

Hepatoprotective Effects of Camel Milk and Urine on Carbon Tetrachloride (CCL₄) induced Liver Damage

Khan Rawoof*, Hussien Mohamed, Gouman Ali, Talib Ahmed, Ghaznwi Ramallah

Dubai Institute for Environmental and Laboratory Research, Dubai, UAE

ABSTRACT

Carbon tetrachloride (CCL₄) is a highly toxic chemical agent and is the most commonly used drug to experimentally induce liver damage. Aim of the study the aim is to investigate the possible protective role of both camel milk and urine on CCL₄ induced liver damage. Method 20 rats were used in the study and they were classified into 4 different groups. Group I control, Group II CCL₄ group, Group III (milk+CCL₄), Group IV (urine+CCL₄). Biochemical parameters in addition to liver enzymes were estimated to evaluate the liver damage. Results the CCL₄ treatment markedly affected the liver-specific enzyme activities. 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 was 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. Concluded from this study that Camel urine has protective effect against CCL₄ induced liver damage more than Camel milk.

Keywords: Carbon Tetrachloride, Camel Milk, Camel Urine, Liver Damage.

I. INTRODUCTION

The liver is a key organ that regulates many important metabolic, detoxification, and secretory functions in the body. Hepatic injury is associated with disruptions of these metabolic functions. Carbon tetrachloride (CCL₄) is a highly toxic chemical agent and is the most commonly used drug to experimentally induce liver damage. Histopathological examination of liver tissue sections indicated that CCL₄ induces fibrosis, cirrhosis, and hepatocarcinoma. The toxic effect of CCL₄ is attributed to the production of trichloromethyl radicals during oxidative stress. The numbers of infiltrated neutrophils, macrophages, Kupffer cells, lymphocytes, and natural killer cells are significantly increased after liver injury induced by hepatotoxins such as CCL₄. CCL₄ induces the activation of resident liver macrophages and (or) the chemoattraction of extrahepatic cells (e.g., neutrophils and lymphocytes).

The activated macrophages are then released and contribute to liver fibrosis, inflammation, and injury. Once the liver is injured, the efficiency of treatment with common drugs is limited. Therefore, interest in using alternative medicines for the treatment of hepatic disease has arisen. The camel is among the animals mentioned in the Quran as a miracle of God. Camel milk has been shown to have medicinal effects; thus, Islamic populations have been encouraged and permitted to drink camel milk in cases in which medical treatment is necessary [1].

Milk of a specific humped camel (*Camelus dromedaries*) has been medically used for centuries in different regions of Arab countries [2]. reported that over 200 proteins were identified by two-dimensional gel electrophoresis and peptide mass mapping and liquid chromatography. The same authors found some known camel milk proteins, including heavy-chain

immunoglobulins, antioxidative peptides, lactoperoxidase, and lactoferrin protein and others exhibiting regions of exact homology with proteins from other species. The peptides and proteins present in camel milk exert biological activities that have beneficial effects on many bioprocesses such as digestion, absorption, growth, and immunity. Camel milk is different from the milk of other ruminant animals; it is low in cholesterol and sugar but high in minerals (sodium, potassium, iron, copper, zinc, and magnesium) and vitamins (A, B2, C, and E), and it contains a high concentration of insulin. Furthermore, camel milk can be stored at room temperature for a longer period than milk from other animals. The most commonly described uses for camel milk are as a drug against autoimmune diseases, dropsy, jaundice, splenomegaly, tuberculosis, asthma, anemia, piles, and diabetes. In addition, camel milk has antitoxic effects against cadmium chloride, CCL₄, cisplatin, paracetamol, and aluminum chloride. Patients who suffer from chronic hepatitis show improved liver function after drinking camel milk [3].

Currently, urine-therapy can only be used as an unconventional or complementary medical practice on the basis of trial and error, and requires significant research to support its use in conventional medicine. 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, elephants, horses, camels and donkeys. The use of cattle urine (cows and oxen) has been reported in India and Tibet [4].

II. METHODS AND MATERIAL

20 Wistar albino rats of both sexes weighing 150 - 250 g were used. They were obtained from the Animal House of Dubai Institute for Environmental Research and Laboratory Analysis, Dubai, U.A.E. After one-week adaptation period, housed in cages, maintained in a light room under normal environmental conditions with temperature (28 °C ± 2 °C) plus relative

humidity (61%), with free access to water and food, they were divided into 4 groups. The research was carried out according to the rules governing the use of laboratory animals as acceptable international.

Fresh milk was collected from female camels of different ages and lactation periods during early morning milking time, while fresh urine from young female camels (Bekra) (6 months up to 3 years old) was collected early morning during normal urination, this was done daily for 35 days during all experiment period.

Experimental rats were randomly divided into four groups of five rats each.

Group 1: served as the control rats, **Group 2:** was administered with CCL₄ only in groundnut oil (1:1) at a dose of 3 ml/kg B. wt. by single intraperitoneal administration. **Group 3:** was administered with Camel Milk + CCL₄ (2.5 ml/kg B. wt./day), **Group 4:** was administered with Camel Urine + CCL₄ (2.5 ml/kg B. wt./day).

Blood samples were collected from the optic vein of rat eye. The markers of liver damages as aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were determined. In addition, serum total protein, Kidney Function Tests were also determined. All samples were analyzed by using commercial kits (Randox Laboratories Ltd., U.K.).

III. STATISTICAL ANALYSIS

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

IV. RESULTS AND DISCUSSION

Effects of CCL₄ and treatments with Camel milk and Urine on liver-specific enzymes The activities of AST, ALT, and ALP were estimated in serum samples as biomarkers of liver function. These results are provided in Table 1. The CCL₄ treatment markedly affected the liver-specific enzyme activities. 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 was 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. However, 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. Effects of CCL₄ and treatments with Camel milk and Urine on complete blood count (CBC). The amount of WBCs, RBCs, hemoglobin, HCT, MCV and MCH were estimated using CBC analyzer there was significance ($p < 0.05$) difference in WBCs in CCL₄ group comparing with both control and camel milk and urine groups Table (2) with values (16.6 ± 1.23) and (7.60 ± 0.41 , 19.6 ± 1.1 , 13.4 ± 2.3 , respectively). For RBCs there was significance ($p < 0.05$) difference in CCL₄ group comparing with both control and camel milk and urine groups with values (7.40 ± 0.99) and (7.2 ± 0.211 , 6.5 ± 0.29 , 7.6 ± 0.63 , respectively). For hemoglobin there was significance ($p < 0.05$) difference in CCL₄ group comparing 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). For HCT there was significance ($p < 0.05$) difference in CCL₄ group comparing with both control and camel milk and urine groups with values (32.7 ± 1.11) and (37.0 ± 0.29 , 32.8 ± 1.96 , 35.8 ± 2.5 , respectively). For MCV there was significance ($p < 0.05$) difference in CCL₄ group

comparing with both control and camel milk and urine groups with values (49.5 ± 0.91) and (50.9 ± 0.47 , 49.7 ± 1.41 , 46.3 ± 3.8 , respectively). For MCH there was significance ($p < 0.05$) difference in CCL₄ group comparing with both control and camel milk and urine groups with values (18.6 ± 0.58) and (36.6 ± 0.47 , 18.5 ± 0.74 , 18.9 ± 0.91 , respectively).

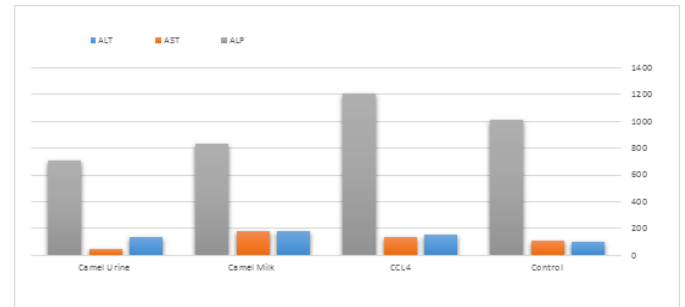


Fig. (1): Liver enzymes in CCL₄, Control, Camel milk and urine groups

Table (1): Liver enzymes in all groups

Parameter	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group I	101 ± 14.6	104 ± 6.9	146.8 ± 11.2
Group II	157 ± 24.3	135 ± 13.6	1209 ± 2.59
Group III	177 ± 27.4	182 ± 46.5	835 ± 89
Group IV	132 ± 3.2	43 ± 4.9	706 ± 0.54
P value	0.000	0.000	0.000

Table (2): Biochemical Parameters in all groups

Parameters	Group I	Group II	Group III	Group IV	P value
Glucose (mg/dl)	64.4 ± 3.19	166.1 ± 30.1	160.2 ± 9.3	140.8 ± 1.0	0.000
Creatinine (mg/dl)	0.3 ± 0.15	0.3 ± 0.0	0.3 ± 0.0	0.36 ± 0.05	0.599
Urea (mg/dl)	27.3 ± 0.53	44.0 ± 10.9	34.9 ± 13.2	20.2 ± 0.1	0.003
Uric acid (mg/dl)	1.6 ± 0.3	1.6 ± 0.33	1.7 ± 0.1	0.6 ± 0.05	0.000
T. Protein (g/dl)	6.1 ± 0.18	15.5 ± 1.3	16.3 ± 1.9	14.9 ± 0.54	0.000
Cholesterol (mg/dl)	59.7 ± 1.03	81.7 ± 16.0	68.8 ± 10.2	74.4 ± 1.09	0.015
Triglyceride (mg/dl)	35.6 ± 0.82	75.4 ± 8.6	56.8 ± 20.7	85.7 ± 0.65	0.000
HDL (mg/dl)	33.9 ± 1.4	45.0 ± 9.4	38.8 ± 1.25	20.9 ± 0.49	0.000
LDL (mg/dl)	18.1 ± 0.39	20.4 ± 6.08	17.8 ± 0.05	31.0 ± 0.4	0.000

Significance $p < 0.05$, insignificance $p > 0.05$

V. DISCUSSION

The liver is a key organ that regulates many important metabolic, detoxification, and secretory functions in the body. Hepatic injury is associated with disruptions of these metabolic functions. Carbon tetrachloride (CCL₄) is a highly toxic chemical agent and is the most commonly used drug to experimentally induce liver damage. Histopathological examination of liver tissue sections indicated that CCL₄ induces fibrosis, cirrhosis, and hepatocarcinoma [5]. The toxic effect of CCL₄ is attributed to the production of trichloromethyl radicals during oxidative stress.

Carbon (CCL₄) inhibits the secretion of the amylase system, which is one of the systems that metabolizes carbohydrates or carbon tetrachloride (CCL₄) may affect the nuclear material of the cells, leading to liver cell degeneration. Some nuclei of this cell characterized by passing stages programmed death has confirmed the negative impact results by observing the presence of necrosis and ascites, And blood hemorrhage. Increased thickness of cell membranes was also observed. This may be due to the effect of carbon tetrachloride (CCL₄) on proteins or lipids forming the cellular membrane and thus increasing the thickness of the cell membrane. Most the cells of the liver have been spotted with fat droplets, possibly due to hepatitis. The present study aimed to clarify the possible beneficial effects of camel milk and urine against CCL₄-induced liver cirrhosis using biochemical assay. Liver enzymes were used to evaluate the liver functions: AST and ALT, and ALP are all reliable indicators of liver function. In the present study, the activities of these enzymes were greatly increased in the rats treated with CCL₄ compared with control rats. The CCL₄ treatment markedly affected the liver-specific enzyme activities. 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 was 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. However, 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. The liver damage induced by CCL₄ was reflected in increased serum ALT, AST, and ALP activities [6].

For group II there was significant positive correlation ($p < 0.05$) between ALT and AST with ($R = 0.999$), Also there was insignificant positive correlation ($p > 0.05$) between ALT and ALP. For group III there was significant positive correlation ($P < 0.05$) between ALT and AST ($R = 1.00$), Also there was significant negative correlation ($p < 0.05$) between ALT and ALP ($R = -1.00$). For group IV there was significant negative correlation ($p < 0.05$) between ALT and AST ($R = -0.667$), Also there was positive correlation ($p < 0.05$) between ALT and ALP. From a biochemical point of view, these disturbances can be considered a direct reflex of marked liver damage caused by the selectively destructive cytotoxic effect of CCL₄ on liver cells [7]. Also, CCL₄ induces lipid peroxidation and subsequently leads to a loss of membrane fluidity, changes in the membrane potential, increases in the membrane permeability, and enzymatic leakage [8]. 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 [9]. From a biochemical point of view, these disturbances can be considered a direct reflex of marked liver damage caused by the selectively destructive cytotoxic effect of CCL₄ on liver cells [10]. Also, CCL₄ induces lipid peroxidation and subsequently leads to a loss of membrane fluidity, changes in the membrane potential, increases in the membrane permeability, and enzymatic leakage. For Lipids there were increasing in both cholesterol and

triglycerides in group II with values (81.7± 16.0 and 75.4 ±8.6, respectively). For group III there were increasing in HDL- Cholesterol and decreasing in LDL- Cholesterol with values (38.8 ±1.25 and 17.8 ±0.05), respectively.

VI. CONCLUSION

From this study we concluded that Camel urine has initial protective effect against hepatocellular toxicity induced by CCL₄ more than Camel milk.

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