively (19).

The amount of $\Delta E > 1$ refers to a difference in the color which is detectable by a minimum of 50% of the observers. Further, $\Delta E > 3.7$ is defined as a clinically unacceptable color change (19).

The normal distribution of data and the homogeneity of variances were evaluated using the Kolmogorov-Smirnov and Levene tests, respectively. In the case of the normal distribution of the data and the equality of variances, the color change in each group at 24, 48, 72, and 96-hour time points was analyzed using the ANOVA test. On the other

Table 1. Composition of the understudy products.

Name	Ingredient/1 mL	Company/Country	Other Properties
Ferbolin	125 mg of ferrous sulfate heptahydrate	Shahr Daru, Sharyar, Iran	Contains 1.5 mg of sodium saccharine
Ferrodrops	125 mg of ferrous sulfate heptahydrate	DonyayeBehdasht, Sharyar, Iran	Contains 1.5 mg of sodium saccharine and 0.01 mg of ascorbic acid
Irovit	75 mg of ferrous sulfate	Vitane, Germany	Favorable taste
Ferroglobin	10 mg of ferrous sulfate	Vitabiotics, UK	contains vitamin B1, B2, B6, B12, C, folic acid, zinc, iron, copper, and calcium

hand, the non-parametric Kruskal-Wallis test was applied in the case of the non-normal distribution of data and the inequality of the variances. Furthermore, the Tukey test, the least significant difference (LSD) test, independent t-test, and the Mann-Whitney test were used for pairwise comparisons.

Moreover, the repeated measures ANOVA was applied to assess the color change of the teeth at the four-time points. To use this test, the normal distribution of data was evaluated using the Kolmogorov-Smirnov test and the sphericity assumption was evaluated using the Mauchly test. Finally, the Friedman test was applied if the assumptions were violated, followed by using the Tukey's and LSD tests for pairwise comparisons.

Results

Given that the color change data in the Ferroglobin group did not have a normal distribution at 24 hours, the Kruskal-Wallis test was used for the comparison of a color change among the 5 groups, which showed a significant difference in this regard ($P < 0.05$). Likewise, the independent t-test was then used for pairwise comparisons. As shown in Table 2, significant differences were observed between the control and Irovit, control and Ferbolin, as well as control and Ferrodrip groups ($P < 0.05$). However, the difference between Irovit and Ferbolin, Irovit and Ferrodrip, and finally, Ferbolin and Ferrodrip groups was not significant ($P > 0.05$).

The results of the Mann-Whitney test (Table 3) revealed a significant difference in the color change between the control and Ferroglobin, as well as Irovit and Ferroglobin groups ($P < 0.05$). On the other hand, no significant difference was found between Ferbolin and Ferroglobin, as well as Ferrodrip and Ferroglobin groups ($P > 0.05$). Considering that both assumptions were met, the ANOVA test was applied to compare the groups at 48 hours, indicating a significant difference in the color change of the five groups at 48 hours ($P < 0.05$). Contrarily, no significant difference was noted in this regard among the five groups at 72 and 96 hours ($P > 0.05$).

Table 4 shows the mean and standard deviation (SD) of the color change in the 5 groups at different time points. At 24 and 72 hours, the minimum color change was observed

Table 2. Results of the independent *t* test for the pairwise comparisons of groups regarding the color change at 24 hours

Groups	P Value
Irovit-control	0.001
Ferbolin-control	0.0001
Ferrodrip-control	0.001
Irovit-Ferbolin	0.460
Irovit-Ferrodrip	0.214
Ferrodrip-Ferbolin	0.303

Table 3. Results of the Mann-Whitney Test for the Pairwise Comparisons of Groups Regarding the Color Change at 24 Hours

Groups	P Value
Control-Ferroglobin	0.010
Irovit-Ferroglobin	0.027
Ferbolin-Ferroglobin	0.050
Ferrodrip-Ferroglobin	0.353

in the control group. Among the experimental groups, the minimum color change was noted in the Ferbolin whereas the maximum color change was found in the Ferroglobin group. At 48 hours, the minimum color change was noted in the control group. Among the experimental groups, the minimum color change was detected in the Irovit group while the maximum color change was noted in the Ferroglobin group. Finally, the minimum color change was observed in the control group at 96 hours. Among the experimental groups, the minimum color change was noted in the Ferbolin whereas the maximum color change was found in the Ferrodrip group.

The mean (SD) of the color change of the teeth at 0, 24, 48, 72, and 96 hours revealed that the color of the primary teeth remained the same at all time points in the control group.

The results demonstrated that long-term use of iron supplements caused a greater color change considering the normal distribution of the data at all time points in the Irovit, Ferbolin, and Ferrodrip groups and the mean color change (Table 4) at different time points. The repeated measures ANOVA showed a significant difference in the color at 0, 24, 48, 72, and 96 hours in the Irovit, Ferbolin, and Ferrodrip groups ($P < 0.05$). Additionally, the pairwise comparisons by the LSD test represented significant differences in this respect at different time points ($P = 0.0001$).

Table 4 presents the mean (SD) of the color change of the teeth at 0, 24, 48, 72, and 96 hours in the Ferroglobin group. The results indicated a greater color change in the long-term use of the Ferroglobin. Based on the results of the Kolmogorov-Smirnov test, the data were not normally distributed in this group at 96 hours. Thus, the Friedman test was applied, which showed a significant difference in the color change of the teeth in the Ferroglobin group at different time points ($P < 0.05$). Therefore, pairwise comparisons were made using the Wilcoxon test, which revealed significant differences in all pairwise comparisons ($P = 0.0001$).

Figure 1 illustrates the mean ΔE in the 5 groups at different time points.

Discussion

Physical and mental development is a dynamic process. In addition, adequate nutrition plays a fundamental role in physical growth and development although nutritional

Table 4. Descriptive Results of the Color Change in the Five Groups at Different Time Points

	Time (h)			
	24	48	72	96
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Control group	7.7688 (3.17586)	7.7688 (3.17586)	7.7688 (3.17586)	7.7688 (3.17586)
Ferbolin group	8.4167 (3.19805)	9.7125 (3.24242)	10.9708 (3.19354)	12.6792 (3.20176)
Irovit group	8.6292 (2.46673)	9.6667 (2.43162)	11.1250 (2.08874)	12.9042 (2.23130)
Ferrodrip group	9.4042 (3.36833)	10.6583 (3.26802)	11.5083 (3.25308)	13.4125 (2.77384)
Feroglobin group	10.1750 (2.84899)	11.0000 (2.87750)	12.2333 (2.47591)	13.3042 (2.51905)
P value	0.0001	0.003	0.167	0.837

Note. SD: Standard deviation.

requirements may vary relying on the body size, physical activity, and health status. Iron is an essential element required by the human body (16) and is found in all viable tissues. Similarly, iron has a confirmed role in the growth and development, as well as the synthesis of the red blood cells and the transfer of oxygen from the lungs to the tissues (2-6). Further, a significant correlation was reported between iron deficiency and dental caries (8,13,19-23). Furthermore, iron supplements in Iran are mainly prescribed as the iron drops. Tooth discoloration is a major complication of using iron drops. On the other hand, edible iron compounds available in the Iranian market are mainly in the form of ferrous sulfate, which has a bad taste and leads to the black discoloration of the primary teeth that is unpleasant for the children and parents (11,15).

Tooth discoloration following iron supplementation may be due to superficial stains, also known as metallic stains, which can be easily removed or may penetrate deep into the tooth structure, leading to a persistent color change, which is not easily removable.

In general, iron leaves brown stains but the iron drops cause black stains due to the deposition of the iron sulfide

(24,25).

Color is composed of red, green and blue lights and color perception depends on the following 3 factors (18): (I) the observer, (II) the object and (III) the light source. Any change in any of these three factors can alter the color perception.

Evidence shows greater tooth discoloration following iron supplementation in areas with demineralized or porous enamel due to caries. Iron can be washed off from the outermost enamel surface thus it appears that this element is physically absorbed by the tooth structure and causes discoloration associated with the biofilm formation (3).

Considering the high prevalence of tooth discoloration following iron supplementation and the parents' concerns in this respect, which may lead to the discontinuation of the use, the present study aimed to find an iron supplement with the minimal discoloration of the primary teeth among four commonly used types of iron drops that are available in the Iranian pharmacopeia.

In this study, 60 extracted primary anterior teeth (120 tooth surfaces) with intact crowns were collected and randomly classified into five groups (one control and four experimental groups). The color of the teeth was measured at baseline (time 0) and 24, 48, 72, and 96 hours after the immersion in solutions containing 250 mL of artificial saliva in the control group and artificial saliva plus iron supplements containing 100 mg of the iron in the experimental groups using the Vita Easy Shade Compact. This device has high accuracy and reliability for color measurement. The other advantages of this device include high precision, the reproducibility of measurements, the provision of objective data, the easy use, the direct transfer of data, and cost-effectiveness (26,27). The results showed that the teeth exposed to Ferbolin for 96 hours experienced less discoloration compared to the other iron supplements. Ferrodrip caused the maximum color change. At 96 hours, the color change increased in the experimental groups but it was not significant. The mean ΔE in Ferrodrip, Feroglobin, Irovit and Ferbolin groups was 5.86, 5.49, 5.24, and 4.91 at 96 hours, respectively.

Some previous studies assessed the effect of iron

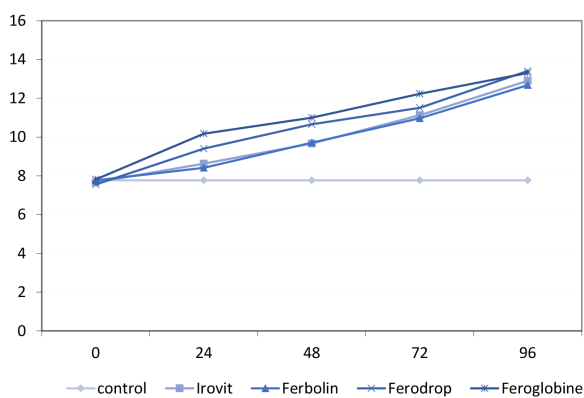


Figure 1. The Mean Color Change in the Five Groups at Different Time Points.

supplementation on the color of primary teeth. For example, Pani et al (2) compared the discoloration potential of the primary teeth exposed to two types of iron syrups, namely, ferrous fumarate (FF) and ferric oxide polymaltose (FOP). They found that using a combination of different forms of iron decreased the severity of staining compared to the equivalent dose of each iron supplement. In addition, FOP caused greater discoloration than FF. The mean ΔE in FF plus FOP, FF, and FOP groups changed by two, three, and four units after 72 hours, respectively. Our findings are in line with the results of the above-mentioned study since the mean ΔE increased in both studies. The mean ΔE was not significantly different among the groups in our study while this difference was significant in the above-mentioned study. Finally, the assessment time points, the device used for color assessment, and the methodology were the same in both studies but different iron supplements were evaluated in our study compared to theirs (2).

Similarly, Mehran et al (16) evaluated the effect of two types of iron drops on the color of the primary teeth and greater than that in the teeth subjected to Fer-in-Sol iron drop. The results of their study indicated an increase in ΔE over time, which corroborates with the results of our study. Both studies used ferrous sulfate iron supplements as well. The mean ΔE was not significantly different among the groups in our study while this difference was significant in the above study, which may be due to the difference in other ingredients present in ferrous sulfate iron drops, methodology, and the method of color assessment in the two studies. In the study by Mehran et al, a restorative dentist evaluated the color change visually. They also used ICP (Vista Pro, Australia) to assess the atomic absorption rate. Eventually, they evaluated structural changes using a scanning electron microscope (16).

In another study, Makarem et al (14) compared the color change of the primary enamel following the use of iron drops and showed that the iron drop manufactured by Mashhad School of Pharmacy caused less color change in the primary enamel. The mean ΔE increased over time in both studies. Further, ferrous sulfate supplements were used in both studies. However, the mean ΔE demonstrated no significant difference among the groups in our study while this difference was significant in their study, which is probably due to the difference in other ingredients present in ferrous sulfate iron drops, methodology, and method of color assessment in the studies. In their study, half of the samples were etched with 37% phosphoric acid and the color change was determined applying the scanning electron microscopy.

Considering that the iron compound was ferrous sulfate in all four evaluated supplements in our study, less color change caused by Ferbolin compared to the other supplements may be attributed to the absence of vitamin C because this vitamin has an erosive property and causes enamel porosity. As the result, the surface energy and the

iron uptake by the enamel surface represents an increase. Eventually, the results showed that Feroglobin, Irovit, and Ferdrop containing vitamin C caused a greater color change.

Conclusions

In general, the color change of the primary teeth was not significantly different following exposure to the four iron drops and the Iranian and foreign-made iron supplements led to similar color changes.

Authors' Contribution

Soudeh Tayebi: Study concept-study design, article design
 Mohammad Esmaeilzade: Study concept-study design
 Loghman Soufi Rezai: article design
 Farnoush Fotovat: Manuscript preparation, Editing and review
 Roya Najafi Vosogh: statistical analysis
 Niloufar Faregh: Laboratory procedure

Ethical Statement

This retrospective study was approved by the Ethics Committee of Hamadan University of Medical Sciences ((Ethical approval code: IR.UMSHA.REC.1396.689)).

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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