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The protective effects of *Hibiscus sabdariffa* extract on CCl_4-induced liver fibrosis in rats

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Abstract

Dried flower *Hibiscus sabdariffa* L. (HSE) extracts, a local soft drink material and medicinal herb, were studied for their protective effects against liver fibrosis induced using carbon tetrachloride (CCl_4) in rats. Male Wistar rats were administered CCl_4 by intraperitoneal injection for 7 weeks and received a normal diet or normal diet with various HSE doses (1–5%) for 9 weeks. HSE significantly reduced the liver damage including steatosis and fibrosis in a dose dependent manner. Moreover, HSE significantly decreased the elevation in plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT). It also restored the decrease in glutathione content and inhibited the formation of lipid peroxidative products during CCl_4 treatment. In the primary culture, HSE also significantly inhibited the activation of the hepatic stellate cells. These results suggested that HSE may protect the liver against CCl_4-induced fibrosis. This protective effect appears due to HSEs antioxidant properties.

Keywords: *Hibiscus sabdariffa* extract; Liver fibrosis

1. Introduction

Oxidative stress, an important factor that induces liver fibrosis, represents a key feature of hepatitis induced by various conditions, including anoxic/reoxygenation injury, autoimmune hepatitis, viral hepatitis and alcoholic hepatitis (Wasmuth et al., 2003). Less severe oxidative stress may sustain fibrosis progression by the activation and morphological change in hepatic stellate cells (HSCs) that include promoting proliferative activity, synthesis and degradation/remodeling of the extracellular matrix, chemotaxis, contractility, proinflammatory activity and retinoid loss (Kershenobich Stalnikowitz and Weissbrod, 2003).

Carbon tetrachloride (CCl_4) is a xenobiotic used extensively to induce oxidative stress. It is assumed to initiate free radical mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products that cause liver injury (Poli et al., 1987; Recknagel et al., 1989) and excess collagen deposition in the liver, resulting in liver fibrosis. A number of investigators have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation (Teselkin et al., 2000), suppressing alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Lin and Huang, 2000), and increasing antioxidant enzyme activity (Kumaravelu et al., 1995).

Hibiscus sabdariffa L. (Malvaceae) has been used in traditional Chinese rose tea and folk medicines to

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protect against hypertension (Haji Faraji and Haji Tarkhani, 1999; Onyenekwe et al., 1999), inflammation (Dafallah and al-Mustafa, 1996), and mutagenicity (Chewonarin et al., 1999). Water-soluble extracts from *Hibiscus sabdariffa* L. (HSE) contain several antioxidants, such as protocatechuic acid (Tseng et al., 1996; Liu et al., 2002) and anthocyanins (Ali et al., 2003; Wang et al., 2000), which prevent peroxidative liver damage. Since hepatic fibrosis has been induced by long-term administration of CCl_4 (Myara et al., 1987), it is unclear if HSE also has protective effects. The purpose of this study was to investigate the effect of HSE on the CCl_4-induced chronic fibrosis in rats. The inhibitory activity of HSE was also compared with silymarin.

2. Materials and methods

2.1. Preparation of HSE

HSE from *H. sabdariffa* (Malvaceae) was prepared as previously described (Chen et al., 2003). The final extract (HSE) was composed of 2.5% anthocyanins, 1.7% polyphenolic acid, and 1.43% flavonoids, similar to that used previously.

2.2. Animals and treatment

Male Wistar rats weighing 200–240 g were housed in conventional cages with free access to water and rodent chow at 20–22 °C with a 12-h light–dark cycle. All procedures involving laboratory animal use were in accordance with the guidelines of the Instituted Animal Care and Use Committee of Chung Shan Medical University (IACUC, CSMU) for the care and use of laboratory animals. Rats were treated intraperitoneally with CCl_4 (8% CCl_4/corn oil; 1 ml/ kg body weight twice a week; Mon and Thu) for 7 weeks to produce slowly reversible cirrhosis, as described by Hernandez-Munoz et al. (Hernandez-Munoz et al., 2001) with some modification. At the same time, the rats were fed with a diet containing HSE (0%, 1%, 2% and 5%), or given silymarin orally (200 mg/kg body weight; four times a week; Tue, Wed, Fri and Sat) (Muriel and Mourelle, 1990; Muriel et al., 1992). After 7 weeks, to present protective efficiency, CCl_4 was withdrawn and the HSE and silymarin treatment was continued for 2 more weeks. The control rats were treated with corn oil and fed a normal diet. Blood samples at 0.2 ml with heparin (10 U/ml) were collected from the tail vein before the first CCl_4 treatment and at the end of the third, sixth, and ninth weeks. At the end of the experiment, blood and livers were immediately obtained after the animals were sacrificed. Liver tissue samples were taken from the left liver lobe, cut into two pieces. One piece was fixed in formalin for pathological examination. The other piece was utilized for the following biological analyses. Liver homogenates (10%, w/v) were obtained in 50 mM phosphate buffer (pH. 7.0) and stored at −80 °C for analysis within 2 weeks.

2.3. Histological examinations

A portion of the liver was fixed in 10% formalin, processed using routine histology procedures, embedded in paraffin, cut in 5 μm sections and mounted on a slide. The samples were stained with hematoxylin and eosin for histopathological examination. Masson stain was used for liver fibrosis. Liver steatosis was graded on a 3-point scale: 1+: hepatocytes in the area of one third of the lobules showed fatty accumulation, 2+ for two thirds and 3+, for all hepatocytes. The criteria used for scoring fibrosis severity were as follows: 0, normal; 1+, fibrosis present (collagen fiber present that extends from portal triad or central vein to peripheral region); 2+, mild fibrosis (the collagen fiber present with extension without compartment formation); 3+, moderate fibrosis (the collagen fiber present with some pseudo lobe formation); and 4+, severe fibrosis (the collagen fiber present with thickening of the partial compartments and frequent pseudo lobe formation).

2.4. Measurement of plasma transaminase activities

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the plasma were measured using Boehringer Mannheim reagents (International Federation of Clinical Chemistry (IFCC) Scientific Committee recommended) (Bergmeyer et al., 1986a,b).

2.5. Determination of glutathione content

The procedure used for determining GSH using OPA (o-phtalaldehyde) was performed by Senft et al. (2000). Liver glutathione levels were determined using 1–10 ml of deproteinized homogenate. OPA-derived fluorescence was measured at 365-nm excitation and 430-nm emission in a F4500 fluorescence spectrophotometer (Hitachi, Japan).

2.6. Measurement of hepatic lipid peroxidation

Lipid peroxidation was determined based on the amount of thiobarituric acid-reactive substances (TBARS) (Fraga et al., 1988). The fluorescence of the samples was detected at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a F4500 fluorescence spectrophotometer (Hitachi, Japan)