

Camel's Milk Protects Against Cadmium Chloride-Induced Hypochromic Microcytic Anemia and Oxidative Stress in Red Blood Cells of White Albino Rats

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Abstract: Problem statement: Cadmium is a heavy metal of wide occupational and environmental contamination. In recent years, however, Cadmium has been implicated in the pathogenesis of several clinical disorders. One of the most frequently described problems in aluminum toxicity is anemia. Therefore, the present study was carried out to determine the effectiveness of Camel's milk in alleviating the toxicity of Aluminum Chloride (AlCl_3) on certain hematological parameters and antioxidant system components in the RBC's of white albino rats. **Approach:** Albino rats of both sexes (6/group) weighting between 230-250 g were divided into three treatment groups: Group one were rats given normal saline and served as control group, group two were rats treated with 2 mL of a solution containing cadmium chloride (10 mg kg^{-1}) and named Cadmium chloride treated rats, group 3 were rat treated with 2 mL of a solution containing Camel's milk in which the same concentration of cadmium chloride was dissolved and named Camel's milk and Cadmium chloride treated rats. Rats were orally administered their respective doses every day for 21 days. Evaluations were made for hematological parameters in the blood and for oxidative stress components in the RBC's. **Results:** Results obtained showed that oral Cadmium chloride treatment caused a significant decrease in total red blood cell counts, Hematocrite (PCV) value, Hemoglobin (Hb) concentration, Meancorpuscular Volume (MVC), Mean Hemoglobin Concentration (MHC) and Mean Corpuscular Hemoglobin Concentration (MCHC). Also oral administration of Cadmium chloride induced free radicals and as a result caused a significant decrease in the activities of Superoxide Dismutase (SOD), Catalase (CAT) and reduced glutathione (SGH) in the RBCs homolysate. The oral administration of Camel's milk with cadmium chloride alleviated its toxic effect. Camel's milk administration resulted in a significant increase in the in total erythrocytes count, blood Hb, PCV, MCV, MHC and MCHC. Camel's milk reduced free radicals production and oxidative stress status in the RBC's noticed by the significant increased activities of SOD and CAT, as well as concentrations reduced glutathione (SGH). **Conclusion:** The above results indicated a protective effect of camel's milk oral administration against cadmium induced anemia and oxidative stress in the RBC's of white albino rats.

Key words: Camel's milk, cadmium chloride, oxidative system, hematological values, rats

INTRODUCTION

Cadmium is a heavy metal of wide occupational and environmental contamination and present in trace levels in seawater and in a broad range of animal and plant species. Relatively large quantities of cadmium are found in commercial phosphate fertilizer, thus the increases in soil and plant cadmium contents may lead to increases in dietary cadmium. Also, cadmium poses a potential environmental hazard due to increased in its industrial use^[1-4]. Recently, the effect of long-term exposure to cadmium compounds has been widely investigated^[5-9].

Cadmium toxicity in human and animals causes a number of pathophysiological disturbances including

proximal tubular dysfunction characterized by proteinuria, aminoaciduria and glucosuria, bone disease^[10], pulmonary emphysema^[11] and liver damage^[12]. Exposure of human through contaminated air, food, water, manufactured goods and occupational hazards^[13] might cause these disturbances. Cigarettes made from tobacco grown in soil containing cadmium is another major source of cadmium intoxication^[14].

In the blood, Cadmium stimulates the formation of metallothioneins^[15] and Reactive Oxygen Species (ROS) thus causing oxidative damage in erythrocytes and in various tissues, which results in a loss of membrane functions^[16]. Cadmium also induces the onset of anemia, decreases the red blood cell count, hemoglobin concentration and hematocrite value as

well as reduced blood iron levels^[17]. Animal experiments have shown that hypochromic microcytic anemia might be developed in rats with cadmium intoxication following exposure to dietary cadmium^[18].

Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported^[18,19]. Fariss^[20] has shown that free radical scavengers and antioxidants are useful in protecting against Cadmium toxicity.

Camel's milk is different from other ruminant milk; having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C, B2, A and E, low protein and high concentrations of insulin^[22]. It has no allergic properties and it can be consumed by lactase deficient persons and those with weak immune systems.

The milk is considered to have medicinal properties. In Sahara, fresh butter is often used as a base for medicines. The products developed also include cosmetics or pharmaceuticals. A series of metabolic and autoimmune diseases are successfully being treated with camel's milk. In India, camel's milk is used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes^[23]. Beneficial role of raw camel's milk in chronic pulmonary tuberculosis patients has been observed^[24]. In repeated trials, it was observed that there was 30-35% reduction in daily doses of insulin in patients of type 1 diabetes receiving raw camel's milk^[25]. It is mentioned in Islam online that Camel's milk and urine have medical effects, so Islam encourages and permits the drinking of camel milk and camel urine is permitted in case of necessary medical treatment.

In our survey, we didn't find any study dealt with the therapeutic effect of Camel's milk against the bad effect of Cadmium on blood of rats, therefore the Therefore the aim of our study was to investigate a possible protective influence of Camel's milk treatment on some hematological parameters and antioxidant defense system in the blood of rats treated with cadmium chloride. The following parameters were determined in the blood to confirm anemia: Red Blood Cells Count (RBCs), Hematocrit value (Hct), Hemoglobin (Hb) and blood indices Also, the following antioxidant components were determined in the RBC's: Reduced glutathione (SGH), Superoxide Dismutase (SOD) and Catalase (CAT).

MATERIALS AND METHODS

Camel's milk samples: Milk samples were collected early morning daily from Camel's farm in Abha area (Southeastern region of Saudi Arabia). Milk was

collected from camels by hand milking as normally practiced by the farmers. The samples were collected in sterile screw bottles kept in cool boxes until transported to the laboratory.

Animals: White albino rats of both sexes (230-250 g) were supplied by the animal house at the medical faculty of King Khalid University. The rats were housed in standard metal cages (6 rats/cage) and were fed a stock diet containing 50% wheat, 21% corn, 20% soybean, 8% concentrated proteins and 1% a mixture of salts, vitamins and dicalcium phosphate. Water was supplied *ad libitum*. These rats were kept at room temperature 22°C before and during the experiments.

Three groups of rats each of 6 male rats were used; all rats were given the treatments by cavage needle for 21 days and were treated as follows:

Group 1: Given a daily dose of 2 mL normal saline (control group).

Group 2: Given a daily 2 mL dose of a solution contains 10 mg kg⁻¹ body weight of cadmium chloride orally.

Group 3: Given a daily dose of a solution contains 10 mg kg⁻¹ body weight of cadmium chloride dissolved in 2 mL of camel milk orally.

Physiological and biochemical analyses: After the treatment, the animals were sacrificed by decapitation always between 8:00 and 10:00 and fresh blood was immediately collected into heparinized test tubes. Some Blood were used for preparation of homolysate for determination of reduced glutathione and the rest of blood was used for the following biochemical measurements: RBCs count and hematocrit value were determined by standard hematological techniques^[26]. The hemoglobin concentration was determined by the cyanmethemoglobin method^[27]. Mean Corpuscular Volume (MCV), Mean Hemoglobin Concentration (MHC) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated. Activity of superoxide dismutase SOD (inhibition rate percent) was assayed in the RBC's cell using Commercial available kits (Biovision, Kit number K335-100), according to manufacture's instruction. Catalase activity (One unit of catalase is the amount of catalase decomposes 1.0 μmol of H₂O₂ min⁻¹ at pH 4.5 at 25°C.) was assay in the RBC's cell using Commercial available kits (Biovision, Kit number K335-100 and K773-100), according to manufacture's instruction.

Preparation of hemolysate: After collecting blood samples in heparinized tubes, centrifugation was performed at 1000 g for 15 min to remove the buffy coat. The packed cells obtained at the bottom were washed thrice with phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4). A known amount of erythrocytes was lysed with hypotonic phosphate buffer. The hemolysate was obtained after removing the cell debris by centrifugation at 3000 g for 15 min and used for determination of reduced glutathione levels.

Estimation of reduced glutathione: The GSH content of the homolysate was measured at 412 nm using the method of Sedlak and Lindsay^[28]. The homogenate was precipitated with 50% trichloroacetic acid and then centrifuged at 1000 rpm for 5 min. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of Tris-EDTA buffer (0.2 M; pH 8.9) and 0.1 mL of 0.01 M 5,5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer. The values were expressed as mmol/100 g of tissues.

Statistical analysis: Data are given as the mean \pm SEM Student's t-test was used to determine if the difference observed among various treatment groups at individual time points was significant.

RESULTS

Table 1 shows the results of RBC's count, hemoglobin concentration, Hematocrite value and red blood cell indices (MCV, MCH and MCHC) in all groups of rat. Rats administered cadmium chloride orally showed decreased values of erythrocyte counts, hemoglobin and hematocrite, as well as decreased MCV, MCH and reduced MCHC. On the other hand oral administration of Camel's milk with cadmium chloride to rats significantly increased these parameters to their normal levels when compared to rats administered cadmium.

Table 2 shows the activity of Superoxide Dismutase (SOD) and Catalase (CAT) as well as reduced glutathione (SGH) levels in RBC's of all groups of rats. Cadmium treated-rats showed a significant decrease in the activity of these enzymes (SOD and CAT) and in the level of SGH, while the addition of Camel's milk to cadmium chloride resulted in a significant increase in the activity of SOD and CAT as well as a significant increase in the levels GSH of the treated rats when compared to cadmium treated rats.

Table 1: Red Blood Cells (RBC's) count, Hematocrite (Hct) value and Hemoglobin (Hb) concentration in the blood of control rats, cadmium treated-rats and camel's milk and cadmium treated rats

Parameters	Control	Cadmium	Cadmium + milk
RBC's ($\times 10^6 \text{ mm}^3$)	7.12 \pm 0.4	6.48 \pm 0.85*	6.95 \pm 13.5*
Hematocrite (%)	49.65 \pm 7.6	36.77 \pm 5.32*	46.00 \pm 6.32*
Hemoglobin (g dL ⁻¹)	14.30 \pm 3.4	8.30 \pm 3.2*	13.45 \pm 3.15*
MCV (fl)	68.80 \pm 3.4	56.74.2.4*	66.18 \pm 3.1*
MCH (pg)	20.08 \pm 2.6	12.80 \pm 3.1*	19.30 \pm 4.2*
MHCH (g dL ⁻¹)	28.80 \pm 3.2	22.60 \pm 2.88*	29.23 \pm 2.7*

Values are given as mean \pm SEM for groups of six animals each. Values are statistically significant at *p<0.05. Cadmium treated rats were compared with control rats; camel's milk cadmium treated rats were compared with cadmium treated rats

Table 2: Superoxide dismutase activity, catalase activity and reduced glutathione levels in RBC's in control rats, cadmium treated-rats and camel's milk and cadmium treated rats

Parameters	Control	Cadmium	Cadmium + milk
SOD (U mL ⁻¹)	9.8 \pm 0.05	2.43 \pm 2.2*	10.05 \pm 1.6*
CAT (U mL ⁻¹)	120.0 \pm 6.2	63.30 \pm 7.5*	122.81 \pm 6.5*
GSH mmol gm ⁻¹	50.5 \pm 6.5	28.50 \pm 4.7*	48.70 \pm 5.2

Values are given as mean \pm SEM for groups of six animals each. Values are statistically significant at *p<0.05. Cadmium treated rats were compared with control rats; camel's milk Cadmium -treated rats were compared with Cadmium rats

DISCUSSION

Cadmium is a very toxic heavy metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium causes poisoning in various tissues of humans and animals^[9,29]. It has been reported that chronic treatment with cadmium induced oxidative damage in erythrocytes of rats, causing destruction of cell membranes and increased lipid peroxidation, as well as alteration of the oxidative enzyme system, energy metabolism and the appearance of anemia^[17,20].

It is essential to evaluate the level of anaemia by the following measurements: RBC's count hemoglobin concentration, hematocrite and red blood cell indices (MCV, MCH and MCHC). In this study, rats administered cadmium chloride orally showed decreased values of erythrocyte counts, hemoglobin and Hematocrite, as well as decreased MCV, MCH and reduced MCHC, suggestive of microcytic and hypochromic anaemia. This finding is consistent with previous studies of anemia in fish, rats and rabbits exposed to cadmium, lead, nickel, copper and zinc^[30,31].

It could be concluded that the decrease RBC's count, hematocrite value and MCV is due to increase in the rate of RBC's destruction or due to decrease synthesis and release of erythrocytes in circulation.

Oxidative stress can disrupt normal physiological pathways and cause erythrocyte destruction.

Glutathione is a major component of RBCs^[32]. It was suggested that the decrease in cellular glutathione content due to cadmium toxicity can affect the physiological responses of cells^[33]. Oxidative stress develops when the levels of antioxidants as glutathione are lowered and the production of Reactive Oxygen Species (ROS) exceeds the capacity of the cell to dispose of them^[34]. As a result of ROS activity, irreversible modifications of biologically fundamental macromolecules have been described^[35].

On the other hand renal failure, developing under the influence of cadmium, results in erythropoietin deficiency which may induced anemia without a proportional increase of reticulocytes^[36,37]. Chun *et al.*^[38] are of the opinion that cadmium ions significantly affect regulatory genes for erythropoietin, which may be the cause of inhibiting its expression. Additionally, the toxic effect of cadmium in various organs particularly, the kidney and liver has been observed^[93,40] and it is conceivable that the activity of this haematopoietic tissues may be suppressed.

Accompanied with the decreased hemoglobin levels are decreased MHC and MCHC. Blood hemoglobin concentration may be diminished because of hemolysis or because of impaired blood formation in bone marrow in rats administrated cadmium chloride compared with hemoglobin levels in blood of control group rats receiving normal saline. Moreover, Cadmium inhibits the bone marrow to make hemoglobin by interfering with several enzymatic steps in the heme synthesis. Also Cadmium has been found to have direct effect on blood hemoglobin by decreasing its formation as results from two basic red cell defects, shortened life span and impaired heme synthesis. The mechanisms by which synthesis of the red cell pigment heme is inhibited by cadmium involves at least two enzymes, a cytoplasm one (delta-aminoleuvinic acid) at the beginning of heme synthesis and a mitochondrial one, ferrochelatase, at the end of heme synthesis^[41]. Also, cadmium may compete with iron, leading to the occurrence of anemia due to iron deficiency.

In our result, oral administration of Camel's milk to rats alleviated the bad effect of cadmium on hematological parameters. As shown in Table 1, oral administration of Camel's milk to rats significantly increased RBC's, Hemoglobin, Hematocrite and blood indices when compared to rats administered cadmium.

In this study we observed that Cadmium chloride caused severe oxidative stress in the erythrocytes of the experimental mice and that oxidative impairment could be prevented by administration of Camel's milk.

Maintenance of normal cellular functions in the presence of oxygen largely depends on the efficiency of

the defense mechanisms against free-radical mediated oxidative stress. Glutathione are considered to be the first line of cellular defense against Cadmium-mediated oxidative damage. GSH functions by detoxifying various xenobiotics as well as scavenging free radicals and is consequently converted to its oxidized form, glutathione disulfide (GSSG). Cadmium induced cellular toxicity includes alterations in the biological activity of thiol-containing proteins via structural modification^[42]. Chiu *et al.*^[43] reported that treatment of erythrocytes with thiol-reactive substances shortened in vivo survival of those cells. Remarkable decreases in the levels of GSH, has been observed (Table 2) in the erythrocytes of the Cadmium intoxicated animals, which may be due in part to the binding ability of this metal ion with various intracellular sulfhydryl groups. In our results, administration of Camel's milk with cadmium chloride significantly increased the reduced glutathione levels in the RBC's to normal levels as compared to cadmium treated rats.

To prevent biological macromolecules from oxidative damage, antioxidant enzymes are considered to be the second line of cellular defense. In the present study, a significant decrease in the activities of antioxidant enzymes was observed in the red blood cells of the toxin-treated animals (Table 2). The reduction in SOD activity in the erythrocytes of the Cadmium-exposed animals may be due to the accumulation of superoxide radical anions as suggested by earlier reports^[44]. Cadmium intoxication also decreased the activity of CAT, an effect potentially explained by their influence on hydrogen peroxide (a product of SOD) as a substrate (formed in excess in the process of the dismutation reaction of the superoxide radical anion). Oral administration of camel's milk with the cadmium chloride significantly increased and normalized the levels of the enzymatic components of antioxidants system (SOD and CAT) in the RBC's of treated rats.

The protective effect of Camel's milk against Cadmium-induced Anemia and the increase in the levels of oxidative stress in this study could be attributed to its antioxidant and possible chelating effects on cadmium. It has been reported that camel's milk contain high levels of Vitamins C, A, B2 and E and very rich in magnesium and Zinc^[33]. These vitamins are antioxidants found to be useful in reducing the oxidative stress caused by toxic agent. Magnesium deficiency (MgD) has been associated with production of reactive oxygen species^[45]. Magnesium protects cells from heavy metals such as aluminum, mercury, lead, cadmium, beryllium and nickel, which explains why re-mineralization is so essential for heavy metal detoxification and chelating. Magnesium protects the

cell against oxyradical damage and assists in the absorption and metabolism of B vitamins, vitamin C and E^[45], which are antioxidants important in cell protection. Recent evidence suggests that vitamin E enhances glutathione levels and may play a protective role in magnesium deficiency-induced cardiac lesions^[46]. Also it has been reported that magnesium is very essential for biosynthesis of glutathione, because the enzyme Glutathione synthetase requires γ -glutamyl cysteine, glycine, ATP and magnesium ions to form glutathione^[47].

Another interpretation for the improvement of the parameters of the present investigation may be due to the decrease of cadmium accumulation in liver and kidneys which are important haematopoietic organs in the rats receiving Camel's milks in addition to cadmium. Camel's milk is rich in Zinc (Zn)^[33]. Zinc is a trace element essential for living organisms. More than 300 enzymes require Zn for their activity. It also plays an important role in the DNA replication, transcription and protein synthesis, influencing cell division and differentiation^[48]. It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system^[49-51]. Zinc is an essential component of the oxidant defense system and functions at many levels^[52]. One study has shown that Zn deficiency in the diet paves the way for cell damage in the rat testis^[53]. Furthermore, Zn deficiency increases lipid peroxidation in various rat tissues, whereas the Zn supplementation corrects the impairment^[51-53]. Interestingly, a protective effect of zinc (Zn) has been reported in vitro against the cellular toxicity due to cadmium, Zinc protection is probably due to an action on oxidative stress and apoptosis^[54,55].

Also, it might occur that Camel's milks reduced the renal uptake of cadmium by competition for a common transporter and demonstrates protective actions against the damages of hepatocytes and renal function during cadmium intoxication in the rats.

CONCLUSION

In conclusion, our results reported that aluminum chloride is capable of caused marked alterations in some hematological parameters, induced anemia and inhibited the activities of antioxidant components in the RBC's. While, Camel's milk administered with cadmium chloride minimized its hazards. Consequently, attention should be paid toward sources of cadmium in foods, water other sources. Furthermore, Drinking Camel's milk could be beneficial in alleviating cadmium toxicity.

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