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Original article

Protective effects of cholestin against carbon tetrachloride-induced hepatotoxicity in rats

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SUMMARY

Background & aims: To investigate the effect of Cholestin against carbon tetrachloride-induced hepatotoxicity.

Methods: The male Wistar rats were randomly divided into seven groups with each consisting of 8 rats. Group A: basal diet, Group B: basal diet containing 1% cholestin, Groups C–F the rats with carbon tetrachloride (CCl_4)-induced liver damage. Group C served as control CCl_4. Groups D–F were administered orally the Cholestin in diet for 1%, 2%, 3%, Group G served as positive control and was given silymarin in diet for 12%.

Results: During 8-weeks experimental period, treatment with Cholestin for 8-weeks significantly reduced the impact of CCl_4 toxicity on the serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The overall potential of the antioxidant system was significantly enhanced by the Cholestin supplements as the plasma and hepatic thiobarbituric acid reactive substances (TBARS) levels were lowered while the hepatic superoxide dismutase (SOD) and catalase (CAT) activities and glutathione peroxidase (GSH-Px) protein level were elevated ($P < 0.05$).

Conclusions: The results indicated that Cholestin has a protective effect against acute hepatotoxicity induced by the administration of CCl_4 was found to be comparable to that of silymarin (200 mg/kg) and have been supported by the evaluation of the liver histopathology in rats. The hepatoprotective effects of Cholestin may be due to both the inhibition of lipid peroxidation and the increase of antioxidant activity.

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1. Introduction

Among the various health problems suffered by people in Taiwan, liver diseases including hepatocellular carcinoma, fibrosis, cirrhosis and hepatitis appear to be one of the most serious.[1] Hepatotoxins, such as ethanol, acetaminophen, and carbon tetrachloride (CCl_4), sparked off liver injury which it is characterised by varying degrees of hepatocyte degeneration and cell death.[1] Vitaglione et al.[2] suggested that reactive oxygen species (ROS) including superoxide and hydroxyl radicals are known to play an important role in liver disease's pathology and progression as well as ROS have been proved to associate with the intoxication by CCl_4.[3] Documented evidences suggested that CCl_4 has been commonly used as hepatotoxins in experimental hepatopathy.[4] Covalent binding of the metabolites of CCl_4, trichloromethyl free radicals, to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell death.[5] Many studies reported that natural antioxidants are efficacious to prevent oxidative stress-related liver pathologies due to particular interactions and synergisms.[2,6] ROS production is linked with oxidative stress which is defined as the imbalance in the generation of oxidants and the antioxidant defense.[7,8] Regarding the central role of ROS in liver disease and pathology, antioxidants might prevent hepatic damage through scavenger activity and increase the activity of intracellular antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). There are a number of evidences indicating that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against hepatic toxicity caused by various toxicants.[8–10]

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A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH-Px, which convert active oxygen molecules into non-toxic compounds. One of such candidates is Cholestin, which was chosen in the present study.

Cholestin is the fermented product of rice on which red yeast (*Monascus purpureus*) has been grown; and it is a dietary staple in many Asian countries with typical consumption ranging from 0.5 to 2 oz/person/day.[11] This product has been used as a food preservative for maintaining taste and colour in fish and meat, and/or as functional medicine.[12]

The medicinal properties of red yeast extract were described by pharmacologists of the Ming Dynasty (1368–1644) as mentioned by Ma et al.[13] Increased levels of cholesterol and triglycerides are known to be risk factors for developing coronary artery diseases. Lipid-lowering agents that inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase are now prominent among the drugs of choice for treating hypercholesterolaemia. It is another effective way to control cholesterol level with diet and food supplements.[14] Cholestin also contains 2–6% fatty acids including palmitic acid, linoleic acid, oleic acid and stearic acid.[15] Some of which have been shown to have the ability to reduce blood-lipid level in animal models and humans.[16] Indeed, diets enriched with Cholestin were effective in reducing cholesterol in high cholesterol rabbits and rats.[17] Recently, Cholestin played an important role in reducing the toxic effect of lipid peroxidation in rats.[17] Cholestin may play an important role in reducing the toxic effect of oxidized cholesterol and oxidized fish oil in rats.[18] Therefore, in the present study, we investigated the activity of Cholestin against CCl_4-induced oxidative stress and hepatotoxicity in the rats for 8 weeks, hepatic GSH-Px and thiobarbituric acid reactive substances (TBARS) levels as well as activities of AST, ALT and ALP in serum and CAT, SOD and GSH-Px in liver tissues were measured to monitor liver injury. The extent of CCl_4-induced liver injury was also analysed through histopathological examination.

2. Materials and methods

2.1. Materials

Carbon tetrachloride (CCl_4), olive oil and silymarin were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals used were in the purest form available commercially.

2.2. Preparation of cholestin[19]

Cholestin (red yeast rice) is described as the fermented product of rice on which red yeast (*M. purpureus*) has been grown. *M. purpureus* strain BCRC 31498 was purchased from the Bioresources Collection and Research Center (Food Industry Research and Development Institute, Taiwan, R.O.C.). The fungus was maintained on malt extract broth (MEB) agar, containing 4 g yeast extract/L, 20 g malt extract/L, 20 g glucose/L, and 20 g agar (pH 7.0)/L. Freshly inoculated cultures were incubated at 28 °C for 5 days, after which stock cultures were kept at 4 °C and transferred to fresh medium monthly.

M. purpureus strain BCRC 31498 was grown in liquid medium by inoculating one loop of stock culture into a 500 mL Erlenmeyer flask containing 50 mL of malt extract broth I (Blakeslee's formula) growth medium (containing 1. Malt extract 20.0 g, 2. Glucose 20.0 g, 3. Peptone 1.0 g, 4. Distilled water 1.0 l, pH 4.7) and incubating the culture at 30 °C on a rotary shaker at 220 rpm. Lovastatin esterase activity was induced by the addition of lovastatin ammonium salt (LAS) to each flask to a final concentration of 0.5 mg/mL. The culture was then allowed to incubate for another day before it was harvested.

Dried red yeast rice was extracted with boiling water at 100 °C for 4 h. The extract was then filtered through a Bűchner funnel and freeze-dried red yeast rice was stored at −20 °C until use.

2.3. Animals

Male Wistar rats, weighing 230–270 g, were purchased from National Laboratory Animal Center and housed individually in stainless steel wire bottom cages with a control environment (25 °C, 50–60% humidity, 12 h light per day) for two weeks. The animals were fed a laboratory diet (PMI Feeds, USA). Tap water was supplied in free access. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline.

2.4. Treatment

Two weeks later, the animals were randomly divided into seven groups with each consisting of 8 rats. Group A basal diet without the addition of cholestin with a formulation based on American Institute of Nutrition (AIN)[20] for a period of 8 weeks, Group B basal diet (with the addition of cholestin) 1% at doses of 16.67 mg/kg for a period of 8 weeks. For inducing hepatotoxicity, animals of groups C–F were given carbon tetrachloride at a dose of 0.1 ml/100 g body weight of CCl_4 (20% v/v in olive oil) twice a week for a period of 8 weeks of Group A and B received olive oil and saline served as vehicle control animals. After CCl_4 intoxication, Group C served as control CCl_4. Groups D–F were administered Cholestin in diet for 1% at doses of 16.67 mg/kg, 2% at doses of 33.33 mg/kg, 3% at doses of 50 mg/kg, Group G served as positive control and was given silymarin in diet for 12% at doses of 200 mg/kg, respectively, daily for a period of 8 weeks. All other groups were fed the basal diet, its components are listed in Table 1. On weeks 2 and 4, blood was obtained by tail vein puncture 6 h after administration. On week 8, the rats were weighed and anesthetized with diethyl ether. Blood was obtained by heart puncture with syringes.

Plasma was collected by centrifugation (1000 $g \times$ 15 min) from blood and analysed using a Merck VITALAB Selectra Biochemical Autoanalyzer (Merck, Germany) to determine blood urea nitrogen (BUN), creatinine, aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP). Livers and kidneys of the rats were quickly excised and weighed. Both relative ratios of liver and kidney weight to body weight were obtained. The liver was stored at −40 °C for glutathione peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS) determinations.

2.5. Antioxidant activities

Appropriate liver tissues were dissected, weighed, immersed in liquid N_2 within 60 s of death, and kept frozen at −70 °C. Prior to enzyme determinations, thawed tissue samples were homogenized in 20 volumes of ice cold 50 mM phosphate buffer (pH 7.4), centrifuged at 3200 × 3 g for 20 min at 5 °C. The supernatant was used for antioxidant enzyme determinations.

2.5.1. CAT activity

The catalase (CAT) activity was measured using Aebi's[21] method with a slight modification. The mitochondria pellet was dissolved in 1.0 mL of a 0.25 M sucrose buffer. Ten μL of the mitochondria solution was added to a cuvette containing 2.89 mL of a 50 mM potassium phosphate buffer (pH 7.4), then the reaction was initiated by adding 0.1 mL of 30 mM H_2O_2 to make a final volume of 3.0 mL at 25 °C. The decomposition rate of H_2O_2 was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction