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Hepatoprotective effect of seaweeds' methanol extract against carbon tetrachloride-induced poisoning in rats

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Abstract

Three species of marine brown macroalgae (seaweeds), *Myagropsis myagroides*, *Sargassum henslowianum* and *S. siliquastrum* collected from Tung Ping Chau, Hong Kong were studied for their curative effects on hepatotoxicity caused by carbon tetrachloride (CCl_4) in male Sprague-Dawley rats. A single suitable oral dose of 1.25 ml kg^{-1} of 20% CCl_4 was used as a model hepatotoxin to produce significantly elevated levels of serum glutamic pyruvic transaminase (SGPT) and glutamic oxaloacetic transaminase (SGOT). Gavage oral administration of 300 mg kg^{-1} of methanol crude extract from *S. siliquastrum* 6 h post-treatment of CCl_4 significantly reduced the CCl_4-induced acute elevation in the levels of SGPT and SGOT in rats. Similar results, though at a less effective level, were achieved for extracts from*S. henslowianum* and *M. myagroides*. These results indicate that these seaweeds may contain some active principles in their methanol extracts which acted as an antidote against the hepatotoxicity induced by CCl_4. Further investigation is necessary to clarify and characterize the active component(s) in the extracts.

Introduction

Carbon tetrachloride (CCl_4) is one of several chemicals which cause liver injury. It has long been well documented as a hepatotoxin (Recknagel, 1967; Klaassen & Plaa, 1969; Harris et al., 1982). It is introduced into the water mainly as industrial wastes from its primarily use in the manufacture of chlorofluorocarbons (Borzelleca et al., 1990). CCl_4 causes centrilobular necrosis and fatty accumulation in the liver. Early studies have proved that CCl_4-induced hepatotoxicity is catalyzed by cytochrome P-450 in the endoplasmic reticulum of hepatocytes (Recknagel, 1967; Slater, 1984). The present study used CCl_4as a model hepatotoxin to induce experimental liver injury. Three species of seaweeds, *Myagropsis myagroides* (Mertens *ex* Turner) Fensholt, *Sargassum henslowianum* C. Ag. and *S. siliquastrum* (Turn.) Ag., abundantly found in Tung Ping Chau, an island located in the northeastern part of Hong Kong, were collected and examined for their curative effects against hepatotoxicity caused by CCl_4 after the gavage oral administration of their methanol extracts in male rats.

Materials and methods

Preparation of methanol extracts of seaweeds

Fresh samples of *Myagropsis myagroides, Sargassum henslowianum* and*S. siliquastrum* were used. For extraction, washed seaweeds were weighed and blended with distilled water. They were kept at 4 °C for 1 d and then filtered through cotton gauze. The filtrates were centrifuged at 23 700 ***g*** for 20 min. After centrifugation, the water extracted supernatant was stored for other uses and the pellets (residues), which could not dissolve in water, were freeze-dried. The resulting pellets were placed in the Soxhlet apparatus for 6 h by using pure methanol (Ajax Chemicals, Australia). The total amount of methanol extract products was combined and evaporated to dryness under vacuum at 40 °C by using the rotor evaporator to extract water-

insoluble extract. The final residue was dissolved in pure methanol and stored in air-tight glass vials for aspiration under nitrogen gas to form dark-green viscous semisolid. The final products were stored in the refrigerator until use.

Experimental protocol

Carbon tetrachloride (CCl_4) induces hepatotoxic effect when taken in suitable dose (1.25 ml kg^{-1}) (Slater, 1966). The CCl_4 (Ajax Chemicals, Australia) was dissolved in corn oil and was introduced into the stomach of the rat by gavage oral administration through an intragastric tube.

Seven to 8 week old male Sprague-Dawley rats, weighing from 150 to 250 g, were provided with tap water and rodent chows *ad libitum*, and housed in a controlled-environment with 12 h day light. These experimental animals were divided into seven groups, each group being consisted of five animals. The first group (the no treatment control group) received no chemical treatment. The second group (the saline vehicle control group) received only the vehicle (6.25 ml kg^{-1}) orally. The vehicle is made of corn oil (Mazola, U.S.A) and normal saline solution (10 ml kg^{-1}). The third group (the CCl_4toxin treatment group) received the suitable dose of CCl_4 to induce chemical hepatitis followed 6 h later by oral saline administration. The fourth group (the DMSO vehicle control group) was treated similarly to Group 3 except that another vehicle, 25% of dimethyl sulfoxide (DMSO) (Ajax Chemicals, Australia) dissolved in 0.9% (v/v) saline, was administered instead of saline 6 h after exposure to CCl_4 to evaluate its effect on CCl_4-induced hepatotoxicity. DMSO was the vehicle used to carry the seaweed methanol extracts used in the experiments. The last three groups were the treatment groups. They were treated similarly to Group 3 (the CCl_4toxin treatment group) except that each seaweed extract was individually administered in each group instead of saline to evaluate their curative effects. All animals in each of these treatment groups received one dose of each seaweed extract (300 mg kg^{-1}, dissolved in 10 ml of 25% of DMSO vehicle) respectively.

Biochemical assays

Enzyme activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) in blood serum served as parameters to illustrate the extent of hepatotoxicity in rats.

The animals were anaesthetized with ether 24 h after the last hepatotoxin treatment (CCl_4) and blood (5 ml) was withdrawn from their posterior vena cava with sterile disposable syringes equipped with hypodermic needles. Serum was separated by centrifugation at 1100 *g* for 15 min. Serum was separated from the cells immediately to prevent interference caused by haemolysis as red blood cells also contain SGOT. The serum was then diluted 10 fold with 0.9% (v/v) saline. The serum enzyme levels of SGPT and SGOT were estimated according to the method of Reitman & Frankel (1957).

Statistical analysis

The Student *t*-test was used to compare the levels of SGPT and SGOT induced by CCl_4 + 25% DMSO (DMSO control group) and the reduced levels due to the effect of individual seaweed extract.

Results

The enzyme assays of the serum transaminases showed that a toxic dose of CCl_4 (1.25 ml kg^{-1}) raised the levels of SGPT in the experimental rats to 1235 $\pm$ 116 (mean $\pm$ S.E.) and that of SGOT to 2119 $\pm$ 89.0 IU l^{-1} (Fig. 1A,B, CCl_4 group). These levels were significantly much higher than those in both the no treatment and saline vehicle control groups with SGPT and SGOT levels being less than 15 and 55 IU l^{-1} respectively (Fig. 1A,B). The DMSO vehicle had no effect on CCl_4-induced hepatotoxicity. The levels of SGPT and SGOT serum enzymes in the DMSO vehicle control group were comparable (Student *t* test, $p > 0.05$) to those of the CCl_4toxin treatment group (Fig. 1A,B).

In general, methanol extracts of the three species of seaweeds significantly reduced the levels of both the SGPT and SGOT in rats exposed to CCl_4. An exception being that for *M. myagroides*, where its methanol extracts did not reduce the level of SGPT significantly (Fig. 1A). Of the three species, the methanol extracts of *S. siliquastrum* appeared to show the most promising hepatoprotective effect. Experimental animals treated with methanol extracts of this species showed a level of SGPT at 492.4 $\pm$ 42.5 and that of SGOT at 904.8 $\pm$ 101 IU l^{-1}. This is a significant reduction of 60% and 59%, respectively, of their levels in the toxin treatment and DMSO control groups (Fig. 1A,B).

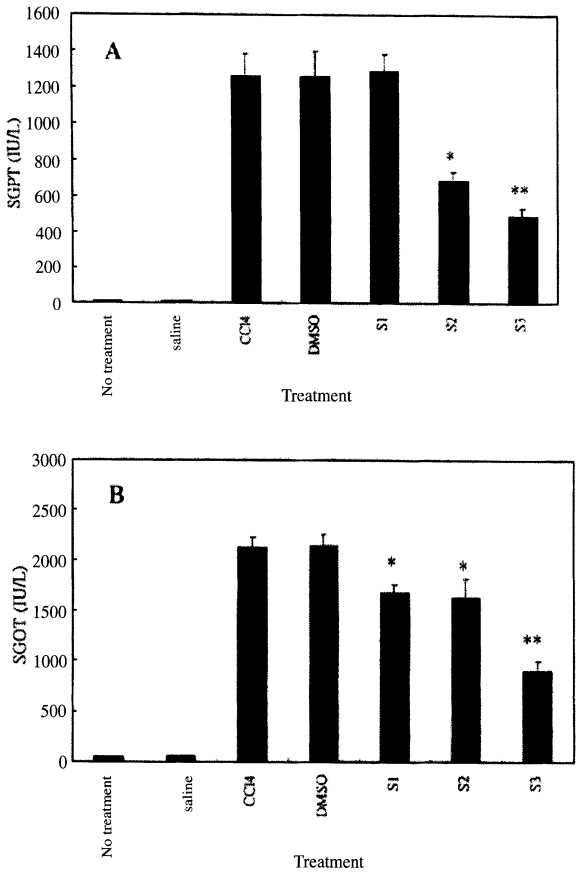

Figure 1. Curative effect of methanol extract (at dosage of 300 mg kg^{-1}) of three species of seaweed on CCl_4-induced elevation of (A) SGPT and (B) SGOT activities in rats. Each value represents the mean ± S.E. of 5 treated rats. Values statistically significantly different from those of DMSO control group are indicated by *(Student's *t*-test, $p < 0.05$) and **($p < 0.005$). Treatment groups are as follow: No Treatment control (Group 1); Saline vehicle control (Group 2); CCl_4 toxin treatment (Group 3); DMSO vehicle control (Group 4); S1 *Myagropsis myagroides* extract treatment (Group 5); S2 *Sargassum henslowianum* extract treatment (Group 6); and S3 *Sargassum siliquastrum* extract treatment (Group 7).

Discussion

CCl_4 is commonly used as a model to study hepatotoxicity (Plaa & Hewitt, 1982; Gilani, & Janbazz, 1995a,b,c). When the liver is injured as a result of the introduction of infectious agents or chemicals, the serum levels of SGPT and SGOT are raised significantly (Recknagel, 1967). The increases in SGPT and SGOT serum levels have been attributed to damage to the structural integrity of the liver (Chenoweth & Hake, 1962). They may be released from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (Sallie et al., 1991). At a suitable dose, CCl_4 causes extensive necrosis in the liver centrilobular regions around the central veins (Walker et al., 1980). It is generally accepted that CCl_4 hepatotoxicity resulted from activation of CCl_4 by the respective specific isozyme of the cytochrome P-450 system in the endoplasmic reticulum (ER) of hepatocytes to form the reactive metabolite, trichloromethyl radical ($CCl_3^{\cdot}$), which covalently binds to macromolecules, protein and lipid, and also interact with O_2 to yield highly reactive trichloromethylperoxy radical ($CCl_3O_2^{\cdot}$). This in turn initiates peroxidative degeneration of membrane lipids of the ER rich in polyunsaturated fatty acids (Slater, 1966; Recknagel, 1967; Klaassen & Plaa, 1969; Packer et al., 1978; Van de Straat et al., 1987). However, the actual mechanisms of how these initial events lead to further degenerative effects, finally bringing about cell necrosis and death, are still obscure (Wang et al., 1996).

Based on the SGPT and SGOT values of the DMSO Control group, the 25% DMSO exhibited no significant influence on CCl_4-induced hepatotoxicity. It demonstrated that the dose of DMSO applied could be used as a vehicle for methanol extracts of seaweed in this experiment. Therefore, any resulting effect of seaweed extracts on the serum enzymes was due to the effect of the extracts.

SGPT and SGOT, especially the former, are highly localized in hepatocyte cytosols (Ooi, 1996). The crude methanol extracts of seaweeds probably acted to preserve the structural integrity of the plasma cellular membrane of the hepatocytes to protect it against breakage by the reactive metabolites produced from exposure to CCl_4. This prevented further damage to more hepatocytes and hence reduced further leakage of SGPT and SGOT due to cell destruction. This may explain the lower levels of these transaminases observed in rats treated with the seaweed extracts after exposure to the toxin. *S. siliquastrum* extracts appeared to have the best overall curative action, followed by those from *S. henslowianum* and *M. myagroides*. However, the ability of methanol extract of *M. myagroides* to reduce the level of SGOT but not that of SGPT remains difficult to explain.

In contrast to the findings of this present study, the aqueous extracts of *S. henslowianum* showed the most promising hepatoprotective effect against CCl_4 exposure than those of the other two seaweeds (Wong et al., 2000). Moreover, those of *S. siliquastrum* showed the least protective effect. It thus appears that both methanol and aqueous extracts of these seaweeds exhibit hepatoprotective effect, but different types of

active ingredients are likely involved. Furthermore, it is noted that the methanol extracts of *S. siliquastrum* and *S. henslowianum* at the dose of 300 mg kg^{-1} showed better results than the aqueous extracts of *S. henslowianum* at the same dose. Therefore, it can be assumed that the possible hepatoprotective effect of methanol extracts of these seaweeds is better than that of the aqueous extracts. The same results could also be shown in the histopathological examinations (Wong, 1999). The possible component(s) in methanol extracts may be phenol or polyphenols which are organic in nature (Lee et al., 1996). They are different from those of the aqueous extracts which are likely to be polysaccharides or glycoproteins (Harada et al., 1997). The hepatoprotective activity of the extracts may also be due to their antioxidant properties. In which case, they may act as scavengers of free radicals, such as superoxide and alkoxy radicals, and protect the liver against liver plasma membrane peroxidative degradation or promote cellular mitosis for the repair of damaged liver cells (Ooi, 1996). Some of these processes may involve active binding sites. This was indicated by the saturation of their effects with methanol extracts of seaweed. The active components in the methanol extracts are currently being isolated and further details of the mechanisms involved in their hepatoprotective effects have yet to be elucidated.

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