Protective effect of ethyl acetate extract of *Ishige okamurae* against carbon tetrachloride-induced acute liver injury in rats

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Abstract: Several compounds and extracts isolated from a brown alga, *Ishige (I.) okamurae*, exhibit anti-oxidant and anti-inflammatory effects. The present study investigated whether the ethyl acetate (EtOAc) fraction of *I. okamurae* (EFIO) could ameliorate carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats. Sprague-Dawley rats were intraperitoneally (i.p.) administered with EFIO at 10 or 50 mg/kg per day for 2 consecutive days before CCl₄ injection (3.3 mL/kg, i.p.). Twenty four hours later, the rats were anesthetized with diethyl ether and dissected. Pretreatment with EFIO significantly reduced the increased serum levels of alanine aminotransferase and aspartate aminotransferase in CCl₄-treated rats. Pretreatment with EFIO also significantly inhibited the reduced activities of superoxide dismutase and catalase in the CCl₄-injured liver. Histopathological evaluations showed that hemorrhage, hepatocyte necrosis, inflammatory cell infiltration, and fatty degeneration induced by CCl₄ treatment were ameliorated by the administration of EFIO. Additionally, liver immunohistochemical analyses revealed the marked reduction in ED1-positive monocyte-like macrophages in EFIO-pretreated rats given CCl₄. These results suggest that EFIO ameliorates CCl₄-induced liver injury, possibly through the inhibition of oxidative stress.

Keywords: antioxidant, carbon tetrachloride, hepatoprotection, *Ishige okamurae*, serum chemistry

Introduction

Carbon tetrachloride (CCl₄) is a well-known hepatotoxicant that induces liver injury in experimental animals. This model has been widely used for the evaluation of the therapeutic potential of drugs as well as study of the mechanisms of liver injury, since it is similar to human liver disease from the standpoint of morphology to biochemical features of the cellular lesions [15].

Biochemically, when the metabolism of CCl₄ is initiated by NADPH-dependent cytochrome P-450 enzyme, trichloromethyl radicals (•CCl₃) that are produced in liver microsomes react with O₂ to form trichloromethyl peroxyl radicals (Cl₃COO•), which peroxidate membrane lipids [13]. The cleavage products of the lipid peroxides: malondialdehyde, 4-hydroxy-2-pentenal, 2,4-hexadienal, 4-hydroxy-2-nonenal, are toxic and cause the breakdown of the rough endoplasmic reticulum (rER) structure, decreased activity of rER enzymes and inhibit protein synthesis, which leads to an alteration in the fatty content of the liver that is linked to hepatocellular necrosis [5].

This CCl₄-induced hepatic injury is characterized by two sequential phases: a direct oxidative stress leading to hepatocyte death in the first phase [13], and secondary...
damage from activated hepatic macrophages (Kupffer cells) through the release of inflammatory mediators (i.e., tumor necrosis factor alpha) [3, 4]. These are morphologically visualized in the form of the central lobular necrosis of the liver. Because of the direct involvement of oxidative stress in CCl_4-induced hepatic injury, it is conceivable that antioxidants may ameliorate CCl_4-induced liver damage.

*Ishige (I.) okamurae* (Phylum Phaeophyta, Class Phaeophyceae, Order Chordariales, Family Ishigeaceae) is an edible brown alga that has been collected from the coast of Jeju island of Korea [12]. Several studies have reported that the extracts and compounds originated from *I. okamurae* possess free radical scavenging activity [7, 8, 16] and anti-inflammatory activity [10, 11]. Thus, we considered that ethyl acetate (EtOAc) fraction *I. okamurae* (EFIO) may be useful in the prevention of various hepatic damages induced by oxidative stress and inflammation. However, little is known about the protective effect of compounds derived from *I. okamurae* against CCl_4-induced hepatic injury.

The aim of this study was to examine whether EFIO protects against CCl_4-induced hepatotoxicity in rats.

**Materials and Methods**

**Fractionation of *I. okamurae***

The brown alga *I. okamurae* was collected from the coast of Sungsanri, Jeju Island, in July 2009. The samples were washed three times with water to remove salt, epiphytes, and sand attached to the surface, and then carefully rinsed with fresh water. The samples were dried at 60°C for 24 h in an oven and then ground in a grinder prior to extraction. The shade-dried whole plant of *I. okamurae* (2,900 g) was extracted with 80% aqueous methanol with stirring for 2 days at room temperature. The filtrate was concentrated under reduced pressure and freeze-dried to create a powder. The powdered extract (311.5 g) was suspended in water (2.0 L) and successively partitioned with n-hexane (n-Hex), methylene chloride (CH_2Cl_2), EtOAc, and n-butanol (n-BuOH). The EFIO was used for this experiment because EFIO contains anti-oxidant diphenothydroxycarmalol [1, 8] as well as fucoxanthin [10].

**Experimental animals**

Female Sprague Dawley rats (150–200 g), 6–10-weeks-old, were purchased from Orient Bio (Korea). The rats were housed in plastic cages and maintained at 23 ± 2°C under a 12 h : 12 h light-dark cycle. Feed was 5L79 rat formula (PMI Nutrition, USA). Feed and water were given *ad libitum*. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Jeju National University, Korea.

**Optimal dose for in vivo preliminary study**

Twenty five healthy female Sprague Dawley rats were randomly assigned to five experimental groups (five rats/each group). EFIO was dissolved in phosphate-buffered saline (PBS) and used for injection. To determine the optimal dose of EFIO, rats were intraperitoneally injected with 0, 10, 50, 100 and 200 mg/kg of EFIO once daily for 2 consecutive days. Body weights of the rats were checked daily for 1 week. Rats treated with over 100 mg/kg of EFIO showed a decreased body weight. In all subsequent experiments, the EFIO dose was 10 and 50 mg/kg.

**Treatment with EFIO prior to CCl_4 injection**

EFIO dissolved in PBS was used for intraperitoneal (i.p.) injection (10 or 50 mg/kg body weight) for 2 consecutive days before CCl_4 injection. As a control, only PBS vehicle was injected into rats that received CCl_4. The rats were divided (n = 5 per group) into a normal control, 10 mg/kg EFIO-treated group, 50 mg/kg EFIO-treated group, vehicle-treated CCl_4 group, 10 mg/kg EFIO-treated CCl_4 group, and 50 mg/kg EFIO-treated CCl_4 group. To induce acute liver injury, a 1 : 1 (v/v) mixture of the CCl_4 and sterile olive oil was injected i.p. (3.3 mL/kg). Rats were fasted and sacrificed 24 h after CCl_4 injection.

**Preparation of serum**

After 24 h after CCl_4 injection, the rats were anesthetized using diethyl ether for the sampling of the blood and liver. Blood samples were collected from heart or infraorbital venous plexus. These were allowed to coagulate at room temperature, and were centrifuged at 3,000 g for 15 min at room temperature to collect the serum fraction. The serum was separated from the blood and stored at 20°C before assay.

**Alanine aminotransferase and aspartate aminotransferase assays**

The serum levels of alanine aminotransferase (ALT)