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Hepatoprotective Effects of *Paecilomyces tenuipes* Against Carbon Tetrachloride-induced Toxicity in Primary Cultures of Adult Rat Hepatocytes

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Paecilomyces tenuipes (PT), one of the *Ascomycetes* family, has been used for medicinal purposes due to its broad pharmacological activities. The present study was undertaken to investigate the hepatoprotective effects of PT water extracts against CCl_4-induced hepatotoxicity in primary cultures of adult rat hepatocytes. When the extract of PT was directly added into the culture medium at 1, 2, and 5 mg/ml, the extracts not only reduce the CCl_4-induced elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, and lipid peroxide, but also protect cultured hepatocytes from CCl_4-induced reduction of reduced glutathione, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, catalase and superoxide dismutase. In addition, the effects of PT water extracts on cytochrome P450 enzymes were relatively marginal, indicating that the hepatoprotective effects of PT extract against CCl_4-induced toxicity might not be due to the inhibition of CCl_4 activation. In conclusion, the PT extracts were effective in protecting against CCl_4-induced hepatotoxicity in hepatocyte cultures, at least in part, by scavenging free radicals, and by modulating enzyme systems involved in cellular oxidative stress.

Key words: *Paecilomyces tenuipes*, Carbon tetrachloride, Cytochrome P450, Hepatocyte, Hepatotoxicity.

INTRODUCTION

Many hepatotoxicants including carbon tetrachloride (CCl_4) require metabolic activation, especially by hepatic cytochrome P450 (CYP) enzymes, to form reactive and toxic metabolites, which in turn produce liver injury in experimental animals and humans (Gonzalez, 1988). CCl_4 requires biotransformation by the hepatic microsomal CYP to produce hepatotoxic metabolites, namely trichloromethyl free radical ($\bullet CCl_3$) and/or $\bullet CCl_3OO$ (Brattin *et al.*, 1985; Williams and Burk, 1990; Brent and Rumack, 1993). Although several isoforms of CYP can metabolize CCl_4, most attentions have been focused on the CYP 2E1, which is an ethanol inducible isoform (Koop, 1992; Zangar *et al.*, 2000). CYP 2E1 is well recognized for its role in the activation of many toxic chemicals and carcinogenic agents (Guengerich *et al.*, 1991; Koop, 1992; Jeong, 1999). Alterations in the activity of this isoform can modulate the susceptibility of hepatic injury to CCl_4 (Kim *et al.*, 1997; Jeong, 1999). Also, CYP 2E1 played a role in the metabolism of small organic molecules including acetaminophen, aliphatic organic alcohols, nitrosamines, benzene, phenol, 4-nitrophenol and pyrazole (Guengerich *et al.*, 1991; Koop, 1992; Lee *et al.*, 1996b).

Trichloromethyl free radicals can react with sulfhydryl groups, such as glutathione (GSH) and protein thiols. The covalent binding of the trichloromethyl free radicals to the cellular proteins is considered to be the initial step in a chain of events that eventually lead to a membrane lipid peroxidation and finally to cell necrosis (Recknagel *et al.*, 1989; Williams and Burk, 1990; Brent and Rumack, 1993; Brautbar and Williams, 2002). CCl_4 is an extensively used xenobiotic to induce lipid peroxidation and toxicity in the liver.

According to the *in vitro* and *in vivo* studies, several

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classical antioxidants have been shown to protect hepatocytes against lipid peroxidation or inflammation, and preventing the occurrence of hepatic necrosis (Yoshikawa *et al.*, 1996; von Herbay *et al.*, 1996). Several antioxidants and/or free-radical trapping agents can protect animals from the hepatotoxic effects of CCl_4. Antioxidants, such as GSH, cysteine, ascorbate, cystamine, ubiquinone, and carotene, terminate free radical chains, scavenge peroxides and help to stop the propagation of autocatalytic lipid peroxidation reactions. Therefore, these antioxidants have protective effects against hepatotoxicity induced by CCl_4.

The fungus, *Paecilomyces tenuipes* (PT), one of the *Ascomycetes* family, has been used for many medicinal purposes. PT is one of the famous Chinese medicinal entomopathogenic fungi together with other fungi such as *Cordyceps sinensis* and *Cordyceps militaris.* Both *Paecilomyces* and *Cordyceps* are the genera of family Clavicipitaceae. The fruit bodies of entomopathogenic fungi are useful as medicinal herbs, owing to their various biological and pharmacological activities (Lee *et al.*, 1996a; Borchers *et al.*, 1999). These include immunostimulating, antitumor, and hypoglycemic activities. It has also been used as dietary supplements for enhancement of stamina and as a medicine for curing coughs and blood circulatory problems or a tonic for promoting longevity and improving quality of life (Kiho *et al.*, 1993; Kuo *et al.*, 1996; Lee *et al.*, 1996a).

The present study was carried out to investigate the hepatoprotective effects of PT extracts against CCl_4-induced toxicity in adult rat hepatocytes. To demonstrate the possible mechanism of hepatoprotection by PT extracts, the effects of PT extracts on the metabolism of GSH and reactive oxygen species (ROS) was evaluated. In addition, the effects of PT extract on CYP enzymes were studied because CCl_4 requires metabolic activation by CYP 2E1 enzyme for its hepatotoxicity.

MATERIALS AND METHODS

Chemicals and reagents. Collagenase, bovine serum albumin, p-nitrophenol, dimethyl sulfoxide (DMSO), pyrogallol, H_2O_2, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, 5,5-dithiobis-2-nitrobenzoic acid, CCl_4 and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Waymouth's MB 752/1 powdered medium, insulin, sodium bicarbonate, sodium oleic acid, gentamycin sulfate, L-serine, L-alanine, L-asparagine, sodium linoleic acid, 5-aminolevulinic acid, α-tocopherol, hydrocortisone, D-thyroxine, estradiol, testosterone and glucagon were purchased from GIBCO (Grand Island, NY, USA). All other chemicals were of reagent grade unless otherwise stated.

Strains and preparation of test materials. PT was obtained from Department of Applied Microbiology in National Institute of Agriculture Science and Technology (Suwon, Republic of Korea). The voucher specimen (No.YU 0405-01) was deposited in the herbarium at Yeungnam University. The stock culture was maintained on potato dextrose agar slants. The slants were incubated at 25°C for 6 days and stored at 4°C. The seed culture was grown in a 500 ml flask containing 250 ml of potato dextrose broth medium and cultured on a rotary shaker incubator at 25°C and 150 rpm for 15 days. The broth and mycelium were homogenized together with a homogenizer (NISSEI, Japan). The homogenate was then filtered, concentrated to 10 ml, and freeze dried. The freeze dried samples were stored at -70°C until use.

Animals. Specific pathogen-free adult male Sprague-Dawley rats were obtained from the Orient (Seoul, Republic of Korea). The animals received at 4 weeks of age were acclimated for at least 2 weeks prior to the experimental procedures. The animal quarters were strictly maintained at 23 ± 3°C and 40~60% relative humidity. A 12 h light/dark cycle was used with an intensity of 150~300 Lux. All rats were provided with food (Orient Co., Seoul, Republic of Korea) and water *ad libitum.*

Isolation and culture of rat hepatocytes. In according to the method of Dickins and Peterson (1980), the hepatocytes were isolated using a two step perfusion. Details of this procedure have been described elsewhere (Yang *et al.*, 1983). The cell suspension was diluted to 1.0×10^6 cells/ml in the culture medium that was prepared by the methods of Dickins and Peterson (1980) and Salocks *et al.* (1981).

Treatment with PT extracts in hepatocytes cultures. PT extracts were dissolved in culture medium and added directly to the culture medium. The final concentrations of PT extracts were 1, 2 and 5 mg/ml of culture medium. CCl_4 was dissolved in DMSO and added directly into the culture medium at 4 mM. The final concentration of DMSO in culture was 0.5%. After hepatocytes had been in primary culture for 24 h, the medium was removed. The attached hepatocytes were scrapped off with a rubber policeman in 2 ml of 0.1 M potassium phosphate buffer, pH 7.4. The scrapped cells were centrifuged at 3000 rpm for 10 min at 4°C. After the supernatants were aspirated, the pellets were harvested with 0.1 M potassium phosphate buffer, pH 7.4, and stored at -70°C until use. The cells were thawed and homogenized. The aspirated medium and homogenate were used for assays.

Hepatotoxicity parameters. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic dehydrogenase (LDH) were determined using a spectrophotometric enzyme assay kit (Asan Pharmaceuticals, Seoul, Republic of Korea) according to the methods suggested by the manufacturer.

GSH and its related enzymes. The level of reduced GSH was determined in respects to the method described previously by Sedlak and Lindsay (1968) with minor modification. Glutathione S-transferase activity was assayed according to the method of Habig *et al.* (1974). Glutathione peroxidase activity was measured specrophotometrically using a technique developed by Paglia and Valentine (1967). Glutathione reductase activity was assayed in refer to the method of Carlberg and Mannervik (1975).

Lipid peroxidation, catalase and superoxide dismutase assay. The concentrations of malondialdehyde (MDA) were estimated by the method of Ohkawa *et al.* (1979). Catalase activity was assessed via a modified method of spectrophotometry (Aebi, 1984). Superoxide dismutase activity was determined by the method of Marklund and Marklund (1974).

p-Nitrophenol hydroxylase (PNPH) assay. The hydroxylation of p-nitrophenol to 4-nitrocatechol (1,2-dihydroxy-4-nitrobenzene) was determined as described by Koop (1986). The reaction mixture (1.0 ml) was composed of 0.1 M potassium phosphate buffer, pH 7.4, containing 100 μM p-nitrophenol, 1 mM NADPH and enzyme source. The reaction was started by adding the substrate. After 30 min incubation at 37°C with frequent vortexing, the reaction was quenched by the addition of 0.5 ml of ice-cold 0.6 N perchloric acid. The mixture was then centrifuged at 3,000 rpm for 10 min at room temperature. The formed 4-nitrocatechol was determined spectrophotometrically in 1.0 ml of supernatant at 546 nm following the addition of 100 μl of 10 N NaOH solution. An extinction coefficient of 9.53 mM^{-1} cm^{-1} was used for the calculation of enzyme activity.

Protein assay. Protein concentration was determined by Lowry's method using bovine serum albumin as a standard (Lowry *et al.*, 1951).

Statistical analysis. All results were expressed as mean ± SE. Differences between the means of the individual groups were assessed by one-way analysis of variance (ANOVA) with Duncan's multiple range tests (SPSS 8.0, SPSS Institute, USA).

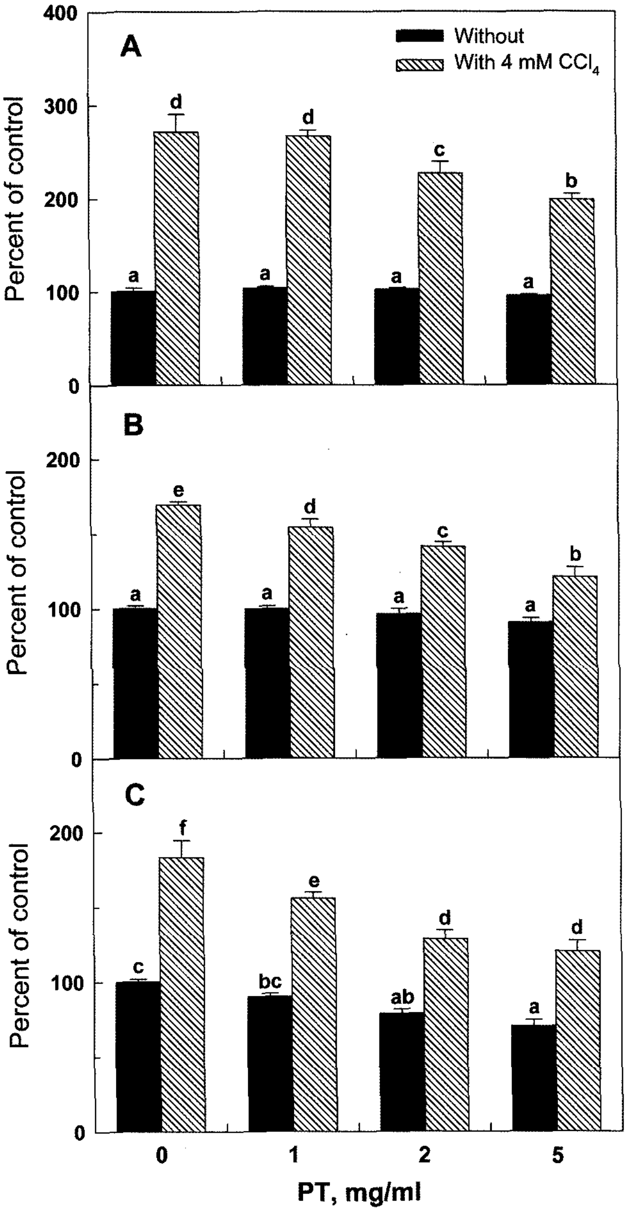

Fig. 1. Protective effects of *Paecilomyces tenuipes* (PT) extracts against leakages of AST (A), ALT (B), and LDH (C) induced by carbon tetrachloride (CCl_4) in cultured hepatocytes. Following that the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then, given concentrations of PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for 24 h. Each bar represents the mean ± SE of triplicate cultures. Mean levels with different alphabets are significantly different at $P < 0.05$.

RESULTS

Effects of PT extracts on CCl_4-induced elevation of AST, ALT, and LDH levels in cultured hepatocytes. The effects of PT extracts on CCl_4-induced elevation of AST, ALT and LDH activities in cultured hepatocytes are shown in Fig. 1. When PT extracts were directly incubated to the culture medium, AST, ALT and LDH levels were maintained or slightly decreased at the level observed in control group. Meanwhile, AST, ALT and LDH levels were significantly different between the CCl_4 + PT extracts groups and CCl_4 groups, indicating the hepatoprotective effects by PT extracts in hepatocyte cultures.

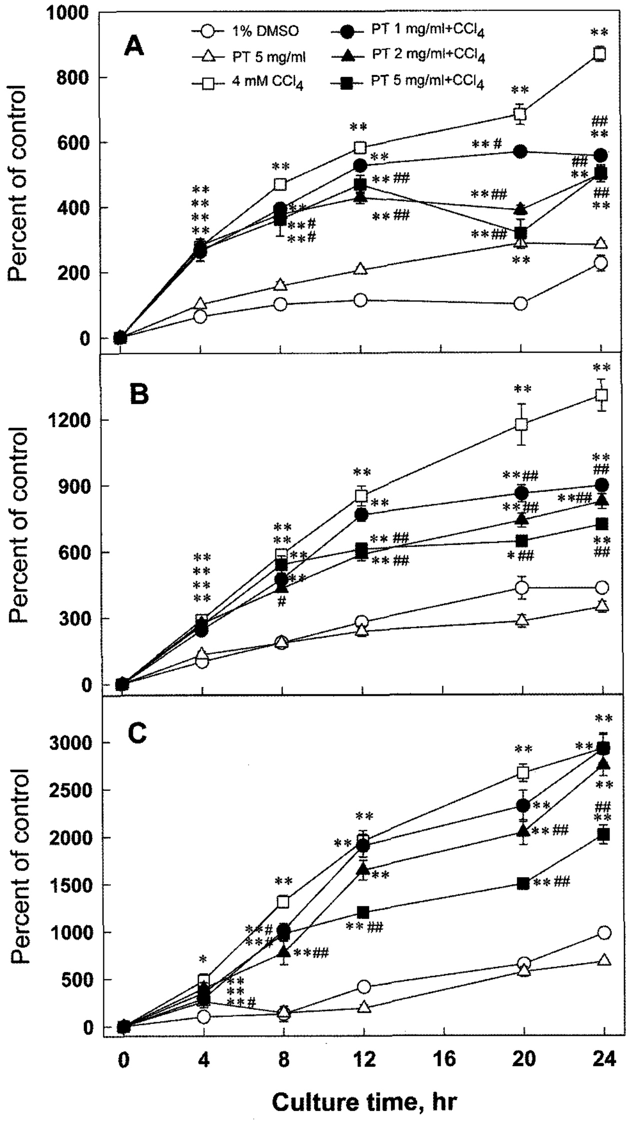

Fig. 2. Time courses of the effects of *Paecilomyces tenuipes* (PT) extracts against carbon tetrachloride (CCl_4)-induced elevations of AST (A), ALT (B), and LDH (C) in cultured hepatocytes. Following that the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then the PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for the given time. Each value represents the mean ± SE of triplicate cultures. * and ** indicate the levels significantly different from DMSO controls at $P < 0.05$ and $P < 0.01$, respectively. # and ## indicate the levels significantly different from CCl_4-treated controls at $P < 0.05$ and $P < 0.01$, respectively.

Fig. 2 shows the time courses of the effects of PT extracts on CCl_4-induced hepatotoxicity. After hepatocytes being in primary culture for 4 h, the medium was changed with fresh medium. Then the PT extracts and 4 mM CCl_4 were added directly to the culture medium. The supernatants were collected at 0, 4, 8, 12, 16, 20, and 24 h following CCl_4 treatment for the determination of AST, ALT, and LDH levels. The hepatocyte injury by CCl_4 was evident as early as at 4 h and increased up to 24 h after CCl_4 treatment. PT extracts reduced the elevation of AST, ALT, and LDH levels induced by CCl_4 in dose-dependent manners.

Protective effects of PT extracts against GSH levels and GSH-related enzymes. Fig. 3 shows the effects of PT extracts on CCl_4-induced depletion of GSH in primary culture of rat hepatocytes. PT extracts only did not affect the GSH level in normal hepatocytes. CCl_4 significantly decreased GSH level to 25% of con-

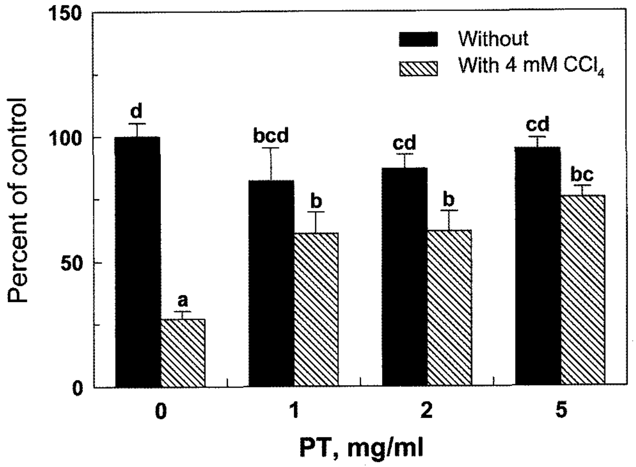

Fig. 3. Protective effects of *Paecilomyces tenuipes* (PT) extracts against glutathione levels reduced by CCl_4 in cultured hepatocytes. After the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for 24 h. Each bar represents the mean percent of control ± SE of triplicate cultures. Mean levels with different alphabets are significantly different at $P < 0.05$.

trol group. PT extracts protected the hepatocytes from CCl_4-induced depletion of GSH in a dose-dependent manner.

Fig. 4A shows the effects of PT extracts on CCl_4-induced suppression of glutathione S-transferase activity in primary cultures of rat hepatocytes. PT extracts could protect the decline in glutathione S-transferase level by CCl_4. In addition, CCl_4 significantly decreased glutathione reductase level by 57% of the control group. PT extracts protected hepatocytes from CCl_4-induced suppression of glutathione reductase in a dose-dependent manner. Meanwhile, the effects of PT extracts against CCl_4-induced suppression of glutathione peroxidase were relatively marginal (Fig. 4B).

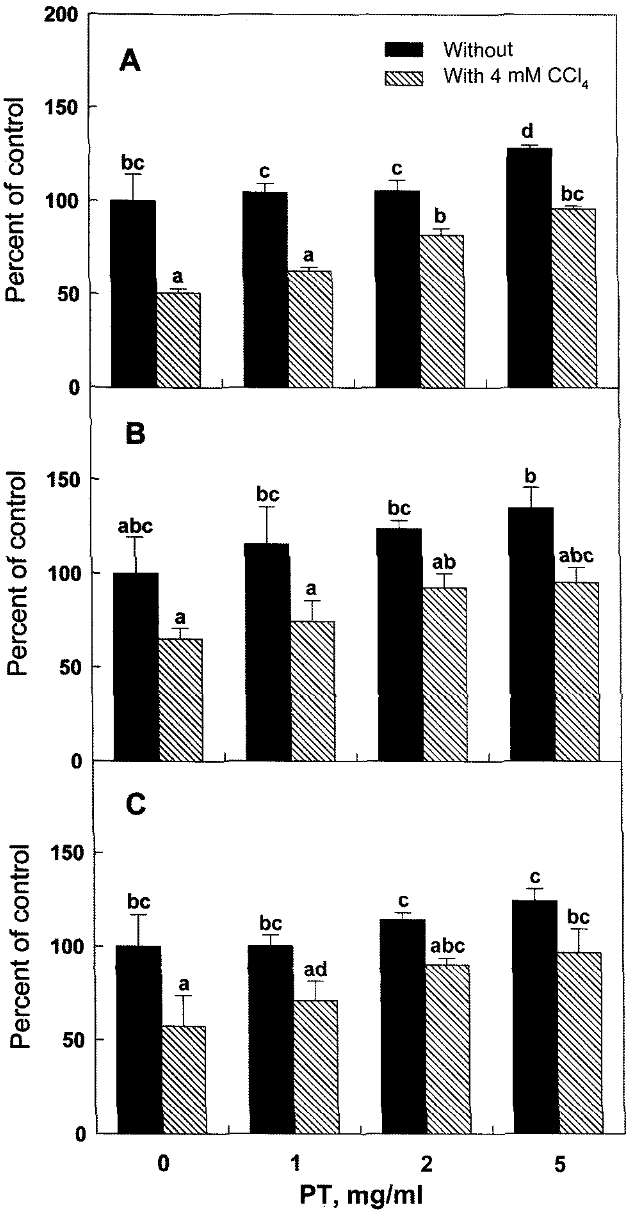

Fig. 4. Protective effects of *Paecilomyces tenuipes* (PT) extracts against glutathione-related enzymes suppressed by CCl_4 in cultured hepatocytes. After the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for 24 h. Each bar represents the mean percent activity of control ± SE of triplicate cultures. Mean levels with different alphabets are significantly different at $P < 0.05$. A, glutathione S-transferase; B, glutathione peroxidase; and C, glutathione reductase.

Protective effects of PT extracts against lipid peroxidation induced by CCl_4 in cultured hepatocytes. Fig. 5 shows the effects of PT extracts on CCl_4-induced lipid peroxidation in primary cultures of rat hepatocytes. PT extracts only did not affect the production of lipid peroxide level in normal hepatocytes. CCl_4 significantly increased lipid peroxide level to 158% of control group. PT extracts protected hepatocytes from CCl_4-induced level of lipid peroxide in a dose-dependent manner.

Fig. 6A shows the effects of PT extracts on CCl_4-induced suppression of superoxide dismutase activity in primary cultures of rat hepatocytes. CCl_4 significantly decreased in the superoxide dismutase activity to 55% of control group. PT extracts seemed to protect hepatocytes from CCl_4-induced suppression of superoxide dismutase. Fig. 6B shows the effects of PT extracts on CCl_4-induced suppression of catalase activity in primary

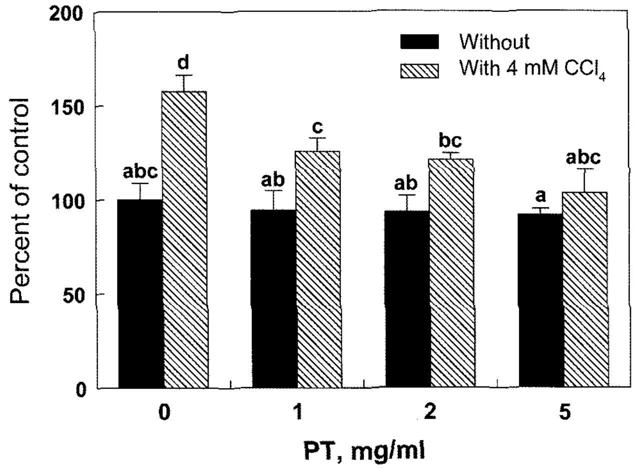

Fig. 5. Protective effects of *Paecilomyces tenuipes* (PT) extracts against lipid peroxide levels induced by CCl_4 in cultured hepatocytes. After the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for 24 h. Each bar represents the mean percent of control ± SE of triplicate cultures. Mean levels with different alphabets are significantly different at $P < 0.05$.

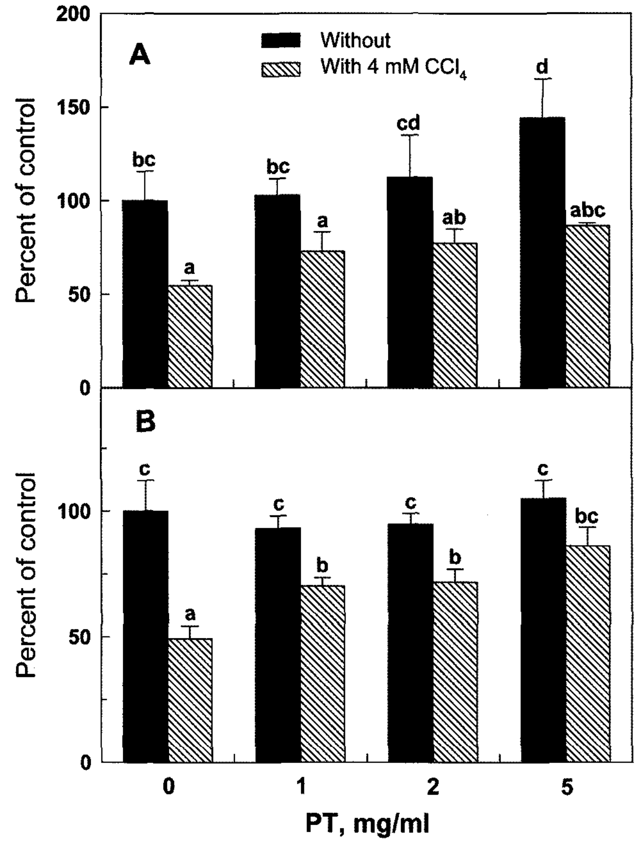

Fig. 6. Protective effects of *Paecilomyces tenuipes* (PT) extracts against superoxide dismutase (A) and catalase (B) activities suppressed by CCl$_4$ in cultured hepatocytes. After the monolayer was obtained by culturing the hepatocytes for 4 h, the medium was changed with fresh medium. Then PT extracts and carbon tetrachloride at 4 mM were incubated directly to the culture medium for 24 h. Each bar represents the mean percent of control ± SE of triplicate cultures. Mean levels with different alphabets are significantly different at $P < 0.05$.

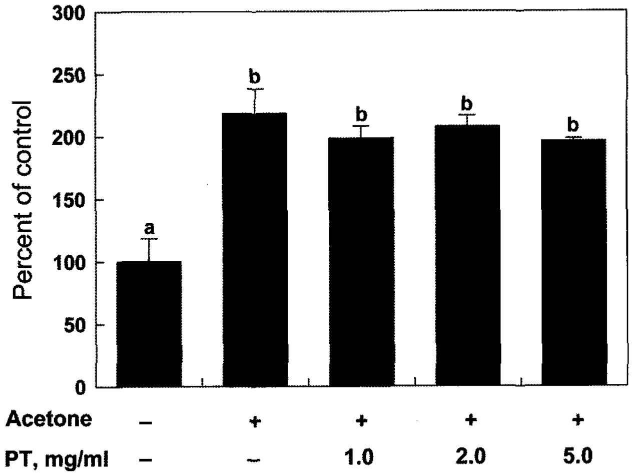

Fig. 7. Effects of *Paecilomyces tenuipes* (PT) extracts on acetone-induced microsomal p-nitrophenol hydroxylase activity. Male SD rats were pretreated with acetone at 5 ml/kg, p.o., once to induce CYP 2E1 enzyme. Two days after the administration, the enriched liver microsomes were isolated. Each bar represents the percent activity of uninduced control ± SE of three determinations. Mean levels with different alphabets are significantly different at $P < 0.05$.

cultures of rat hepatocytes. CCl$_4$ significantly decreased in the catalase activity to 49% of control group. PT extracts protected hepatocytes from CCl$_4$-induced suppression of catalase activity in a dose-dependent manner.

Effects of PT extracts on CYP enzymes in cultured hepatocytes. Fig. 7 shows effects of PT extracts on acetone-induced microsomal PNPH activity. Acetone-induced microsome significantly increased PNPH activity by 220% of that in un-induced group. Meanwhile, the PNPH activity wasn't inhibited by PT extracts in acetone-induced microsomes.

DISCUSSION

Various hepatotoxins may induce hepatic damage varying from asymptomatic hepatic functional disturbance to widespread liver necrosis. One of these chemicals is CCl$_4$ which is a xenobiotic that induces hepatotoxicity in humans as well as in animals (Stacey and Priestly, 1978). Many studies have demonstrated that ROS, including oxygen free radicals, might be causative in the etiology of degenerative diseases (Ames *et al.*, 1993; Poli, 1993). ROS have also been shown to modify and damage proteins, carbohydrates, and DNA in both *in vitro* and *in vivo* (Halliwell *et al.*, 1995). Therefore, biomembrane and bioactive molecules are disturbed or inactivated by aggressive oxidative stress. Furthermore, microsomes, mitochondria and the nuclei of hepatocytes are also impaired by peroxidation products, ultimately being destroyed and becoming necrotic (Comporti, 1985).

In accordance to these findings, CCl$_4$ treatment caused the elevation of AST, ALT, and LDH activities (Fig. 1). The present study also demonstrated that the treatment of hepatocyte cultures with PT extracts at 1, 2, and 5 mg/ml are protected against CCl$_4$-induced hepatotoxicity in a dose-dependent manner. In time course studies, CCl$_4$ treated group increased the AST, ALT, and LDH levels rapidly starting from 4 h onwards. However, the increased levels of enzymes were considerably protected by treatment with PT extracts, implying that PT extracts tend to prevent hepatocyte damage and suppress the leakage of enzymes through damaged cellular membranes.

Identification of new antioxidants remains a highly active research area because antioxidants may reduce the risk of various chronic diseases caused by free radicals. Cooperative defense systems that protect the body from free radical damage include the nutrients and antioxidant enzymes. The antioxidant enzymes include superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase and glutathione reductase. Their roles as protective enzymes are well established and have been investigated extensively both *in vivo* and *in vitro* model systems (Eaton, 1991).

The first three enzymes directly catalyze the transformation of peroxides and superoxide to nontoxic species. Glutathione reductase reduces oxidized GSH (i.e., GSSG) to GSH, a substrate for glutathione peroxidase. The outcomes of oxidative stress are serious, and, in many cases, are manifested by decreased activities of enzymes involved in reactive oxygen detoxification.

GSH constitutes the first line of defense against free radicals. GSH is involved in numerous cellular processes, including cell protection against the damaging effects of lipid peroxidation. Conjugation of toxic metabolites with GSH is one of the major pathways for the detoxification of toxic metabolites. To prevent lipid peroxidation, it is very important to maintain the level of GSH. PT extract significantly inhibited lipid peroxidation by CCl_4 (Fig. 5) and recovered the decreased hepatic GSH level induced by CCl_4 (Fig. 3).

GSSG is reduced to GSH by glutathione reductase, which is NADPH-dependent. It plays a role in maintaining adequate amounts of GSH (Recknagel *et al.*, 1991). Glutathione reductase is one of the most important hepatic enzymes for the detoxification of lipid peroxide or ROS. CCl_4 suppressed the glutathione reductase activity, whereas the PT extract protected the glutathione reductase activity suppressed by CCl_4 (Fig. 4).

Glutathione S-transferase aids in the protection of cells from the lethal effects of toxic and carcinogenic compounds (Ketterer, 1988). Inducers of glutathione S-transferase are generally considered as protective compounds against carcinogens. One of the hypotheses explaining the mechanism of chemopreventive activity of antioxidants against carcinogens is that it activates the detoxification system, such as glutathione S-transferase (Hatono *et al.*, 1996). Glutathione S-transferase level was significantly reduced in CCl_4-treated hepatocytes and the protection was observed by the treatment with higher concentration of PT extracts (Fig. 4).

Glutathione peroxidase plays a pivotal role in H_2O_2 catabolism (Eaton, 1991) and the detoxification of endogenous metabolic peroxides and hydroperoxides, which oxidize GSH. Glutathione peroxidase activity was reduced by CCl_4 treatment when compared to control. The marginal reversal of the glutathione peroxidase activity to normal after treatment with PT extracts are possibly due to antioxidant activity scavenging or detoxifying the endogenous metabolic peroxides generated after CCl_4 treatment.

Lipid peroxide, a type of oxidative degradation of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation. The present findings showed that CCl_4 had a marked oxidative impact as evidenced by the significant production of free radicals and/or a decrease in antioxidant status (Fig. 5). These free radicals trigger cell damage though two mechanisms, namely covalent binding to cellular macromolecules and lipid peroxidation which affects the ionic permeability of the membrane preventing the disintegration and stabilization of membrane structure. The diminished lipid peroxide level following treatment with the PT extracts may be attributed to the antioxidant activity by scavenging the $\cdot CCl_3$ radical generated due to the metabolic transformation of CCl_4. Although the precise mechanism of action of PT extracts has not been elucidated in the present studies, it could be safely assumed that the lowering of enzyme levels by CCl_4 might be responsible for cell injury and that protecting these enzymes by PT extracts might be responsible for hepatoprotective activity.

Catalase activity plays a central role in defending cell against oxidative stress (Rahman *et al.*, 1996). Presumably, a decrease in catalase activity could be attributed to cross-linking and inactivation of the enzyme protein in the lipid peroxidation. Decreased catalase activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by CCl_4. The catalase activity was protected to normal by treatment with PT extracts, evidently indicating the antioxidant property of the PT extracts against oxygen free radicals.

The superoxide dismutase activity is significantly reduced in CCl_4-intoxicated rats. The superoxide dismutase activity was brought to near normal after treatment with the higher concentration of PT extracts in CCl_4-intoxicated primary cultured hepatocytes (Fig. 6). Superoxide dismutase and catalase are the major enzymes which catalyze ROS in most cells. Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotics in liver tissues. It was suggested that catalase and superoxide dismutase are easily inactivated by lipid peroxide or ROS. It can be concluded that the PT extracts might have antioxidant activities either through stabilization of cellular membrane or antioxidative activity.

The hepatotoxic effects of CCl_4 is considered to be resulted from the reductive dehalogenation by the CYP isoforms to the highly reactive trichloromethyl radical. Removal of hydrogen atoms from unsaturated fatty acids by such a radical creates carbon-centered lipid radicals. These lipid radicals also react rapidly with molecular oxygen to form lipid peroxyl radicals, thereby, initiating the process of lipid peroxidation. Unless scavenged by radical scavengers, these lipid peroxyl radicals in turn remove hydrogen atoms from other lipid molecules, thus propagating the process of lipid peroxidation (McCay *et al.*, 1984 ; Recknagel *et al.*, 1989).

Inhibition of CYP enzymes by certain chemicals can also modulate the pharmacological effects or toxicities of many compounds. In the present studies, the possibility of inhibitory effects of PT extracts on CYP 2E1 enzyme was determined to characterize whether the protection by PT extracts against CCl_4-induced hepatotoxicity is derived from the possible inhibitory effects of PT extracts on CYP 2E1 enzyme involved in the metabolic activation of CCl_4 to its hepatotoxic reactive metabolites. PT extracts didn't inhibit microsomal activity of CYP 2E1 enzyme, indicating that PT extracts may not modulate CCl_4 metabolism.

Information on active constituents in PT has been very limited. Recently, Nam *et al.* (2001) reported on the constituents of the PT. Two cytotoxic components were isolated from methanolic extracts of the carpophores of the fungus that was cultivated artificially. Spectral analyses of the cytotoxic components showed that they were ergosterol peroxide (5α,8α-epidioxy-24(R)-methylcholesta-6,22-dien-3β-ol) and acetoxyscirpenediol (4β-acetoxyscirpene-3α,15-diol) that were isolated for the first time from this fungus. Therefore, the effects of these two compounds on hepatoprotection should be further investigated in the near future.

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