Food and Chemical Toxicology 50 (2012) 1377–1383

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Camel's milk alleviates alcohol-induced liver injury in rats

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ARTICLE INFO

Article history:
Received 4 August 2011
Accepted 10 January 2012
Available online 18 February 2012

Keywords:
Ethanol
Liver toxicity
Camel's milk
Oxidative stress
Tumor necrosis factor-alpha
Caspase-3

ABSTRACT

Alcoholic liver disease (ALD) represents a spectrum of clinical illness and morphological changes that range from fatty liver, hepatic inflammation and necrosis to progressive fibrosis. For the etiology of ALD, oxidative stress, increased expression of proinflammatory cytokines and apoptosis have been described. The present study aimed to investigate the effectiveness of camel's milk (CM) in alleviating alcohol-induced hepatotoxicity as a model of clinical liver illness. Male rats were grouped into four groups from which one group received normal saline and served as control. Groups from 2 to 4 received a daily oral dose of 56% ethanol for 4 weeks. Group 2 served as untreated control while groups 3 and 4 were respectively treated with CM either in a prophylactic or a curative approach. Alanine transaminase, aspartate transaminase, alkaline phosphatase, triglycerides, as well as cholesterol levels were estimated in the serum. Malondialdehyde, total antioxidant capacity, and tumor necrosis factor-alpha levels along with caspase-3 activity were determined in liver tissue homogenate. A histopathological analysis of liver tissue was also achieved. Results showed amelioration of all tested parameters following administration of CM. Conclusively, treatment with camel's milk alleviates alcohol-associated hazards and protects hepatic tissue from alcohol-induced toxicity.

1. Introduction

Alcohol is a dietary component which is usually consumed for its psychophysical and mood-altering effects. Long-term alcohol consumption may cause damage to vital organs including cardiovascular, endocrine, gastrointestinal, and central nervous systems (Niemela, 2001; Preedy et al., 1999). According to the World Health Organization report of 2005, approximately 2 billion people worldwide consume alcohol, and about 76 million of them have been estimated to be suffering from alcohol consumption disorders. The most extensively investigated aspect of ethanol on health is alcoholic liver disease (ALD), which is one of the major causes of illness and death worldwide (Liu et al., 2010). ALD morphological features include fatty liver (steatosis), hepatitis, and alcoholic cirrhosis (Smathers et al., 2011).

In the last several decades, significant progress has been made in understanding the cellular and molecular mechanisms contributing to the pathogenesis of ALD (O'Shea et al., 2010). These mechanisms include direct hepatotoxicity, production of reactive oxygen species (ROS) induced by ethanol and its metabolites, activation of innate immunity and complement system with subsequent production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) (Arteel, 2003; Breitkopf et al., 2009; Zhao et al., 2008).

Apoptosis, known as programmed cell death, is a biological process that plays a crucial role in the normal development and tissue homeostasis (Jang et al., 2002). Caspases, a family of cysteine proteases, are integral parts of the apoptotic pathway. Caspase-3, in particular, has many cellular targets and its activation produces the morphologic features of apoptosis (Jang et al., 2002; Omezzine et al., 2003). Minana et al. (2002) showed that ethanol and its metabolite, acetaldehyde, have induced apoptosis in hepatocytes after chronic ethanol feeding via elevating Fas ligand levels with subsequent caspase-8 and caspase-3 activation.

Prevention of ALD is a therapeutic challenge and identifying inexpensive natural agents which can achieve the goal is further a higher challenge. Camel's milk (CM) might represent such a potential candidate. CM is different from other ruminant milk; it is lower in cholesterol, protein and sugar, but higher in minerals, vitamins, and insulin (Yousef, 2004). It has no allergic properties and can be consumed by lactose-intolerant individuals (Cardoso et al., 2010). It also contains a relatively large amount of polyunsaturated fatty acids and linoleic acids, which are essential for human nutrition (Gorban and Izzeldin, 2001).

Additionally, CM exhibits a wide range of biological activities; antimicrobial, antioxidative, antithrombotic, antihypertensive,

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doi:10.1016/j.fct.2012.01.016

and immuno-modulatory effect (FitzGerald and Meisel, 2000; Kohonen and Pihlanto, 2003; Saltanat et al., 2009). Hence, it was therapeutically used to treat jaundice, splenic problems, asthma, anemia, piles, and diabetes (Knoess, 1979; Rao et al., 1970).

In view of that, the present study was conducted to investigate the potential protective and/or therapeutic effects of feeding CM on ethanol-induced liver injury in rats. The study also aimed to explore the underlying mechanisms of such effects.

2. Materials and methods

2.1. Chemicals

Ethyl alcohol (absolute ethanol) was purchased from Sigma–Aldrich chemicals co. (St. Louis, MD, USA). CM was obtained from Milk of Ebel Matrouh El sahrawy company, Egypt. Other chemicals were from analar grade or from the purest grade available. Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) kits were provided from Vitro Scient, Egypt. Triglycerides and cholesterol kits were obtained from Stanbio, TX, USA. Total antioxidant capacity (TAC) kit was purchased from Biodiagnostic, Egypt. Rat TNF-α was determined using enzyme-linked immunosorbent assay (ELISA) kit provided by ID Labs Biotechnology Inc., USA. Caspase-3 activity was estimated by the colorimetric assay kit supplied by R&D systems, USA.

2.2. Animals

Male albino rats of Wistar strain, weighing 100–150 g, were obtained from the farm of the National Institute for Vaccination, Helwan, Egypt. Rats were housed in standard plastic cages in an environmentally controlled room (constant temperature 25–27 °C with 12 h light/dark cycle) before and during the experiment. They were allowed to receive standard chow diet and *ad libitum* water throughout the experimental period. The study was carried out according to The European Communities Council Directive of 1986 (EC 86/609) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

2.3. Experimental design

After 1 week acclimatization, rats were randomly divided into four groups as follows:

- Group I (control group) received a daily oral dose of 2 ml normal saline.
- Group II (ethanol-treated group) received a daily oral dose of ethanol. The initial dose of ethanol (56%) was 6 g/kg/day, and the dose was progressively increased during week 1 to a maintenance dose of 8 g/kg/day that was continued for another 3 weeks (Yuan et al., 2006).

- Group III (prophylactic group) was treated daily with an oral dose of 2 ml CM (Dallak, 2009) followed 10 min later by an oral administration of ethanol given by the same schedule mentioned in group II. Administration of camel's milk started 1 week before ethanol and continued simultaneously with ethanol throughout the 4 weeks-treatment period.

- Group IV (therapeutic group) was concomitantly treated with both CM and ethanol as in group III but milk intake started 2 weeks after the initiation of ethanol administration.

The animals in all groups were weighed twice per week.

2.4. Blood and tissue collection

At the end of the experimental period, rats were killed by decapitation, Blood samples were allowed to clot, and the sera were isolated by centrifugation at 1000*g* for 10 min and kept at −20 °C till determination of liver function enzyme activities (ALT, AST, and ALP) as well as triglycerides and cholesterol levels.

Meanwhile, the abdominal cavity was dissected immediately; the liver was separated, weighed and divided into three portions: the first portion was homogenized in 10 volumes of ice-cold double distilled water. An aliquot of this homogenate was mixed with 2.3% KCl and centrifuged at 600*g* for 15 min at 4 °C. The resulting supernatant was used for the determination of malondialdehyde (MDA) level. Another aliquot of the 10% homogenate was centrifuged at 600*g* for 10 min at 4 °C. The resultant cell pellet was further homogenized in 2 ml cold phosphate buffer (5 mM potassium phosphate, pH 7.4, containing 0.9% sodium chloride and 0.1% glucose) and subsequently centrifuged at 800*g* for 15 min at 4 °C. The obtained supernatant was used for the determination of TAC.

The second portion of the liver was weighed (~50 mg) and homogenized in 1 ml lysis buffer (25 mM HEPES, 5 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X100, 5 mM DTT, 1 mM pefablock, pH 7.4). The lysate was centrifuged at 10, 000*g* for 30 min and the supernatant was taken for estimation of TNF-α level and caspase-3 activity. Finally, the small remaining portion of the liver was used for the histopathological examination.

2.5. Biochemical assays

2.5.1. Determination of liver function enzymes activities, triglycerides, and cholesterol levels

Spectrophotometric diagnostic kits were used for estimation of ALT, AST, and ALP activities (Moss, 1982; Young, 1990) as well as triglycerides (Wahlefeld, 1974) and cholesterol (Stein, 1986) levels.

2.5.2. Determination of MDA level

MDA level as index of lipid peroxidation was measured according to the method of Uchiyama and Mihara (1978). MDA reacts with thiobarbituric acid in acid medium giving a pink-colored complex that can be measured spectrophotometrically at 520 and 535 nm, using 1,1,3,3-tetramethoxypropane as standard. MDA level was expressed as nmol/g tissue.

2.5.3. Determination of TAC

The TAC was determined according to the method of Koracevic et al. (2001). This method depends upon the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H_2O_2) causing its decomposition. The residual H_2O_2 is determined by an enzymatic reaction which involves the conversion of 3,5-dichloro-2-hydroxybenzensulphonate to a colored product measured colorimetrically at 505 nm. TAC level was expressed as nmol/g tissue.

2.5.4. Determination of TNF-α level

TNF-α levels were quantified with a commercially available rat TNF-α ELISA kit (Taylor, 2001). A murine monoclonal antibody (capture antibody) specific for rat TNF-α was precoated onto a microplate. Standards and samples were added to the wells, and any rat TNF-α was captured by the immobilized antibody. The wells were washed and a biotinylated polyclonal anti-rat TNF-α (detection antibody) was added. After a second wash, avidin-horseradish peroxidase was added, producing an antibody–antigen–antibody sandwich. All unbound material was then washed away and a substrate was added, producing a blue color in direct proportion to the amount of TNF-α in the sample. Finally, an acid stop solution was added to terminate the reaction. This resulted in converting the endpoint color to yellow. The intensity of the color was measured at 450 nm using a microtitration plate reader (Stat Fax® 2100, Awareness Technology, Inc., FL 34990, USA). TNF-α level (pg/mg protein) was calculated from a standard calibration curve.

2.5.5. Determination of caspase-3 activity

Caspase-3 activity was measured by using colorimetric assay kit according to the procedure (Fernandes-Alnemri et al., 1994) supplied by the manufacturer. Briefly, after homogenization of the liver in cell lysis buffer, an aliquot of the supernatant (containing approximately 50–200 μg protein) was incubated with labeled substrate DEVD-pNA (acetyl-Asp-Glu-Val-Asp *p*-nitroanilide). The cleavage of the peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. Caspase-3 activity was expressed as optical density.

2.5.6. Determination of protein content

Protein was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

2.6. Histopathological examination

Liver specimens were fixed with 10% formaldehyde and processed routinely for embedding in paraffin. Sections of 5 μm were stained with hematoxylin and eosin (H&E) for routine histopathological examination. Other sections were stained with Crossman's stain for the detection of collagenous fibers (Banchroft et al., 1996). They were then examined under the light microscope.

2.7. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Differences among were tested for statistical significance by one-way analysis of variance (ANOVA). When differences were significant, Tukey–Kramer's test was used for multiple comparisons between groups. Statistical significance was considered when $p < 0.05$. Linear regression test and Pearson's correlation test were carried out to analyze the correlation between TNF-α and caspase-3 in all animals studied.