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# Efficacy Studies of Natural and Synthetic Iron Sources among Anemic Pregnant Women in Community of Faisalabad-Pakistan

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**Abstract** In present study, different treatments enriched with natural and synthetic Iron sources along with control were developed and evaluated for proximate composition, mineral content, calorific values and sensory attributes fortnightly for sixty days and their efficacy was tested among anemic pregnant women for ninety days. Blood of 787 pregnant women was investigated for Hb status. Among them 38.50 % were found anemic (Hb < 11 g/dL) whose blood was further analyzed for complete blood count (CBC), serum Ferritin, serum Fe, total Fe binding capacity (TIBC), transferrin saturation (TS) and mean corpuscular volume (MCV) to find IDA victims. Hence, 70.29 % pregnant females were identified as IDA sufferer. Finally, 200 volunteers were assorted randomly into 4 treatment groups. As regards product development, all treatments provided 50 % RDA (13.5 mg) of Fe except placebo ($T_0$). The significant variations (*P-value* <0.05) in anthropometrics, energetics and dietary intakes were observed during the study. Hematological indices were improved significantly by $T_2$ in 90 days as RBC count (4.60±0.027 M/uL), Hb (11.01±0.039 g/dL), Hct (33.57±0.091 %), MCV (75.00±0.117 fL), MCH (24.96±0.089 pg), MCHC (30.10±0.101 g/dL), Serum Fe (47.72±0.219 ug/dL), TIBC (373.42±1.154 ug/dL) and TS (10.20±0.093 %). However, serum Ferritin had maximum uplift (8.28±0.234 ng/mL) by $T_1$. Liver function tests (LFTs) and renal function tests (RFTs) of volunteers showed safety of intervention. A significant positive correlation (*P-value* <0.05) was also found amongst indices of IDA. The outcomes of the current research work showed that natural iron sources like blackstrap molasses, dried apricots, dried dates and rape seeds are cost effective and healthful foods having high bio-available Fe as compared to synthetic Iron sources (Ferrous sulphate) in combating IDA among pregnant women.

***Keywords:*** *synthetic, natural iron sources, pregnant women, IDA*

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## 1. Introduction

Anemia is a pathological condition which is established when Hb < 11 g/dL in pregnant women. It is a global health problem that affects individuals of all ages and economic classes especially among women of reproductive age [1]. Hb deficiency and IDA directly correlate with socio-graphic conditions [2]. Anemia is declared as a public health problem and its prevalence is classified as mild (5-19.9%), moderate (20-39.9%) and severe (≥ 40 %) [3]. Globally 41.8 %, in Asia 41.6 % and in Pakistan 39.1% pregnant women are anemic. In Pakistan and Punjab province, Hb deficiency in pregnant ladies was 51.2 and 49.6 %, respectively [2]. Similarly, about 30 % of world population is Fe deficient whereas pregnant women in industrialized world, developing nations, Pakistan and Punjab province were 14, 56, 25 and 27 % IDA victim, respectively. In Pakistan and Punjab province, pregnant mothers with low Ferritin levels (< 12 ng/dL) have also been found as 38.2 and 40.5%, respectively [2]. Major contributors of anemia among women in developing countries are malnutrition, physiological blood losses, hemoglobinopathies, inflammatory problems and multiple para [4]. Lethargy, dizziness, fatigue, shortness of breath, ringing in ears, pallor, pale conjunctivae, taste disturbances, brittle nails, pica and glossitis are indicative symptoms of IDA which also disturbs metabolic activities [5]. Iron, vitamins A, B6, B12 and C and folic acid are integral to hematopoiesis process [6] and have considerable contribution in Hb level if consumed in ample proportion [7]. Iron bounds to Transferrin (Tf) through surface receptors (TfR) according to its complexing capability. However, the toxicity may develop when excessive Fe is accumulated in vital organs [8] such as labile cellular Fe in red blood cells (RBC) [9]. Similarly, erythroid precursors may also uptake excessive Fe [10]. Iron absorption may be inhibited by polyphenols and tannins, therefore, the regular use of vitamin C , α-Tocopherols and organic acids enhances the absorption of Fe particularly under the situation when plant sources are consumed [11]. Blackstrap molasses, dates (*Phoenix dactylifera* L.), apricots (*Prunus armeniaca* L.) and rape (*Brassica rapa* L.) seeds have 250, 5.8, 400 and 100 mg Fe/ Kg [5,12,13,14] whereas 3.07 mg dried ferrous sulphate contains 1 mg Fe [15]. Blackstrap, being an economical, easily available source of iron and a waste in sugar manufacturing, provides more than 95 % Fe in available form due to the presence of fumaric acid. The study was, therefore, designed to exploit the comparative efficacy potential of natural source of blackstrap with ferrous sulphate to combat anemia in pregnant women and discourse their impact on IDA.

## 2. Materials and Methods

Dietary interventions play a vital role to combat micronutrient deficiencies like IDA which is most common among women of childbearing age. This study was comprised of two phases, a product development phase and efficacy studies phase. In first phase, four treatments/samples have been developed by using natural and synthetic source iron, as detailed in Table 1. The treatments so prepared were then evaluated for proximate composition, minerals content, calorific values and sensory attributes fortnightly for 60 days. In the second phase, the efficacy study of the treatments was carried out for a period of 90 days, on pregnant women suffering from IDA.

### 2.1. Product Development

Different treatments were prepared by using natural and synthetic sources of iron as given in Table 1.

The proximate analysis for all treatments was carried out with fortnight interval for 60 days of storage at room temperature. Minerals like Fe, Ca, K, Mg and Cu in all the treatments were analyzed by using AOAC Method No. 985. 35 [16] after the same intervals during storage. All the treatments were subjected to Oxygen Bomb Calorimeter (Model: 1341, Parr Instrument Company, Werke IKA) and calorific value of all the treatments was determined by following the recommended procedure. Sensory evaluation of treatments for color, flavor, taste, texture and overall acceptability was also conducted fortnightly during 60 days of storage by a trained panel of judges through 9-point hedonic scale [17].

### 2.2. Efficacy Study

#### 2.2.1. Ethical Review and Criteria of Research Project

The project was presented to Departmental Review Committee for Ethics (DRCE) Institute of Home and Health Sciences (IHFS), GC University Faisalabad, for its ethical review. The DRCE approved the project unanimously. The pregnant females (15-49 years) having hemoglobin level < 11.0 g/dL, serum Ferritin < 12 ug/L [1], serum Fe < 60 ug/dL, TIBC > 350 mg/dL, Transferrin saturation (TS) < 15 % [18] and MCV < 80 fL [19] were selected for this study, whereas, women with hepatic and renal diseases, hemorrhoids and or excessive menstrual bleeding were excluded from the studies. Written informed consent was taken from the females who wanted to be the part of the research project. The selected volunteers were dewormed by providing an anthelmintic drug (Praziquantel) at 40 mg/ Kg body weight [20] followed by a fortnight booster dose prior to baseline. Various variables including independent (demographics, socioeconomic status, food intake and eating habits), dependent (anthropometrics and blood tests) and confounding variables (life style pattern and family back ground) of the respondents, have been investigated at both baseline and during intervention in this study.

#### 2.2.2. Study Locale, Target Population and Sampling Technique

The study design for the research project was randomized controlled clinical trial. Twenty Population Welfare Centers (PWCs), Department of Population Welfare, Faisalabad, Government of Punjab, were the study sites of research work. Study site was selected after approval from District Population Welfare Officer, Department of Population Welfare, Faisalabad, and Government of Punjab. The anemic pregnant females (15-49 years) were the target population. The selection of the human subjects was carried out in accordance to Two Stage Non-Probability Sampling Methods i.e. Convenience and Purposive Sampling Techniques. The sample size for pregnant women was calculated by the formula of Magnani [21]; considering estimated prevalence of variable of interest (anemia), required level of confidence (95%) and 5 % margin of error. By using the above mentioned formula, required sample size was 2359 women. By considering the economy and convenience, 787 women (1/3rd of required sample size) were then randomly selected for further studies [22].

#### 2.2.3. Screening and Assortment of Pregnant Women

Initially 787 pregnant women were screened for Hb level through B-Hemoglobin Photometer (Model: K061047 HEMOCUE AB, Angelholm, Sweden). Among them, 303 (38.50 %) were found to be anemic (Hb < 11 g/dL). The blood samples of those women were drawn through disposable syringe (BD Luer-Lok, Becton Dickinson Pakistan Private Limited) from suitable vein after skin disinfection (Alcohol swab-BD, Becton Dickinson Pakistan Private Limited) according to standard procedure. The blood was collected in pre-coded tubes. Tube-I was EDTA-K3 tube (Guangzhou Improve Medical Instruments Co., Ltd), whereas, Tube-II was gel and clot activator tube (Guangzhou Improve Medical Instruments Co., Ltd). The tubes of blood samples were wrapped in Aluminum foil, kept in an ice box and shifted to Food and Nutrition Laboratory for further analysis. The blood of tube-I was analysed for ABO blood grouping (Anti-A, Anti-B and Anti–A-B) using reagents and complete blood count. Complete blood count (CBC) included hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Erythrocyte sedimentation rates (ESR) and platelets (Plt) were determined through the reported procedure. White blood cell (WBC) count and its indices like neutrophiles, lymphocytes, monocytes, eosinophiles and basophiles determination was also done. All these analysis were carried out through fully Automatic Blood Analyser Nihon Kohden, Japan. Serum was extracted from blood of tube-II through centrifugation by using centrifugal machine (Model: 800, Centrifugal Machine, China) at 4000 rpm for six minutes [23]. Extracted serum was used for the determination of hepatitis B and C infection, Liver Function Test (LFT) and Renal Function Test (RFT), which were carried out by using commercial kits (Microlab-300, Merck Germany). Hepatitis B and Hepatitis C were screened by HBs Ag and by HCV by using one step test device, respectively. During LFT investigation, serum samples were utilized for billirubin total, direct and indirect, by the method reported by Jendrassik-Grof, SGPT by IFFC-method and alkaline phosphatase by ALP-DGKC procedure. In case of RFT, separated serum sample was used for the determination of urea by GLDH and creatinine by Jaffe procedure. Serum iron and total iron-binding capacity (TIBC) were estimated by Colorimetric method (Microlab-300, Merck Germany) while serum Ferritin was analyzed by Immunoassay technique [24] through Access II and Transferrin saturation was determined by the formula of Gibson [25]. Hence, 213 (70.29 %) women were found to be victim of IDA and 12 (5.62 %) more women that had social constraints and suffering from HBV, HCV, LFT, RFT and Hemorrhoids were excluded from the study. In this way, 200 women were selected at the start of intervention and randomly assorted into four treatment groups of 50 volunteers each. During intervention, 12 (6 %) more subjects had also been dropped out due to some unavoidable circumstances on the part of volunteers (Figure 1).

**Table 1. Different treatments of Fe**

| Treatments | Composition | Dose | Fe Content (mg) |
|---|---|---|---|
| $T_0$ | 250 mg Lactose as filler in capsule (control) | 3 Capsules | 00.00 |
| $T_1$ | 23.63 g Blackstrap molasses (viscous liquid; net wt: 24 g in sachets) | 3 Sachets | 13.50 |
| $T_2$ | 17.10 g Blackstrap molasses + 3.0 g dried Dates + 3.0 g dried Apricots + 3.0 g Rape seeds (semi solid paste; net wt: 26.50 g in sachets) | 3 Sachets | 13.50 |
| $T_3$ | 13.84 mg Ferrous sulphate + 236.16 mg Lactose in Capsule | 3 Capsules | 13.50 |

*Pregnant women were provided treatments to meet 50 % of their RDA for Fe.

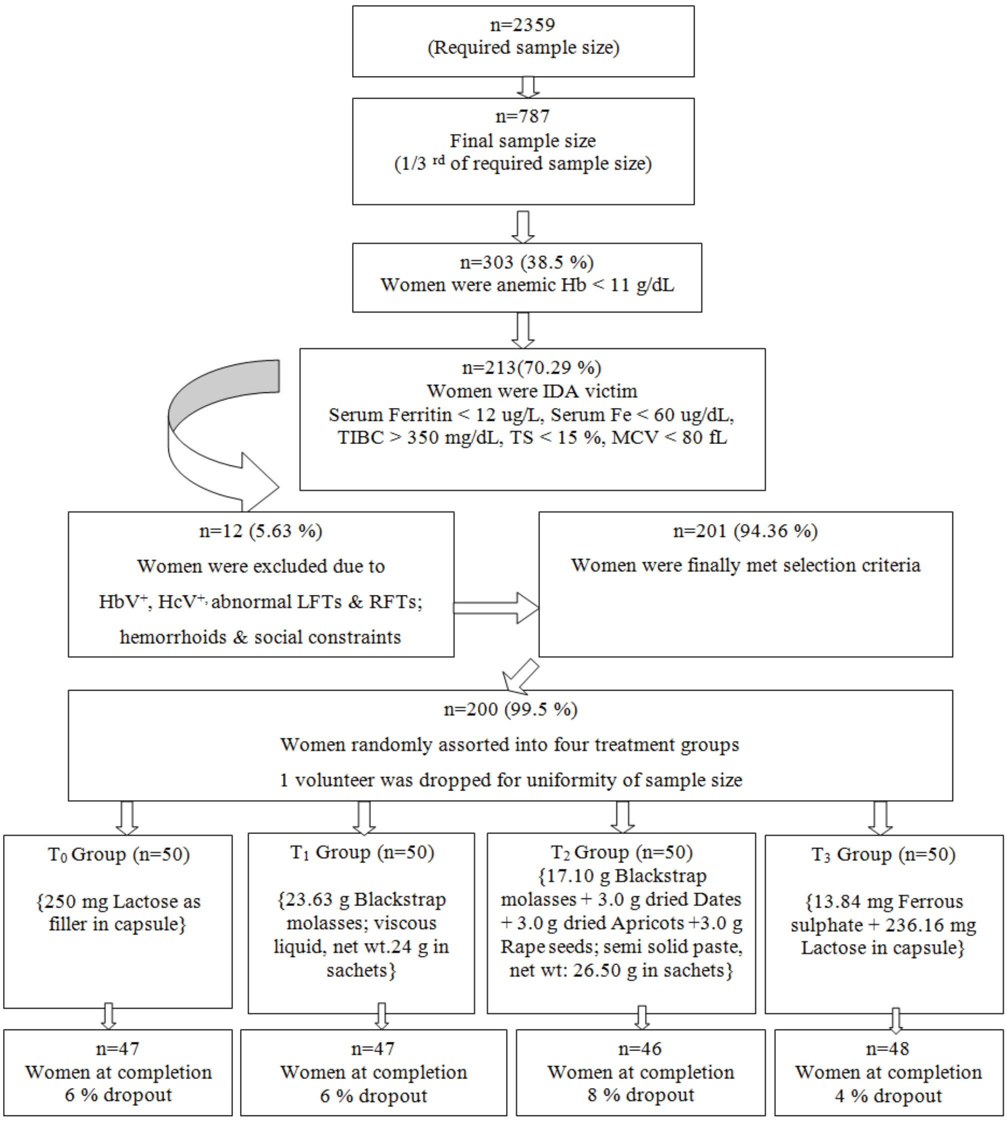


**Figure 1.** Flow diagram of volunteers' screening and assortment

#### 2.2.4. Demographics, Anthropometrics and Dietary Intakes of Volunteers

Demographic attributes such as volunteers' name, age, gender, qualification, occupation, income, contact number, family diseases and physical activity level were collected. The anthropometric measurements of the selected subjects as height, weight, body composition, body mass index, basal metabolic rate and active metabolic rate were recorded to assess their nutritional status. The dietary intakes history Performa was filled up by all of the volunteers at baseline, thrice a week during the intervention.

#### 2.2.5. Provision and Distribution of the Treatments

The pregnant women were randomly and separately assorted into T0, T1, T2 and T3 groups. Each volunteer had been given daily three capsules/sachets of assigned treatment to meet their 50 % RDA (13.5 mg) of Fe except placebo. Every volunteer visited PWC on weekly basis and collected her respective treatment in a packet containing 7 doses and submitted Food Diary and empty packets. At completion of study, all volunteers had visited PWCs for their anthropometrics; energetics and vital signs recording followed by blood samples collected for further analyses as described earlier in detail.

#### 2.2.6. Statistical Analysis

Data collected was analyzed with the help of statistical software SPSS-20. Descriptive statistics was run to check the distribution and frequency of data and data was further processed by one way analysis of variance (ANOVA) technique and LSD Test was used to find out significance level ($p \leq 5$ %) between groups. The results so obtained of this intervention have been interpreted on logical grounds and conclusions were drawn.

## 3. Results and Discussions

Iron deficiency anemia (IDA) is a public health problem which is most common among females of reproductive age residing in developing countries. At most it is caused by malnutrition, blood losses, hemoglobinopathies, multiple para and inflammatory problems [4]. Since poverty is the main cause of poor nutritional status, therefore, exploration of cheaper sources of natural Fe becomes very important in the management of IDA. Blackstrap molasses is the by-product of sugar industry and easily available in sugarcane belt of Pakistan. Interventions in terms of dietary and micro-nutrients aspects play an imperative role to alleviate IDA and other nutritional deficiencies. Being a demographics factor, the results regarding volunteers' family income indicated that 86.40 % of pregnant women had low monthly income or low socioeconomic status (SES), 11.40% had medium level of income, while only 2.20 % possessed a high family income. These results are corroborated by the studies of other scientists who already have carried out the research work on IDA. In one of the studies [26] it was observed that 67.14 % women facing chronic energy malnutrition had an income of Rs. 2001-4000. However, 25.04 % that belonged to income group of Rs. 4000 were highly malnourished. In another study [27] it was reported that 102 pregnant women with Hb < 10.5 g/dL; 12-16 weeks gestational age and 20-35 years old had income was less than 1500 NIS/month in both 47.1 % experimental and 60.8 % control group. In the current study, volunteers were taking only appropriate servings from bread, cereal, rice and pasta group while number of servings of remaining food groups was less as compared to the standard servings per day, recommended by Food Guide Pyramid [28]. However, a highly significant variation in intake of number of servings from various food groups during study period of 90 days was investigated (Table 2) that might be due to dietary counseling and education. According to Kaur & Kaur [29] positive association was found between Hb levels and intake of cereals, milk and milk products. A study [30] reported that 91 % of college students thought that they had good health status but their actual intake was 7 % of the recommended daily allowance of fruits and vegetables.

Huma [31] also worked on whole wheat flour that was fortified with either $FeSO_4$ (FS) at 30 ppm Fe or Ferrous sulfate plus EDTA (FSE) at 20 ppm Fe plus 20 ppm EDTA and elemental Fe (EI) at 60 ppm Fe.

Intake of milk and meat was less than tea and lassi (local fermented food) among the study participants, which is strengthened by the results of Croezen *et al*.[32], who had documented that rise in unhealthy dietary habits and increased consumption of sweetened beverages by young people had an important role in pathogenesis of under nutrition or obesity. In another study [33] it was declared that utilization of dairy products reduced the chances of anemia. Rausch reported [34] that low Fe level is a common feature of diet with excessive junk foods. These results are also supported by the research work of other scientists [35,36] who delineated that insufficient dietary intakes were significantly associated with increased risk of anemia.

**Table 2. Intake of water and no. of servings of volunteers**

| Food Group n = 184 | | Days 0 Mean | Days 90 Mean | P.value |
|---|---|---|---|---|
| Water intake (L/Day) | | 1.009±0.035 | 1.119±0.032 | 0.004 |
| a | No of servings | 8.80±0.001 | 10.04±0.061 | 0.003 |
| b | No of servings | 0.86±0.001 | 1.02±0.017 | 0.005 |
| c | No of servings | 1.87±0.001 | 2.01±0.017 | 0.002 |
| d | No of servings | 1.77±0.001 | 1.91±0.024 | 0.001 |
| e | No of servings | 1.75±0.001 | 1.8±0.010 | 0.005 |
| f | No of servings | 3.75±0.001 | 4.00±0.022 | 0.003 |
| g | No of servings | 1.91±0.024 | 1.77±0.001 | 0.000 |

a= Bread, Cereal, Rice and Pasta Group, b=Fruit Group, c= Vegetable Group, d=Meat, Fish, Poultry, Beans, Eggs and Nuts Group, e= Milk, Yogurt and Cheese Group, f= Fats, Oils and Sweets Group, g= Junk Food Group, SEM= Standard error of mean, Data is expressed as mean values (SEM), *P* value of all food groups is < 0.05 at 90 days of study

Anthropometrics and energetics of the volunteers under study followed a similar pattern of variation as reported in dietary intake. Body weight (Kg), BMI (Kg/m2), BMR (Kcal) and AMR (Kcal) varied significantly after 90 days of study span (Table 3). These results were supported by the scientific outcomes of other authors. Khalid *et al*. [36] worked on 160 anemic pregnant women that were provided Fe supplements for three months. After the study period, body mass index was found to be 22.45±0.39 $Kg/m^2$ in the daily supplemented group followed by 21.95±0.37 Kg/m2 in the twice weekly supplemented group. Al *et al*. [37] stated that 90 anemic pregnant women of 26 - 34 gestational weeks with Hb from 8.0 - 10.5 g/dL and serum Ferritin concentration less than 13 ug/L were supplemented with Fe. After the study, rise in weight of 58.2 Kg was observed in the oral Fe group and 56.0 Kg in the intravenous Fe group. Similarly, it was reported [38] that some obese persons depicted an increase in weight although their intake was less. The reason behind this increase in weight was due to the basal metabolic rate. BMR and body composition parameters were investigated in 24 healthy women during lactation and after the cessation of breastfeeding at 18 or 24 months postpartum [39]. BMR changed from 5.57±0.48 to 5.65±0.60 MJ and body fats changed from 20.8±7.7 to 19.2±8.1 Kg. Another study showed [35] that dietary energy (Kcal) of non-anemic and anemic women was 1546.7±580.4 and 1473.3±584.8, respectively. Although in many studies, large doses of oral and intravenous Fe supplementation had been used to improve the Hb levels and Fe status of patients but sometime they induce severe complications. However, some studies also quoted good improvement effects regarding patients Fe deficiency anemia with low doses of Fe supplementation producing less or no complications at all. Therefore, food based approaches are always preferred over synthetic sources to cope with such nutritional deficiencies. In this regard some studies using natural Fe sources are available and reported in literature but according to country like Pakistan where poverty is the main dilemma, a study was therefore, designed and conducted to pin down economical and rich source of natural Fe.

**Table 3. Anthropometrics and energetic of volunteers**

| Indicators (n = 184) | Days | | **P.value** |
|---|---|---|---|
| | 0 | 90 | |
| | Mean | Mean | |
| Wt (Kg) | 58.11±0.802 | 62.39±0.798 | 0.000 |
| BMI (Kg/m$^2$) | 23.65±0.291 | 25.40±0.296 | 0.005 |
| BMR (Kcal) | 1357.8±8.24 | 1400.2±8.10 | 0.003 |
| AMR (Kcal) | 1464.9±9.66 | 1514.5± 9.34 | 0.001 |

Wt= weight, BMI= body mass index, BMR= basal metabolic rate, AMR= active metabolic rate, Data are presented as mean values (SEM), SEM= standard error of mean, *P* value of all indicators is < 0.05 at 90 days.

**Table 4. Indices of IDA of volunteers**

| Biomarkers n=184 | Treatments | Days | | P.value |
|---|---|---|---|---|
| | | 0 | 90 | |
| | | Mean | Mean | |
| Total red blood cells (M/uL) | $T_0$ | 4.18±0.039 | 4.00±0.044 | 0.004 |
| | $T_1$ | 4.15±0.014 | 4.37±0.012 | 0.003 |
| | $T_2$ | 4.26±0.027 | 4.60±0.027 | 0.000 |
| | $T_3$ | 4.20±0.017 | 4.40±0.017 | 0.004 |
| Hemoglobin (g/dL) | $T_0$ | 9.41±0.136 | 8.67±0.102 | 0.005 |
| | $T_1$ | 9.27±0.135 | 11.00±0.036 | 0.002 |
| | $T_2$ | 9.09±0.175 | 11.01±0.039 | 0.000 |
| | $T_3$ | 9.86±0.123 | 11.07±0.043 | 0.004 |
| Hematocrit (%) | $T_0$ | 30.98±0.081 | 29.01±0.088 | 0.006 |
| | $T_1$ | 30.49±0.058 | 32.52±0.050 | 0.005 |
| | $T_2$ | 31.05±0.089 | 33.57±0.091 | 0.001 |
| | $T_3$ | 30.47±0.046 | 31.98±0.042 | 0.006 |
| Mean corpuscular volume (fL) | $T_0$ | 73.14±0.088 | 71.16±0.093 | 0.004 |
| | $T_1$ | 73.11±0.237 | 74.68±0.122 | 0.003 |
| | $T_2$ | 72.95±0.116 | 75.00±0.117 | 0.001 |
| | $T_3$ | 72.98±0.149 | 73.92±0.138 | 0.005 |
| Mean corpuscular hemoglobin (pg) | $T_0$ | 23.93±0.091 | 22.58±0.086 | 0.004 |
| | $T_1$ | 24.07±0.141 | 24.64±0.086 | 0.004 |
| | $T_2$ | 23.97±0.076 | 24.96±0.089 | 0.001 |
| | $T_3$ | 24.11±0.078 | 24.49±0.075 | 0.003 |
| Mean corpuscular hemoglobin concentration (g/dL) | $T_0$ | 28.98±0.090 | 27.55±0.094 | 0.006 |
| | $T_1$ | 29.06±0.109 | 29.61±0.109 | 0.004 |
| | $T_2$ | 29.12±0.119 | 30.10±0.101 | 0.003 |
| | $T_3$ | 29.15±0.137 | 29.69±0.137 | 0.005 |
| Serum iron (ug/dL) | $T_0$ | 46.23±0.310 | 45.40±0.241 | 0.004 |
| | $T_1$ | 46.70±0.248 | 47.58±0.128 | 0.003 |
| | $T_2$ | 46.55±0.250 | 47.72±0.219 | 0.001 |
| | $T_3$ | 46.40±0.201 | 46.84±0.192 | 0.003 |
| Serum total iron binding capacity (ug/dL) | $T_0$ | 387.87±0.892 | 402.50±0.821 | 0.005 |
| | $T_1$ | 389.84±0.895 | 376.58±0.970 | 0.004 |
| | $T_2$ | 392.22±1.076 | 373.42±1.154 | 0.002 |
| | $T_3$ | 387.90±0.843 | 374.21±0.916 | 0.004 |
| Serum ferritin (ng/mL) | $T_0$ | 7.98±0.179 | 7.62±0.165 | 0.002 |
| | $T_1$ | 7.60±0.232 | 8.28±0.234 | 0.001 |
| | $T_2$ | 8.42±0.134 | 8.92±0.138 | 0.000 |
| | $T_3$ | 7.95±0.114 | 8.08±0.123 | 0.002 |
| Serum transferrin saturation (%) | $T_0$ | 9.19±0.084 | 8.07±0.101 | 0.003 |
| | $T_1$ | 9.16±0.082 | 9.67±0.082 | 0.001 |
| | $T_2$ | 9.16±0.080 | 10.20±0.093 | 0.000 |
| | $T_3$ | 8.99±0.072 | 9.19±0.083 | 0.004 |

Day 0= At baseline before intervention, Day 90= After intervention of 90 days.

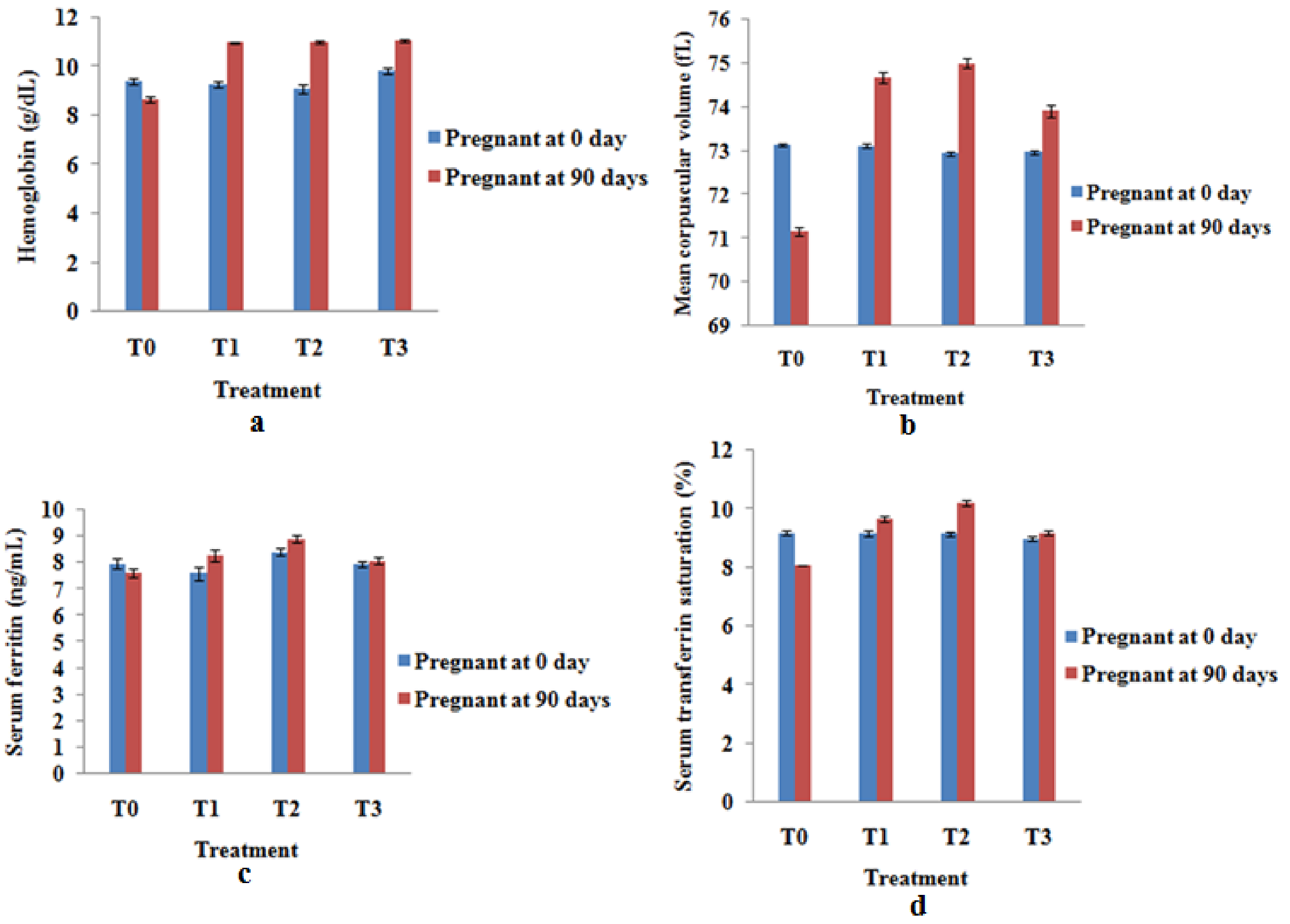


**Figure 2.** Column graphs of mean change in Hemoglobin (2a) serum ferritin (2b) mean corpuscular volume (2c) and serum transferrin saturation (2d) of pregnant volunteers at base line (0 day) and after the study period (90 days) due to intervention of various iron treatments of $T_0$ (control), $T_1$, $T_2$ and $T_3$. Data points in column indicate mean values ; error bars indicate 95% confidence intervals of the mean. *P < 0.05* for all treatments at post study periods as compared to base line stage. *P- value* is from paired t-test

In the current study, the use of blackstrap molasses at the ratio of 50% RDA of Fe (13.5 mg Fe) during pregnancy explored very encouraging results on patients Hb level and iron status. Hematological indices of pregnant volunteers were improved significantly by T2 in 90 days which is proved by following results (Table 4); total red blood cells (TRBC) 4.26±0.027c →4.60±0.027a M/uL, Hb 9.09±0.175d →1.01±0.039a g/dL, hematocrit (Hct) 31.05±0.089d →33.57±0.091a %, mean corpuscular volume (MCV) 72.95±0.116c →75.00±0.117a fL. corpuscular hemoglobin (MCH) 23.97±0.076c →24.96±0.089a pg, mean corpuscular hemoglobin concentration (MCHC) 29.12±0.119c →30.10±0.101a g/dL, serum iron (s. Fe) 46.55±0.250b →47.72±0.219a ug/dL and transferrin saturation (TS) 9.16±0.080c →10.20±0.093a %, respectively. A different trend regarding serum Ferritin was explored in pregnant women in whom T1 showed maximum uplift (7.60±0.232d →8.28±0.234b ng/mL).

A highly significant increase in TRBC count (7.39 %), Hb (17.44 %), Hct (7.51 %), MCV (2.73 %), MCH (3.97 %), MCHC (3.26 %), s. Fe (2.45 %), and TS (10.20 %) whereas 4.79 % decrease in TIBC was noted among volunteers ingesting T2 during 90 days (Table 4 & Figure 2a,2c,2d). However, the highest boost up (5.61 %) in serum Ferritin (Table 4 & Figure 2b) was recorded by treatment T1 that was composed of blackstrap molasses alone. T2 showed the highest increase in Hb level (11.01±0.039) than all other treatments while Hb of T1 the The results indicated that pregnant women grouped as T1 (11.00±0.036) was statistically similar to T2 (11.07±0.039), whereas T3 had greater increase in Hb level that might be due to the mixture of blackstrap molasses, apricots, dates and rape seeds, being a good source of Fe. US Department of Agriculture [40] indicated that blackstrap molasses was used as a major ingredient for iron fortification. In one of the similar studies, a patient used blackstrap molasses regularly for three months and his Hb level raised to 12 g/dL [12]. Some researchers investigated [41] an increase of 14.14, 13.15, 9.17, 18.44 and 17.84 % in Hb level of women by consuming buffalo liver, blackstrap molasses, watercress, aubergine and black dates, respectively. Dietary intake of buffalo liver, blackstrap molasses, watercress, aubergine and black dates increased 42.66, 32.69, 25, 50.56 and 42 % serum Fe of women, respectively. Similarly, serum Ferritin increased by 57.02, 48.33, 52.77, 61.94 and 51.61 % by respective use of buffalo liver, blackstrap molasses, watercress, aubergine and black dates. It was also explored by some scientists [42] that Fe supplementation during pregnancy exerts significant positive effects ($p < 0.05$) on hematological parameters such as Hb, Hct, TRBC and MCV. It was also found that Hb, Hct, MCH and MCHC have been increased to 18, 13.51, 10.20 and 7.39 %, respectively. In another study [43], Hb was found to have significantly positive correlation with RBC, Hct and MCV. It was also revealed that RBC had significant positive correlation with Hct and

MCV. Another interventional study depicted 7.32% decrease in TIBC [44,45].

# 4. Conclusions

All treatments provided 50 % RDA of Fe except placebo (T0). The significant variations in anthropometrics and energetic of pregnant women were observed. A significant positive impact of study span regarding dietary intakes was identified among all treatment groups. Hematological indices of volunteers were improved significantly by treatment T2. LFTs and RFTs of volunteers depicted the safety of the dietary intervention. A significant positive correlation was also found amongst indices of IDA except TIBC that had negative association. From the investigation of the current study, it may be concluded that that natural Iron sources are cost effective and healthier having high bio-available Fe in combating IDA among pregnant women. It is recommended that the same study should be conducted on large scale.

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# Conflict of Interest

This project was self funded and there are no any conflicts of interest by any author.

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