Hepatoprotective Effects of Camel Milk against CCl₄-induced Hepatotoxicity in Rats

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ABSTRACT

Camel milk has been widely used in a number of countries as a food additive and for curing some commonly occurring diseases. Recently, camel milk has been deeply studied for its special properties because of higher hepatoprotective, insulin like and antibacterial activities. These properties distinguish camel milk from milk of other animals. The present study was carried out to investigate the protective effects of camel milk against CCl₄-induced hepatotoxicity which lead to biochemical alterations in liver function of male albino wister rats. White albino male rats (200-250 g) were divided into 5 groups, a normal control water group, a control camel milk group and three CCl₄-intoxicated groups treated with or without camel milk. Protective roles of camel milk were analyzed by assaying the liver function parameters as serum aminotransferases, alkaline phosphatase, serum proteins and cholesterol levels. Histopathological examinations were also studied in all groups of rats by microscopy. Data showed that intraperitoneal administration of CCl₄ (1 mL kg⁻¹ b.wt.) resulted in statistically significant increase in the serum levels of aminotransferases and change in serum protein, albumin and cholesterol levels which approach to normal levels after the treatment with raw camel milk. Furthermore, histopathological studies reveal that camel milk treatments significantly reduce the incidence of liver lesions induced by CCl₄. Our findings demonstrate that CCl₄ exposure alters liver function biochemical parameters, which shift towards normal values after treatment with camel milk. So camel milk has a good potent for curing some liver diseases.

Key words: Aminotransferases, carbon tetrachloride hepatotoxicity, camel milk, histopathology

INTRODUCTION

The liver is the organ for metabolism and detoxification of various components that enter into the body. It is involved in wide range of functions and hence it is exposed to toxic substances and drugs absorbed from the intestine. Apart from the toxins and drugs, viral infections (hepatitis A, B, C, D, etc.) and microbial infections also cause damage to the hepatocytes (Nunez, 2006).

Carbon tetrachloride (CCl₄) is a highly toxic chemical agent, widely used to elicit experimental liver damage. The effects of CCl₄ 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 (Stoyanovsky and Cederbaum, 1999; Recknagel et al., 1989).
However, few approaches which delineate the comprehensive metabolic disorders and metabolic syndromes of COCl₂ 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). Therefore, there has been considerable interest in role of complementary and alternative medicines for the treatment of liver disease. Developing therapeutically effective agents from natural products may reduce the risk of toxicity when the drug is used clinically.

Camel milk is different from other ruminant milk as it is low in cholesterol, sugar and protein but high in minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamin A, B₉, C and E and contains a high concentration of insulin and immunoglobulins (Kamal et al., 2007; Al-Hashem, 2009). Further, camel milk has a high storage room temperature capacity as compared to milk from other animals (Omer and Etiltay, 2009). It has no allergic properties and can be consumed by lactase-deficient individuals and those with a weakened immune system (Inayat et al., 2003; Yateem et al., 2008). In fact, this milk is believed to have medicinal properties. In Sahara, fresh butter made from camel milk is often used as a base for medicines. Other products also developed from camel milk include cosmetics or pharmaceuticals. A series of metabolic and autoimmune diseases are successfully being treated with camel milk. Furthermore, in India, camel milk is used therapeutically to treat dyspepsia, jaundice, problems of spleen, tuberculosis, asthma, anemia, piles and diabetes (Rao et al., 1970). Furthermore, a beneficial effect of raw camel milk has been observed in chronic pulmonary tuberculosis patients (Mal et al., 2001). Also, in repeated trials, a 30-55% reduction in daily insulin dose required by patients with type I diabetes was observed in response to treatment with raw camel milk (Agarwal et al., 2002).

In the present study, we investigated the treatment and protective effects of camel milk against COCl₂-induced hepatotoxicity in rats by assaying liver function test enzymes (ALT, AST), alkaline phosphatase (ALP) and protein, albumin, lipid synthetic functions of liver.

MATERIALS AND METHODS

This study was carried out in four months from Jan.-Apr., 2010

Chemicals and kits: Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase (ALP), albumin, total protein and Cholesterol were purchased from Biosystems, Barcelona (Spain). Paraffin oil was purchased from Winlab, UK and carbon tetrachloride purchased from E. Merck, Darmstadt, Germany. All other chemicals and solvents were of highest grade commercially available.

Camel milk: Camel milk samples were collected daily early in the morning from Alsalman camel milk farms in the Buraidah area of Qassim (central Saudi Arabia). 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 (400 mL/24 h/cage) as such without any further treatment.

Animals and treatment: Male albino wistar rats (200-250 g) were obtained from College of Pharmacy, King Saud University, Riyadh 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. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health.

Experimental groups and protocol: The rats were divided randomly into 5 groups comprising eight rats in each group as follows:

Group I : Control rats fed only with diet and tap water
Group II : Rats fed with standard diet and camel milk
Group III : Disease control group intoxicated with CCl₄ and fed with tap water and diet
Group IV : Rats intoxicated with CCl₄ on first two days of the experimental month and then treated with camel milk
Group V : Rats fed with camel milk and diet and intoxicated with CCl₄ on the last two days of the experimental month

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 the month and were given tap water and standard rat feed for one month of experimental course. Similarly, Group IV rats received CCl₄ injection on first two days of the experimental month but were fed with fresh and raw camel milk (400 mL/day/cage) to study the protective role of camel milk. One more group, Group V rats were fed with camel milk first for one month and then were injected with CCl₄ on last two consecutive days to study how much this group resists the toxic effects caused by CCl₄ intoxication. Group I and II were injected with paraffin oil only as a vehicle.

Blood and tissue collection: At the end of day 30, 24 h after the last CCl₄ injection, 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 1000x g for 15 min at 4°C and were saved in aliquots and stored at -80°C for further analysis. The liver was also quickly removed and washed with cold normal saline, cut and preserved in 10% neutral formalin for the pathological studies for microscopy.

Serum biochemistry: ALT, AST and ALP serum activities were measured to assess hepatotoxicity by CCl₄. Protein; albumin and cholesterol activities were also measured using spectrophotometric diagnostic kits as previously described.

Histopathological examinations: Liver tissues were cut in small pieces and placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques (Bancroft and Gamble, 2002). The extent of CCl₄-induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E), using standard techniques.

Statistical analysis: Results were expressed as Means±standard deviation of three replicates. The significance of differences was calculated by using student t-test p<0.05 was considered statistically significant.
RESULTS

CCl₄ is a frequently used model substance for hepatotoxicity studies (Weber et al., 2003). The acute toxicity study exhibited no mortality up to a dose level of 1000 µL kg⁻¹ b.wt. The hepatoprotective effects of camel milk feeding against CCl₄ induced hepatotoxicity was evaluated in rats. Group III rats exhibited significant increased ALT level, which received CCl₄ only without post treatment with camel milks (disease control group). The level of ALT in this group was observed to reach up to a level of 225±12.9 U L⁻¹. Post treatments of rats after receiving CCl₄ intraperitoneal injections, with fresh and raw camel milk (Group IV) resulted in dramatically decrease in ALT level but still its level was almost double than the control water (Group I) rats (Fig. 1). In group II rats, which were fed with camel milk only, no significant change in ALT and AST levels were observed when compared to control (Group I) rats (Fig. 1, 2). Group V rats were selected to study the immunity enhancement with camel milk prior to CCl₄ intoxication. We observed that camel milk results in enhanced immunity against CCl₄ intoxication (Fig. 1, 2). So In group V rats, pre-treatment with camel milk controlled the dramatic rise of aminotransferase level after CCl₄ exposure which clearly indicates that camel milk protects the hepatocellular integrity.

AST level was also found to follow the similar pattern of results like ALT. AST level was significantly high in group III rats as compared to control (Group I) rats. Its level was significantly controlled in pre- and post-treated camel milk rats (Fig. 2). In group II rats also no change in AST level was found. The level of ALP increased significantly in CCl₄ alone (Group III) animals (167.75±13.84 U L⁻¹) when compared to normal control (88.97±7.68 U L⁻¹) (Fig. 3). The total otein and albumin levels decreased considerably in the toxic group (Group III) when compared to normal control group I rats. Induction of hepatotoxicity in rats with CCl₄ reduced the production of total proteins. But the rats, which were pretreated with camel, milk after taking the injection of CCl₄ its level regained (Table 1). Similarly, the level of serum albumin production significantly

Fig. 1: Alanine aminotransferase level in serum of different groups of rats. Changes in ALT level in different groups of rats intoxicated with CCl₄ and treated with camel milk. Group I (normal control fed on tap water only), Group II (rats fed on camel milk only), Group III (disease control received CCl₄ and fed on tap water) Group IV and V (post-and pre-treated with camel milk after CCl₄ injections). Data are presented as the Mean±standard deviation for the three independent values (n = 8)
Fig. 2: Aspartate aminotransferase level in serum of different groups of rats. AST level in different groups of rats intoxicated with CCl$_4$ and treated with camel milk. Group I (normal control fed on tap water only), Group II (rats fed on camel milk only), Group III (disease control received CCl$_4$ and fed on tap water) Group IV and V (received CCl$_4$ and post- and pre-treated with camel milk). Data are presented as the Mean±standard deviation for the three independent values (n = 8)

Fig. 3: Alkaline phosphatase level in serum of different groups of rats. ALP levels in different groups of rats intoxicated with CCl$_4$ and treated with camel milk. Group I represents normal control fed on tap water only, Group II represents rats fed on camel milk only, Group III represents rats intoxicated with CCl$_4$ and fed with water only, Group IV and V represents rats post- and pre-treated with camel milk after CCl$_4$ dose. Data are present as the Mean±SD of three independent values (n = 8)

dropped in rats (group III) that received CCl$_4$ only. The level of serum albumin was found to approach normal values in group IV rats (Table 1). Furthermore, the cholesterol level in control
Table 1: Effect of Camel milk on serum protein, albumin and cholesterol in rats intoxicated with CCl₄

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g L⁻¹)</td>
<td>66.5±4.50</td>
<td>67.3±4.30</td>
<td>44.6±3.60</td>
<td>54.5±2.40</td>
<td>58.0±6.60</td>
</tr>
<tr>
<td>Albumin (g L⁻¹)</td>
<td>25.9±2.10</td>
<td>27.2±1.00</td>
<td>15.0±2.30</td>
<td>24.0±2.10</td>
<td>25.0±2.00</td>
</tr>
<tr>
<td>Cholesterol (mmol L⁻¹)</td>
<td>1.64±0.26</td>
<td>1.66±0.30</td>
<td>2.26±0.28</td>
<td>1.87±0.34</td>
<td>1.92±0.30</td>
</tr>
</tbody>
</table>

Results are Means±SD, n = 8, *p<0.05

Fig. 4: Photomicrographs of the histopathological studies of livers of various groups of rats. Photomicrographs (original magnification 100x) of the histopathological studies of livers of various groups stained with hematoxylin and eosin (a) normal architecture of rat liver (b) architecture of camel milk only fed rats (c) necrosis, fatty changes and hepatocellular degeneration of CCl₄ intoxicated groups (d) marked reduction in fatty changes, inflammatory cell infiltration and necrosis (e) less damage of hepatocytes and low index of necrosis in camel milk pre treated rats

The group (group I) was found to be 1.64±0.26 mmol L⁻¹ and the cholesterol levels in the serum were increased in the rats treated with CCl₄ by about 37%. Camel milk treatment caused the fall in this level to 1.87±0.34 mmol L⁻¹ in Group IV rats (Table 1).

The histopathological studies also supported the protective roles of camel milk. The areas of necrosis and ballooning degeneration of hepatocytes were observed in the toxic group with vacuolar fatty change and mild inflammatory cell infiltration (Group III). The group of animals post treated with camel milk showed a marked protective effects with decreased necrotic zones and hepatocellular degeneration (Group IV). The liver of rats pretreated with camel milk (group V) showed moderate necrosis with low presence of inflammatory cells (Fig. 4e). The photomicrographs of the liver sections of all the groups is shown is Fig. 4a-e.
DISCUSSION

The liver is the major organ responsible for the metabolism of drugs and toxic chemicals and therefore is the primary target organ for nearly all toxic chemicals (Kaplowitz, 2000; Bissel et al., 2001). Liver injury induced by CCl₄ is the best characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening the anti-hepatotoxic and hepatoprotective activity of agents (Recknagel et al., 1989; Brautbar and Williams, 2002). A number of drugs, chemicals and viruses have been reported to cause severe liver necrosis, which sometimes becomes difficult to be managed by medical therapies. So it is important to search for compounds that can be used for better management of the hepatic failure due to severe necrosis.

Literature overview shows that most of the studies on camel milk show that it is a high quality drink and since ancient times people have used this product for curing a number of diseases (Shabo et al., 2005; Agarwal et al., 2007; Redwan and Tabll, 2007). The protective effects of camel milk could be attributed to its antioxidant activity and it may possibly have chelating effects on toxicants (Al-Humaid et al., 2010). It has been reported that camel milk contains high levels of vitamins A, B2, C and E and is very rich in magnesium (Mg) and other trace elements (Knoess, 1979). These vitamins are antioxidants that have been found in camel milk and are useful in preventing tissue injury caused by toxic agents. Mg protects cells from heavy metals such as aluminum, mercury lead, cadmium, beryllium and nickel, which explains why re-mineralization is so essential for heavy metal detoxification and chelating. In fact, Mg deficiency has been associated with production of Reactive Oxygen Species (ROS) (Martin et al., 2003). Additionally, Mg protects cells against oxynradical damage and assists in absorption and metabolism of vitamin B, C and E (Barbagallo et al., 1999). Also, it has been reported that Mg is essential for biosynthesis of glutathione because the enzyme, glutathione synthetase, requires γ-glutamyl cysteine, glycine, ATP and Mg ions to form glutathione (Minnich et al., 1971).

Additionally, camel milk is rich in zinc (Zn) (Knoess, 1979), which is a trace element essential for living organisms. More than 300 enzymes require Zn for their activity. It also plays an important role in DNA replication, transportation and protein synthesis, influencing cell division and differentiation (Frederickson, 1989). It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system (Powell, 2000; Çezurk et al., 2003; Ozdemir and Inanc, 2005). Zinc is an essential component of the oxidant defense system and function at many levels (Sato and Brennner, 1993). One study has shown that a diet deficient in zinc paves the way for cell damage in the rat testis (Cai et al., 2001). Furthermore, Zn deficiency increase lipid peroxidation in various rat tissues, whereas the Zn supplementation corrects this increase (Shaheen and El-Fattah, 1965).

In the present study, we used the murine model of CCl₄-induced liver injury to investigate the hepatoprotective effects of camel milk. Since, many cases of acute toxic liver damages are triggered by free radical formation and further driven by local inflammatory response, potent antioxidant and inflammatory properties of camel milk seems to be protective during these conditions.

The results of the present study show that CCl₄ administration causes severe acute liver damage in rats demonstrated by remarkable elevation of serum ALT, AST and ALP levels (Fig. 1-3). The increased serum levels of AST and ALT have been attributed to a damaged structural integrity of the liver. This is because they are intracellular enzymes, released into circulation after hepatocyte damage or necrosis (Sallie et al., 1991). These findings were also confirmed by histological observations (Fig. 4c). The changes mostly include hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells infiltration and other histological manifestations which were also consistent with the findings of other authors (Brattin et al., 1985; Sun et al., 2001).
Pre-treatment and post-treatment with camel milk could ameliorate CCl₄-induced hepatotoxicity in rats, as demonstrated by the lower serum aminotransferase activities. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987).

Our results were in agreement with Kumar et al. (2009) who found that the groups of rats which received the pre-treatment of *T. cucumerina* (a curcurbitaceae medicinal plant) extract significantly controlled the change in the biochemical parameters. The extract exhibited a sharp decrease in the serum enzyme levels and the effect was comparable with the standard group. Pretreatment of rats with camel milk prior to CCl₄ intoxication (group V) (Fig.1,2,3) were found to have less rise in serum aminotransferases and alkaline phosphatase levels after CCl₄ intoxications. So camel milk consumption has some protective effects and enhances the defence system. Our results were also in agreement with Cucureanu et al. (2009) who found that the groups of rats, which received pre-treatment of montelukast sodium before the administration of CCl₄ intoxication, exhibited statistically significant lower levels of ALT, AST, TB and MDA in blood and liver homogenate as compared to the groups that received CCl₄ only. Similarly, Shen et al. (2009) reported that *Z. jujube* fruit administration prior to CCl₄ intoxication significantly decreased ALT and AST and attenuated histopathology of hepatic injury and ameliorated the oxidative stress in hepatic tissue as compared to the groups of rats who received CCl₄ intoxication only.

CONCLUSION

In conclusion, camel milk shows a significant role in hepatoprotective effects on acute liver toxicity induced by CCl₄. Camel milk seems to be a beneficial drink for the prevention of acute liver toxicity, although further studies are necessary.

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