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Effects of *Callophyllis japonica* powder on carbon tetrachloride-induced liver injury in rats

Dal-soo Park[1], Ki-hyun Lee[1], Hee-chul Kim[1], Mee-jung Ahn[1], Chang-jong Moon[1], Myung-soon Ko[1], Kyoung-kap Lee[1], Gyung-min Go[2] and Tae-kyun Shin[1,*]

[1]Department of Veterinary Medicine, Graduate School, Cheju National University, Jeju City, Jeju-do, 690-756, South Korea; [2]Provincial Fisheries Resources Research Institute, Jeju-do 699-810, South Korea

SUMMARY

A limited number of studies have been conducted on the bioactivity of *Callophyllis japonica (C. japonica)*, which is a red seaweed that is traditional in the oriental diet. In this report, the hepatoprotective effect of *C. japonica* was studied in a carbon tetrachloride (CCl_4)-induced hepatotoxicity model in rats. A single intraperitoneal injection of 1.25 ml/kg of 20% CCl_4 in olive oil produced an elevated level of serum glutamic pyruvic transaminase (SGPT) and increased enzyme activity of serum glutamic oxaloacetic transaminase (SGOT). Pre-treatment with *C. japonica* (150 mg/kg/d) for 3 days in CCl_4-injected rats reduced the levels of SGPT and SGOT compared with control levels ($P < 0.05$), while higher doses (300 and 600 mg/kg) were ineffective. The administration of *C. japonica* (150 mg/kg/d) for 3 days after the CCl_4 injection in rats was ineffective at reducing SGPT and SGOT. The histopathological findings in each group largely agreed with the biochemical data. The results of this study support the suggestion that *C. japonica* has a hepatoprotective effect on chemical-induced liver injury.

Key words: *Callophyllis japonica*; Carbon tetrachloride; Red seaweed; Hepatoprotection

INTRODUCTION

The red seaweed, *Callophyllis japonica (C. japonica)*, has been traditionally eaten in salads by seaside communities in Korea, but few studies on the bio-functional effects of *C. japonica* have been reported. Kang *et al.* (2005) found that *C. japonica* extract can function as a radical scavenger, and other types of seaweed are known to protect the liver against injury (Wong *et al.*, 2000).

The liver performs the vital biochemical functions of detoxification and synthesis (Achliya *et al.*, 2004). Carbon tetrachloride (CCl_4) has been used as a model substance to evaluate hepatotoxicity (Gilani and Janbazz, 1995; Slater, 1966). When the liver is injured as a result of infectious agents or chemicals, the serum levels of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) become significantly elevated (Klaassen and Plaa, 1969; Recknagel *et al.*, 1989). CCl_4 causes centrilobular necrosis and fatty accumulation in the liver.

The aim of the present study was to investigate the effect of *C. japonica* on a CCl_4-induced liver injury model. We examined the histopathological findings of the affected liver and the serum levels of SGOT and SGPT because both enzymes are

*Correspondence: Tae-kyun Shin, Department of Veterinary Medicine, Graduate School, Cheju National University, Jeju City, Jeju-do, 690-756, South Korea. Tel: +82-64-754-3363; Fax: +82-64-756-3354; E-mail: shint@cheju.ac.kr

representative of the extent of liver damage.

MATERIALS AND METHODS

Preparation of seaweed

Fresh *C. japonica* plants were obtained from a local marine market on Jeju Island, South Korea. The plants were confirmed to be *C. japonica* by a taxonomist (Prof. Y. P. Lee, Cheju National University), and specimens were kept for reference.

The experimental material was washed several times with distilled water, cut into small pieces, shade dried, and ground into a powder. The *C. japonica* powder was used for oral administration to mimic oral intake.

Experimental protocol

Sprague-Dawley male rats, 7-8 weeks old and 150-250 g each, were fed a commercial diet and water *ad libitum* during the experiments. CCl_4 hepatotoxicity was induced by the intraperitoneal (i.p.) injection of 1.25 ml/kg of a 20% (v/v) solution of CCl_4 (Oriental Chemicals, Seoul, South Korea) dissolved in olive oil (Slater, 1966; Raju *et al.*, 2003; Wong *et al.*, 2003).

In the pre-treatment experiment, the experimental animals were divided into three groups: 1) vehicle-treated control rats, 2) saline (3 days)- and CCl_4-treated rats, and 3) *C. japonica* (3 days)- and CCl_4-treated rats. In brief, the rats in the vehicle-treated control group (10 rats) were treated with normal saline (10 ml/kg, PO) for 3 days and were then given olive oil (1.25 ml/kg, i.p.) 6 h before sacrifice. The rats in the second group (10 rats) received saline for 3 days and were then injected with CCl_4 (1.25 ml/kg, i.p.) to induce chemical hepatitis 6 h prior to sacrifice. The third group was given an oral administration of *C. japonica* (150 mg, 300 mg, and 600 mg/kg/d) for 3 days and was then given an i.p. injection of CCl_4 6 h prior to sacrifice.

The rats were given i.p. injections of CCl_4 in olive oil followed by oral treatments with *C. japonica* powder (150 mg/kg/d) for 3 days to examine the effect of *C. japonica* on damaged liver tissues. This dose of *C. japonica* was selected because it was shown to ameliorate liver injury induced by CCl_4. Saline was substituted for *C. japonica* in the vehicle-treated control group.

Biochemical assays

The animals were anaesthetized with ether, and blood (5 ml) was withdrawn from the heart with sterile disposable syringes equipped with hypodermic needles. The serum was separated from the cells immediately by centrifugation at 3000 × g for 10 min to avoid interference caused by the hemolysis of red blood cells. The serum was then diluted to 10-fold with 0.9% (v/v) saline and was analyzed using an automatic clinical analyzer (Chiron).

Histopathological examination

A portion of the median lobe of the liver was dissected and fixed in 10% formalin for 48 h. The fixed samples were dehydrated in ethanol and cleared in xylene. The liver specimens were then embedded in paraffin, sectioned to 5 μm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination under a light microscope (Olympus).

Statistical analysis

Student's *t*-test was used to compare the levels of SGPT and SGOT between the experimental groups.

RESULTS

Pre-treatment with *C. japonica* ameliorates the hepatotoxicity induced by CCl_4

The enzyme assays of the serum transaminases showed that a toxic dose of CCl_4 (1.25 ml/kg) significantly increased the levels of SGPT and SGOT to 1847 ± 344 and 2794 ± 427 IU/L (mean ± SEM) (n = 10), respectively, compared with the normal control (saline + vehicle) levels, which had

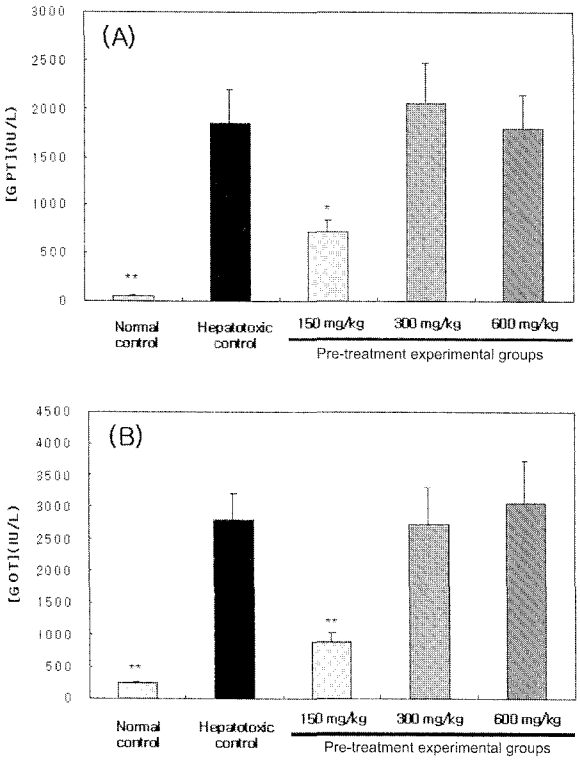

Fig. 1. Effect of *C. japonica* (150, 300, and 600 mg/kg) on the CCl_4-induced elevation of: (A) SGPT and (B) SGOT activities (pre-treatment test). Each value represents the mean ± SEM of ten treated rats. A significant difference from the CCl_4 group according to Student's *t*-test is marked by an asterisk ($^{*}P < 0.01$; $^{**}P < 0.001$).

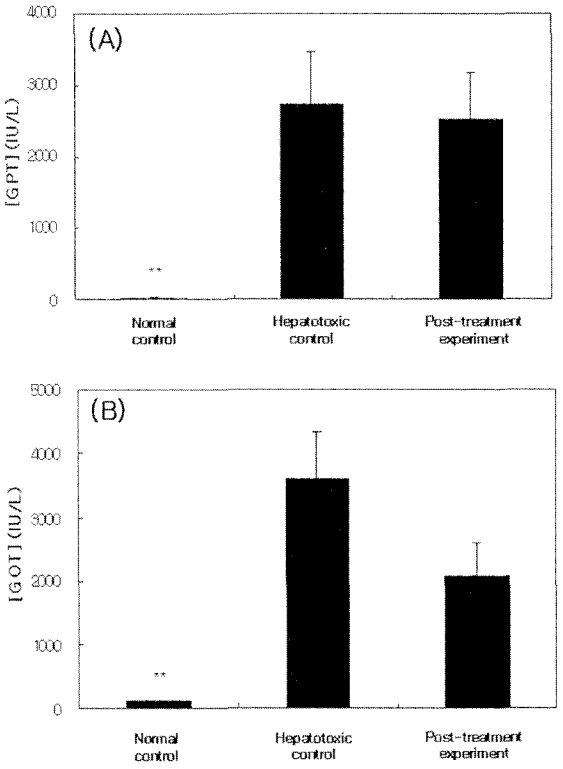

Fig. 2. Effect of *C. japonica* (150 mg/kg) on the CCl_4-induced elevation of: (A) SGPT and (B) SGOT activities (post-treatment test). Each value represents the mean ± SEM of ten treated rats. A significant difference from the CCl_4 group according to Student's *t*-test is marked by an asterisk ($^{*}P < 0.01$; $^{**}P < 0.001$).

corresponding values of 52 ± 6.3 and 230 ± 38.2 IU/L, respectively (Fig. 1).

In the *C. japonica* powder-treated group, the transaminase values were 715 ± 129 IU/L SGPT and 892 ± 159 IU/L SGOT for the 150 mg/kg powder group. The levels of SGPT ($P < 0.01$) and SGOT ($P < 0.001$) were significantly suppressed in the *C. japonica*-treated rats that were injected with CCl_4 (Fig. 1), which suggests that *C. japonica* is hepatoprotective at a dose of 150 mg/kg when administered orally prior to liver injury.

In contrast to the amelioration by *C. japonica* (150 mg/kg), the values of SGPT and SGOT were 2050 ± 414 and 2743 ± 566 IU/L, respectively, for the 300 mg/kg powder group and were not significantly different from the CCl_4-treated hepatotoxicity group. Furthermore, there was no improvement when the dose of *C. japonica* was 600 mg/kg/d for 3 days. The values of SGPT and SGOT were 1788 ± 357 and 3073 ± 652 IU/L, respectively, for the 600 mg/kg powder group. These findings suggest that a higher dose (300 - 600 mg/kg) of *C. japonica* has no effect on the levels of SGPT and SGOT in a rat model.

Post-treatment with *C. japonica* powder has no effect on the levels of SGPT and SGOT in CCl_4-induced hepatotoxicity

The values of SGPT and SGOT in the *C. japonica* powder-treated group were 2517 ± 647 and 2069 ± 530, respectively, for the 150 mg/kg powder group

This suggests that even the low dose of *C. japonica* (when administered after the CCl_4 injection) does not produce a protective effect against CCl_4-induced hepatotoxicity because both SGPT and SGOT were found to be increased to levels comparable to the those of the saline-treated CCl_4-injected group (Fig. 2).

Histopathological examination

The livers of the vehicle-treated control group contained well-distributed, healthy hepatocyte cords and sinusoids around the central vein (Fig. 3A). A single injection of CCl_4 at 1.25 ml/kg caused extensive centrilobular necrosis around the central vein in the liver (Fig. 3B). The massive necrotic zone with damaged cells was concentrated around the central vein and extended to the portal triad, leaving only a rim of apparently normal cells. Only moderate necrosis was found in the livers of rats pre-treated with *C. japonica* powder (150 mg/kg/d for 3 days) before the injection of CCl_4 (Fig. 3C). However, necrosis similar to that found in the CCl_4-treated control rats was observed in animals treated with higher doses of *C. japonica* (300 mg/kg and 600 mg/kg) (data not shown).

DISCUSSION

The increased levels of SGPT and SGOT have been attributed to structural damage to the liver (Sallie *et al.*, 1991). In the present study, the administration of CCl_4 was found to elevate the levels of the marker enzymes SGPT and SGOT, as shown in previous studies (Sallie *et al.*, 1991; Gilani and Janbaz, 1995).

We observed the protective effect of *C. japonica* against liver injury caused by CCl_4 in rats. The *C. japonica*-treated (150 mg/kg) group exhibited lower levels of SGPT and SGOT compared with the CCl_4-treated hepatotoxic group. The mechanism of decreased liver damage is unknown and remains to be studied, but *C. japonica* is known to function as a scavenger of radicals (Kang *et al.*, 2005).

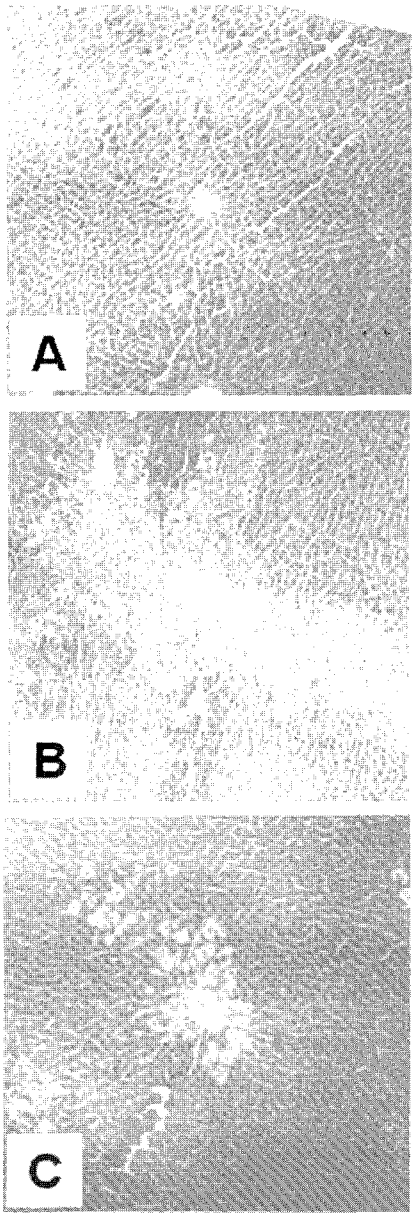

Fig. 3. (A) Histological finding of livers with vehicle-saline treatment, (B) CCl_4 treatment, and (C) *C. japonica* (150 mg/kg) treatment. (A) Shows the normal appearance of liver. (B) Shows centrilobular fatty changes in the liver. (C) Shows a decrease in the necrotic changes in the liver. A-C: H&E stain; magnification: ×75.

The protective effect of *C. japonica* powder against CCl_4 could also be seen in the histopathological examination of the liver sections. The scarcity of

necrotic cells accompanied by the presence of a regeneration zone and mitosis indicates that *C. japonica* powder was effective in reducing liver damage. A dose-response relationship was evident in both the biochemical and histopatho-logical analyses.

The reason why higher doses of *C. japonica* powder were ineffective for the amelioration of hepatotoxicity remained further study. We only postulate that higher doses of certain sea weeds power interrupt a homeostatic balance in the liver affected by CCl_4, although *C. japonica* powder has an antioxidant capacity in immune cells (Kang *et al.*, 2005). Or we do not exclude a possibility that the anti-oxidant activity of *C. japonica* overwhelms the beneficial hepatoprotective effect, and that excess elimination of oxidation in the liver may synergistically incorporates CCl_4 - induced liver injury as well.

In conclusion, *C. japonica* powder prevents the acute elevation of transaminases (SGOT and SGPT) and reduces the hepatic necrosis caused by a toxic dose of CCl_4. The hepatoprotective effect of *C. japonica* was greatest in the pre-treatment experiment, in which *C. japonica* powder treatment was provided before the administration of CCl_4. However, the treatment with *C. japonica* powder after CCl_4 administration was shown to be ineffective based on the levels of SGPT and SGOT, suggesting that pre-treatment with a diet containing radical scavenging substances pre-conditions liver cells to avoid injury.

The data in this study suggest that the regular consumption of red seaweed, *C. japonica,* has a protective effect on hepatocytes and probably protects against CCl_4-induced liver injury by scavenging radicals, as shown in our previous study (Kang *et al.*, 2005).

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