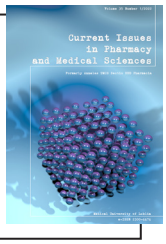
DOI: 10.2478/cipms-2024-0005 Curr. Issues Pharm. Med. Sci., Vol. 37, No. 1, Pages 26-32

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Comparative evaluation of anti-anemic effect of Sucrosomial iron in experimental model of iron deficiency anemia in Wistar rats

Manoj Arajanbhai Suva, Pravin Rambhai Tirgar*

Department of Pharmacology, RK University, School of Pharmacy, Gujarat, India

ARTICLE INFO

Received 15 March 2023
Accepted 04 December 2023

Keywords:
iron deficiency anemia,
bloodletting,
conventional oral iron,
sucrosomial iron,
iron store indices.

ABSTRACT

Anemia is a grave public health issue that affects 25% of the global population. Conventional iron formulations used in treatment have drawbacks such as poor bioavailability and gastric intolerability. The current study aimed to evaluate the anti-anemic effects of different iron salts in Wistar rats with iron deficiency anemia (IDA).

IDA was induced by the validated pre-clinical model by retro-orbital bloodletting (1 ml) for 21 days along with an iron-deficient diet in Wistar rats. The rats (n=48) were assigned into 8 groups: Control group, IDA rats, IDA rats receiving either vehicle or different iron salts (ferrous sulfate, ferrous ascorbate, ferrous fumarate, and Sucrosomial iron) for 21 days at a dose of 30 mg/kg p.o. Hematological parameters and iron store indices were assessed at each visit.

Anemia induction markedly reduced hemoglobin levels in all IDA groups on day 21. In contrast, iron supplements showed significant improvement in hematological profile after 21 days of treatment. Interestingly, the Sucrosomial iron-supplemented group (group 8) showed significantly higher improvement in hemoglobin levels and hematocrit than did conventional iron supplements such as ferrous sulfate (group 5), ferrous ascorbate (group 6) and ferrous fumarate (group 7) ($p < 0.05$ for each group, respectively). Sucrosomial iron also showed slightly better improvement in iron store indices (serum iron & ferritin levels, total iron binding capacity and transferrin saturation [%]) when compared with other iron supplements (non-significant difference).

Authors concluded that Sucrosomial iron has a significant potential to improve IDA in Wistar rats compared to conventional iron salts. Sucrosomial iron can be useful for the management of IDA either prophylactically or therapeutically.

INTRODUCTION

Anemia is a grave public health concern that affects ~25% of the world's population (>1.6 billion people) [1]. Hemoglobin (Hb) levels that are two standard deviations below the mean for the patient's age are considered anemic. Chronic infections, genetics and dietary deficiencies such as hemoglobinopathies, iron insufficiency, folate inadequacy and vitamin B12 deficiency may all be contributing causes of anemia. Iron insufficiency has a significant impact on a baby's brain development and is a major cause of iron deficiency anemia. In addition to having an impact on behavior, memory, learning and sensory systems, iron is a crucial component of neuronal myelination, metabolism, neurotransmission and neurogenesis [2-5].

The most prevalent nutritional deficit in the world, iron deficiency anemia (IDA), can impair mental and physical development, promote lipid peroxidation and weaken anti-oxidant defenses, impair immunological function and cause diseases of the neurological system [6-9]. Conventional oral iron salts such as ferrous fumarate, ferrous sulfate and ferrous gluconate are used to treat iron deficiency anemia, but they have significant limitations, including low bioavailability (10% to 15%) and even further lower bioavailability for ferric iron salts or ferric iron complexes (polysaccharide, amino acids, ovo-albumin, etc.).

* **Corresponding author**
e-mail: pravin.tirgar@rku.ac.in

Apart from the aforementioned, conventional oral iron salts have a poor tolerability profile (50% of all patients reported gastrointestinal side effects) leading to poor compliance and poor clinical outcome. Additionally, the concurrent use of antacids or proton pump inhibitors, food consumption, and concomitant chronic inflammatory disorders (higher hepcidin limiting further iron absorption) may lower the iron absorption from standard oral iron salts and reduce its utilization. This might cause a delay in reaching the desired hematological condition or perhaps make the therapy ineffective. Furthermore, gastrointestinal adverse effects were noted by 50% of all patients using oral iron supplements, which might indicate poor compliance with oral iron supplementation [10]. As a result, research has focused on high bioavailability iron supplements with a favorable tolerability profile. To optimize iron use and reduce side effects, new iron supplements must be formulated [11].

Sucrosomial® iron constitutes a novel oral iron-containing formulation in which ferric pyrophosphate is protected by a phospholipid bilayer membrane made from sunflower lecithin and sucrester matrix (surfactant derived from the esterification of fatty acids with sucrose [sucrose esters]). Tricalcium phosphate and starch provide further stability to the structure, forming the "sucrosome" and providing Sucrosomial iron its gastro-resistant properties and good gastric tolerability profile [12-14]. *In vitro* studies suggest that Sucrosomial iron is absorbed in Peyer's patches of the small intestine via M-cells, para-cellular and trans-cellular pathways. Therefore, increased hepcidin levels that are prevalent during chronic inflammatory situations do not affect its uptake or usage. Sucrosomial iron, as opposed to conventional oral iron salts, assures increased bioavailability, better tolerance and compliance and better clinical outcomes by avoiding the hepcidin-dependent iron absorption pathway [10].

Although evidence of its efficacy is available in a few studies, no preclinical studies have compared the hematological state and iron storage indicators of Sucrosomial iron with conventional oral iron salts. The present study aimed to assess the comparative anti-anemic effects of Sucrosomial iron with conventional oral iron salts in a standardized validated experimental model of iron deficiency anemia induced by retro-orbital bloodletting and iron deficient diet in Wistar rats. This method of inducing iron deficiency anemia in Wistar rats is well-established through different pre-clinical studies. Several pre-clinical models have used two insults, bloodletting, and inadequate iron intake to induce anemia in rats. Numerous pre-clinical studies have validated that animals undergoing retro-orbital bloodletting for 21 days along with an iron-deficient diet effectively induce anemia (hemoglobin levels fall below 11 g/dl) in rats. Hence, this experimental model was used to effectively induce iron deficiency anemia in the present study followed by treatment with different iron salts for 21 days [15-17].

MATERIALS AND METHODS

Animals

For at least 7 days before and during the investigation, adult Wistar rats of either sex, weighing between 180 and 250 g and of 6-8 weeks of age, were kept under tightly regulated conditions of temperature (22±5°C), humidity (30-70%), and a 12-hour light-dark cycle. The animals were distributed at random into 8 groups (n = 6) as shown in Table 1 below, and they were kept in polypropylene cages with autoclaved rice husk for bedding and a stainless top grill with feeding bottles attached. Study groups received a prescribed diet, as well as unlimited access to filtered and UV-purified water.

Table 1. Animal groups

Group	Description	Dose
1	Control group	-
2	Disease Control (IDA) group	-
3	Disease Control (IDA) group received vehicle	Carboxymethylcellulose 0.5% p.o.
4	Control animal group received Sucrosomial iron	30 mg/kg/day p.o.
5	Disease Control (IDA) group treated with Ferrous sulfate	
6	Disease Control (IDA) group treated with Ferrous ascorbate	
7	Disease Control (IDA) group treated with Ferrous fumarate	
8	Disease Control (IDA) group treated with Sucrosomial iron	

Anemia induction

The Institutional Animal Ethics Committee approved the study (ARL/PT/390/2021) and it complied with CPCSEA criteria. The hematological status and ferritin levels were assessed on Day 0 in all animal groups. The normal control group (group 1) and group 4 (positive control) received a normal rat diet and water ad libitum. In this animal model of inducing iron deficiency anemia, the rats in the other group underwent daily (1 ml) retro-orbital sinus punctures and were fed a milk diet (600 ml/cage/day/6 rats) instead of a normal diet for 21 days. To corroborate IDA, an assessment of hemoglobin levels was made on day 21 [15]. On day 21, when IDA was confirmed, they were divided into different treatment groups (n = 6 rats/group). Group 1 rats (control group) received a normal diet throughout the study period. Group 2 served as the disease control group (IDA); Group 3 served as the vehicle control (carboxymethyl cellulose [CMC] 0.5%); Group 4 served as the positive normal control group receiving Sucrosomial iron; groups 5, 6, and 7 received conventional standard iron supplements such as ferrous sulfate, ferrous ascorbate and ferrous fumarate at a dose 30 mg/kg bodyweight p.o., respectively. Group 8 received the test iron supplement Sucrosomial iron at a dose of 30 mg/kg body weight p.o. The dose of 30 mg/kg body weight for all oral iron supplements was selected based on the previously validated pre-clinical models of iron deficiency anemia.[18-20] The vehicle/iron supplements continued for 21 days of the treatment period. The bleeding from the retro-orbital sinus was stopped during the treatment period, however, only milk was provided as the sole food. This animal model closely resembles the features of iron deficiency anemia in humans. The rats were classified as anemic when their hemoglobin levels fell below 12 g/dL. On day 0, day 21, and day 42, blood samples were taken from a retro-orbital plexus puncture and placed in an ethylenediaminetetraacetic acid tube.

Hematological tests

To compare the anti-anemic effects of various oral iron salts, hematological parameters including hemoglobin, hematocrit, red blood cells (RBCs), mean corpuscle volume (MCV), mean cell hematocrit (MCH) and mean corpuscle hemoglobin concentration (MCHC) were measured after treatment on specific days using an automatic blood analyzer (Horiba ABX, MICROS 60) [21,22]. By way of a semi-auto AU480 analyzer from Beckman Coulter, immunoassay experiments were performed to measure the levels of serum iron (SI) concentration, total iron binding capacity (TIBC), serum ferritin (SF) and transferrin saturation (TS%). Transferrin saturation (TS) % was calculated from the ratio of the SI concentration to the TIBC as follows [23]:

$$\text{Transferrin saturation (\%)} = \frac{\text{SerumIron}(\mu g/dL)}{\text{TIBC}(\mu g/dL)} \times 100\%$$

Statistical analysis

All the hematological data and iron store indices data were expressed as the man values ± Standard Error of Mean (n = 6). Data analysis employed univariate data by applying the Shapiro-Wilk test to see the distribution of the data. The obtained data was evaluated through one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, to examine the differences in the groups. Results were regarded as statistically significant when $p < 0.05$. GraphPad Prism V.9.3.1 software was employed to perform the statistical analysis.

RESULTS

Anemia induction in animal groups

The hematological parameters of the rats at the baseline (day 0) exhibited no significant group differences ($p > 0.05$) as shown in Table 2. Anemia induction with bloodletting and milk as the sole diet for 21 days led to a significant reduction in hemoglobin levels (<11 g/dl) in the disease control (IDA) group (group 2), IDA+ Vehicle (CMC 0.5%) receiving group (group 3), groups receiving conventional oral iron supplements such as ferrous sulfate (group 5), ferrous ascorbate (group 6), and ferrous fumarate (group 7) and also the Sucrosomial iron group (group 8) ($p < 0.0001$ for each group – as shown in Table 3). This indicated the successful induction of anemia in all disease-control animal groups. The iron store biomarker serum ferritin levels were found to be significantly reduced on day 21 in the disease control (IDA) group (group 2), IDA+ Vehicle (CMC 0.5%) receiving group (group 3), groups receiving conventional oral iron supplements such as ferrous sulfate (group 5), ferrous ascorbate (group 6), ferrous fumarate (group 7) and the Sucrosomial iron group (group 8) ($p < 0.0001$ for each group, compared to the control group – as shown in Table 3). The RBCs count was found to be significantly reduced on day 21 in the disease control (IDA) group (group 2), groups receiving conventional oral iron supplements such as ferrous sulfate (group 5), ferrous ascorbate (group 6), ferrous fumarate (group 7) and the Sucrosomial iron group (group 8) ($p < 0.0001$ for each group, compared to the control group, Table 3). The Mean corpuscular volume (MCV) was found to be significantly reduced in the disease control (IDA) group (group 2) ($p < 0.0001$) on day 21, while other groups showed no significant differences. The other hematological parameters (MCH and MCHC) showed no significant differences in any group on day 21 compared to the control group ($p > 0.05$ for each group, Table 3).

Effect of different iron supplements on hematological parameters

Conventional oral iron supplements such as ferrous sulfate, ferrous ascorbate, ferrous fumarate and novel iron formulation Sucrosomial iron were administered at 30 mg/kg body weight/day dose orally for 21 days to assess their comparative anti-anemic potential. After a treatment period of 21 days, hemoglobin levels were significantly increased in the ferrous sulfate group (13.14±0.14; $p = 0.0015$), ferrous ascorbate group (13.00±0.26; $p < 0.011$), ferrous fumarate

Table 2. Hematological parameters on baseline (day 0) of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs (count)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1: Control	15.55±0.33	49.32±0.41	6.63×10^6 ±0.61	73.97±0.77	15.55±0.33	28.64±0.16
Group 2: Disease Control (IDA)	15.54±0.35	49.29±0.35	6.46×10^6 ±0.48	73.63±0.77	15.54±0.35	28.26±0.22
Group 3: IDA + Vehicle (CMC 0.5%)	15.53±0.25	49.24±0.42	6.48×10^6 ±0.74	74.35±1.05	15.53±0.25	28.49±0.16
Group 4: Control + Sucrosomial iron	15.59±0.14	49.37±0.42	6.30×10^6 ±0.77	73.98±1.13	15.59±0.14	28.40±0.34
Group 5: IDA + Ferrous sulfate	15.43±0.15	49.36±0.12	6.46×10^6 ±0.64	73.21±1.09	15.43±0.15	28.51±0.27
Group 6: IDA + Ferrous ascorbate	15.41±0.09	49.38±0.24	6.39×10^6 ±0.53	73.36±0.91	15.41±0.09	28.33±0.25
Group 7: IDA + Ferrous fumarate	15.55±0.18	49.44±0.30	6.48×10^6 ±0.57	73.38±0.66	15.55±0.18	28.35±0.29
Group 8: IDA + Sucrosomial iron	15.50±0.23	49.34±0.11	6.45×10^6 ±0.52	73.52±0.47	15.50±0.23	28.31±0.29

IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test.

Table 3. Hematological parameters on day 21 of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs (count)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1: Control	15.53±0.14	49.58±0.28	6.37×10^6 ±0.80	73.38±1.09	18.37±0.14	28.94±0.23
Group 2: Disease Control (IDA)	10.85±0.27*	29.75±0.30*	4.31×10^6 ±0.60*	67.84±0.84*	17.48±0.18	27.74±0.27
Group 3: IDA + Vehicle (CMC 0.5%)	10.91±0.14*	29.61±0.18*	4.55×10^6 ±0.53	69.16±0.54	17.65±0.24	28.05±0.20
Group 4: Control + Sucrosomial iron	15.66±0.15	49.68±0.22	6.40×10^6 ±0.82	71.33±0.58	17.63±0.31	28.43±0.12
Group 5: IDA + Ferrous sulfate	10.80±0.22*	29.80±0.40*	4.35×10^6 ±0.84*	68.07±0.66	17.56±0.15	27.91±0.22
Group 6: IDA + Ferrous ascorbate	10.87±0.18*	29.92±0.21*	4.51×10^6 ±1.15*	68.30±0.71	17.50±0.22	27.97±0.19
Group 7: IDA + Ferrous fumarate	10.94±0.11*	29.82±0.30*	4.48×10^6 ±1.01*	68.53±0.55	17.47±0.35	27.94±0.15
Group 8: IDA + Sucrosomial iron	10.85±0.14*	29.77±0.18*	4.57×10^6 ±0.87*	68.19±0.56	17.43±0.14	27.95±0.07

IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test
Non-significance at P value >0.05; Statistical significance at P value <0.05
*p<0.0001 as compared to the control group (group 1) on day 21

Table 4. Hematological parameters on day 42 of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs (count)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1: Control	15.28±0.08	49.88±0.26	6.23×10^6 ±1.11	72.95±0.85	18.39±0.28	28.78±0.09
Group 2: Disease Control (IDA)	12.22±0.17	30.80±0.34	4.50×10^6 ±0.92	68.45±0.59	17.33±0.07	27.96±0.23
Group 3: IDA + Vehicle (CMC 0.5%)	12.34±0.09	31.37±0.38	4.54×10^6 ±0.63	69.24±0.46	17.38±0.17	28.09±0.23
Group 4: Control + Sucrosomial iron	15.62±0.12	50.60±0.21	6.43×10^6 ±0.46	72.68±0.84	17.81±0.27	28.55±0.10
Group 5: IDA + Ferrous sulfate	13.14±0.14*	38.35±0.36	5.09×10^6 ±0.85	70.07±0.50	17.86±0.18	28.38±0.10
Group 6: IDA + Ferrous ascorbate	13.00±0.26†	38.66±0.27	5.10×10^6 ±0.67	72.46±0.34	18.08±0.52	28.32±0.09
Group 7: IDA + Ferrous fumarate	13.14±0.09‡	38.51±0.29	5.08×10^6 ±0.84	72.28±0.63	17.84±0.25	28.37±0.09
Group 8: IDA + Sucrosomial iron	13.84 ±0.15§‖¶**	40.34 ±0.39††‡‡§§	5.45×10^6 ±0.50	72.89±0.52	18.17±0.20	28.63±0.10

IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test

Non-significance at P value >0.05; Statistical significance at P value <0.05

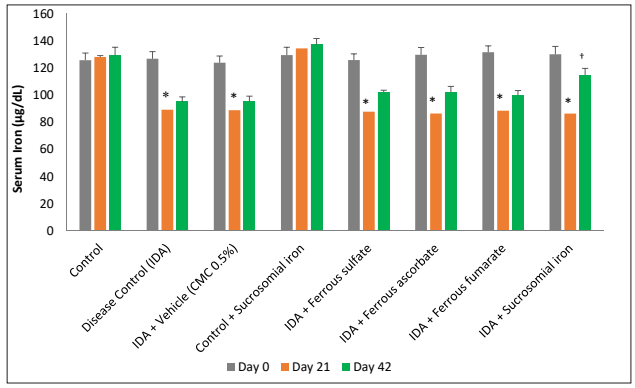
*p=0.0015, as compared to disease control group; †p=0.011, as compared to disease control group; ‡p=0.0017, as compared to disease control group; §p<0.0001, as compared to disease control group; ||p=0.0326, as compared to IDA + ferrous sulfate group, ¶p=0.005, as compared to IDA + ferrous ascorbate group; **p=0.03, as compared to IDA + ferrous fumarate group; ††p=0.005, as compared to IDA + ferrous fumarate group; ‡‡p=0.0132, as compared to IDA + ferrous ascorbate group; §§p=0.0018, as compared to IDA + ferrous sulfate group

group (13.14±0.09; p = 0.0017) and novel iron formulation Sucrosomial iron group (13.84±0.15; p <0.0001), as compared to the disease control group (group 2) on day 42 of the study Table 4). Interestingly, the Sucrosomial iron receiving group showed significantly higher improvement in hemoglobin levels than conventional oral iron supplements such as ferrous sulfate group p = 0.0326), ferrous ascorbate group p = 0.005, and ferrous fumarate group p = 0.03) on day 42 of the study. Hematocrit levels were significantly increased with all iron supplement groups as compared to the disease control group (group 2) on day 42 of the study p <0.0001) with no significant inter-group difference. RBCs count was found to be significantly increased with all iron supplement groups, as compared to the disease control group (group 2) on day 42 of the study p <0.0001 for ferrous sulfate, ferrous ascorbat, and Sucrosomial iron group, respectively, and p = 0.0001 for the ferrous fumarate group). There was no significant inter-group difference in RBCs count on day 42 of the study p >0.05). RBC morphology showed irregular cell walled and hypochromic cells in all disease control (IDA) groups as compared to normal control (group 1) on day 21 of the study-– and it was only corrected in the Sucrosomial iron receiving group on day 42 of the study. All iron-supplemented groups showed a positive trend in improving hematological parameters such as MCV, MC, and MCHC on day 42 without any significant difference p >0.05). Interestingly, the Sucrosomial iron-supplemented group (group 8) showed slightly better improvement than other oral iron supplements (non-significant difference; p >0.05).

Effect of different iron supplements on serum iron levels

The total quantity of iron in serum is represented by the serum iron concentration [21]. Serum iron levels were found to be significantly reduced in all disease control (IDA) groups on day 21 of the study (p <0.0001). After a treatment period of 21 days, only the Sucrosomial iron-supplemented group showed significantly higher improvement in serum iron levels (p <0.05) on day 42 of the study. There was no significant improvement observed in serum iron levels with conventional oral iron supplements: ferrous sulfate, ferrous ascorbate and ferrous fumarate (p>0.05 for each group, respectively) – as shown in Figure 1.

The maximum quantity of iron necessary to bind to all the transferrin in serum is referred to as the 'total iron binding capacity' (TIBC). When the serum iron concentration is low, the TIBC rises, and when the serum iron concentration is high, the TIBC falls [21]. Total iron binding capacity was found to be significantly increased in all disease control (IDA) groups on day 21 of the study (p <0.0001). Higher total iron binding capacity indicates low

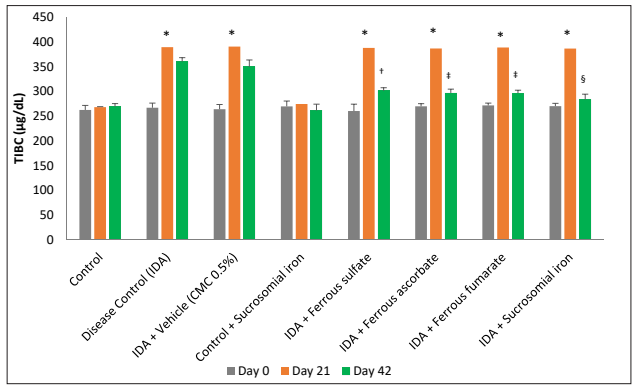


IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test

Non-significance at P value >0.05; Statistical significance at P value <0.05
*p<0.0001, as compared to the control group on day 21, †p<0.05, as compared to the Disease Control (IDA) group on day 42
Effect of different iron supplements on Total iron binding capacity (TIBC)

Figure 1. Serum iron levels on Baseline (Day 0), Day 21, and Day 42 in different iron-supplemented groups



IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

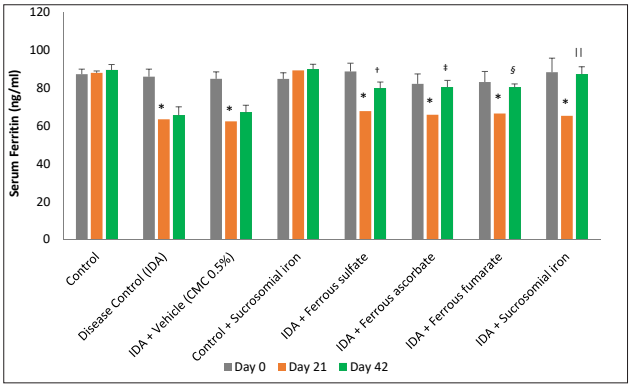
The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test
Non-significance at P value >0.05; Statistical significance at P value <0.05
*p<0.000, as compared to the control group on day 21, †p = 0.0017, ‡p = 0.006 and §p<0.0001, as compared to the Disease Control (IDA) group on day 42

Figure 2. TIBC levels on Baseline (Day 0), Day 21, and Day 42 in different iron-supplemented groups

serum iron concentration and demonstrates iron deficiency anemia. After a treatment period of 21 days, all iron supplement groups: ferrous sulfate (p = 0.0017), ferrous ascorbate (p = 0.006), ferrous fumarate (p = 0.006) and Sucrosomial (p <0.0001) showed a significant reduction in TIBC on day 42 of the study – as shown in Figure 2. There was no significant difference observed in the different groups (p >0.05).

Effect of different iron supplements on Serum ferritin levels

Serum ferritin levels were found to be significantly reduced in all disease control (IDA) groups on day 21 of the study (p = 0.0003). Lower serum ferritin levels indicate diminished iron reserve and reveal iron deficiency anemia. After a treatment period of 21 days, all iron supplement groups: ferrous sulfate (p = 0.0038), ferrous ascorbate (p = 0.0012), ferrous fumarate (p = 0.008) and Sucrosomial (p = 0.0018) showed a significant improvement in serum ferritin levels on day 42 of the study – as shown in Figure 3. There was no significant difference observed in the different groups (p >0.05). however, the Sucrosomial iron receiving group showed marginally higher improvement in serum ferritin levels, when compared to conventional oral iron supplements (non-significant difference). This result indicates the higher hematinic potential of Sucrosomial iron for the management of iron deficiency anemia as achieved by normalizing the serum ferritin levels.



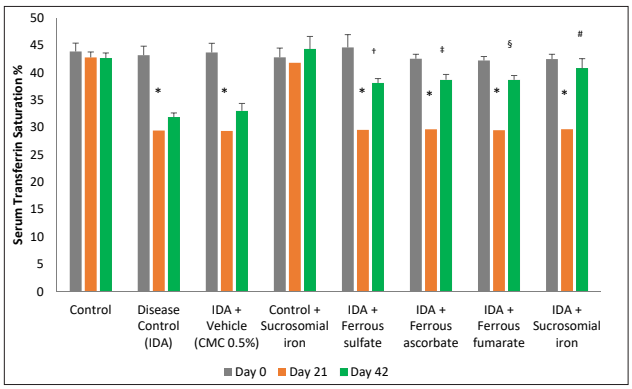
IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test
Non-significance at P value >0.05; Statistical significance at P value <0.05
*p=0.0003, as compared to the control group on day 21, †p=0.0038, ‡p=0.0012, §p=0.008 and ||p=0.0018, as compared to the Disease Control (IDA) group on day 42

Figure 3. Serum ferritin levels on Baseline (Day 0), Day 21, and Day 42 in different iron-supplemented groups

Effect of different iron supplements on serum transferrin saturation (%)

Serum transferrin saturation (%) refers to the amount of iron present in the body in bound form [21]. Serum transferrin saturation was found to be significantly reduced in all disease control (IDA) groups on day 21 of the study (p <0.0001). Lower serum transferrin saturation (%) indicates iron deficiency anemia. After a treatment period of 21 days, all iron supplement groups: ferrous sulfate (p = 0.0357), ferrous ascorbate (p = 0.0157), ferrous fumarate (p = 0.016) and Sucrosomial (p = 0.0007) showed a significant improvement in serum ferritin levels on day 42 of the study – as shown in Figure 4. There was no significant difference observed in the different groups (p>0.05).



IDA: Iron-deficiency anemia, CMC: carboxymethyl cellulose

The data are expressed as mean±SEM and analyzed by ANOVA and Tukey's multiple comparison test
Non-significance at P value >0.05; Statistical significance at P value <0.05
* p<0.0001, as compared to the control group on day 21, †p = 0.0357, ‡p = 0.0157, §p = 0.016 and #p = 0.0007, as compared to the Disease Control (IDA) group on day 42

Figure 4. Serum Transferrin saturation (%) on Baseline (Day 0), Day 21, and Day 42 in different iron-supplemented groups

DISCUSSION

Iron deficiency is the most prevalent dietary deficiency in the world. It is associated with reduced size and number of red blood cells. Iron depletion, which causes no physiological defects, and iron deficiency anemia, which compromises the operation of several organ systems, are two different types of iron deficiency. If the hemoglobin concentration or hematocrit value increases after receiving therapeutic iron supplementation, iron deficiency anemia can be identified [24]. The results of the present study showed that all iron supplements (ferrous sulfate, ferrous ascorbate, ferrous fumarate and Sucrosomial iron) demonstrated significantly improved hemoglobin levels and enhanced hematocrit profile. Interestingly, the Sucrosomial iron receiving group showed significantly higher improvement in hemoglobin levels and hematocrit profile – indicating its stronger hematinic potential for the management of iron deficiency anemia when compared with conventional oral iron supplements.

According to the literature, continuous iron delivery in anemic rats induces intestinal iron build-up. In rats supplemented with conventional iron salts low ceruloplasmin activity and high mucosal ferritin levels impeded intestinal iron mobilization, increasing peroxidative stress [25]. Ceruloplasmin, Divalent metal transporter 1, hepcidin and hephaestin are proteins that are crucial for both iron absorption from the gut and iron efflux from enterocytes [10,22]. Hence, the absorption and utilization of iron from conventional iron supplements are impacted by such factors. The Sucrosomial iron gets absorbed through the transcellular route, paracellular route and M-cells in the small intestine thereby bypassing the divalent metal transporter 1 pathway and hepcidin-dependent pathway. Thus, Sucrosomial iron exerts a superior hematinic effect than conventional oral iron supplements. The uptake of radiolabelled Sucrosomial iron through the M-cells of the Peyer's patches in the intestine was observed in several ex-vivo studies using

immunofluorescence analysis and microscopic examination of excised rat intestinal tissues. This uptake was followed by uptake in CD68+ macrophages, which also serve as a temporary storage site for Sucrosomial iron [26].

In another *in vitro* investigation, Sucrosomial iron was found to be more readily absorbed than conventional oral iron salts such as ferrous sulfate, ferrous bis-glycinate, ferrous ascorbate and ferrous edetate in the presence of M cells (RajiB cells). Sucrosomial iron is absorbed through the intestinal epithelium via a DMT-1 independent process that is unaffected by the divalent iron chelator bathophenanthroline disulfonic acid, according to ex-vivo permeation tests using an excised rat intestine model [10]. This finding supports the recognized mechanism for Sucrosomial iron absorption through M-cells and avoiding the DMT-1-dependent route, hence absorption and utilization are not hampered by higher hepcidin levels. The bioavailability of iron (Fe^{+3}) with Sucrosomial iron was found to be much higher than that with ferric pyrophosphate after 5 hours of administration. Moreover, Sucrosomial iron supplementation compared to treatment with ferric pyrophosphate, as investigated through several organs from a sacrificed rat (including the liver, spleen and bone marrow), revealed substantially increased Fe^{+3} concentration. Furthermore, the Pharmacokinetic profiles demonstrated a significantly higher area under the curve (AUC) and maximal plasma concentration of iron (C_{max}) for Sucrosomial iron than those for ferric pyrophosphate.

These data suggest that Sucrosomial iron has higher bioavailability, and the excess iron amount required, apart from the hematopoiesis and metabolic processes, is mainly stored in the hepatocytes [26-28]. Sucrosomial iron also showed higher accumulation of serum ferritin levels within enterocytes compared to ferrous sulfate and ferric pyrophosphate (3-fold higher) and phospholipid containing ferric pyrophosphate or micronized, dispersible ferric pyrophosphate (3.5-fold higher) in an experimental model using CACO-2 cell culture.

Previous experimental animal studies in mice and piglets reported equal therapeutic efficacy of Sucrosomial iron and conventional oral iron supplements for correction of iron deficiency anemia [10]. However, the present pre-clinical study results showed the superiority of Sucrosomial iron over conventional oral iron supplements in improving hemoglobin levels and iron store indices in Wistar rats. A recent pre-clinical study conducted by us showed that Sucrosomial iron significantly improved hematinic parameters when compared to other oral iron salts in Wistar rats with haloperidol-induced anemia. However, the iron store indices were not studied in our previous study [18]. In accordance with the previous study, the present study revealed significant improvement in the hematological profile, along with improvement in iron store indices (serum iron, total iron binding capacity, serum transferrin saturation [%] and serum ferritin) with the use of Sucrosomial iron, as compared to conventional oral iron formulations such as ferrous sulfate, ferrous ascorbate and ferrous fumarate.

The use of conventional oral iron salts is associated with gastrointestinal side effects which further reduce its clinical adherence thereby reducing clinical outcome, while Sucrosomial iron is considered relatively safe. Therefore, the results of the current investigation suggest that a novel Sucrosomial iron supplement can turn out to be a potent option for the treatment of iron deficiency anemia in anemic individuals, either prophylactically or therapeutically, as it restores normal hemoglobin levels as well as iron store indices such as serum ferritin levels. Thus the product can be a good therapeutic option not only for the management of iron deficiency anemia, but also in the management of anemia of chronic inflammatory diseases in which conventional iron supplements are not very useful due to their reduced bioavailability and utilization profile because of higher hepcidin levels.

CONCLUSION

Based on the findings of the study, it can be concluded that, in comparison to other conventional oral iron formulations, the novel oral iron formulation Sucrosomial iron displayed a potent anti-anemic effect by improving hematological parameters and iron store indices such as serum iron levels, serum ferritin levels, TIBC and TSAT% in a validated experimental model of iron-deficiency anemia in Wistar rats. The greater bioavailability of Sucrosomial iron may be the cause of this benefit, although additional research is required to confirm this finding. Sucrosomial iron's therapeutic potential needs to be further validated in different pre-clinical models and clinical studies.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

SOURCES OF FUNDING OF THE WORK

Nil

APPROVAL OF RESEARCH ETHICS COMMITTEE (BIOETHICS COMMITTEE)

The Institutional Animal Ethics Committee approved the study (ARL/PT/390/2021) and it complied with CPCSEA criteria.

ORCID iDs

Manoj A. Suva https://orcid.org/0000-0003-0484-7375
Pravin R. Tirgar https://orcid.org/0000-0002-4877-7201

REFERENCES

1. Li Q, Liang F, Liang W, Shi W, Han Y. Prevalence of anemia and its associated risk factors among 6-months-old infants in Beijing. *Front Pediatr.* 2019;7:286.
2. Ortiz E, Pasquini JM, Thompson K, Felt B, Butkus G, Beard J, et al. Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. *J Neurosci Res.* 2004;77:681-9.
3. Lozoff B. Iron deficiency and child development. *Food Nutr Bull.* 2007;28:S560-71.

4. Sheikh NA, Desai TR, Tirgar PR. Investigation into iron chelating and antioxidant potential of melilotus officinalis in iron dextran induced iron overloaded sprague dawley rat model. *Drug Res* (Stuttg). 2016;66(12):618-27.
5. Desai TR, Tirgar PR. Investigation into iron chelating activity of Triticum aestivum (wheat grass) in iron-dextran induce iron overload model of thalassemia. *J Pharm Res.* 2011;4(9):1-4.
6. Hassan TH, Badr MA, Karam NA, Zkaria M, El Saadany HF, Rahman DM, et al. Impact of iron deficiency anemia on the function of the immune system in children. *Medicine*. 2016;95:e5395.
7. Zakaurrab Z, Adnan M, Ahmad SM, Islam N. Effect of oral iron on markers of oxidative stress and antioxidant status in children with iron deficiency anaemia. *J. Clin Diagn Res*. 2016;10:SC13-SC19.
8. Gupta PM, Perrine CG, Mei Z, Scanlon KS. Iron, anemia, and iron deficiency anemia among young children in the United States. *Nutrients*. 2016;8:1-4.
9. Jougleux JL, Rioux FM, Church MW, Fiset S, Suretteet ME. Mild maternal iron deficiency anemia during pregnancy and lactation in guinea pigs causes abnormal auditory function in the offspring. *J. Nutr.* 2011;141:1390-5.
10. Gómez-Ramírez S, Brilli E, Tarantino G, Muñoz M. Sucrosomial® Iron: A new generation iron for improving oral supplementation. *Pharmaceuticals* (Basel). 2018;11(4):97.
11. Bovellbenjamin AC, Viteri FE, Allen LH. Iron absorption from ferrous bis-glycinate and ferric trisglycinate in whole maize is regulated by iron status. *Am J Clin Nutr.* 2000;71:1563-9.
12. Kis L, Szuts A, Otomo N, Szabó-Révész P, Deli MA. The Potential of sucrose esters to be used as oral absorption enhancers. *Sci Pharm*. 2010;78:716.
13. Kiss L, Hellinger E, Pilbat AM, Kittel Á, Török Z, Füredi A, et al. Sucrose esters increase drug penetration, but do not inhibit p-glycoprotein in Caco-2 intestinal epithelial cells. *J Pharm Sci*. 2014;103:3107-19.
14. Fabiano A, Brilli E, Fogli S, Beconcini D, Carpi S, Tarantino G, et al. Sucrosomial® iron absorption studied by in vitro and ex-vivo models. *Eur J Pharm Sci*. 2018;111:425-31.
15. Gawde SR, Patel TC, Rege NN, Gajbhiye S, Uchil D. Evaluation of effects of Mandurabhasma on structural and functional integrity of small intestine in comparison with ferrous sulfate using an experimental model of iron deficiency anemia. *Ancient Sci Life*. 2015;34:134-41.
16. Goldberg L, Martin E. Iron containing hematinics. In: Laurence DR, Bacharach AL, (ed). *Pharmacometrics, evaluation of drug activities*. London: Academic Press; 1964:535-83.
17. Markandeywar NR. *Evaluation of efficacy and safety of an ayurvedic hematinic formulation in an experimental model of iron deficiency anaemia*. University of Mumbai; 2011.
18. Suva MA, Tirgar PR. Comparative assessment of anti-anemic effect of Sucrosomial iron in haloperidol-induced iron deficiency anemia in Wistar rats. *J Integr Sci Technol*. 2022;10(3):204-8.
19. Baqa K, Waris N, Butt A, Nazim U, Abbasi SR, Naz A. Comparative liver function assessment of natural and available drug (Ferrous sulfate) for iron-deficiency anemia in rat model. *Pak J Pharm Sci*. 2019;32(3):973-9.
20. Moazedi AA, Shooshtari MK, Ali G. Dose-dependent effects of iron supplementation on short-term and long-term memory in adult male wistar rats. *J Biol Sci*. 2010;10(7):648-52.
21. He H, Huang Q, Liu C, Jia S, Wang Y, An F, et al. Effectiveness of AOS–iron on iron deficiency anemia in rats. *RSC Adv*. 2019;9: 5053-63.
22. Trivedi A, Mishra S. Evaluation of hematinic potential of a herbomineral Formulation (HMF-TE) in Haloperidol-induced anemic rats. *Pharmacognosy Res*. 2009;1(4):192-6.
23. Lazarte CE, Soto A, Alvarez L, Bergenståhl B, Medrano N, Granfeldt Y, et al. Nutritional status of children with intestinal parasites from a tropical area of bolivia, emphasis on zinc and iron status. *Food Nutr Sci*. 2015;6:399-411.
24. Srivastava V, Sarkar S, Pattanayak C, Mishra M, Jena J. Comparative analysis of hematinic effect of Dhatryarista with standard Ferrous sulfate in Tannic acid-induced iron deficiency anemia in albino Wistar rats. *Indian J Public Health Res Dev.* 2020;11(6):1047-53.
25. Medappa N. Iron absorption and its implications on strategies to control iron deficiency anemia. *ICMR Bulletin*. 2000;30(2):1-6.
26. Fabiano A, Brilli E, Mattii L, Testai L, Moscato S, Citi V, et al. Ex vivo and in vivo study of sucrosomial iron intestinal absorption and bioavailability. *Int J Mol Sci*. 2018;19:2722.
27. Rishi G, Subramaniam VN. The liver in regulation of iron homeostasis. *Am J Physiol. Gastrointest Liver Physiol*. 2017;313: G157-G165.
28. Brilli E, Lipinski P, Barnadas R, Camacho M, Giordano G, Tarantino G. Sucrosomial® iron absorption involves m cells interaction. *Blood*. 2017;130(1):2217.