Research Paper

The Role of Camel's Milk against Some Oxidant-Antioxidant Markers of Male Rats Treated With CCl4

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ABSTRACT

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 CCL4 induced oxidative stress in male white albino rats. White 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 CCl4 on first two days of the experimental 14 days and fed with tap water and diet, and the fourth group intoxicated with CCl4 on first two days of the experimental 14 days and then treated with camel milk . Intrapretoneal injection of male rats with CCl4 induced lipid peroxidation in the liver, which was indicated by a significant increase in lipid peroxidation biomarkers (TBARS). Also the data showed that the treated of male rats with CCl4 caused a significant increases in the serum levels of iron and ceruloplasmin, while the levels of transferrin and albumin were decreased significantly in male rats treated with CCl4. In all rats treated with camel's milk after given CCl4, oxidant-antioxidant markers were altered compared with male rats treated with CCl4 only.

Key Words: CCl4, camel's milk, Oxidant-antioxidant markers, rats.

INTRODUCTION

The liver regulates many important metabolic functions, detoxification, and secretary functions in the body. Hepatic injury is associated with distortion of these metabolic functions (Gupta,1984). Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies. Despite, considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations (Recnagel,1983). So, the search for new medicines is still ongoing. Because liver performs many vital functions in the human body and damage of liver causes unbearable problems(Saravana *et al*.,2009) .

Carbon tetrachloride (CCl_4) is a highly toxic chemical agent, widely used to elicit experimental liver damage. The effects of CCl_4 on hepatocytes are manifested histologically as hepatic steatosis, fibrosis, hepatocellular death and carcinogenicity (Junnila *et al*.,2000). Its toxic effect is believed to be due to trichloromethyl radical which is formed by an unstable metabolic intermediate under the presence of oxidative stress(Recknagel *et al*.,1989;Stoyanovsky and Cederbaum,1999). However, few approaches which delineate the comprehensive metabolic disorders and metabolic syndromes of CCl_4 induced hepatotoxicity have been investigated in literature.

Acute and chronic liver diseases constitute a global concern and medical treatments for these diseases are often difficult to handle and have limited efficiency (Lee *et al*.,2007). There fore, there has been considerable interest in role of complementary and alternative medicines for the treatment of liver diseases. Developing therapeutically effective agents from natural products may reduce the risk of toxicity . When the drug is used clinically.

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(Knoess,1979). 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. Aseries 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 *et al*.,1970). A beneficial effect of raw camel's milk has been observed in chronic pulmonary tuberculosis patients(Mal *et al*.,2001). Also, in repeated trials, a 30-35% reduction in the daily insulin dose required by patients with type 1 diabetes was observed in response to treatment with raw camel's milk(Agrawal *et al*.,2002).

MATERIALS AND METHODS

Chemicals and Kits

Thiobarbutaricacid (TBA), Trichloroaceticacid (TCA), Butanol,Para-Phenylenediamine (PPD), Sodium acetate trihydrate and Sodium azide were purchased from BDH , England . Diagnostic kits for serum Iron (Fe) ,Total Iron Binding Capacity (TIBC) and Albumin (Alb) were purchased from Biolabo, France. Paraffin oil was purchased from Riedel dehaenage (Germany) and Carbon tetrachloride purchased from E. Merck, Darmstadt, Germany. All other chemicals and solvents were of highest grade commercially available.

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 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: Male albino wister rats (150-250 g) were obtained from College of Science, Thi-Qar University. and acclimated for at least 7 day before starting the experiment. All animals were housed in standard aluminum cages (4 rats cage-1), 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/dark cycle.

Experimental Groups Protocol

White albino male rats weighting (150-200 g), and aged 5-6 weeks were supplied by the animal house of biology department, College of Science, University of Thi-Qar. The rats were housed in standard plastic cages (6 rats / cage) in an environmentally controlled room with a constant temperature of 23-25 C and 12 h light/dark cycle. The rats ere fed a standard lab die and given *ad libitum* access to food and water. The rats were divided randomly into 4 groups comprising six rats in each group as following:

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 CCl4 on first two days of the experimental 14 days and fed with tap water and diet
Group IV:	Rats intoxicated with CCl4 on first two days of the experimental 14 days and then treated with camel milk

Induction of hepatotoxicity by CCl4

Liver disease was induced by the intraperitoneal injection of CCl4 (1 mL kg-1 b.wt.), 1:1 diluted with paraffin oil for two successive days of the experiment. Group III received CCl4 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 CCl4 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 was fed with standard diet and 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.

Serum biochemistry

Malondialdehyde and Ceruloplasmin serum activities were measured to assess hepatotoxicity by CCl4. Iron, Transferrin and Albumin activities were also measured using spectrophotometric diagnostic kits as previously described.

RESULTS AND DISCUSSION

Table (1) showed the results of the effect of camel's milk against some oxidant-antioxidant markers of

male rats treated with CCl4. The results indicated a significant increase in concentration of serum MDA in group(III) in comparison with group(I) ($p<0.05$). This elevation in serum MDA may be due to the association with a lose of balance between pro-oxidation and antioxidation , energy depletion and accelerated aging in the target organs such as heart, kidney and brain (Romero and Roche,1996). In the same table there is no significant differences in concentration of serum MDA in group (II) in comparison with group(I)($p<0.05$).Whereas there is a significant differences can be observed in group(IV) as compared to control group(I), also there is a significant decrease in concentration of serum MDA in group (IV) in comparison with group(III) and there is a significant differences in concentration of serum MDA in group(IV) in comparison with group (II)($p<0.05$).

It could be concluded that the protective effect of camel milk against CCl4-induced oxidative stress in the rat is due to its antioxidant properties ,camel milk was found to contain high concentrations of vitamins A,B2,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(Yousef,2004). Magnesium protects the cell against damage from oxyradicals and assists in the absorption and metabolism of vitamins B,C and E, which play a big role in cell protection from free radicals by functioning as antioxidants(Barbagallo,1999).

The results showed a significant increase in concentration of serum iron in group(III) in comparison with group(I) ($p<0.05$) and there is a significant increase ($p<0.05$) in concentration of serum iron in group (III) in comparison with groups(II and IV). The increase of iron levels which were reported in the present study agree with the finding of (Wood et al., 2007) and showed that the toxic free radical types are superoxide radical anion (O-2), the presence the latter in high amount leads the releasing of free iron circulatory system because (O^{-2}) attack to ferritin.

While no significant differences can be observed between each one of groups (II,IV and I) after having been treated for (14) days with (1mL/Kg B.W) of camel milk .The levels of serum iron decreased significantly in (II and IV) groups after they were treated for (14)days with (1mL/Kg B.W) of camel milk compared to group(III). The decreases of iron levels which were reported in this study as aresult of treatment camel milk (as anti oxidants) can cause deficiency of oxidation processes ,disruption of heme biosynthesis and low oxygen transfer might be resulted in a compensatory increase in the rate of red blood cell(RBC) production (Golalipour *et al*., 2007).

Also, the results presented a significant decrease in concentration of serum transferrin in group(III) in comparison with group(I) ($p<0.05$).Also, there is a significant decrease in concentration of serum transferrin in group (III) comparison with groups (II and IV). The lower degree serum transferrin level which was reported in the present study this may be due to the higher concentrations of iron in these patients made Tf saturated this leads to decrease its concentrations (Gonzalez and Guerrero,2006). However a significant differences can be observed among animal groups (II and IV) as compression with control group (I) after having been treated with (1mL /kg B.W) of camel milk for (14) days. Whereas a significant increase ($P < 0.05$) in serum Tf can be observed in group (II and IV) compared to group(III). On the other hand, the result showed that the serum Tf levels after having been treated with camel milk appeared no significant when compare with control group (I), this may be due to the decrease of serum iron levels after treatment ,our results is compatible with the previous study of (Biessels *et al*., 2004) which showed that the decrease in serum iron levels associated with the increase of the binding capacity of transferrin to iron.

Ceruloplasmin was increase significantly ($p<0.05$) in group(III) in comparison with group (I)($p<0.05$). This increasing could also cause vascular injury by generating free radicals ,such as hydrogen peroxide ,ceruloplasmin is thought to be a scavenger(Kingston and Kingston,2007).While a significant decrease in concentrations of serum ceruloplasmin in groups (II and IV) in comparison with group(III) ($p<0.05$).Also there is noa significant differences in concentrations of serum ceruloplasmin in groups(II and IV) in comparison with group(I). The decrease in concentration of serum ceruloplasmin in groups (II and IV) compared to control group after having been treatment with(1 mL/kg B.W) of camel milk this may be due that camel milk decrease an activity of numerous proteins associated with oxidative stress (Senra,2008). Also the reduction in Cp concentration could be to counter balance of the ROS generation radicals generated in the lipid peroxidation processes and presence of iron or copper ions (Saraymen *et al*, 2004).

Albumin was decreased significantly ($p<0.05$) in group(III) in comparison with group(I) and there is no a significant differences in concentration of serum albumin in group(III) in comparison with group(IV). Albumin is the most abundant protein in human plasma, representing 55-56% of total protein. It synthesized in the liver at a rate that is dependent on protein intake subject to feedback regulation by the

plasma level .Little albumin is filtered through the kidney glomerali and most of that is reabsorbed by proximal tubule cells and degraded by their lysosomal enzymes into fragment that are returned to the circulation .In this study there was a significant decrease in serum albumin of rats treated with carbon chloride tetra alone as compared to the control rats received normal saline .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, other interpretation may cadmium caused loss protein in urine ,due to nephritic syndrome ,chronic glomerulonephritis (Shibutani *et al*.,2001). On the other hand , a significant increase in concentration of serum albumin in group (II and IV) in comparison with group(III) ,Also there is no a significant differences in concentration of serum albumin in group (II and IV) in comparison with group (I). The increase albumin concentration after treatment with (1mL/kg B.W) of camel milk may be due 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 (Al-Hashem,2009).

Table 1: Levels of MDA, Iron, Transferrin, Ceruloplasmin and Albumin in serum of control and experimental male rats

BIOCHEMICAL PARAMETERS (MEAN ± SD)						
Groups	**n**	**MDA (nmol/mL)**	**Iron (µmol/L)**	**Transferrin (g/L)**	**Ceruloplasmin (g/L)**	**Albumin (g/L)**
Group(I)	4	87.87 ±1.86^{c}	46.70 ±3.18^{b}	1.10 ±0.43^{a}	1.82 ±0.07^{b}	47.71 ±6.90^{a}
Group(II)	4	87.00 ±2.58^{c}	44.86 ±7.16^{b}	0.79 ±0.12 ^{b}	2.20 ±0.76^{b}	45.36 ±2.76^{a}
Group(III)	4	256.50 ±13.36^{a}	69.43 ±2.49^{a}	0.48 ±0.04 ^{c}	3.31 ±1.27^{a}	35.08 ±5.94^{b}
Group(IV)	4	142.43 ±9.49^{b}	48.00 ±9.34^{b}	0.83 ±0.10 ^{b}	2.03 ±0.51^{b}	41.61 ±3.88^{a}

- Each value represents mean ± SD values with non identical superscript (a , b or c …etc) were considered significantly differences (P <0.05).
- 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 CCl4 and fed with tap water and diet.
- Group (IV) = Rats intoxicated with CCl4 on first two days of the experimental 14 days and then treated with camel milk.

CONCLUSION

CCl4 has adverse effects on human health. Our results demonstrate that CCl4 is capable of inducing marked alterations in biochemical parameters and oxidative damage, and inhibiting the function of antioxidant enzymes. Camel's milk, administered after benzene exposure, minimized CCl4-associated hazards. Therefore, drinking camel's milk could be beneficial for alleviating CCl4 toxicity.

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