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HEPATOPROTECTIVE PROPERTIES OF CAMEL'S MILK AGAINST BENZENE INDUCED TOXICITY IN MALE RATS

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ABSTRACT

Benzene is commonly used in daily life but it can be potentially toxic. Therefore, the present study was carried out to investigate the protective effects of camel's milk against benzene induced biochemical alterations and oxidative stress in the liver of male white albino rats. White albino male rats (150-200 g) were divided into three groups of 6 rats: a control group treated orally with 1mL normal saline for two weeks, the benzene-treated group and the camel's milk-benzene-treated group. The benzene treated group received 0.5 ml kg-1 of benzene intrapretonealy at the first day from each week for two weeks. The camel's milk-benzene-treated group was injected intrapretonealy with 0.5 ml kg-1 at the first day from each week for two weeks + 1 mL/day of fresh camel's milk for two weeks. Lipid peroxidation was determined by the serum concentrations of thiobarbituric acid reactive substances (TBARS). Liver biochemical serum parameters were analyzed. Oral administration of benzene induced lipid peroxidation in the liver, which was indicated by a significant increase in lipid peroxidation biomarkers (TBARS). Also the data showed that the administration of benzene resulted in statistically significant increases in the serum levels of cholesterol, triglycerides, aspartae aminotransferase (AST) and alanine aminotransferase (ALT) and a significant decrease in total protein and albumin. In all rats treated with camel's milk after given benzene, lipid peroxidation and other biochemical parameters were altered compared with male rats treated with benzene only.

KEYWORDS: Benzene, camel's milk, liver, rats, toxicity.

INTRODUCTION

Benzene is a ubiquitous environmental pollutant being used for industrial purposes. It is used in manufacturing products such as rubber, lubricants, detergents, drugs and pesticides. It is a clastogenic and carcinogenic agent. The most common exposures occur through auto-exhaust, industrial emissions, cigarette and smoke (ASTDR-2007). Benzene exposure suppresses bone marrow function, causing blood changes and CNS depression (U.S EPA). In addition to this, it has been shown benzene exposure affect the variety of organs such as kidney, liver and brain (Dundaroz *et al*., 2003).

Exposure to benzene linked with a variety of leukemia in humans (Aksoy, 1989). It is generally accepted that benzene requires metabolism to induce its toxic effects (Cooper and Snyder, 1988). Benzene administration to rats has been shown to induce lipid peroxidation in both liver and bone marrow of rats. Oxidative metabolites of benzene cause destruction of cytochrome CYP4502E1 (Gut, 1983). CYP4502E1 was shown to yield very high levels of reactive oxygen species, which are thought to damage DNA (Snyder *et al.*, 1993).

Camel's milk is different from other ruminant milk, having low cholesterol, law sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium) high vitamin C, B2, A and E low protein and high concentration of insulin (Knoess, 1979). The milk is considered to have medicinal properties, A series 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). The positive effect of camel milk on diabetic patients has been shown (Agrawal *et al.*, 2002; Mnati, 2010). Afifi (2010) demonstrated the renoprotective potential of camels milk against cisplatin induced oxidative stress and renal dysfunction in mice. Other studies indicated a protective effect of camels milk against aluminum and cadmium induced toxicity in laboratory animals (Al-Hashem, 2009a, b; Al –Hashem *et al*., 2009).

In our survey, we didn't find any study dealt with the effect of camel's milk against the bad effect of benzene, therefore in this study, we tested the effect of oral administration camel's milk in male rats by examining its

protective effects against benzene induced toxicity in male rats.

MATERIALS AND METHODS

Camel's milk samples: Daily milk samples were collected early in the morning from camel farm in the Al-Nassiriya city, Thi-Qar Province, Iraq. Milk was collected from camel 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.

Experimental design: 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. Three groups of 6 rats were used for the experiments as following: Group 1, the control group, received orally a daily dose of 1 ml normal saline for 14 days; Group II, the benzene treated group, received 0.5 ml/kg body weight from benzene (I.P) at the first day from each week for two weeks; Group III, the Camel's milk-benzene treated group was treated with 0.5 ml/kg of body weight from benzene(I.P) at the first day from each week for two weeks +1 ml/day of a Camel's milk for two weeks.

Collection of blood serum: At the end of 14 days, the animals were subjected to over-night fasting before being sacrificed by cervical dislocation and blood samples were collected directly into tubes and were centrifuged at 3000 rpm for 10 minutes to obtain serum.

Biochemical serum parameters analyses: According to the method by Buege and Aust (1976), a colorimetric method that measures the reaction product of thiobarbituric acid (TBA) with aldehyde such as malondialdehyde (MDA) formed by lipid peroxidation was used for this analysis. Cholesterol, triglyceride, albumin and protein levels were evaluated as described previously by (References). Additionally, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were assayed using commercial kits ( ).

Statistical analysis

Statistical analysis of the results was performed by SPSS test, data are presented as Mean ± Stander Error.

RESULTS

Intrapretoneal injection of male rats with benzene resulted in statistically significant increases MDA when compared to the serum from saline-treated rats (table 1). However, the oral administration of camel's milk prior to treatment with benzene caused a significant decrease of this parameter compared with benzene treated group.

Table (1): Effect of camel's milk on MDA level of male rats treated with benzene.

Treatments	No. of male rats	MDA level
Control group	6	78.90±3.29 b
Benzene group	6	102.11±7.43 a
Camel's milk+ benzene group	6	91.35±5.74 b

- Abbreviation: MDA: Malondialdehyde.
-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at P<0.05.

Evaluation serum levels of AST and ALT demonstrated that there was a highly significant increase in the activities of these enzymes in the serum of rats treated with benzene compared to control rats, while rats treated with camel's milk and benzene showed normal activities of these enzymes (table 2).

Table (2): Effect of camel's milk on AST and ALT levels of male rats treated with benzene.

Treatments	No. of male rats	AST	ALT
Control group	6	125.33±6.06 b	69.33±4.26 b
Benzene group	6	182.12±7.14 a	83.14±2.19 a
Camel's milk + benzene group	6	122.83±8.14 b	64.93±4.17 b

- Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase
-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at P<0.05.

Furthermore, the levels of cholesterol and triglycerides in the serum of benzene-treated male rats were significantly higher than in the control-treated animals (table 3). Administration of camel's milk prior to benzene treated group resulted in a highly significant reduction to near normal levels for serum lipid profiles when compared to benzene treated rats (table 3).

Table (3): Effect of camel's milk on lipid profile levels of male rats treated with benzene

Treatments	No. of male rats	Cholesterol	Triglyceride
Control group	6	50.83±1.85 b	71.90±1.33 b
Benzene group	6	62.55±0.42 a	91.71±3.46 a
Camel's milk + benzene group	6	55.68±6.80 b	65.37±2.89 b

-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at P<0.05.

Oral administration of benzene for two weeks resulted in statistically significant decrease in albumin concentrations when compared to the serum from saline-treated rats (table 4). However, the oral administration of camel's milk prior to treatment with benzene maintained this parameter within normal level.

Table (4): Effect of camel's milk on albumin level of male rats treated with benzene.

Treatments	No. of male rats	Albumin
Control group	6	57.82±1.42 a
Benzene group	6	46.00±1.71 b
Camel's milk + benzene group	6	53.19±2.09 a

-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at P<0.05.

DISCUSSION

Benzene, a toxic aromatic hydrocarbon can alter the microanatomy and physiology of different organs including liver (Ozturk *et al*., 1997). Benzene is widely used in different industries like rubber, drugs, detergents etc (Fatima *et al.*, 2004). The present study investigated the protective effects of camel's milk against the harmful effects of benzene on the liver of white albino rats through the analysis of lipid peroxidation and multiple biochemical serum parameters. It has been reported that animals exposed to benzene displayed hepatic necrosis, which was indicated by increasing of lipid peroxidation and serum levels of liver-specific enzymes including AST and ALT and decrease of albumin. Dere *et al.*, (2003) showed that benzene causes some changes (first increases then decreases) in LDH, ALP, ALT, AST, PK enzyme activities and estradiol, testosterone levels in different tissues of rats. Increased lipid peroxidation and oxidative degeneration of poly unsaturated fatty acids of hepatocytic membranes due to generation of free radicals via benzene toxicity was also reported in previous reviews (Barale *et al.*, 1990). Increased level of MDA level is a marker of lipid peroxidation (Del *et al.*, 2005). Our results demonstrated that I.P. injection of benzene caused a significant increase in serum cholesterol and triglycerides, which may indicate a loss of membrane integrity, disturbance of lipid metabolism and/or liver dysfunction.

Benzene being lipid soluble is transported in the blood and absorbed by red cell membrane. It tends to accumulate in tissues with high lipid content and about 50% of the absorbed dose may be eliminated unchanged while the remaining is metabolized in liver, primarily by Cyt-P450 systems (Hannumantharao *et al*., 2001). Benzene microsomal metabolism plays a critical role in benzene toxicity (Sukhodub and Padalko, 1999). There are indications that benzene, or its metabolites, can metabolize during transportion to target tissues and again in the tissue themselves, causing a reaction with nucleophilic parts of cell macromolecules. Metabolites perform their reactions with enzymes with the aid of reactive groups in them, such as epoxcite, oxepin, quinine and aldehyde. The metabolism of quinone compounds includes the reduction of one or two electrons. The reduction of a single electron leads to the appearance of semiquinone radical. This radical reduces the molecular oxygen immediately, and superoxide radicals form, followed by the creation of other reactive oxygen products (Arfellini *et al*., 1985; Smith *et al.*, 1989; Witz *et al.*, 1996).

The present study demonstrated that treatment of benzene-male rats with camel's milk protects the liver from benzene toxicity. The protective effect of camel's milk could be attributed to its antioxidant activity and it may possibly have chelating effects on benzene. It has been reported that camel's 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 to be 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 heavymetal detoxification and chelating. In fact, Mg deficiency has been associated with production of ROS (Martin *et al*., 2003). Additionally, Mg protects cells against oxyradical damage and assists in the absorption and metabolism of vitamins B, C and E (Barbagallo, 1999), which are antioxidants important in cellular protection. Recent evidence suggests that vitamin E enhances glutathione levels and may play a protective role in Mg deficiency induced cardiac lesions (Barbagallo, 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 (Virginia, 1971). Additionally, camel's milk is rich in zinc (Zn) (Knoess, 1979), 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. Our study demonstrated that treatment of rats with camel's milk prior to benzene resulted in decreased cholesterol and triglyceride levels in the serum compared to the benzene intoxicated group. It can be concluded that camel's milk can act in several ways to lower serum cholesterol and triglycerides. First, uptake of cholesterol and triglycerides in the gastrointestinal tract could be inhibited; second, LDL-cholesterol could be eliminated from the blood via the LDL receptor and finally, the activity of cholesterol-degrading enzymes could be increased (Hashem, 2009).

CONCLUSION

Benzene has adverse effects on human health. Our results demonstrate that benzene 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 benzene-associated hazards. Therefore, drinking camel's milk could be beneficial for alleviating benzene toxicity. Further studies are required, using a human population, to confirm these protective effects.

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