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Naringin prevents carbon tetrachloride-induced acute liver injury in mice

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ABSTRACT

The protective effects and mechanisms of action of naringin (Nar) against carbon tetrachloride (CCl_4)-induced hepatic injury in mice were investigated. The results showed that oral administration of Nar significantly decreased the levels of alanine transaminase and alanine transaminase in serum, nitric oxide, inducible nitric oxide synthase and thiobarbituric acid reactive substances in hepatic tissue, and markedly increased the levels of superoxide dismutase, catalase, glutathione peroxidase and glutathione/oxidized glutathione ratio compared with the model group. Transmission electron microscopy and histopathology assay also showed the hepatoprotective effect of Nar against the damage. In addition, Nar markedly decreased cytochrome P4502E1 expression, suppressed oxidative stress, inflammation and apoptosis, and decreased phosphorylation levels of mitogen-activated protein kinases caused by CCl_4. These results imply that Nar has perfect effect against CCl_4-induced liver injury in mice, which should be developed as an effective food and healthcare product for the treatment of liver injury in the future.

1. Introduction

Functional foods containing bioactive components can provide positive impacts on health (Sang, 2014), and influence physiological or cellular activities in animals and humans (Oh & Jun, 2014). Naringin (Nar, 4',5,7-trihydroxyflavonone-7-rhamno glucoside) has been found in many kinds of fruits and medicinal foods including *Citrus paradise, Citrus sinensis, Citrus unshiu, Citrus nobilis, Citrus tachibana, Citrus junos, Artemisia selengensis, Artemisia stolonifera*, *Cudrania cochinchinensis var. geronatogea*, *Thymusherba barona*, *Pon cirus* species, *Mabea fistulifera* and *Swartiza polyphylla*, (Jagetia & Reddy, 2005). Citrus fruits are greatly consumed worldwide due to their unique sensory attributes and desirable nutritive value. Previous studies have shown that increased dietary consumption of Nar appeared to be associated with reduced risk of certain chronic diseases and increased survival of affected patients (Chen et al., 2002). Jung et al. (2003) reported that Nar at a dose of 400 mg/day lowered plasma lipids in hypercholesterolaemic subjects. In addition, some investigations have shown that Nar has anti-inflammatory, cardiovascular, anti-atherosclerotic, antioxidant, neuroprotective, anti-diabetic, hepatoprotective and anti-cancer (Benavente-García & Castillo, 2008; Chanet, Milenkovic, Manach, Mazur, & Morand, 2012; Jeon et al., 2001; Kroyer, 1986) activities. Moreover, Nar has been empirically proven to render no side effects because humans have been ingesting grapes and citrus fruits for a long time (Choe, Kim, Jeong, Bok, & Park, 2001). Thus, Nar has received more and more attention as a dietary supplement. However, to the best of our knowledge, there is no report about the protective effect of Nar against CCl_4-induced liver injury.

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Liver injury can be induced by various factors including inadequate nutrition, alcohol and drug abuse, viral infections, incidental poisoning and autoimmune attack of hepatocytes (Domitrović et al., 2013; Peng et al., 2009). Acute liver injury, one common damage, can be easily caused by various toxicants (Oumi et al., 2012), and carbon tetrachloride (CCl_4) has been widely used for experimental induction of acute liver injury in laboratory animals (Brautbar & Williams, 2002). CCl_4 is catalyzed by cytochrome P4502E1 (CYP2E1) to produce the unstable free radicals of trichloro-methyl radical ($\cdot CCl_3$), proxyl trichloromethyl ($\cdot OOCCl_3$), and reactive oxygen species (ROS) (Kuzu et al., 2007; Recknagel, Glende, Dolak, & Waller, 1989). These radicals and ROS can cause lipid peroxidation, release inflammatory mediators and induce cell apoptosis and necrosis (Kuo et al., 2010; Park et al., 2008). In order to prevent CCl_4-caused injury, an antioxidant defence system involving nonenzymatic and enzyme antioxidants, including glutathione, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) has been developed in living organisms (Valko et al., 2007). Thus, chemicals or drugs with antioxidant properties may be used to prevent or alleviate CCl_4-induced damage (Ghaffari, Ghassam, & Prakash, 2012; Kang et al., 2013).

Therefore, the aim of the present study was to study the hepatoprotective effect of Nar against CCl_4-induced liver injury in mice, and to offer the possible mechanisms of its action.

2. Materials and methods

2.1. Chemicals and reagents

Nar (purity > 98%), silymarin and 4′,6′-diamidino-2-phenylindole (DAPI) were procured from Sigma Chemicals Co. (St. Louis, MO, USA). CCl_4 was purchased from Kaixing Chemical Industry Co., Ltd. (Tianjin, China). Detection kits including aspartate transaminase (AST), alanine transaminase (ALT), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GSH-Px), nitric oxide (NO), inducible nitric oxide synthase (iNOS) and thiobarbituric acid-malondialdehyde (TBA-MDA) were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Tissue protein extraction kit was provided by KeyGEN Biotech. CO., LTD. (Nanjing, China). Enhanced bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). In Situ Cell Death Detection Kit, POD was obtained from Roche Diagnostics (Roche Diagnostics Gmbh, Mannheim, Germany). RNAiso Plus, PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) and SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China).

2.2. Animals

Male Kunming mice (18–22 g) were obtained from the Experimental Animal Center of Dalian Medical University, Dalian, China (Quality certificate number: SCXK (Liao) 2008-0002). After 1 week of acclimatization, all animals were housed in a polypropylene plastic cage covered with metal grids under standard conditions of 12-h light/dark cycle at a temperature of 21 ± 3 °C and a humidity of 60 ± 5% until the end of the experiments. The mice had free access to tap water and standard chow diet (Xietong Organism Institute, Nanjing, China). The animal experiment was approved (No.: 0001612) by the Laboratory Animal Center of Dalian Medical University (Dalian, China), and approved by ethical committee for Laboratory Animals Care and Use of Dalian Medical University. All procedures involving animals complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals.

2.3. Experimental design

Animals were randomly divided into seven groups ($n = 10$) including Group I (Nar control group, 120 mg/kg), Group II (normal control group), Group III (model group), Groups IV–VI (Nar-treated groups at the doses of 30, 60 and 120 mg/kg) and Group VII (silymarin-treated group at the dose of 200 mg/kg). Nar was dissolved in 0.5% sodium carboxymethyl cellulose solution (CMC-Na) and orally administrated to mice by intragastric intubation once daily for seven consecutive days. Mice in Groups II and III were given 0.5% CMC-Na. Two hours after the last treatment, the mice in Groups III–VII were treated with 0.3% CCl_4 (10 mL/kg, dissolved in olive oil), intraperitoneally (i.p.). The animals in Groups I and II received vehicle (i.p.). Twenty-four hours after CCl_4 or vehicle challenge, the animals were sacrificed. Blood was collected and livers were removed. The fresh liver was weighed to calculate the relative liver weight (relative liver weight (%) = liver weight/body weight × 100). The right lobe of liver was preserved in 10% formalin solution for histology and immuno-fluorescence, and the remaining parts were frozen in liquid nitrogen and stored at –80 °C for other assays.

2.4. Serum biochemistry

Blood sample of each animal was centrifuged at 2500 *g* for 10 min at 4 °C to produce serum. Then the activities of ALT and AST in serum were analyzed using the detection kits according to the manufacturer's instructions.

2.5. Histopathological examination

The liver tissues randomly chosen from five animals in each group were fixed in 10% buffered formalin for 24 h, then washed with tap water, dehydrated in alcohol and embedded in paraffin. The sections with 5 μm thickness were cut, transferred onto glass slides and stained with haematoxylin and eosin (H&E) for histopathological examination.

2.6. Determination of antioxidant markers in liver tissue

Partial liver tissues of the 10 animals in each group were prepared to make 1:10 (w/v) homogenates with cold Tris–HCl (5 mmol/L containing 2 mmol/L EDTA (ethylenediaminetetraacetic acid), pH = 7.4) using a homogenizer. The homogenate was centrifuged at 2500 *g* for 10 min at 4 °C, and the supernatant was produced. Total protein concentrations in the homogenate were measured using BCA protein assay kit according to the instruction manual. Then, the levels of CAT, SOD, GSH, GSSG, GSH-Px, NO and iNOS were determined