The edible thorny oyster, *Spondylus varius* (Mizuiri-shoujou), was found to suppress the carbon tetrachloride-induced increase in serum aspartate and alanine aminotransferase activities in mice. Significant suppressive effects on these enzyme activities were found in the fraction eluted with 75% ethanol from polystyrene gel in a dose-dependent manner. These results suggest that *S. varius* exerts a protective effect against liver injury.

**Key words:** thorny oyster; *Spondylus varius*; hepatoprotective effect; carbon tetrachloride; functional food

Products from marine sources have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods (functional supplements). Supplements derived from marine foods have been shown to have various functions in animal and clinical experiments. For example, unsaturated fatty acids,1,2) carotenoids,3) and functional peptides4,5) are being increasingly used to treat and prevent a wide variety of lifestyle-related diseases and to improve the quality of life. In this study, we focused on the hepatoprotective effect of the thorny oyster, *Spondylus varius*. This edible oyster is a sessile bivalve that measures over 30 cm in diameter and is distributed in shallow coastal waters of the sub-tropical and tropical West Pacific. This oyster is used as a food in the islands of Okinawa; in particular, the “soup” from this oyster is used as a folk medicine to maintain hepatic function. However, there has not been any scientific investigation of this functional food.

We investigated in the present study the hepatoprotective action of a hot-water extract of the edible thorny oyster, *S. varius*, on chemical-induced hepatitis in mice.

The parameters analyzed included the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

Specimens of *Spondylus varius* Sowerby (Fig. 1) were collected from the waters around the Okinawa Islands. Fresh whole bodies away from the shells were stored at −20 °C until needed. After thawing, the shelled oysters (2.0 kg) were coarsely chopped and homogenized with a mixer. The homogenate was extracted with hot water (70–80 °C) for 3 hr and allowed to cool to room temperature. After filtration with Celite powder and filter paper, the filtrate was used as the crude extract of *S. varius* in the present experiments. The procedure
used to fractionate the crude extract on TSK G-3000S polystyrene gel (Tosoh Co., Osaka, Japan) as shown in Fig. 2.

Male ICR mice (5–6 weeks old) from SLC (Hamamatsu, Japan) were used for the experiments. The mice were kept in an isolated room at a constant temperature (23 ± 2°C) and fed on standard laboratory feed (type MF, Oriental Yeast, Tokyo, Japan). The mice had free access to a water bottle. Three separate experiments were performed. In experiment 1, the effect of a crude extract of *S. varius* on carbon tetrachloride (CCl₄)-induced liver injury were investigated. In experiments 2 and 3, the effects of five fractions (Fr. I–V, Fig. 2) were evaluated. Mice were pretreated orally with each sample solution (10 ml/kg). One hour later, 10 ml/kg of CCl₄ was given intraperitoneally to the mice to induce liver injury. The CCl₄ content of the solution was adjusted with mineral oil to 5% (v/v) for experiment 1 and to 10% (v/v) for experiments 2 and 3. Control mice received distilled water or mineral oil as a vehicle instead of the sample solution or CCl₄, respectively. At 24 hr after the CCl₄ treatment following an overnight fast, blood was taken from the vena caudalis of the mice. The serum was separated from whole blood by centrifugation and stored at 4°C until being analyzed. This study was carried out in accordance with the Guidelines for Animal Experimentation of Nagoya University.

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum were determined with an assay kit (TA-LN; Kainos, Tokyo, Japan) according to the manufacturer’s protocol. Enzyme activity is expressed as I.U. (μmol/min/1 of serum at 25°C). Each data value is expressed as the mean ± S.E. The significance of differences was evaluated by Student’s t-test, and *p* < 0.05 is considered significant.

The CCl₄-treated mice showed a substantial increase in the serum levels of ALT (normal, 47 ± 5; control, 1122 ± 110) and AST (normal, 24.3 ± 3; control, 1603 ± 96). Pretreatment with the crude extract of *S. varius* (1 g/10 ml/kg, p.o.) before the injection of CCl₄ significantly reduced this elevation in the serum levels of ALT and AST (191 ± 50 and 314 ± 83, respectively). Since an increase in these enzyme activities in the serum is known to reflect an increase in liver damage, these results show that *S. varius* could prevent the liver damage induced by CCl₄. One of the mechanisms in this experimental model is considered to be biotransformation of CCl₄ to the toxic trichloromethyl radical by the cytochrome P450 enzyme system, which causes lipid peroxidation and liver injury.⁶,⁷ A preliminary experiment *in vitro* showed that the crude extract of *S. varius* scavenged the DPPH radical in a concentration-dependent manner (IC₅₀ = 1.37 mg/ml). Thus, the radical-scavenging action of *S. varius* may contribute to its hepatoprotective action in CCl₄-induced liver toxicity. The trichloromethyl radical or lipid peroxides generated by the CCl₄ treatment may be scavenged by *S. varius* in the suppression of liver injury.

The effects of five fractions prepared from *S. varius* (Fr. I–V) on the CCl₄-induced elevation in the serum levels of ALT and AST are shown in Fig. 2. With the CCl₄ treatment, the serum AST and ALT activities were increased to 4303% and 3370%, respectively. Fraction I, which did not interact with polystyrene gel under our chromatographic conditions, had no significant effect on these enzyme activities. This fraction accounted for 86% by weight of the crude extract and may include mainly water-soluble compounds, *i.e.* minerals, amines, organic acids, mucopolysaccharide, water-soluble vitamins and...
amino acids. On the other hand, Fractions III, IV and V significantly suppressed the elevation in the serum levels of AST and ALT. The suppressive effect of Fraction IV on the serum AST activity was found to be in a dose-dependent manner (Fig. 3). In addition, with the further purification of fraction IV by gel filtration chromatography (Sephadex LH-20, Amersham Bioscience), significant suppressive activity against these enzyme activities was found in later fractions that included small-molecular-size compounds (data not shown). These results suggest that the active principles in S. varius were low-molecular-weight compounds that were relatively lipid soluble.

Hepatoprotective effects have been reported for foods and herbs such as nutmeg Myristica fragrans, avocado Persea americana, some mushrooms and Okinawan herb Crassocephalum crepidioides. However, this is the first study to show thorny oyster Spondylus varius having a hepatoprotective effect as a folk remedy for hepatic injury in experimental animals. These results reveal that this edible oyster may be suitable as a source of functional food for the prevention of liver diseases. Further studies on the mode of action and characterization of the active components in S. varius are now in progress.

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