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Prebiotics and iron fortification among women of reproductive age group - Is there an association with liver and renal function tests?

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Abstract

Iron fortification compounds are of special interest to treat iron deficiency anemia, however, the dose-response effects of these fortificants on liver and renal functions have not been extensively reported in human subjects. The present study determines the effects of prebiotics and iron fortificants on liver function tests (LFTs) and renal function tests (RFTs) among women of reproductive age (WRA). A double-blind randomized controlled trial was performed for the duration of 90 days. A total of 75 iron-deficient women were selected and randomly divided into 5 groups (4 treatment groups and 1 control group). For this purpose, four different types of fortified wheat flour were prepared using two iron fortificants (NaFeEDTA and $FeSO_4$) and two prebiotics (Inulin and Galacto oligosaccharides) were given to four treatment groups, while control groups were only given iron-fortified flour without the addition of prebiotics. Blood samples were collected every month to evaluate Liver Function Tests, including Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), total bilirubin and Renal Function Tests, including serum urea and creatinine. Our results found that prebiotic and iron-fortified diets increased ALT, AST and total bilirubin levels among WRA. For AST, ALP and total bilirubin, our results found the highest increase in the treatment groups treated with prebiotics and iron fortificants at 963 mg/kg GOS + 15 ppm $FeSO_4$. Moreover, the highest values of ALT and serum creatine were seen in groups treated with 963 mg/kg Inulin + 20 ppm NaFeEDTA, while maximum value for serum urea could be seen in the group given 963 mg/kg GOS + 30 ppm $FeSO_4$. The study concluded that prebiotic and iron-fortified diets increased ALT, AST and total bilirubin levels among WRA.

Keywords: LFTs, RFTs, Prebiotics, Iron Fortification, Women of reproductive age

1. Introduction

Iron is a vital trace element actively involved in various biological functions, including oxygen transport, DNA synthesis, and production of and defence against free radicals [1]. Similar to other micronutrients, importance of iron for human survival cannot be denied as it is one of the primary components of hemoglobin is an oxygen transporter and is involved in energy production [2]. Despite its importance, an excessive amount of iron might have serious consequences leading to cell damage and organ impairment (Isidori et al., 2018). High iron intake is directly related to caustic injury to the gastrointestinal mucosa, leading to nausea, vomiting, abdominal distress, and diarrhea [3]. Correspondingly, iron overload might be responsible for adverse outcomes in patients with advanced kidney and chronic liver diseases [4].

At a systemic and cellular level, therefore, iron homeostasis mechanism in the body helps to maintain metabolic needs for iron and to minimize the risks posed by iron's toxicity. The iron homeostasis system functions through the regulation of the iron delivery to circulating transferrin, along with the action of hepcidin [5]. Liver is the main body organ involved in iron hemostasis via producing several proteins, including hepcidin and transferrin. Thus, transferrin has the ability to reversibly bind with iron and consequently serves as a cellular iron donor or iron acceptor, while hepcidin, through ferroprotein, regulates iron availability to the plasma and blockage of dietary iron absorption [6]. Under physiologic conditions like iron deficiency anemia, the transcription of hepcidin is suppressed, leading to reduced hepcidin production [7]. On the other hand, elevated plasma hepcidin levels due to inflammation are responsible for inhibiting duodenal iron absorption, sequestering iron in macrophages and ultimately iron deficiency anemia in patients with chronic kidney disease [8].

Iron homeostasis is regulated in the body by the hepatic, peptide hormone hepcidin (HEPC). The mechanistic approach shows that HEPC is released when body iron stores increase and also due to infection and inflammation

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to reduce serum iron concentrations. It is accomplished through the binding to the iron exporter, ferroportin 1, expressed on the surface of cells responsible for absorbing and storing iron, its internalization and degradation [9]. However, iron surplus in the blood i) saturates the buffering capacity of serum transferrin and ii) stimulates non-transferrin-bound iron (highly reactive forms) responsible for organ damage, promotes fibrogenesis and carcinogenesis in the kidneys, adrenal glands, liver, spleen, and pancreas [10]. Moreover, increased iron in the cytosol and mitochondria stimulates the production of highly toxic reactive oxygen species, and hydroxyl radicals increase oxidative stress in the body [11]. Few studies suggested that iron supplementation was able to considerably decrease 8-isoprostane which is a marker of oxidative stress and might be positively associated with liver health among anemic subjects, while other studies reported the adverse effect of iron supplementation on liver biomarkers [12].

Iron fortification compounds are of special interest, influencing iron bioavailability and absorption to treat iron deficiency anemia [13]. Also, it has been suggested that prebiotics such as Galacto oligosaccharides and Inulin can significantly enhance iron absorption in anemic subjects [14]. Previously, prebiotics (Xylo oligosaccharides) rich diet coupled with iron fortificants has been reported to improve the iron absorption transferrin saturation, Hemoglobin (Hb), and Total Iron Binding Capacity (TIBC) levels in Sprague Dawley rats. It is postulated that prebiotics help to produce more short-chain fatty acids in the colon, which stimulate the absorption of iron in the proximal colon and duodenum [15]. This particular attribute of such prebiotics in combination with iron fortificants could therefore be exploited to overcome iron deficiency anemia [16]. Many studies reported the harmful effects of excessive iron intake, including liver and renal functions [12]. However, very few studies have focused on the effect of prebiotics and iron fortificants on liver function biomarkers such as Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), total bilirubin and renal function biomarkers including serum urea and creatinine. Similarly, most studies evaluating the combined effects of prebiotics and iron fortificants in treating iron deficiency anemia have involved animal models. Considering the scarcity of literature and limited studies on human subjects, the current research was therefore planned and aimed to determine the effects of prebiotics and iron fortificants on liver function tests (LFTs) and renal function tests (RFTs) among women of reproductive age (WRA).

2. Materials and methods

2.1. Study Design and Participants

The current study was conducted on university-going iron-deficient female adults aged 18-25 years. It was a double-blind, randomized controlled trial (RCT) whereby n = 75 women were recruited in the study based on initial assessment for iron deficiency anemia and consent to participate. A physician also performed a comprehensive medical examination of the participants to ascertain the presence of any chronic diseases.

Women without any chronic diseases who gave their written consent were included. Women with diabetes and hypertension were excluded from the study.

2.2. Prebiotics and Iron-Fortified Diets

For the current study, we used two iron fortificants, i.e., NaFeEDTA and $FeSO_4$, and two prebiotics, namely Inulin and Galacto oligosaccharides. The dose for prebiotics was kept at 963 mg/kg body weight, while iron fortificants were used at doses of 10 ppm and 20 ppm for NaFeEDTA and 15 and 30 ppm for $FeS0_4$. The human equivalent dose equation (HED) was used to determine the dosage for prebiotics (Nair et al., 2018).

$$\text{HED (mg/kg)} = \text{Animal Dose in mg/kg} \times \frac{(\text{Animal Weight in kgs})^{0.33}}{(\text{Human Weight in kgs})}$$

The study participants were weighed individually for the exact calculation of prebiotics dose. Changes in body weight at the end of each week were also considered for calculating the dose of prebiotics for the subsequent week. We divided the study participants into 5 groups, each having 15 individuals as per the provision of iron fortificants and prebiotics. G0 was the control group which was only given iron fortificants without prebiotics. Groups G1, G2, G3 and G4 were the treatment groups that were given varying dosages of prebiotics and iron fortificants (Table 1).

2.3. Study Trials

Study participants were given wheat flour on a weekly basis for 90 days. Blood samples were collected from overnight fasted women on a monthly basis at four different times, that is, zero day, 30th day, 60th day and 90th day.

2.4. Analysis of Blood Samples

LFTs and RFTs were performed on the collected blood samples, according to their standard protocols.

2.4.1. Liver Function Tests (LFTs)

LFTs including AST, ALT, ALP and total bilirubin levels were analyzed according to their respective methods. To determine total bilirubin levels, a test tube containing the recommended amounts of blood serum, distilled water, diazo reagent and methanol was mixed properly and left for 30 minutes. The reading was then taken at 540 nm for measurement of total bilirubin. The spectrophotometric assay was used for ALT, AST and ALP [17].

Table 1. Treatment Plan (Iron Fortificants & Prebiotics Based Diet).

Groups	Diet Plan
G_0	Control (no prebiotic given)
G_1	963 mg/kg Inulin + 10 ppm NaFeEDTA
G_2	963 mg/kg Inulin + 20 ppm NaFeEDTA
G_3	963 mg/kg GOS + 15 ppm $FeSO_4$
G_4	963 mg/kg GOS + 30 ppm $FeSO_4$

2.4.2. Renal Function Tests (RFTs)

For RFTs, serum urea was calculated using the Glutamate Dehydrogenase (GLDH) method, while serum creatinine was determined using the Jaffe method. In this regard, a working reagent was made by transferring one urea powder to the bottle having two urea buffers (2 mL) for the GLDH method. The contents are then mixed to dissolve thoroughly and left for 15 minutes before use. This working reagent, standard and sample were then transferred to a pipette, mixed and a difference in absorbance between 20 and 80 seconds for standard and test was noted. Urea (mg/dL) was then calculated using the formula.

Urea (mg/dL) = ΔA/minute x Factor [18]

For the Jaffe method, 1mL each of distilled water, sodium tungstate reagent and sulfuric acid were added to control and sample tubes and mixed thoroughly, followed by adding serum sample or control to the tubes. These were mixed and centrifuged for 5 minutes at 1500 rpm. After that, recommended amounts of distilled water, working standard, filtrate, NaOH and picric acid as per the standard protocol were added to the tubes, mixed and allowed to stay at room temperature for 15 minutes. The contents were transferred to the cuvette and absorbance was read at 510 nm against the blank. Creatinine values for control and samples were determined by making use of standard absorbance and concentration [19].

2.5. Statistical Analysis

SPSS version 23.0 was used to analyze the collected data. Factorial design was used in the study to determine the significance level. P – value was considered significant at < 0.05 [20]. We presented the study results as means ± standard deviations.

3. Results

3.1 Effect of Prebiotic and Iron-Fortified Diet on Liver Function Tests

Table 2 below reveals that there was a significant variation for ALT, AST, and Total Bilirubin with respect to groups, study intervals as well and their interaction. However, ALP showed a non-significant trend.

3.1.1. Alanine Transaminase (ALT)

Mean values for ALT shown in Table 3 revealed that maximum values were recorded in group G2 (13.53±1.50IU/L), followed by G3 (12.85±1.52IU/L), G1 (10.96±0.65IU/L), G0 (10.74±0.36IU/L) and G4 (10.28±1.01IU/L). However, a steady decline in ALT values was observed during the efficacy trials (Table 3).

3.1.2. Aspartate Aminotransferase (AST)

For Aspartate Aminotransferase, the highest value was observed in group G3 which was 18.81±0.06IU/L, followed by G2 (17.27±0.55IU/L), G0 (16.71±0.13IU/L), G4 (15.92±0.15IU/L) and G1 (15.76±0.13IU/L). Across the modeling trials, a steady decrease in trait values was observed shown in Table 3.

3.1.3. Alkaline Phosphatase (ALP)

Maximum value for the trait was observed in group G3 (74.78±0.19IU/L), followed by G2 (73.56±0.35IU/L), G1 (72.45±2.91IU/L), G4 (68.27±0.15IU/L) and G0 (65.61±0.16IU/L). During the feed modeling trials, there was a slight increase in Alkaline Phosphatase levels (Table 3).

3.1.4. Total Bilirubin

Among the groups, the maximum value for the trait was observed in group G3 (0.52±0.08mg/dL), while the minimum value was attained by group G4 (0.22±0.11mg/dL). Across the modeling trials, total bilirubin levels decreased steadily from initiation to the termination of trials (Table 3).

3.2. Effect of Prebiotic and Iron-Fortified Diet on Renal Function Tests

It can be seen from Table 2 that a non-significant variation existed for serum urea with regards to groups, study intervals as well as their interaction while significant variations were recorded for serum creatinine with respect to groups, study intervals and also their interaction.

3.2.1. Serum Urea

The maximum value for serum urea could be seen in group G4 (21.58±0.04mg/dL), while the minimum value was observed in group G1 (17.78±0.14mg/dL). Group G2 attained a value of 19.49±0.26mg/dL, while group G3 showed a value of 21.03±0.22mg/dL. Control group G0 had a mean serum urea value of 18.40±0.37mg/dL. Across the modeling trials, values of serum urea decreased slightly (Table 4).

3.2.2. Serum Creatinine

A maximum serum creatinine value was attained by group G2 (0.92±0.03mg/dL), while the minimum value was recorded in group G3 (0.73±0.10mg/dL). During the efficacy trials involving anemic women, it was observed that the value for the trait increased slightly (Table 4).

Table 2. Liver Functions & Renal Function Tests for Anemic Women Fed with Prebiotic and Iron Fortified Diet (Mean Squares).

SOV	df	LFTs				RFTs	
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Serum Urea (mg/dL)	Serum Creatinine (mg/dL)
Groups	4	122.31*	91.09*	900.07^{ns}	0.941*	160.65^{ns}	0.35*
Study Intervals	3	57.70*	2.52*	6.86^{ns}	0.442*	0.49^{ns}	0.26*
Groups x Study Intervals	12	8.60*	0.72*	1.09^{ns}	0.014*	0.73^{ns}	0.04*
Error	280	0.27	0.29	1.61	0.002	0.41	0.09
Total	299						

* = Significant (P-value < 0.05), ^{ns} = Non-Significant

Table 3. Effect of Fortified Diets on Liver Function Tests among Anemic Women.

Treatments/Groups	Days 0	30	60	90	Means
ALT Levels (IU/L)					
G_0	10.51 ± 0.10	10.61 ± 0.23	10.59 ± 0.24	11.28 ± 0.60	10.74 ± 0.36
G_1	11.80 ± 0.18	11.10 ± 0.35	10.69 ± 0.40	10.27 ± 0.40	10.96 ± 0.65
G_2	15.18 ± 0.44	14.31 ± 0.52	12.85 ± 0.76	11.81 ± 0.84	13.53 ± 1.50
G_3	14.73 ± 0.08	13.24 ± 0.45	12.32 ± 0.75	11.13 ± 0.89	12.85 ± 1.52
G_4	11.59 ± 0.31	10.54 ± 0.56	9.75 ± 0.70	9.27 ± 0.66	10.28 ± 1.01
Means	12.76 ± 2.07	11.96 ± 1.71	11.24 ± 1.29	10.75 ± 1.00	
AST Levels (IU/L)					
G_0	16.83 ± 0.15	16.81 ± 0.14	16.68 ± 0.13	16.55 ± 0.11	16.71 ± 0.13
G_1	15.93 ± 0.53	15.76 ± 0.54	15.73 ± 0.55	15.62 ± 0.56	15.76 ±0.13
G_2	18.07 ± 0.11	17.13 ± 0.19	17.04 ± 0.22	16.84 ± 0.25	17.27 ± 0.55
G_3	18.77 ± 0.14	18.90 ± 0.82	18.80 ± 0.85	18.78 ± 0.90	18.81 ± 0.06
G_4	16.09 ± 0.26	15.99 ± 0.21	15.87 ± 0.27	15.75 ± 0.32	15.92 ± 0.15
Means	17.13 ± 1.24	16.91 ± 1.24	16.82 ± 1.23	16.70 ± 1.27	
ALP Levels (IU/L)					
G_0	65.84 ± 1.54	65.63 ± 2.03	65.51 ± 1.41	65.48 ± 1.38	65.61 ± 0.16
G_1	69.35 ± 0.76	71.07 ± 0.72	73.34 ± 0.73	76.06 ± 0.71	72.45 ± 2.91
G_2	73.92 ± 1.33	73.77 ± 1.32	73.42 ± 1.36	73.14 ± 1.35	73.56 ± 0.35
G_3	74.99 ± 1.25	74.86 ± 1.23	74.72 ± 1.24	74.55 ± 1.19	74.78 ± 0.19
G_4	68.43 ± 1.31	68.37 ± 1.30	68.19 ± 1.24	68.12 ± 1.24	68.27 ± 0.15
Means	70.50 ± 3.85	70.74 ± 3.81	71.03 ± 3.97	71.47 ± 4.48	
Total Bilirubin Levels (mg/dL)					
G_0	0.31 ± 0.02	0.31 ± 0.03	0.26 ± 0.04	0.19 ± 0.06	0.26 ± 0.06
G_1	0.44 ± 0.02	0.41 ± 0.03	0.36 ± 0.05	0.27 ± 0.07	0.37 ± 0.07
G_2	0.47 ± 0.02	0.43 ± 0.03	0.41 ± 0.04	0.28 ± 0.07	0.39 ±0.08
G_3	0.61 ± 0.05	0.55 ± 0.07	0.51 ± 0.08	0.43 ± 0.06	0.52 ± 0.08
G_4	0.35 ± 0.03	0.20 ± 0.04	0.13 ± 0.05	0.11 ± 0.03	0.22 ± 0.11
Means	0.43 ± 0.12	0.38 ± 0.13	0.33 ± 0.15	0.25 ± 0.12	

where, G_0 = Control Group (no prebiotic given), G_1 = 963mg/kg Inulin+10ppm NaFeEDTA, G_2 = 963mg/kg Inulin+20ppm NaFeEDTA,G_3 = 963 mg/kg GOS+15ppm $FeSO_4$, G_4 = 963mg/kg GOS+30ppm $FeSO_4$

Table 4. Effect of Fortified Diets on Renal Function Tests among Anemic Women.

Treatments/Groups	Days 0	30	60	90	Means
Serum Urea (mg/dL)					
G_0	18.86 ± 0.13	18.53 ± 0.20	18.23 ± 0.21	18.01 ± 0.10	18.40 ± 0.37
G_1	17.59 ± 0.19	17.77 ± 0.18	17.86 ± 0.17	17.92 ± 0.20	17.78 ± 0.14
G_2	19.60 ± 0.33	19.63 ± 0.34	19.65 ± 0.35	19.10 ± 2.51	19.49 ± 0.26
G_3	21.02 ± 0.46	21.01 ± 0.44	21.06 ± 0.47	21.04 ± 0.55	21.03 ± 0.22
G_4	21.55 ± 0.27	21.56 ± 0.29	21.59 ± 0.31	21.65 ± 0.35	21.58 ± 0.04
Means	19.72 ± 1.61	19.70 ± 1.60	19.67 ± 1.66	19.54 ± 1.72	
Serum Creatinine (mg/dL)					
G_0	0.77 ± 0.02	0.76 ± 0.03	0.78 ± 0.03	0.80 ± 0.02	0.77 ± 0.02
G_1	0.83 ± 0.02	0.86 ± 0.03	0.90 ± 0.03	0.93 ± 0.03	0.88 ± 0.04
G_2	0.89 ± 0.01	0.93 ± 0.02	0.95 ± 0.02	0.92 ± 0.03	0.92 ± 0.03
G_3	0.62 ± 0.02	0.70 ± 0.04	0.77 ± 0.05	0.86 ± 0.06	0.73 ± 0.10
G_4	0.65 ± 0.02	0.74 ± 0.03	0.84 ± 0.05	0.94 ± 0.03	0.79 ± 0.13
Means	0.75 ± 0.12	0.79 ± 0.09	0.84 ± 0.08	0.89 ± 0.06	

where, G_0 = Control Group (no prebiotic given), G_1 = 963mg/kg Inulin+10ppm NaFeEDTA, G_2 = 963mg/kg Inulin+20ppm NaFeEDTA, G_3 = 963 mg/kg GOS+15ppm $FeSO_4$, G_4 = 963mg/kg GOS+30ppm $FeSO_4$

4. Discussion

Our study determined the effect of iron fortificants on ALT, AST and total bilirubin levels when anemic subjects were administered with prebiotics and iron-fortified diets. Our results found an increase in ALT, AST and total bilirubin levels in the treatment groups compared to the control group.

For AST, ALP and total bilirubin, the highest increase was in the treatment group administered with prebiotics and iron fortificants at 963 mg/kg GOS + 15 ppm $FeSO_4$. Furthermore, the highest values of ALT levels were seen in group treated with 963 mg/kg Inulin + 20 ppm NaFeEDTA. Correspondence to our study results, a previous study found an increase in the ALP levels when treated with iron and folic acid supplementation [12]. Bottari et al., in contrast to our results, found a lower level of ALT during the evaluation of iron supplementation on blood adenine deaminase activity and oxidative stress in rats [21].

Regarding renal function biomarkers, higher serum creatinine levels were found in most of the iron-treated groups. The highest values of serum creatine were seen in group treated with 963 mg/kg Inulin + 20 ppm NaFeEDTA, while the maximum value for serum urea could be seen in the group given 963 mg/kg GOS + 30 ppm $FeSO_4$.

Iron supplementation and fortification is crucial for the treatment of anemia of chronic kidney disease (CKD), preferring intravenous iron for patients with CKD receiving dialysis [22], while other studies reported that Iron complexes that contain dextran or dextran-derived ligands can cause dextran-induced anaphylactic reactions in patients with CKD [23]. Regarding the effects of iron supplementation on renal function in anemic women of reproductive age, we found no previous studies in this regard, which warrant the need for further studies showing the mentioned relationship.

We recruited women since iron deficiency anemia is extremely common among women of reproductive age group in developing countries such as Pakistan. However, since iron deficiency anemia is not only limited to the female population, it might be considered a limitation of our study. Moreover, we only catered to one sub-group of female population, that is, the reproductive age group (and that too using only a narrower range of 18 to 25 years, as this cohort was most easily accessible to us). This can be considered as another limitation of our study, as iron deficiency anemia is also common to other subgroups. Moreover, limited literature and previously conducted studies were found, therefore, more studies in this regard are needed to generalize the results and to take preventive measures in terms of the use of iron fortificants for women of reproductive age.

5. Conclusion

In conclusion, our study revealed that prebiotic and -iron-fortified diets increased ALT, AST and total bilirubin levels among WRA. Hence, treatment with prebiotics along with iron fortificants has good efficacy to increase iron bioavailability and absorption; however, the increase in ALT, AST, and total bilirubin levels warrants further studies to investigate the adverse effects.

Conflict of Interests

The authors have no conflicts with any step of the article preparation.

Consent for publications

The authors have read and approved the final manuscript for publication.

Ethics approval and consent to participate

The current study was approved by the IRC (Institutional Review Committee) for Biomedical Research of the University of Veterinary & Animal Sciences, Lahore (Reference No. 037/IRC/BMR). The trial was registered at clinicaltrials.gov with ID number "NCT03894449" (https://clinicaltrials.gov/ct2/show/NCT03894449). The study was conducted exactly in accordance with the guidelines laid down in the Declaration of Helsinki.

Informed Consent

Informed written consent was taken from all the study participants.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Sehar Iqbal: Data Analysis and Final Write-up. Waqas Ahmed: Conception and Supervision. Saira Zafar: Data Analysis. Umar Farooq: Editing the Final Draft. Juweria Abid: Final Proofreading and Preparing the Manuscript for Submission. Abdul Momin Rizwan Ahmad: Conception, Data Collection and Initial Write-up.

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