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Original Article

Extrinsic Primary Tooth Staining Due to Iron Syrup Medication: Effects of Dilution and Tooth Brushing - An *In Vitro* Study

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Abstract

Objective: Iron deficiency, a leading cause of anemia in children under five, is treated by iron supplementation. However, iron syrups prescribed for children aged 6–24 months, may cause extrinsic staining of primary teeth, raising esthetic concerns. This *in vitro* experimental study aimed to evaluate the impact of syrup dilution and tooth brushing on iron-induced staining of primary anterior teeth.

Materials and Methods: After ethical approval, 90 anterior primary teeth with intact crowns were collected, disinfected, and mounted in acrylic resin molds. Samples were randomly assigned to six groups (n=15). Groups either received undiluted or diluted ferrous sulfate syrup (PediaFer®, 15 mg/mL elemental iron), with or without brushing using a children's fluoride toothpaste (500 ppm) (Aquafresh® milk teeth, GSK Consumer Healthcare, Brentford, UK). Artificial saliva served as control. Immersion simulated three years of clinical exposure by incubating samples for 36 days at 37°C. Color measurements were recorded at baseline and after 12, 24 and 36 days using the CIE Lab* system and a spectrophotometer (VITA Easyshade®, VITA Zahnfabrik, Bad Säckingen, Germany). Color change (ΔE) was calculated and interpreted according to ISO/TR 28642:2016 standards. Analysis was performed using RStudio (version 2024.12.1). Repeated-measures ANOVA or the Friedman tests assessed within-group time effects; t-tests or Wilcoxon tests examined brushing effects; and ANOVA or Kruskal-Wallis tests analyzed dilution effects. Significance was set at $p \leq 0.05$.

Results: Teeth exposed to concentrated iron syrup without brushing showed the highest ΔE values, rising significantly from T1 to T3 ($p<0.001$), indicating progressive staining. Diluted syrup without brushing caused significant discoloration ($p=0.015$). Brushing reduced early staining only in the diluted group at T1 ($p=0.002$), but this effect was not sustained. Pooled analysis showed no significant effect of brushing by T3 ($p=0.08$), while syrup concentration had an overall strong and consistent effect ($p<0.001$). Concentrated syrup led to significantly higher ΔE values than diluted and control groups (adjusted $p<0.001$). Mean ΔE values for concentrated syrup exceeded the clinical visibility threshold ($\Delta E>6$) confirming its marked staining potential.

Conclusion: These findings suggest that dilution is a more consistently effective strategy than brushing alone for minimizing iron-induced staining in primary teeth.

Keywords: Deciduous teeth, iron deficiencies, pigmentation, spectrophotometry, tooth discoloration

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Introduction

Iron is an essential mineral involved in numerous physiological processes, including hemoglobin formation, oxygen transport, immune function, and neurodevelopment.[1] Iron deficiency (ID) is the most prevalent micronutrient disorder globally, affecting over two billion people. Iron-deficiency anemia (IDA) remains the leading cause of anemia in children, especially during the rapid growth phases in the first five years of life.[2,3] To address this, the World Health Organization (WHO) recommends, as a first-line intervention, universal iron supplementation-commonly in the form of syrups or drops- for children aged 6–24 months to support healthy motor and cognitive development.[4] However, the use of iron syrups in children under three years old, coinciding with the eruption of primary teeth, often leads to an undesirable yet common side effect: Extrinsic staining of the teeth.[5,6] This concern is frequently raised by parents and poses an esthetic challenge in pediatric dentistry. Given the increasing esthetic expectations in dental care and the psychosocial impact of visible tooth discoloration on young children, especially in social settings like daycare and preschool, managing such staining is of clinical relevance.[7] Previous *in vitro* studies confirm the staining potential of various iron formulations. Tayebi et al[8] demonstrated that four different types of iron supplements all caused noticeable tooth discoloration, with no significant differences in staining potential among them. Additionally, Pani et al[9] found that while ferrous fumarate (FF) causes less staining than ferric hydroxide polymaltose (FOP), it may not provide adequate iron supplementation; notably, a combination of FF and FOP resulted in significantly less staining than either alone. Abbasi et al[10] demonstrated that a liposomal nano-encapsulated ferrous sulfate drop led to significantly less tooth discoloration compared to commercial iron drops, suggesting nano-encapsulation as a cost-effective alternative. Furthermore, Tüzüner et al[11] reported that iron syrups cause prominent staining on restorative materials, particularly composites, while glass ionomer cements showed greater resistance. Limited research has explored the influence of dilution or preventive oral hygiene measures such as tooth brushing on this staining effect.

Thus, the present study aimed to evaluate the extrinsic staining effects of iron syrup on anterior primary teeth *in vitro*, focusing on two variables: Dilution of the syrup and the effect of tooth brushing. The primary objective was to assess whether dilution reduces staining potential, while the secondary objective was to determine the additional impact of tooth brushing. The primary null hypothesis (H0) posited no significant difference in staining between diluted and undiluted syrups, while the secondary null hypothesis (HS0) suggested no difference between brushed and unbrushed samples.

Materials and Methods

The study is approved by the Saint-Joseph University of Beirut (No: USJ-2024-99, Date: 18/04/2024) and conducted according to Declaration of Helsinki.

Minimal sample size calculation

The present study used an *in vitro* experimental model.

The minimum required sample size was calculated using the G*Power software, assuming a large effect size (f=0.4). With a significance level (α) of 0.05 and six independent groups, a total of 90 units was determined to achieve a statistical power of 80% for detecting differences among groups: This corresponds to 15 units per group.

Sample preparation

Ninety anterior primary teeth were collected, none of which were extracted for reasons related to the present study; all were either recently exfoliated or extracted due to space discrepancy, mobility, or trauma. There were no developmental anomalies, enamel hypoplasia, restorations, extrinsic nor intrinsic stains in the selected teeth. The teeth were sterilized by immersion in 10% formalin for 24 hours and stored in distilled water until usage. Each tooth was thoroughly rinsed and cleaned by a prophylaxis paste (SpectraTM Prophylaxis paste, Prevest DenPro Limited, India) and brush (FlydentTM, China). Each tooth was dissected at the cemento-enamel junction and the pulpal residues were completely removed from the pulp chamber using an excavator (1020/127-128, Carl Martin GmbHTM, Solingen, Germany). Each tooth was then embedded in an acrylic resin mold at the cemento-enamel junction level so that the crown was positioned perpendicularly to the mold (Fig. 1). The teeth were all immersed in artificial saliva containing sodium chloride, potassium chloride, calcium chloride, sodium dihydrogen phosphate, sodium sulfide, and urea with the pH adjusted to 6.75 using potassium hydroxide (KOH).

The teeth were then randomly divided into six groups of 15 each, as follows:



Figure 1. Sample preparation

Group 1: No tooth brushing

Group 2: Tooth brushing

Group 3: Iron supplement without tooth brushing

Group 4: Iron supplement with tooth brushing

Group 5: Iron supplement and water without tooth brushing

Group 6: Iron supplement and water with tooth brushing.

The iron-supplemented solutions contained 6.6 mL of ferrous sulfate oral solution (15 mg iron/mL), equivalent to 100 mg of elemental iron according to the label. The supplement used was a commercially available pediatric iron syrup (PediaFer®, EURO-PHARM International Canada Inc., Montréal, Québec, Canada).

In Groups 5 and 6, 37 mL of bottled drinking water was added to represent the typical daily intake for toddlers. This quantity was based on the American Academy of Pediatric Dentistry (AAPD) recommendation of a minimum daily water intake of 5 oz (148 mL) for children aged 0–3 years, proportionally scaled to reflect consumption patterns throughout the day.

The different solutions containing the samples were placed in an incubator at a temperature of 37°C which represents the human body temperature. To simulate three years of exposure to the iron syrup, the solutions were placed in the incubator for 36 days given the fact that a 24-hour immersion in colorant solutions corresponds approximately to one month of clinical aging.[12] For the groups that required tooth brushing (groups 2, 4 and 6) the teeth were first rinsed with running water before brushing. Tooth brushing was performed three times in each 12-day interval for a duration of two minutes using a manual toothbrush, following the method outlined by Ren et al[13].

Brushing was performed on the labial surface of each specimen according to the modified Bass brushing technique. In this technique, the toothbrush bristles were positioned at approximately a 45° angle to the gingival margin and moved with short vibratory strokes, followed by a gentle sweeping motion away from the gingival margin.[14] Each specimen was brushed individually while maintaining consistent pressure and stroke frequency to simulate routine pediatric toothbrushing conditions.

A toothbrush with extra-soft bristles, suitable for children under three years of age (Aquafresh® Milk Teeth, GSK Consumer Healthcare, Brentford, UK), was utilized. Additionally, a children's toothpaste containing the recommended fluoride concentration (500 ppm) was used (Aquafresh® milk teeth, GSK Consumer Healthcare, Brentford, UK). In accordance with the American Academy of Pediatric Dentistry (AAPD) guidelines, a rice-grain-sized amount of toothpaste was applied for brushing. After brushing, the teeth were rinsed with running water, returned to the solutions and placed in the incubator. All brushing procedures were performed by a single operator to ensure consistency and minimize operator-related variability.

Measurement of color parameters

Prior to all color measurements, all the specimens were rinsed with tap water for 5 seconds and blotted dry using absorbent paper. Color evaluations were conducted using the CIE L*a*b* color space, an internationally recognized color-opponent system defined by the International Commission on Illumination (CIE). Color values were obtained using the Vita Easyshade Spectrophotometer (VITA Easyshade®, VITA Zahnfabrik, Bad Säckingen, Germany) (Fig. 2).



Figure 2. Vita Easyshade® Advance spectrophotometer

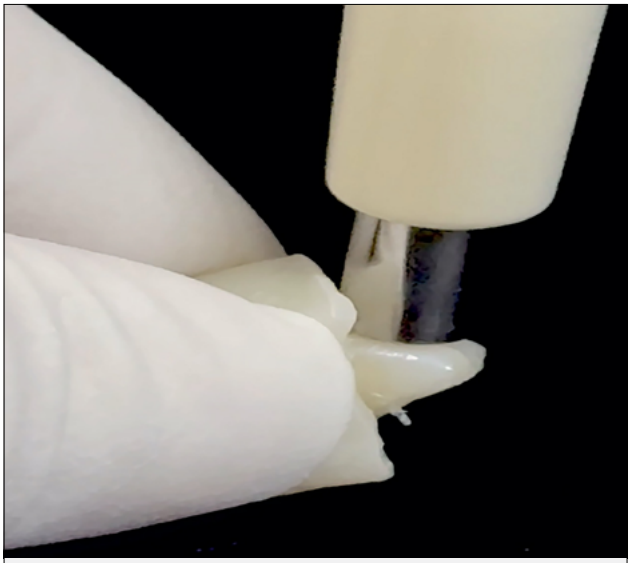

Figure 3. Positioning of the spectrophotometer tip on the labial surface of the tooth



Figure 4. Values obtained by the spectrophotometer

The spectrophotometer was calibrated following the manufacture's guidelines. All the measurements were performed by a single operator under standardized conditions. Measurements were performed on the labial surface with the device tip positioned perpendicularly at the center of the crown's middle third (Figs. 3, 4). For each specimen, three independent measurements were obtained. The mean value was calculated by summing these measurements and dividing them by three, thereby providing a representative value for subsequent analysis. Measurements were recorded at four time points: T0: Baseline (prior to immersion in the solution), T1 (after 12 days), T2 (after 24 days) and T3: Final evaluation (after 36 days). The interval between each time point was set to 12 days to simulate the effects of approximately one year of clinical aging. In groups assigned to tooth brushing (groups 2, 4, and 6), color assessment was conducted after brushing procedures at each time point. Following every measurement session, specimens were returned to freshly prepared solutions.

Color change calculation

To quantify color changes over time, the total color difference (ΔE) between time points was calculated using the standard formula:

$$\Delta E = [(\Delta a)2 + (\Delta b)2 + (\Delta L)2]\ ½$$

Where:

- ΔL denotes the difference in lightness, with L indicating the degree of lightness/darkness on a scale from 0 (black) to 100 (white)
- Δa represents the difference in the red-green axis (a*), where a* is the chromaticity coordinate along the red-green direction, ranging from -128 (green) to +128 (red)
- Δb represents the difference in the yellow-blue axis (b*), where b* is the chromaticity coordinate along the yellow-blue direction, ranging from -128 (blue) to +128 (yellow)

Interpretation of color differences was based on the ISO/TR 28642:2016 standards: [15]

- a color change of ΔE<1.2 is considered imperceptible to the human eye

Table 1. Descriptive statistics for ΔE by group and time point

Group	Brushing	Dilution	ΔE T1† (Mean±SD)	ΔE T2† (Mean±SD)	ΔE T3† (Mean±SD)	Clinical interpretation‡
G1	No	Control	10.01±5.23	9.56±3.27	10.13±4.09	Clinically perceptible
G2	Yes	Control	8.29±4.06	7.70±5.09	8.87±5.06	Clinically perceptible
G3	No	Concentrated	22.98±14.46	33.86±17.86	44.90±19.39	Clearly noticeable and severe
G4	Yes	Concentrated	31.62±9.96	34.39±12.32	40.90±11.83	Clearly noticeable and severe
G5	No	Diluted	17.58±5.79	23.48±7.54	26.41±9.67	Clinically perceptible
G6	Yes	Diluted	10.14±5.98	13.04±7.76	11.53±7.72	Clinically perceptible but substantially reduced

ΔE: Color difference measured using the CIE Lab system. †: T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. ‡: "Clinically perceptible" and "clearly noticeable" interpretations follow the thresholds defined by the International Commission on Illumination (CIE).

Table 2. Time effect within each group

Group	Test used	Statistic	p	Interpretation
G 1	rm-ANOVA	F(2,28)=0.098	0.907	No change
G 2	rm-ANOVA	F(2,28)=0.233	0.793	No change
G 3	rm-ANOVA	F(2,28)=9.61	<0.001*	Significant increase
G 4	rm-ANOVA	F(2,28)=2.56	0.095	Trend toward significant increase
G 5	Friedman Test	Chi^2=8.4	0.015*	Significant increase
G 6	Friedman Test	Chi^2=0.533	0.766	No change

(*) indicates statistically significant difference at p<0.05.

- a color change of ΔE=3.7 is considered the acceptability threshold, where approximately 50% of observers perceive the difference as acceptable
- a color change of 3.7<ΔE<6.0 is considered clinically perceptible (mild to moderate)
- a color change ΔE>6.0 is considered clearly visible and aesthetically concerning

Statistical Analysis

Statistical analyses were conducted using RStudio (version 2024.12.1 Build 563). The main outcome was the color change (ΔE) between each post-treatment time point and baseline (T0). Normality was tested using the Shapiro-Wilk test and homogeneity of variances using Levene's test. Depending on these assumptions, the appropriate test (repeated-measures ANOVA or Friedman test for time effect; t-test or Wilcoxon test for brushing; ANOVA or Kruskal-Wallis for dilution) was selected. Statistical significance was set at p≤0.05.

Results

Descriptive statistics

Descriptive statistics for ΔE values at time points T1, T2, and T3 were calculated for all six groups. Table 1 shows the mean ± SD for each group.

Effect of time

To evaluate whether the degree of discoloration statistically increased over time within each group, intra-group comparisons of ΔE values were conducted across the time points. Depending on the results of normality testing, either repeated-measures ANOVA or the non-parametric Friedman test was applied (Table 2).

Effect of tooth brushing

To evaluate the role of brushing, ΔE values were compared between brushed and unbrushed subgroups within each dilution level. These comparisons were performed independently at T1, T2, and T3 using t-tests or Wilcoxon tests depending on distribution (Table 3).

Pooled brushing comparison

To evaluate the overall effect of brushing regardless of syrup concentration, ΔE values were compared between all brushed (groups 2, 4, 6) and unbrushed (groups 1, 3, 5) teeth at each time point. The results are presented in Table 4.

Effect of dilution across groups by time point

To determine the influence of iron syrup concentration on extrinsic staining, comparisons were made across the three dilution levels at each time point, separately for brushed and unbrushed teeth. Due to significant heterogeneity of variances, a Kruskal-Wallis test was used to evaluate the overall group effect (Table 5) and Dunn's Post Hoc was used to show the significant pairwise comparisons within each brushing condition and time point (Table 6).

Table 3. Brushing effect between paired groups

Group pair	Time	Test used	Statistic	p	Interpretation
1 vs 2	T1	t-test	t=1.01	0.32	No effect
1 vs 2	T2	t-test	t=1.19	0.24	No effect
1 vs 2	T3	t-test	t=0.75	0.46	No effect
3 vs 4	T1	t-test	t=-1.90	0.07	Trend toward significance
3 vs 4	T2	t-test	t=-0.09	0.93	No effect
3 vs 4	T3	t-test	t=0.68	0.50	No effect
5 vs 6	T1	t-test	t=3.46	0.002*	Significant effect
5 vs 6	T2	Wilcoxon	W=195	0.12	No effect
5 vs 6	T3	Wilcoxon	W=197	0.15	No effect

T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. (*) indicates statistically significant difference at p<0.05.

Table 4. Pooled comparison of ΔE between brushed and unbrushed teeth

Time	No Brushing (ΔE±SD)	Brushing (ΔE±SD)	p
T1	16.86±10.72	16.68±12.76	0.943
T2	22.30±14.98	18.38±14.55	0.211
T3	27.15±19.00	20.43±16.94	0.08

ΔE: Color difference measured using the CIE Lab* system. T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively.

Table 5. Kruskal-Wallis results: Effect of dilution by brushing

Brushing	Time	Chi²	p	Interpretation
No	T1	10.6	0.005*	Significant
No	T2	26.6	<0.001*	Significant
No	T3	29.2	<0.001*	Significant
Yes	T1	28.3	<0.001*	Significant
Yes	T2	26.8	<0.001*	Significant
Yes	T3	29	<0.001*	Significant

T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. (*) indicates statistically significant difference at p<0.05.

Pooled dilution comparison

To evaluate the overall effect of dilution regardless of brushing, ΔE values were compared between the three syrup concentrations at each time point using the Kruskal-Wallis test (Table 7).

To clarify where these overall differences occurred, Table 8 summarizes the pairwise comparisons between dilution levels at each time point, pooling data across all groups regardless of brushing.

Across all time points, concentrated syrup consistently caused significantly more discoloration than both con-

Table 6. Summary of significant pairwise comparisons (Dunn's post hoc)

Brushing	Time	Significant Comparisons	Adjusted p-value
No	T1	Concentrated > Control	0.0105*
No	T2	Concentrated > Control Diluted > Control	<0.001* <0.001*
No	T3	Concentrated > Control Diluted > Control	<0.001* <0.001*
Yes	T1	Concentrated > Control & Diluted	<0.001*, <0.001*
Yes	T2	Concentrated > Control & Diluted	<0.001*, <0.01*
Yes	T3	Concentrated > Control & Diluted	<0.001*, <0.001*

T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. (*) indicates statistically significant difference at p<0.05.

Table 7. Pooled ΔE (Mean±SD) by dilution level and Kruskal-Wallis p-values

Time	Control (ΔE±SD)	Concentrated (ΔE±SD)	Diluted (ΔE±SD)	p
T1	9.15±4.68	27.30±12.97	13.86±6.91	<0.001*
T2	8.63±4.31	34.13±15.08	18.26±9.20	<0.001*
T3	9.50±4.56	42.90±15.91	18.97±11.45	<0.001*

ΔE: Color difference measured using the CIE Lab* system. T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. (*) indicates statistically significant difference at p<0.05 as determined by Kruskal-Wallis test.

Table 8. Summary of pooled comparisons (Dunn's post hoc)

Time	Comparisons	Adjusted p-value
T1	Concentrated > Control	<0.001*
	Concentrated > Diluted	<0.01*
	Diluted > Control	0.07
T2	Concentrated > Control	<0.001*
	Concentrated > Diluted	<0.01*
	Diluted > Control	<0.001*
T3	Concentrated > Control	<0.001*
	Concentrated > Diluted	<0.001*
	Diluted > Control	0.0127*

T1, T2, T3: Time points corresponding to 12, 24 and 36 days of immersion, respectively. (*) indicates statistically significant difference at p<0.05.

trol and diluted syrups (p<0.01). The diluted syrup showed intermediate staining and became significantly different from control at T2 and T3, while the difference at T1 remained marginal (p=0.07).

The extrinsic staining observed on primary teeth for each dilution level - control, concentrated, and diluted pediatric iron syrups - regardless of brushing, is shown in (Fig. 5). The differences in staining intensity among the groups are visibly evident, with the concentrated syrup group exhibiting the most pronounced discoloration.

Discussion

The present study explores the extrinsic staining effects of pediatric iron supplements, on primary anterior teeth, focusing on the influence of syrup dilution and tooth brushing. The WHO recommends iron supplementation in the form of drops for children under the age of three to prevent iron deficiency anemia.[1] However, a well-documented side effect is tooth discoloration, which has been reported in over half of children receiving oral iron supplements, leading to concerns among parents.[6] Despite this, limited research has explored the combined effects of syrup concentration, dilution, and brushing on tooth staining, especially in primary teeth. Aiming to fill that gap, the main objectives of this study were to determine whether dilution and brushing mitigate the staining effects of iron syrup and how staining progresses over time.

In this study, the CIELab* color space was employed to objectively evaluate extrinsic staining, as it offers a standardized and widely accepted framework in dental research for quantifying tooth color changes. Color was measured using the L* (lightness), a* (green-red), and b* (blue-yellow) coordinates, while the ΔE value was used to represent the overall color difference over time. This objective method provides greater precision and consistency compared to traditional visual assessments or shade guides. Similar methodologies have been used in dental research, including studies evaluating the color stability of various dental materials subjected to different staining agents, reinforcing the reliability of the CIELab* system in controlled *in vitro* settings.[16,17] To ensure accuracy and reproducibility of the measurements, the VITA Easyshade® spectrophotometer - a device validated in both clinical and laboratory contexts - was utilized. In fact, the use of spectrophotometers in dental research has gained prominence due to their ability to provide objective, reproducible color measure-

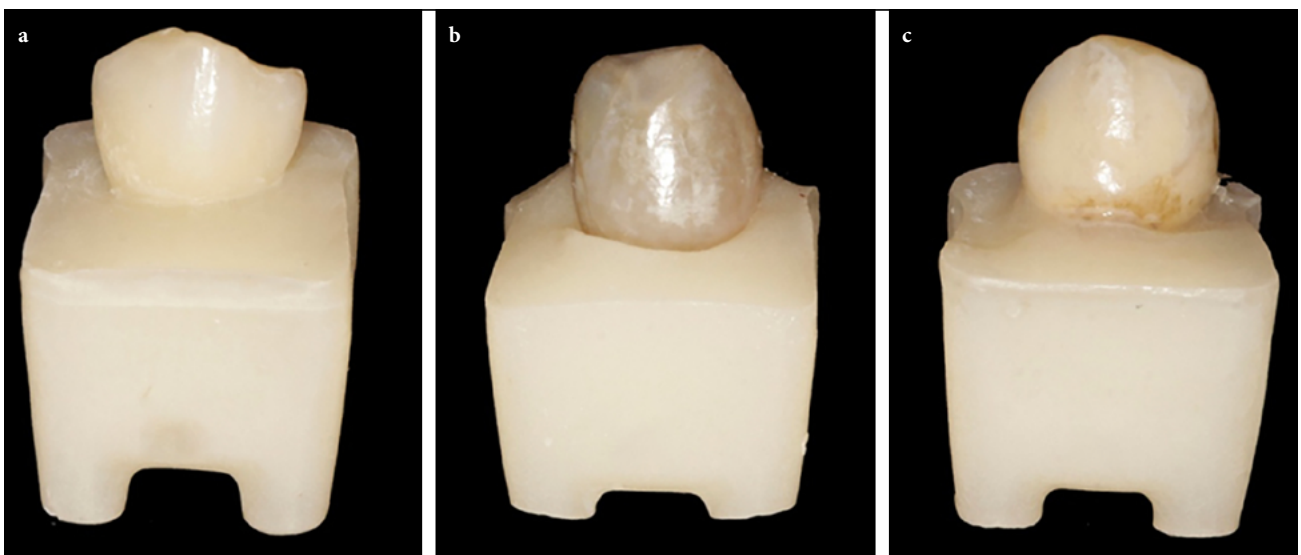

Figure 5. Representative images of primary teeth showing varying degrees of extrinsic staining following exposure to different dilution levels of iron syrup solutions. (a) Control group. (b) Concentrated iron syrup. (c) Diluted iron syrup.

ments, making them especially valuable in studies evaluating extrinsic staining on primary teeth. Recent systematic reviews have emphasized the importance of accurate shade selection and evaluation methods, highlighting spectrophotometry as a reliable tool in both clinical and research settings.[18]

The study's findings revealed a clear progression of extrinsic staining over the three time points, with significant differences between groups. Group 3 (unbrushed, concentrated) exhibited the most severe staining, with ΔE values increasing significantly from T1 to T3 ($p<0.001$). A less intense but still significant increase in staining was observed in Group 5 (unbrushed, diluted), where ΔE values showed an increase from T1 to T3 ($p=0.015$). In contrast, no significant staining progression was seen in the control group (Groups 1 and 2), nor in Group 6 (brushed, diluted), where ΔE values remained relatively stable. These results suggest that the iron syrup concentration played an important role in staining severity, with diluted syrup causing less pronounced discoloration, in line with the results of several studies evaluating the effect of different types of iron drops on the color of primary teeth. For example, Pani et al[9] 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 the mean ΔE in all groups increased over time and the difference was significant similarly to our study. Another study by Mehran et al[19] evaluated the effect of two types of iron drops on the color of the primary teeth. The results of their study indicated an increase in the mean ΔE over time with statistically significant differences, which corroborates with the results of the present study. Both studies used ferrous sulfate iron supplements but the assessment time points and the immersion protocols differ from the present study.

Further statistical analysis confirmed that syrup concentration significantly influenced staining severity. At all-time points, the concentrated syrup groups (Groups 3 and 4) exhibited the highest mean ΔE values whereas the diluted syrup groups (Groups 5 and 6) had lower but still elevated ΔE values. The control group maintained ΔE values around 9.15 to 9.50, showing minimal discoloration. These differences were statistically significant across all time points (Kruskal-Wallis $p<0.001$), with post hoc tests confirming that the concentrated syrup caused significantly more staining than both the diluted and control groups (adjusted p-values<0.01). These results suggest that while dilution reduces discoloration compared to the concentrated syrup, it does not fully prevent it. Therefore, the primary null hypothesis (H0) is rejected, indicating that iron syrup concentration plays a critical role in staining severity. To the best of our knowledge, this is the first study to evaluate the effect of dilution on iron-induced staining of primary anterior teeth. Previous research has largely focused on the effects of different factors such as different types of iron syrups or the impact of tooth brushing, the role of dilution remaining unexplored. By addressing this gap, our findings allow novel insights into addressing this side effect, highlighting a key strength and innovation of the present study. Proven effective to reduce iron-staining, dilution of iron supplements can be incorporated into the preventive measures given to the parents during the first dental visit. More specifically, special needs children such as children with autism spectrum disorder (ASD) can benefit from this approach as they face difficulty cleaning their teeth or enduring in –office dental prophylaxis due to their poor behavioral activities and complex physiological state.[20]

The effect of brushing on tooth discoloration appeared to be limited and dependent on both iron concentration and duration of exposure. In the diluted syrup group, brushing significantly reduced staining at T1 ($p=0.002$), suggesting an early benefit from mechanical pigment removal. However, this effect was not sustained at T2 ($p=0.12$) or T3 ($p=0.15$), indicating a diminished protective role over time. In contrast, brushing had no significant impact in the concentrated syrup group at any time point. Pooled analysis of all samples supported this trend, showing no overall statistical difference between brushed and unbrushed teeth ($p>0.05$), though a non-significant reduction in staining among brushed samples was noted at T3. These findings suggest that brushing may help limit early pigment buildup when staining intensity is low, as in diluted formulations, but becomes less effective as iron exposure increases and pigments penetrate more deeply into enamel. Thus, the secondary null hypothesis (HS_0) is largely retained, with partial rejection only at T1 in the diluted group where brushing had a measurable effect.

The study also compared its findings to existing literature. Heidari et al[21] examined the removal of iron-induced stains on extracted primary teeth and found that prophylaxis treatment was the most effective in removing iron stains. The present study differed in its use of manual brushing, whereas Heidari et al[21] used an automatic brushing machine, which could explain some of the observed differences in the results. Moreover, the

use of the VITA Easyshade spectrophotometer in this study provided a more precise and reliable measure of color change compared to the Photoshop and iColor software used in Heidari's study.

Further comparisons with studies on restorative materials, such as the one by Almutairi et al[22], revealed similar results to our study, indicating that brushing may have limited impact on staining when exposure is prolonged or when the staining is more intense.

In clinical terms, the ΔE values obtained in this study indicated that all groups experienced clinically perceptible staining, with values exceeding the commonly accepted threshold of ΔE=3.7 used in various studies. [16,18] In some cases, ΔE values surpassed 6.0 representing staining that is clearly visible and likely to be of aesthetic concern. The concentrated syrup groups showed the most severe staining, while the diluted syrup group exhibited moderate discoloration. Interestingly, brushing significantly reduced discoloration in the diluted syrup group, particularly in the early stages, but did not prevent staining from becoming clinically noticeable over time.

The limitations of the present study include its *in vitro* design, which does not fully replicate the dynamic oral environment. Factors such as saliva composition, flow and other oral hygiene habits were not accounted for, which could influence stain formation and removal in real-life scenarios. Additionally, the brushing protocol used in this study was not standardized to the same degree as in some other studies, which may have introduced some variability in the results.

Conclusion

The present study found that the concentration of iron syrup plays a significant role in extrinsic tooth discoloration, with concentrated iron syrup causing the most severe staining. Diluting the syrup reduced staining but did not completely prevent discoloration. Tooth brushing provided only limited, short-term benefits suggesting that while brushing may offer modest protection, especially with diluted formulations, it cannot fully counteract iron-induced staining. Dilution of iron supplements emerges as an effective strategy for reducing discoloration in primary teeth. Pediatricians and pediatric dentists should advise parents to dilute iron supplements and emphasize the importance of early, effective tooth brushing to help minimize staining and maintain oral health.

Disclosures

Ethics Committee Approval: The study was approved by the Saint-Joseph University of Beirut Ethics Committee (no: USJ-2024-99, date: 18/04/2024).

Informed Consent: Informed consent was obtained from all participants.

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