TOXICOLOGICAL EVALUATION OF A NEW ANTIANEMIC FORMULATION FROM NATURAL PRODUCT DRY TROFÍN®

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Summary

Iron deficiency is the most important nutritional disorder all over around the world. For prevention and treatment of iron deficiency anemia iron salts obtain for synthetic pathway have a wide use in spite the adverse effects and low bioavailability. On the contrary, heme iron containing in the blood haemoglobin has been a little use in spite the high bioavailability and it doesn't produce adverse reactions. Combination of both type of iron to obtain a drug or a nutritional supplement is newness. The aim of this work was to evaluate the toxicity of a new formulation with both heme iron of dry Trofín® and non heme iron of ferrous fumarate, in both acute and repeated doses by oral route during 28 days in Sprague-Dawley rats. In acute toxicity assay, doses evaluated were 6, 12 and 40 mgFe/Kg of corporal weight/day. In the repeat toxicity assay doses evaluated were 3, 6 and 12 mgFe/Kg of corporal weight/day and one group received the assayed substance doses of 12 mgFe/Kg of corporal weight/day and was analyzed during 14 days after the last administration (satellite). In both studies control groups received saline solution or dry Trofín®. Clinical observations were done every days and corporal weight was measured weekly. Macroscopical necropsies were done in both assays and in the repeat doses assays was done too histological preparations. In both assay 100% of rats survived and corporal weight of animals were increased. In the stomach of rats treated with low and high doses of substance assayed a lightly reddish coloration, gastric mucous erosions and independent hemorrhages were observed. Microscopical preparations showed hyperemic blood vessels with many congestion and point-like erosions. None of these reactions were observed in satellite group. Adverse reactions provoked by the assayed substance are characteristic adverse reactions due to utilization of iron salt in humans.

Keyword: Toxicology, iron, rat, anemia

Introduction

Iron deficiency is one of the major nutritional problems and it affects more than 2000 millions of persons around the world. The half of persons with iron deficiency are anemic.(1) For the prevention and treatment of the iron deficiency, iron salts obtained by synthetic pathway are broadly used. These products have a low bioavailability and they cause adverse reactions in 25% of patients.(2,3) Heme iron, whose fundamental source is the hemoglobin of the blood, is another alternative, but fewer used that the iron salts, in spite of the advantages like its biggest bioavailability and it doesn't produce adverse reactions.(4)

Trofín® is a natural product that contain heme iron from the hemoglobin of bovine blood, honey bee and propolis.(5) This product has been evaluated how antianemic and restorative in different population groups, with satisfactory results as well as absence of adverse reactions.(6-8) Recently a group of researchers have shown evidence of existence of two different receptors in the duodenum for the absorption of both heme and non heme iron.(9,10) That's why a product containing both types of iron could stimulate the two pathways of iron absorption at the same time and it could be more efficient than products containing only one type of iron. It doesn't exist in the market any product with both iron types. Our group has developed different formulations in two pharmaceutical forms, oral suspension and pills, from dry Trofín® as source of heme iron and ferrous fumarate as source of non heme iron.

Due to reports of the adverse reactions caused by the ferrous fumarate in humans,(2,3) the aim of this work was to evaluate the toxicity of one formulations in the pharmaceutical form of oral suspension, for repeated dose during 28 days in rats Sprague-Dawley (Cenp:SPRD).

Materials and Methods

The studies were carried out through the recommendations of OECD,(11,12) as well as the established regulations for care and use of laboratory animals.(13)

Substance assayed is a powder of reddish brown color that contains 50% of the iron from hydrolyzed iron-protein Trofín, and the remaining percent contributed by ferrous fumarate. Concentration of ferrous iron is 50.0mg/(g of substance assayed), according to potentiometric titration Redox with Cerium IV Sulfate,(5) corresponding to 50% the non heme iron coming from the Ferrous fumarate and the remaining percent belongs to heme iron of dry Trofín (T+F50%).

Adult Sprague Dawley rats of both sexes were obtained from National Center for Breeding of Laboratory Animal (CENPALAB, Havana Cuba), and adapted during 5 days to experimental conditions. The rats were single housed and had free access to water and food during the study. Environmental conditions were controlled during the entire experiment: Temperature 22 ± 3 ˚C, humidity 60-70%; 12h light/dark cycles. The study was carried out with approval from CENPALAB's Institutional Animal Care and Use Committee.

In acute toxicity assay control groups were administered with saline solution or a suspension of 6mgFe/Kg of corporal weight/day of dry Trofin containing 5mgFe/mL. Three other groups were treated with doses of. 6, 12 and 40 mgFe/Kg of corporal weight/day through a suspension of substance assayed. In repeat doses assays control groups were administered with saline solution or a suspension of 12mgFe/Kg of corporal weight/day. Three other groups were treated with doses of. 3, 6 and 12 mgFe/Kg of corporal weight/day. A sixth group was administered with 12 mgFe /Kg of corporal weight/day of substance assay and 14 days after the last administration they were analyzed (satellite group). In acute toxicity assay administration was done for unique doses by gastric gavages in the first day of assay. In repeat doses assay administration was done daily by gastric gavages for 28 days. Animals were fasted for 12 hours, prior each administration which extended 4 hours after each administration.

Each animal was observed twice daily, during 14 days in acute toxicity assays and for 28 days in repeat doses assay. Body weights were recorded weekly. In repeat doses assay blood sampling for analysis of hematological and clinical chemistry parameters were taken from the right retroorbital plexus on days 0 and 28. At the end of both assays macroscopical necropsies were done, paying special attention in gastrointestinal tract. In repeat doses assays histopathologycal study was carried on day 28, after completion of all study procedures, for the five groups treated until this one day and on 42 days for satellite group.

Statistical evaluation was performed by a randomized completely analysis of variance design with significance assessed at $p<0.05$ level. Student test was used for the analysis of hematological and clinical chemistry parameters for the satellite group when concluding substance assay administration and 14 days later.

Results

Both studies concluded with 100% of survival. No clinical symptoms of toxicity were observed. Corporal weight had a tendency to increase, in both sexes. Didn't exist significant differences, between corporal weight of groups at the end of study for $p<0.05$. Water and food consumption in both sexes showed a tendency to increase in most of animals.

Statistical analysis of hematological parameters for repeat doses assay showed differences between groups for the LEUC which were significantly bigger in the group treated with T+F50% to the high dose group, and control group treated with a suspension of Trofín and groups treated with T+F50% to the low and medium dose groups, for $p<0.05$. Differences for LEUC between groups didn't show a relationship doses/answer. Significantly bigger differences were obtained for ALB and PT between females from the group treated with the high dose of T+F50%, and the rest of groups.(Tale 1)

Table1. Hematological and biochemistry parameters with statistical differences between groups. Females

Substance administrated (Kg/corporal weight/day)	Leuc (X103µg/mL)	Alb (g/L)	PT (g/L)
Saline solution	6.13 ± 1.85	39.3 ± 0.60	81.2 ± 1.92
Dry Trofín 12mgFe	5.30 ± 0.60	38.7 ± 0.92	81.0 ± 2.91
T+F50% 3mgFe	5.20 ± 1.30	37.4 ± 2.47	80.4 ± 4.61
T+F50% 6mgFe	4.50 ± 0.50	38.5 ± 1.65	82.6 ± 4.16
T+F50% 12mgFe	10.58 ± 0.71	38.06 ± 1.85	82.8 ± 4.92
T+F50% 12mgFe (Satellite group)	5.4 ± 0.70	42.5 ± 2.23	89.4 ± 1.82
Historical range for rats Cenp:SPRD (X ±2DE)	2.21-11.14	25.9-52.6	55.5-83.2

Serum iron levels were higher for all the groups than control group treated with Saline solution, additionally group treated with high dose of T+F50% showed differences statistically higher, in both sexes, than the rest of groups.(Table 2)

Satellite group hematological parameters as HB, HTO, VCM AND CHCM were higher for females 14 days after the last administration, regarded the determinations carried out when concluding substance assayed administration for $p<0.05$. A biochemical determination as PT was significantly lower for the females. Besides, PT and ALB for males were lower 14 days after the last administration. Serum iron levels didn't show significant between the last administration and 14 days later.

Important macroscopical changes were observed at the glandular stomach, in groups treated with T+F50% to both low and high doses. We observed at gastric mucous a light reddish coloration, with brilliant and tumefaction appearance, digested blood deposits blended with snot forming a viscous and dark brown substance.

Table 2. Differences between serum iron levels for both sexes

Substance administrated (Kg/corporal weight/day)	Serum iron (µmol/L)			
	Female		Male	
	T=0	T=28d	T=0	T=28d
Saline solution	68.9 ± 0.80	69.1 ± 0.80	81.7 ± 2.10	81.7 ± 2.10
Dry Trofín 12mgFe	69.0 ± 5.20	75.9 ± 3.20	81.5 ± 3.30	86.4 ± 1.80
T+F50% 3mgFe	69.8 ± 1.00	75.7 ± 2.00	81.60 ± 1.90	88.2 ± 1.90
T+F50% 6mgFe	69.9 ± 1.10	79.5 ± 1.50	80.9 ± 1.00	89.6 ± 2.20
T+F50% 12mgFe	68.9 ± 2.90	81.7 ± 1.20	80.80 ± 1.00	94.6 ± 1.40
T+F50% 12mgFe (Satellite group)	69.4 ± 1.50	81.5 ± 0.90	80.3 ± 2.40	94.5 ± 1.30

Macroscopical necropsies showed too gastric mucous erosions and independent hemorrhage, like dark and point-like blots, on the hyperemic mucous, or associated to the erosion. Microscopical observations showed reddish coloration in the stomach. Hyperemic and congested blood vessels were abundant, as well as disseminated and point-like erosions. Erosions showed the loss of superficial epithelium generating a defect in the mucous that didn't cross the *mucosal muscularis*. Microscopical observations of satellite group 14 days after the last administration of the substance assayed weren't observed changes in the normal appearance of gastric mucous.(Figure1)

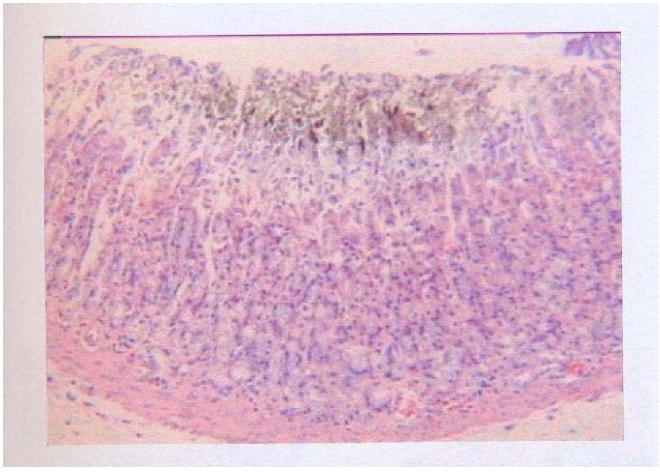

Figure 1 Superficial erosions with necropsies in gastric mucous.

Discussion

The LEUC values, water and foods consumption for repeat doses assay were between ranges reported for the species and together to the increasement of corporal weight evidenced the good state of health of animals during substance assayed administration.

Serum iron values for all groups treated with T+F50% and control group treated with a dry Trofín suspension were higher than normal value reported for the specie.(14) These results suggest that T+F50% and Trofín, stimulate iron absorption in the rat. Results of clinical assay carried out with Trofín in different population groups have demonstrated the increment of serum iron levels.(6-8) Iron absorption in rat depends of iron serum levels however in man it depends of the balance between iron both used and stored.(15) The increased of hematological parameters as HB, HTC, VCM and CHCM in satellite group 14 later days to the last administration should have a relationship with serum iron level, because of iron is necessary for the hemoglobin synthesis.(16)

Macroscopical changes and microscopical observations could be related in great measure with the lingering fast that were subjected the animals before each one substance assayed administration, because of the empty stomach could help the irritating effect of the iron salts. Adverse reactions of iron salt reported for human include gastritis, ulcers at the gastric mucous, abdominal pain, nauseas, vomits, constipation and diarrhea, related in great measure with high doses necessary of these products, due to their low bioavailability.(2,3) Normal appearance of gastric mucous of satellite group 14 days after the last administration suggest the reversibility of adverse effects helped with the quick renovation of the mucous gastric epithelium in rats, every three days.(17)

Adverse reactions described in the experimental conditions of this study, could be caused for the ferrous fumarato and we suppose that in human these reactions will be lower than adverse reactions reported for iron salt in human. In this new formulation iron salts constitutes only the half of doses recommended for prevention and treatment of ferropenic anemia, (3-6 mg/Kg of weight corporal/day).(3) Protein composition of dry Trofin could help the solubility of the iron coming from the ferrous fumarato, and diminish their insolubilization in the gastric mucous associated with most of adverse reactions, causing in most of the cases treatment interruption and intolerance.

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