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RESEARCH ARTICLE

Biochemical Study in Rats Hepatotoxicity with Carbon Tetrachloride and Treated with Camel Milk

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Abstract

Objective: Carbon tetrachloride (CCl_4) is a highly toxic chemical agent. Therefore, the present study was carried out to investigate the protective effects of camel's milk against CCL_4 induced toxicity in male rats. Materials and methods: Albino male rats (150-200 g) were divided into four groups of 6 rats: a control group fed only with diet and tap water for two weeks, the second group fed with standard diet and camel milk, the third group intoxicated with CCl_4 on first two days of the experimental 14 days and fed with tap water and diet, and the fourth group intoxicated with CCl_4 on first two days of the experimental 14 days and then treated with camel milk. Results: The results indicated that the CCl_4 caused a significant increased in the level of serum total cholesterol(TC) , triglycerides(TG), very low density lipoprotein (VLDL)and low density lipoprotein(LDL), lactate dehydrogenase(LDH), creatine kinase(CK)and aspartate aminotransferase (AST). Also, there was a significant decreased in the level of serum high density lipoprotein (HDL).Protective activity of camel's milk against toxicity of CCl_4 observed in decreasing of TC, TG, VLDL, LDL, LDH, CK, AST and increased in the HDL levels. Conclusion: These findings strongly prove that beneficial effects of Camel Milk clearly shown through the reduction of the CCl4 induced related damages and oxidative stress

Keywords: *Camel milk, Carbon tetrachloride, Lipid profile, Rats.*

Introduction

The liver is responsible for metabolism and detoxification of the most of components that enter the body [1]. Liver diseases remain one of the major threats to public health and a worldwide problem [2]. The World health organization estimates that 46% of global disease and 59% of mortality are due to chronic disease [3] and the management of liver disease remains a significant concern of modern medicine since this latter has little to offer as alleviation of hepatic ailments [4].

Liver is the main organ, which is responsible for several metabolic functions. It is the organ in charge of many important life functions; including food digestion, glycogen storage, and control of metabolism, drug detoxification and hormone production [5].

The toxic effect of CCl4 is attributed to trichloromethyl radical produced during oxidative stress [6]. The number of infiltrated neutrophils, macrophages, Kupffer cells, lymphocytes and natural killer cells are significantly increased after liver injury induced by hepatotoxins such as CCl_4. It induced activation of liver resident macrophages and/or chemo attraction of extrahepatic cells (e.g. neutrophils and lymphocytes; [7]. The activated macrophages are released and contributed to liver fibrosis, inflammation and injury [8]. Once the liver became injured, its efficient treatment with famous chemical drugs is limited [9]. Therefore, interest concerned the use of alternative medicines for the treatment of hepatic disease has been arisen.

The presence of peptides and proteins in camel's milk exhibits its biological activities that have beneficial effect on many bioprocesses as digestion, absorption, growth and immunity [10, 11]. Furthermore, camel's milk can be conserved at room temperature longer period than other animals' milk [12].

The most known uses of camel's milk are as drug against autoimmune diseases, dropsy, jaundice, splenomegaly, tuberculosis, asthma, anemia, piles and diabetes [13].

Also, camel's milk is characterized by antitoxic effect in the face of 4 cadmium chloride [14, 15], CCl4 ([16], Cisplatin [17], Paracetamol [18], Aluminum chloride [19]. Although, Althnaian *et al*. [20] confirmed that Camel milk had a protective effect against CCl4 induced hepatotoxicity.

Therefore, in the present study, we investigated the protective effects of camel's milk against CCl_4- induced hepatotoxicity in rats by assaying lipid profiles and heart functions.

Materials and Methods

Chemicals and Kits

Diagnostic kits for serum total cholesterol, triglycerides, high density lipoprotein(HDL), lactate dehydrogenase (LDH) and creatine kinase (CK) and) were purchased from Biolabo(France), aspartate amino transferase (AST) was purchased from Randox (England). Carbon tetrachloride purchased from E. Merck, Darmstadt, Germany. All other chemicals and solvents were of highest grade.

Camel's Milk

Camel milk samples were collected daily early in the morning from a herd of camels, return to one of native, in Sid Dekheel region , about 25 km eastern of Nasiriyah city/ Thi-Qar/ Iraq province. Milk was collected from camels by hand milking as normally practiced by the farmers.

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 with oral delivery (1mL/animal) as such without any further treatment.

Animals and Treatment

A total of 24 albino male rats (200–250 g) were obtained from the animal house of biology department, College of Science, University of Thi-qar. Animal acclimated for 10 days before starting the experiment. All animals were housed in standard cages (6 rats/cage), feeding 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 12 h light/12 h dark cycle.

Induction of Hepatotoxicity by CCl_4

Liver disease was induced by the intraperitoneal injection of CCl_4 (1 mL kg^{-1} b.wt.), 1:1 diluted with paraffin oil for two successive days of the experiment. Group III received CCl_4 injections on first two successive days of 14 days and were given tap water and standard rat feed for 14 days of experimental course.

Similarly, Group IV rats received CCl_4 injection on first two days of the experimental 14 days but were fed with fresh and raw camel milk (1mL/animal) to study the protective role of camel milk. Group I was fed only with diet and tap water, and Group II were fed with standard diet and camel milk.

Experimental Groups and Protocol

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 I: Control rats fed only with diet and tap water.

Group II: Rats fed with standard diet and camel milk.

Group III: Disease group intoxicated with CCl_4 on first two days of the experimental 14 days and fed with tap water and diet

Group IV: Rats intoxicated with CCl_4 on first two days of the experimental 14 days and then treated with camel milk.

Blood Collection

At the end of day 14, the animals were sacrificed by cervical dislocation 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 3000x g for 15 min and were saved in aliquots and stored at -80°C for further analysis.

Statistical Analysis

Data were statistically analyzed using Package Social Sciences (SPSS) for Windows version 12.0 software. All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by the least significance difference (LSD) method. The ($p \leq 0.05$) level of probability was used as the criteria of significance.

Results

Effects of CCl_4 and Camel Milk on Lipid Profile in Rats

The results showed a significant increase ($p \leq 0.05$) in the concentration of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in group (3) in comparison with group (1). There was non-significant difference in the concentration of serum total cholesterol(TC), triglycerides(TG), very low density lipoprotein(VLDL) and low density lipoprotein (LDL) in group (2) in comparison with group(1). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum total cholesterol (TC), triglycerides (TG), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in group (4) in comparison with group (3). While there was a significant decrease ($p \leq 0.05$) in the concentration of high density lipoprotein (HDL) in group (3) in comparison with group (1).Also, there were non-significant differences in the concentration of serum high density lipoprotein (HDL) in group (2) in comparison with group (1).Table (1).

Table 1: Effects of CCl_4 and camel milk on lipid profile in rats

Groups/ Parameters	TC(mg/dL)	TG(mg(dL)	HDL(mg/dL)	VLDL(mg/dL	LDL(mg/dL)
Group I	64.80± 1.13 ^{C}	38.51± 1.49^{c}	44.8± 0.71^{a}	7.70± 0.30 ^{c}	12.30± 0.51^{c}
Group II	65.43± 0.98 ^{c}	40.07± 1.17 ^{c}	44.3± 0.84 ^{a}	8.02± 0.23 ^{c}	11.79± 0.98 ^{c}
Group III	78.78± 0.87^{a}	48.03± 0.94 ^{a}	36.66± 1.05^{c}	9.61± 0.18 ^{a}	32.51± 1.17 ^{a}
Group IV	72.5± 3.61^{b}	44.07± 0.98 ^{b}	41.65± 0.73 ^{b}	8.81± 0.20 ^{b}	22.04± 0.73 ^{b}
LSD	5.21	2.61	2.47	0.53	6.34

Each value represents the mean± standard division of 6 rats.
Different letter refer to a significant difference at ($p \leq 0.05$)

Effects on the Activities of Serum Enzymes Related to Heart Function

The results showed a significant increase ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) in group (3) in comparison with group (1). There was non-significant difference in the concentration of serum lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) in group (2) in comparison with group(1). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) in group (4) in comparison with group (3).Table (2).

Table 2: Effects of CCl_4 and camel milk on LDH, CK, AST in rats

Groups/ Parameters	LDH(U/L)	CK(U/L)	AST(U/L)
Group I	234.59± 34.61 ^{C}	215.44± 44.06 ^{c}	50.66± 3.13 ^{b}
Group II	207.78± 18.04 ^{c}	213.80± 37.31^{c}	47.51± 2.65 ^{b}
Group III	1744± 29.96 ^{a}	624.78± 89.58 ^{a}	75.16± 4.58 ^{a}
Group IV	1221.93± 54.02 ^{b}	388.11± 64.13 ^{b}	57.00± 3.06 ^{b}
LSD	65.54	177.24	9.04

Each value represents the mean± standard division of 6 rats.
Different letter refer to a significant difference at ($p \leq 0.05$)

Discussion

Effects of CCl_4 and Camel Milk on Lipid Profile in Rats

The results of the present study have also established that, the CCl_4 treatment could have affected the lipid metabolism (cholesterol and triglyceride levels) of liver. This is evidenced from the present observations that, CCl_4 caused a significant ($p \leq 0.05$) increase in the levels of lipid parameters. [21] stated that CCl_4 intoxication is similar to hepatitis in case of the triglycerides catabolism. This situation could be also attributed to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis [22]. On the other hand, it can be assumed that hypercholesterolemia in CCl_4 intoxicated rats was resulted from damage of hepatic

parenchymal cells that lead to disturbance of lipid metabolism in liver [23]. However, rats treated with camel milk showed a significant ($p \leq 0.05$) decline in triacylglycerol, cholesterol, VLDL and LDL values but it is increased the HDL levels compared to CCl4-intoxicated rats. The mechanism of lipid lowering effects of camel milk might be attributed to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease in vitro. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver [24].

Effects on the Activities of Serum Enzymes Related to Heart Function

The examination of heart was done through the estimation of the LDH, CK and AST activities in serum that are enzymes originally present at elevated concentration in the cytoplasm. In case of cardiotopathy, such enzymes escape into the blood stream in accordance with the extent of heart damage [25]. Our results showed that the administration of CCl4 induced a significant elevation of enzyme levels in comparison with normal control. The elevated level of this parameter has been seen in rat receiving CCl4 leading to increased cell damage, permeability and cardiac cell necrosis [26]. Notably, preventive treatment with CM was proved to reduce the rise of serum LDH, CK and AST activities induced by CCl_4 treatment in mice. This finding implies that CM faces the challenge to protect cardiac tissue from CCl4 injury. Preventive treatment of CM can repair and protect cardiac tissues by stabilizing the membrane and the preventing intracellular enzyme leakages.

Conclusion

In conclusion, camel milk caused a protective effect against CCl_4-induced liver damage and improved the biochemical parameters. Also, camel milk has a hepatoprotective effect against injury in the liver of CCl4-treated rats. Therefore, camel milk may be used to protect against toxic effects of CCl4 and other chemical agents in liver. In the future, examination of the liver protective effect of camel milk against CCl_4 in dose dependant manner could be investigated.

References

1. Nunez M (2006) Hepatotoxicity of antiretrovirals: Incidence, mechanisms and management. J. Hepatol., 44:132-139.
2. Adewusi EA, Afolayan AJ (2010) A review of natural products with hepatoprotective activity. J Med Plants Res, 4: 1318-34.
3. Williams R (2006) Global challenges in liver disease. J. Hepatology, 44:21-6.
4. Wagh AE, Yeotkar US, Nimbhorker MG (2010) JAM-A regulates permeability and inflammation in the intestine *in vivo*. J. Orient Phann., 10:111-5.
5. Si-Tayeb K, Lemaigre FP, Duncan SA (2010) Organogenesis and development of the liver. J. Dev. Cell, 18:175-89.
6. Stoyanovsky D, Cederbaum AI (1999) Metabolism of carbon tetrachloride to trichlo-romethyl radical: an ESR and HPLC-EC study. Chem. Res Toxicol., 12:730-736.
7. Saile B, Ramadori G (2007) Inflammation, damage repair and liver fibrosis-role of cytokines and different cell types. Z Gastroenterol., 45:77-86.
8. Canbay A, Feldstein A, Baskin-Bey E (2004) The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. J. Pharm. Exp. Ther., 3:1191-1196.
9. Lee C H, Park S W, Kim YS, Kang S S, Kim J A, Lee SH, Lee SM (2007) Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. Biological and Pharmaceutical Bulletin, 30**:** 1898-1904.
10. Korhonen H, Pihlanto A (2001) Food-derived bioactive peptides opportunities for designing future foods. *Curr. Pharm. Des.*, 9:1297-1308
11. Al-Fartosi KG, Majid A, Auda MA, Hussein MH (2012) The Role of Camel's Milk against Some Oxidant-Antioxidant Markers of Male Rats Treated With CCl4. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3: 385-389.
12. Omer R, Eltinay AH (2009) Antimicrobial Effects of Camel Milk against Some Bacterial Pathogens. J. Pak J. Nutr., 8:607-10.
13. Rao M B, Gupta RC, Dastur NN (1970) Camels' milk and milk products. Indian Journal of Dairy Science, 23**:** 71-78.

14. Al-Hashem F (2009 a) Camel milk protects against aluminum chloride- induced normocytic normocromic anemia, lipid peroxidation and oxidative stress in erythrocytes of white albino rats. J. Am J. Biochem. Biotechnol., 5:127-136.
15. Nelson L, Schwaner R (2009) Transdermal fentanyl: pharmacology and toxicology. Journal of medical toxicology, **5:** 230
16. Yagil R, Saran A, Etzion Z (1984) Camel's milk: for drinking only. Comp Biochem. Physiol., 78:263-266.
17. Afifi ME (2010) Effect of camel's milk on cisplatin-induced nephrotoxicity in Swiss Albino mice. Am J. Biochem. Biotechnol., 6:141-147.
18. Al- Fartosi KG, Khuon, OS, Al- Tae HI (2011) Protective role of Camel's Milk against Paracetamol Induced Hepatotoxicity in Male Rats. International Journal of Research in Pharmaceutical and Biomedical Sciences, 4: 1795-1799.
19. Al-Hashem F (2009b) S-Allyl-cysteines reduce amelioration of aluminum induced toxicity in rats. J. Am J. Biochem. Biotechnol., 5:98-108.
20. Althnaian T, Albokhadaim I, El-Bahr S M (2013) Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk. Springer Plus, **2:** 57.
21. Muller P, Fellin R, Lambrecht J, Agostini B, Wieland H, Rost W, Seidel D (1974) Hypertriglyceridaemia secondary to liver disease. European Journal of Clinical Investigation, 4: 419-428.
22. Jahn CE, Schaegfetr EJ, Taam LA (1985) Lipoprotein abnormalities in primary biliary cirrhosis association with hepatic lipase inhibition as well as altered cholesterol esterification. Gastroenterol., 89:1266-1278.
23. Havel RJ (1986) Functional activities of hepatic lipoproteins receptors. Ann Rev Physiol 48:119-134
24. Matsuda K (1994) ACAT inhibitors as antiatherosclerotic agents: compounds and mechanisms. Med Res Rev., 14:271-305.
25. Manna P, Sinha M, Sil PC (2008) Arsenic induced oxidative myocardial injury: protective role of arjunolic acid. Archives of Toxicology, 82: 137-149.
26. Kurian GA, Philip S, Varghese T (2005) Effect of aqueous extract of the Desmodium gangeticum DC root in the severity of myocardial infarction. Journal of Ethnopharmacology, 97: 457-461.

