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)

Navid Babaei¹, Tina Molaei¹, Saeid Belyad², Somayeh Hekmatfar³

¹Dental Students, Student Committee of Research, Faculty of Dentistry, Ardabil University of Medical Sciences, ²Department of Pediatric Dentistry, Faculty of Dentistry, Ardabil University of Medical Sciences, Ardabil, ³Nursing and Midwifery Care Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

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

INTRODUCTION

Iron deficiency is the most widespread and prevalent nutritional deficiency that affects more than two billion people worldwide.¹ The prevalence of iron-deficiency anemia in children under 5 years of age in Iran has been reported as 18%–38%.² Iron deficiency in children leads to decreased work productivity, poor cognitive performance, delayed psychomotor development, and increased morbidity due to infectious diseases.³,⁴

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.⁵

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Babaei N, Molaei T, Belyad S, Hekmatfar S. 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). Dent Res J 2021;18:7.
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. 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. Several studies have pointed to the protective effect of iron on enamel demineralization. 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.

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. 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. These products may penetrate fissures and proximal areas of teeth; consequently, their harmful effects increase in direct contact with teeth.

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 cementoenamel 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 H₂O (equal to 25 mg Fe²⁺) 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 H₂O (equal to 25 mg Fe²⁺) 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 1st week (T1) (\(\Delta E\) 1st week), between T1 and 2nd week (T2) (\(\Delta E\) 2nd 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. 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 of other groups \((P < 0.05)\). Moreover, the lowest \(\Delta E\) values were detected in the Liposofer 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. 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. 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.[19]

As illustrated by the results, the Ferbolin group displayed a significant change in ΔE 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, Liposofer drop showed the lowest discoloration.

In addition, liposomal iron (Liposofer) 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.[20] Oral liposomal iron was highly compatible and tolerated, as compared to other oral iron salts.[21] 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.[22-24] 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.[25] 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 Shabzendehdar 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.[26]

Low endogenous pH of iron drops greatly contributes to dental erosion and could reduce the hardness of deciduous enamel.[27-29] 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.[30] 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.[31] Xavier investigated the physicochemical characteristics of some pediatric medications and suggested the cariogenic and erosive potential of these medicines.[32] 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.[33] Pasdar reported on the reduction of hardness caused by iron and multivitamin drops.[33]

Therefore, iron drop takers should be recommended to rinse their mouth immediately after iron drop ingestion and delay toothbrushing.

Martinho 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.[9]

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.[34] Some studies have previously pointed to the inhibitory effect of iron on caries development, 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.[15,36] 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 in vitro 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. ΔE 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.

**Acknowledgments**

Authors’ deepest appreciation and thanks go to the Research Deputy of Ardabil University of Medical Sciences for financing the current research project.

**Financial support and sponsorship**

Nil.

**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.

**REFERENCES**

1. Pani SC, Alenazi FM, Alotain AM, Alanazi HD, Alasmari AS. Extrinsic tooth staining potential of high dose and sustained release iron syrups on primary teeth. BMC Oral Health 2015;15:90.

2. Bahrami M. Malnutrition and its effects on development in Iranian children. J Pediatr Dis 2004;14:149-56.

3. Gupta PM, Perrine CG, Mei Z, Scanlon KS. Iron, anemia, and iron deficiency anemia among young children in the United States. Nutrients 2016;8:330.

4. Desalegn A, Mossie A, Gedefaw L. Nutritional iron deficiency anemia: Magnitude and its predictors among school age children, Southwest Ethiopia: A community based cross-sectional study. PLoS One 2014;9:e114059.

5. de-Regil LM, Jeffers ME, Sylvestsky AC, Dowswell T. Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. Cochrane Database Syst Rev 2011;11:CD009085.

6. Talebi M, Parisay I, Mokhtari N. The parents’ knowledge and behavior towards the effects of using iron supplements on tooth staining and dental caries in Mashhad, Iran. Dent Res J (Isfahan) 2012;9:715-8.

7. Adcock KG, Hogan SM. Extrinsic iron staining in infant teeth from iron-fortified formula and rice cereal. J Pediatr Pharmacol Ther 2008;13:162-5.

8. Christofides A, Asante KP, Schauer C, Sharieff W, Owusu-Agyei S, Zlotkin S. Multi-micronutrient Sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: A randomized clinical trial. Matern Child Nutr 2006;2:169-80.

9. Martinhon CC, Italiani FM, Padilha PM, Bijella MF, Delbem AC, Buzalaf MA. Effect of iron on bovine enamel and on the composition of dental biofilm formed in situ. Arch Oral Biol 2006;51:471-5.

10. Pecharki GD, Cury JA, Leme AF, Tabchoury CP, Del Bel Cury AA, Rosalen PL, et al. Effect of sucrose containing iron (II) on dental biofilm and enamel demineralization in situ. Caries Res 2005;39:123-9.

11. Eskandarian T, Motamedifar M, Hekmatfar S, Tamaddon AM. Comparison of the effect of three types of iron drops on surface roughness of deciduous teeth in a simulated cariogenic. Environ J Dent Sch 2013;31:15-22.

12. Hekmatfar S, Piranhe H, Jafari K. Evaluation of the relationship between pH and titratable acidity of five different of iron supplements with the absorption of iron ions in the anterior primary teeth (an in vitro study). Dent Res J (Isfahan) 2018;15:367-71.

13. Lv C, Zou D, Qin M, Meng W, Cao Y, Wang W. Hydrodynamic force depends not only on the viscosity of solution but also on the molecular weights of viscoscengs. Langmuir 2013;29:10624-9.

14. Valinoti AC, Pierro VS, da Silva EM, Maia LC. In vitro alterations in dental enamel exposed to acidic medicines. Int J Paediatr Dent 2011;21:141-50.

15. Valinoti AC, da Costa LC Jr., Farah A, de Sousa VP, Fonseca-Gonçalves A, Mata LC. Are pediatric antibiotic formulations potentials risk factors for dental caries and dental erosion? Open Dent J 2016;10:420-30.

16. Cairns AM, Watson M, Creanor SL, Foye RH. The pH and titratable acidity of a range of diluting drinks and their potential effect on dental erosion. J Dent 2002;30:313-7.

17. Afzali BM, Ghasemi A, Mirani A, Abdolazimiz Z, Baghban AA, Kharazifard MJ. Effect of ingested liquids on color change of composite resins. J Dent (Tehran) 2015;12:577-84.

18. Kumar A, Kumar V, Singh J, Hooda A, Dutta S. Drug-induced discoloration of teeth: An updated review. Clin Pediatr (Philad) 2012;51:181-5.

19. Maguire A, Baqir W, Nunn JH. Are sugars-free medicines more erosive than sugars-containing medicines? An in vitro study of paediatric medicines with prolonged oral clearance used regularly and long-term by children. Int J Paediatr Dent 2007;17:231-8.

20. Pisani A, Riccio E, Sabbatini M, Andreucci M, Del Rio A, Visciano B. Effect of oral liposomal iron versus intravenous iron for treatment of iron deficiency anaemia in CKD patients: A randomized trial. Nephrol Dial Transplant 2015;30:645-52.

21. Charytan C, Qunibi W, Bailie GR. Comparison of intravenous iron sucrose to oral iron in the treatment of anemic patients with chronic kidney disease not on dialysis. Nephrol Dial Transplant 2015;30:645-52.

22. Mehran M, Basir MM, Jafari S. Effect of two kinds of iron drops on the discoloration, atomic absorption and structural changes of primary teeth enamel. J Dent Med 2009;21:290-9.

23. Bandon D, Chabane A, Le Gall M. Les colorations dentaires...
Babaei, et al.: Relationship of pH and the viscosity of iron supplements with the absorption of iron ions and enamel discoloration

24. Shojaipour R. Adsorption rate of iron onto primary incisor teeth following the application of three iron drops. J Kerman Univ Med Sci 2010;17:42-8.

25. Kim-Pusateri S, Brewer JD, Davis EL, Wee AG. Reliability and accuracy of four dental shade-matching devices. J Prostheth Dent 2009;101:193-9.

26. Shahzendehtdar M, Makarem A, Orafai H, Khashayarmanesh Z, Ebrahimizadeh S. Comparsion of primary enamel discoloration caused by the use of three different iron drops (an in vitro study). J Mashhad Dent Sch 2006;30:247-54.

27. Neves BG, Farah A, Lucas E, de Sousa VP, Maia LC. Are paediatric medicines risk factors for dental caries and dental erosion? Community Dent Health 2010;27:46-51.

28. Babu KL, Rai K, Hedge AM. Pediatric liquid medicaments – Do they erode the teeth surface? An in vitro study: Part I. J Clin Pediatr Dent 2008;32:189-94.

29. Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential in vitro. J Dent 2005;33:569-75.

30. Mittal S, Singh BP, Sharma AK, Mittal K, Justa A, Vaid P. Surface changes of primary tooth enamel by commonly used pediatric liquid medicaments: A scanning electron microscope study. J Pediatr Dent 2017;5:14.

31. Kazoullis S, Seow WK, Holcombe T, Newman B, Ford D. Common dental conditions associated with dental erosion in schoolchildren in Australia. Pediatr Dent 2007;29:33-9.

32. Xavier AF, Moura EF, Azevedo WF, Vieira FF, Abreu MH, Cavalcanti AL. Erosive and cariogenicity potential of pediatric drugs: Study of physicochemical parameters. BMC Oral Health 2013;13:71.

33. Pasdar N, Alaghehmand H, Mottaghi F, Tavassoli M. Experimental study of iron and multivitamin drops on enamel microhardness of primary tooth. J Int Soc Prev Community Dent 2015;5:518-24.

34. Sales-Peres SH, Pessan JP, Buzalaf MA. Effect of an iron mouthrinse on enamel and dentine erosion subjected or not to abrasion: An in situ/ex vivo study. Arch Oral Biol 2007;52:128-32.

35. Eshghi A, Kowsari-Isfahan R, Rezaiefar M, Razavi M, Zeighami S. Effect of iron containing supplements on rats’ dental caries progression. J Dent (Tehran) 2012;9:14-9.

36. Kale YJ, Nalwade AV, Dahake PT, Dadpe MV, Kendre SB. Effect of different pediatric drug formulations on color stability of composite, zirconia-reinforced glass ionomer cement, and glass ionomer cement. J Indian Soc Pedod Prev Dent 2019;37:151-6.