Effect Camel's milk in male albino mice exposed to ferrous sulfate toxic

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Abstract

This study was aimed to search the effect of Camel milk on males albino mice exposed to ferrous sulfate toxic 30 mg / kg body weight for period 30 days. The animals were randomly divided into 3 groups, each group comprising 5 animals that weigh 26-27 gm. The results of oral dose were shown with ferrous sulfate a significant decrease in (P <0.05) in body weight, MON, GRAN, HCB, HCT. And significant increase (P˂0.05) in kidney, spleen, LYM, MCHC, AST, ALT, Urea. And no significant difference in the heart, liver, WBC, RBCs, MCV, MCH, Protein and Albumin compared with the control group.

As for the results of oral dosage of Camel's milk + ferrous sulfate appeared a beneficial effect of these values compared to the group given ferrous sulfate. The oral dose of ferrous sulfate causes pathological histopathological changes in the kidneys and liver of male mice. The tissue is Separation of central venous wall from Hepatocyte and the Existence of congestion in the liver and Clear hemorrhage within the kidney tissue with a clear contraction of renal glomerulosclerosis in the kidneys. The treatment with Camel's milk + ferrous sulfate showed the return of central vein and hepatocytes to a semi-natural condition with congestion remaining within the central vein in the liver and showing Glomerulus and urinary tubules are almost normal in the kidneys. Showed an effect to reduce damage or damage completely in certain areas of the tissue compared to ferrous sulfate group.

Key word: Camel's milk, ferrous sulfate, haematological, histological.

1. Introduction

Iron is one the microminerals that is classified as an essential nutrient supplement [1]. It is an essential element within the body, being found in functions of both myoglobin, cytochromes, hemoglobin, enzymes with iron sulphur complexes and other iron-dependent enzymes, increase iron in the body is associated with toxic influences and possess health problems [2]. And Iron is fundamental for many biological processes, can be toxic when present at high levels in a free form [3]. Iron overdose accumulation of iron within the body and Liver influenced by iron overload because it is the more active site of iron storage in the body [4]. may lead to fibrosis, cirrhosis, liver failure and hepatocellular carcinoma [5], that rapid accumulation of excessive iron negatively affects the hematopoietic system by damaging hematopoietic cells and hematopoietic microenvironment [6]. iron can lead to intra lysosomal storage of iron in the kidney and other sites of body [7]. iron toxic indicating an oxidative stress situation [8]. And as a redox active transitional metal, iron generates reactive oxygen species [ROS] via the Fenton and Haber–Weiss reactions. ROS react directly with lipids, proteins, nucleic acids and induce oxidative stress by depleting cellular stores of antioxidants. ROS also effect multiple cell signaling pathways important to cell survival, proliferation and death [9, 10]. Camel's milk is characterized by a white liquid that is opaque, smells good and tastes salty and has different qualities and percentages than the rest of the milk [11]. It is a natural substance that possesses many elements with anti-living cells [12]. It contains proteins that possess many functions of these functions it's antioxidant [13]. And used as an antioxidant because it contains high amounts of the elements that stimulate the antioxidant enzymes. These elements include magnesium, zinc, vitamin E, B and C [14, 15], Have medicinal properties used to treated autoimmune diseases, problems of the spleen, asthma, piles, diabetes, jaundice and tuberculosis [16]. The study aims to know effect of Camel's milk on blood parameters, some chemical changes, histological changes of liver and kidneys in male albino mice exposed to ferrous sulfate toxic.
2. Materials and methods

Camel’s milk samples: Daily milk samples were collected early in the morning from camel farm in the (Sharqat city, Salah Al-deen Governorate, Iraq). Milk was collected from camels by hand milking as normally practiced by the farmers. Then samples were placed in sterile screw bottles and kept in cool boxes until transported to the laboratory.

2.1 Experimental animals:

This study was conducted on uses albino male mice, the weights were in 26-27g. The animals were obtained from the animal house of Veterinary Medicine College in Tikrit University. Wing banded and housed in heated battery brooders under 12 hours fluorescent lighting daily with feed and water provided ad libitum. Rats were fed the optimal formula according to [17, 18]. And randomly divided the animals into 3 groups containing each group 5 animals. The distribution of experimental totals was as follows:

1-Group control
2-Administered orally ferrous sulfate 30 mg/kg of body weight daily for period 30 days.
3-Administered orally Camel’s milk 1 ml a day + ferrous sulfate 30 mg/kg of body weight daily for period 30 days.

2.2 Blood samples:

After 30 days from the experimental, all the animals fasted for 10 hours, but the water was free. Then weighted and anesthetized by chloroform and sacrificed by cutting jugular vein. Then take of blood from each animal about 0.5 ml from blood in tubes containing anticoagulant Ethylene diamine tetraacetic acid (EDTA) to do haematological analysis. concerning the remaining part of blood put in tubes without anticoagulant that centrifuged by using Centrifuge at 3000 round minute for 15 minute to obtained the serum that stored at temperature -20°C until to do the biochemical tests, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), Protein, Albumin and Urea, using (Kits) manufactured by BIOLABO SA, France and the analysis done by using Japanese Spectrophotometer [19]. Complete blood counts (CBCs) (whole blood analysis). The haematological analysis of White blood cells (WBCs) count, lymphocyte (LYM), monocyte (MON), granulocyte (GRAN), Red blood cells (RBCs) count, haemoglobin concentration (HGB), haematocrit (Hct), mean cellular volume (MCV), mean corpuscular haemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined by using automated haematology analyzer (Syamex model; K-1000 Japan [20]. In addition to, the most of blood tests asserted by used modified methods [21].

2.3 Histo-physiopathological study:

anesthetized of the animal by chloroform and take the blood from each mice, laparotomy to opened abdominal cavity and chest (sternum) in form (T) inverted shape, then, quickly extirpated the liver, kidneys, heart, spleen and tasticular, from each animal, then the weighed, afterwards taken liver and kidneys for a histological examination using the preparation method he used [22, 23].

2.4 statistical analysis:

Data were analyzed by the ANOVA analysis, using the general linear model of the Statically Analysis System (SAS Institute, 2001). Significant treatment differences were evaluated using Duncan’s multiple-range test [24]. All statements of significance are based on the 0.5 level of probability.

3. Results and Discussion

Table 1. shows effect the treatment of Camel’s milk in body weight and organs weight for mice exposed to ferrous sulfate toxic at a concentration of 30 mg / kg body weight for 30 days. The oral administration with ferrous sulfate resulted a significant decrease (P<0.05) in final body weight and significant increase (P<0.05) in kidney and spleen. And no significant difference in the heart and liver compared with control group. The oral dose Camel’s milk + ferrous sulfate significantly increased the final body weight, and significant decrease in kidney and spleen, no significant difference in the heart and liver compared to the group given ferrous sulfate.
Table 1. Effect the oral dose from Camel's milk in body weight and organs weight of male mice exposed to ferrous sulfate toxic.

| Treatment type                  | Initial body weight | Final body weight | Increase in weight | Kidneys | Heart | Liver | Spleen |
|---------------------------------|---------------------|-------------------|--------------------|---------|-------|-------|--------|
| control                         | 26.00 a             | 36.00 a           | 10.00 a            | 0.44 c  | 0.15 b| 1.20 a| 0.17 c |
|                                 | 0.57±               | 0.57±             | 0.09±              | 0.02±   | 0.03± | 0.11± | 0.04±  |
| ferrous sulfate                 | 26.83 c             | 30.33 c           | 3.50 c             | 0.64 a  | 0.16 ab| 2.00 a| 1.30 a |
|                                 | 0.16±               | 0.33±             | 0.28±              | 0.03±   | 0.05± | 0.57± | 0.05±  |
| Camel's milk + ferrous sulfate  | 26.60 a             | 34.00 b           | 7.40 b             | 0.54 b  | 0.17 a| 1.50 a| 0.50 b |
|                                 | 0.55±               | 0.57±             | 0.57±              | 0.05±   | 0.05± | 0.23± | 0.05±  |

Different letters in a column mean significant differences at the probability level 0.05.

Table 2. shows effect the oral dose of Camel's milk on total and differential white blood cells in male albino mice exposed to ferrous sulfate toxic for 30 days. The results were found that the oral dose with ferrous sulfate resulted a significant increase in LYM and significant decrease in MON and GRAN. And no significant WBC compared with control group. The oral dose Camel's milk + ferrous sulfate resulted in a significant increase significant in MON and GRAN and significant decrease in LYM. And no significant WBC compared to the group given ferrous sulfate.

Table 2. Effect the oral dose from Camel's milk on total and differential white blood cells in male albino mice exposed to ferrous sulfate toxic.

| Treatment type                  | Measured Standards |
|---------------------------------|--------------------|
|                                 | WBC \(10^9/L\) | LYM (%) | MON (%) | GRAN (%) |
| Control                         | 7.50 a            | 83.10 a | 9.70 a  | 7.20 a   |
| ferrous sulfate                 | 5.80 a            | 91.40 a | 6.10 c  | 2.50 c   |
|                               | 0.57±             | 0.01±   | 0.02±   | 0.03±    |
|                               | 0.01±             | 0.01±   | 0.01±   | 0.05±    |
|                               | 0.57±             | 0.02±   | 0.03±   | 0.05±    |

Different letters in a column mean significant differences at the probability level 0.05.

Table 3. shows effect the oral dose of Camel's milk on some blood parameters in male albino mice exposed to ferrous sulfate toxic for 30 days, led oral administration with ferrous sulfate decreased significantly in HCB and HCT, and significant increase in MCHC. And no significant RBCs, MCV and MCH compared with control group. The oral dosage of Camel’s milk + ferrous sulfate significantly increased in HCB and HCT. And no significant RBCs, MCV, MCH and MCHC compared to the group given ferrous sulfate.

Table 3. Effect the oral dose from Camel's milk on some blood parameters in male albino mice exposed to ferrous sulfate toxic.

| Treatment type                  | Measured Standards |
|---------------------------------|--------------------|
|                                 | HCB   | HCT   | MCHC  | RBCs | MCV | MCH  |
| Control                         |       |       |       |      |     |      |
| ferrous sulfate                 |       |       |       |      |     |      |
|                               |       |       |       |      |     |      |
|                               |       |       |       |      |     |      |
|                               |       |       |       |      |     |      |

Different letters in a column mean significant differences at the probability level 0.05.
Table 3. Effect the oral dose from Camel's milk on some blood parameters in male albino mice exposed to ferrous sulfate toxic.

| Treatment type          | RBCs 10^12/L | HCB g/l | HCT % | MCV fl  | MCH g  | MCHC g/Dl |
|-------------------------|--------------|---------|-------|---------|--------|-----------|
| control                 | 8.40 a       | 12.90 a | 38.40 a | 51.00 a | 16.30 a | 28.53 b   |
| ferrous sulfate         | 6.90 a       | 10.66 b | 36.60 b | 49.00 a | 15.50 a | 31.20 a   |
| Camel's milk + ferrous sulfate | 7.89 a | 12.10 a | 37.70 a | 50.00 a | 16.10 a | 30.50 a   |
| control                 | 31.20 a      | 15.50 a | 49.00 a | 36.60 b | 10.66 b | 6.90 a    |
| ferrous sulfate         | 30.50 a      | 16.10 a | 50.00 a | 37.70 a | 12.10 a | 7.89 a    |
| Camel's milk + ferrous sulfate | 30.50 a | 16.10 a | 50.00 a | 37.70 a | 12.10 a | 7.89 a    |

Different letters in a column mean significant differences at the probability level 0.05.

Table 4. Effect the oral dose from Camel's milk on some parameters biochemical in male albino mice exposed to ferrous sulfate toxic for 30 days. The oral dose of ferrous sulfate significantly increased (P<0.05) in the efficacy values of ALT, AST and Urea. No significant difference in the Protein and Albumin compared with control group. Treatment with Camel's milk + ferrous sulfate significantly reduced (P<0.05) in ALT, AST and Urea compared to control with ferrous sulfate.

| Treatment type          | ALT IU/L | AST IU/L | Protein g/dl | Albumin g/dl | Urea mg/dl |
|-------------------------|----------|----------|---------------|---------------|------------|
| control                 | 39.61 c  | 75.02 c  | 4.30 a        | 3.10 a        | 26.20 c    |
| ferrous sulfate         | 75.42 a  | 120.64 a | 2.90 a        | 1.89 a        | 43.10 a    |
| Camel’s milk + ferrous sulfate | 47.30 b | 84.12 b  | 4.00 a        | 2.81 a        | 39.10 b    |
| control                 | 0.57±    | 0.58±    | 0.55±         | 0.57±         | 0.58±      |
| ferrous sulfate         | 0.57±    | 1.17±    | 0.57±         | 0.58±         | 1.15±      |
| Camel’s milk + ferrous sulfate | 1.32±  | 1.15±    | 0.56±         | 0.56±         | 0.57±      |

Different letters in a column mean significant differences at the probability level 0.05.

4. Histopathological:

In this study, several tissue changes were identified in the histological sections taken from the liver and kidneys of male mice exposed to ferrous sulfate toxic and Camel's milk treatment for 30 days compared to the sections taken from healthy male mice. The effects varied from one tissue to another and from one region to another. The same fabric as in the following:

A microscopic examination of the tissue sections of the male mice liver of control group, explain the central vein surrounded by Hepatocytes arranged in a regular radiograph. Sinusoids are naturally observed as in Figure 1. The histological sections of the Male mice kidney of control group, explain the natural shape of the renal Glomerulus and proximal and distal urinary bulb Figure 2.

showed Figure 3. a microscopic examination of the liver tissue sections of animals treated with ferrous sulfate. Separation of central venous wall from Hepatocytes and the presence of congestion. And the microscopic examination of sections of kidney tissues of animals treated with ferrous sulfate show Clear hemorrhage within the kidney tissue with a clear contraction of renal glomerulosclerosis Figure 4. While the liver tissue of the treated group of Camel's milk + ferrous sulfate showed the return of central vein and hepatocytes to a semi-natural condition with congestion remaining within the central vein (Fig.5).

Explains the (Fig.6) Histological section of renal tissue of a mice treated with Camel's milk + ferrous sulfate showing Glomerulus and urinary tubules are almost normal.

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Effect ferrous sulfate on body weight loss mice may be due to attributed to increased metabolic costs and reduced food consumption [25]. The decreased significantly in haematological of albino mice exposed to ferrous sulfate poisoning. May be effect the ferrous sulfate on bone marrow hematopoiesis damage [26]. Or due exposure for ferrous sulfate to the hemolytic effect induced by the release of oxidative stress. And effect damage altered liver membrane permeability on liver and kidneys. Perhaps it may be the reason decreased ability of the kidney to secrete erythropoietin. Erythropoietin stimulates the bone marrow to produce red blood cell. The reason for the increased enzymes may be Increase in ALT, AST an indicator of hepatocytes harm have been found in response to iron overload induced oxidative stress [27]. Iron excess induces cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation. The reason for the increase in urea may be due to ferrous sulfate effect on the kidneys, which causes a defect in kidney function and induces the expression of nitric oxide, releases the nitric oxide which combines with superoxide anions to shape peroxynitrite, a very poisonous mediator of lipid peroxidation and oxidative damage to cellular membrane [28]. The critical role of iron in the formation of reactive oxygen species that ultimately reason peroxidative injury to vital cell structures [29]. ferrous sulfate induced histological damages liver and kidney functional disorders in rats [30]. Camel milk contains proteins that have many functions and these functions are antioxidants that work to remove the harmful effect of free radicals [31]. And Camel milk contains on minerals, magnesium, zinc and vitamin B2, C, E ingredients that stimulate the body's antioxidant enzymes [15, 32, 33]. It also contains the element copper, which performs important functions in the body and works as an antioxidant, stopping the work of oxygen atoms active, which act as free radicals and lead to cell damage [34]. This is constituents an effective therapeutic as antioxidant activity approach can play a double role in reducing the rate of oxidation one by sequestering and chelating cellular iron stores and other as radical trap, which significantly reduced the oxidative stress leading to reduction of pathological changes and restoration of normal physiological functions.

**Figure 1.** Male mice liver of control group, explain the Central vein (CV) , Hepatocyte (HC) and Sinusoids (S). H & E 400X.

**Figure 2.** Male mice kidney of control group, explain the natural shape of the renal Glomerulus (G) and proximal and distal urinary bulb(UT). H & E 400X.
4. Conclusion

In conclusion, this study evidences that the administration of Camel's milk that may be linked to its active antioxidant components that help to improve blood parameters and protect different body tissues from damage, as a result of the effect of toxic with ferrous sulfate that causes oxidative stress in male albino mice.
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