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Protective effect of *Lycium chinense* fruit on carbon tetrachloride-induced hepatotoxicity

Ki-Tae Ha[a], Sang-Ju Yoon[a], Dall-Yeong Choi[a], Dong-Wook Kim[b], June-Ki Kim[a,*,1], Cheorl-Ho Kim[a,*]

[a] *Department of Pathology, Biochemistry and Molecular Biology, College of Oriental Medicine, Dongguk University, 707 Suk-Jang Dong, Kyungju City, Kyungbuk 780-714, Korea*
[b] *Department of Medicinal Plant Resources, Mokpo National University, Muan, Jeonnam 534-729, Korea*

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Abstract

In the present study, we investigated the protective effect of *Lycium chinense* Miller (Solanaceae) fruit (LFE) against CCl_4-induced hepatotoxicity and the mechanism underlying these protective effects in rats. The pretreatment of LFE has shown to possess a significant protective effect by lowering the serum aspartate and alanine aminotransferase (AST and ALT) and alkaline phosphatase (ALP). This hepatoprotective action was confirmed by histological observation. In addition, pretreatment of LFE prevented the elevation of hepatic malondialdehyde (MDA) formation and the depletion of reduced glutathione (GSH) content and catalase activity in the liver of CCl_4-injected rats. The LFE also displayed hydroxide radical scavenging activity in a dose-dependent manner (IC_{50} = 83.6 μg/ml), as assayed by electron spin resonance (ESR) spin-trapping technique. The expression level of cytochrome P450 2E1 (CYP2E1) mRNA and protein, as measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot analysis, was significantly decreased in the liver of LFE-pretreated rats when compared with that in the liver of control group. Based on these results, it was suggested that the hepatoprotective effects of the LFE might be related to antioxidative activity and expressional regulation of CYP2E1.

Keywords: *Lycium chinense*; Fruit; CCl_4-Induced hepatotoxicity; Cytochrome P450 2E1 (CYP2E1); Antioxidative activity; Hepatoprotection; CCl_4

1. Introduction

Carbon tetrachloride (CCl_4) is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury. CCl_4-induced liver injury depends on a toxic agent that has to be metabolized by the liver NAPDH-cytochrome P450 enzyme system to a highly reactive intermediate. It has been suggested that this toxic intermediate is the trichloromethyl radical ($CCl_3^{\bullet}$) producing maximum damage to liver (Recknagel et al., 1989; Koop, 1992). The free radicals can react with sulfhydryl groups, such as glutathione (GSH) and protein thiols. The covalent binding of trichloromethyl free radicals to cell protein is considered the initial step in a chain of events, which eventually leads to membrane lipid peroxidation and finally cell necrosis (Brattin et al., 1985; Recknagel et al., 1989, 1991). Although several isoforms of cytochrome P450 may metabolize CCl_4, attention has been focused largely on the cytochrome P450 2E1 (CYP2E1) isoform, which is ethanol-inducible (Koop, 1992; Raucy et al., 1993; Zangar et al., 2000). Alterations in the activity of CYP2E1 affect the susceptibility to hepatic injury from CCl_4 (Kim et al., 1997; Jeong and Park, 1998; Jeong, 1999).

Abbreviations: LFE, *Lycium chinense* fruit extract; CCl_4, carbon tetrachloride; $CCl_3^{\bullet -}$, trichloromethyl radical; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; MDA, malondialdehyde; GSH, reduced glutathione; ESR, electron spin resonance; CYP2E1, cytochrome P450 2E1; RT-PCR, reverse transcriptase-polymerase chain reaction; EtBr, ethidium bromide

* Corresponding author. Tel.: +82 54 770 2663; fax: +82 54 770 2281.
E-mail addresses: graywolf@dongguk.ac.kr (J.-K. Kim), chkim-bio@dongguk.ac.kr (C.-H. Kim).
[1] Tel.: 82 54 770 2371; fax: 82 54 770 2281.

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Herbs have recently become attractive as health-beneficial foods (physiologically functional foods) and as a source material for the development of drugs. Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action (Matthews et al., 1999). *Lycium chinense* Miller (Solanaceae) is widely distributed in east Asia and the fruit has been used traditionally for anti-aging purposes (Xiao et al., 1993). Recently, the hepatoprotective activity of *Lycium chinense* fruit (LFE) has been reported by Kim et al. (1999), but the underlying mechanism of protective action is not fully understood.

The present study was undertaken to evaluate the protective effect of LFE on CCl_4-induced hepatotoxicity and to elucidate the mechanism underlying these protective effects in rats. The analyzed parameters included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, alkaline phosphatase (ALP), lipid peroxidation, hepatic GSH content, catalase activity, and the histopathology of liver damage. The active oxygen scavenging activity and inhibition of CYP2E1 expression were also investigated.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats were purchased from the Jung-Ang Laboratory Animal Inc. (Seoul, Korea) and were kept for 1 week on a commercial diet under environmentally controlled conditions (room temperature 22 °C ± 3 °C, relative humidity 55% ± 5%) with free access to food and water. A controlled 12 h light/12 h dark cycle was maintained. Rats weighting 180–230 g were used for CCl_4-induced hepatotoxicity. All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Dongguk University.

2.2. Plant source and preparation of crude drug extract

The LFE is the aqueous extract from the the fruits of *Lycium chinense* Miller, which was collected from Yeongchun, Korea, on October 2001 and authenticated by a local botany expert. The voucher specimen of the plant drug studied in this work was kept in the Drug Museum of the Unit of Pharmacology and Pharmacognosy, College of Oriental Medicine, Dongguk University. The sample was chopped and dried at 60 °C for 24 h. A total of 200 g of sample was decocted with 1 l of boiling water for 2 h. The decoction was filtered, mixed, concentrated and lyophilized, and the yield of dried residue corresponded to 18.5% of the original dry fruits weight. The lyophilized sample powder was dissolved in sterilized distilled water before oral administration to the experimental animals.

2.3. Chemicals and reagents

Bovine serum albumine, 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), sodium dodecyl sulfate (SDS), thiobarbituric acid (TBA), sulfosalicylic acid, potassium phosphate, malondialdehyde tetrabutylammonium salt were purchased from Sigma (St. Louis, USA). CCl_4 was obtained from Merck (Frankfurt, Germany). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was supplied by Labotec (Tokyo, Japan). Assay kits of AST, ALT and ALP were purchased from Asan Pharmacology Co. (Seoul, Korea). Cartimox™-14 RNA isolation kit and AMV RNA PCR kit were purchased from Takara Co. (Kyoto, Japan). The rabbit polyclonal anti-rat CYP2E1 antibody was purchased from Chemicon International Inc. (Temecula, USA), and mouse monoclonal anti-rat GAPDH antibody, anti-rabbit IgG and anti-mouse IgG were supplied by Santa Cruz Biotechnology (Santa Cruz, USA). Hydrogen peroxide, *n*-butanol, ethanol, methanol and acetic acid were obtained from Junsei (Tokyo, Japan).

2.4. CCl_4-induced hepatotoxicity in the rats

The liver injury was induced by CCl_4 according to methods described previously (Shibayama, 1989; Miyazawa et al., 1990; Yoshitake et al., 1991). Liver damage was induced in rats with a 1:1 (v:v) mixture of CCl_4 and olive oil, administered subcutaneously at a dose of 0.5 ml/kg body weight. The animals were allotted into three groups of six rats each. The first group received olive oil (0.5 ml/kg, s.c.) as normal control. The second group was injected with CCl_4:olive oil alone (0.5 ml/kg, s.c.). The third group was preadministered with the LFE (100 mg/kg, p.o.), for 7 days before the injection of CCl_4:olive oil.

2.5. Assay of hepatoprotective activity

All animals were anaesthetized with ether and then blood was withdrawn from the carotid artery, after 24 h from CCl_4 injection. The blood was centrifuged at 3000 rpm at 4 °C for 10 min to separate the serum. ALT and AST activities were measured according to the method previously described (Reitman and Frankel, 1957). Serum ALP was estimated following the method of Kind and Kings (1976).

2.6. Histopathological observation

Fresh liver tissues, previously trimmed to approximately 2 mm thickness, were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. Fixed tissues were processed routinely, and then embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. The extent of CCl_4-induced necrosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin, using standard techniques.