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Differences between the original iron sucrose complex Venofer and the iron sucrose similar Sucrofer

Shah GB*, Chorawala MC, Trivedi VR, and Deshpande SS

K.B.Institute of Pharmaceutical Education and Research, Gh/6 Sector-23, Gandhinagar-382 023

ABSTRACT

Sucrofer (an Iron Sucrose complex) is one of the widely used Indian preparations for treatment of Iron deficiency anemia. The study was conducted to determine the possible efficacy and safety differences between the innovator's Venofer and Indian similar (Sucrofer). Thirty rats were divided into three groups and assigned to receive Sucrofer, Venofer or isotonic saline solution (control). Five single intravenous doses of iron (40 mg iron/kg) or saline (equivalent volume) were administered every 7 days for 4 weeks. Blood and urine samples were collected for biological assessment prior to sacrifice (day 28) after which kidney, liver, and heart homogenates were collected for determination of antioxidant enzyme levels. Immmunohistochemistry techniques were used to identify tissue ferritin, TNFα and IL-6. Systolic blood pressure, creatinine clearance as well as proteinuria were not significantly altered by sucrofer as compared to Venofer throughout the study. Post 28 days treatment, serum levels of AST, ALT and ALP were also not altered by sucrofer as compared to both vehicle and Venofer group. Liver, heart and kidney catalase, thiobarbituric reactive species, CuZn-superoxide dismutase and glutathione peroxidase activity, and reduced to oxidized glutathione ratio were also not significantly altered by sucrofer as compared to venofer at 4 weeks of treatment. Serum iron and percentage transferrin saturation were elevated in both treatment groups (except control) and no differences in haemoglobin concentration were observed. Liver, kidney and heart TNFα and IL-6 were not significantly altered in both treatment groups as compared to control group on day 28. These findings suggest that both Venofer and Sucrofer have similar safety and efficacy profile.

Keywords: Anemia; intravenous; iron sucrose; iron sucrose similar; oxidative stress; rats.

Corresponding author

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INTRODUCTION

Intravenous iron supplements compared to oral iron provide rapid repletion of body iron stores and have lesser chances of producing gastrointestinal and liver disorders. In addition, high doses can be administered in severe cases. Iron sucrose is reported to have better safety profile compared to iron dextran and ferric gluconate and therefore preferred in anemia due to chronic kidney diseases.(1) Hypersensitivity reactions are also rare compared to other available iron preparations(2). The stability of the iron sucrose complex can influence the safety of the product. Weakly bound iron may dissociate easily from the complex and catalyze the generation of reactive oxygen species(3). Thus small differences in the structure of the iron sucrose complex may affect the stability of the iron preparation and, thus, determine the severity of oxidative stress and consequent tissue damage. Thus it is worthwhile to compare the safety and efficacy of different iron sucrose preparations. Venofer has been used in clinical practice for decades with proven favorable safety and efficacy profile. The physicochemical properties and pharmacological activity of iron complexes are highly dependent on the manufacturing process which may vary from product to product. Any new compound therefore entering the market has to be thoroughly accessed for both efficacy and safety (4). Earlier studies with other iron sucrose preparations have shown significant hemodynamic, tissue and functional variation in comparison with Venofer(3,5). Sucrofer is however relatively new and its specifications closely resemble to Venofer. It is therefore important to assess the effects that slight structural modifications might have on safety and efficacy.

The present study examines potential differences in hemodynamic and oxidative stress parameters between the original iron sucrose complex Venofer and Sucrofer in rats.

MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Ethics Committee formed under the direction of CPCSEA, INDIA. Fifteen male and fifteen female 2-month-old Sprague-Dawley rats weighing 210–230 g were randomised into three groups of 10 with an equal male/female distribution. Animals assigned to the "control" group received isotonic saline solution, those assigned to the "reference" group received iron sucrose (Venofer, Vifor [International], Switzerland) and those assigned to the TEST group received Sucrofer(Claris Life Sciences, INDIA)

Rats were housed in metabolic cages in a temperature-controlled room (22±2°C) and subjected to 12 hour light/dark cycles. All animals received free access to tap water and were fed standard rat chow *ad libitum* throughout the study. Rats from each experimental group received a single IV dose by tail vein injection of the corresponding iron compound (40 mg iron/kg) or saline (equivalent volume) at the same time every 7 days for 4 weeks (total of five applications). Doses were adjusted each week according to the body weight of each animal.

Blood samples were obtained for biochemical assessment of haemoglobin (Hb), serum iron and percentage transferrin saturation (TSAT) after each IV iron dose at baseline (after 24

hours), and every 7 days for 4 weeks. Urine was also collected for 24 hours every 7 days. Rats were sacrificed on day 28 by subtotal exanguination under anesthesia (sodium thiopental 40 mg/kg intraperitoneal) according to institutional guidelines for animal care and use. The liver, heart and kidneys of each rat were perfused with ice cold saline solution through the abdominal aorta until they were free of blood and then removed for oxidative stress evaluation, microscopy and immunohistochemical study.

Blood pressure: Systolic blood pressure was measured by tail cuff plethysmography every 7 days for 4 weeks using pneumatic pulse transducer. Three measurements were taken and average of three readings was calculated.

Blood Hemoglobin, Serum iron and percentage transferrin saturation (TSAT) determination: 24 hours prior to first dose and after 24 hours of each dosing the 0.5 ml blood sample was collected for measurement of serum iron, serum Hb and percentage transferrin saturation determination.

Hemoglobin measurement: Hemoglobin content was measured using Sahli's technique. Twenty micro litre of blood was placed in a calibrated tube containing 1.0 ml 0.1N HCl. After thorough stirring the tube was placed in hemoglobinometer having comparator colour glasses on both side of tube. Drop by drop distilled water was added with stirring until the tube solution colour and the comparator glass colour exactly matches. The Hb gm% was read on the tube and recorded.

Serum iron and percentage transferrin saturation (TSAT) determination: TSAT, serum iron and Total iron binding capacity were determined using the procedure described below. TSAT was calculated using the following formula:

$$\text{TSAT} = \text{Serum Fe}/\text{Serum TIBC} \times 100\%$$

Serum iron determination: To 0.1 ml of serum 0.1 ml of 0.1 M sodium sulphite and 0.1 ml of dipyridyl reagent (2,2'- Dipyridyl 0.1% in 3% v/v acetic acid) was mixed in a tube with ground glass neck. The mixture was then heated in a boiling water bath for 5 min and then cooled. One ml. of Chloroform was added and the tube was stoppered and shaken violently for 30 sec. The tube was then centrifuged at 3000 rpm for 5 min. The optical density of the supernatant was measured at 520 nm.

Total Iron Binding Capacity (TIBC): To 0.1 ml of serum 0.2 ml of Ferric Chloride solution was added (5 µg Fe/ml in N/200 HCl). After 5 min 20 mg $MgCO_3$ was added. The mixture was agitated frequently and thoroughly during next 30 min and centrifuged at 3000 rpm for 5 min. The supernatant was separated in a dry test tube and 0.05 ml of sodium sulphite (0.2M) and 0.05 ml dipyridyl reagent (0.2% in 6%v/v acetic acid) was added. The tube was tightly stoppered and heated in boiling water bath for 5 min and then cooled. 1 ml. of chloroform was added and shaken violently for 30 sec. The tube was then centrifuged at 3000rpm for 5 min. The optical density was measured at 520 nm.

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Creatinine clearance and proteinuria: Every 7 days the urine samples were collected for 24 hours and urine creatinine was determined. Using this data Creatinine clearance was calculated. Urine creatinine, plasma creatinine and urine protein content was determined using Creatinine diagnostic kit (Span Diagnostics) and Protein determination kit (Span Diagnostics). Creatinine Clearance was determined using following formula:

$$C_{Cr} = \frac{U_{Cr} \times \text{24-hr volume}}{P_{Cr} \times 24 \times 60}$$

C_{cr} = Creatinine clearance, U_{cr} = urine Creatinine, P_{cr} = Plasma creatinine

Serum AST, ALP and ALT determination: Serum AST, ALP and ALT was determined 24 hours prior to dose administration and then on the 28th day using standard diagnostic kits(Span Diagnostics) by spectrophotometric methods.

Oxidative stress parameters in heart, liver and kidney (Malondialdehyde, Catalase activity, CuZn SOD activity, GSH peroxidase activity, GSH: GSSG activity)

a) **Isolation of Tissues:** After 28 days of treatment (total 5 doses) after collection of the blood sample the animals were sacrificed. The abdomen was opened and abdominal aorta was cannulated and perfused with ice cold saline to make heart liver and kidney free from blood. Later these organs were isolate and used for oxidative stress evaluation, microscopy and immunohistochemical study.
b) **Determination of oxidative stress parameters: (Evaluation of oxidative stress parameters in liver, heart and kidney)**

i) **Preparation of Samples:** Samples of the liver, heart and kidney was homogenised (1:3, w:v) in ice-cold 0.25 M sucrosesolution. Glutathione (GSH) levels were determined in the 10,000 ×g supernatant. Further samples of the corresponding perfused tissues was homogenised (1:10, w:v) in 0.05 M sodium phosphate buffer solution (pH 7.4) and used for the determination of malondialdehyde to evaluate lipoperoxidation by thiobarbituric reactive species (TBARS). The remaining homogenate was centrifuged at 4°C for 15 min at 9,500 ×g and the supernatant was used to measure catalase activity. Finally, the remaining tissue samples was homogenised (1:3, w/v) in ice-cold sucrose solution (0.25 M). The supernatant obtained after centrifugation at 105,000 ×g for 90 min was used to measure CuZnsuperoxide dismutase (CuZnSOD), GSH peroxidase(GPx) activity and the ratio of reduced to oxidized glutathione (GSH:GSSG). Enzyme units (U) were defined as the amount of enzyme producing 1 nmol of product or consuming 1 nmol of substrate under the standard incubation conditions. Specific activity (Sp Act) was expressed as U/mg protein. One unit of CuZnSOD was defined as the amount of CuZnSOD capable of inhibiting the rate of NADH oxidation measured in the control by 50%.

ii) **Malondialdehyde measurement:** One ml sample was mixed with 0.2 ml 4% *(w/v)* sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 1.5 ml 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85°C for 1 hr. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The amount of malondialdehyde (TBA reactive material) was calculated using molar extinction -coefficient 1.56X $10^{-5}M^{-1}cm^{-1}$ and was reported as nmoles of *MDA/mg* protein.

iii) **Catalase activity:** Decomposition of H_20_2 in presence of catalase was followed at 240 nm A 50 µl sample was added to buffered substrate (50 mM phosphate buffer, pH 7.0 containing 10 mM H_20_2) to make total volume 3 ml and decrease in the absorbance was monitored at 37°C for 2.5 min at an interval of 15 sec. The catalase activity was calculated using extinction coefficient of H_20_2 , 0.041 /µ mole/cm^2 at 240 nm and results was expressed as µmoles of H_20_2utilized/min/mg protein.

iv) **CuZnsuperoxide dismutase (CuZnSOD) measurement:** SOD was estimated by following procedure: Assay mixture contained sodium pyrophosphate buffer (0 .052 M, pH 8 .3), Phenazine methosulfate (PMS, 6 .2 M), Nitroblue tetrazolium (NBT, 30 M), Potassium cyanide (KCN 10 µM*,* pH 7.0) and 0 .2 ml samples. Samples were preincubated for 5 min at 36°C prior to the addition of reduced nicotinamide adenine dinucleotide (NADH, 52 µM*).* Mixture was further incubated for 120 sec at 37° in a water bath and the reaction was stopped by adding 1 ml glacial acetic acid (17.4 M). The violet colour developed was extracted in 4 .0 ml of n-butanol and absorbance of the butanol layer was measured at 560 nm against reagent blank. Potassium cyanide 10 µM was added to inhibit mitochondrial SOD. Superoxide dismutase inhibits chromogen formation due to the NADH mediated PMS depended reduction of NBT and its activity was expressed in terms of *U/min/mg* protein. One unit of enzyme activity is defined as the enzyme concentration required to inhibit the chromogen production by 50% in one min under the defined assay conditions.

v) **GSH peroxidase (GPx) activity:** This assay is based on the oxidation of reduced glutathione by glutathione peroxidase coupled to oxidation of NADPH by glutathione reductase. The rate of NADPH oxidation was monitored photometrically. The one ml of reaction mixture will contain 50 mM phosphate buffer (pH 7.2), 1 mM EDTA, 1 mM NaN_3), 0.2 mM NADPH, 1mM GSH, 0.24 U glutathione reductase 0 .25 mM $H_20_2$and 25 µL sample (1:200 dilution) was added to initiate the reaction . Change in the absorbance at 37°C was recorded at 340 nm at 15 sec. and interval for 2.5 min. The enzyme activities were calculated using extinction coefficient of NADPH 6.22x $10^6M^{-1}cm^{-1}$. Results were expressed as µmole of NADPH oxidised *I* min/mg protein.

vi) **Measurement of Ratio of Reduced and oxidized Glutathione (GSH/GSSG ratio):** GSH contents in tissue homogenates was measured after precipitation of protein

with 10% *(w/v)* chilled trichloroacetic acid. Samples was kept in ice bath and was centrifuged after 30 min at 1000g for 10 min at 4°C. GSH levels were measured in the supernatant. Supernatant (0.5 ml) was mixed with 2.0 ml 0.3 M disodium hydrogen phosphate solution and 0.3 M disodium hydrogen phosphate solution and0.25 ml 5,5'-dithiobis-2-nitrobenzoic acid (40 mgll00ml in 1% *(w/v)*sodium citrate] was added just before measuring the absorbance at 412nm. Different concentrations of GSH will also be processed similarly to prepare a standard curve (5-25 µg) simultaneously .Results was expressed as µmole of GSH/g tissue.

vii) **Oxidised Glutathione measurement:** To 200 µL 1-methyl-2-vinylpyridinium trifluoromethane sulfonate (M2VP) in a micro centrifuge tube, 100 µL sample was added to the bottom of the centrifuge tube and mixed gently. To it 200 µL cold 5% MPA (50 µL Metaphosphoric acid in 700µL buffer) was added. The tube will then be vortexed for 15-20 seconds and centrifuged at 1000 x *g* or greater for 10 minutes. To 200µL of supernatant in cuvette 200 µL of 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) (40 mgll00 ml in 1% *(w/v)* sodium citrate was added. To this mixture 200 µL of Glutathione reductase was added. Later to this 200 µL of NADPH was added and change in absorbance at 412 nm for 3 min was recorded.

GSSG (Oxidised Glutathione) concentration was determined by following equation

$$GSSG = \frac{\text{Change in absorbance/ min x 30}}{0.1475}$$

GSH/GSSG ratio was determined by following formula:

$$\text{Ratio} = \frac{GSH - 2(GSSG)}{GSSG}$$

Light microscopy and immuno histochemical study: Heart, Liver and Kidney were collected from all thirty animals were fixed in 10% Phosphate buffered formaldehyde(pH 7.2) and embedded in paraffin. Three micron sections were cut and processed for immuno histochemical study. The sections were immuno-labelled using a modified avidin-biotin-peroxidase technique (Vectastain ABC kit, Universal Elite, Vector Laboratories, CA). The sections were washed in phosphate buffer saline (PBS) for 5 min following deparaffinization and rehydration and then incubated for 30 min in 1% hydrogen peroxide in methanol to quench endogenous peroxidase activity. After washing in PBS (pH 7.2) for 20 min, the sections were incubated with blocking serum for a further 20 min. Thereafter, the sections were rinsed in PBS and incubated with Biotynilated Universal Antibody for 30 min. After washing in PBS a final time, the sections were incubated for 40 min with Vectastain Elite ABC reagent (Vector Laboratories, CA) and exposed for 5 min to 0.1% diaminobenzydine and 0.2% hydrogen peroxide in 50 mM Tris buffer (pH 8). Tissue ferritin was quantified using anti-ferritin monoclonal antibody. Pro-inflammatory markers were quantified using monoclonal antibodies against rat tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) at dilutions of 1:50 and 1:100

respectively using PBS as diluting agent. Using the image analyzer software at 100 X and 400X magnification, immunostained area were measured for ferritin, TNFα and IL-6.

Statistical Analysis:

All the values obtained in the study were expressed as mean ± SD. For parameters with a Gaussian distribution, comparisons among groups was performed using analysis of variance (ANOVA). A value of $p<0.05$ was considered significant.

RESULTS

Chemical analysis revealed that Sucrofer fully complies with the specifications of the Venofer as well as the US Pharmacopeia (USP) Monograph for iron sucrose (see Table I).

Table 1: Chemical analysis of Sucrofer samples by the quality control laboratory of Claris Life Sciences INDIA (Lot. A070704, A090630 and 9017005) compared with the Venofer® iron sucrose injection])

Parameter	USP specifications	Claris iron sucrose	Innovator/Originator
Characteristics	Dark brown, opaque aqueous Solution	Complies	Complies
pH	10.5-11.1	10,85	10.98
Titrable alkalinity	0.5-0.8	0.60	0.70
Turbidity Point	4.4-5.3	4.62	4.92
Mw(Da)	49997	49997	46097
Mn(Da)	34,000-60,000	32755	31214
P(Mw/Mn)	≥1.2	1.50	1.40
Kinetic Degradation T_{75}		10.70 min	9.0 min

Blood Pressure: The systolic blood pressure recorded every week did not show significant change during the five weeks of observation in either of test, control or standard group animals.(Table. 2)

Table 2: Systolic blood pressure recordings of rat treated with Claris sucrofer and Originator's Iron sucrose preparation.

Groups	Day0	Day7	Day14	Day21	Day28
	Systolic B.P. mm/Hg				
Control	118.8±36.35	120.1±34.7	121.9±33.1	121.6±30.99	117.5±27.24
Claris Sucrofer	113±5.37	118.4±8.75	119.7±7.76	116.7±8.03	118.5±6.08
Originator	117.4±7.93	118.2±8.63	116.5±7.63	118.8±6.01	116.7±8.14

The data are represented as mean ± SD of 10 observations

The differences in the blood pressure recordings of test and standard group animals compared to that observed with control group animals were not significantly different.

Blood Hemoglobin, Serum iron and percentage transferrin saturation (TSAT)

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i) **Blood Hemoglobin content:** The amount of hemoglobin(Hb) in the blood was recorded every week for 5 weeks in animals treated with either the vehicle or iron sucrose preparations(Claris sucrofer or Originator's preparation). The hemoglobin content was not significantly altered in any of the group of animals. However, a small rise in hemoglobin content was observed in both test and standard drug treated animals.(See table 3)

Table 3: Hemoglobin levels in rats treated with Claris sucrofer and Originator's Iron sucrose preparation.

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Hb(g/dl)					
Control	13.62±1.462	13.12±1.711	13.23±1.221	13.14±1.726	13.53±1.852
Claris Sucrofer	13.19±0.621	13.32±0.932	13.37±1.176	13.89±0.78	14.01±1.126
Originator	12.81±1.041	12.67±1.072	13.13±1.194	13.53±1.114	13.75±1.183

The data is represented as mean ± SD of 10 observations

ii) **Serum Iron:** Serum Iron concentration was significantly increased in both standard and test group as compared to control and remained higher for all five weeks.(See Table 4)

Table 4: Effect of iron sucrose treatment on serum iron content in rats for 5 weeks of treatment with iron sucrose preparations (Claris sucrofer and originator's).

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Serum Iron (µg/dl)					
Control	375±119.717	326.7±106.595	348.9±111.102	342.9±105.422	324±94.831
Claris Sucrofer	442.7±60.037	480.48±83.581	490.99±64.666	439.1±68.971	470.49±46.65
Originator	477.28±48.901	448.89±66.781	480.32±84.722	531.73±77.784	450.55±84.005

Each value represent mean ± SD of 10 observations

iii) **Serum % Transferrin saturation (TSAT):** Serum % transferrin saturation was significantly elevated in both groups treated with iron sucrose injections. However, there was no significant difference between standard and teat groups. (See table 5)

Table 5: Effect of iron sucrose treatment on serum % transferrin saturation(TSAT) in rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
	%TSAT				
Control	40.812±3.982	41.68±1.59	46.6±4.041	43.92±4.207	46.4±3.951

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Claris Sucrofer	41.268±3.72	48.006±5.469	51.934±3.771	52.032±3.088	55.6±5.109
Originator	43.928±3.543	47.628±3.587	52.302±5.413	51.744±4.386	57.65±3.895

Each value represents mean ± SD of 10 observations

iv) **Creatinine clearance and proteinuria:** Creatinine clearance was not significantly altered in either of claris's sucrofer treated animals or originator's preparation treated animals when compared with control group animals.(See table 6)

Table 6: Creatinine clearance in rats treated with Claris sucrofer and Originator's Iron sucrose preparation.

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
	Creatinine Clearance ml/min				
Control	3.9±0.55	3.976±0.507	4.124±0.424	3.788±0.404	3.996±0.532
Claris Sucrofer	3.6112±0.44	3.7629±0.37	3.7333±0.493	3.7999±0.433	3.9072±0.454
Originator	3.577±0.614	3.724±0.348	3.798±0.664	3.8±0.381	3.907±0.59

The data is represented as mean ± SD of 10 observations

v) **Proteinuria:** Protein excretion in urine was not significantly affected by either of test or standard drug treatment.(See table 7)

Table 7: Effect of iron sucrose treatment on protein excretion in urine

	Protein excretion in urine per day (mg/day)				
Days→	**0**	**7**	**14**	**21**	**28**
Test	**1.8536±0.6429**	**4.4769±1.5442**	**4.6891±2.0117**	**4.6175±1.6049**	**4.2353±1.5384**
Originator	**1.9755±1.067**	**4.0723±2.7892**	**4.6149±1.4021**	**3.6226±1.2241**	**4.4437±1.1877**
Control	**1.9719±1.0424**	**2.5077±1.936**	**4.3733±1.2975**	**3.0315±1.7122**	**4.6673±1.4219**

Each value in the table represents mean ±SD of 10 observations

vi) **Serum AST, ALP and ALT determination:** Serum AST, ALP and ALT enzyme levels were not significantly altered by iron sucrose treatment in either of standard or test group of animals when compared with control group of animals. (See table 8)

Table 8: Effect of iron sucrose administration on serum AST,ALT and ALP levels:

	AST		**ALT**		**ALP**	
DAYS→	**0**	**28**	**0**	**28**	**0**	**28**
Control	**152.85±10.31**	**153.15±11.06**	**92.925±12.1**	**84.375±13.46**	**479.82±84.13**	**565.598±69.82**
Claris Sucrofer	**158.25±9.73**	**166.83±15.9**	**87.45±12.55**	**94.945±11.13**	**489.427±94.15**	**535.513±65.1**
Originator	**153.45±13.86**	**165.3±14.49**	**87.225±9.46**	**108.68±13.52**	**481.308±64.18**	**542.624±62.78**

Each value in the table represents mean ± SD of 10 observation

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Oxidative stress parameters in heart, liver and kidney (Malondialdehyde, Catalase activity, CuZn SOD activity, GSH peroxidase activity, GSH: GSSG activity)

i) Malondialdehyde (TBARS):

The malondialdehyde content of the liver, heart and kidney after five once weekly dosing of iron sucrose preparations were obtained as described in the table 9 shown below:

Table 9: Malondiladehyde content of rat liver, heart and kidney after iron sucrose treatment:

	Liver	Heart	Kidney
	nmols of MDA/mg protein		
Standard	69.18±7.63	59.35±6.29	74.9±7.82
Test	70.86±7.85	58.08±7.14	77.07±8.12
Control	71.16±7.12	63.86±7.99	76.09±7.15

The results are expressed as mean of 10 observations ± Standard deviation.

The malondialdehyde content in all these tissues obtained from animals subjected to test drug was not significantly different from that obtained from animals subjected to either vehicle treatment or standard iron sucrose preparation.

ii) Catalase activity:

The catalase activity of the liver, heart and kidney after five once weekly dosing of iron sucrose preparations were obtained as described in the table and graph shown below:

Table 10: Catalase activity of rat liver, heart and kidney after iron sucrose treatment:

	Liver	Heart	Kidney
	U/mg protein		
Standard	287.56±32.04	61.22±6.49	229.32±29.08
Test	287.04±27.93	60.12±6.68	230.58±25.33
Control	261.12±30.14	55.5±4.26	219.4±27.26

The results are expressed as mean of 10 observations ± Standard deviation.

The catalase activity in all these tissues obtained from animals subjected to test drug was not significantly different from that obtained from animals subjected to either vehicle treatment or standard iron sucrose preparation.

iii) Cu/Zn SOD activity:

The Cu/Zn SOD activity of the liver, heart and kidney after five once weekly dosing of iron sucrose preparations were obtained as described in the table 11.

Table 11: Cu/Zn SOD activity of rat liver, heart and kidney after iron sucrose treatment:

	Liver	Heart	Kidney
	U/mg protein		
Standard	9.78±0.6	14.94±0.91	7.96±0.48
Test	9.89±0.69	15.05±0.87	8.13±0.51
Control	9.01±0.56	13.18±0.84	7.06±0.4

The results are expressed as mean of 10 observations ± Standard deviation.

The Cu/Zn SOD activity in all these tissues obtained from animals subjected to test drug was not significantly different from that obtained from animals subjected to either vehicle treatment or standard iron sucrose preparation.

iv) Glutathione peroxidase activity:

The Glutathione peroxidase activity of the liver, heart and kidney after five once weekly dosing of iron sucrose preparations were obtained as described in the table below:

Table 12 Glutathione peroxidase activity of rat liver, heart and kidney after iron sucrose treatment:

	Liver	Heart	Kidney
	U/mg protein		
Standard	236.04±25.61	209.79±25.73	128.62±10.61
Test	233.1±17.79	200.54±21.06	126.06±14.69
Control	224.07±26.21	202.58±21.21	122.76±14.98

The results are expressed as mean of 10 observations ± Standard deviation.

The Glutathione peroxidase activity in all these tissues obtained from animals subjected to test drug was not significantly different from that obtained from animals subjected to either vehicle treatment or standard iron sucrose preparation.

v) GSH/GSSG ratio:

The GSH/GSSG ratio of the liver, heart and kidney after five once weekly dosing of iron sucrose preparations were obtained as described in the table 13:

Table 13: GSH/GSSG ratio of rat liver, heart and kidney after iron sucrose treatment:

	Liver	Heart	Kidney
Standard	5.01±0.21	6.04±0.33	4.89±0.33
Test	5.1±0.31	6.12±0.32	4.97±0.17
Control	4.94±0.31	5.98±0.35	5.07±0.33

The results are expressed as mean of 10 observations ± Standard deviation.

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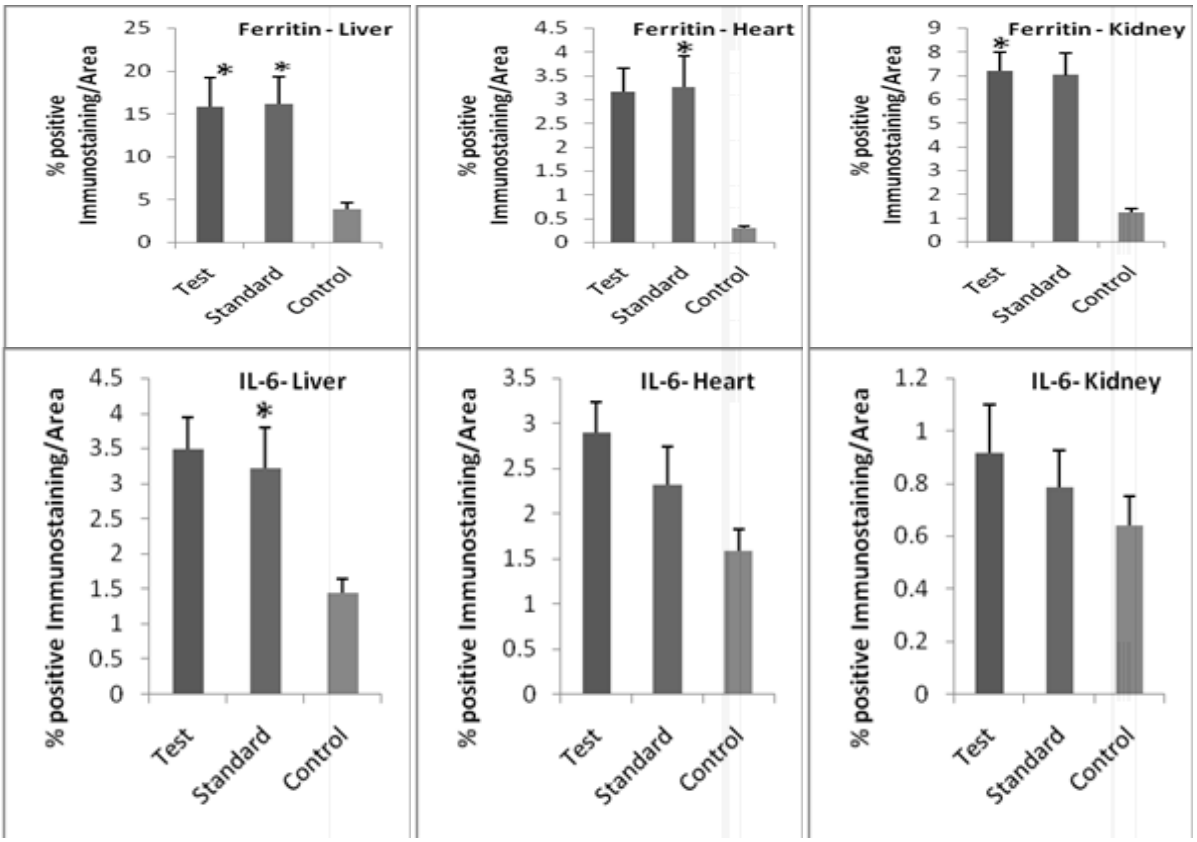
The GSH/GSSG ratio in all these tissues obtained from animals subjected to test drug was not significantly different from that obtained from animals subjected to either vehicle treatment or standard iron sucrose preparation.

Light microscopy and immuno histochemical and morphometric study:

Immunohistochemical studies revealed that Iron sucrose treatment caused a significant increase in deposition of ferritin in heart, liver and kidney. Also a small elevation in the levels of TNF alpha and IL-6 in these tissues was observed. This elevation was small but significant and found in both standard and test group animals. There was no significant difference in serum ferritin deposition, TNF alpha and IL-6 levels between test and standard group. (See figure 1)

Figure 1: Effect of iron sucrose administration on ferritin deposition and levels of inflammatory markers TNFα and IL-6 in heart, liver and kidney described as % positive immunostaining in unit area using specific antibodies to each of them. Each bar represents mean ± SD of 10 observations.



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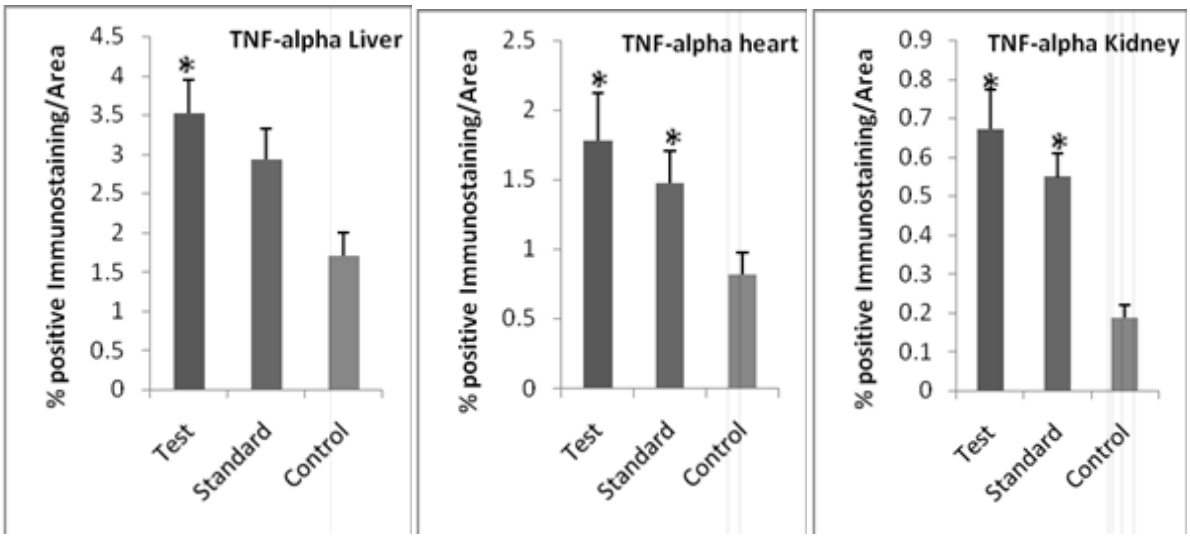

DISCUSSION

The study was conducted with an objective to compare efficacy and safety of test (Claris sucrofer) and standard iron sucrose preparation (Venofer). Both the preparations passed the Pharmacopoeial requirements. However, the safety and efficacy profile is dependent on many other factors, which needs to be assessed. The efficacy study included the effect on hemoglobin levels, serum iron levels, % transferrin saturation and serum ferritin deposition in heart, liver and kidney. The safety parameters included effect on systolic blood pressure, oxidative stress parameters, serum ALT,AST and ALP levels, Creatinine clearance, proteinuria and finally levels of TNF alpha and IL-6 in heart, liver and kidney. The observations obtained from the study suggest that both of the formulations caused a significant increase in serum iron and %TSAT levels .Also ferritin deposition in heart, liver and kidney were significantly elevated. Thus both of these preparations are effective equally in producing their desirable effects.

With respect to safety parameters the systolic blood pressure, creatinine clearance and serum levels of AST,ALT and ALP were not significantly affected by both the formulations. Also protein content of urine was not significantly affected. Iron sucrose is known to produce proteinuria(6,7). This is associated with free iron induced oxidative damage. Both Venofer and Sucrofer did not produce proteinuria suggesting a stable complex as desired for safe therapy. This suggests both of these formulations do not produce any significant adverse effect on heart liver and kidney. In addition the oxidative stress parameters were not significantly altered. Though small increase in glutathione peroxidase and catalase activity were observed the overall oxidative stress was not increased as observed from GSH/GSSG ratio. GSH(reduced glutathione) scavenges free radicals generated due to oxidative stress and in turn it gets converted to oxidised glutathione(GSSG). In case of oxidative stress more of reduced glutathione gets converted to oxidised glutathione and thus the ratio of GSH/GSSG decreases(8,9). In the present study, the ratio was not significantly altered suggesting the oxidative stress, if at all, was not that significant to affect clinically. Also IL-6 and TNF alpha levels were higher in liver

heart and kidney obtained from iron sucrose treated animals, suggesting that both the iron preparations have potential to induce small amount of inflammation. However, both the formulations were alike in this regard. The levels of these inflammatory markers were bit higher in test group animals but this difference was not statistically significant. Small increase in inflammatory markers is not likely to induce clinical safety issues.

During the entire study, the general conditions such as body weight, food, intake, water intake general behavior etc. remained normal in all groups of animals. There was no mortality observed in any of the groups.

CONCLUSION

The study reveals that both the formulations are similar with respect to their efficacy and safety. There is no observable difference between the two formulations as far as this study is concerned. The oxidative stress and resultant damage to various target organs was minimal with both the formulation. Thus, the test formulation Sucrofer is as effective and safe as the reference product Venofer in rats.

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