ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

In Vitro Evaluation of the Effect of Mixing Iron Supplements with Orange Juice on the Surface Properties and Discoloration of Primary Teeth

Demir Takviyelerinin Portakal Suyu ile Karıştırılmasının Süt Dişi Yüzey Özellikleri ve Renk Değişimine Etkisinin Değerlendirilmesi

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Abstract

Introduction: Tooth discoloration is a common side effect of liquid iron supplements used in pediatric patients. This in vitro study aimed to evaluate the effects of ferric polymaltose, ferrous sulfate, and Lipofer® iron supplements, when mixed with orange juice, on the color change and enamel surface roughness of primary teeth.
Materials and Methods: Sixty primary canine teeth were embedded in acrylic resin and randomly divided into six groups. Each supplement was diluted with either orange juice or distilled water. Samples were immersed in the solutions for 5 minutes daily over 28 days. Color change (ΔE_{00}) was measured with a spectrophotometer, and surface roughness was assessed using a profilometer on days 0, 7, 14, 21, and 28.
Results: Among the iron supplement groups, the Lipofer® group showed significantly less discoloration effect ($p<0.05$). Mixing iron supplements with orange juice had no statistically significant effect on primary teeth discoloration and enamel surface roughness ($p>0.05$).
Conclusions: Lipofer® supplementation caused less discoloration compared to other iron preparations. Based on the findings of our study, the administration of iron supplements mixed with orange juice may be recommended to enhance taste tolerance in pediatric patients.

Keywords
Primary teeth, iron supplements, tooth discoloration

Öz

Giriş: Diş renklenmesi, çocuk hastalarda kullanılan sıvı demir takviyelerinin yaygın bir yan etkisidir. Bu in vitro çalışma, ferrik polimaltoz, ferröz sülfat ve Lipofer® içerikli demir takviyelerinin portakal suyu ile karıştırıldığında süt dişlerinin renk değişimi ve mine yüzey pürüzlülüğü üzerindeki etkilerini değerlendirmeyi amaçlamıştır.
Gereç ve Yöntem: Altmış adet süt kanin dişi akrilik rezine gömülerek rastgele altı gruba ayrıldı. Her demir takviyesi, portakal suyu veya saf su ile seyreltilerek kullanıldı. Örnekler 28 gün boyunca her gün 5 dakika boyunca bu solüsyonlara daldırıldı. Renk değişimi (ΔE_{00}) spektrofotometre ile, yüzey pürüzlülüğü ise profilometre kullanılarak 0., 7., 14., 21. ve 28. günlerde değerlendirildi.
Bulgular: Demir takviyesi grupları arasında, Lipofer® grubu anlamlı derecede daha az renklenme etkisi gösterdi ($p<0,05$). Demir takviyelerinin portakal suyu ile karıştırılması, süt dişlerindeki renk değişimi ve mine yüzey pürüzlülüğü üzerinde istatistiksel olarak anlamlı bir etki göstermedi ($p>0,05$).
Sonuç: Lipofer® takviyesi, diğer demir preparatlarına kıyasla daha az renk değişimine neden oldu. Çalışmamızın bulgularına dayanarak, demir takviyelerinin portakal suyu ile karıştırılarak verilmesi, çocuk hastalarda tat toleransını artırmak amacıyla önerilebilir.

Anahtar kelimeler
Süt dişi, demir takviyeleri, diş renklenmesi

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Introduction

Iron deficiency and the resultant iron deficiency anaemia represent a considerable public health problem, affecting a substantial segment of the global population (1). Oral iron supplements are widely used for the prophylactic prevention and treatment of iron deficiency anaemia. In children, ferrous (Fe^{+2}) iron salts, ferric polymaltose (FP; Fe^{+3}) iron complexes and supplements containing sucrosomial and liposomal ferric pyrophosphate are most commonly used to treat the condition (2).

Tooth discoloration and erosion that may occur due to the use of iron supplements in the form of drops and syrups constitute a significant adverse effect of these supplements in children (3,4). In vitro studies have shown that iron supplements containing ferrous sulphate (FS) and FP possess the potential to discolour primary teeth (3,5).

Ascorbic acid (vitamin C) increases the absorption of iron supplements. Therefore, it is recommended that iron-containing drops and syrups be consumed with fruit juices containing vitamin C (6,7). Orange juice (OJ) has a high ascorbic acid content and influences iron absorption (8). Studies on the effect of iron supplements on the physical properties of primary teeth are available in the literature (4,9,10). However, no study has evaluated the effect of combining different iron supplements with OJ on the enamel surface properties and discoloration of primary teeth.

This study aimed to evaluate the effect of FP, FS and Lipofer® (LF) iron supplements mixed with fresh OJ and distilled water (DW) on the colour change and enamel surface roughness of primary teeth in vitro. The null hypothesis of this study was that mixing different iron supplements with orange juice would not have a significant effect on enamel discoloration or surface roughness of primary teeth compared to distilled water.

Materials and Methods

Approval for this in vitro experimental study was obtained from the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (decision no: E-41997688-050.99-130396, date: 11.03.2024).

The power analysis of the study was performed using the G. Power programme (HHU, Düsseldorf, Germany). As established in a preceding study, it was determined that a minimum of eight samples should be incorporated into each group, in accordance with the 95% confidence level (1-α), 95% test power level (1-β), an effect size (d) of 2.017 and a two-way independent samples t-test power analysis (11). The tooth samples were randomly divided into six groups, with 10 teeth in each group. The distribution of the study groups is illustrated in Figure 1.

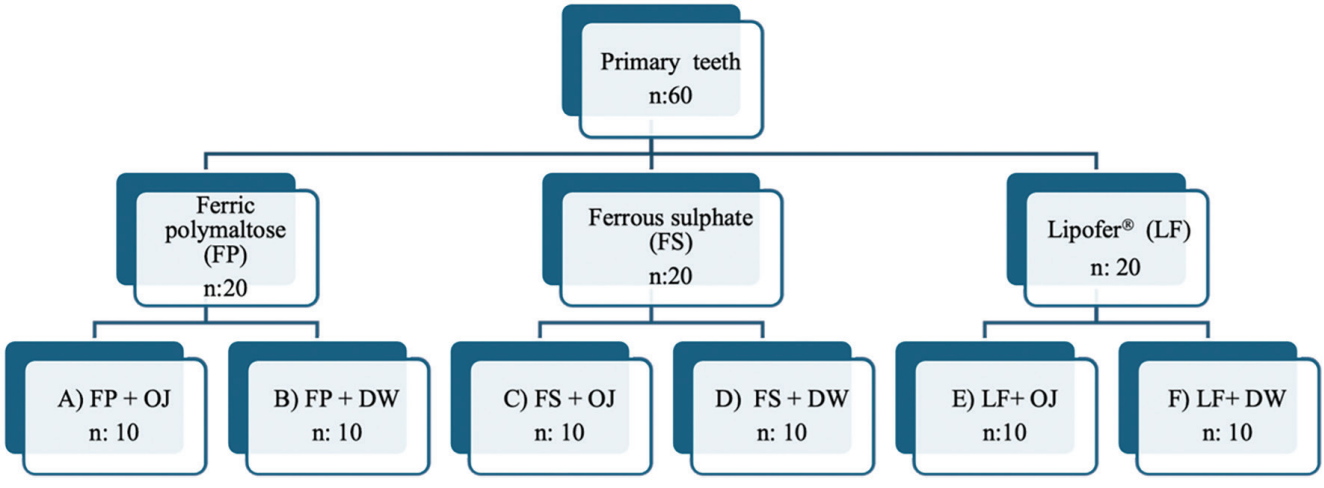

Figure 1. Flow chart. (FP: ferric polymaltose, FS: ferrous sulphate, LF: Lipofer® OJ: orange juice, DW: distilled water)

Specimen Preparation

Sixty primary canine teeth extracted within the last 3 months from patients referred to the clinic and indicated for extraction were used in this study; informed consent for using the samples in the study was obtained from the patients. The teeth were examined under a microscope (Zumax OMS2380; Suzhou, China) and those exhibiting visible caries, cracks or hypomineralised surfaces were excluded from the study. The root tissues were removed with a diamond saw under submerged and water-cooling conditions, exposing the enamel-cementum junction. Thereafter, the soft tissue residues on the crown of the tooth were removed with a periodontal curette and polishing rubber. The teeth were then fixed in acrylic resin (Integra®, Turkey) using round plastic moulds (one cm in diameter) with the buccal crown surface facing upwards.

Iron Supplements

Three distinct iron supplements, each comprising a unique active ingredient, were employed in the treatment of iron deficiency anaemia in paediatric patients (Table 1). Pure, sterile DW (Botofarma®, Turkey) and freshly squeezed OJ, prepared daily, were used to dilute the iron supplements. The samples were stored in artificial saliva between immersion cycles. The artificial saliva consisted of [NaCl, KCl, $CaCl_2$, NaH_2PO_4, urea, and distilled water; pH ≈ 6.8], and was refreshed daily. Samples were maintained at 37°C to simulate intraoral conditions.

Colour Measurement

The colour of the samples was measured at the beginning (t0) and on the 7th (t7), 14th (t14), 21st (t21) and 28th (t28) day using a spectrophotometer (Vita Easy Shade Advance 4.0, Germany). The measuring tip of the device was allowed to contact the samples at an angle of 90° and three measurements were taken from a 5-mm-diameter area and averaged.

The CIEDE2000 colour difference formula was utilised to assess the impact of iron supplements mixed with OJ or DW on the discoloration of primary teeth (12). This formula measures the colour difference by evaluating the effects of lightness, chroma and hue and the interaction between chroma and hue. The measurement was performed using the following equation:

$$\Delta E_{00} = [(\Delta L'/KLSL)^2 + (\Delta C'/KCSC)^2 (\Delta H'/KHSH)^2 + RT (\Delta C'/KC\ SC) (\Delta H'/KHSH)]^{1/2},$$

where ΔL′, ΔC′ and ΔH′ represent the differences in lightness, chroma and hue, respectively. The weighting functions (SL, SC, SH) adjust the total colour variation relative to the location in the L′, a′, b′ colour space. The parametric factors (KL, KC, KH) are the correction terms for the experimental conditions. Finally, RT corresponds to the interaction of differences between chroma and hue in the blue region (12).

Measurement of the Surface Roughness (Ra)

The Ra of the samples was measured at t0 and on t7, t14, t21 and t28 using a mechanical profilometer (Taylor Hobson The Surtronic S 128® S-128®, Leicester, UK). The arithmetic means of the data obtained by measuring three times from the same surface of each tooth sample were recorded.

Scanning Electron Microscopy

For SEM analysis, one representative sample from each group (n=6) was randomly selected after 28 days. The images presented are representative of the observed surface characteristics. The enamel surfaces were examined using

Table 1. Iron supplements used in the study

Medication Content	Trade name and batch no	Active ingredient	Auxiliary ingredients
Ferric Polymaltose (Fe^{+3}) (FP)	Ferrum Hausmann® Drops (Abdilbrahim Turkey) 23T234	Iron (III) Hydroxide Polymaltose Complex	Sugar (Sucrose) Sorbitol 70% Methyl hydroxybenzoate Propyl hydroxybenzoate cream essence, Sodium Hydroxide
Ferrous Sulphate (Fe^{+2}) (FS)	Ferro Sanol B® Syrup (ADEKA Turkey) H008	Iron (II) glycine sulphate complex	Sorbitol, saccharin sodium, sulphuric acid, orange essence (with ethanol), purified water
Lipofer® (LF)	Ocean Mikrofer® Drops (Orzax Turkey) 092310127	Ferric pyrophosphate	Deionised water, grape molasses, lipofer (corn starch, ferric pyrophosphate, hydroxypropyl, methylcellulose, lecithin) thickener: xanthan, gum, preservative: (potassium sorbate, sodium benzoate).

an SEM (LEO 1430 VP model, Germany) at 100×, 500× and 1000× magnifications.

Statistical Analysis

Data obtained from the study were analysed using the statistical software package SPSS (IBM SPSS Statistics, version 25; IBM Corp., Armonk, NY, USA). The effects of the drugs and mixing solutions on colour change and surface roughness were examined by two-way analysis of variance (ANOVA). The main effects were compared using the Bonferroni test and multiple comparisons in interactions were analysed using the Duncan test. A repeated measures two-way ANOVA test was employed to evaluate differences between the specified times. Pairwise comparisons were evaluated using the post hoc Tukey's test. The results of the analyses are presented as mean ± standard deviation. The significance level was set at $p < 0.05$.

Results

Mean Colour Change (ΔE_{00}) Values

The primary effect of iron supplements on the ΔE_{00} value was significant. The Lipofer® (LF) group demonstrated a significantly lower colour change compared to the FP and FS groups at all-time points ($p<0.05$). No statistically significant difference was observed between the FP and FS groups at any point. The primary effect of combining solutions and the interaction of iron supplements with these solutions (iron*solution) did not exhibit any statistically significant impact on the ΔE_{00} value (Table 2).

The $L^* a^* b^*$ Values

The $L^* a^* b^*$ values of the iron supplements and mixture solutions are presented in Table 3. The primary effect of iron supplements on the L^* value was found to be statistically significant ($p < 0.05$). No statistically significant differences in L^* value were observed among the drug groups on day 7. However, statistically significant differences were observed between the supplement groups on the 14th, 21st and 28th day ($p < 0.05$). The LF group demonstrated a significantly lower L^* value change than the FP and FS groups on the 14th, 21st and 28th day ($p < 0.05$).

The primary effect of iron supplements on the a^* value was also statistically significant ($p<0.05$). The FP group demonstrated significantly higher a^* values than the FS and LF groups at all-time points ($p<0.05$).

Table 2. CIEDE (ΔE00) Values of iron supplements and mixture solutions at all times

	Day	Iron Supplements	Mixing Solutions		Total
			Orange Juice	Distilled Water	
CIEDE (ΔE_{00})	7	FP	5.67 ± 0.69	6.13 ± 1.57	**5.90 ± 1.18^{A}**
		FS	4.39 ± 1.64	6.50 ± 1.63	**5.45 ± 1.91^{A}**
		LF	3.49 ± 1.39	3.47 ± 0.76	**3.48 ± 1.07^{B}**
		Total	4.51 ± 1.53	5.37 ± 1.90	**4.94± 1.75**
	14	FP	5.61 ± 0.71	7.36 ± 1.73	**6.48 ± 1.56^{A}**
		FS	6.40 ± 2.62	7.19 ± 2.40	**6.80 ± 2.43^{A}**
		LF	3.05 ± 1.75	3.47 ± 0.63	**3.26 ± 1.27^{B}**
		Total	5.02 ± 2.29	6.01 ± 2.46	**5.51 ± 2.40**
	21	FP	5.76 ± 1.71	8.14 ± 1.92	**6.95 ± 2.13^{A}**
		FS	6.93 ± 2.74	8.24 ± 2.49	**7.59 ± 2.59^{A}**
		LF	3.33 ± 1.91	3.08 ± 1.25	**3.21 ± 1.54^{B}**
		Total	5.34 ± 2.55	6.49 ± 3.08	**5.91 ± 2.85**
	28	FP	5.70 ± 2.42	8.54 ± 1.58	**7.12 ± 2.45^{A}**
		FS	8.17 ± 2.22	9.39 ± 3.027	**8.78 ± 2.61^{A}**
		LF	3.72 ± 2.22	3.94 ± 1.65	**3.83 ± 1.86^{B}**
		Total	5.86 ± 2.85	7.29 ± 3.21	**6.58 ± 3.08**

FP: Ferric polymaltose. FS: Ferrous sulphate. LF: Lipofer®
$^{A-B}$: There is no statistically significant difference between columns with the same letter ($p>0.05$).
Two-way ANOVA. Bonferroni

Table 3. L*a*b* values of iron supplements and mixture solutions at all times					
	Day	Iron Supplements	Mixing Solutions		Total
			Orange Juice	Distilled Water	
L* Value	7	FP	-8.38 ± 6.39	-4.35 ± 3.44	**-6.71 ± 4.10**
		FS	-2.98 ± 5.52	-6.95 ± 4.70	**-4.96 ± 5.31**
		LF	-2.48 ± 2.14	-2.83 ± 1.54	**-2.65 ± 1.79**
		Total	-4.61 ± 5.47	-4.71 ± 3.71	**-4.66 ± 4.60**
	14	FP	-6.71 ± 4.10	-4.95 ± 3.21	**-5.83 ± 3.63^{A}**
		FS	-4.03 ± 5.50	-6.20 ± 7.20	**-5.11 ± 6.21^{A}**
		LF	-1.83 ± 1.45	1.66 ± 3.15	**-0.08 ± 2.97^{B}**
		Total	-4.19 ± 4.32	-3.16 ± 5.81	**-3.67 ± 5.07**
	21	FP	-9.55 ± 4.76	-11.98 ±4.60	**-10.76 ± 4.64^{A}**
		FS	-7.91 ± 6.07	-8.81 ± 5.58	**-8.36 ± 5.58^{A}**
		LF	-3.66 ± 2.65	1.48 ± 2.82	**-1.09 ± 3.74^{B}**
		Total	-7.04 ± 5.11	-6.43 ± 7.26	**-6.74 ± 6.19**
	28	FP	-8.68 ± 5.40	-8.53 ± 5.16	**-8.60 ± 5.04^{A}**
		FS	-10.75 ± 4.47	-12.9 ± 5.75	**-11.82 ± 5.04^{A}**
		LF	-4.51 ± 3.67	0.45 ± 3.50	**-2.03 ± 4.29^{B}**
		Total	-7.98 ± 5.05	-6.99 ± 7.34	**-7.48 ± 6.23**
a* Value	7	FP	5.13 ± 2.54	3.51 ± 2.40	**4.32 ± 2.50^{A}**
		FS	1.10 ± 0.95	2.18 ± 1.17	**1.64 ± 1.16^{B}**
		LF	2.00 ± 1.58	1.71 ± 0.56	**1.85 ± 1.14^{B}**
		Total	2.74 ± 2.46	2.47 ± 1.67	**2.60 ± 2.08**
	14	FP	3.35 ± 2.82	2.66 ± 2.22	**3.00 ± 2.45^{A}**
		FS	-1.61 ± 0.97	-1.30 ± 0.74	**-1.45 ± 0.83^{B}**
		LF	-0.46 ± 0.78	-1.41 ± 0.85	**-0.94 ± 0.92^{B}**
		Total	0.42 ± 2.75	-0.01 ± 2.37	**0.20 ± 2.54**
	21	FP	3.35 ± 2.78	4.96 ± 2.02	**4.15 ± 2.47^{A}**
		FS	-1.55 ± 1.94	-0.71 ± 0.73	**-1.13 ± 1.46^{B}**
		LF	-0.36 ± 1.02	-0.81± 0.98	**-0.59 ± 0.98^{B}**
		Total	0.47 ± 2.88	1.14 ± 3.06	**0.81 ± 2.95**
	28	FP	1.63 ± 2.09	1.93 ± 1.43	**1.78 ± 1.72^{A}**
		FS	-1.21 ± 1.33	0.36 ± 0.64	**-0.42 ± 1.29^{B}**
		LF	-0.56 ± 0.58	-1.98 ± 1.28	**-1.27 ± 1.20^{B}**
		Total	-0.05 ± 1.86	0.10 ± 1.99	**0.02 ± 1.90**
b* Value	7	FP	13.41 ± 1.68^{bc}	10.33 ± 1.73^{b}	**11.87 ± 2.29^{A}**
		FS	5.56 ± 2.53^{ab}	10.13 ± 1.89^{abc}	**7.85 ± 3.19^{B}**
		LF	6.03 ± 2.24^{ab}	5.66 ± 2.23^{a}	**5.85 ± 2.14^{B}**
		Total	8.33 ± 4.22	8.71 ± 2.88	**8.52 ± 3.57**
	14	FP	3.83 ± 5.39	1.43 ± 3.56	**2.63 ± 4.53^{A}**
		FS	-3.91 ± 2.90	-4.11 ± 3.02	**-4.01 ± 2.83^{B}**
		LF	-8.76 ± 10.98	-5.85 ± 3.91	**-7.30 ± 8.01^{B}**
		Total	-2.95 ± 8.66	-2.84 ± 4.59	**-2.89 ± 6.83**
	21	FP	3.61 ± 4.57	10.2 ± 4.17	**6.90 ± 5.40^{A}**
		FS	-0.65 ± 5.97	-2.05 ± 4.29	**-1.35 ± 5.01^{B}**
		LF	-3.76 ± 4.68	-2.95 ± 5.17	**-3.35 ± 4.72^{B}**
		Total	-0.26 ± 5.72	1.73 ± 7.51	**0.73 ± 6.66**
	28	FP	1.05 ± 4.35	4.00 ± 3.40	**2.52 ± 4.02^{A}**
		FS	1.90 ± 6.30	2.68 ± 4.32	**2.29 ± 5.17^{A}**
		LF	-3.81 ± 2.79	-7.75 ± 5.69	**-5.78 ± 4.74^{B}**
		Total	-0.28 ± 5.12	-0.35 ± 6.90	**-0.32 ± 5.99**

FP: Ferric polymaltose, FS: Ferrous sulphate, LF: Lipofer®

[a-b] There is no statistically significant difference between columns with the same letter ($p > 0.05$).

Iron supplements also demonstrated a statistically significant effect on the b^* values ($p<0.05$), with significant differences observed among the groups ($p<0.05$). The b^* value in the FP group was significantly ($p<0.05$) higher than those in the other groups on the 7th, 14th and 21st day. However, on day 28, the FP and FS groups demonstrated significantly higher b^* values than the LF group ($p<0.05$).

The primary effect of the solution and the interaction between the solution and iron exhibited no statistically significant impact on the $L^*a^*b^*$ values.

Representative images of the tooth samples at the initial time point (day 0) and after 28 days are shown in Figures 2, 3, and 4.

Surface Roughness

No statistically significant differences in surface roughness were seen among the groups at all-time points (Table 4). When the iron supplements were used, the FP group exhibited the highest surface roughness at the conclusion of the 28 days compared to the other groups (0.88±0.6).

Scanning Electron Microscopy Results

Figures 5,6 and 7 presents the SEM images of the tooth samples obtained from each group on the 28th day of the study.

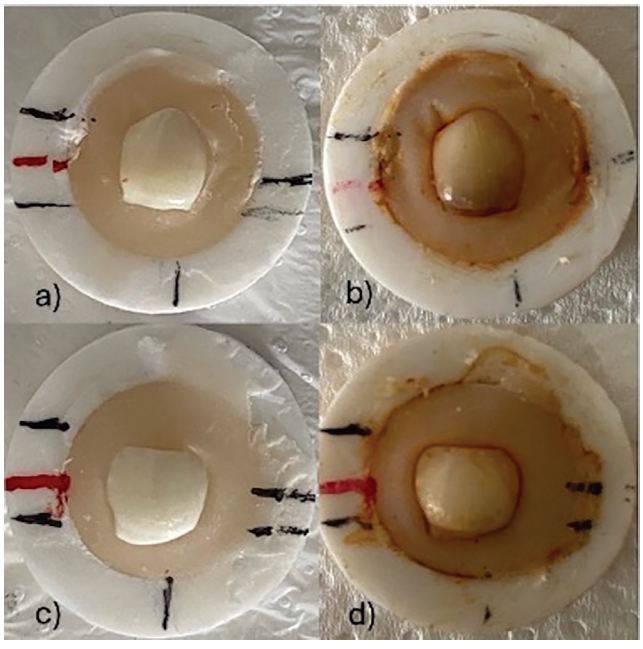

Figure 2. Representative images of tooth samples from the FP+OJ (a, b) and FP+DS (c, d) groups at baseline (a, c) and day 28 (b, d). (FP: ferric polymaltose, OJ: orange juice, DW: distilled water)

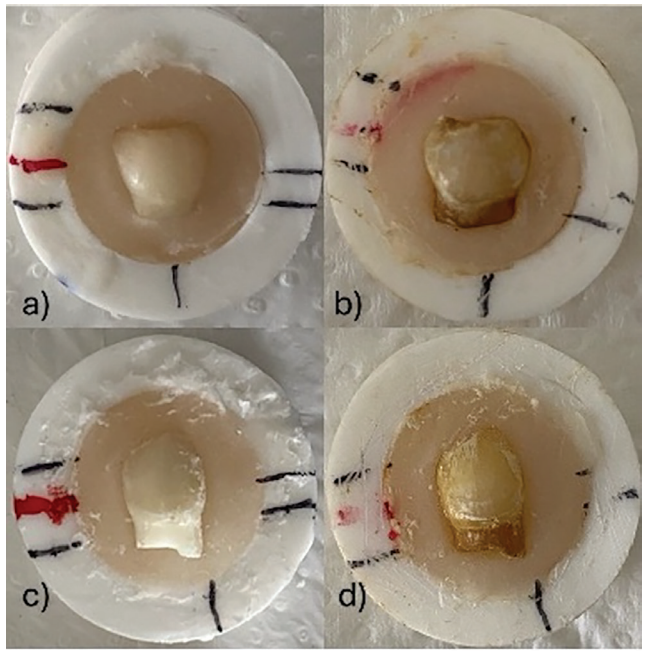

Figure 3. Representative images of tooth samples from the FS+OJ (a, b) and FS+DW (c, d) groups at baseline (a, c) and day 28 (b, d). (FS: ferrous sulphate, OJ: orange juice, DW: distilled water)

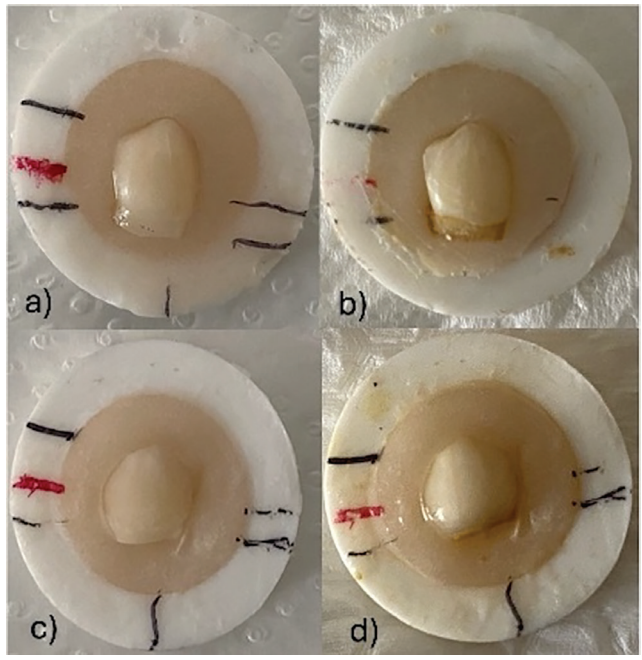

Figure 4. Representative images of tooth samples from the LF+OJ (a, b) and LF+DW (c, d) groups at baseline (a, c) and day 28 (b, d). *(LF: Lipofer®, OJ: orange juice, DW: distilled water)*

Table 4. Surface roughness results of iron supplements and mixture solutions at all times					
	Day	Iron Supplements	Mixing Solutions		Total
			Orange Juice	Distilled Water	
Surface Roughness	7	FP	0.01 ± 0.48	0.03 ± 0.19	0.02 ± 0.35
		FS	-0.33 ± 0.89	0.40 ± 0.68	0.05 ± 0.84
		LF	0.40 ± 0.26	-0.03 ± 0.42	0.18 ± 0.40
		Total	0.04 ± 0.64	0.13 ± 0.49	0.09 ± 0.56
	14	FP	-0.01 ± 0.73	0.26 ± 0.56	0.12 ± 0.63
		FS	0.10 ± 0.62	0.21 ± 0.85	0.15 ± 0.71
		LF	0.60 ± 0.58	0.30 ± 0.63	0.26 ± 0.54
		Total	0.22 ± 0.66	0.33 ± 0.61	0.30 ± 0.64
	21	FP	0.32 ± 0.50	0.48 ± 0.22	0.40 ± 0.38
		FS	-0.16 ± 0.88	0.35 ± 1.18	0.09 ± 1.03
		LF	0.62 ± 0.64	-0.2 ± 0.81	0.41 ± 0.73
		Total	0.26 ± 0.73	0.34 ± 0.79	0.30 ± 0.75
	28	FP	0.88 ± 0.88	0.88 ± 0.45	0.88 ± 0.66
		FS	-0.05 ± 1.19	1.12 ± 0.94	0.53 ± 1.19
		LF	0.68 ± 0.65	0.43 ± 1.52	0.56 ± 1.12
		Total	0.51 ± 0.97	0.81 ± 1.04	0.66 ± 1.00

Two-way ANOVA. Bonferroni
FP: Ferric polymaltose. FS: Ferrous sulphate. LF: Lipofer®

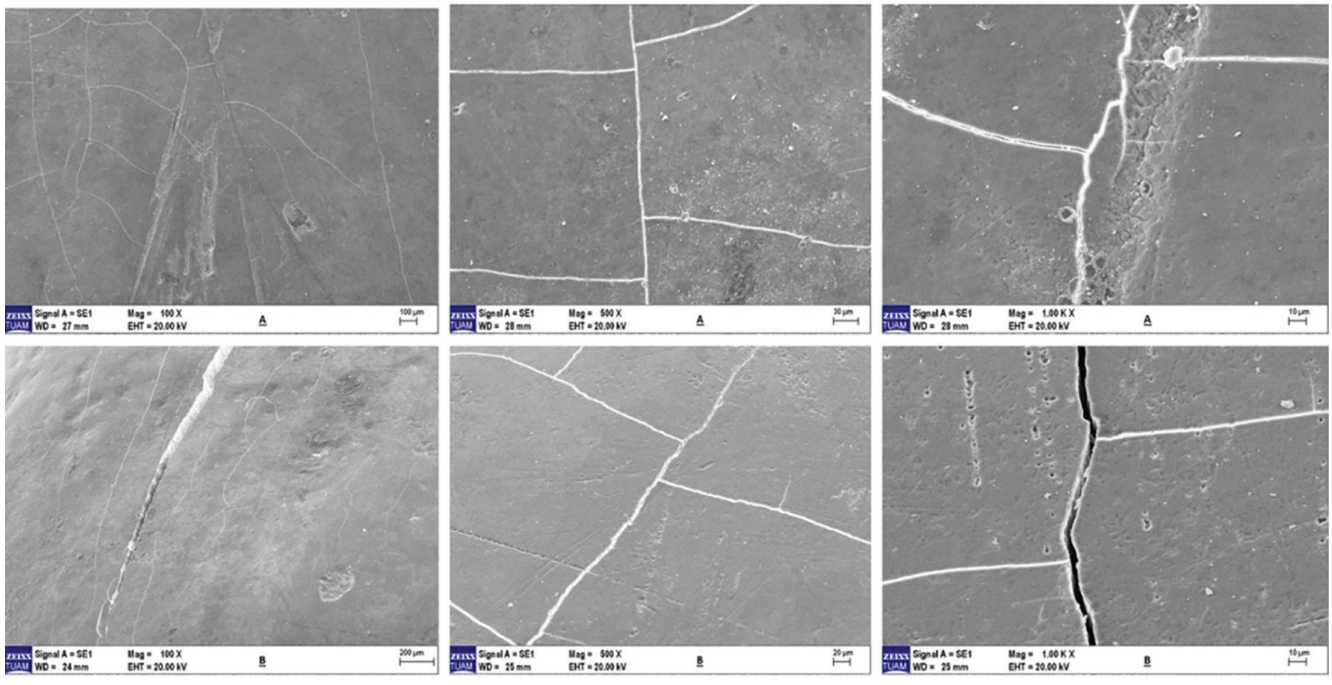

Figure 5. SEM images of tooth samples from the FP+OJ (A) and FP+DW (B) groups at 100×, 500×, and 1000× magnifications. (FP: ferric polymaltose, OJ: orange juice, DW: distilled water)

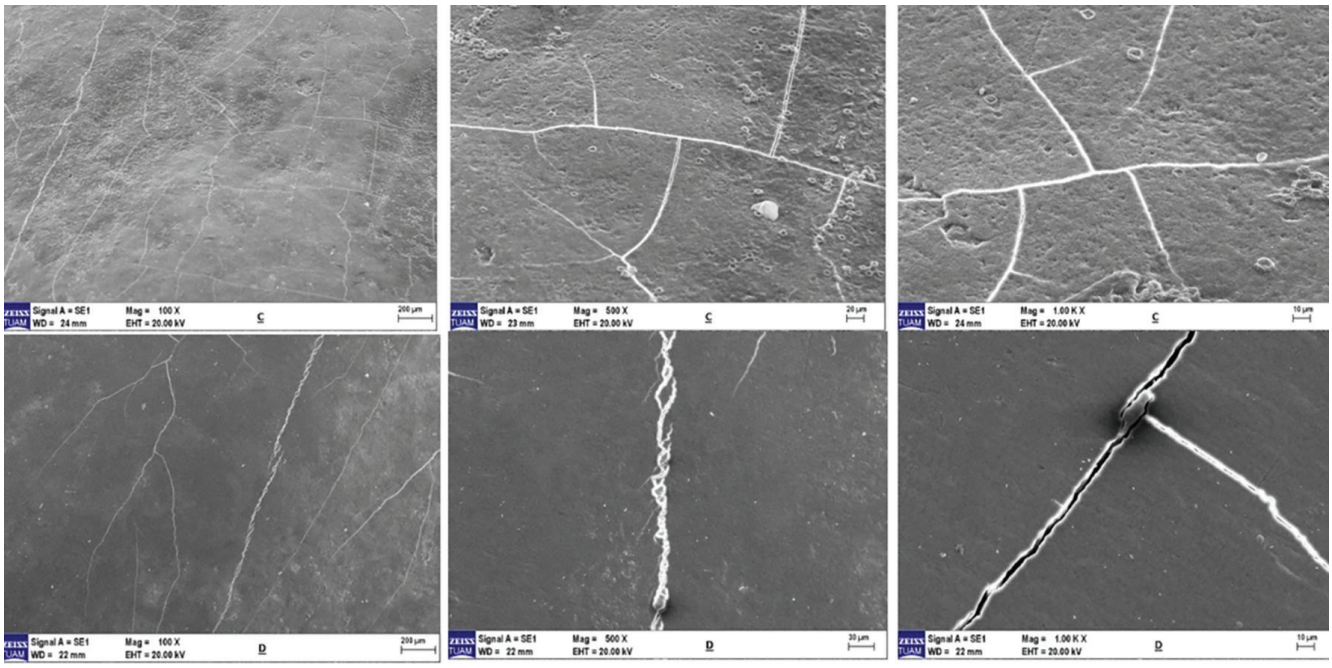

Figure 6. SEM images of tooth samples from the FS+OJ (C) and FS+DW (D) groups at 100×, 500×, and 1000× magnifications. (FS: ferrous sulphate, OJ: orange juice, DW: distilled water)

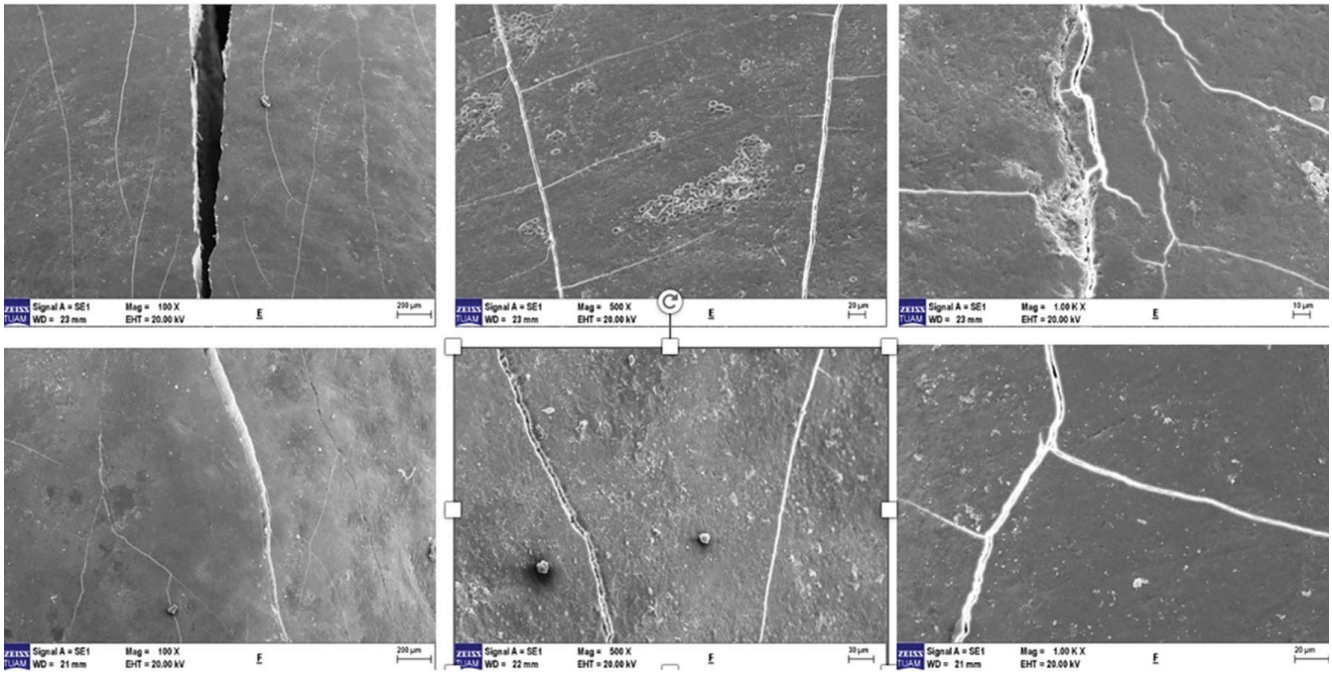

Figure 7. SEM images of tooth samples from the LF+OJ (E) and LF+DW (F) groups at 100×, 500×, and 1000× magnifications. (LF: Lipofer®, OJ: orange juice, DW: distilled water)

Discussion

This study investigated the effect of FS, FP and Lipofer® containing iron supplements mixed with OJ on the colouring and surface roughness of the primary dentition enamel in vitro.

Oral iron supplements are widely utilised for the prophylactic prevention and treatment of iron deficiency anaemia. They are available in the form of Fe^{+2} salts or Fe^{+3} complexes (2). Sucrosomial and liposomal iron preparations containing ferric pyrophosphate were recently introduced (13,14). Studies have shown that iron preparations of various forms and contents can cause tooth tissue erosion and discoloration (3-5). Tooth discoloration, a significant side effect of iron-based medications in paediatric patients, has been associated with parental anxiety and reluctance to continue treatment (15,16).

Various studies have been conducted to improve the bioavailability and absorption of iron during the treatment of iron deficiency anaemia. Various fruit acids, such as ascorbic acid (vitamin C), can augment iron absorption (6). Therefore, it is recommended that iron supplements be ingested concomitantly with OJ to facilitate both absorption and tolerance in children (7).

Oral iron supplements are commonly available in Fe^{+2} and Fe^{+3} forms. The body exhibits a high level of absorption of Fe^{+2}. FS preparations demonstrate optimal absorption and high bioavailability; however, they may be accompanied by systemic side effects such as gastrointestinal symptoms, nausea, vomiting, and abdominal pain complaints (2). The Fe^{+3} form is preferred to reduce the gastrointestinal side effects of Fe^{+2} preparations. Sucrosomial and liposomal iron preparations introduced in recent years are better tolerated systemically and have fewer undesirable side effects than traditional iron salts (2,14). A review of the extant literature revealed that FS, FP and Lipofer® (liposomal ferric pyrophosphate) supplements are the most prevalent iron preparations in contemporary use (17). Consequently, these commonly used iron supplements were selected for the current study. Given the necessity for precise dose adjustment of iron drug salts and the greater accessibility of commercially available iron supplements, these were the preferred options in the present study (16).

Iron supplements comprising three distinct active ingredients exhibited varying absorption and bioavailability rates in the present study (17). The daily doses recommended by paediatricians were used as a reference for adjusting the daily dose of iron supplements. In the event of dosage adjustment, the quantity of medicine required for each iron supplement was calculated in millilitres, with the dosage calculated based on a child with an average weight of 20 kg.

A significant challenge in the management of iron deficiency anaemia pertains to the absorption of inorganic iron once ingested by the body. Various fruit acids, such as ascorbic acid, citric acid, malic acid and tartaric acid, have been reported to be effective in increasing iron absorption in the body. Ascorbic acid is one of the main inducers in increasing iron absorption and has a dose-dependent effect on iron absorption (6). Orange, one of the fruits with the highest ascorbic acid content, has been shown to increase iron absorption because it contains various organic acids, such as citric acid, alongside ascorbic acid (8). Given the structure of ascorbic acid (a fruit acid) and its capacity to enhance iron absorption, we decided to use freshly squeezed OJ, prepared daily, in this study. This decision was made in view of the variability of the additives present in commercially available fruit juices (6).

Iron supplements cause discoloration of tooth tissue, raising concerns for parents (3,5,16). Spectrophotometers provide reliable and accurate results when measuring colour changes in teeth (18) and have been used to investigate the effect of iron supplements on tooth discoloration (3,19). In the current study, a digital spectrophotometer was utilised to evaluate the effect on colour change.

Colour systems are used to define and distinguish colours. The CIE L^* a^* b^* colour system is predicated on calculating the tristimulus values that indicate how the human visual system responds to a particular colour. In this system, the L^* coordinate corresponds to the degree of lightness/darkness, ranging from 0 (black) to 100 (white); the a^* coordinate represents the redness ($a>0$) or greenness ($a<0$); and the b^* coordinate represents yellow ($b>0$) or blue ($b<0$) variation (20). The CIEDE2000 colour system was developed to overcome the deficiencies in the CIE L^* a^* b^* colour system and to facilitate a more effective colour change analysis (12). The CIEDE2000 colour system was utilised to analyse the colour change of primary teeth in the present study.

The severity of tooth staining is contingent upon the duration of exposure of the tooth surface to the drug and the type and dosage of the supplement (3,19). In a study evaluating the erosive effect of iron supplementation and antitussive and bronchodilator drugs on dental tissue over a 28-day period, a significant decrease in microhardness was reported by day 28 (21). In the present study, the alterations observed at days 7, 14, 21 and 28 were measured to evaluate the effect of the time factor. In accordance with the extant literature the DE00 value was found to increase with time

in the present study; the highest colour change recorded was 6.58 on the 28th day (19). The immersion protocol of 5 minutes per day was designed to simulate the cumulative short-term exposure of iron supplements in the oral cavity, as previously described in similar in vitro studies (4,21).

As has been emphasised in a considerable number of studies, the type of iron supplement is one of the most important factors in tooth discoloration (3,5,15,19). Studies have shown that sucrosomial iron and specially synthesised liposomal iron supplements have a lower discoloration effect than FS preparations (5,15); similar findings were observed in the present study. Examination of the ΔE_{00} values in the FS, FP and LF supplement groups at different time intervals revealed a significantly lower colour change in the LF group than in the FS and FP groups at all-time points. In the present study, OJ did not cause an increase in the colouring potential of iron supplements. Thus, mixing supplements with OJ to enhance iron absorption and improve the taste for children did not negatively affect tooth discoloration.

In studies examining the effect of iron supplements on tooth discoloration, colour analysis was conducted using the mean discoloration value (DE) (5,19). The L^*, a^* and b^* parameters are utilised to evaluate the effect of colour change (22). Studies evaluating the effect of iron supplements on the discoloration of primary teeth in terms of $L^* a^* b^*$ parameters are lacking. Thus, the current study is the first to evaluate the effect of iron supplements on colour change in primary teeth using the $L^*a^* b^*$ colour parameters. In terms of the L^* value, teeth in the LF group showed a lighter colour appearance than those in the FS and FP groups. In terms of the a^* value, teeth in the FP group exhibited a redder colour appearance than those in the FS and LF groups; this colour difference may be attributable to the dark red colour of the iron drop containing FP. In terms of the b^* value, teeth in the FS and FP groups exhibited a more yellow colour appearance than those in the LF group.

Several factors influence the colour and appearance of teeth; for example, an increase in the roughness of the tooth surface can facilitate the attachment of chromogenic pigments and bacterial biofilm (23). Many studies have shown that the low pH of iron supplements can cause demineralisation of tooth surfaces and tooth erosion (9,24,25). In vitro studies using microhardness and surface roughness analyses have evaluated whether and to what extent dental hard tissues are affected by erosion (9,21,26). Various instruments equipped with optical or mechanical sensors are utilised to measure the surface roughness and topography. Profilometers are preferred for examining the surface properties of dental materials. In the present study, changes in enamel surface roughness were evaluated using a mechanical profilometer. Surface roughness analysis performed by quantitative methods, such as a profilometer and supported by qualitative measurements (like SEM, as utilised in the present study) contributes to the reliability of the results (27,28).

The findings of the present study revealed no significant difference in surface roughness among the groups examined. In another study employing a similar methodology, FS-containing iron drops with erosive potential were diluted with natural apple juice, reducing the adverse effects on the tooth surface (4). In the present study, the combination of iron supplements with OJ had no substantial impact on the enamel surface roughness. This may be attributed to the dilution of the supplements, which minimises the negative effects.

The present study utilised extracted primary canine teeth to evaluate the effect of different iron supplement groups on primary teeth. The study was conducted under in vitro conditions. However, in vitro studies face challenges in accurately reflecting the intraoral environmental conditions, making it impossible to mimic the oral environment completely (29,30). Therefore, the tooth samples were stored in an artificial saliva solution during the immersion cycles to simulate the oral environment for evaluating the erosive activity (30).

In the present study, the most prescribed preparations of iron supplements were preferred and their results were evaluated. However, each preparation contains varying amounts of preservative additives and by-products (31). Pure iron salts were not preferred because they are difficult to dose and supply.

Study Limitations

Despite the results being interpreted in relation to the type of iron supplements administered, the by-products contained within the supplements may have exerted some influence, which could represent a significant limitation of the study.

Iron supplements containing Lipofer® caused less tooth discoloration than those containing FP and FS. The administration of iron supplements mixed with OJ did not increase the colouring potential of iron on the teeth. Therefore, it may be recommended to mix iron supplements with OJ to increase absorption and enhance the taste of the medicine for children. When administered in conjunction with OJ or DW, the three distinct iron supplements did

not induce any surface texture alterations. Although a mechanical profilometer device was used to evaluate the surface roughness in this study, an optical profilometer or atomic force microscopy may provide more reliable results in the future.

Conclusion

The results of this study may serve as a practical guide for healthcare professionals working with children, such as pediatric dentists, pediatricians, and family physicians, as well as for parents. Moreover, this is the first study to examine the effects of ferrous sulfate, ferric polymaltose, and Lipofer®-containing iron preparations combined with orange juice on the enamel of primary teeth, specifically in terms of discoloration and surface roughness. These findings provide a scientific basis for future research evaluating the combined use of iron supplements and supportive agents such as orange juice. Within the limitations of this in vitro study, mixing iron supplements with orange juice did not increase discoloration or surface roughness. However, clinical studies are needed to confirm these findings before making definitive recommendations.

Ethics

Ethics Committee Approval: Approval for this in vitro experimental study was obtained from the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (decision no: E-41997688-050.99-130396, date: 11.03.2024).

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Footnotes

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Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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