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Original article

Beneficial effects of fermented camel milk by *lactococcus lactis subsp cremoris* on cardiotoxicity induced by carbon tetrachloride in mice

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ABSTRACT

Carbon tetrachloride (CCl_4) is a xenobiotic present in the environment, can cause harmful effects on human health. In the present study, we attempted to elucidate the cardiopreventive potential of the fermented camel milk by *lactococcus lactis subsp cremoris* (FCM-LLC) against the toxic effects of acute exposure to CCl_4 on heart tissue of mice. Twenty-eight mice's were divided into four groups of seven each: group (C) served as control; group (FCM-LLC) received only 100 mg L of FCM-LLC/kg body weight daily for 15 days; group (CCl_4) was administered by a single dose of CCl_4 (10 mL/kg in 0.3% olive oil, i.p) at day 14 and group (FCM-LLC + CCl_4) pretreated with FCM-LLC and received a single dose of CCl_4 on day 14. The exposure to a single dose of CCl_4 caused cardiotoxicity expressed by an increase in lipid peroxidation (TBARS), protein carbonyls (PC) levels and in antioxidant markers (superoxide dismutase (SOD), catalase (CAT), gluthathione peroxidase (GPx), glutathione (GSH) and Vitamin C levels) in the CCl_4-treated group when compared with the untreated group. Furthermore, treatment with CCl_4 significantly elevated the cardiac toxicity markers while increasing of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB (CKMB) and Troponin I activities. The pre-treatment of experimental mice's with FCM-LLC has allowed an improvement through lowering oxidative stress and attenuating cardiac toxicity. These modifications were further evident through histopathological aspects of the heart. Overall, the present data provide evidence of the beneficial effects of fermented camel milk by *lactococcus lactis subsp creemoris* clearly revealed through the reduction of the CCl_4 induced heart oxidative damages.

1. Introduction

It is known that various substances cause cardiac damage, and one of them is carbon tetrachloride (CCl_4) [1]. In the body, CCl_4 decomposes into trichloromethyl and trichloromethyl peroxyl radicals highly toxic by the enzyme cytochrome P450 [2], which cause damage to lipids, proteins and DNA are inducing cellular dysfunction evidenced by changes in biochemical and hematological parameters [3]. Induction of cardiac injury by CCl_4 has been used as a model for the study of producing chemical tissue toxicity [4,5]. The heart is a primary target of mitochondrial and bioenergetic failure due to the low number of Antioxidant defenses and high oxygen content.

Indeed, CCl_4 stimulate reactive oxygen production which is a process preventing the growth of tissue peroxidative damage [6]. While, excessive production of free radicals can cause oxidative stress which produces major interrelated rearrangements of cellular metabolism, increase in intracellular free calcium, altered biological membranes and damage of the cells by lipid peroxidation [7]. The implication of oxidative stress is largely associated with cardiotoxicity, and it leads to a loss of structural myocardial integrity and a deficiency of cardiomyocytic function [6].

The oxidant–antioxidant balance paly a crucial role to heart

Abreviations: AI, atherogenic index; ALT, alanine aminotransferase; b.w, body weight; CAT, catalase; CCl_4, carbon tetrachloride; CK, creatine phosphokinase; CK-MB, creatine phosphokinase-MB; FCM-LLC, fermented camel milk by *lactococcus lactis subsp cremoris*; GPx, glutathione peroxidase; HDL-Ch, high density lipoproteins of cholesterol; LDH, lactate dehydrogenase; LDL-Ch, low density lipoproteins of cholesterol; LPO, lipid peroxidation; PCO, protein carbonyl; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TBS, tris-buffer saline; T-Ch, total cholesterol; TG, triglycerides; Vit C, vitamin C

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protection and to maintain the myocardial performance [8]. The cardiotoxic effect of CCl_4 is partially circumvented by antioxidant compounds including vitamins C, E, and melatonin as non-enzymatic antioxidants [9,10]. Therefore, interest in the benefits of alternative medicines as a treatment of heart disease has arisen.

In recent years, there has been an explosive interest in the use of antioxidant nutritional supplements [11]. Epidemiologic evidence has suggested that the intake of some vitamins, minerals, and other food constituents may help to protect the body against heart disease, cancer, and the aging process and those antioxidants may have a protective effect in preventing these diseases or lessening their severity [12]. Several activities of the antioxidants are mediated by inhibition of reactive oxygen species, which are generated during the oxidative burst. Thus, the usefulness of antioxidants in protecting cellular components against oxidative stress is well established [13].

Fermented milk has attracted the attention of several research studies and consumers. In fact, previous studies have reported that consuming fermented milk products provides various health benefits such as its antihypertensive, hypolipidemic antipathogenic and antiinflammatory proprieties, that assists the defense systems against oxidative stress and treating heart diseases [14,15]. Also, some strain of lactobacilli has been shown to modulate the risk of oxidative damage accumulated during ingestion [14]. *Lactococcus lactis* is one of the most frequent bacteria that is used in probiotic preparations of fermented dairy products [16]. Additionally, previous research has reported that fermented camel milk by *Lactococcus lactis subsp cremoris* exhibited a good inhibition of angiotensin converting enzyme (ACE) in vitro [17]. This enzyme showed a capacity to improve the heart fibrosis [18].

As far as we know, such cardioprotective effect of FCM-LLC has not yet been elucidated. Thus, the aim of the present study was to evaluate the plausible cardiopreventif effect of fermented camel milk by *lactococcus lactis subsp cremoris* against cardiotoxicity induced by CCl_4 in mice.

2. Methods and materials

2.1. Bacterial strains and growth conditions

Lactococcus lactis subsp. cremoris was isolated in a previous study. Standard cultures were prepared by inoculation of 10 mL M17 broth (Biokar Diagnostics) or MRS (Accumix) with 100 μL of frozen strains stock and incubated for 24 h at 37 °C. Then, 100 μL of the 24 h grown strain was reinoculated into 10 mL MRS broth for 24' h at 37 °C. To adapt bacteria to milk fermentation conditions, strains working cultures were prepared by inoculating (1%) fresh precultures in sterile (100 °C, 20 min) camel milk and incubated (12 h, 37 °C).

2.2. Batch culture material and preparation

A sample of fresh camel milk was collected from a local farm in May 2016 (Sfax, Tunisia). A sample was cooled immediately at 4 °C and then blended to obtain a homogenous sample followed by the pasteurization process. Fresh milk heated at 80 °C for 20 min followed by cooling at 43 °C as described by [19].

Batch culture experiments were performed in the pasteurized fresh milk pure and mixed cultures were carried out without any mechanical agitation. After, 24 h of fermentation, the samples were centrifuged for 15 min at 15,000 × *g* and supernatant used in the assays.

2.3. Reagents

The present study used reagents of analytical grade. Carbon tetrachloride was obtained from SD Fine Chemicals, Bhoisar, Mumbai, India. 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), L-Glutathione (reduced form), and all current chemicals were purchased from Sigma Chemical Co., (St. Louis, MO, USA)

2.4. Ethics statement

Approval for mice experiments was obtained from the Ethical Committee at Sciences Faculty of Sfax with ethics approval number 1204 and all the experimental procedures were in accordance with the Natural Health Institute of Health Guidelines for Animal Care and approved by the "Institute Ethical Committee Guidelines" [20].

2.5. Experimental design

In this study, Female Wistar Mice weighing 29 to 32 g were purchased from Central Pharmacy (SIPHAT, Tunisia). The animals were kept in an air-conditioned room (temperature 21 ± 1 °C and relative humidity of 40%) with a 12 h light/dark cycle. All mice had free access to drinking water and standard diet. The pelleted diet for mice was 15% protein and supplied by the Industrial Society of Concentrate (SICO, Sfax, Tunisia).

Twenty-eight mice were randomly and equally divided into four groups of seven animals each as follows.

- Group (C): control mice received distilled water and standard laboratory diet.
- Group CCl_4: (a CCl_4cardiotoxicity model) was given a single dose of CCl_4 (10 mL/kg in 0.3% olive oil. ip) [21] on the 14th day.
- Group FCM-LLC: daily administrated by oral gavage of fermented camel milk by *lactococcus lactis subsp cremoris* (100 mg/kg, b.w) during 15 days.
- Group FCM-LLC + CCl_4: pretreated with FCM-LLC and intoxicated with CCl_4 on the 14th day.

The fermented camel milk dose was found to be 100 mg/kg after some preliminary experiments. During the period of treatment, all animals survived and weighed every day. Twenty-four hours after the CCl_4 challenge, all animals were weighed and then sacrificed.

2.6. Organ sampling

At the end of the experiment period (15 days), control and treated mice were sacrificed after anesthesia by intra-abdominal injection with chloral hydrate. Blood samples were collected with heparin from brachial artery of animals. Plasma samples were drawn from blood after centrifugation at 2500 × *g* for 15 min. They were kept at −80 °C until analysis. Heart tissues were dissected, cleaned and weighed. Some samples were rinsed, homogenized (1:2, w/v) in 50 mMTris buffer (pH 7.4) containing 150 mMNaCl using an Ultra-Turrax device and centrifuged (5000 × *g* for 25 min at 4 °C). The resulting supernatants were collected and kept at −80 °C until biochemical analysis. Other samples were fixed in 10% buffered formalin solution and embedded in paraffin for histological examination.

For the biochemical and the histological experiments, samples (heart tissue, plasma) were taken from seven mice in each group. All samples were analyzed in triplicate.

2.7. Biochemical assay

2.7.1. Protein quantification

Protein content was measured according to Lowry et al. [22] using bovine serum albumin (BSA) as a standard.

2.7.2. Evaluation of heart lipid peroxidation

The level of lipid peroxidation in heart was estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS), according to the method of Yagi [23]. In brief, 0.5 mL of homogenate was treated with 2 mL (1:1:1 ratio) TBA–TCA–HCl reagent (thiobarbituric acid, 0.37%, 0.25 N HCl, 15% TCA) placed for 15 min in a water bath and cooled. The absorbance of the clear supernatant was measured