

Effect of different doses of ferrous sulfate drug on some haematological parameters in male albino rat

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Abstract:

The study was designed to investigate the effect of different doses and different durations of ferrous sulfate drug administration on some blood criteria, using 48 males of albino rats. The animals were divided into four main groups (12 males for each group), the first group control and the other three groups were orally intragastric administrated with (50,75,100) mg/kg bw for three periods (4,6,8) weeks. The blood samples were collected to measure the haematological criteria that include Red Blood Cells count (RBCs), blood haemoglobine (Hb), Packed Cell Volume (PCV), Mean Corpuscle Haemoglobine Concentration (MCHC), Mean Corpuscular Haemoglobine (MCH) and Mean Corpuscle Volume (MCV).

Results of this study revealed, that ferrous sulfate administration causes a significant elevation for all haematological parameters which proportionally increased with the dosage levels and dosage durations. It was also observed a significant effect of interaction between different doses and periods on most haematological parameters except MCH and MCHC. All results compared with control group and between treated groups.

The results suggested that the administration of ferrous sulfate drug in high dosing level and long durations may causes defect in haemopoiesis especially erythropoiesis that indicated by increased blood parameters level rather than treatment of iron deficiency anemia because of iron supply increase.

Introduction

Iron is a nutrient that is related to health and immunity (Heidarpouret *et al.*, 2008). It is the most common element on earth, unfortunately iron is chemically unstable and easily oxidized into an insoluble ferric form, ferric iron is unavailable in most biological systems (Mohriet *et al.*, 2006). Iron is an essential component of haemoglobin, myoglobin and several enzymes such as catalase, peroxidase, cytochrome oxidase and ribinucleotide reductase (Harvey, 2000). Iron of organic (heme) or inorganic (non-heme) origin is a physiologically important element that plays vital role in erythropoiesis, oxygen transport, oxidative energy production and mitochondrial respiration (Coplin *et al.*, 2000).

Iron deficiency anemia (IDA) is the most prevalent nutritional deficiency worldwide and it is often associated with trace iron change, it is a major public health affects over two billion people (WHO/UNICEF/UNU, 2001). The main cause of iron deficiency is the low iron bioavailability of the diet that's lead to depletion of iron in haemoglobin and reduction in the numbers of Red Blood Cells (RBCs), the consequences of iron deficiency are many and serious, affecting not only individuals health but also the development of societies and countries (Yip, 1994). The main treatment of iron deficiency anemia include treatment with ferrous iron as ferrous sulfate (FeSO₄) which is much better absorbed than ferric iron e.g. ferric citrate (Davidsson *et al.*, 2000). But, numerous studies concluded that oral administration of FeSO₄ for long durations may be lead to defect in haemopoiesis provided by an increase in haematological parameters in human (Frykman *et al.*, 1994; Conrad, 2006) and animal (Gygax *et al.*, 1993; Kume and Tanabe, 1996;

Bosted *et al.*,2000). Therefore , this study designed to clarify the effect of different doses and periods of oral administration of FeSo₄ on some haematological parameters in albino rat.

Materials and Methods

Animals:

Forty eight healthy adult males of albino rat weighing (193±11) g and approximately age (10-11) weeks, maintained in the animal house of College of Veterinary Medicine / Kufa University were used in the present study. Water was supplied *ad libitum*. They were fed a normal commercial stock diet which contained 35%wheat grains, 35% corn grains, 18% Soya, 10% protein and 2% minerals and vitamins. The animals were housed under a 12h:12h light/dark cycle and maintained in controlled temperature (25±2°C).

Experimental design:

These forty eight males of albino rat were divided in four main groups, with 12 animals in each one , and then each of these groups were divided into three groups (4 males of each). The first group served as a control and received tap water, other three groups were exposed for administration of (50 , 75 and 100) mg ferrous sulfate (FeSo₄) / kg bw by orally intragastric intubations for (4 , 6 , 8) weeks . At the end of each three administration periods sacrifice (4 rats) for each four subdivided groups and used to collection of samples.

Collection of samples:

5 ml of blood was collected from each rat by cardiac puncture using sterile disposable syringe and put in a test tube containing ethylene-diamine-tetraacetic acid (EDTA) and used for haematological examinations.

Haematological examinations:

All haematological examinations were performed in the haematology center of Al-Sader teaching hospital in Al-Najaf province. Hemoglobin was measured by the cyanomethaemoglobin method using Randox kits, Randox: Laboratories, USA (Dacie and Lewis, 1975). Hematocrite was measured by centrifugation of blood collected into heparinized microcapillary tubes no. 563 supplied by Bio Merieux and calculate the percentage of PCV by particular ruler (Hillman and Ault, 2002). Red blood cells count (RBCs) was counted manually (Monica, 2004). Mean cell hemoglobin concentration (MCHC) was calculated using the equation: $MCHC = [(Hb \times 100) / PCV]$. Mean cell hemoglobin (MCH) was calculated using the equation: $MCH = [(Hb \times 10) / RBC]$. Mean red cell volume (MCV) was calculated using the equation: $MCV = [(PCV \times 10) / RBC]$ (Hillman and Ault, 2002).

Statistical analysis

All results were expressed as the mean ±SD. Statistical analysis was performed with statistical package for the social science for windows (SPSS, version 10). One-way ANOVA was used to find the effect of ferrous sulfate according to the dose and period of treatment on measured haematological parameters .Also one-way ANOVA use to find the interaction between the dose and period .Differences between observations were considered significant at $P < 0.05$.

Results:

Effect of different doses of ferrous sulfate administration on haematological parameters in albino rats:

Table (1) showed that RBCs levels were increased significantly ($p < 0.0001$) in G1, G2, and G3 groups when compared with the control group, also RBCs increased significantly ($p < 0.01$, $p < 0.05$) in G3 when compared with G1 and G2 respectively. This table also indicates that Hb levels was increased significantly ($p < 0.0001$) in G1, G2, and G3 groups when compared with the control, In contrast, Hb level increased significantly ($p < 0.0001$, $p < 0.005$) in G3 when compared with G1 and G2 respectively. In respect to PCV, the result pointed out significant increase ($p < 0.0001$) in G1, G2, and G3 groups when compared with the control, whereas increased significantly ($p < 0.01$, $p < 0.0001$) in G2 and G3 respectively when compared with G1, and ($p < 0.005$) in G3 when compared with G2.

This table also showed that MCH and MCHC levels elicited a significant increase ($p < 0.001$) in G3 when compared with C, while don't obvious any significant changes in other groups. In respect to MCV, the results demonstrated significant increase ($p < 0.0001$) in G2 and G3 when compared with control, whereas increased significantly ($p < 0.001$) in these groups when compared with G1.

Effect of different durations of ferrous sulfate administration on haematological parameters in albino rats :

In this approach of analysis, the effect of dosing level is neglected and the data was collected taking in our consideration the result of administration periods of the 1ST (G1), 2nd (G2), and 3rd (G3) that mean 4, 6, 8 weeks. Table (2) showed that RBCs and Hb levels was increased significantly ($p < 0.0001$, $p < 0.005$) in G3 when compared with G1 and G2 respectively. In contrast PCV levels increased significantly ($p < 0.001$, $p < 0.0001$) in G2 and G3 when compared with G1, also increased significantly ($p < 0.001$) in G3 when compared with G2.

This table also indicated that were no significant increase in MCH and MCHC levels when compared treated groups, while MCV levels elicited a significant increase ($p < 0.05$) in G2 and G3 when compared with G1.

Effect of interaction between doses and durations on haematological parameters:

The results were demonstrated a significant effect ($p < 0.0001$) for the interaction between doses and durations on RBCs, Hb and PCV levels, that mean when increased doses and durations increase these parameters. On the other hand there were a significant increase ($p < 0.001$) for the interaction between doses and durations on MCV levels. While in MCH and MCHC don't obvious clearly the effect of interaction on these parameters levels.

Table 1: Effect of different doses of ferrous sulfate administration on haematological parameters in albino rats:

Parameters	Group	FeSo4 (mg/kg)	SD	± Mean
RBCs (x10 ⁶ /μl)	C	0	0.14	±8.03
	G1	50	0.69 a	±9.12
	G2	75	0.70 a	± 9.20
	G3	100	0.86 abc	±9.55
Hb (g/dl)	C	0	0.27	±8.57
	G1	50	0.41 a	±9.80
	G2	75	0.65 a	± 10.08
	G3	100	0.82 abc	± 10.73
PCV (%)	C	0	0.56 ±	26.21
	G1	50	1.53 a	± 30.29
	G2	75	1.35 ab	± 31.43
	G3	100	1.86 abc	± 32.74
MCH (Pg)	C	0	0.23	±10.67
	G1	50	0.44 ±	10.74
	G2	75	0.39	±10.95
	G3	100	0.21 a	± 11.23
MCV (fl)	C	0	0.66±	32.64
	G1	50	0.61±	33.21
	G2	75	0.72 ab	± 34.16
	G3	100	0.98 ab	± 34.28
MCHC (%)	C	0	0.72	±31.93
	G1	50	0.91	±32.35
	G2	75	1.08 ±	32.07
	G3	100	1.16 a	± 32.77

C: control, G1 G3: treated groups.

a: means there are a significant difference between treated groups and control group.

b: means there are a significant differences between (G2 ,G3) and G1.

c: means there are a significant differences between G3 and G2. number of animals:12/group.

Table 2: Effect of different durations of ferrous sulfate administration on haematological parameters in albino rats:

Parameters	Group	Duration (weeks)	SD ± Mean
RBCs (x10 ⁶ /μl)	G1	4	0.29 ±8.42
	G2	6	0.63 ±8.89
	G3	8	0.57 ^{ab} ±9.56
Hb (g/dl)	G1	4	0.38 ±9.13
	G2	6	0.65 ±9.85
	G3	8	1.07 ^{ab} ± 10.63
PCV (%)	G1	4	1.32 ±28.54
	G2	6	2.00 ^a ±30.63
	G3	8	2.23 ^{ab} ±32.98
MCH (Pg)	G1	4	0.41±10.84
	G2	6	0.55±11.07
	G3	8	0.30±11.11
MCV (fl)	G1	4	0.78 ±33.89
	G2	6	1.13 ^a ±34.45
	G3	8	1.29 ^a ±34.49
MCHC (%)	G1	4	1.04±31.99
	G2	6	1.11±32.15
	G3	8	0.95±32.23

a: means there are a significant differences between (G2 ,G3) and G1.

b: means there are a significant differences between G3and G2.

number of animals:16/group.

Discussion:

Effect of different doses and durations of ferrous sulfate administration on some haematological parameters:

Iron an essential component of a number of protein involved in oxygen transport and utilization, one of these proteins is haemoglobin (Atyabi *et al.*,2006). Thus, iron supply necessary for production of RBCs by erythropoiesis process (Heidarpour *et al.*, 2008). RBC parameters are used most commonly to monitor erythropoiesis, these parameters include RBCs count, Hb, PCV, MCHC, MCH and MCV (Miyata *et al.*,1994) .

Regarding the effect of the dosing level of FeSo₄ administration on RBCs parameters, FeSo₄ elicited a significantly increases in RBCs count , Hb concentration , PCV, MCHC, MCH and MCV in treated groups when compared with contral and between it , these increases were eminent when the dosing level elevated, hence the variation being significant in those groups of animals administered higher levels of FeSo₄. These results were in agreement with previous studies concluded that iron administration as FeSo₄ provided an increase in RBCs parameters in rats (Wenzlf and Erhardt ,1991 ;Rajora *et al.*, 1995 ; Kume and Tanabe 1996; Bosted *et al.*,2000; Mohri *et al.*,2004; Heidarpour *et al.*, 2008). Similar results have been also reported by several studies Mehrdad *et al.*, (2004) who utilized forty male rats were given as120 mg/daily for 45 days. Adel *et al.*, (2010) who use twenty rats treated with 150 and 250 mgFeSo₄/day for 28 days. Other findings use more than one type of orally iron administration were consistent with our results such as Jahan *et al.*, (2007)that use twenty four male mice to study haematological changes following administering of different haematinics 60mg Cuso₄/mice/day and 120mg FeSo₄ /mice/day for one month, and Soliman *et al.*, (2010) that participatedforty male rats ,some of one treated with 40mgFeSo₄/kg bw for 6 weeks and others administrated with 40mg chelating amino acid /kg bw for the same period . Also they founded FeSo₄ more effective than other types of iron therapy .

The analysis of the results of the durations dependency of RBCs parameters revealed approximately comparable data of those obtained for the dosing level. RBCs count and Hb level were significantly increased in the groups of animals treated for 8 weeks(G3) relative to those of 4 weeks(G1) and 6weeks group (G2). Such increased was also demonstrated for MCH and MCHC but the variation was insignificant. PCV was significantly increased in all durations and MCV was raised in G2 and G3 groups in comparison with G1 animals. It seemed that the duration of administration plays a prominent role in directing the RBCs parameters variation in the treated rats. Similar findings were reported by other researches (Baustad and Tollersrud ,1996 ; Gyax *et al.*, 1993; Mohri *et al.*,2006). Other researches were in agreement with the result of this study as Juan *et al.*, (2009) that used 60 rats administrated 80mg/daily for 1,2,3 months. Mohri *et al.*, (2009) who observed increased in these parameters in twenty neonatal calves gave 1 g FeSo₄ in diet for 14 days.

Increased of RBCs count may be attributed to increased iron level in the serum and body storages , as the result of this increase, Hypoxia-inducible factor (HIF) which orchestrates erythropoiesis by mediating genes is increased this lead to HIF raised promotes erythropoeitin hormone (EPO) secretion from the kidney and other non-renal sources (e.g., liver) and up-regulates EPO receptor (EPO-R) in the bone marrow then, increased of targeting colony forming unit-erythroid (CFU-E) that promote increase of erythroblast numbers . HIF also activates factors that improve iron absorption from the gut, mobilization from storage sites, and transport to the bone marrow (e.g., transferrin, transferrin receptor, ferroportin, ceruloplasm, DMT, and DcytB) (Porth,1998).

It is also believed that several enzymes also either contain iron or are activated by iron because of iron overload stimulate heme production by interfering with enzymes involved in heme biosynthesis such as ferrochelatase that inserts iron into the ring structure of protoporphyrin IX to produce heme, hence raised Hb synthesis ,RBCs count and other haematological parameters (Tandon *et al.*, 2002). Also may be result from increased of other enzymes that contain iron in their structures and have vital role in Hb synthesis like Hemeoxygenase (Labbeet *al.*,1999) and Levulinic Acid Dehydrogenase which has an essential role in first step of Hb synthesis and Aryl Sulphatase A, B, C (Howard and Hamilton,1999).

Increased of hemoglobin and red blood cells could also be due to sufficiency of protein synthesis that mainly induces increase of an essential amino acids and long age of energy source of protein synthesis incorporated in hemoglobin production (Bersenyi *et al.*, 2003 ; Lavicoli *et al.*, 2003).

References :

- **Adel M.**, Elgebaly E. and Elgebaly L.(2010) .Effect of ferrous sulfate on haematological , biochemical and immunological parameters in albino rats.Veterinaria Italiana .46(3):329-335.
- **Atyabi N.**, Gharagozloo M. and Nassiri M.(2006).The necessity of iron supplementation for normal development of commercially reared sucking calves.Comp Clin Pathol.15:165-168.
- **Baustad B.** and Tollersrud S. (1996). Experiments with injection of cobalt and iron dextran in calves. Medlemsbl. Norske. Vet. Foren18: 419-423
- **Bersenyi A.**, Fekete S., Szoes Z. and Be'rta E. (2003). Effect of ingested iron on haematology and serum biochemistry in rabbits acta vet. Hung. 51 (3): 297-304.
- **Bosted H.**, Hospes R.,Wehrend A. and Scheramel P.(2000).Effect of parenteral administration of iron preparation in rats. Tierztl Umsch.55:305.
- **Conrad M.**(2006). Toxicity of Iron. American Chemical Society J. 44: 87-102.
- **Coplin M.**, Schuette S., Leichtmann G. and Lashner, B. (2000). Tolerability of iron: A comparison of bisglycino iron II and ferrous sulphate. Clinical Therapeutics .13:606-612.
- **Dacie J.** and Lewis S.(1975). Basic hematology techniques. In: Dacie J, Lewis S (Eds). Practical hematology. Churchill Livingstone: London. pp 21–96.
- **Davidsson L.**, Kastenmayer P. and Szajewska H. (2000). Iron bioavailability in infants from an infant cereal fortified with ferric pyrophosphate or ferrous fumarate. Am. J. Clin. Nutr. 71:1597–602.
- **Frykman E.**, Bystrom M., Jansson U., Edberg A. and Hansen T. (1994). Side effects of iron supplements in blood donors; superior tolerance of heme iron. J. Laborat. and Clin. Medi. 123: 561-564.
- **Gygax M.**,Hirn H. and Zwahlen R. (1993).Immune function of male rats fed low amount of iron .J Vet and Med (Ser A).40:345-358.
- **Harvey J.**(2000). Microcytic anemia.In Schalms verterinaryhematology,5th Ed.Lippincott,Williams and Wilkins,Philadelphina,201-204.
- **Heidarpour B.**, Mohri M., Seifi H. and Tabatabaee A.(2008). Effect of parenteral supply of iron and copper on hematology , weight gain and health in rat . Vet Res Commun. 32: 553-561.
- **Hillman R.** and Ault K. (2002). Hematology in Clinical Practice. 3rd ed., McGraw-Hill. pp. 46-47.
- **Howard M.** and Hamilton P. (1999): Hematology. Churchill Living stone . pp. 4-25.
- **Jahan M.**, Ahmad N. and Myenuddin M.(2007). Effect of certain Haematinics on body weight and haemato-biochemical changes in laboratory mice. Bangl. J. Vet. Med. 5 (1 & 2): 103–105

- **Juan F.**, Pérez D ., Fernando R. and Gaspar R.(2009). Iron absorption and haemoglobin status of rats fed a ferrous sulfate in diet . J. Sci. Food and Agricul.89(12).2107-2114.
- **Kume S.** and Tanabe S. (1996).Effects of supplemental lactoferrin and ferrous sulfate on iron status of newborn calves.J Dairy Sci.67:1256-1236.
- **Labbe R.**, Ureman H. and Stevenson D. (1999).Zinc protoporphyrin; A metabolite with a mission. Clin. Chem. 42(12): 2060-72.
- **Lavicoli I.**, Cavelli G., Stanek E. , Castellino N. and Calabrese E. (2003). Effect of low doses of dietary Pb ,Hg and Fe on red blood cell production in male and female mice. Toxicol. Lett., 3. 137(3): 193-199.
- **Mehrdad M.** , **Hesam A.** and **Farzaneh N.**(2004).Effects of oral iron supplementation on some haematological parameters and iron biochemistry in rats. [Compar. Clin.Patho.](#)13(1)39-42.
- **Miyata Y.**, Furugouri K. and Shijimaya K.(1994).Developmental changes in serum ferritin concentration in rats.J Dairy Sci. 67:1256-1263.
- **Mohri M.**, Sarrafzadeh F., Seifi A. and Farzanch N. (2004). Effect of oral iron supplementation on some hematological parameters and iron biochemistry in rats.Comp Clin Pathol. 13:39-42.
- **Mohri M.**, Sarrafzadeh F. and Seifi H. (2006) . Effect of parenteral supply of iron on RBCs parameters in albinos rats. Bio Trace Elem Res . 136: 33-39.
- **Mohri M.**, Poorsina S. and Sedaghat R.(2009). Effects of Parenteral Supply of Iron on RBC Parameters, Performance, and Health in Dairy Calves.Bio.Trac.Elem.Res.136(1):33-39.
- **Monica C.**(2004).District laboratories practice in tropical countries. Part 2. Cambridge Low-Price Edition. PP: 308-318.
- **Porth C. (1998).** pathophysiology : 5th ed .,P 133 – 137 Lippincott.
- **Rajora V.**, Pachauri S. and Gupta G. (1995). Use of iron preparation in anemia associated with anorexia in male rats. Indian J. Vet Med. 15:1-4.
- **Soliman G.**, Mahfouz M and. Emara I.(2010) . Effect of Different Types of Oral Iron Therapy Used for the Treatment of Iron Deficiency Anemia and Their Effects on Some Hormones and Minerals in Anemic Rats.J. Americ. Scie. 6(6): 109-118.
- **Tandon S.**, Singh S., Prasas S., Srivastava S. and Siddigui M. (2002). Reversal of some metals - induced oxidative stress by chelating agent, antioxidant or their combination in the rat .Environ. Res. 90(1): 61-6.
- **Wenzl O.** and Erhardt G. (1991). Effect of parenterally administered iron dextran on erythrocyte parameters and growth rate in lambs of rats. Berliner and Munchener Tierarztliche Wochenschrift. 103 (7): 239-244.
- **WHO/UNICEF/UNU.** (2001). Iron deficiency anemia: Assessment, prevention and control. A guide for program managers.
- **Yip R.** (1994). Iron deficiency: contemporary scientific issues and international programmatic approaches. J. Nutr. 124: 1479S –1490S.

تأثير الجرعة المختلفة لعقار كبريتات الحديد على بعض المعايير الدموية في ذكور الجرذ الأبيض

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الخلاصة:

صممت الدراسة الحالية لمعرفة تأثير الجرع وفترات التجريع المختلفة من عقار كبريتات الحديد على بعض معايير الدم . إذ استخدم 48 ذكرا من الجرذ الأبيض قسمت الى أربعة مجاميع رئيسية (12 ذكر لكل مجموعة) المجموعة الأولى سيطرة والمجاميع الثلاثة الأخرى جرعت بوساطة التجريع داخل المعدي (100,75,50) ملغم/كغم من وزن الجسم لثلاث فترات (8,6,4) أسابيع . جمعت عينات الدم لقياس المعايير الدموية التي تتضمن تعداد كريات الدم الحمر , نسبة هيموغلوبين الدم , حجم خلايا الدم الحمر المتكدسة , متوسط تركيز الهيموغلوبين في كريات الدم الحمر , متوسط الهيموغلوبين الخلوي ومعدل حجم كريات الدم الحمر.

أظهرت نتائج هذه الدراسة إن تجريع كبريتات الحديد يسبب ارتفاع معنوي في كل معايير الدم متناسبا مع ازدياد الجرع وفترات التجريع. كما لوحظ تأثير معنوي للتداخل بين الجرع والفترات المختلفة على معظم معايير الدم عدا متوسط تركيز الهيموغلوبين في كريات الدم الحمر و متوسط الهيموغلوبين الخلوي.

تقترح هذه النتائج بان تجريع عقار كبريتات الحديد بجرع عالية وفترات طويلة ممكن ان يسبب خلا في عملية تكوين الدم خصوصا عملية تكوين كريات الدم الحمر وهذا يتضح من خلال ازدياد مستوى معايير الدم بدلا من علاج هذا العقار لفقر الدم بنقص الحديد بسبب زيادة تجهيز الحديد.