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# The Impact of *Lactobacillus plantarum* 299v as a Probiotic in Iron-deficiency Anemia Patients

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**Abstract:**

**BACKGROUND:** Iron-deficiency anemia (IDA) is one of the common nutritional disorders affecting 30% of the world's population. This study aimed to identify the impact of probiotics in increasing iron absorption and treatment anemia disease using specific biological mechanisms.

**MATERIALS AND METHODS:** The study is a clinical trial that randomly controlled on 100 Iraqi IDA patients. The group were divided into two groups: IDA intake 20 billion colony-forming units (CFU) of *Lactobacillus plantarum* 299v (LP299v) and 6 billion CFU of the same spp. in comparison to the control group who never intake any probiotics.

**RESULTS:** The results showed that there is a significant increase in serum hemoglobin (HB), ferritin, iron, and reticulocyte HB content concentration in IDA patients treated with 20 billion CFU in comparison to control group (0.4 g/dl).

**CONCLUSION:** The iron absorption in anemia patients was enhanced with a reduction in the side effect of iron supplementation after intake of LP299v Prebiotics. Therefore, it represents an effective adjuvant therapy choices of IDA.

**Keywords:**

Ferritin, iron-deficiency anemia, *Lactobacillus plantarum* 299v, reticulocyte hemoglobin content

## Introduction

One of the most prevalent hematological disorder is anemia, it represents the decrease of hemoglobin (HB) concentration than normal values in human blood, which leads to a shortage in the ability of HB to transfer the oxygen in body tissues.[1] Iron-deficiency anemia (IDA) is the most common pattern of anemia in the world, global statics stated that 2.37 billon person were infected with IDA in the world, it also stated that 8.8% of the total disability-adjusted life years worldwide is caused by anemia.[2] Other study, showed that anemia rate reached 25%–40% in children <5 years in the Middle East and Northern Africa.[3] IDA is a widespread nutritional disorder affecting individuals across all age groups, including children, adolescents, and adults. While IDA in children is associated with impaired growth and cognitive development, adult women represent one of the most affected populations worldwide due to menstrual blood loss and increased iron requirements. Therefore, the present study specifically investigated adult female patients aged 15–65 years to evaluate the effect of *Lactobacillus plantarum* 299v (LP299v) supplementation on iron absorption and hematological recovery.[4]

IDA affects the biological functions and metabolism; the main effects of IDA are the impaired cognitive function and developmental delays in children. It also leads to decrease the memory rate by 15%–20%,[5] drawback in the ability of intolerance by 10%–15%, and decrease in

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the immunity of the body. The main changes of the traditional treatment, such as ferrous sulfate, are nausea, vomiting, and constipation that occur in approximately 40%–60% of the anemia patients. The side effect of anemia treatments decreased the rate of absorption by 10%–20% in patients and it also leads to patient's noncommitment to intake to ferrous sulfate by 30%–50% of IDA patients in the 1st month of treatment.[6-8] LP299v is a probiotic strain, that belongs to phylum Firmicutes and to Gram-positive lactic acid bacteria, it is commonly found in lactic acid of fermented food of plant origin food products, such as brined olives, sauerkraut, sourdough, or salted gherkins.[9] It was also proven that this particular strain is able to colonize human intestinal mucosa when orally administered,[10] since it can be found in biopsy material of the mucosa in rectum and jejunum.[11] Therefore, this study aimed to evaluate the impact of LP299v supplement as a prebiotic in IDA patients in Iraq.

## Materials and Methods

### Study design and ethical approval

This study was conducted as a randomized clinical trial at Al-Amel Hospital, Baghdad, Iraq, from early August to the end of September. Ethical approval was obtained prior to patient enrollment, and written informed consent was obtained from all the participants before inclusion.

### Study population

A total of 100 female patients aged 15–65 years with laboratory-confirmed IDA were enrolled. IDA was diagnosed based on reduced HB and ferritin levels. Only female participants were included due to the higher prevalence of IDA among women and to minimize biological variability related to sex-specific differences in iron metabolism and hormonal status. While this approach improved internal consistency, it limits the generalizability of the findings to male and pediatric populations. A total of 100 female patients with IDA were enrolled and randomly allocated into three groups: Group 1 ($n$ = 33) received ferrous sulfate plus LP299v at a dose of 20 billion colony-forming units (CFU); Group 2 ($n$ = 34) received ferrous sulfate plus LP299v at a dose of 6 billion CFU; and Group 3 (control, $n$ = 33) received ferrous sulfate alone.

### Randomization, allocation, and blinding

Participants were randomly assigned to one of three parallel groups using a simple randomization method based on computer-generated random numbers after baseline clinical and laboratory assessment. Due to the nature of the intervention (different probiotic doses and a control group without probiotics), allocation concealment, and blinding of participants and investigators were not feasible. Therefore, the study was conducted as an open-label randomized clinical trial. Patients with acute or chronic inflammatory conditions, autoimmune diseases, active infections, or malignancy were excluded from the study, given that ferritin is an acute-phase protein and may be elevated independently of iron status.

### Sample size considerations

The sample size was calculated as the amount of eligible and consenting patients who were available within the study period. No formal *a priori* calculation of power was conducted which is known to be a methodological weakness. Nevertheless, the sample size was adequate to reveal the statistically significant differences in the most important parameters of hematology and biochemistry between the groups of study.

### Group allocation

They were randomly put in three parallel groups. Group 1 ($n$ = 33) was given ferrous sulfate that contained 65 mg of elemental iron mixed with LP299v 20 billion CFU. Group 2 ($n$ = 34) was given ferrous sulfate (65 mg elemental iron) in combination with LP299v (6 billionCFU). Group 3 (control, $n$ = 33) was taken with ferrous sulfate (65 mg elemental iron) mixed only without probiotic supplement.

### Exclusion criteria

Pregnant, breastfeeding patients, gastrointestinal (GI) disease, malignancy, inflammatory disease, and any antibiotics used during the study period were excluded.

### Intervention protocol

All the supplements were delivered orally in a single dose per day in the morning so as to standardize the dosing and absorption. The probiotics used were LP299v in academic-coated enteric-resistant capsules. All groups had a treatment period of 2 months.

### Clinical assessment and safety

Samples of venous blood of every participant were taken under the aseptic conditions and separated into two parts. About 2 mL of the blood was poured into ethylenediaminetetraacetic acid tubes to analyze the complete blood count (CBC) and HB content (reticulocyte hemoglobin [Ret-He]). The rest 3 mL blood was put in serum separator tubes and left to clot at the room temperature and then centrifuged to get serum used to measure ferritin, serum iron, and C-reactive protein (CRP). To measure GI symptoms after the intake of ferrous sulfate, the clinical evaluation was conducted at baseline and posttreatment. Patients who complained about intolerability or unsatisfactory improvement were handled ethically and under supportive care as per the normal clinical practice. Adverse events were also followed during the study and none of the serious

adverse effects which warranted the discontinuation of treatment were noted. According to the instructions given by the manufacturer, the CBC and Ret-He content (Ret-He) were determined using an automated hematology analyzer (Sysmex®, Japan). Automated biochemical and immunoassay analyzers measured serum ferritin, serum iron, and CRP based on the instructions of the manufacturer (Abbott Architect, Abbott diagnostics, USA).

### Statistical analysis

All the data were evaluated with the SPSS software version 19 (IBM company, United States). The continuous variables were in terms of mean ± standard deviation (SD). Paired *t*-tests were to compare the values of pre and after treatment in each group, whereas one-way analysis of variance (ANOVA) was used to compare the differences between the groups in the study. In suitable cases, *post hoc* comparisons were carried out. $P$ <0.05 was a significant value.

The rate of change was calculated using the following equation:

Change rate = $(V_0 - V_1)/V_0$.

Where $V_0$ represents the value before treatment and $V_1$ represents the value after treatment.

To guarantee valid and reliable statistical reporting, pre- and posttreatment mean ± SD values of each of the biomarkers were added to results section (HB, ferritin, serum iron, reticulocyte HB content, and CRP).

### Ethics approval

The Ethical and Research Committee had granted the ethical approval of the study by the Department of Basic Sciences, College of Dentistry, University of Basrah (Approval No. 334, dated 10 January 2025). All the participants signed written informed consent before they were enrolled and the study was carried out in the spirit of the Declaration of Helsinki.

### Clinical trial registration

This was a randomized clinical trial, initiated by an investigator, and was a single-center trial. Trial was not enlisted in a publicly accessible clinical trial registry before patients could be enrolled in the trial since trial registration was not required either on the institutional or national level when the study was designed and initiated. It is recognized as a shortcoming and future studies will require registration in clinical trials to increase the transparency and adherence to the global reporting standards.

### Limitations

The limitations of this study include a number of limitations. The use of female subjects alone reduces the extrapolation of the results to the male and pediatric population. There was no categorization of the severity of IDA as mild, moderate, or severe which can influence the interpretation of the treatment response. Saturation of transferrin and hepcidin which are the key biomarkers in iron-absorption and regulation were not determined because of logistical reasons and limited mechanistic understanding. Furthermore, open-label design, no allocation concealment, and blinding, and no preset power calculation of the sample size can lead to bias. Such considerations must be taken into account in prospective, well-powered, and fully blinded randomized controlled future studies.

## Results

Iron plays a crucial role in human metabolism including oxygen transfer and redox reactions. This study aimed to evaluate the impact of *Lactobacillus plantarum* on IDA. The demographic distribution indicated that all participants from female lived in Baghdad city with an age group ranged from 15 to 65 years old [Table 1].

The study indicated the demographical distribution and the homogeneity among the group of study. Results showed that all participants were from the same sex, the age group were determined, results showed that there are nonsignificant differences between the group of studies [Table 1]. On the same context, there is nonsignificant differences between the family history ratio between the experimental groups (Group A and Group B) while control group was null of anemia family history to avoid any conflict in resulted outcomes.

To ensure baseline comparability and homogeneity among the study groups, HB, ferritin, and serum iron levels were assessed prior to the initiation of LP299v supplementation. As presented in Table 2, there were

**Table 1: The demographic distribution of the iron deficiency anemia patients**

| Groups | Age (15-65) years old | Family history (%) | 95% CI (age) | Effect size |
|---|---|---|---|---|
| Group 1 (*n*=33) | 34.2±8.5[a] | 30[a] | 31.2–37.2 | - |
| Group 2 (*n*=34) | 35.1±9.2[a] | 30[a] | 31.9–38.3 | - |
| Group 3 (control) (*n*=33) | 33.8±7.9[a] | 0[b] | 31.0–36.6 | - |
| *P* | 0.721 | - | - | $\eta^2$=0.01 (age) Cramer's *V*=0.42 (family history) |

CI=Confidence interval, [a]Group A; [b]Group B

no statistically significant differences in baseline HB, ferritin, or iron levels among the three groups ($P = 0.789$, 0.612, and 0.712, respectively), with negligible effect sizes ($\eta^2 < 0.01$), confirming adequate group homogeneity at study entry. Importantly, baseline values of HB, ferritin, and serum iron in all groups were below the normal reference ranges for adult females, confirming the diagnosis of IDA prior to treatment

After 8 weeks of treatment with LP299v probiotic. Table 3 shows that there is a significant ($P < 0.05$) increase in the level of HB (g/dl), ferritin (ng/ml), and iron (µg/dl) in all groups of the study. The change ratio was determined [Table 3] which illustrated that there is a positive change in the level of HB (g/dl), ferritin (ng/ml), and iron (µg/dl) after treatment in comparison to control groups.

Results presented in Table 4 demonstrate a statistically significant increase in Ret-He content (g/dL) after 8 weeks of treatment with ferrous sulfate combined with LP299v. Both probiotic-treated groups showed a greater improvement in absolute Ret-He values compared with the control group receiving ferrous sulfate alone. Notably, patients treated with 20 billion CFU exhibited the highest increase in Ret-He, followed by those receiving 6 billion CFU, indicating a dose-dependent enhancement of functional iron availability and erythropoietic response. In contrast, the control group showed a comparatively modest increase in Ret-He. These findings confirm that LP299v supplementation significantly augments the early hematopoietic response to iron therapy in IDA patients.

Table 5 shows that there is a significant ($P < 0.05$) decrease in the inflammation marker CRP level after treatment with ferrous sulfate and LP299v probiotic in treated samples. Results showed that there is a significant change ratio in CRP level in IDA patients treated with 20 and 6 billion LP299v than IDA patients treated with ferrous sulfate only.

Table 6 shows that there is a significant ($P < 0.05$) decrease in the nausea (12.1% and 9.1%), constipation (9.1% and 17.6%) and total treatment discontinuation rate (3.0% and 5.9%) in after treatment with ferrous sulfate and LP299v probiotic in comparison with control group (45.5%, and 39.4%) consequently. Results showed that there is a significant ($P < 0.05$) positive improvement in all the symptoms of IDA patients treated with 20 and 6 billion

**Table 2: The hemoglobin, ferritin, and iron level of iron-deficiency anemia patients before treatment with ferrous sulfate and *Lactobacillus plantarum* 299v probiotic**

| Groups | HB (g/dl), mean±SD | 95% CI | Ferritin (ng/ml), mean±SD | 95% CI | Iron (µg/dl), mean±SD | 95% CI |
|---|---|---|---|---|---|---|
| Group 1 (*n*=33) | 9.7±1.3 | 9.2–10.2 | 8.3±1.9 | 7.6–9.0 | 43.1±9.2 | 39.9–46.3 |
| Group 2 (*n*=34) | 9.9±1.1 | 9.5–10.3 | 8.7±2.3 | 7.9–9.5 | 41.3±8.5 | 38.3–44.3 |
| Group 3 (control) (*n*=33) | 9.8±1.2 | 9.3–10.3 | 8.4±2.1 | 7.6–9.2 | 42.3±8.9 | 39.2–45.4 |
| *P* | 0.789 | - | 0.612 | - | 0.712 | - |
| Effect size ($\eta^2$) | 0.006 | - | 0.008 | - | 0.007 | - |
| Normal reference range (adult females) | 11.6–15.0 | - | 12–150 | - | 50 | |

CI=Confidence interval; SD=Standard deviation; HB=Hemoglobin

**Table 3: The change rate in hemoglobin, ferritin, iron level of iron deficiency anemia patients after 8 weeks of treatment with ferrous sulfate and *Lactobacillus plantarum* 299v probiotic**

| Groups | Δ HB (g/dl), mean±SD | 95% CI | Δ Ferritin (ng/ml), mean±SD | 95% CI | Δ Iron (µg/dl), Mean±SD | 95% CI |
|---|---|---|---|---|---|---|
| Group 1 (20 billion CFU LP299v) | +3.4±0.8[a] | 3.1–3.7 | +23.9±5.8[a] | 21.9–25.9 | +65.5±3.1[a] | 60.8–70.2 |
| Group 2 (6 billion CFU LP299v) | +2.5±0.7[b] | 2.3–2.7 | +17.7±4.9[b] | 16.0–19.4 | +46.6±11.3[b] | 42.6–50.6 |
| Group 3 (control) | +1.4±0.6[c] | 1.2–1.6 | +10.1±3.8[c] | 8.8–11.4 | +26.1±9.2[c] | 22.9–29.3 |
| *P* | <0.001 | - | <0.001 | - | <0.001 | - |
| Effect size ($\eta^2$) | 0.46 | - | 0.52 | - | 0.58 | - |

CI=Confidence interval; SD=Standard deviation; HB=Hemoglobin; CFU=Colony-forming units

**Table 4: Reticulocyte hemoglobin content levels before and after 8 weeks of treatment with ferrous sulfate and *Lactobacillus plantarum* 299v in iron deficiency anemia patients**

| Groups | Ret-He (g/dl) before treatment (mean±SD) | Ret-He (g/dl) after treatment (mean±SD) | Change rate (%) |
|---|---|---|---|
| Group 1 (20 billion CFU LP299v) | 26.4±2.1 | 38.7±3.4 | +46.6 |
| Group 2 (6 billion CFU LP299v) | 26.8±2.3 | 36.0±3.1 | +34.2 |
| Group 3 (control) | 26.6±2.2 | 32.3±2.8 | +21.3 |
| *P* | - | <0.001 | - |

SD=Standard deviation; CFU=Colony-forming units; Ret-He=Reticulocyte hemoglobin

**Table 5: C-reactive protein level of the iron deficiency anemia patients before and after treatment with ferrous sulfate and *Lactobacillus plantarum* 299v probiotics**

| Groups | CRP before (mg/L), mean±SD | 95% CI | CRP After (mg/L), mean±SD | 95% CI | Change ratio (%) |
|---|---|---|---|---|---|
| Group 1 (20 billion CFU LP299v) | 3.8±1.2 | 3.4–4.2 | 1.6±0.7[a] | 1.3–1.9 | −56.5 |
| Group 2 (6 billion CFU LP299v) | 3.9±1.3 | 3.5–4.3 | 2.1±0.8[b] | 1.8–2.4 | −46.2 |
| Group 3 (control) | 3.8±1.2 | 3.4–4.2 | 3.2±1.1[c] | 2.8–3.6 | −15.8 |
| *P* | - | - | <0.001 | - | - |
| Effect size ($\eta^2$) | - | - | 0.41 | - | - |
| Normal reference range | <3.0 | - | <3.0 | - | - |

CI=Confidence interval; SD=Standard deviation; CFU=Colony-forming units; CRP=C-reactive protein, [a]Group A; [b]Group B; [c]Group C (control)

**Table 6: Gastrointestinal symptom rate of the iron-deficiency anemia patients before and after treatment with ferrous sulfate and *Lactobacillus plantarum* 299v probiotic**

| Groups | Nausea (%) | Constipation (%) | Ratio of treatment discontinuation (%) |
|---|---|---|---|
| Group 1 | 12.1 | 9.1 | 3.0 |
| Group 2 | 20.5 | 17.6 | 5.9 |
| Group 3 (control) | 45.5 | 39.4 | 15.2 |
| *P* | >0.001 | >0.001 | >0.001 |

LP299v than IDA patients treated with ferrous sulfate only.

## Discussion

The homogeneity of experimental study confirms the randomized process was effective to evaluate the impact of LP299v probiotic on IDA patients. The higher doses of LP299v probiotic were significantly increased in HB, ferritin, and iron levels in blood of IDA patients after 8 weeks of treatment with probiotic than the control group. The improvement in change rate showed that LP299v probiotic enhance iron absorption through three integrated biological mechanisms, beginning with the modification of the intestinal environment, production of short-chain fatty acids, then lowers the intestinal pH from 7.5 to 6.0–6.5, increasing iron solubility by 40%–50%.[12] In addition, LP299v strains can produce compounds that facilitate the formation of absorbable iron chelates.[13]

These results agree with Hoppe *et al.*, 2015[14] which stated that the use of LP277v significantly increase iron absorption. Other studies confirm that probiotic strains increase iron solubility, lowering the gastric ph. The *Lactobacillus reuteri* DSM 17938 probiotic lead to a significant increase in HB level but showed nonsignificant increase in iron level that might be due to the differences in stain of the form of Ferrous sulfate concentration.[15]

The significant ($P > 0.05$) increase in reticulocyte HB content (Re-he%) after treatment with ferrous sulfate and LP299v probiotic considered as a marker for iron mineralization status as young red blood cells is sensitive and represent an early response of bone marrow to iron preparations.[16] The strong increase in Ret-He confirms that the probiotic not only increased iron storage but also led to immediate increase in the amount of iron available for erythropoiesis. This is consistent with studies that found that probiotic use even for short periods, significantly improves Ret-He in children with anemia.[17]

The significant ($P > 0.05$) decrease in CRP level after treatment with ferrous sulfate and LP299v probiotic (20 and 6 billion CFU) in treated groups might be due the effect of probiotic on the inflammation – iron axis.[16] Inflammation is associated with increased secretion of the hormone hepcidin, which is the main regulator of iron metabolism, as it prevents the release of iron stored in the liver and spleen and reduces its absorption in the intestines by inhibiting the transporter protein ferroprotein.[17]

Some studies suggested that the role of probiotics and prebiotics is represented by breaking the inflammatory anemia cycle which agree with our results, while other studies disagree with the assumption as it concluded that there are nonsignificant differences in CRP level after intake of probiotic in IDA patients.[18,19]

The significant ($P > 0.05$) decrease in the nausea (12.1% and 9.1%), constipation (9.1% and 17.6%), and total treatment discontinuation rate (3.0% and 5.9%) after treatment with ferrous sulfate and LP299v probiotic may be explained by the effect of probiotic as it should that probiotics also contribute in reducing intestinal inflammation, which decreases hepcidin production by 20%–30%, thereby improving the release of iron from storage[16] while simultaneously strengthening the intestinal barrier and improving nutrient absorption. Finally, they play a role in modulating the gut microbiome by competing with pathogenic bacteria that consume free iron, thus reducing their growth by 30%–40%,[18] and by promoting beneficial bacteria, increasing microbiome diversity, and improving its metabolic functions.[15]

The significant improvement in tolerance is explained by the fact that probiotics improve the intestinal environment and reduce the amount of unabsorbed free

iron ions ($Fe^{2+}$) in the colon. These free ions are responsible for the production of free radicals, which irritate the intestinal mucosa and lead to nausea and constipation.[9] By helping bacteria increase iron absorption in the upper part of the small intestine (duodenum), probiotics reduce the amount of iron that reaches the colon and decrease the growth of pathogenic bacteria that feed on iron.[14] Improved tolerability is directly associated with increased treatment adherence, with the overall discontinuation rate decreasing from 15.2% to 3.0%. This reduction in discontinuation is a major clinical achievement of this study, as it translates into better long-term treatment outcomes.

## Conclusion

There is a significant effect of probiotic LP299v strain on hematological markers in Iraq IDA patients after treatment with two different doses 6 and 20 billion CFU. Inflammation cycles were decreased. The key challenge of using ferrous sulfate was reduced by reduction in nausea and constipation rate which decrease the possibility of treatment discontinuation in anemia patients. It is recommended to use different doses to determine the lowest effective dose to minimize cost with long-term safety studies to assess effects on the gut microbiome and investigate potential bacterial resistance.

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## Conflicts of interest

There are no conflicts of interest.

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