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Effect of different doses of ferrous gluconate drug on some biochemical parameter in male rat

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ABSTRACT: The study was designed to investigate the effect of different doses and different durations of ferrous gluconate drug administration on some blood criteria, using 36 males of rats. The animals were divided into four main groups (9 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, and had no signefecant effect in ALT, AST, bilirubin, creatinin.

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

KEYWORDS: Iron deficiency anemia, ferrous gluconate.

ملخص: صممت الدراسة الحالية لمعرفة تأثير الجرع وفترات التجريع المختلفة من عقار كلوكونات الحديد على بعض المعاير الكيموحيوية اذا استخدم 36 ذكر جرذ قسمت الى اربعة مجاميع رئيسية (9 ذكر لكل مجموعة). المجموعة الاولى سيطرة والمجاميع الثلاثة الاخرى جرعت بواسطة التجريع داخل المعدي (100,75,50) ملغم/ كغم من وزن الجسم لثلاث فترات (8,6,4) أسابيع . جمعت عينات الدم لقياس المعايير الكيموحيوية التي تضمنت تعداد كريات الدم الحمر ,نسبة هيموكلوبين الدم ,حجم خلايا الدم الحمر المتكدسة , متوسط تركيز الهيموكلوبين في كريات الدم الحمر , متوسط الهيموكلوبين الخلوي ومعدل حجم كريات الدم الحمر.

أظهرت نتائج هذه الدراسة ان تجريع كلوكونات 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 الهيموكلوبين في كريات الدم الحمر ومتوسط الهيموكلوبين الخلوي. اما انزيمي ALT, AST والكرياتنيين والبليروبين فليس هناك تأثير معنوي.

تقترح هذه النتائج بان تجريع عقار كلوكونات الحديد بجرع عالية ولفترات طويلة ممكن ان يسبب خللا في عملية تكوين الدم خصوصا عملية تكوين كريات الدم الحمر وهذا يتضح من خلال ازدياد مستوى معايير الدم.

1 INTRODUCTION

Iron is a nutrient that is related to health and immunity(1). 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 system(2). Iron is an essential component of haemoglobin , myoglobin and several enzymes such as catalase, peroxidase , cytochrome oxidase and ribinucleotide reductase (3).Iron of organic (heme)or inorganic (non- heme) origin is physiologically important element that plays vital role in erythropoiesis , oxygen transport , oxidative energy production and mitochondrial respiration (4).

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Iron deficiency anemia 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 (5). 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 (6). The main treatment of iron deficiency anemia include treatment with ferrous iron as ferrous gluconate which is much better absorbed than ferric iron e.g. ferric citrate (7). But, numerous studies concluded that oral administration of iron for long durations may be lead to defect in haemopoisis provided by an increase in haematological parameters in human (8 ; 9)and animal (10). Therefore , this study designed to clarify the effect of different doses and periods of oral administration of ferrous gluconate on some biochemical parameters in rat.

2 MATERIALS AND METHODS

2.1 ANIMALS

thirty six healthy adult males of rat weighing (216±18) g and approximately age (12-14) weeks,. 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 14h:10h light/dark cycle and maintained in controlled temperature (25±2ºC).

2.2 EXPERIMENTAL DESIGN

These thirty six males of rat were divided in four main groups, with 9 animals in each one , and then each of these groups were divided into three groups (3 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 gluconate / kg B.W by orally intragastric intubations for (4 , 6 , 8) weeks . At the end of each three administration periods sacrifice (3 rats) for each four subdivided groups and used to collection of samples.

2.3 COLLECTION OF SAMPLES

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

2.4 BIOCHEMICAL EXAMINATIONS

All haematological examinations were performed in the haematology center of Al-Diwanya teaching hospital in Al-Diwanya city. Hemoglobin was measured by the cyanomethaemoglobin method using Randox kits, Randox: Laboratories, USA (11). 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 (12). Red blood cells count (RBCs) was counted manually (Monica, 2004). Mean cell hemoglobin concentration (MCHC) was calculated using the equation: MCHC = [(Hb*100) / PCV]. Mean cell hemoglobin (MCH) was calculated using the equation: MCH = [(Hb*10) / RBC]. Mean red cell volume (MCV) was calculated using the equation: MCV = [(PCV*10) / RBC] (12).

Estimation of total bilirubin concentration in serum as in (13) Estimation of creatinine concentration in serum from (Biolabo company)as in (14) Estimation of alanine amino transferase (ALT) activity from Biomerieux companyas in (15) Estimation of aspartate amino transferase (AST) colored method from Biomerieux companyas in (15)

2.5 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 $P<0.05$.

3 RESULTS

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

Parameters	Group	Ferrous gluconate (mg/kg)	SD ± Mean
RBCs ($x10^6/\mu l$)	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	0.41a±9.80
	G2	75	0.65a± 10.08
	G3	100	0.82 abc± 10.73
PCV (%)	C	0	0.56 ± 26.21
	G1	00	1.53a± 30.29
	G2	75	1.35ab± 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	0.21a± 11.23
MCV (fl)	C	0	0.66± 32.64
	G1	50	0.61± 33.21
	G2	75	0.72ab± 34.16
	G3	100	0.98ab± 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:9/group.

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

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

Table 3:Effect of different doses of ferrous gluconate administration on biochemical paramrters in rat

Parameters	Group	Ferrous gluconate (mg/kg)	SD ± Mean
ALT	C G1 G2 G3	0 50 75 100	2.17 ± 44.9 2.23±70.0 2.31±66.9 2.7±51.2
AST	C G1 G2 G3	0 50 75 100	1.87±70.0 1.97±83.4 2.39±88.4 2.08±79.0
Bilirubin	C G1 G2 G3	0 50 75 100	0.05±0.63 0.7±0.69 0.08±0.74 0.13±0.74
cereatinin	C G1 G2 G3	0 50 75 100	6.11±98.98 7.27±110.1 5.47±87.82 6.81±89.1

Iron an essential component of a number of protein involved in oxygen transport and utilization, one of these proteins is haemoglobin (16). Thus, iron supply necessary for production of RBCs by erythropoiesis process (17). RBC parameters are used most commonly to monitor erythropoiesis, these parameters include RBCs count, Hb, PCV, MCHC, MCH and MCV (18). 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 (6). Similar results have been also reported by several studies (19) who utilized forty male rats were given as120 mg/daily for 45 days. (20) 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 (21)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,. 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 (22, 2). Other researches were in agreement with the result of this study as (23) that used 60 rats administrated 80mg/daily for 1,2,3 months. (24) who observed increased in these parameters in twenty neonatal calves gave iron 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) (24).

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 (25). Also may be result from increased of other enzymes that contain iron in their structures and have vital role in Hb synthesis like Hemeoxygenase (26) and Levulinic Acid Dehydrogenase which has an essential role in first step of Hb synthesis and Aryl Sulphatase A, B, C (27).

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 (28,29).

Biochemical parameters (ALT , AST , Bilirubin , cereatinin) are slightly increase (no significant effect) that mean the different doses of **ferrous gluconate had no** significant effect on **biochemical paramrters.**(30).

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