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Protective Effect of Camel Milk Against Carbon Tetrachloride Hepatotoxicity in Rats

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Abstract: A total of 20 rats was divided into four equal groups to study the hepatoprotective effect of camel milk against carbon tetrachloride toxicity. Rats of the first group and second groups were injected i/p with paraffin oil and drank either tap water (control 1) or camel's milk (control 2), respectively. Rats of the third and fourth groups were injected i/p with CCl_4 and drank either tap water or camel's milk, respectively. At the end of the experiment (5 weeks), liver samples were collected from each group and liver tissues were cut into small pieces and immersed in neutral buffered formalin 10% for histopathology. In addition, blood samples were collected and the harvested sera were used for determination of liver injuries biomarkers enzymes. The present findings revealed that a great amount of mononuclear cells infiltration, necrotic cells and few fibroblasts were observed in liver of CCl_4 group. The liver of carbon tetra chloride-intoxicated rats and treated with camel milk exhibited clear hepatic recovery characterized by a complete regeneration of hepatocytes and the hepatic tissue appeared more or less normal in most cases. CCl_4 challenge elevated serum alanine and aspartate aminotransferase but these effects were prevented by the treatment of rats with camel milk. The present study concluded that camel milk treatment may play a protective role by improving the changes in histological structure against CCl_4-induced liver damages and enhancing liver enzyme activities in rats.

Key words: Camel Milk · Ccl_4 · Liver Tissues · Histopathology · Enzymes

INTRODUCTION

Histological sectioning of liver tissues has indicated that CCl_4 induces fibrosis, cirrhosis and hepatocarcinoma [1, 2]. The toxic effect of CCl_4 is attributed to trichloromethyl radical produced during oxidative stress [3]. The number of infiltrated neutrophils, macrophages, Kupffer cells, lymphocytes and natural killer cells increases significantly after liver injury induced by hepatotoxins such as Ccl_4, alcohols, d-galactosamine, etc., most of which causes activation of liver resident macrophages and/or chemo attraction of extra hepatic cells (e.g. neutrophils and lymphocytes) [4]. The activated macrophages are release to cell death ligand (tumour necrosis alpha) and contributed to liver fibrosis, inflammation and injury [5-7].

Once the liver became injured, its efficient treatment with famous chemical drugs is limited [8]. Therefore, interest concerned the use of alternative medicines for the treatment of hepatic disease has been arisen. The camel milk has been used as drug against autoimmune diseases, tuberculosis, asthma and diabetes [9] with antimicrobial activities [10] and antitoxic effect [11-14]. Few publications have investigated the protective effect of camel milk against CCl_4 induced hepatotoxicity in rats. Therefore, the present study investigated the protective effects of camel's milk against CCl_4-induced hepatotoxicity in rats by biochemical assaying and histopathology of liver tissues.

MATERIALS AND METHODS

Chemicals and Kits: Diagnostic kits for serum total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine were purchased from ELIPSE, United diagnostic industry, UDI, Dammam, Saudi Arabia). Paraffin oil, carbon tetrachloride (Spectrosol® BHD chemicals Ltd pool, England) and other chemicals and solvents used in the study were of highest grade and commercially available.

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Camel's Milk: Camel's milk samples were collected daily early in the morning from camel farm in Camel Research Center, King Faisal University, Al-Ahsa (Western Province), Saudi Arabia.. Milk was collected from camels by hand milking. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory. The rats were given this fresh milk (100mL/24h/cage) as such without any further treatment.

Animals: A total of 20 albino rats (200-250 g) was obtained from Laboratory House of College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia and acclimated for 10 days before starting the experiment. All animals were housed in standard cages (5 rats/cage), fed with 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 12h light/12hdark cycle. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by ethics of scientific research committee of king Faisal University, Saudi Arabia.

Induction of Hepatotoxicity by Ccl_4: Liver toxicity was induced by the intraperitoneal injection of CCl_4 (1 ml/kg b.wt.), 1:1 diluted with paraffin oil, for two successive days of the experiment [15].

Experimental Groups and Protocol: The rats were divided randomly into 4 groups comprising 5 rats in each group and fed the same diet throughout the experimental period.

Group 1: Control rats fed only with diet and tap water and injected I/p with paraffin oil, group 2: Rats were injected i/p with paraffin oil and received camel's milk (Positive control; (100mL/24h/cage) as their sole source of drinking water.

Group 3: Diseased control group intoxicated with CCl_4 (1 ml/kg b.wt.), 1:1 diluted with paraffin oil in first two days of the experiment and drank tap water and group 4: Rats intoxicated with CCl_4 (1 ml/kgb.wt.), 1:1 diluted with paraffin oil in first two days of the experiment and then treated with camel's milk (100mL/24h/cage) as their sole source of drinking water.

Blood and Tissue Collection: At the end of the experiment, the overnight fasted animals (the control and experimental animals) were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture before incision of the abdomen; 5 ml of blood samples were collected in plain tubes, serum was collected and frozen at -30°C until the time of analysis.

Histopathological Examination: Liver tissues were cut in small pieces and immersed in neutral buffered formalin 10% for 24 h. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques [16]. The extent of CCl_4-induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E), using standard techniques.

Biochemical Analysis: Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of total proteins (EP56-660), albumin (EP03-570), ALT (EP07-500), AST (EP15-500) and creatinine (EP33K-660) on ELIPSE full automated chemistry analyzer (Rome, Italy). Concentration of the biochemical constituents was calculated according to the manufacture instruction.

Statistical Analysis: Results were expressed as Means ± standard error of mean (SEM). The significance of differences was calculated by using student t-test, $p<0.05$ was considered statistically significant.

RESULTS

Histopathological Findings: Examination of liver of control rats revealed that hepatocytes, portal triads and vasculature appear normal. The liver of camel milk only treated rats did not reveal any pathological alterations (necrosis, inflammation or fibrosis) (Fig. 1a). The liver of carbon tetra chloride-intoxicated rats showed massive fatty change and centrilobular necrosis in most cases. Additionally, single hepatic cell necrosis (apoptosis) was clearly observed in some cases (Fig. 1b). Hepatitis characterized by mononuclear cells infiltration mostly macrophages and lymphocytes around central veins and in portal areas was also noticed in most cases of carbon tetrachloride intoxicated rats (Fig.1c).

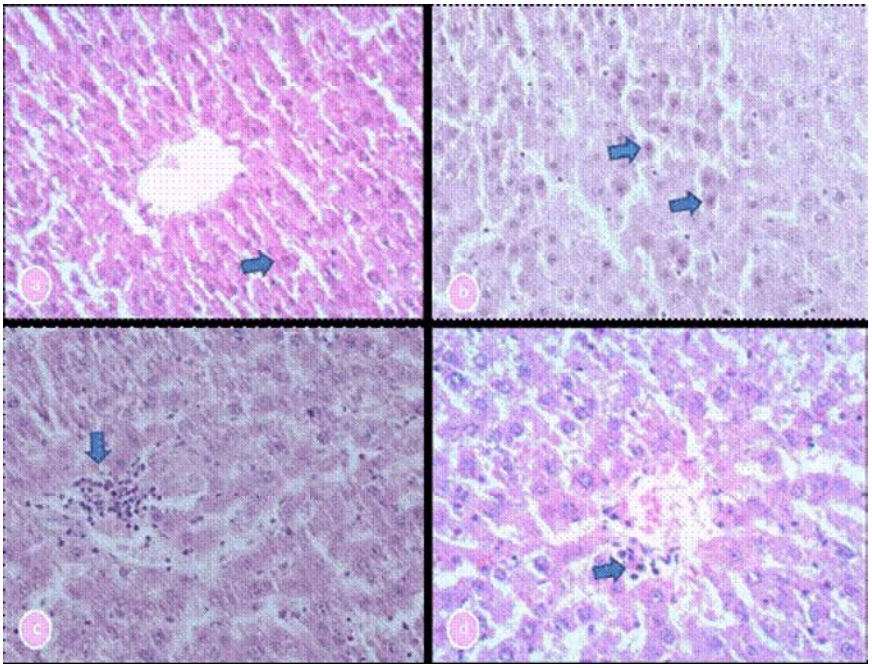

Fig. 1: Histopathological changes induced by CCl4 and camel milk treatments (H&E X400). (a): Liver of camel milk only treated rats showing normal central veins and normal hepatocytes (arrow). (b): Liver of carbon tetra chloride-intoxicated rats showing massive number of apoptotic hepatocytes (arrows). (c): Liver of carbon tetra chloride-intoxicated rats showing mononuclear cells infiltration in portal area (arrow). (d): Liver of camel milk + CCl4 treated rats showing few numbers of mononuclear cells around central veins.

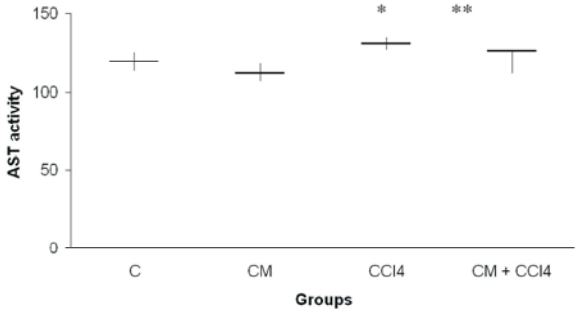

Fig. 2: Effect of CCl_4 and camel milk on serum AST activity in rats.
*Significantly different from control group (p<0.05).
**Significantly different from CCl_4 group (p<0.05).

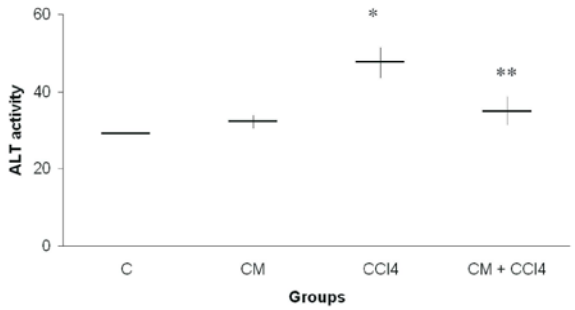

Fig. 3: Effect of CCl_4 and camel milk on serum ALT activity in rats.
*Significantly different from control group (p<0.05).
**Significantly different from CCl_4 group (p<0.05).

The liver of carbon tetra chloride-intoxicated rats and treated with camel milk exhibited clear hepatic recovery characterized by a complete regeneration of hepatocytes and the hepatic tissue appeared more or less normal in most cases (Fig. 1d).

Biochemical Findings: The activities of AST (Fig. 2) and ALT (Fig. 3) were estimated in serum samples as the liver function biomarkers. The CCl_4 treatment markedly affected the liver specific enzymes. It was found that a significant increase in serum AST and ALT activities of rats given alone CCl_4 (p<0.05). However a significant decrease was observed in the respective serum activities of rats given camel milk + CCl_4 compared with the alone CCl_4 treated groups (p<0.05). A significant decrease (p<0.05) in serum total proteins (Fig. 4) and albumin (Fig. 5) of rats given

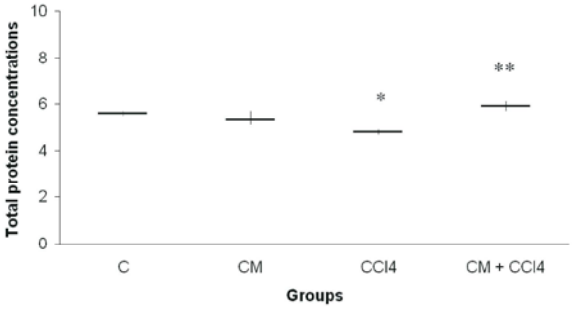

Fig. 4: Effect of CCl_4 and camel milk on serum total proteins in rats
*Significantly different from control group (p<0.05).
**Significantly different from CCl_4 group (p<0.05).

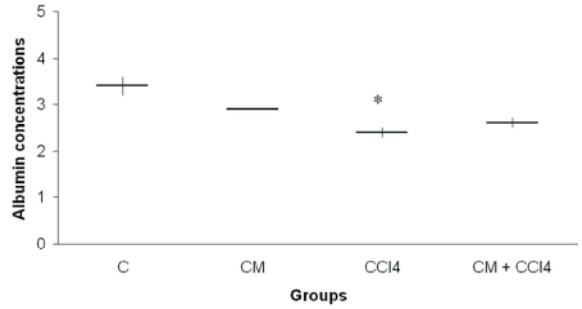

Fig. 5: Effect of CCl_4 and camel milk on serum Albumin in rats
*Significantly different from control group (p<0.05).
**Significantly different from CCl_4 group (p<0.05).

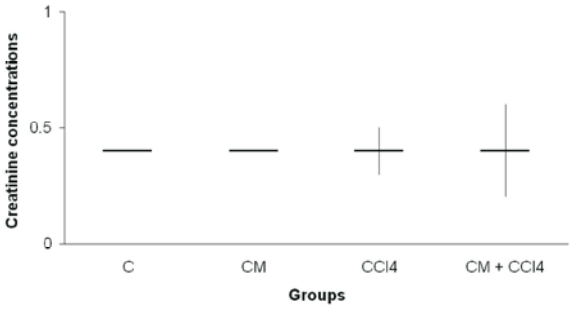

Fig. 6: Effect of CCl_4 and camel milk on serum creatinine in rats

alone Ccl_4 (p<0.05) was observed. However, camel milk restored the normal values of such parameters corrected these values towards the control values in Ccl_4 intoxicated rats As shown in Fig. 6, creatinine level remained unchanged in all treated group (p>0.05).

DISCUSSION

Histopathological sectioning revealed that the liver of CCl_4 intoxicated rats showed massive fatty changes and centrilobular necrosis in most cases. These findings come in accordance with previous researches [2, 17-19]. The same dose of the current experiment was used and induced the same effect reported above [15]. The current study reported hepatitis with mononuclear cell infiltration around the central vein in portal area in rats intoxicated with CCl_4. Theses results come in accordance with previous researches [2, 15, 17-19]. The hepatic recovery and complete regeneration of hepatocytes in rats treated with camel milk agree with those obtained by other researcher [15] who reported that camel milk reduce the incidence of liver lesions induced by CCl_4.

The histopathological findings were also confirmed by some biochemical observations. In the present study serum hepatic markers, AST and ALT activities were greatly increased in rats with the CCl_4 treatment alone compare to control groups. The increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are placed in cytoplasmic area of the cell and are released into circulation in case of cellular damage [20, 21]. The CCl_4 induced the increase of serum ALT and AST levels which result from cell membrane and mitochondrial damages in liver cells [22]. There are many authors' [23-26] reports that these enzymes activities were significantly elevated after CCl_4 treatment.

The first reports about of hepatotoxic effects by CCl_4, are lipid peroxidation origin and are largely due to its active metabolite CCl_3 (This metabolite can abstract hydrogen from fatty acids, initiating the lipid per oxidation), leading to cell injury and finally liver damage [27, 28]. On the other hand, treatment with camel milk was found to significantly suppress the increase in serum AST and ALT activities induced by CCl_4 in rats. This finding implies that camel milk challenge to protect liver tissue from CCl_4 injury. The reversal of increased serum enzymes in CCl_4-induced liver damage by camel milk may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [29]. Several studies [14, 15, 30] have provided a considerable support for evidencing the protective effects of camel milk on liver damage. Also, these studies declared that the protective effect of camel milk against CCl_4-induced

oxidative stress in the rat is due to its antioxidant properties, camel milk was found to contain high concentrations of vitamins A, B_2, C and E and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury [31].

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Camel milk decreased CCl4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytes cell membrane or regeneration of damaged liver cells [32].

As in the current experiment, previous experimental studies have shown that $CC1_4$ increase significantly serum ALP levels [15] and decrease urea [15, 33], total proteins [15, 34] and albumin [15, 34] levels. However, there is a controversy about the effect of $CC1_4$ on serum creatinine level. While some investigators [35] found a decrease in serum creatinine in CCl_4 toxicity, parallel to the present study others [15, 36, 37] didn't find any significant changes.

In this study there was a significant decrease in serum albumin of rats treated with Ccl_4 alone (group 3) as compared to the control rats either received tape water (group 1) or drank camel milk (group 2). Indicating poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition, or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis [14]. On the other hand, a significant increase in concentration of serum albumin in rats fed standard diet and camel milk (group 2) and rats intoxicated with CCl^4 and treated with camel milk (group 4) in comparison with rats treated with CCl_4 alone (group 3). The increase of albumin concentration after treatment with camel milk may be due to that camel milk could induce decrease in lipid peroxidation processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human [11, 14].

The present study concluded that camel milk treatment may play a protective role by improving the changes in histological structure against CCl_4-induced liver damages and enhancing liver enzyme activities in rats. Therefore, camel milk may be used to protect against toxic effects of CCl_4 and other chemical agents in liver.

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