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Research Article

Real-World Effectiveness of Livogen®Z an Oral Iron, Folic Acid, Plus Zinc Fixed Dose Combination Supplement in Pregnant and Non-Pregnant Women with Iron Deficiency Anaemia: Insights from a Phase IV Observational Study in India (LIBERTY)

Parag Biniwale[1], Lakshmi Swetha Karlapudi[2], Nimisha Pagare[3], Roger D Gibb[4], Sue Aspley[5], Poonam Sule[6*]

[1]Biniwale Women's Clinic, Pune, India; [2]Sanjeevani Hospital, Pune, India; [3]Srushti Hospital and Maternity Hospital, Aurangabad, India; [4]Personal Health Care, The Procter and Gamble Company, Cincinnati, Ohio, United States of America; [5]Department of Clinical Development, Procter and Gamble Technical Centres, Berkshire, United Kingdom; [6]Department of Clinical Development, Procter and Gamble Health Limited, Mumbai, India

ABSTRACT

Background: Iron Deficiency Anaemia (IDA) is a major health concern in India. Livogen®Z (manufactured by Procter and Gamble Health Ltd.) combines ferrous fumarate (152mg), folic acid (750mcg) and zinc sulphate (61.8mg) for management of anaemia, but real-world data from India is scarce.

Methods: A phase IV, prospective observational study was conducted at four sites in India in pregnant and non-pregnant women with IDA aged 18-55 years. Subjects received Livogen®Z tablet twice daily for 90 days between June-2022 and October-2023. The primary objective was to evaluate the Change from Baseline (CFB) in Hemoglobin (Hb) after 90 days of treatment. CFB at Days 21 and 60, CFB in ferritin at end-of-study. IDA symptoms and Adverse Events (AE) were secondary endpoints.

Results: Of the 102 recruited subjects, data for 69 women were included (62.3% nonpregnant, 11.6% first trimester, 26.1% second trimester) in the final analysis after excluding data for 33 subjects at one site that was non-compliant with good clinical practices. At Day 90, the mean Hb CFB was 2.2 g/dL ($p<0.0001$). Mean Hb CFB was 1.1 g/dL ($p<0.0001$) on Day 21 and 1.9 g/dL ($p<0.0001$) on Day 60. At Day 90, median ferritin CFB was 12.0 ng/mL ($p<0.0001$) and 29.5% of women were symptom-free *vs.* 2.9% at study start. The treatment was well tolerated with no AEs reported by 49.3% of subjects and no serious AEs reported.

Conclusion: Livogen®Z improves iron status, alleviates symptoms in women with IDA and is well-tolerated. Clinically significant improvements in Hb and ferritin levels with accompanying meaningful symptom benefits with relatively few AEs highlight Livogen®Z treatment as a favorable IDA management option.

The study was registered on the Clinical Trial Registry of India (CTRI) http://ctri.nic.in/Clinicaltrials/login.php (CTRI No. CTRI/2023/04/051785).

Keywords: Iron deficiency anaemia; Anaemia in pregnancy; Serum ferritin; IDA symptoms; Real-world study; Iron supplement; Ferrous fumarate

INTRODUCTION

Anaemia is a public health concern impacting approximately 1.62 billion people worldwide, affecting women more commonly than men [1,2]. Though there are various forms of anaemia, Iron Deficiency Anaemia (IDA) is the most common form, which affects millions annually [3]. The World Health Organization (WHO) defines anaemia in women based on hemoglobin blood concentrations (<120 g/L for non-pregnant and <110 g/L for pregnant women) [4]. In India, the National Family Health Survey-5 (NFHS-5) data reports high rates of anaemia among

Correspondence to: Poonam Sule, Department of Clinical Development, Procter and Gamble Health Limited, Mumbai, India, E-mail: sule.p@pg.com

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pregnant women (52%), non-pregnant non-lactating women (57%), adolescent girls (59%) aged 15-19 years and children (67%) aged 6-59 months [5]. According to the Indian Council of Medical Research (ICMR), between 1990 and 2016, dietary iron deficiency remained the leading cause of disability in the general population (11% of all disability) [6].

IDA arises primarily from inadequate intake of essential nutrients such as iron, zinc, vitamin B12 and folic acid. Heavy menstrual bleeding, recurrent pregnancy, regular blood donation and Crohn's disease contribute to IDA. Poverty and resultant malnutrition, infections such as hookworm and malaria are also known to be endemic in the developing countries and act as contributing factors to IDA.

Pregnancy exacerbates iron requirements due to the expansion of maternal blood volume. Insufficient iron levels can hinder this process, leading to adverse pregnancy outcomes such as preterm labour, low birth weight and increased maternal mortality [7,8]. Despite the heightened requirement, 51%-83% of pregnant women in India do not meet the recommended daily iron intake of 15-18 mg and have a median intake of 13.7 mg/day [9].

Iron deficiency is often associated with zinc and folate deficiency [10,11]. Zinc stimulates immature erythroblast growth, which regulates hematopoietic stem cells and megakaryocytes and aids erythropoiesis [11,12]. According to Abdelhaleim et al., iron together with zinc supplementation could be more effective than iron replacement alone for iron deficiency [11]. Folate deficiency leads to impaired DNA synthesis and increased death of hematopoietic cells, eventually resulting in anaemia due to ineffective erythropoiesis [12].

WHO recommends daily oral iron and folic acid supplementation, especially as part of antenatal care, since pregnant women are vulnerable to anaemia, to reduce the risks of low birth weight, maternal anaemia and iron deficiency [13]. The initial therapy phase in IDA management requires iron supplementation until hemoglobin values normalize. Administering iron orally, typically with ferrous salts- fumarate, lactate, succinate, gluconate, or sulphate, proves to be a convenient, cost-effective and efficient method [14,15].

The fixed-dose combination of ferrous fumarate, zinc sulphate and folic acid represents a promising therapy for IDA. Currently, there is limited real-world evidence available regarding the use of this triple combination in either pregnant or non-pregnant women, especially in India.

This post-marketing observational study aimed to evaluate the gap in evidence for the effectiveness of the ferrous fumarate, folic acid, and zinc sulphate fixed-dose combination (Livogen®Z (manufactured by Procter and Gamble Health Ltd.)) in the management of IDA in diverse subgroups of Indian women.

MATERIALS AND METHODS

Study design

This was a single-arm, phase IV, open-label, real-world, multicenter, prospective, observational study conducted at four sites in India to assess the effectiveness of Livogen®Z in pregnant and non-pregnant women with IDA. The study was conducted between June 2022 and October 2023.

The study was conducted following International ethical guidance, including the Declaration of Helsinki, National ethical guidelines for biomedical and health research involving human participants, 2017 by ICMR and other applicable local regulations and guidelines. The study was registered on the Clinical Trial Registry of India (CTRI) portal https://ctri.nic.in/Clinicaltrials/pmaindet2.php?EncHid=Nzk5OTA=&Enc=&userName= (CTRI No. CTRI/2023/04/051785).

The study was approved by the Royal Pune Independent Ethics Committee (registration no. ECR/45/Indt/MH/2013/RR-19) in India.

All subjects were required to provide written informed consent before enrolment into the study.

Study population

Pregnant and non-pregnant women aged between ≥18 and ≤55 years of age, who provided written informed consent, were diagnosed with IDA as per WHO criteria were recruited in the study. Subjects with severe anaemia requiring blood transfusions, pernicious anaemia, thalassemia, sickle cell or aplastic anaemia, active peptic ulcer, regional enteritis, ulcerative colitis, haemorrhoids, oesophageal varices, helminthiasis, megaloblastic anaemia and porphyria cutanea tarda were excluded. Detailed inclusion, exclusion, withdrawal and termination criteria are summarised in Table S1 in the supplementary materials.

Sample size

The sample size target was one hundred (100) subjects who fulfilled the study eligibility criteria. A similar study design with a different iron salt showed significant effects in hemoglobin changes as early as 14 days' post-baseline with a mean ± Standard Deviation (SD) of 1g/dl ± 1.24 [16]. Applying the criteria above with alpha=0.05, two-sided testing and 90% power, the sample size would reflect 19 subjects to complete. This study was extremely well-powered to detect mean change from baseline in hemoglobin concentration. The sample size was expanded to a target of 100 to enable exploration of secondary endpoints, including IDA symptom relief and relevant population subgroups.

Study objectives and endpoints

The primary objective of the study was to evaluate the change in haemoglobin in subjects prescribed Livogen®Z with IDA after 90 days of treatment or the last post-baseline haemoglobin assessment. Therefore, the primary endpoint was the change from baseline in haemoglobin concentration at Day 90 or the last post-baseline haemoglobin assessment.

The study's secondary endpoints included changes from baseline in haemoglobin concentration at Days 21 and 60, serum ferritin concentration at the end of the study, IDA symptoms assessment using Visual Analogue Scale (VAS) at Days 21, 60 and 90 and evaluation of adverse events, gastrointestinal tolerability and product acceptability.

Assessments

The study was conducted in an outpatient setting, following routine clinical practice at four study centres in India.

Subjects who participated in this study completed four visits to the centre.

- Baseline/Screening Visit 1 (Day 0),

- Visit 2 (Day 21 ± 7 days),
- Visit 3 (Day 60 ± 3 days),
- End-of-Study Visit 4 (Day 90 ± 3 days).

Subjects who were prescribed Livogen®Z, each tablet consisting of ferrous fumarate (152 mg equivalent to 50 mg elemental iron), folic acid 750 mcg and zinc sulphate (61.8 mg equivalent to elemental zinc 22.5 mg), manufactured by: Procter and Gamble Health Ltd. Subjects were instructed to take one tablet twice a day and were followed up for up to three months (90 days). At each visit, the subject's laboratory parameters were evaluated and documented, including a Complete Blood Count (CBC), while serum ferritin levels were evaluated at Baseline and Day 90 (or EOS). Subjects completed a VAS for each of the 16 IDA symptoms assessed. VAS scale was presented to the subject at each visit by the investigator and explained that 0 means no symptom and 100 on the scale means the symptom is most severe. For each symptom present, the subject marked on the scale with a perpendicular straight line, and the score was captured in the column VAS score (Supplementary Figure S1). Additionally, subject input on product acceptability and satisfaction was collected. Any prior and concomitant medications consumed by subjects were recorded in the study by the investigator. No follow-up was conducted beyond Day 90.

Statistical analysis

Primary efficacy and safety analyses were conducted in the Intent-To-Treat (ITT) population. The data were summarized with suitable descriptive statistics, e.g., number of participants, mean, standard deviation, median, minimum, maximum, etc. for continuous data and frequency counts and percentages for categorical data. For continuous data with a skewed distribution or outliers, the descriptive statistics included the median, Median Absolute Deviation (MAD) or Interquartile Range (IQR). Missing data were not imputed, except in the case of haemoglobin where the Last Observation Carried Forward (LOCF) approach was applied to subjects with at least one post-baseline observation. Of the 69 subjects enrolled and analyzed, 64 attended at least one post-baseline visit and had LOCF applied. The number of subjects with LOCF-imputed haemoglobin data was two at Day 60 and three at Day 90. The statistical significance of the mean change from baseline was assessed for continuous endpoints with a paired-difference t-test. For highly skewed distributions, the Wilcoxon Signed Rank Test was used. Unless indicated otherwise, all statistical tests were conducted at the 5% significance level. SAS software version 9.4 was used for the analysis.

RESULTS

Demographic characteristics

A total of 102 Subjects were enrolled from four centres in West and South India (Pune, Chhatrapati Sambhaji Nagar (Aurangabad) and Bengaluru (Bangalore)). Data for all 33 participants enrolled at site 04 were excluded, based on the site's non-compliance with Good Clinical Practices (GCP) (mostly laboratory data credibility issues). Thus, the final analyses were conducted on data from 69 subjects enrolled at the other three centres. Table 1 summarizes the demographic characteristics, baseline medical history and dietary and exercise patterns of the analysed population. The ITT population included 69 female subjects with a mean ± SD age of 29.3 ± 7.64 years. Among them, 62.3% were non-pregnant, 11.6% were pregnant in the first trimester and 26.1% were pregnant in the second trimester. Baseline anaemia severity varied, with 27.5% presenting with mild anaemia, 68.1% with moderate anaemia, 2.9% with severe anaemia and 1.4% with no anaemia. The average ± SD Body Mass Index (BMI) was 23.5 ± 4.01 kg/m^2, ranging from 15.0 to 39.0 kg/m^2. Menstrual patterns in non-pregnant participants revealed 34.8% with normal flow, 20.3% reporting heavy flow and 7.2% experiencing less-than-normal flow.

Table 1: Demographic findings of the study population.

Demographic	Overall
Age (yrs)	
Mean (SD)	29.3 (7.64)
Median	28
Min-Max	18.0-46.0
Sex[a]	
Female	69 (100.0%)
Pregnancy statusa	
Not Pregnant	43 (62.3%)
Pregnant, 1st Trimester	8 (11.6%)
Pregnant, 2nd Trimester	18 (26.1%)
Iron deficiency anaemia statusa	
None	1 (1.4%)
Mild	19 (27.5%)
Moderate	47 (68.1%)
Severe	2 (2.9%)
Weight (kg)	
Mean (SD)	55.9 (10.75)
Median	55
Min-Max	34.0-95.0
Height (cm)	
Mean (SD)	154.4 (6.12)
Median	155
Min-Max	140.0-168.0
BMI (kg/m^2)	
Mean (SD)	23.5 (4.01)
Median	24
Min-Max	15.0-39.0
Duration of menses (days)	
Mean (SD)	4.1 (1.67)
Median	4
Min-Max	1.0-10.0

Menses amount in non-pregnant women[a]	
Less than Normal	5 (7.2%)
Normal blood flow	24 (34.8%)
Heavy Blood flow	14 (20.3%)
Vegetarian diet[a]	
No	40 (58.0%)
Yes	29 (42.0%)
Consumes food containing iron absorption enhancers[ab]	
Vitamin C containing Fruits and Vegetables	63 (91.3%)
Meat	39 (56.5%)
Fish	21 (30.4%)
Poultry	30 (43.5%)
Consumes food containing iron absorption inhibitors[ab]	
Phytic Acid in Bran products, Bread, Grains, Cereals, Oats, Rice, Legumes	69 (100.0%)
Soy Protein from Soya products	39 (56.5%)
Egg protein from whole eggs and yolk	37 (53.6%)
Calcium containing milk products like milk, yogurt, cheese	52 (75.4%)
Polyphenols in Coffee, Cocoa, Tea and Wine	6 (8.7%)
Exercise[a]	
No	30 (43.5%)
Yes	39 (56.5%)

Note: a: Number and percentage of participants; b: Participants were allowed to select one or more items; BMI: Body Mass Index; SD: Standard Deviation; WHO definition of anaemia: <120 g/L for non-pregnant and <110 g/L for pregnant women at sea level

Dietary patterns showed 58.0% as non-vegetarians and 42.0% following a vegetarian diet. Vitamin C-rich foods, which enhance iron absorption were consumed by 91.3% of participants, alongside meat (56.5%), fish (30.4%) and poultry (43.5%). However, all participants also consumed foods containing inhibitors of iron absorption, with 56.5% consuming soy protein, 53.6% egg protein, 75.4% dairy products and 8.7% polyphenols. Regarding lifestyle habits, 56.5% engaged in regular physical activity while 43.5% reported no exercise.

In the ITT population (N=69), 88.40% of subjects completed the study, while 11.60% discontinued. The discontinuations were primarily due to lost to follow-up (87.5%) or protocol violations (12.5%). (Supplementary Table S2).

Measurements of treatment compliance

Compliance to treatment, measured by counting returned tablets and the number taken, was expressed as a percentage of those dispensed across intervals: Baseline to Day 21, Day 21 to Day 60 and Day 60 to Day 90. Over these 3 intervals, 76.6%, 74.2% and 100% of subjects achieved ≥75% compliance, respectively. Overall, 84.4% of subjects had ≥75%, highlighting a generally favorable adherence to treatment protocols (Supplementary Table S3).

Primary endpoint haemoglobin change at day 90

At baseline, the mean ± Standard Error (SE) haemoglobin level was 9.9 ± 0.13 g/dL, ranging from 5.1 to 11.9 g/dL. By Day 90, the mean haemoglobin level increased to 12.1 ± 0.14 g/dL, reflecting a mean change from the baseline of 2.2 ± 0.18 g/dL ($p<0.0001$) (Table 2, Figure 1a and 1b).

Subjects with mild baseline IDA (n=19): In subjects with mild baseline IDA and post-baseline data (n=19), the baseline mean haemoglobin level was 11.1 ± 0.14 g/dL. By Day 21, there was a slight increase in mean haemoglobin of 0.4 ± 0.16 g/dL. This trend continued at Day 60 and Day 90, with mean haemoglobin levels further increasing by 1.0 ± 0.20 g/dL and 1.2 ± 0.15 g/dL, respectively. The mean changes observed at Days 21, 60 and 90 were all statistically significant ($p\leq0.013$, Table 2).

Subjects with moderate baseline IDA (n=45): In subjects with moderate to severe baseline IDA and post-baseline data (n=45), the baseline mean haemoglobin level was 9.4 ± 0.12 g/dL. By Day 21, mean haemoglobin increased by 1.4 ± 0.14 g/dL. At Day 60 and Day 90, haemoglobin levels increased by 2.3 ± 0.20 g/dL and 2.6 ± 0.22 g/dL, respectively. The mean changes at each post-baseline visit were statistically significant ($p<0.0001$, Table 2).

Haemoglobin summary by pregnancy status

Haemoglobin levels significantly increased over time in both non-pregnant and pregnant groups. In the non-pregnant group, the mean ± SE haemoglobin level rose from 10.0 ± 0.17 g/dL at baseline to 12.3 ± 0.16 g/dL by Day 90, with a significant change from baseline at all post-baseline visits ($p < 0.0001$). For pregnant participants, the mean haemoglobin increased from 9.7 ± 0.21 g/dL at baseline to 11.7 ± 0.24 g/dL by Day 90, with statistically significant improvements at all post-baseline time points ($p <0.0001$). These changes are depicted in Figure 2.

Table 2: Haemoglobin (g/dL) efficacy analysis by visit, ITT population (LOCF) (N:69).

		Baseline	Post-baseline	Change from Baseline		
Anaemia Status / Visit	**N**	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**Mean 95% CI**	**p-value[a]**
			All subjects			
Day 21	64	9.9 (0.13)	11.0 (0.12)	1.1 (0.12)	(0.9, 1.4)	<.0001
Day 60	64	9.9 (013)	11.8 (0.14)	1.9 (0.17)	(1.6, 2.3)	<.0001

Day 90	64	9.9 (0.13)	12.1 (0.14)	2.2 (0.18)	(1.8, 2.6)	<.0001
Mild iron deficiency anaemia at baseline[b]						
Day 21	19	11.0 (0.14)	11.5 (0.23)	0.4 (0.16)	(0.1, 0.8)	0.0131
Day 60	19	11.0 (0.14)	12.0 (0.26)	1.0 (0.20)	(0.5, 1.4)	0.0001
Day 90	19	11.0 (0.14)	12.2 (0.18)	1.2 (0.15)	(0.9, 1.5)	<.0001
Moderate to severe iron deficiency anaemia at baseline						
Day 21	45	9.4 (0.12)	10.8 (0.13)	1.4 (0.14)	(1.1, 1.7)	<.0001
Day 60	45	9.4 (0.12)	11.7 (0.16)	2.3 (0.20)	(1.9, 2.7)	<.0001
Day 90	45	9.4 (0.12)	12.1 (0.18)	2.6 (0.22)	(2.2, 3.1)	<.0001

Note: ITT: Intent-To-Treat; LOCF: Last Observation Carried Forward; N: Number of subjects in the indicated population; n: Number of subjects with non-missing values; SE: Standard Error; CI: Confidence Interval; a: Paired t-test 2-sided p-value

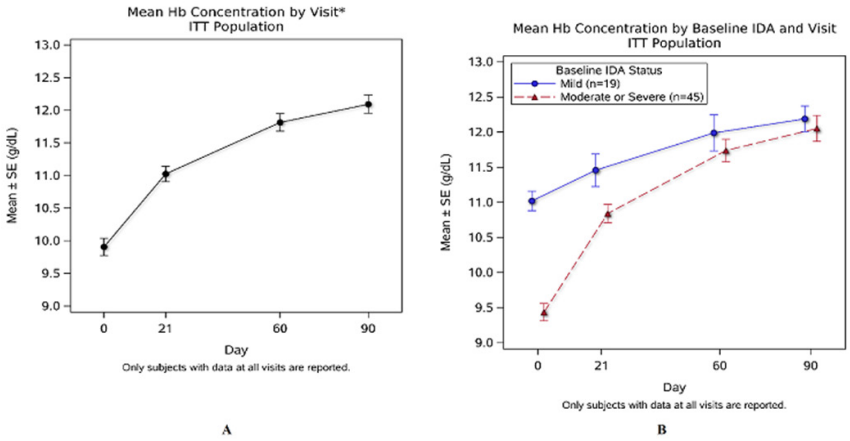

Figure 1: A) Mean change in Hemoglobin (Hb) from baseline by visit (Day 21, 60 and 90)-ITT population; B) Individual haemoglobin changes from baseline to day 90-ITT population.

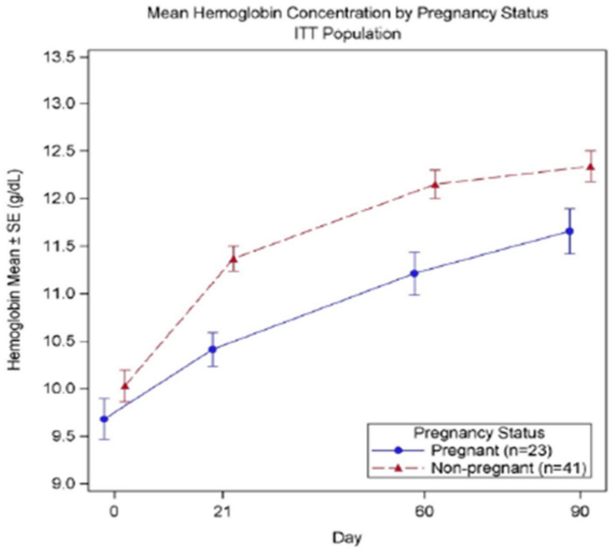

Figure 2: Haemoglobin concentration by pregnancy status. **Note:** ITT: Intent-To-Treat.

IDA status by visit (ITT Population)

At baseline, most subjects had moderate IDA (68.1%), followed by mild anaemia (27.5%), severe anaemia (2.9%) and a small percentage were non-anaemic (1.4%). By Day 21, the percentage of subjects with moderate anaemia decreased significantly to 28.1%, while those with mild anaemia increased to 42.2%. Additionally, 29.7% of subjects were non-anaemic at Day 21. By Day 60, the trend continued with a significant decrease in moderate anaemia (9.4%), a notable increase in the non-anaemic group (57.8%), 32.8% of subjects having mild anaemia and no subjects with severe anaemia. By Day 90, there was a substantial increase in the non-anaemic group (76.6%), a decrease in mild anaemia (14.1%) and moderate anaemia reported for only 9.4% of subjects, as shown in Supplementary Figure S3.

Ferritin changes at day 90 (ITT population)

In the ITT population with non-missing post-baseline ferritin data (n=61), the baseline median ± MAD ferritin level was 20.0 ± 14.2 ng/mL. By Day 90, the median ferritin level had increased to 33.0 ± 12.0 ng/mL.

The median ferritin change from baseline at Day 90 was 12.0 ± 9.0 ng/mL and the change was highly statistically significant (p<0.0001) (Figure 3a).

Subjects with mild baseline IDA (n=17): At baseline, the median ferritin level was 29.0 ± 16.0 ng/mL. At Day 90, subjects showed an increase in the median ferritin concentration to 40.0 ± 12.0 ng/mL. The median change from baseline in ferritin level was 12.0 ± 9.0 ng/mL (Figure 3b).

Subjects with moderate to severe baseline IDA (n=44): At baseline, the median ferritin level was 16.5 ± 12.2 ng/mL. At Day 90, the median ferritin level increased to 29.6 ± 11.4 ng/mL. The median change from baseline in ferritin level was 10.8 to 9.6 ng/mL (Figure 3b).

IDA symptoms assessment using the VAS

Symptoms were recorded at baseline and follow-up visits on Day 21, Day 60 and Day 90. Substantial improvements in all 16 IDA symptoms were observed across all time points throughout the study, all of which were statistically significant (p=0.004) by Day 90. IDA symptom improvement at Day 90 ranged from 70.4% to 100%. For example, among the 14 subjects who reported having brittle nails at baseline and completed the study, mean VAS decreased from 13.2 at baseline to 2.5 by Day 90, a reduction of 81.1% (p=0.0001). Cold hands or feet (n=9) showed a dramatic 89.9% reduction, with mean VAS dropping from 22.0 at baseline to 2.2 at Day 90. Difficulty sleeping (n=11) improved by 98.5% and dizziness (n=23) showed a 96.4% reduction. (Figure 4a and Supplementary Table S4).

The percentage of IDA symptom-free subjects increased from 2.9% at baseline to 29.5% at Day 90, highlighting the effectiveness of the intervention in alleviating IDA symptoms (Figure 4b). The number of IDA symptoms per subject also decreased progressively from baseline to Day 90. At baseline, most subjects had between 5 to 10 symptoms, but by Day 90 most subjects had between zero and three symptoms, demonstrating a marked improvement in the overall symptom burden (Figure 4c).

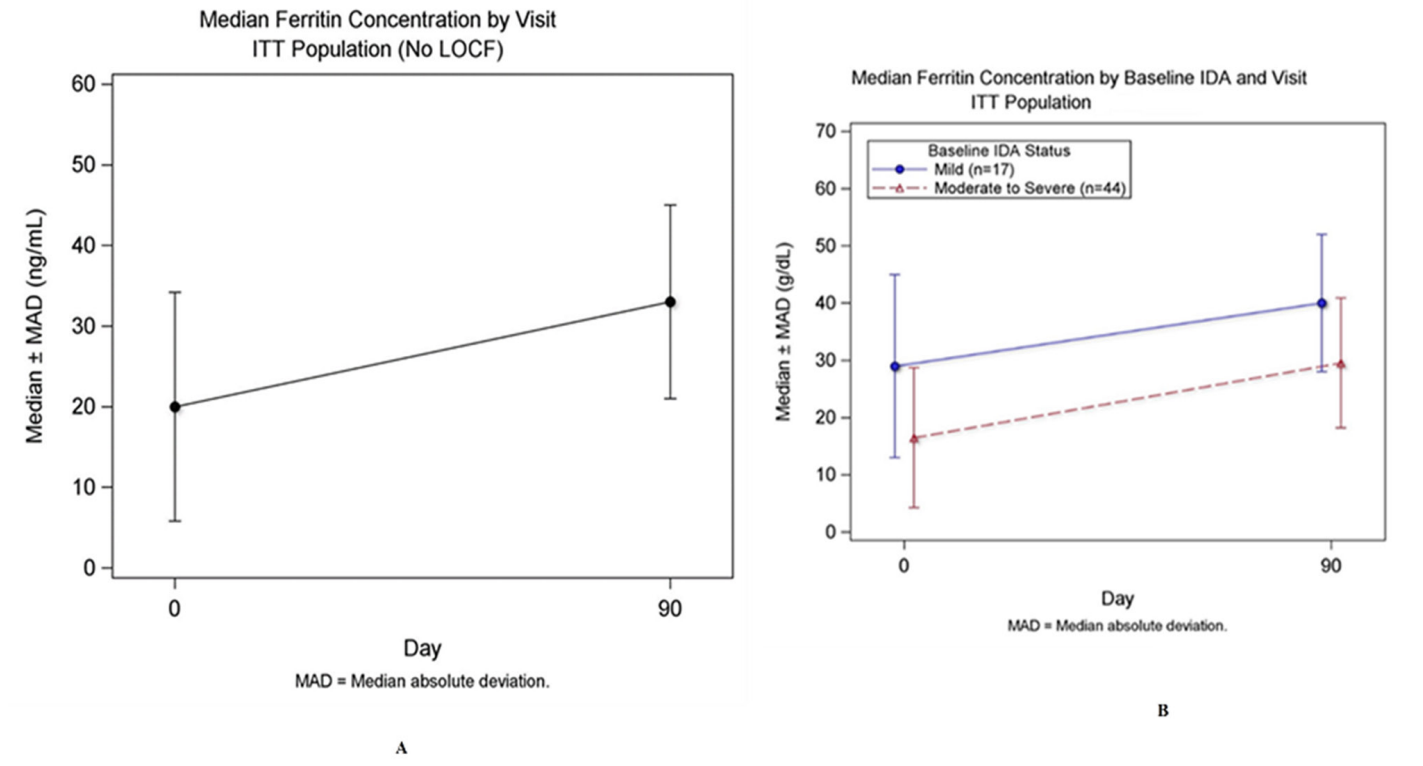

Figure 3: Effect on ferritin concentration: A) Median ferritin concentration by visit; B) Median ferritin concentration by baseline IDA status. **Note:** IDA: Iron Deficiency Anaemia; ITT: Intent-To-Treat; LOCF: Last Observation Carried Forward.

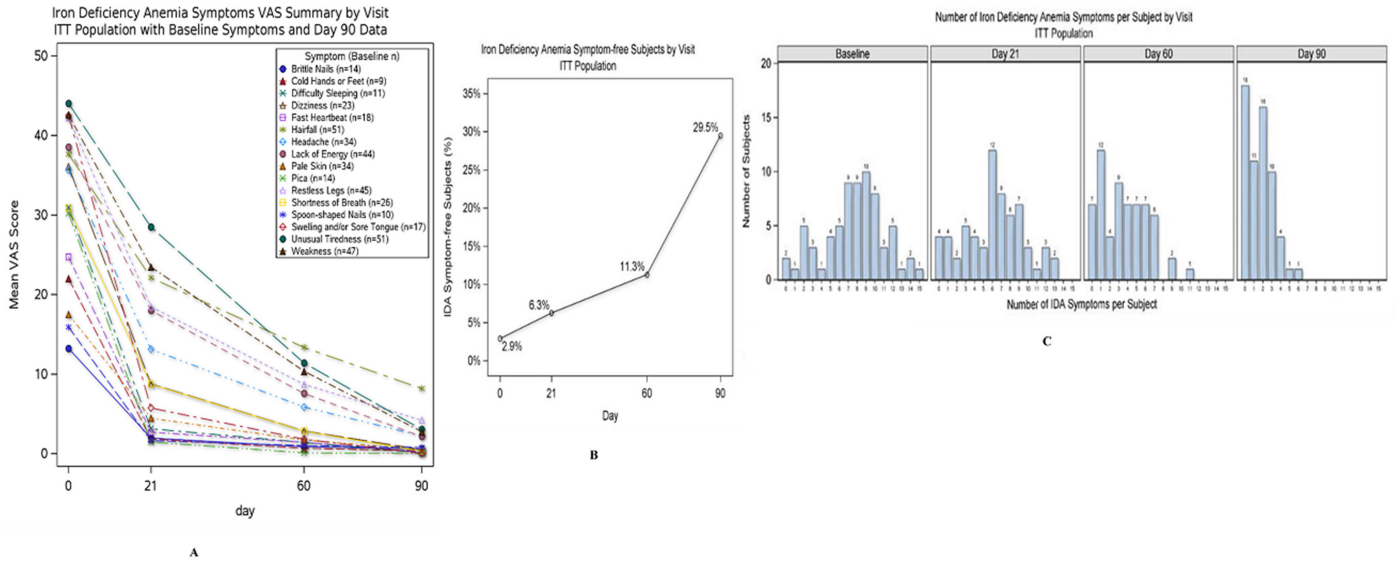

Figure 4: Effect on IDA symptoms: A) IDA symptoms VAS summary by visit; B) symptom-free subjects; C) Distribution of IDA symptoms by visit.
Note: IDA: Iron Deficiency Anaemia; ITT: Intent-To-Treat; VAS: Visual Analogue Scale.

Table 3: Summary of adverse events.

Intent to treat Population (N=69)			
	n	(%)	**nAE**
Participants			
With any adverse events (AE)	35	-50.70%	108
With serious AE	0	0.00%	0
Who withdrew from study due to AE	0	0.00%	0
Who Died	0	0.00%	0
Participants			
Reporting 0 AE	34	-49.30%	
Reporting 1 AE	13	-18.80%	
Reporting 2 AE	6	-8.70%	
Reporting >2 AE	16	-23.20%	
AE Severity	**nAE**	**(%AE)**	
Mild	107	-99.10%	
Moderate	1	-0.90%	
Overall	108	-100.00%	
AE Causality	**nAE**	**(%AE)**	
Unrelated	39	-36.10%	
Possible	47	-43.50%	
Definite	22	-20.40%	
Overall	108	-100.00%	
Mean number of AE per enrolled participant		1.6	
Mean number of AE per participant with AEs		3.1	

Note: N:Number of participants in the indicated population; n (%): Number and percentage of participants who reported adverse events; nAE: Number of adverse events within specified treatment group; % AE: Percentage of adverse events within specified treatment group: (nAE/Overall nAE)*100.

Adverse events

In the study, 49.3% of subjects (n=34) reported no AEs, while 50.7% (n=35) experienced at least one AE, totaling 108 AEs. No serious AEs, withdrawals, or deaths were reported due to AEs. Of those who experienced AEs, 18.8% (n=13) reported one AE, 8.7% (n=6) reported two AEs and 23.2% (n=16) reported more than two AEs. Regarding causality, 36.1% of AE (n=39) were deemed unrelated to the study treatment, 43.5% (n=47) were considered possible and 20.4% (n=22) were definite. On an average, there were 1.6 AEs per enrolled subject and 3.1 AEs per subject who experienced any AE. Causality assessment revealed that 29% of AEs were considered possibly related to the study drug, which included gastrointestinal events like epigastric discomfort (7.2%) and nausea (2.9%), while 14.5% of AE were classified as unrelated to the drug. Table 3 summarizes the AE profile reported in the study. A summary of all AEs by severity can be found in the supplementary material (Supplementary Table S5).

Gastrointestinal (GI) tolerability and product acceptance

The GI tolerability of Livogen®Z was assessed in the ITT population (n=69) at three visits: Day 21, Day 60 and Day 90. Most GI symptoms were either absent or mild. The most common symptom reported was black-colored stools, reported by 20.3% of subjects on Day 21, with a decrease to 8.2% by Day 90. Other symptoms such as bloating, constipation and nausea were relatively rare, impacting fewer than 10% of subjects by Day 90. Most symptoms were mild and related to the treatment, such as black-colored stools and metallic taste. Notably, symptoms like bloating and vomiting were completely absent by Day 60 and Day 90, indicating good GI tolerability over time (Supplementary Table S6).

Product acceptance (ITT population (n=69))

All 61 subjects (100%) reported that the study product was easy to consume, with no issues reported. Additionally, 100% of the subjects found the taste and smell of the product to be acceptable. Regarding overall product acceptability, 43 (70.5%) of participants were very satisfied and 18 (29.5%) were satisfied. No participants were neutral, dissatisfied, or very dissatisfied with the overall product acceptability (Supplementary Table S7).

DISCUSSION

This phase IV observational study showed that Livogen®Z, a supplement containing iron along with folic acid and zinc, increased mean haemoglobin levels by 2.2 g/dL over 90 days. Ferrous fumarate aids in addressing iron deficiency anaemia, while folic acid is important for the absorption of iron. Furthermore, zinc sulphate supports immune function and cell growth.

According to a Delphi consensus from the Asia-Pacific region, oral ferrous iron is recommended as the first-line therapy for iron deficiency and IDA in both pregnant and non-pregnant adult women, including peri-menopausal women without additional comorbidities [17]. WHO recommends that iron supplements should contain 30-60 mg of elemental iron, with the higher dose preferred in settings where anaemia in pregnant women is a severe public health problem, along with 400 µg of folic acid [18]. However, the Indian Guidelines published by the National Health Mission recommend 100 mg elemental iron and 500 mcg of folic acid every day for at least 100 days in pregnant women with IDA [19]. These recommendations were assessed in a study conducted in India, which demonstrated that, in pregnant women, the average increase in haemoglobin was 2.70 g/dL and 3.53 g/dL for moderate and severe anaemia, respectively. While the average increase in haemoglobin over three months was 1.17 g/dL, 2.06 g/dL and 3.28 g/dL for those with baseline mild, moderate and severe anaemia, respectively, for non-pregnant women [20]. Recent research from the ICMR-National Institute of Nutrition (NIN) demonstrated that iron plus folic acid treatment reduced IDA by 48% at Day 90 [21]. A systematic review conducted in low-middle-income countries demonstrated that iron therapy improved haemoglobin and ferritin levels in women of reproductive age [22]. Additionally, Tiwari et al., found that iron and folic acid supplementation significantly improved haemoglobin levels in pregnant women with anaemia [23]. Another study highlighted that iron-zinc supplementation effectively increased haemoglobin and ferritin levels in women with early pregnancy iron deficiency but had no impact on those with adequate iron levels [24]. The observations of the present study are consistent with the previously reported results. In this study, subjects with mild IDA demonstrated a mean increase of 1.2 g/dL and subjects with moderate IDA experienced a 2.6 g/dL increase in mean haemoglobin levels at Day 90, as compared to baseline. These findings reflect the substantial benefits of iron supplementation in improving haemoglobin levels in women in India.

In the present study, significant improvements were observed in haemoglobin levels, with the mean haemoglobin levels increasing significantly by Day 90 (change from baseline 2.2 g/dL for all subjects and 2.0 g/dL for pregnant subjects). These results are significant as the mean Hb level for pregnant women was corrected from anaemic to non-anaemic (>110 g/L or 11.0 g/dL) at Day 90. Furthermore, a proportionate impact on ferritin levels was also observed. Studies have shown the serum ferritin thresholds to be ~25 µg/L (or 25 ng/ml) in the first and ~20 µg/L (or 20 ng/ml) in the second and third trimesters, respectively [25]. In the present study, serum ferritin improved from a median of 20.0 ng/ml at baseline to 33.0 ng/ml at Day 90. Ferritin is an indicator of total body stores of iron and extremely low ferritin levels are typically seen only in cases of iron deficiency. During pregnancy, ferritin levels drop gradually and reach their lowest point around weeks 35-38 of the pregnancy. The lowest point reached is worse without iron supplements (approximately 15 ng/ml) and slightly better with supplementation (20 ng/ml) [26,27]. Typically, with iron supplementation, serum ferritin does not increase until the haemoglobin levels are normalised [28,29]. Ferritin is also considered a more sensitive and accurate marker for diagnosing iron deficiency, as compared to serum iron for the diagnosis of IDA [26,30]. This indicates the treatment's effectiveness in managing IDA.

Low dietary iron, low iron absorption, prolonged blood loss, pregnancy and post-partum are the major causes of IDA in developing countries [31]. Pregnancy significantly decreases the haemoglobin concentration in pregnant women reaching the lowest point during labour and delivery [32]. Pregnancy results in increased blood volume and causes haemodilution, which exacerbates the iron deficiency [33]. In a longitudinal study conducted in a resource-limited setting, pregnant women were reported to have progressively lower haemoglobin concentration through the trimesters, reaching the lowest level at 6 weeks' post-partum [32]. Iron supplements are shown to lower the incidence of low birthweight and preterm births. Studies indicate that women who take iron supplements during pregnancy are less likely to deliver low birth weight babies and babies small for gestational age, contributing to improved

neonatal outcomes [34-36]. A study by Pena-Rosas et al. evaluated daily oral iron supplementation during pregnancy, alone or with folic acid and other nutrients. Results showed that preventive iron supplementation reduced maternal anaemia at term by 70%, iron deficiency anaemia by 67% and iron deficiency by 57%. In the present study, a mean change in haemoglobin from baseline at 90 days was found to be 2.2 g/dL for all participants and was found to have increased by 2.0 g/dL in pregnant participants. These benefits highlight the importance of iron supplementation in antenatal care, emphasizing its role in reducing complications and promoting healthy pregnancy outcomes [34].

IDA is associated with various symptoms such as unusual tiredness, weakness, hair fall, restless legs, lack of energy, headache, dizziness, shortness of breath, swelling and/or sore tongue, among others. These symptoms can be attributed to low delivery of oxygen to the tissues [37]. The symptoms of IDA are thought to initiate before the onset or diagnosis of IDA and may continue beyond the resolution of clinical IDA [38]. In the present study, all symptoms improved by Day 21. Substantial improvements were noticed in the most commonly reported and debilitating symptoms such as dizziness (-96.4%), breathlessness (-97.8%), weakness (-91.6%) and tiredness (-91.8%), reinforcing the clinical significance of the IDA improvements seen in this study. The resolution of these symptoms is in line with the changes observed in a previous study [39].

Zinc deficiency is associated with gastrointestinal disturbances such as diarrhoea and glossitis, dermatological conditions including alopecia and dermatitis, nail dystrophy, restless leg syndrome, cardiovascular symptoms [40,41]. Folic acid is essential for cellular DNA synthesis and its deficiency has been shown to result in megaloblastic anaemia, glossitis, angular stomatitis, and oral ulcers and may also result in neuropsychiatric manifestations, including depression, irritability, insomnia, cognitive decline, fatigue, and psychosis [42]. Together with IDA, these concomitant deficiencies aggravate the epithelial barrier dysfunction in the skin and gastrointestinal tract. The current study demonstrated significant improvement in all anaemia-related symptoms in both pregnant and non-pregnant sub-groups, aligning with findings from the studies conducted by Kelkitli et al conducted in Turkey [41] and Soliman et al in Egypt [40]. A possible explanation for these could be the pathophysiology of the individual deficiencies. All these three deficiencies share common risk factors such as poor dietary intake, pregnancy and malnutrition [41,42].

The compliance in the study was seen to improve between Day 60 and Day 90 of treatment. It could be speculated that this improvement may be attributable to improvements in IDA symptoms observed by Day 60. Furthermore, the resolution of GI symptoms by Day 60 may have also contributed to improved compliance.

In this study, all the participants agreed that the product was acceptable regarding taste, smell and ease of consumption. The palatability of the medication is a significant factor contributing to adherence to the treatment. Physical barriers surrounding medicine intake, such as difficulty swallowing the medicine, unpleasant taste or pain while swallowing may result in poorer adherence to treatment, leading to suboptimal treatment outcomes [43,44].

In this study, the treatment was well-tolerated, with no serious AE reported. The most common AEs were mild gastrointestinal symptoms, such as nausea and constipation, which decreased in frequency over the study period. This suggests an adaptation to the supplement over time. The safety profile of the study supplement was consistent with the adverse events commonly associated with iron and folic acid supplements. In previous studies, gastrointestinal side effects have been recognized as significant factors contributing to low adherence to iron supplementation [45-47]. In this study, "black colored stools" was recorded as one of the GI tolerability symptoms. However, the normal metabolic process is to excrete excess, unabsorbed iron from the body and does not demonstrate any adverse pathological process due to the consumption of the iron supplement.

This study shows that Livogen®Z is effective and well-tolerated for improving iron status and alleviating symptoms in women with IDA. Clinically significant improvements in haemoglobin and ferritin levels, resolution of common and distressing symptoms and minimal AE underscore the treatment's potential as an important IDA management option. However, continued research and larger studies are necessary to validate these findings further and assess the long-term benefits and safety of the treatment.

The study demonstrated several notable strengths. The collection of detailed demographic, medical history and baseline characteristic data provided a thorough understanding of the study population, which may facilitate subgroup analyses in the future. This comprehensive data collection enabled the identification of specific factors influencing treatment outcomes. Furthermore, the high treatment compliance observed in the study indicates that the treatment regimen is acceptable to patients. These strengths collectively enhance the robustness of the study's conclusions, providing valuable insights into the effectiveness of oral iron supplementation for IDA in Indian women.

The study has a few limitations. It was designed as a single-arm study without a comparator, which limits the ability to attribute improvements solely to treatment. Future research should incorporate control groups to better establish causality, longer-term studies are needed to evaluate the sustained efficacy and safety of the treatment. A further limitation was that data from one study site could not be utilized due to GCP non-compliance that was discovered after the in-clinic completion of the study. The generalizability of the study findings is an important consideration. Despite the lack of comparator arm, and reduced sample size, the findings remain highly generalizable to populations with characteristics similar to those of our study group comprising diverse subgroups of Indian women. The study size was adequate to successfully detect statistically significant improvements in hemoglobin, ferritin, and IDA symptoms, both overall and within various population subgroups.

This study provides valuable insights into the real-world effectiveness of oral iron supplementation for iron deficiency anaemia in in various diverse subgroups of women in India.

CONCLUSION

This Phase IV study provides evidence of the real-world effectiveness of Livogen®Z a fixed-dose combination of ferrous fumarate, zinc sulphate and folic acid in managing IDA among both pregnant and non-pregnant women in India. The study demonstrated that treatment with Livogen®Z results in clinically significant improvements in haemoglobin, ferritin levels and IDA symptoms across various baseline IDA statuses and the improvements were noted as early as 21 days. The safety profile of Livogen®Z was consistent with expectations, with mild gastrointestinal symptoms

being the most common adverse effects, which diminished over time. The findings align with established guidelines recommending oral ferrous iron as the first-line treatment for IDA, to boost iron levels and improve overall health. Additional research on comparative and long-term studies is warranted to validate the findings in larger groups.

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DECLARATION OF INTEREST

PB, LSK and NP declare no conflicts of interest.

RG, SA and PS are employees of Procter & Gamble and own stocks and shares in the company.

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DATA AVAILABILITY STATEMENT

All relevant data for this study are present in the manuscript and supplementary files. Further data generated during and/or analysed during the study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the study conception, design, writing, review and approving the final draft. PS and AS contributed to methodology, project supervision, resource planning and provision of funding. RG and PS had full access to datasets and contributed to data curation, validation data analysis and visualization.

ETHICS APPROVAL AND INFORMED CONSENT

The study was conducted following International ethical guidance including the Declaration of Helsinki, National ethical guidelines for biomedical and health research involving human participants, 2017 by ICMR and other applicable local regulations and guidelines. The study was registered on the Clinical Trial Registry of India (CTRI) portal http://ctri.nic.in/Clinicaltrials/login.php (CTRI No. CTRI/2023/04/051785).

The study was approved by the Royal Pune Independent Ethics Committee (Registration no. ECR/45/Indt/MH/2013/RR-19) in India.

All subjects were required to provide written informed consent before enrolment into the study.

DATA PRESENTED AT CONFERENCE

Results related to the primary endpoint were presented at the AICOG (All India Conference of Obstetrics and Gynaecology) as an oral presentation.

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