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Comparative assessment of anti-anemic effect of Sucrosomial iron in haloperidol-induced iron deficiency anemia in Wistar rats

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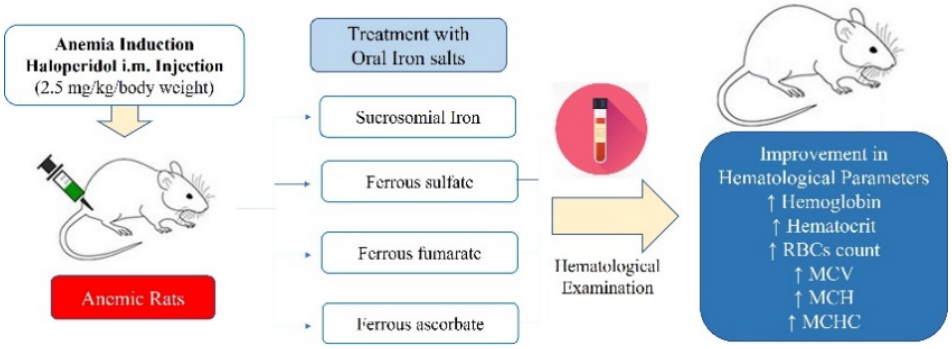
ABSTRACT



Anemia affects almost one-third of the world's population. Conventional oral iron salts have limitations such as poor bioavailability and poor tolerability. Sucrosomial iron gets absorbed through the hepcidin independent pathway; hence overcoming all these limitations. The present study assessed the anti-anemic effect of various iron salts on hematological parameters in haloperidol-induced iron deficiency anemia in Wistar rats. Iron deficiency anemia was induced by haloperidol injection (2.5 mg/kg body weight/day i.m.) for initial 5 days along with an iron-deficient diet throughout the study. Disease control animals received ferrous sulfate, ferrous ascorbate, ferrous fumarate, and Sucrosomial iron at a dose of 30 mg/kg p.o for 15 days. On day 20, animals treated with all iron-supplemented groups showed significant improvement in hematological parameters. Interestingly, the Sucrosomial iron group showed significantly higher improvement in hemoglobin levels and hematocrit parameters than other oral iron salt groups ($P<0.05$). This effect may be due to the higher bioavailability of Sucrosomial iron. We concluded that Sucrosomial iron exhibited a potent anti-anemic effect against haloperidol-induced iron deficiency anemia in Wistar rats compared to other conventional oral iron salts.

Keywords: Iron deficiency anemia, Haloperidol, Conventional oral iron, Sucrosomial iron, Hemoglobin

INTRODUCTION

Iron is an essential element for every living organism, and it is a component of human hemoglobin, myoglobin, and different enzymes. Iron is essential for oxygen transfer, DNA production, mitochondrial electron transport, and energy metabolism. A shortage of iron in the body can cause iron-deficiency anemia (IDA) and physical impairment.[1] Anemia affects 1/3rd of the world's population of all ages and is caused mostly by iron deficiency.[2] Iron deficiency can interfere with mental health and physical growth, increase lipid peroxidation, weaken antioxidant defence, and impair the immune system and nervous system.[1]

Providing adequate iron is the most fundamental approach to avoiding iron deficiency.[3] The correction of iron deficiency anemia with currently available conventional oral iron salts such as ferrous sulfate, ferrous fumarate, ferric iron complexes (amino acids, polysaccharide, and ovalbumin), etc. is associated with many drawbacks such as poor bioavailability (less than 15%). In addition, the concomitant administration of antacids or proton pump inhibitors, food intake, and any chronic inflammatory disease condition (higher hepcidin levels which impact iron absorption and utilization) may further reduce the absorption of conventional oral iron salts. This may result in a delay in achieving the target hematological status or treatment remains even ineffective. Moreover, half of the patients receiving oral iron supplements reported gastrointestinal side effects which may lead to poor compliance with the oral iron supplementation.[4] As a result, high bioavailability iron supplements with a good tolerability profile have been the subject of study.[5] New iron supplements must be developed to maximize iron usage while minimizing negative effects. Sucrosomial iron is a novel oral iron formulation that is absorbed through M-cells, para-cellular and trans-cellular routes in

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Peyer's patches in the small intestine. Hence, its absorption and utilization do not get affected by higher hepcidin levels present during chronic inflammatory conditions. Therefore, Sucrosomial iron bypasses the normal intestinal mechanism of iron absorption (hepcidin-dependent pathway) and ensures higher bioavailability, better tolerability & compliance, and better clinical outcome than conventional oral iron salts.[4] Although evidence of its efficacy and tolerability are available in a few studies, the comparative preclinical data with the most preferred conventional oral iron salts are lacking. The present study aimed to assess the comparative anti-anemic effects of various oral iron salts (ferrous sulfate, ferrous ascorbate, and ferrous fumarate) in haloperidol-induced iron deficiency anemia in Wistar rats.

MATERIALS AND METHODS

Adult Wistar rats of either sex, weighing range 190-225 g were maintained under well-controlled conditions of temperature (22 ± 3°C), humidity (30-70%), and a 12-hour light-dark cycle for at least 7 days before and throughout the study. Animals were housed in groups of six animals/cages in polypropylene cages with bedding of autoclaved paddy husk, and a stainless top grill with attached feeding bottles. Study groups received a specified diet and filtered & UV purified water ad libitum. Rats were randomly assigned to 8 groups (n=6) as given below (Table 1).

Table 1. Animal Groups

Gr. No.	Description
1	Control
2	Disease Control (IDA)
3	Disease Control (IDA) received vehicle (Carboxymethylcellulose 0.5% p.o.)
4	Control animal received Sucrosomial iron (30mg/kg/day p.o.)
5	Disease Control (IDA) treated with Ferrous sulfate (30mg/kg/day p.o.)
6	Disease Control (IDA) treated with Ferrous ascorbate (30mg/kg/day p.o.)
7	Disease Control (IDA) treated with Ferrous fumarate (30mg/kg/day p.o.)
8	Disease Control (IDA) treated with Sucrosomial iron (30mg/kg/day p.o.)

Iron deficient diet preparation:

An iron-deficient diet was developed in the laboratory according to Dytes Co.'s AIG-93, which included 35 ppm of iron, a very little amount in feed. The diet contains 640 gm potato starch, 210 gm casein, 80 mL groundnut oil, 40 gm vitamin mix and 30 gm mineral mix.[6] During the study, animals were fed a meal of 10-20 gm/rat/day, and deionized water was provided ad libitum. The experiment followed CPCSEA guidelines and was authorized by the Institutional Animal Ethics Committee (ARL/PT/390/2021).

Anemia induction:

To lower hemoglobin levels in animals, haloperidol injection (2.5 mg/kg/day i.m.) was given to all groups for the initial 5 days. Throughout the trial, they were fed an iron-deficient diet. This animal model of inducing iron deficiency anemia by Haloperidol i.m. injection in rats replicates the aspects observed in iron deficiency anemia in humans. When the rats' hemoglobin levels went below 12g/dL, they were declared anemic. Blood samples were withdrawn from retro-orbital plexus puncture and added to a tube containing ethylenediaminetetraacetic acid on day 1, day 5, and day 20. After treatment on specified days, the hematological parameters like Hemoglobin concentration, Haematocrit value, Red blood cells (RBCs) count, Mean corpuscle volume (MCV), Mean Cell Hemoglobin (MCH), and Mean Corpuscle Hemoglobin Concentration (MCHC) were evaluated using an automatic blood analyzer (Horiba ABX, MICROS 60) to assess the comparative anti-anemic effect of different oral iron salts.[7]

Statistical analysis

All the data were tabulated as mean values of six determinants with ± Standard Error of Mean (SEM) and were analyzed by one-way ANOVA followed by Tukey's post hoc test. Nonparametric statistical methods were used to evaluate significance with the probability level at which the null hypothesis was rejected set at $P>0.05$ (GraphPad Prism V.9.3.1).

RESULTS AND DISCUSSION

As shown in Table 2, the hematological parameters of the rats in the beginning (day 1) exhibited no significant differences ($P>0.05$). Anemia induction with Haloperidol i.m. injection (2.5 mg/kg body weight/day) for initial 5 days led to a significant reduction in hemoglobin levels and it fall below 11 g/dl in group 2, group 3, and group 5-8 on day 5 ($P<0.0001$) (Table 3).

Table 2. Hematological parameters on day 1 of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	15.11 ±0.38	51.02 ±0.65	6.57×10^{6} ±0.89	73.89 ±1.30	15.11 ±0.38	28.75±0.23
Disease Control (IDA)	15.37 ±0.32	49.73 ±0.75	6.40×10^{6} ±0.57	72.99 ±0.73	15.37 ±0.32	28.67±0.16
IDA + Vehicle (CMC 0.5%)	15.35 ±0.23	51.24 ±0.86	6.48×10^{6} ±0.74	74.99 ±1.14	15.35 ±0.23	28.62±0.17
Control + Sucrosomial iron	15.40 ±0.27	50.18 ±0.70	6.31×10^{6} ±0.83	74.81 ±0.76	15.40 ±0.27	28.63±0.09
IDA + Ferrous sulfate	15.45 ±0.23	49.76 ±0.87	6.47×10^{6} ±0.77	73.59 ±1.12	15.45 ±0.23	28.66±0.22
IDA + Ferrous ascorbate	15.31 ±0.27	49.81 ±0.78	6.49×10^{6} ±0.60	73.85 ±0.69	15.31 ±0.27	28.70±0.24
IDA + Ferrous fumarate	15.33 ±0.32	50.13 ±0.75	6.44×10^{6} ±0.64	73.68 ±0.68	15.33 ±0.32	28.63±0.28

IDA + Sucrosomial iron	15.25 ±0.25	49.29 ±0.81	5.29×10^{6} ±0.15	73.35 ±0.99	15.25 ±0.25	28.59±0.08

All the values are the mean of 6 estimations with ±SEM. IDA: Iron-deficiency anemia

Table 3. Hematological parameters on day 5 of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	15.24±0.21	51.10±0.59	6.45×10^{6} ±0.99	73.96±0.60	18.30±0.49	28.51±0.06
Disease Control (IDA)	10.83±0.28*	29.38±0.17*	4.33×10^{6} ±0.65*	69.38±0.83$	16.49±0.29^	28.13±0.23
IDA + Vehicle (CMC 0.5%)	10.55±0.24	30.82±0.79*	4.49×10^{6} ±0.75*	70.31±0.44#	16.99±0.24	28.36±0.14
Control+ Sucrosomial iron	15.61±0.23	51.65±0.77	6.38×10^{6} ±0.76	73.82±0.55	17.61±0.41	28.58±0.10
IDA + Ferrous sulfate	10.57±0.14*	32.28±0.62*	4.58×10^{6} ±0.37*	70.66±0.80#	16.85±0.21#	28.38±0.11
IDA + Ferrous ascorbate	10.55±0.16*	32.88±0.59*	4.61×10^{6} ±0.93*	70.26±0.71#	16.57±0.41#	28.36±0.07
IDA + Ferrous fumarate	10.80±0.14*	32.33±0.59*	4.61×10^{6} ±0.76*	70.63±0.50#	16.77±0.09#	28.39±0.11
IDA + Sucrosomial iron	10.82±0.18*	33.01±0.70*	4.65×10^{6} ±0.77*	70.29±0.98#	16.73±0.26#	28.40±0.09

All the values are the mean of 6 estimations with ±SEM.
Significance level: *P<0.0001, $$P$=0.0009, #$P$<0.05, ^$P$=0.0061 as compared to control group.

Different Iron supplements like ferrous sulfate, ferrous ascorbate, ferrous fumarate, and Sucrosomial iron at 30mg/kg body weight/day dose were given orally for 15 days to assess their comparative hematinic potential. Hemoglobin levels were significantly increased in the ferrous sulfate group (12.75±0.20; P=0.0004), ferrous ascorbate group (12.82±0.20; P<0.0002), ferrous fumarate group (12.76±0.14; P=0.0003) and Sucrosomial iron (13.77±0.12; P<0.0001) as compared to the disease control group on day 20 of the study (Table 4) (figure 1). The Sucrosomial iron receiving group showed higher improvement in hemoglobin levels than other oral iron salts (non-significant difference P value >0.05).

Table 4. Hematological parameters on day 20 of the study

Group	Hemoglobin (g/dl)	Hematocrit (%)	RBCs	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	15.28 ±0.14	51.44 ±0.63	5.92×10^{6} ±0.23	73.84±0.49	18.34 ±0.34	28.51±0.24
Disease Control (IDA)	11.29 ±0.33	32.05 ±0.51	4.44×10^{6} ±0.14	70.91±0.70	16.94 ±0.35	28.40±0.34
IDA + Vehicle (CMC 0.5%)	11.40 ±0.18	33.04 ±0.62	4.70×10^{6} ±0.19	72.40±0.57	17.35 ±0.33	28.52±0.13
Control + Sucrosomial iron	15.90 ±0.27	53.07 ±0.46	6.31×10^{6} ±0.23	75.01±0.64	17.97 ±0.34	28.79±0.29
IDA + Ferrous sulfate	12.75 ±0.20$	37.61 ±0.31*	4.94×10^{6} ±0.22	72.29±0.62	17.90 ±0.40	28.64±0.13
IDA + Ferrous ascorbate	12.82 ±0.20#	37.84 ±0.42*	4.99×10^{6} ±0.17	72.98±0.36	18.04 ±0.68	28.73±0.11
IDA + Ferrous fumarate	12.76 ±0.14@	37.80 ±0.62*	4.94×10^{6} ±0.15	72.74±0.45	17.89 ±0.36	28.75±0.13
IDA + Sucrosomial iron	13.77 ±0.12*	40.35 ±0.74*	5.29×10^{6} ±0.15^	73.04±0.70	18.42 ±0.30	28.88±0.13

All the values are the mean of 6 estimations with ±SEM.
Significance level: $$P$=0.0004 as compared to the disease control group; #P<0.0002 as compared to the disease control group; @P=0.0003 as compared to the disease control group; *P<0.0001 as compared to the disease control group; ^P<0.05 as compared to the disease control group.

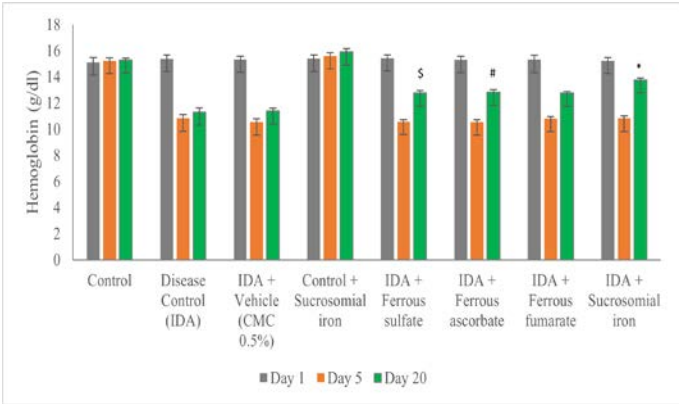

Figure 1. Hemoglobin levels on Baseline (Day 1), Day 5 and Day 20 in different groups
*All the values are the mean of 6 estimations with ± SEM. Significance level: $P=0.0004; #P<0.0002; *P<0.0001.*

Hematocrit levels were also significantly increased with all iron salts groups as compared to the disease control group on day 20 of the study (P<0.0001). The Sucrosomial iron receiving group showed higher improvement in hematocrit levels than other oral iron salts (non-significant difference P value >0.05) (figure 2).

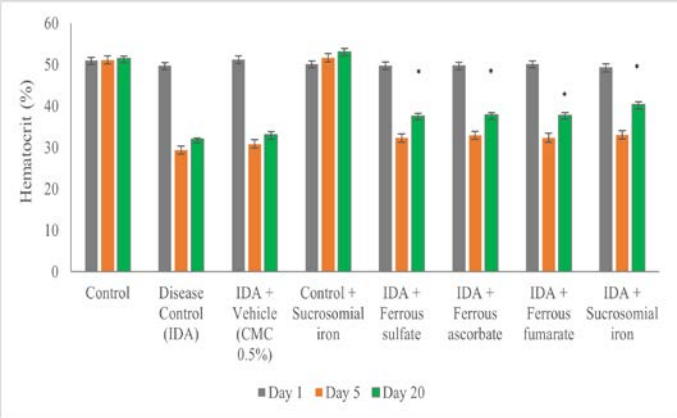

Figure 2. Hematocrit levels on Baseline (Day 1), Day 5 and Day 20 in different groups

*All the values are the mean of 6 estimations with ± SEM. Significance level: *P<0.0001.*

RBC count was significantly reduced in all iron-supplemented groups on day 5 and RBC count was found to be significantly increased only in the Sucrosomial iron group (P<0.05 as compared to the disease control group). RBC morphology showed irregular cell walled and hypochromic cells on day 5 of the study by haloperidol treatment as compared to control and it was corrected with the Sucrosomial iron treatment on day 20 of the study. All iron-supplemented groups showed a positive trend in improving blood parameters such as MCV, MCH, and MCHC on day 20; moreover, Sucrosomial iron-supplemented group showed better improvement than other oral iron supplements.

The most prevalent type of dietary deficit overall in the world is iron deficiency. Red blood cell size and number are lowered in iron deficiency. Iron insufficiency can range from iron depletion, which produces no physiological abnormalities, to iron-deficiency anemia, which impairs the functioning of many organ systems. Iron deficiency anemia can be diagnosed if the hemoglobin concentration or hematocrit value rises following therapeutic iron supplementation. In terms of hemoglobin decrease, which is suggestive of anemia, animals are identical to humans. All indicators, particularly hemoglobin, are significantly lowered in iron deficiency anemia.[8] Neuroleptic medication such as haloperidol causes iron deficiency anemia including anisocytosis and hypochromic cells in rats.[9,10] Literature suggests that chronic iron administration in anemic rats led to iron accumulation in the intestine. Low ceruloplasmin (ferrioxidase) activity and high mucosal ferritin levels impaired intestinal iron mobilization leading to increased peroxidative stress in iron-supplemented rats.[11] The proteins like ceruloplasmin (ferrioxidase), divalent metal transporter-1 (DMT-1), hepcidin, and hephaestin play important role in iron absorption in the intestine as well as in iron efflux from enterocytes.[4,7] Experimental data from several ex-vivo studies using immunofluorescence analysis and microscopic examination of excised rat intestinal tissues revealed the uptake of radiolabeled Sucrosomial iron through microfold cells (M-cells) of the Peyer's patches in the intestine followed by uptake in CD68+ macrophages which also acts as a transient storage site of Sucrosomial iron.[12] In vitro experiment using a co-culture system of CACO2/RajiB revealed higher absorption of Sucrosomial iron in the presence of M cells (RajiB cells) compared to conventional oral iron salts like ferrous sulfate, ferrous bis-glycinate, ferrous ascorbate, and ferrous edetate. Ex-vivo permeation investigations utilizing an excised rat intestine model revealed that Sucrosomial iron is absorbed across the intestinal epithelium via a DMT-1 independent mechanism that is unaffected by the divalent iron chelator bathophenanthroline disulfonic acid (BPDS).[4] This evidence confirms the established mechanism of absorption of Sucrosomial iron through M cells and bypassing the DMT-1-dependent pathway. Time-courses of plasma iron (Fe^{+3}) concentration up to 5 hours after Sucrosomial iron and ferric pyrophosphate revealed the significantly higher bioavailability of iron with Sucrosomial iron as represented by a higher area under the curve (AUC) and Cmax compared to ferric pyrophosphate. Moreover, the different tissues like liver, spleen, and bone marrow from a sacrificed rat after 5 hours of treatment with Sucrosomial iron showed significantly higher Fe^{+3} content compared to treatment with ferric pyrophosphate.[12] Experimental data using CACAO-2 cell culture showed a higher accumulation of ferritin compared to Ferrous sulfate (3-fold higher) and phospholipid containing ferric pyrophosphate or micronized, dispersible ferric pyrophosphate (3.5-fold higher). Animal studies with piglets and mice suggested that the therapeutic efficacy of Sucrosomial iron was comparable with all forms of oral iron salts.[4] However, in the present study, it was found that Sucrosomial iron showed significant improvement on hematinic parameters than other oral iron salts in haloperidol-induced anemia in Wistar rats. The findings of the present study indicate that a novel Sucrosomial iron supplement can serve as a potent choice of therapy for iron deficiency anemia either prophylactically or therapeutically in anemic patients. The therapeutic potential of Sucrosomial iron needs to be further validated in different pre-clinical models.[13-16]

CONCLUSION

Based on study results we concluded that the novel oral iron formulation Sucrosomial iron exhibited a potent anti-anemic effect in terms of improving hematological parameters against haloperidol-induced iron-deficiency anemia in Wistar rats compared to other conventional oral iron salts. This effect may be due to the higher bioavailability of Sucrosomial iron, and it needs to be validated in further studies.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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