Does iron-fortified chewing gum influence the biochemical profile of school-going children (6–10 yrs.)?

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Abstract

Iron deficiency has become a common nutritional problem of developing countries, especially in children. This study approached to tackle the issue of iron deficiency by inexpensive fortified food such as chewing gums, which is commonly consumed by children. In this study, iron-fortified chewing gums were prepared by adding ferrous sulfate ($FeSO_4$) and sodium iron EDTA (NaFeEDTA) 30 mg/100 g. An efficacy trial was conducted to determine the impact of iron-fortified chewing gums on the blood profile and iron status of school-going children (n = 300). Results showed maximum increase in blood profile and iron status that is, serum ferritin (10.43%), hemoglobin (3.22%), hematocrit (3.42%), red blood cells (3.05%), mean cell volume (1.55%), mean cell hemoglobin (5.43%), total white blood cells count (9.09%), and platelets count (4.40%) as compared with control whereas decrease in mean cell hemoglobin concentration (1.90%) and neutrophils (3.33%) was also observed. The study concluded that $FeSO_4$ and NaFeEDTA (1:1) fortification of chewing gums is an appropriate approach for mitigating iron deficiency among the target population.

Keywords: complete blood count (CBC), daily value (DV), ferrous sulfate ($FeSO_4$), iron deficiency anemia (IDA), sodium iron EDTA (NaFeEDTA)

Introduction

Iron (Fe) deficiency anemia (IDA) is known as a predominant nutritional deficiency worldwide. It has depicted substantial economic losses and health problems to distress a massive segment of global population (Petry *et al.*, 2016). IDA causes maternal hemorrhage, reduced productivity, decreased school performance, and innumerable mortalities in vulnerable population. Nearly 700 to 800 million people worldwide are affected by the IDA, which severely distresses 60–70 million people in developing countries. Approximately 65% of pregnant women in South Asia suffer from IDA (Siddiqui *et al.*, 2007).

Numerous reports have revealed that 65–78% of children aged <5 years are suffering from IDA; their hemoglobin (Hb) levels were found below 11 g/dL (Akhtar *et al.*, 2013; Menon and Yoon, 2015). According to another report, the occurrence of anemia was estimated as 82.90%, 85%, 83%, and 78% among children, adolescent girls, pregnant, and lactating women, respectively (Akhtar *et al.*, 2013).

Survey of semi-urban areas of Abbottabad and Peshawar exhibited the occurrence of iron deficiency among children aged <2 years as 68% and 69%, respectively (Idris and Anis-ur-Rehman, 2005). Similarly, in urban slums of Karachi, occurrence of IDA has been depicted as up to 61% (Hb < 11 g/dL) and low hematocrit (PCV) (63.80 % in children aged 6–60 months). The prevalence of anemia among rural-based pre-school children of Karachi has shown highly significant results (Molla and Khurshid, 1992). Likewise, according to another survey conducted in Pakistan, approximately 69% children aged <2 years, 40–50% of pre-school and primary school children, 39% of adolescents, 30% of adult females, and 54% of young girls were reported to be affected by IDA (Khor, 2005). In Pakistan, IDA has also been demonstrated as a severe health problem in pre-school children, followed by pregnant and nonpregnant women (World Health Organization [WHO], 2011).

The WHO has recommended four basic strategies to control and prevent IDA, which include increased iron intake, control of infection, supplementation, and food fortification (Prentice *et al*., 2017). A lot of iron fortification success stories have been reported in literature, that is, countries of the Middle East, Mongolia, Kyrgyz Republic, Kazakhstan, Tajikistan, Azerbaijan, North Africa, and Uzbekistan have successfully implemented iron fortification in wheat and corn flour (Mclean *et al.,* 2008). In fortification of food, either voluntarily or mandatorily, in order to achieve optimum results and to avoid side effects, concentration of iron fortificants should be carefully monitored. Additionally, extensive research has to be practiced to preserve flavor, color, and appearance of food (Kuong *et al*., 2016). Predominant constraints for the success of any fortification depend upon the types and appropriate concentration of fortificants, fortified food, and viability of plans. Isotopic studies indicated that iron absorption from NaFeEDTA was possibly two to three times higher than the other form of iron used as a fortificant (Hurrell, 2002). Huo *et al*. (2002) investigated that NaFeEDTA-fortified soy sauce intervention within concentrations of 5 and 20 mg Fe/day significantly improved the iron status in children (aged 11–17 years). Davidsson *et al*. (2002) evaluated bioavailability of iron in foods based on corn tortillas and black bean paste fortified with ferrous sulfate ($FeSO_4$), ferrous fumarate, and Na-FeEDTA, and concluded that NaFeEDTA retains the higher geometric mean bioavailability (9.0%) than $FeSO_4$ (5.5%). Bouhouch *et al*. (2016) investigated and concluded that the influence of wheat flour biscuits fortified with iron and EDTA resulted in improved iron status significantly, but failed to show any positive influence on cognitive scores. In another study, maize flour, fortified with iron such as NaFeEDTA at in high and low concentrations (56 and 28 mg/kg) along with electrolyte iron (56 mg/kg) was fed to school children (aged 3–8 years) in Kenya. Its findings revealed that only high concentration of NaFeEDTA improved the iron status in children (Andang'o *et al*., 2007). Arcanjo *et al*.(2010) used $FeSO_4$-fortified drinking water with different concentrations of $FeSO_4$ (5, 7.5, and 10 mg/L) for a period of four months, and suggested that 7.5 mg/L of $FeSO_4$-fortified drinking water enhanced the hemoglobin level along with significant reduction in anemia. Moretti *et al*. (2006) advocated the influence of extruded rice fortified with ferric pyrophosphate in Indian school children and showed that IDA reduced from 15 to 30% within the group using iron-fortified meals whereas the control group indicated nonsignificant difference ($P > 0.05$). Indeed, bioavailability of iron relies on the composition of meals, and presence of enhancers and inhibitors during iron absorption (Davidsson *et al*., 2002). Besides selecting suitable vehicles during fortification, processing of food is also crucial. Traditionally, in many countries, cereal products are used as vehicles for fortification. Other vehicles include fish sauce, sugar, common salt, and cookies (Sari *et al*., 2001). However, chewing gums are selected because they are more preferred by children in every economic segment of population. Currently, $FeSO_4$ and NaFeEDTA are being used to fortify food products. Presently, stability, acceptability, and bioavailability of iron compounds in the final product are critical issues and need to be explored to a greater extent.

The objective of the present study was to prepare the cheaper fortified chewing gums and to elucidate changes in proximate composition, minerals, and texture; its impact on the blood profile and iron status of school-going children was also explored.

Materials and Methods

Materials

Ingredients required for the preparation of chewing gums were procured from the local market. All reagents (analytical) were procured from Merck (Merck KG_a A, Darmstadt, Germany) and Sigma-Aldrich (Sigma Aldrich, USA). All other chemicals and reagents used were of analytical grade.

Preparation of iron-fortified chewing gums

Water (10 kg) and glucose (30 kg) were mixed, followed by the addition of 50-kg sucrose and 24 kg gums. The mixture was heated at 154°C followed by the removal of vacuum to get a better color, texture, and flavor. Furthermore, vacuum application decreased mixing to wrapping time. Other ingredients such as color (0.9 g), flavor (4.5 mL), black salt (2.5 g), and citric acid (2.5 g)

were added per kilogram of the above mixture and mixed uniformly. Successively, after the approval of ethical committee, $FeSO_4$ (30 mg) and NaFeEDTA (100 g) were added in 1:1 ratio. After cooling, the mixture was rolled into a sheet and passed through roller press to get chewing gums of uniform size (5 g). Wrapping was done through a central seal machine to remove air and to extend shelf life of the product.

Proximate composition

Chewing gum samples were analyzed on a dry weight basis for crude protein (Method No. 46-30), crude fat (Method No. 30-25), crude fiber (Method No. 32-10), ash (Method No. 08-01), and nitrogen-free extract (NFE) according to their respective procedures. Minerals' analysis was done using the procedure described by Azeem *et al*. (2019).

Textural analysis

Hardness of samples was measured using a texture analyzer (TA-XT2, Plus, Stable Microsystems, Surrey, UK) interfaced with a computer. To compare the hardness of chewing gums, 2-mm cylinder probe (P/2) with a 5-kg load was used. For data analysis, Texture Expert program, version 4.0.9.0, was used.

Energy value

Energy value of chewing gums was determined by taking 0.5 g of sample using Oxygen Bomb Calorimeter (C2000 Basic, IKA®-Werke GMBH & Co., KG, Staufen, Germany) as described by Krishna and Ranjhan (1981).

Sensory acceptability

Sensory acceptability was assessed using a 9-point hedonic scale system, commencing from 1 = dislike extremely up to 9 = like extremely, for sensory attributes like color, flavor, mouthfeel, stickiness, texture, hardness, and overall acceptability by following the instructions of (Meilgaard *et al*., 1999).

Efficacy trial

Healthy school-going children (boys) aged 6–10 years, with a hemoglobin concentration of <11 g/dL, from low- to moderate-income families were selected for efficacy study after the approval of the ethical committee of Allied Hospital, affiliated to the Department of Medicine (No. 09-0234), Punjab Medical College, Pakistan. An efficacy trial was carried out from 10 March 2016 to 15 April 2016 in two middle schools of district Jhang, Pakistan. Parents and children were invited in a seminar to introduce them the purpose, procedure, potential risks, and benefits of the study, and parents' written consent was taken. Complete enrollment and randomization procedure was explained. Parents of 318 children showed willingness to get enrolled in the study. An acceptability trial was conducted prior to efficacy studies to familiarize the children with sensory properties of the proposed iron-fortified chewing gums. Children were examined by a physician to determine whether they are healthy enough to be included in the study. Eighteen children were excluded, who were found diseased, infected, stunted, and malnourished. Finally, 300 healthy children, free from any chronic and acute disease, were stratified by computer-generated random numbers into four groups (T_1–T_4) with 75 children in each group. Fortified chewing gums (5 g) were given during the recess time to each selected child for 45 days continuously. In case of absence of child from school, missed chewing gums were compensated by provision of one extra chewing gum on the following day with advice to consume at midnight. The consumption and distribution of chewing gums were monitored by the authors and teachers. The blood samples were collected at baseline and after 45 days of intervention by a well-trained phlebotomist in the presence of a medical doctor through venipuncture procedure. Each group consumed chewing gums according to the following pattern.

T1: Group of children served with chewing gums without fortificants (control group); **T2**: Group of children served with chewing gums fortified with $FeSO_4$ (100%); **T3**: Group of children served with chewing gums fortified with $FeSO_4$ (50%) and NaFeEDTA (50%), and **T4**: Group of children served with chewing gums fortified with NaFeEDTA (100%). T_1 group was a placebo group. During the period of intervention, parents were advised to remain consistent with their children's dietary habits, constraining them from using any supplements. During pretrial, students complaining diarrhea were managed by decreasing the concentration of iron to the required level (30 mg/100 g). Since placebo group was considered as a control group, parents knew that their children were receiving chewing gums but were not aware whether chewing gums possessed iron or not.

Complete blood count (CBC)

Blood samples (5 mL) of each child were collected at the baseline and after 45 days of intervention through venipuncture procedure by a well-trained phlebotomist. Each 5-mL blood sample was divided into two equal parts and transferred into an erythrocyte sedimentation rate (ESR) tube and a gel tube (anticoagulant-free tube) to collect serum. Immediately after sample collection, CBC was carried out in ESR tubes separately using a CBC analyzer (Sy-Bh192, Sunny Medical Equipment Limited.,

Guangzhou, China), which analyzed the following parameters: hemoglobin, hematocrit, red blood cells (RBCs), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), total white blood cells count (TWBCC), neutrophils, platelets count (PLT), and erythrocyte sedimentation rate. Samples collected in gel tubes were centrifuged to isolate serum that was placed in refrigerator at –20°C for further tests.

Serum ferritin and iron

Serum ferritin (SF) concentrations were measured according to the method described by Sari *et al.* (2001) within 1 h of blood collection by using the enzyme immunoassay method (IMx System; Abbott, Abbott Park, IL). Iron concentration was performed according to the method described by Schoorl *et al.* (2012) using semi-automatic clinical chemistry analyzer (YSTE 168, Guangzhou Yueshen Medical Equipment Co. Ltd., Guangzhou, China).

Statistical analysis

All samples were analyzed in triplicate, and the results were presented as mean ± standard deviation. The statistical analyses were performed using the one-way analysis of variance (ANOVA) to evaluate differences between treatments followed by the Tukey test used to check differences between mean values with SPSS version 17.0 (SPSS Inc., USA). $P < 0.05$ was considered statistically significant.

Results and Discussion

Proximate and mineral composition

Compositional analysis of the iron-fortified chewing gums revealed significant differences for ash- and nitrogen-free extract but nonsignificant results were found for moisture, crude protein, and crude fat (Table 1). The highest ash content was found in T_3 (2.60 ± 0.09%), followed by T_4 (2.56 ± 0.05%), while the lowest ash content was found in T_2 (2.30 ± 0.05%). The overall ash content of iron-fortified chewing gums was in the range of 2.30 ± 0.05–2.60 ± 0.09% whereas the ash content of non-fortified (control) chewing gums was 1.30±0.05%. This increase in ash content was possibly due to the addition of fortificants. It was found in another study that the ash content of flour was increased with the fortification of iron from 1.63 to 1.76% (Akhtar *et al.*, 2005). The highest nitrogen content was found in T_2 (95.55 ± 0.70%), followed by T_4 (95.42 ± 0.60%) whereas the lowest nitrogen content was found in T_3 (95.12 ± 0.10%). Overall, the nitrogen content of iron-fortified chewing gums was in the range of 95.12±0.1–95.55±0.70%, while the nitrogen content of non-fortified (control) chewing gums was 96.64±0.90%. Besides, mean values of moisture, protein, fiber, and fat were found in the range of 1.3±0.26–1.4±0.17%, 0.33±0.12–0.60±0.22%, 0.01±0.05–0.03±0.04%, and 0.49±0.16–0.53±0.17%, respectively, whereas mean values of moisture, protein, fiber, and fat of non-fortified chewing gums were 1.7±0.17, 0.27±0.17, 0.2±0.005, and 0.60±0.21%, respectively. A similar pattern of the contents was found in another study, where tomato-based candy was prepared using 40% sugar solution. The results found similarity with the findings of Manjula and Suneetha (2014), who investigated that crude fiber in a hard candy prepared from pumpkin juice ranged from 1.20 to 2.24%.

Mean values for the iron content of iron-fortified chewing gums revealed significant results (Table 1). The highest iron content (334.03 mg/kg) was found in T_4, followed by T_2 (333.36 mg/kg) whereas the minimum iron content was found in T_2 (330.43 mg/kg). Current results showed similarities with the findings of Manjula and Suneetha (2014).

Texture analysis

Mean values for the hardness of iron-fortified chewing gums were nonsignificant among treatments ($P > 0.05$). Overall hardness content of iron-fortified chewing gums remained in the range of 36.27–39.59 (N) (Table 1). The texture of chewing gums was the same due to the addition of the same ingredient except for iron salt, which showed that addition of salt does not affect the texture. Textural values of chewing gums were comparable with that of tomato candy prepared from 40% sugar solution (Kamruzzaman *et al.*, 2014).

Energy value

Mean values of energy of iron-fortified chewing gums revealed a significant difference among treatments ($P < 0.05$) (Table 1). Mean values of iron-fortified chewing gums revealed that the highest energy value was found in T_3 (326.0 kcal/100 g), followed by T_2 (325.50 kcal/100 g). The lowest energy was found in T_4 (324.63 kcal/100 g). The overall energy of iron-fortified chewing gums was in the range of 324.63–326.0 kcal/100 g. T_1 had less energy value, probably because of missing iron salt whereas other treatments have differences because of salt characteristics.

Sensory evaluation

Sensory evaluation of iron-fortified chewing gums revealed significant difference in flavor, mouthfeel,

Table 1. Mean values for the effect of treatments on physiochemical characteristics of iron-fortified chewing gums.

Treatment	Moisture (%)	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Ash (%)	NFE (%)	Energy (kcal/100 g)	Fe (mg/kg)	Texture (N)
T_1	1.7 ± 0.17	0.267 ± 0.17	0.02 ± 0.05	0.60 ± 0.21	1.30 ± 0.05^d	96.64 ± 0.9^a	322.14 ± 3.26^d	0.01 + 0.25^d	38.40 ± 0.81
T_2	1.3 ± 0.26	0.43 ± 0.17	0.01 ± 0.05	0.53 ± 0.17	2.30 ± 0.05^c	95.55 ± 0.7^b	325.50 ± 1.35^b	330.43 + 0.24^c	39.59 ± 0.12
T_3	1.3 ± 0.12	0.60 ± 0.22	0.02 ± 0.02	0.50 ± 0.12	2.60 ± 0.09^a	95.12 ± 0.1^d	326.0 ± 2.73^a	333.36 + 0.17^b	37.91 ± 0.68
T_4	1.4 ± 0.17	0.33 ± 0.12	0.03 ± 0.04	0.49 ± 0.16	2.56 ± 0.05^b	95.42 ± 0.6^c	324.63 ± 4.44^c	334.03 + 0.13^a	36.27 ± 0.45

Different letters in the same column indicate significant differences ($P < 0.05$).
T_1: 0% $FeSO_4$ and 0% NaFeEDTA; T_2: 100% $FeSO_4$; T_3: 50% $FeSO_4$ and 50% NaFeEDTA; T_4: 100% NaFeEDTA.

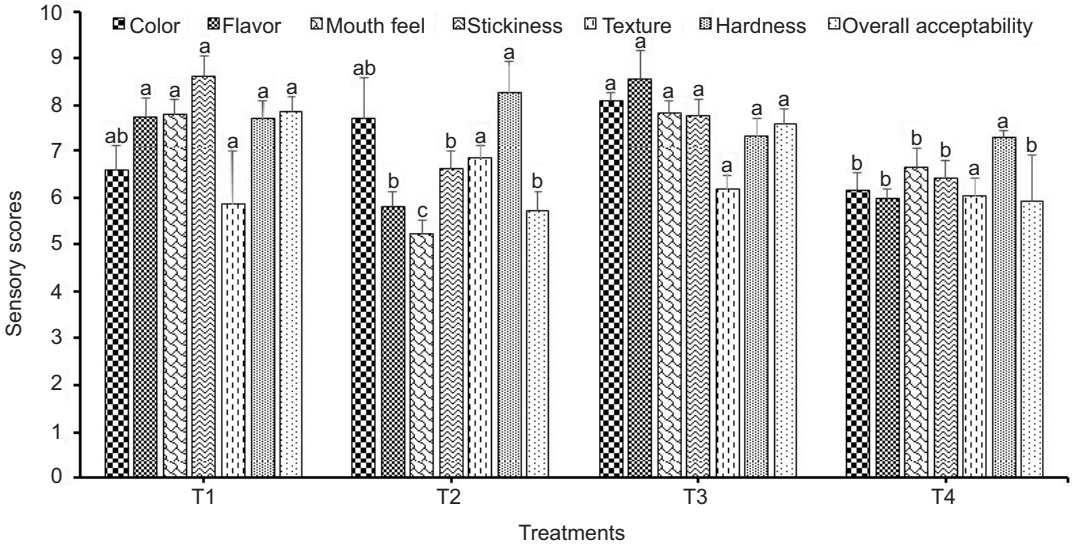

Figure 1. Sensory profile of $FeSO_4$- and NaFeEDTA-fortified chewing gums. Different letters on the same respective parameter bar indicate significant differences between mean values ($P < 0.05$).

stickiness, and overall acceptability but nonsignificant difference for color, texture, and hardness (Figure 1). The best flavor was found in T_3 (8.54 ± 0.62), followed by T_4 (5.99 ± 0.19) whereas the lowest acceptable flavor was observed in T_2 (5.81 ± 0.31). The flavor of non-fortified (control) chewing gums was 7.74 ± 0.40. T_3 (7.84 ± 0.26) secured the highest scores for mouthfeel, followed by T_4 (6.63±0.41) whereas the lowest scores for the stated trait was found in T_2 (5.23 ± 0.29). The score for mouthfeel of non-fortified (control) chewing gums was 7.79 ± 0.33. The highest stickiness was found in T_3 (7.76 ± 0.37), trailed by T_2 (6.61 ± 0.38); however, the lowest stickiness was found in T_4 (6.40 ± 0.39). The stickiness of non-fortified (control) chewing gums was 8.62 ± 0.42. The highest overall acceptability was found for T_3 (7.61 ± 0.30), followed by T_4 (5.91 ± 0.98), while the lowest overall acceptability was shown by T_2 (5.72 ± 0.41). However, the score of overall acceptability of non-fortified (control) chewing gums was 7.87 ± 0.30. Mean values for color and hardness were found in the range of 6.59 ± 0.55–8.10 ± 0.16 and 7.31 ± 0.39–8.27 ± 0.42, respectively, while mean values for color and hardness of non-fortified chewing gums were 6.59 ± 0.55 and 7.71 ± 0.39, respectively. The highest sensory scores for texture was found in T_2 (6.86 ± 0.28), followed by T_3 (6.19 ± 0.25), while the lowest value was found in T_4 (6.03 ± 0.39). The score for texture of non-fortified (control) chewing gums was 5.87 ± 1.12. Indeed, the overall acceptability of food products was affected by fortificant types (Chadare *et al.*, 2019; Davidsson *et al.*, 2005). Current outcomes were consistent with the findings of Durrani *et al.* (2011), who elucidated the color scores of honey-based candy as 7.56–9.23. The flavor's sensory scores reported in the previous study on the quality evaluation of aonla (amla) candy prepared from steep preserved fruits ranged from 6–8%, which were in agreement with the recent findings. The mouthfeel scores in the present study ranged from 5–6%, which showed similarities to the scores reported by Bhattacherjee *et al.* (2013). The maximum score was given to T_3 because of the combination of $FeSO_4$ and NaFeEDTA, as NaFeEDTA

has the capability to mask the off-flavor produced by $FeSO_4$. The lowest score was given to T_2 because of bitter aftertaste imparted by $FeSO_4$.

Efficacy study

Hemoglobin

Hemoglobin level was found significantly increased among the children fed with fortified chewing gums (Figure 2). Maximum increase in hemoglobin (3.22%) was observed in children consuming chewing gums fortified with 100% NaFeEDTA, which ranged from 11.05 to 11.80 g/dL (Table 2) whereas an increase in control group was 0.96% during the study trial of 45 days. Minimum increase (2.31%) was noted in children fed with 100% $FeSO_4$-fortified chewing gums. Overall, a significant improvement in the hemoglobin level of subjects was observed during the study period. It is concluded from the present exploration that the consumption of iron-fortified chewing gums is helpful to increase blood hemoglobin levels in school-going children. This increase was due to iron fortification, which is the basic component of hemoglobin, so an increase in iron in diet is directly related to the serum hemoglobin level (Longfils *et al.*, 2008).

Hematocrit

Hematocrit percentage in school-going children revealed a significant increase after the consumption of iron-fortified chewing gums (Figure 2). This fortification induced improvement in hematocrit by up to 3.42% in children consuming chewing gums fortified with 100% NaFeEDTA that range from 35.92 to 38.12% as compared to the control group (0.98%). The least increase (1.03%) was found in the diet group fed with 100% $FeSO_4$-fortified chewing gums. It is concluded that iron-fortified chewing gums are helpful to increase hematocrit percentage in the blood. The results of hematocrit are related to the findings of hemoglobin. In another study, fortification was done using the micro-capsulated iron pyrophosphate (500 mL/d) providing 18 mg of iron in fruit juice and the influence was determined in menstruated women for eight weeks. Finding indicated that after the intervention, hematocrit increased by 3% as compared to control (Blanco-Rojo *et al.*, 2011). Similarly, in another study

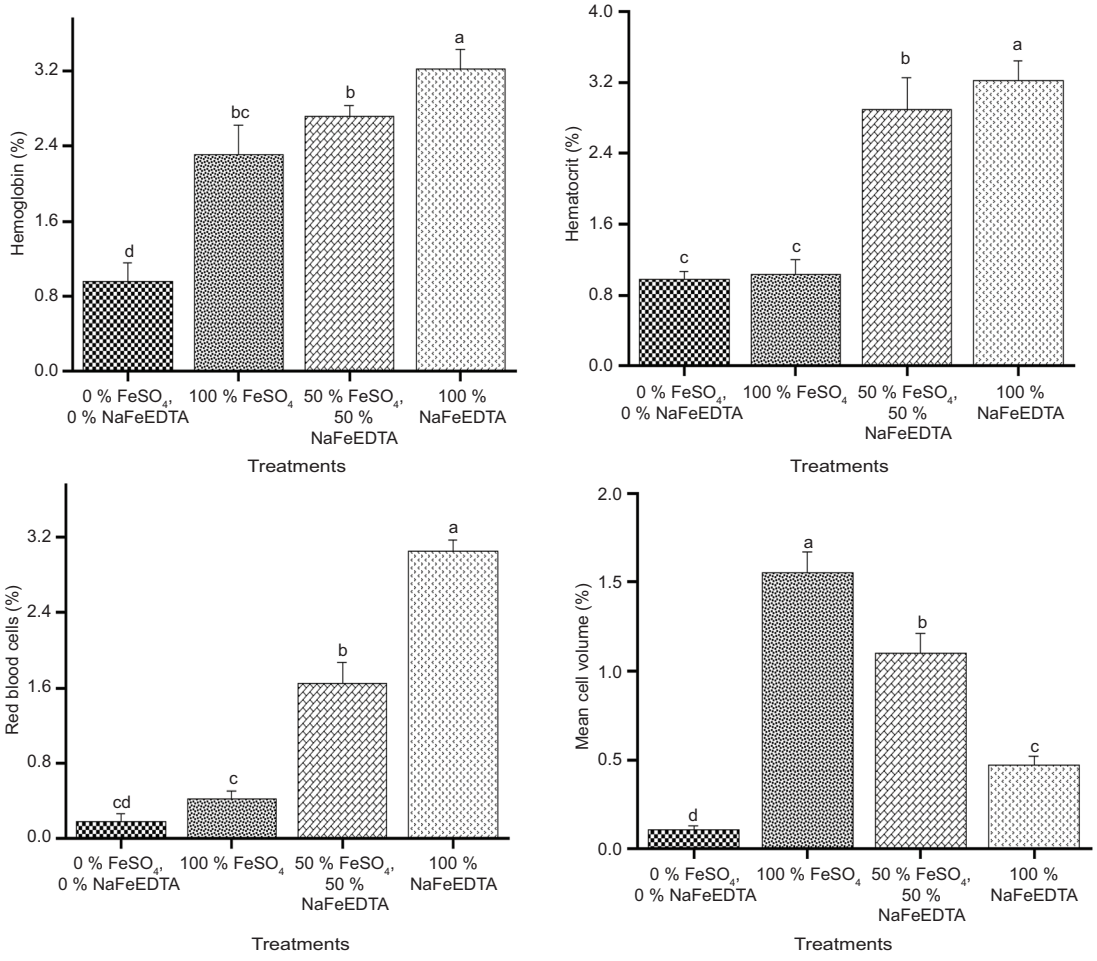

Figure 2. Percentage of hemoglobin, hematocrit, red blood cells, and mean cell volume deviation in school-going children influenced by the consumption of $FeSO_4$- and NaFeEDTA-fortified chewing gums.

hematocrit increased by 2% as compared to control after consumption of iron-fortified food (Patil *et al*., 2013).

Red blood cells

Changes in red blood cell count in children who consumed iron-fortified chewing gums are shown in (Figure 2). This fortification induced the highest red blood cells (3.05%) in children consuming chewing gums fortified with 100% NaFeEDTA, which increased from 4.12 to 4.38 M/UI, followed by the control group (0.18%). The lowest increase in red blood cells (0.42%) was found in the group fed with 100% $FeSO_4$-fortified chewing gums. This increase in the levels of red blood cells is related to improvement in the levels of hemoglobin. In another study, the fortification was done using the micro-capsulated iron pyrophosphate (500 mL/d) by providing 18 mg of iron in fruit juice to menstruated women for four weeks. This resulted in increase of red blood cells by up to 2% as compared to the control group (Blanco-Rojo *et al*., 2011).

Mean cell volume

Deviation in percentage values of MCV in school-going children is described in Figure 2. This fortification induced the highest MCV (1.55%) in children consuming chewing gums fortified with 100% NaFeEDTA that improved from 82.95 to 85.91 femtoliters (fL), followed by the control group (0.11%). The lowest increase in MCV (0.47%) was found in the diet group fed with 100% $FeSO_4$-fortified chewing gums. It is obvious from the present results that recent fortificants are effective in increasing the MCV. The results of MCV are directly related to hemoglobin because hemoglobin is an iron-based protein in red blood cells. In another study done in India, tolerability and cost of three iron fortificants, such as ferrous fumarate (100 mg), ferrous biglycinate (100 mg), and carbonyl iron (100 mg), among pregnant women were determined for 60 days; MCV concentration increased by 0.60% as compared to the control group (Patil *et al*., 2013).

Mean Cell Hemoglobin

Percentage values of MCH in school-going children by the consumption of iron-fortified chewing gums are presented in Figure 3. This fortification induced the highest MCH (5.43%) in children consuming chewing gums fortified with 100% NaFeEDTA that range from 23.47 to 26.17 picograms (pg), followed by the control group (0.98%) (Table 2). The lowest increase in MCH (0.98%) was found in the group fed with 100% $FeSO_4$-fortified chewing gums. The findings of MCH are related to the findings of hemoglobin.

Total White Blood Cells Count

Percentage changes in TWBCC by the consumption of iron-fortified chewing gums by school-going children are shown in Figure 3. This fortification induced the highest TWBCC (7.98%) in children consuming chewing gums fortified with 50% $FeSO_4$ and 50% NaFeEDTA, followed by the control group (3.35%). The lowest increase in TWBCC (6.90%) was found in the diet group fed with 100% NaFeEDTA, followed by (6.90%) chewing gums fortified with 100% $FeSO_4$. It is obvious from the present findings that the consumption of iron-fortified chewing gums may be helpful in increasing TWBCC.

Platelet Count

Percentage changes in platelet count by consumption of iron-fortified chewing gums by school-going children are presented in Figure 3. This fortification induced the highest platelet count (4.40%) in children consuming chewing gums fortified with 100% NaFeEDTA that increased from 136.75 to 89.50 thousands per cubic milliliter (K/uL), followed by the control group (0.59%) (Table 2). The lowest increase in platelet count (0.61%) was found in the diet group fed with 100% $FeSO_4$ followed by the diet group fed with 50% $FeSO_4$ and 50% NaFeEDTA (2.3%). The results showed that fortificants were effective in increasing the platelet count in normal individuals. Furthermore, increase in platelet count is mainly done in the bone marrow and affected by alcohol, drugs, hepatitis, medication, and anemia. Current results are consistent with the finding of Kulnigg-Dabsch *et al*. (2012).

Serum Ferritin

The results of increase in serum ferritin in school-going children after consuming iron-fortified chewing gums are presented in Figure 3. This fortification induced the highest increase in serum ferritin (7.43%) in children consuming chewing gums fortified with 50% NaFeEDTA and 50% $FeSO_4$, followed by the control group (2.08%) (Table 2). The lowest increase in serum ferritin (6.52%) was found in the group fed with 100% NaFeEDTA-fortified chewing gums, followed by 100% $FeSO_4$-fortified chewing gums (5.3%). In another study, impact of different iron fortificants, such as NaFeEDTA (60 mg/kg) and $FeSO_4$ (20 mg/kg), in wheat were observed in anemic school children and concluded that concentration of serum ferritin increased significantly by 3% as compared to the control group (Huang *et al*., 2009).

Iron

Percentage of iron consumption changes with the consumption of iron-fortified chewing gums in normal school-going children as shown in Figure 4. This fortification induced the highest percentage of iron (29.82%) in children consuming chewing gums fortified with 100% NaFeEDTA, followed by the control group (16.14%). The minimum increase in iron (23.47%) was found in the diet group fed with 50% NaFeEDTA- and 50% FeSO4-fortified chewing gums. It is clear from the result that iron-fortified chewing gums may be supportive in children fed

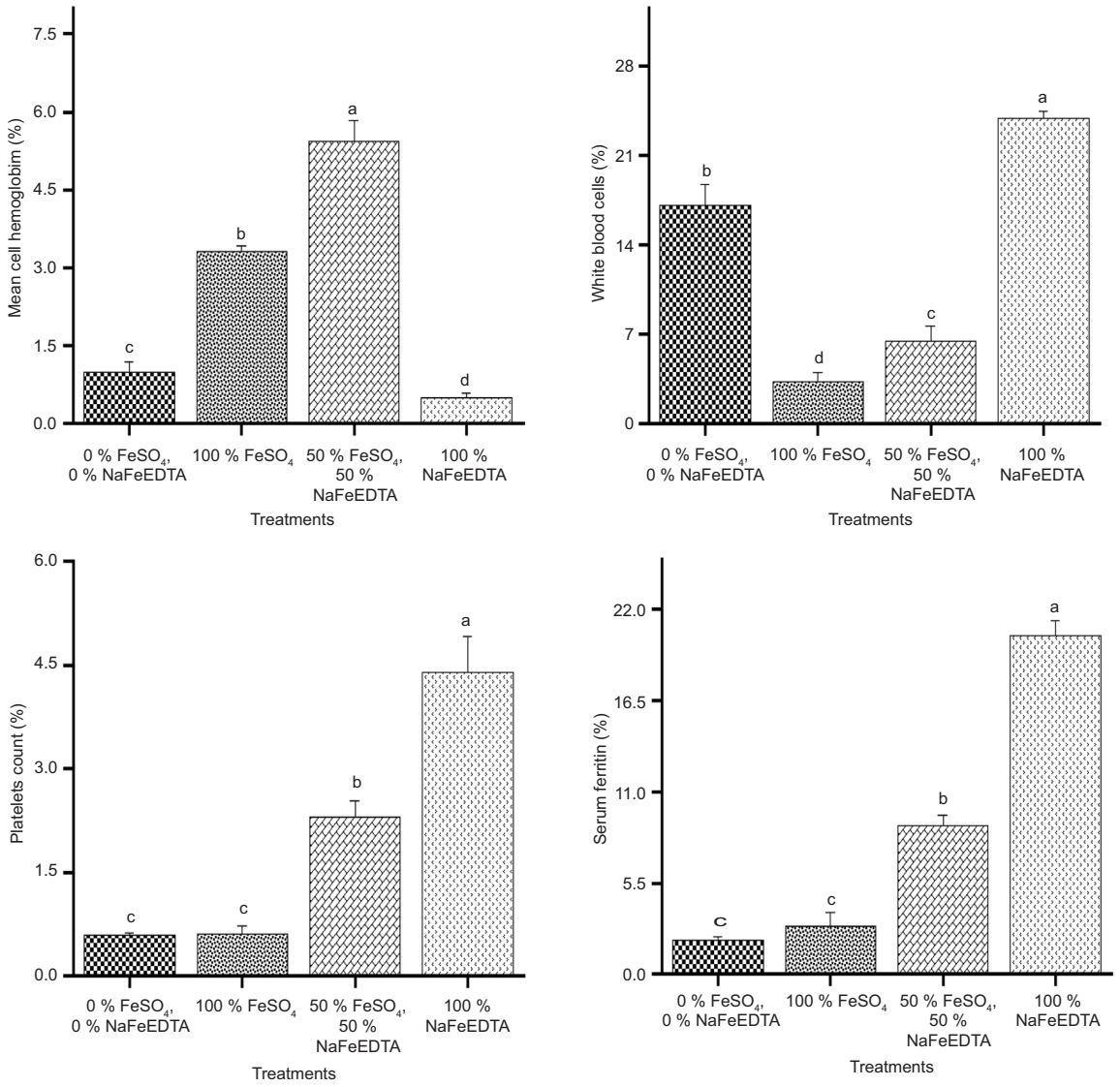

Figure 3. Percentage mean cell hemoglobin, total white blood cell count, platelets count, and serum ferritin deviation in school-going children affected by the consumption of $FeSO_4$- and NaFeEDTA-fortified chewing gums.

for enhancing iron consumption. The current findings were consistent with the findings of Ma *et al.* (2016).

Conclusion

Synthesized evidence revealed that consumption of iron-fortified chewing gums were found effective to improve hemoglobin, serum ferritin and iron status in school-going children aged of 6–10 years, and this is an inexpensive way to combat iron deficiency in low- to middle-income population. Chewing gums fortified with $FeSO_4$ and NaFeEDTA (1:1) obtained highest sensory scores. Proximate composition and texture showed nonsignificant differences ($P > 0.05$), except ash, which might be due to the involvement of different iron salts. Conclusively, the findings of the current study infer that chewing gums fortified with $FeSO_4$ and NaFeEDTA (1:1) possess significant potential to ensure nutritional security. The cost of chewing gums was calculated as PKR 2 per child per day. Diarrhea, widespread malabsorption, and enteropathy may be a barrier to this approach for the acquisition of maximum results. Further research in the future is needed to explain the mechanism underlying these outcomes along with rigorous randomized trials needed to resolve clinical and safety effectiveness.

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Table 2. Mean values of blood biochemical profile of school-going children.

Treatments	T_1		T_2		T_3		T_4	
Days	0	45	0	45	0	45	0	45
Hb (g/dL)	11.25 ± 0.32^b	11.47 ± 0.37^a	13.70 ± 0.26^b	14.35 ± 1.74^a	11.65 ± 0.26^b	12.30 ± 0.22^a	11.05 ± 0.50^b	11.80 ± 0.35^a
PCV (%)	34.32 ± 1.55^b	36.07 ± 0.86^a	42.85 ± 2.04^b	43.75 ± 0.17^a	34.37 ± 0.10^b	36.42 ± 1.44^a	35.72 ± 0.74^a	35.92 ± 0.74^a
RBC (M/uL)	5.27 ± 0.44^a	5.29 ± 0.53^a	4.70 ± 0.15^a	4.66 ± 0.08^a	4.17 ± 0.15^b	4.31 ± 0.13^a	4.12 ± 0.35^b	4.38 ± 0.43^a
MCV (fL)	90.40 ± 1.0^a	90.20 ± 0.80^b	83.20 ± 0.60^b	84.0 ± 0.30^a	71.50 ± 0.90^b	73.10 ± 0.10^a	82.95 ± 0.50^b	85.91 ± 0.40^a
MCH (pg)	26.87 ± 0.88^b	27.15 ± 0.16^a	29.75 ± 0.78^a	29.17 ± 0.45^b	27.15 ± 0.49^b	29.02 ± 0.63^a	23.47 ± 1.74^b	26.17 ± 0.10^a
TWBC (K/uL)	7.91 ± 0.21^b	8.46 ± 0.31^a	9.37 ± 0.70^b	10.70 ± 0.11^a	10.67 ± 0.80^b	12.52 ± 0.87^a	7.62 ± 0.70^b	8.75 ± 0.24^a
PLT (K/uL)	280.17 ± 1.80^a	276.83 ± 1.0^b	286.10 ± 1.10^a	285.75 ± 0.90^b	260.75 ± 0.71^a	249.0 ± 0.40^b	136.75 ± 0.79^b	189.50 ± 0.90^a
SF (ng/mL)	21.30 ± 0.15^b	22.57 ± 0.76^a	25.45 ± 0.90^b	28.35 ± 1.02^a	24.40 ± 1.83^b	28.32 ± 0.73^a	28.95 ± 0.34^b	32.97 ± 1.46^a
ESR (mm/h)	26.0 ± 15.03^b	28.17 ± 22.04^a	9.15 ± 4.12^b	10.57 ± 4.87^a	12.5 ± 4.15^b	20.0 ± 6.04^a	26.25 ± 3.39^b	51.42 ± 7.41^a
Fe (ug/dL)	88.8 ± 44.94^b	123.00 ± 23.54^a	60.85 ± 19.24^b	119.35 ± 30.98^a	51.75 ± 17.71^b	83.50 ± 20.33^a	53.92 ± 16.94^b	99.75 ± 8.25^a

Different letters in treatment (consecutive two rows) indicate significant differences ($P < 0.05$).
Hb: hemoglobin, PCV: hematocrit, RBC: red blood cells, MCV: mean cell volume, MCH: mean cell hemoglobin, TWBCC: total white blood cells count, PLT: platelet count, MCHC: mean cell hemoglobin concentration, SF: serum ferritin, ESR: erythrocyte sedimentation rate, Fe: iron.
T_1 = 0% $FeSO_4$ and 0% NaFeEDTA, T_2 = 100% $FeSO_4$; T_3 = 50% $FeSO_4$ and 50% NaFeEDTA; T_4 = 100% NaFeEDTA.

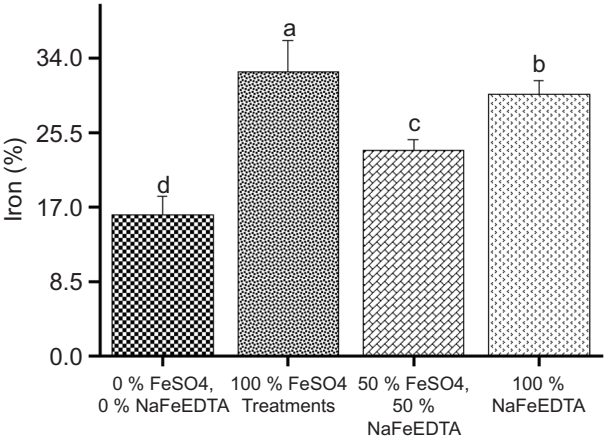

Figure 4. Percentage of iron deviation in school-going children affected by the consumption of $FeSO_4$- and NaFeEDTA-fortified chewing gums.

Conflict of Interest

The authors declared no conflict of interest.

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