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Camel Milk Regulates Gene Expression and Activities of Hepatic Antioxidant Enzymes in Rats Intoxicated with Carbon Tetrachloride

^{1,2}S.M. El-Bahr

¹Department of Physiology, Biochemistry and Pharmacology (Biochemistry), College of Veterinary Medicine and Animal Resources, King Faisal University, Saudi Arabia

²Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

ABSTRACT

The objective of the present study was to investigate the effect of camel milk on activities and gene expression of hepatic antioxidant enzymes in rats intoxicated with carbon tetrachloride (CCl₄). Therefore, twenty four rats were used in the current study. Rats were divided into four groups, the first and second groups were received water and camel's milk, respectively whereas rats of both third and fourth groups were injected with CCl₄ and received water and camel's milk, respectively. After 5 weeks, liver tissues were collected for biochemical analysis of the activities and gene expression of antioxidant enzymes. Rats supplemented with camel milk alone showed no significant difference in all examined parameters compared to control rats. Liver damage and oxidative stress were evident in untreated CCl₄ intoxicated rats as indicated by significant elevation of hepatic enzymes, significant elevation in thiobarbituric acid reactive substance (TBARS), significant reduction in reduced glutathione level (GSH), significant reduction in the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione-S transferase (GST) and finally significant down-regulation of antioxidant enzymes gene expression compare to control. Administration of camel milk along with CCl₄ caused amelioration in CCl₄-induced effects compare to the untreated CCl₄ intoxicated rats via up-regulation of antioxidant enzyme gene expression, activation of the expressed genes and increasing the availability of GSH. Conclusively, camel milk exerted its protective effect against CCl₄ induced hepatic toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system at activity and gene expression levels.

Key words: Real time PCR, Biomarkers, toxins, oxidative stress, liver function, serum enzymes

INTRODUCTION

CCl₄ is one of the most toxic agent to the liver tissue and its hepatotoxicity is due to trichloromethyl radical which produced during oxidative stress (Stoyanovsky and Cederbaum, 1999). These radicals stimulate the release of hepatic macrophages, kupffer cells and accompanied increase of infiltrated neutrophils and lymphocytes (Ramadori and Saile, 2004). The activated macrophages are released and contributed to liver fibrosis, inflammation and injury (Canbay *et al.*, 2004; Saile and Ramadori, 2007) with inefficient remedy (Lee *et al.*, 2007). Although, progress in the treatment of liver disease by chemical drugs has been reported, searching for natural drug is still ongoing (Recknagel, 1983). Currently, providing therapeutically effective natural drug for the treatment of liver diseases is the main duty of complementary and alternative medicines. Bioactive peptides and proteins contents of camel's milk are important for maintain effective bioprocesses such

as digestion, growth and immune responses (Yagil *et al.*, 1984; Korhonen and Pihlanto, 2003). In addition, camel's milk has the advantage of being stored at room temperature for longer period than other milk (Omer and Eltinay, 2009). The most described uses of camel's milk are reported as drug against autoimmune diseases, dropsy, jaundice, splenomegaly, tuberculosis, asthma, anemia, piles, diabetes (Rao *et al.*, 1970) and as antimicrobial agent (El Agamy *et al.*, 1992). In addition, camel's milk has antitoxic effect against cadmium chloride (Al-Hashem *et al.*, 2009; Dallak, 2009), CCl₄ (Khan and Alzohairy, 2011), Cisplatin (Afifi, 2010), Paracetamol (Al-Fartosi *et al.*, 2011), Aluminum chloride (Al-Hashem, 2009). It has been reported that, camel milk ameliorates CCl₄ induced oxidative stress via regulation of antioxidant enzyme activities (Al-Fartosi *et al.*, 2012). However, publications reported the gene expression regulation of these enzymes by camel milk are lack so far. Therefore, the aim of the present study was to investigate the effect of camel milk on oxidative damage and oxidative stress related gene expression and activities in rat liver intoxicated with CCl₄.

MATERIALS AND METHODS

Chemicals and kits: Paraffin oil, CCl₄ (Spectrosol® BHD chemicals ltd pool, England) and other chemicals and solvents were of highest grade commercially available. EGTA, EDTA, sucrose, Tris, butanol, mannitol, metaphosphoric and H₂O₂ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RIPA buffer was provided by Cayman chemical company, USA. All other chemicals were of analytical grade. Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate amino transferase (AST) were purchased from ELIPSE, United diagnostic industry, UDI, Dammam, Saudi Arabia.

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, 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 fresh milk (100 mL 24 h⁻¹ cage⁻¹) (Althnaian *et al.*, 2013) as such without any further treatment.

Animals and treatment: A total of 24 albino rats (200-250 g) were 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 (6 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/12 h dark 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 CCl₄: Liver toxicity 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 (Khan and Alzohairy, 2011).

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 follow:

- Group 1:** Rats fed only with basal diet and water and injected i/p with Paraffin oil, this group was served as control 1 group
- Group 2:** Rats fed normal basal diet, injected i/p with Paraffin oil and treated with camel's milk (100 mL 24 h⁻¹ cage⁻¹) (Althnaian *et al.*, 2013) as their sole source of drinking water, this group was served as control 2 group
- Group 3:** Rats fed basal diet and water and intoxicated with CCl₄ (1 mL kg⁻¹ b.wt.), 1:1 diluted with paraffin oil on first two days of the experiment (Khan and Alzohairy, 2011)
- Group 4:** Rats fed basal diet and intoxicated with CCl₄ (1 mL kg⁻¹ b.wt.), 1:1 diluted with paraffin oil on first two days of the experiment and then treated with camel's milk (100 mL 24 h⁻¹ cage⁻¹) as their sole source of drinking water

Sample collection: After 5 weeks, all rats were anesthetized with diethyl ether. 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 of liver enzymes using commercial assay kits according to the manufacture instruction. The liver tissues were removed and liver fragments were immediately frozen in liquid nitrogen and stored at -80°C for molecular and biochemical analysis of antioxidant enzymes. Because of the same liver tissues of the previously published work (Althnaian *et al.*, 2013) were used, the methodology of liver histogram was not mentioned in the current work.

Assessment of liver damage: Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of ALT (EP07-500) and AST (EP15-500) on ELIPSE full automated chemistry analyzer (Rome, Italy). The histopathological picture of the previous work (Althnaian *et al.*, 2013) was taken in consideration.

Determination of hepatic antioxidant enzymes, thiobarbituric acid reactive substances (TBARS) and reduced glutathione: One gram of liver tissues was homogenized in 5 mL of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose. After centrifugation (1500xg/5 min) at 4°C, the supernatant was removed and stored frozen at -80°C until the time of analysis of superoxide dismutase (SOD). Another one gram of liver tissues was homogenized in 5 mL of cold buffer of 50 mM potassium phosphate buffer, pH 7, containing 1 mM EDTA. After centrifugation (10.000xg/15 min) at 4°C, the supernatant was removed and stored frozen at -80°C until the time of analysis of catalase (CAT), glutathione peroxidase (GPX), glutathione-S transferase (GST) and reduced glutathione (GSH). The extent of lipid peroxidation in terms of TBARS formation was measured by mixing one gram of liver tissues with RIPA buffer (Item No. 10010263, Cayman chemical company, USA). After homogenization, sonication and centrifugation (1600xg/10 min), the supernatant was removed and stored frozen at -80°C until the time of analysis. The activities of CAT (nmol min⁻¹ g⁻¹ tissue; Cayman Chemical Company, USA, Catalog No. 707002), GPX (nmol min⁻¹ g⁻¹ tissue; Cayman Chemical Company, USA, Catalog No.703102), SOD (U g⁻¹ tissue; Cayman Chemical Company, USA, Catalog No. 706002), GST (nmol min⁻¹ g⁻¹ tissue; Cayman Chemical Company, USA, Catalog No. 703302) and concentrations of GSH (µM; Cayman Chemical Company, USA, Catalog No. 703002) and TBARS (µM; Cayman Chemical Company, USA, Catalog No. 10009055) were determined by ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA). Results were calculated according to the manufacture instructions.

Total RNA isolation and real time RT-PCR of hepatic antioxidant enzymes: Liver tissues (approximately 1 g of tissue per sample) were immediately added to 1 mL of TriZol reagent (Invitrogen, Carlsbad, CA) and homogenized using homogenizer (Tissue Ruptor, Qiagen GmbH, Germany). One milliliter of the tissue homogenate was transferred to a microfuge tube and total RNA was extracted by adding 0.2 mL chloroform. Afterwards, samples were vortexed vigorously for 15 sec and incubated at room temperature for 3 min. After centrifugation (12,000 g 15 min⁻¹) at 4°C, the aqueous phase containing RNA was transferred into new tubes. RNA was precipitated by mixing the aqueous phase with 0.5 mL isopropyl alcohol and incubated at room temperature for 10 min. After centrifugation at 12,000 g for 10 min at 4°C, RNA pellets were washed by mixing and vortexing with 1ml of 75% ethanol. After centrifugation (7,500 g 5 min⁻¹) at 4°C, RNA pellets were resuspended in nuclease free water (Life Technologies, USA). The purity of RNA at 260/280 OD ratio and RNA integrity was evaluated using Multi-Mode Microplate reader (SYNERGY Mx, BIO-TEK, Winooski, Vermont, USA). Only high purity samples (OD260/280 >1.8) were subjected to further manipulation. cDNA was prepared from RNA samples according to Revers Transcription System Kit (Promega, Madison, USA) by using Bio-Rad Thermal Cycler (T100™, Foster city, California, USA). Briefly, total RNA were activated at 70°C for 10 min and 20 µL reaction mix were made of 4 µL MgCl₂, 2 µL of reverse transcription 10X buffer, 2 µL of dNTP mixture (10 mM), 0.5 µL of random primers, 0.75 µL of AMV reverse transcriptase enzyme, 1ng RNA and nuclease-free water to a final volume of 20 µL. Then the reaction was incubated at 42°C for 60 min followed by incubation at 94°C for 5 min. cDNA was diluted up to 100 µL with nuclease-free water for PCR amplification. Real time RT-PCR was performed using QuantiFast™ SYBR Green PCR Master Mix kit (QIAGEN, Hilden; Germany). The 25 µL reaction for each examined gene was prepared from 12.5 µL of master mix; 2 µL forward primer (10 pmol); 2 µL reverse primer (10 pmol); 2 µL cDNA of the sample and 6.5 µL of nuclease-free water. Cycling parameters were, 50°C for 2 min, 95°C for 15 min, 40 cycles of 95°C for 10 sec, followed by 55°C for 30 sec and 72°C for 10 sec with final melting at 95°C for 20 sec. For each gene examined, duplicate samples from each cDNA analyzed by real time RT-PCR using the Bio-Rad CFX Manager 3.0 Software of the C1000 Touch thermal cycler-CFX96 Real time PCR(BIO-RAD, Foster city, California, USA). The β -actin mRNA fragment was used as housekeeping gene to normalize the expression data. The primer sequences are described in Table 1.

Statistical analysis: All data was presented as Mean±Standard error of mean by using student-ttest. All tests were performed using computer package of the statistical analysis system (SAS, 2002). The relative gene expression of target genes in comparison to the β -actin reference gene was calculated using the Bio-Rad CFX Manager 3.0 Software of the C1000 Touch thermal cycler-CFX96 Real time PCR(BIO-RAD, Foster city, California, USA).

Table 1: Details giving primer sequences and expected product size for the genes amplified

Gene	Forward primer sequence	Reverse primer sequence
β -actin	5'-AGC CAT GTA CGT AGC CAT CC-3'	5'-CTC TCA GCT GTG GTGGTG AA-3'
SOD	5'-AGG ATT AAC TGA AGG CGA GCA T-3'	5'-TCT ACA GTT AGC AGG CCA GCA G-3'
CAT	5'-ACG AGA TGG CAC ACT TTG ACA G-3'	5'-TGG GTT TCT CTT CTG GCT ATG G-3'
GPx	5'-AAG GTG CTG CTC ATT GAG AAT G-3'	5'-CGT CTG GAC CTA CCA GGA ACT T-3'
GST	5'-GCT GGA GTG GAG TTT GAA GAA-3'	5'-GTC CTG ACC ACG TCA ACA TAG-3'

SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GST: Glutathione-S transferase

RESULTS

Assessment of liver function: The activities of AST and ALT were estimated in serum samples as the liver function biomarkers. These results are given in Table 2. The CCl₄ treatment markedly affected the liver specific enzymes. It was found that a significant ($p < 0.05$) increase in serum AST ($130.8 \pm 0.1.7 \text{ U L}^{-1}$) and ALT ($47.4 \pm 4.0 \text{ U L}^{-1}$) activities of CCl₄ treated rats compare to control ($118.9 \pm 0.6.0$ and $29.2 \pm 0.1 \text{ U L}^{-1}$), respectively. This result suggests that these hepatic biomarkers are elevated in the serum due to release of the enzymes from damaged liver. However a significant decrease ($p < 0.05$) was observed in the respective serum activities of rats given Camel milk+CCl₄ (125.6 ± 2.0 and $34.7 \pm 1.5 \text{ U L}^{-1}$) compared with CCl₄ treated group ($130.8 \pm 0.1.7$ and $47.4 \pm 4.0 \text{ U L}^{-1}$), respectively. As this work is a continuation to the previous published work (Althnaian *et al.*, 2013), inclusion of histopathological picture in this study was not necessary because the same liver samples were used. The previous study had demonstrated that, liver of CCl₄-intoxicated rats showed massive fatty change and centrilobular necrosis in most cases. Hepatitis characterized by mononuclear cells infiltration mostly macrophages and lymphocytes around central veins and in portal areas was also noticed in most cases of CCl₄ intoxicated rats. In addition, the liver of CCl₄-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.

Effect of treatments on hepatic lipid peroxidation: Results in Table 3 shows that the TBARS level was significantly ($p < 0.05$) increased in the liver of CCl₄ intoxicated rats ($33.3 \pm 1.01 \mu\text{M}$)

Table 2: Effect of administration of CCl₄ and/or camel milk for five weeks on serum biomarker enzymes activities of AST (U L^{-1}) and ALT (U L^{-1})

Parameters	Experimental groups			
	Control	Camel milk	CCl ₄	CCl ₄ +camel milk
AST	118.9±6.0	111.6±5.0	130.8±1.7*	125.6±2.0**
ALT	29.2±0.1	32.2±1.7	47.4±4.0*	34.7±1.5**

ALT: Alanine aminotransferase, AST: Aspartate amino transferase. Values are expressed as Mean±SE, n = 6 for each group; Significance was calculated at $p < 0.05$. *Significant as compared to control animals. **significant as compared to CCl₄ treated animals

Table 3: Effect of administration of CCl₄ and/or camel milk for five weeks on levels of TBARS (μM) and reduced glutathione (μM) and activities of CAT ($\text{nmol min}^{-1} \text{g}^{-1}$ tissue), SOD (U g^{-1} tissue), GPX ($\text{nmol min}^{-1} \text{g}^{-1}$ tissue) and GST ($\text{nmol min}^{-1} \text{g}^{-1}$ tissue) in rats liver tissue

Parameters	Experimental groups			
	Control	Camel milk	CCl ₄	CCl ₄ +camel milk
TBARS	28.3±0.93	26.1±0.81	33.3±1.01*	28.3±1.02**
CAT	27.0±0.20	26.7±1.10	23.2±1.10*	27.1±1.20**
SOD	8.0±0.02	8.0±0.01	6.0±0.02*	8.0±0.01**
GPX	290.1±0.90	290.8±0.70	272.1±2.10*	291.4±2.20**
GST	186.0±1.00	185.1±1.00	140.1±1.02*	188.8±2.01**
GSH	5.0±0.10	5.1±0.10	4.0±0.10*	6.0±0.20**

TBARS: Thiobarbituric acid reactive substance, CAT: Catalase, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, GST: Glutathione-S transferase and GSH: Reduced glutathione. Values are expressed as Mean±SE, n = 6 for each group. Significance was calculated at $p < 0.05$, *significant as compared to control animals, **significant as compared to CCl₄ treated animals

compare to the control ($28.3 \pm 0.93 \mu\text{M}$). Treatment of CCl_4 intoxicated rats with camel milk caused significant ($p < 0.05$) decrease in TBARS level ($28.3 \pm 1.02 \mu\text{M}$) of liver tissue compared to that in liver from untreated CCl_4 intoxicated rats ($33.3 \pm 1.01 \mu\text{M}$). Administration of camel milk only did not affect significantly ($p > 0.05$) the level of TABARS in rat liver compare to the control.

Effect of treatments on GSH and hepatic antioxidant enzyme activities: GSH concentration and antioxidant enzyme activities (CAT, SOD, GPX and GST) are shown in Table 3. The concentration of GSH was reduced significantly ($p < 0.05$) in CCl_4 intoxicated rats ($4.0 \pm 0.10 \mu\text{M}$) compare to the control ($5.0 \pm 0.10 \mu\text{M}$). However, camel milk administration reserves GSH values in CCl_4 intoxicated rats ($6.0 \pm 0.20 \mu\text{M}$) compare to untreated CCl_4 intoxicated rats ($4.0 \pm 0.10 \mu\text{M}$) and control ($5.0 \pm 0.10 \mu\text{M}$). All the antioxidant enzyme activities were reduced significantly ($p < 0.05$) in CCl_4 intoxicated rats (CAT: $23.2 \pm 1.10 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ tissue}$; SOD: $6.0 \pm 0.02 \text{ U g}^{-1} \text{ tissue}$; GPX: $272.1 \pm 2.10 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ tissue}$ and GST: $140.1 \pm 1.02 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ tissue}$) compare to the control (27.0 ± 0.20 ; 8.0 ± 0.02 ; 290.1 ± 0.90 ; 186.0 ± 1.00), respectively. However all activities of these enzymes showed a significant ($p < 0.05$) recovery in response to camel milk administration to CCl_4 intoxicated rats (27.1 ± 1.20 ; 8.0 ± 0.01 ; 291.4 ± 2.20 ; 188.8 ± 2.01), respectively compare to untreated CCl_4 intoxicated rats (23.2 ± 1.10 ; 6.0 ± 0.02 ; 272.1 ± 2.10 ; 140.1 ± 1.02), respectively. The activities of hepatic antioxidant enzymes of rats treated only with camel milk were comparable to that of the control (Table 3).

Effect of treatments on gene expression of antioxidant enzymes: The mRNA expression of SOD, CAT, GPX and GST were measured by real time PCR (Fig. 1). The results showed that, the expression of all of the antioxidant enzymes were reduced significantly ($p < 0.05$) in CCl_4 intoxicated rats compare to the control. However, the expression of all of the antioxidant enzymes were increased significantly ($p < 0.05$) in CCl_4 intoxicated rats treated with camel milk compare to untreated CCl_4 intoxicated rats. The expression of hepatic antioxidant enzymes of rats treated only with camel milk was comparable to that of the control (Fig. 1).

DISCUSSION

In the current study serum liver biochemical markers, AST and ALT activities were increased significantly ($p < 0.05$) in rats treated with CCl_4 compare to control. This increase in serum levels of liver function biomarkers might be attributed to the liver damage as these enzymes are cytoplasmic in location and its released into circulation indicated damage to cell membrane (Zimmerman *et al.*, 1965; Brent and Rumack, 1993; Recknagel *et al.*, 1989). Similar results (Trible *et al.*, 1987; Wang *et al.*, 1997; Mehmetcik *et al.*, 2008; Arici and Cetin, 2011; Althnaian *et al.*, 2013) demonstrated that, ALT and AST enzymes activities were significantly increased following CCl_4 administration. The hepatotoxic effect of CCl_4 is due to active metabolite Ccl_3 which has the ability to eliminate hydrogen from fatty acids and initiating the lipid peroxidation, hepatocytes injury and liver damage (Forni *et al.*, 1983; Park *et al.*, 2005). However, in the current study, camel milk was found to ameliorate ($p < 0.05$) the leakage of AST and ALT induced by CCl_4 treatment in rats. This suggests a membrane stabilizing activity of camel milk because it has been accepted that, serum levels of transaminases might be return to normal whenever healing of hepatic parenchyma and hepatocytes regeneration have been achieved (Thabrew *et al.*, 1987). Supporting to the present findings, several reports (Khan and Alzohairy, 2011; Hamad *et al.*, 2011; Al-Fartosi *et al.*, 2012; Althnaian *et al.*, 2013) demonstrated the hepatoprotective effect of camel milk. These studies

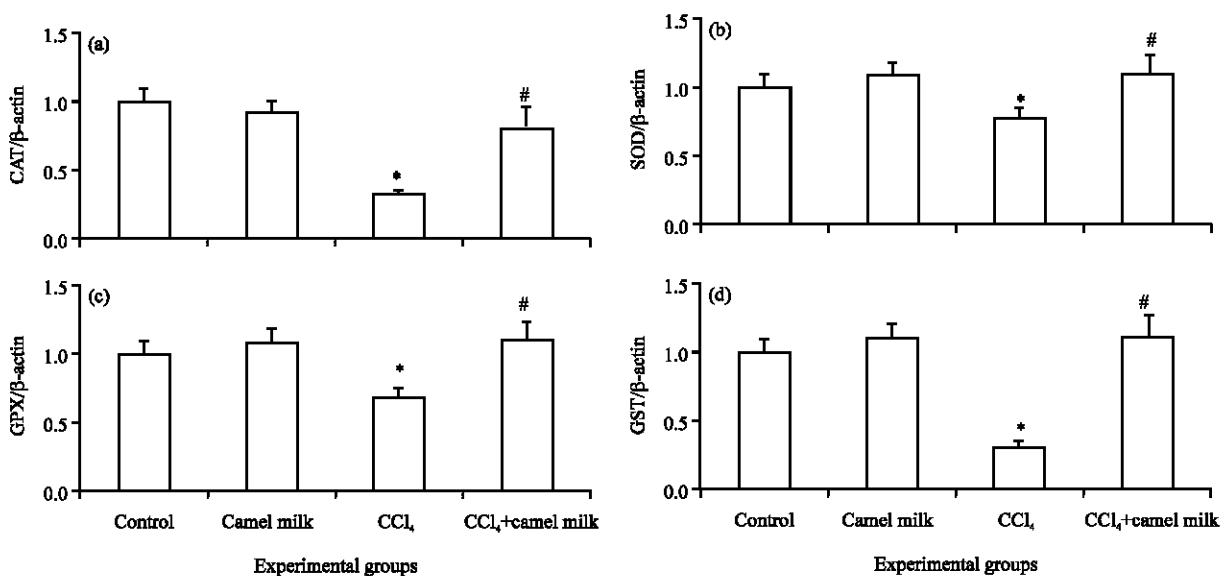


Fig. 1(a-d): Real time PCR analysis of mRNA gene expression of hepatic catalase, (a) CAT, (b) Superoxide dismutase, SOD, (c) Glutathione peroxidase, GPX and (d) Glutathione-S transferase, GST of control non-treated, camel milk treated, CCl₄ treated and CCl₄+camel milk treated rats. Liver tissues were homogenized in TriZol reagent and RNA was extracted by chloroform. After preparation of cDNA, real time RT-PCR was performed using QuantiFast™ SYBR Green PCR Master Mix kit. The relative gene expression of target genes in comparison to the β -actin reference gene was calculated using the Bio-Rad CFX Manager 3.0 Software of the C1000 Touch thermal cycler-CFX96 Real time PCR. Values are expressed as mean \pm SEM. *Values are significantly different ($p < 0.5$) compare to control. #Values are significantly different ($p < 0.5$) compare to CCl₄ intoxicated group

elucidated that, the ameliorative effect of camel milk against CCl₄-induced oxidative stress in rats was attributed to the antioxidant effect of camel milk contents such as vitamins (A, B₂, C and E) and trace elements (Yousef, 2004). The efficient hepatoprotective drugs can be defined as, drug which able to reduce the harmful effect or restore the normal hepatic function that has been distributed by toxin. Camel milk reduced the levels of liver enzymes that has been elevated as results of CCl₄ toxicity. This indicated that, the structural integrity of hepatocytes cell membrane has been protected and/or damaged hepatocytes have been regenerated (Palanivel *et al.*, 2008).

The significant increase of TBARS in liver of untreated CCl₄ intoxicated rats suggests enhanced peroxidation leading to tissue damage and the failure of the antioxidant mechanisms in preventing of excessive free radicals (Romero-Alvira and Roche, 1996). This confirmed by the results of the current study which reported a significant decrease of both GSH content and the activities of antioxidant enzymes (SOD, GPX, CAT, GST) in CCl₄ intoxicated rats compare to control which impose in elevation of TBARS levels. These findings were found to be in agreement with other previous studies in rats liver intoxicated with CCl₄ (Yousef, 2004; Aranda *et al.*, 2010; Al-Fartosi *et al.*, 2012; Bona *et al.*, 2012; Pirinccioglu *et al.*, 2012). The significant decrease of TBARS in liver of rats intoxicated with CCl₄ and treated with camel milk suggests the protective

effect of camel milk against CCl_4 -induced oxidative stress. This protective effect of camel milk is due to contents of essential vitamins and minerals (Barbagallo *et al.*, 1999).

GSH plays an important role in antioxidant system of the body. It maintains normal structure and function of the cells via redox and detoxification reaction. In the present study a significant decrease of GSH values were observed in CCl_4 intoxicated rats. The decrease of GSH is perhaps due to direct requisition of GSH by GPX to scavenge free radicals that formed during the metabolism of CCl_4 . Similar results were reported in rats intoxicated with CCl_4 (Bona *et al.*, 2012). The ameliorative effect of camel milk against CCl_4 induced toxic effect on GSH level was attributed to the ability of camel milk to scavenge free radicals and restore the antioxidant status which underlined by increasing the activities and gene expression of antioxidant enzymes as discussed below. Camel milk restored the GSH values in rat liver intoxicated with aluminum chloride (Al-Hashem, 2009), cadmium chloride (Al-Hashem *et al.*, 2009) and alcohol (Darwish *et al.*, 2012).

The oxidative stress refers to the imbalance between prooxidants and antioxidants in biological systems. Therefore, the significant increase in lipid peroxidation could be due to significant reduction in the activities of enzymatic antioxidants such as CAT, SOD, GPX and GST as well as non-enzymatic antioxidants such as GSH in the liver of CCl_4 -intoxicated rats, as compared to the control. Superoxide radical is converted to H_2O_2 by SOD. Furthermore, H_2O_2 is transferred to molecular oxygen and water by CAT and GPX. Therefore, SOD, CAT, GPX and GST constitute the principal components of the antioxidant system and their deficiencies leads to oxidative stress. Therefore, the significant reduction in these enzyme activities in liver of CCl_4 intoxicated rats (Table 3) could be responsible for increased lipid peroxidation observed as reflected on high level of TBARS along with low level of GSH in these animals compared to the control during CCl_4 -induced hepatotoxicity. Significant reductions in GPX, SOD and CAT have been reported in CCl_4 -intoxicated rat liver (Palanivel *et al.*, 2008; Bigoniya and Rana, 2010), kidney (Adewole *et al.*, 2007; Ganie *et al.*, 2011) and lung (Ganie *et al.*, 2011). Previous study has reported that camel milk is a potent inducer of these detoxifying enzymes and thereby prevents the toxicity induced by aluminum chloride (Al-Hashem, 2009), cadmium chloride (Al-Hashem *et al.*, 2009; Dallak, 2009) and alcohol (Darwish *et al.*, 2012).

At gene expression level, the current findings indicated that, CCl_4 induced down regulation of all examined antioxidant enzymes (SOD, CAT, GPX and GST) gene expression. Similar results (Chen *et al.*, 2013) was obtained in CCl_4 -induced liver fibrosis in mice. The present findings also reported that, camel milk augmented the antioxidant status via up-regulation of CAT, SOD, GPX and GST gene expression (Fig. 1). Similar results (Afifi, 2010) demonstrated that camel milk regulated gene expression of these antioxidant enzymes in lung tissues of rats intoxicated with cisplatin. The author studied gene expression of antioxidant enzymes in lung tissues of rats by conventional PCR. The conjugation of reactive xenobiotic metabolites with GSH as mentioned above is an important step in detoxification mechanism. This conjugation process is mediated by GST. CCl_4 depleted the GSH through conjugation process (Table 3). The down regulation of GST gene expression in CCl_4 intoxicated rats compare to control as presented in the current study (Fig.1c) could limit the ability of hepatic tissues to conjugate the reactive metabolites which alleviated by Camel milk.

CONCLUSION

Camel milk may exert its protective effect against CCl_4 induced hepatic toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system at activity and gene expression levels.

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