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| RESEARCH ARTICLE

CCL4-Induced Hepatotoxicity: Study in Rats Intoxicated with Carbon Tetrachloride and Treated with Camel Milk and Urine

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| ABSTRACT
The liver is responsible for the metabolism and detoxification of the most of components that enter the body. Carbon tetrachloride (CCl4) is a highly toxic chemical agent, the most famous drug used to induce liver damage experimentally. Camel milk has been deeply studied for its special properties because of its higher hepatoprotective, insulin and antibacterial activities. The present study was designed to examine the preventive effects of camel milk (CM) and camel urine against the toxic effects of acute exposure to carbon tetrachloride (CCl4) on the liver tissue of mice. Administration of a single dose of CCl4 caused liver toxicity as monitored by an increase in liver enzymes, including ALT, AST and ALP. A total of 24 albino rats (200–250 g) were divided randomly into 4 groups comprising 6 rats in each group, G1 The first group is untreated control, G2 was the positive CCl4, (G3) Rats fed with Camel milk (100 ml/24 h/cage) injected with CCl4, (G4) Rats fed with Camel Urine (100 ml/24 h/cage) injected with CCl4. A significant ($P < 0.05$) increase in serum AST, ALT, and ALP activities was observed in the CCl4-treated rats compared with those of the control rats, respectively. Based on this study, Camel milk and camel urine have a protective effect against CCL4-Induced Hepatotoxicity.

| KEYWORDS

CCl4; camel milk; hepatotoxicity

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1. Introduction

The liver is the largest gland and vital organ in the body due to its functionality, and without its presence, survival is impossible [Ben-Dan, 2008]. It performs more than 500 tasks and plays a major role in carbohydrates, proteins, fats, steroids and medicines metabolism. Besides, it is involved in the activation, storage and transport of vitamins, minerals and nutrients along with the synthesis of non-essential amino acids [Frühbeck, 2018]. The liver produces and excretes bile, converts ammonia to urea, and operates as a filter by removing bacteria and debris from the blood through phagocytosis by Kupffer cells. Hepatocytes execute functions like glycolysis (break down of glucose), glycogenesis (storage of glucose as glycogen), glycogenolysis (catabolism of glycogen) and gluconeogenesis (production of new glucose from non-carbohydrates molecules) [Hall, 2015]. Carbon tetrachloride (CCl4) is widely used to induce hepatotoxicity in experimental animals. CCl4 hepatotoxicity is characterized by hepatocellular necrosis with fat deposition. At acute toxic doses of CCl4, when hepatocellular necrosis exceeds the regenerative capacity of the liver, fatal liver failure often ensues. High doses of CCl4 result in nonspecific toxicity, including central nervous system depression and respiratory failure resulting in death. CCl4 belongs to the class of hepatotoxins, which act after metabolic activation. It is metabolized in the endoplasmic reticulum by cytochrome p450 enzymes (mostly CYP2E1) to the highly reactive trichloromethyl radical ($CCl_3•$). $CCl_3•$ rapidly reacts with oxygen to form the highly reactive trichloromethyl peroxyl radical ($CCl_3OO•$), which rapidly reacts with lipids to form lipid peroxidation products. Polyunsaturated fatty acids or PUFA of the ER and mitochondria are more susceptible to oxidation by the free radicals. Free radical-mediated lipid peroxidation is one of the main mechanisms of hepatic injury by CCl4 [Weber et al., 2003]. Camel's milk is different from other ruminant milk; it is low in cholesterol, sugar and protein but high in minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamins A, B2, C and E and contains a high

concentration of insulin. It has no allergic properties and can be consumed by lactase deficient individuals and those with a weakened immune system; in fact, this milk is believed to have medicinal properties. In Sahara, fresh butter made from camel's milk is often used as a base for medicines. Other products also developed with camel's milk include cosmetics or pharmaceuticals. A series of metabolic and autoimmune diseases are successfully being treated with camel's milk. Furthermore, in India, camel's milk is used therapeutically to treat dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes [Rao, 2016].

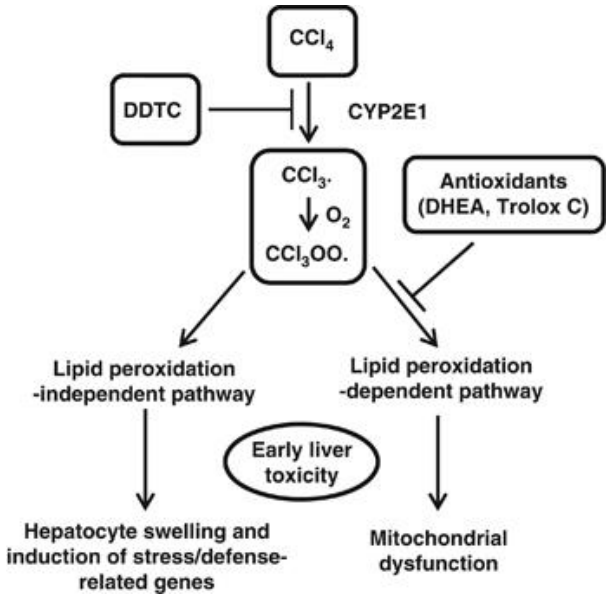

Figure 1. Mechanism CCL4-Induced Hepatotoxicity

2. Materials

2.1 Camel's milk and urine

Camel's milk and Camel's Urine samples were collected daily early in the morning from camel farm, United Arab Emirates. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory.

2.2 Animals

A total of 24 albino rats (200–250 g) were obtained from the Laboratory house of Dubai Pharmacy College, United Arab Emirates and acclimated for 10 days before starting the experiment. All animals were housed in standard cages (6 rats/cage), feeding with a standard laboratory diet and tap water ad libitum. The experimental animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity and kept on a 12 h light/12 h dark cycle. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the ethics of research committee of Dubai Medical College, Dubai, United Arab Emirates.

2.3 Experimental groups

The rats were divided randomly into 4 groups comprising 6 rats in each group and fed the same diet throughout the experimental period. The experimental design is described as fellow: Group 1 The first group is the untreated control group and was Control, rats fed only with diet and tap water. Group 2 was the positive CCl4 control group and received only CCl4 (1 ml/kg body weight): olive oil (1:1) on the first and fourth day of every week intraperitoneally injected for 4 weeks. This group represents the positive control. Group 3 Rats fed with Camel milk (100 ml/24 h/cage) and normal diet and intoxicated with CCl4 on the last two days of the experimental month, injected with CCl4 [(1 ml/kg body weight): olive oil (1:1)], which is tested for hepatoprotective effect. Group 4 Rats fed with Camel Urine (100 ml/24 h/cage) and normal diet and intoxicated with CCl4 on the last two days of the experimental month, injected with CCl4 [(1 ml/kg body weight): olive oil (1:1)], which is tested for hepatoprotective effect.

2.4 Blood collection

At the end of day 30, 24 h after the last CCl4 injection, the animals were sacrificed, and the blood samples were collected directly into tubes, and it was allowed to clot at room temperature for 30 min, and the serum was separated by centrifugation at 1000x g for 15 min at 4°C and was saved in aliquots and stored at -80°C for further analysis. Serum biochemistry: ALT, AST and ALP serum activities were measured to assess hepatotoxicity by CCl4. albumin and cholesterol were also measured using spectrophotometric diagnostic kits.

3. Statistical

Data were entered and analyzed using SPSS statistical package. Numerical data were expressed as means and standard deviation. The significance of the difference between means was tested by one-way ANOVA, depending on the number of compared groups, with a p-value of≤0.05 considered statistically significant.

4. Results

Table 1. Biochemical parameters in all groups

Biochemical Parameters	Group 1	Group 2	Group 3	Group 4	P value
ALT (IU/L)	101 ± 14.6	157 ± 24.3	177 ± 27.4	132 ± 3.2	P< 0.05
AST (IU/L)	104 ± 6.9	135 ± 13.6	182 ± 46.5	43 ± 4.9	P< 0.05
ALP (IU/L)	146.8±11.2	1209 ± 2.59	835 ± 89	706 ± 0.54	P< 0.05
Glucose (mg/dl)	64.4± 3.19	166.1 ±30.1	160.2± 9.3	140.8 ±1.0	P< 0.05
Creatinine (mg/dl)	0.3 ±0.15	0.3 ±0.0	0.3± 0.0	0.36 ±0.05	P< 0.05
Urea (mg/dl)	27.3± 0.53	44.0± 10.9	34.9 ±13.2	20.2± 0.1	P< 0.05
Uric acid (mg/dl)	1.6± 0.3	1.6 ±0.33	1.7 ±0.1	0.6 ±0.05	P< 0.05
Total Protein (mg/dl)	6.1 ±0.18	15.5± 1.3	16.3± 1.9	14.9 ±0.54	P< 0.05
Cholesterol (mg/dl)	59.7± 1.03	81.7 ±16.0	68.8 ±10.2	74.4± 1.09	P< 0.05
Triglycerides (mg/dl)	35.6± 0.82	75.4± 8.6	56.8 ±20.7	85.7 ±0.65	P< 0.05
HDL (mg/dl)	33.9 ±1.4	45.0 ±9.4	38.8± 1.25	20.9± 0.49	P< 0.05
LDL (mg/dl)	18.1± 0.39	20.4± 6.08	17.8 ±0.05	31.0± 0.4	P< 0.05

AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatases; TG, Triglycerides; Ch, cholesterol; HDL, High density lipoproteins of cholesterol; LDL, Low density lipoproteins of cholesterol

Table 2. Complete blood count and blood indices in all groups

Blood Indices	Group 1	Group 2	Group 3	Group 4	P value
WBC Cells per cubic millimeter	7.60 ± 0.41	16.6 ± 1.23	19.6 ± 1.1	13.4 ± 2.3	0.000
RBC Million cells per cmm	7.2 ± 0.211	7.40 ± 0.99	6.5 ± 0.29	7.6 ± 0.63	0.052
Hbg grams per deciliter (g/dL)	13.0 ± 046	12.6 ± 0.61	12.6 ± 0.44	14.3 ± 1.30	0.012
HCT %	37.0 ± 029	32.7 ± 1.11	32.8 ± 1.96	35.8 ± 2.5	0.002
MCV Femtoliters	50.9 ± 047	49.5 ± 0.91	49.7 ± 1.41	46.3 ± 3.8	0.019
MCH Picograms	36.6 ± 047	18.6 ± 0.58	18.5 ± 0.74	18.9 ± 0.91	0.000

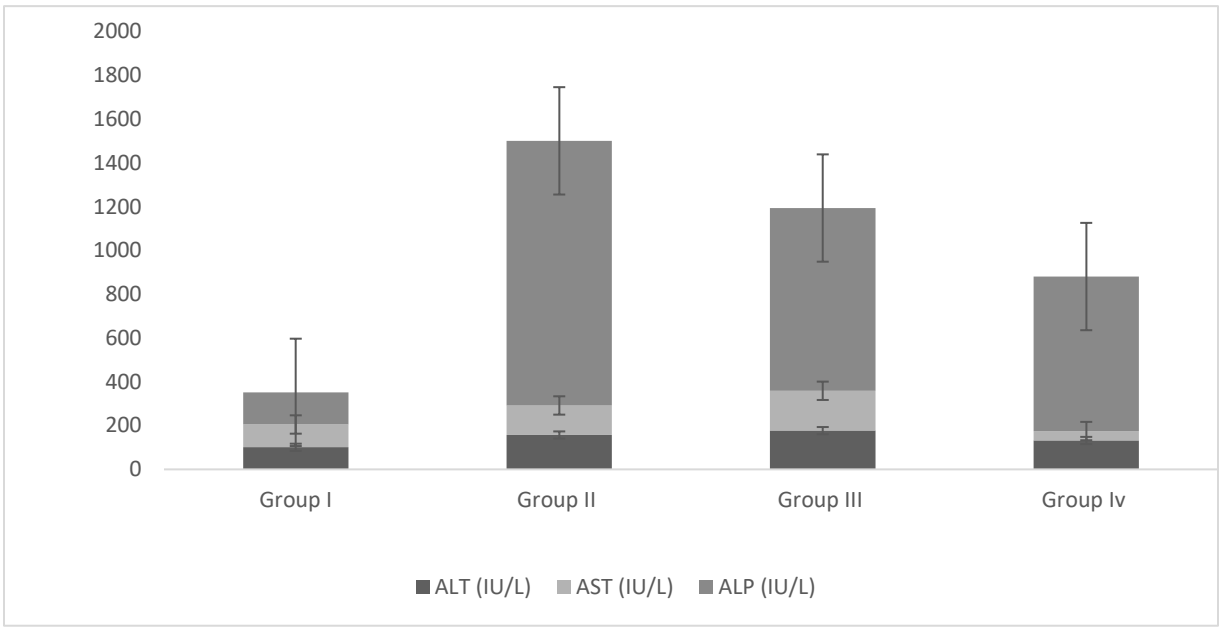

Figure 2. Liver enzymes activity in all groups

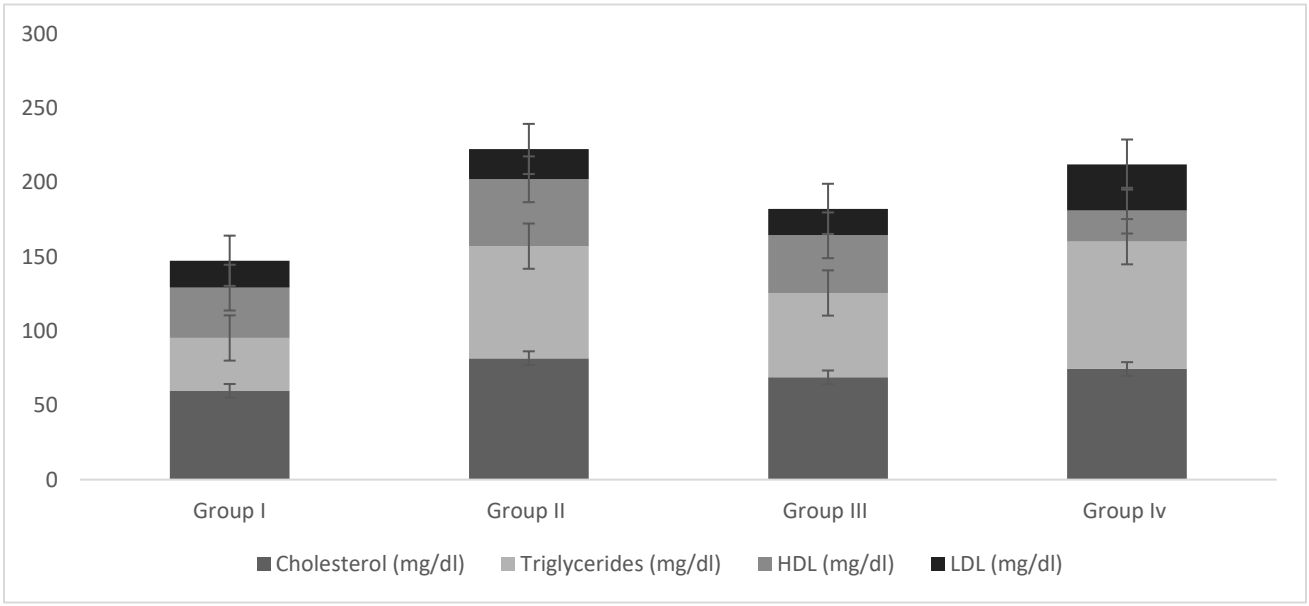

Figure 3. Lipid panel concentration in all groups

5. Discussion

The liver is the largest gland and vital organ in the body due to its functionality, and without its presence, survival is impossible [1]. CCl4 hepatotoxicity is characterized by hepatocellular necrosis with fat deposition. At acute toxic doses of CCl4, when hepatocellular necrosis exceeds the regenerative capacity of the liver, fatal liver failure often ensues [5, 6]. In the present study, serum hepatic biomarkers, ALT activities in groups I, II, III and group IV were 101 ± 14.6, 157 ± 24.3, 177 ± 27.4 and 132 ± 3.2; respectively. AST activities in groups I, II, III and group IV, were 104 ± 6.9, 135 ± 13.6, 182 ± 46.5 and 43 ± 4.9; respectively, and ALP activities in groups I, II, III and group IV were 146.8±11.2, 1209 ± 2.59, 835 ± 89 and 706 ± 0.54; respectively. A significant ($P < 0.05$) increase in serum AST (135± 13.6 IU/L), ALT (157± 24.3 IU/L), and ALP (1209 ± 2.59 IU/L) activities were observed in the CCl4-treated rats compared with those of the control rats (104 ± 6.9 IU/L, 101±14.6 IU/L, 146.8±11.2 IU/L) respectively. These results suggest that these hepatic biomarkers were elevated in the serum due to a release of enzymes from the damaged liver. The increased serum levels of hepatic markers have been attributed to the liver injury because these enzymes are placed in the cytoplasmic area of the cell and are released into circulation in case of cellular damage [8, 9]. a significant decrease ($P < 0.05$) was observed in the respective serum activities of the rats that were treated with camel urine compared with rats treated with camel

milk (43±4.9 IU/L, 132 ±3.2 IU/L, 706 ±0.54 IU/L), (182 ± 46.5 IU/L, 177 ± 27.4 IU/L, and 835±89 respectively) compared with those of the CCl4-treated rats. Results of the present study have also established that the CCl4 treatment could have affected the lipid metabolism of the liver (triglyceride and cholesterol levels). Cholesterol (mg/dl) in groups I, II, III and group IV was 59.7± 1.03, 81.7 ±16.0, 68.8 ±10.2 and 74.4± 1.09; respectively. Triglycerides (mg/dl) in groups I, II, III and group IV was 35.6± 0.82, 75.4± 8.6, 56.8 ±20.7 and 85.7 ±0.65. This is evidenced from the present observations that CCl4 caused a significant ($p < 0.05$) increase in the levels of lipid parameters. Muller et al. stated that CCl4 intoxication is similar to hepatitis in the case of the triglyceride's catabolism. In our study, Urea (mg/dl) in groups I, II, III and group IV were 27.3± 0.53, 44.0± 10.9, 34.9 ±13.2 and 20.2± 0.1; respectively. Table 1. Figure 2, 3. The protective effect of camel milk could be attributed to its antioxidant activity. It has been reported that camel milk contains high levels of vitamins A, B2, C, and E, and it is very rich in magnesium (Mg), manganese, zinc (Zn), copper, and other trace elements. These vitamins are antioxidants that are useful in preventing tissue injury caused by toxic agents [11]. Urine-therapy can only be used as an unconventional or complementary medical practice on the basis of trial and error. Many diseases, such as abdominal tumors, tuberculosis, haemorrhoids, leprosy, dropsy, abdominal enlargement, flatulence, colic and anemia, have been treated with the urine of animals, including goats, sheep, buffalo and camels [12]. Hbg (g/dL) were 13.0 ± 046, 12.6 ± 0.61, 12.6 ± 0.44 and 14.3 ± 1.30; respectively. For haemoglobin, there was a significance ($p<0.05$) difference in the CCL4 group compared with both control and camel milk and urine groups with values (12.6 ± 0.61) and (13.0 ± 0.46, 12.6 ± 0.61, 14.3 ±1.30, respectively)— table 2.

6. Conclusion

CCl4 has adverse effects on human health. Our results demonstrate that CCl4 is capable of inducing marked alterations in biochemical parameters. Camel's milk administered minimized CCl4-associated hazards. Therefore, drinking camel's milk could be beneficial for alleviating CCl4 toxicity. From this study, we concluded that camel urine has a protective effect against toxicity induced by CCL4.

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