Journal of Biochemistry and Molecular Biology, Vol. 34, No. 2, March 2001, pp. 144-149

JOURNAL OF
Biochemistry and Molecular Biology

The Stimulatory Effect of *Garnoderma lucidum* and *Phellinus linteus* on the Antioxidant Enzyme Catalase

Jinseu Park, Byung Ryong Lee, Li Hua Jin[†], Choong Kwon Kim[†], Kyung Soon Choi[†], Jae Hoon Bahn, Kil Soo Lee[‡], Hyeok Yil Kwon[§], Hyun Woo Chang[§], Nam-In Baek[‖], Eunjoo Hwang Lee[#], Jung Hoon Kang[**], Sung-Woo Cho[††] and Soo Young Choi*

Institute of Environment & Life Science, Department of Genetic Engineering,
[†]Graduate Program in Gerontology, [‡]Department of Biology, Division of Life Sciences, Hallym University,
[§]Department of Physiology, College of Medicine, Hallym University, Chunchon,
[‖]Department of Life Sciences, [#]Graduate School of East-West Medical Science, Kyung Hee University, Suwon,
[**]Department of Genetic Engineering, Chongju University, Chongju,
[††]Department of Biochemistry, University of Ulsan College of Medicine, Seoul, Korea

Received 16 December 2000, Accepted 11 January 2001

Antioxidant enzymes, scavengers of the reactive oxygen intermediate (ROI), are involved in numerous defense systems in cells. In the present study, we investigated the effects of the hot-water extracts of two medicinally potent mushrooms (*Ganoderma lucidum* and *Phellinus linteus*) on the activity and expression of antioxidant enzymes *in vitro* and *in vivo*. The mushroom extracts stimulated the catalase activity in a dose-dependent manner *in vitro*, whereas the other antioxidant enzymes (such as superoxide dismutase (SOD), glutathione peroxidase (GPx)) were unaffected by the extracts. The catalytic activity of catalase in the liver and brain was significantly increased after the oral treatment of the mushroom extracts (2.5 g/kg) to ICR mice for 2 months. Western blot analysis of the liver and brain tissues revealed that the expression level of catalase in the mice, treated with both mushroom extracts, was significantly increased compared to that of the control mice. However, the level of the SOD expression in the mice treated with the natural product extracts was unchanged under the same experimental conditions. Although the mechanisms for the stimulatory effect of the catalase expression by these extracts remains unclear, these results suggest that the ingredients of the *Ganoderma lucidum* and *Phellinus linteus* extracts act as an activator of catalase, and regulate the expression of catalase at the translational or transcriptional level.

Keywords: Antioxidant enzyme, Catalase, Stimulatory effect, *Ganoderma lucidum*, *Phellinus linteus*

*To whom correspondence should be addressed.
Tel: 82-33-240-1463, Fax: 82-33-241-1463
E-mail: sychoi@sun.hallym.ac.kr

Introduction

Reactive oxygen species, which include superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), organic peroxide radicals (ROO·), and singlet molecular oxygen ($^1O_2$), are constantly generating intracellularly in aerobic organisms, and released extracellularly during the respiratory burst of phagocytes (Fridovich, 1978; Mason and Chignell, 1983). They are thought to be the major mediators of oxygen cytotoxicity (Beauchamp and Fridovich, 1970). Among the reactive oxygen species, hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Therefore, the cells dispose of hydrogen peroxide by antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase.

Among antioxidant enzymes, catalases are ubiquitous heme enzymes found in aerobic organisms ranging from bacteria to higher plants and animals. Functionally, catalases are related to peroxidases: both promote H_2O_2 oxidation by mechanisms that involve ferryl intermediates (Deisseroth and Dounce, 1970; Dawson, 1988). Catalases differ from peroxidases, with the exception of chloroperoxidase and myeloperoxidase (Ikeda-Saito *et al.*, 1985), in their ability to utilize H_2O_2 as both an electron acceptor and electron donor that yields a disproportionate reaction. Due to this catalytic activity, catalases are believed to be involved in the protective destruction of H_2O_2 generated in respiring cells. The cDNA clone for murine catalase and three-dimensional structure was also identified (Fita *et al.*, 1985; Shaffer *et al.*, 1987). Catalase (EC.1.11.1.6; Hydrogen peroxide oxidoreductase) from bovine liver is a homotetrameric enzyme located primarily in the peroxisomes (Chance *et al.*, 1979). This enzyme catalyzes

the breakdown of H_2O_2 to O_2 and H_2O, thus protecting the cells from toxic effects caused by hydrogen peroxide.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The role of catalase in deterring tumor promotion, and increasing cell survival during periods of oxidative stress, supports the antioxidative function of this enzyme (Jones *et al.*, 1985; Lewis, 1985; Agar *et al.*, 1986). Catalase can also oxidize a variety of compounds such as alcohols, alkyl peroxides, and formic acid. This peroxidatic activity requires a hydrogen donor other than hydrogen peroxide:

$$H_2O_2 + AH_2 \rightarrow 2H_2O + A$$

Ganoderma lucidum and *Phellinus linteus* are commonly known as medicinally potent mushrooms, which have been widely used in China and other oriental countries for hundreds of years for the treatment of various diseases, including cancer. These mushrooms are reported to have various biological activities, such as antitumor, antibacterial and antiviral activities (Yoon *et al.*, 1994; Wang *et al.*, 1997; el-Mekkawy *et al.*, 1998; Eo *et al.*, 2000). It was also reported that they have an anti-inflammatory and liver-protective effect in rats (Lin *et al.*, 1993; Lin *et al.*, 1995). It is now well established from *in vitro* and animal studies that the polysaccharide fraction of *G. lucidum* and *P. linteus* are largely responsible for its anti-tumor efficacy (Wang *et al.*, 1997). Although there are as yet no controlled clinical trials in humans for *G. lucidum* and *P. linteus* against cancer, the indications for its supplemental use can be indirectly supported with the clinical trial data from comparable fungal polysaccharides.

In this study, we examined the effects of *G. lucidum* and *P. linteus* on the activity and expression levels of antioxidant enzymes.

Materials and Methods

Materials Hydrogen peroxide, xanthine, xanthine oxidase, cytochrome c, glutathione, NADH, NBT, mannitol, NADPH, glutathione reductase, superoxide dismutase, glutathione peroxidase were purchased from the Sigma Chemical Co. (St. Louis, USA). Male ICR strain mice, weighing about 25 g, were supplied from the experimental animal center at Hallym University. 100 g of mushroom was extracted with boiling water for 12 h and the extract was lyophilized. 13 g and 17 g of powders were obtained from 100 g of *G. lucidum* and *P. linteus*, respectively.

Preparation of bovine liver catalase and enzymatic assay Bovine liver catalase was purified by the methods previously published (Browett and Stillman, 1979; Eglinton *et al.*, 1983), and the catalase concentration was calculated from the equation of $A_{406} = 3.24 \times 10^5\ M^{-1}cm^{-1}$ (Samejima and Yang, 1963). Catalase activity was assayed spectrophotometrically by measuring the disappearance of H_2O_2 at 240 nm (Cohen *et al.*, 1970; Cho *et al.*, 2000). One unit of enzyme activity was defined as 1 μmol of H_2O_2 decomposed per minute at 25°C. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Assay of other antioxidant enzymes and free radicals Activity of superoxide dismutase was assayed as described previously (McCord and Fridovich, 1969; Eum *et al.*, 1998). Glutathione peroxidase was assayed by the coupled enzyme using hydrogen peroxide as the substrate (Stults *et al.*, 1977). The production of superoxide radical was assayed by the method of Auclair and Voisin (1984) as follows. The reaction mixture containing 0.01 mM NBT, 0.2 mM mannitol, 50 mM Tris-HCl, pH7.4, and 50 μl sample was prewarmed at 30°C. The effect of calcium on the superoxide radical production was assayed by adding stepwise increments of calcium, and the reaction was started by the addition of NADH to a final concentration 0.2 mM. The change of absorbance was monitored at 560 nm and the amount of superoxide radical production was expressed as the extent of NBT reduction.

Effects of *G. lucidum* and *P. linteus* on antioxidant enzyme activity Powders of *G. lucidum* and *P. linteus* extracts were dissolved in H_2O at high a concentration (100 mg/ml) and stored at 4°C. These stock solutions were diluted with distilled water. Antioxidant enzymes (10 μM) were preincubated with the *G. lucidum* and *P. linteus* extracts (20 μg/ml) in a 10 mM potassium phosphate buffer, pH 7.0, for 30 min at 37°C, respectively. An equal volume of H_2O was used in the control experiments. Aliquots were withdrawn and assayed for enzyme activities by adding the standard assay mixture.

Treatment of *G. lucidum* and *P. linteus* extracts to mice Five male ICR mice were used in each group. The animals were housed in a controlled environment of 23±1°C temperature, 55±10% humidity with a 12 hr light-dark cycle and given regular food throughout the experimental period. Powders of mushroom extracts were mixed uniformly into tap water and daily supplied to mice at a dose of 2.5 g/kg/day for 2 months. Control mice received tap water only. All operations were carried out at 4°C unless otherwise indicated. The liver was isolated quickly at the time of sacrifice and then, sliced liver tissue was homogenized in a 10 mM potassium phosphate buffer, pH 7.0 containing 1 mM β-mercaptoethanol, 0.1 mM EDTA. The homogenate was centrifuged at 10,000 × g for 30 min and the pellet was discarded. A supernatant solution was used to assay enzyme activity.

Immunoblot analysis For Western blotting, liver homogenates were subjected to a 10% SDS-polyacrylamide gel electrophoresis (PAGE) and electrophoretically transferred to Immobilon membranes (Towbin *et al.*, 1979; Lee *et al.*, 2000). Then, the blots were blocked with BLOTTO for 1 hr. After rinsing with TBS, the blots were incubated in anti-catalase and anti-SOD monoclonal antibodies for 1 hr. The blots were treated with HRP-conjugated goat anti-mouse IgG for 1 hr and visualized by chemiluminescence (ECL; Amersham, Arlington Heights, USA).

Results

Activation of catalase activity by extracts of *G. lucidum* and *P. linteus in vitro* We investigated the effect of *G.*

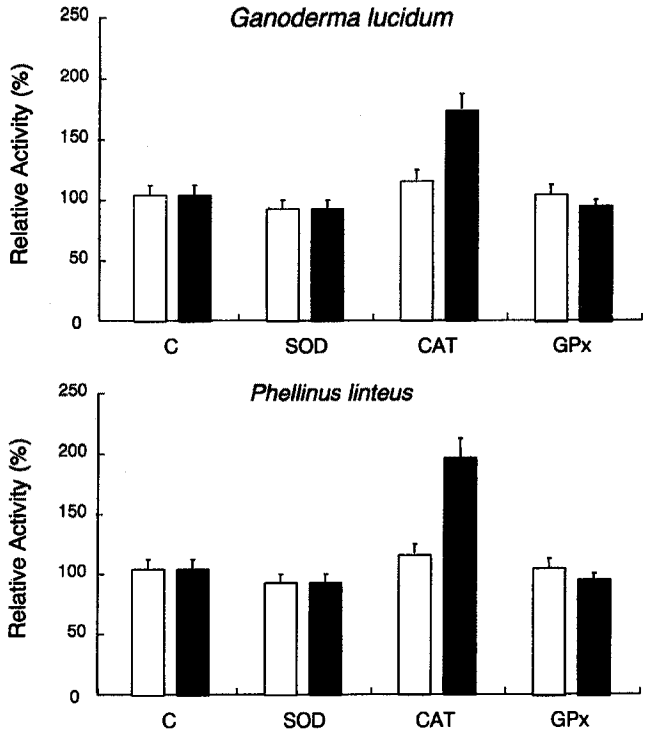

Fig. 1. Effects of *G. lucidum* and *P. linteus* extracts on antioxidant enzymes purified from bovine liver and brain. Various antioxidant enzymes purified from bovine liver and brain were preincubated with *G. lucidum* and *P. linteus* extracts at a concentration of 20 μg/ml for 30 min in a 0.1 M potassium phosphate buffer, pH 7.0, respectively. Aliquots withdrawn from the incubation mixtures were assayed for enzyme activities as described in Materials and Methods. Data are representative of three independent experiments. (❑: bovine brain, ■: bovine liver)

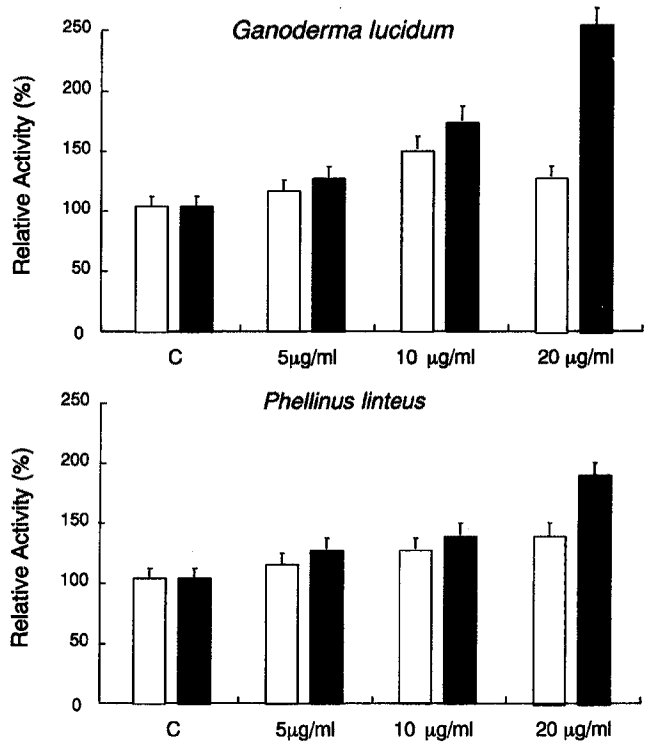

Fig. 2. Dose-dependent analysis of *G. lucidum* and *P. linteus* extracts on catalase activities. Bovine liver and brain catalase (10 μM) were preincubated with *G. lucidum* and *P. linteus* extracts at various concentrations for 30 min in 0.1 M potassium phosphate buffer, pH 7.0. (❑: bovine brain, ■: bovine liver), respectively. Aliquots withdrawn from the incubation mixtures were assayed for catalase activity using H_2O_2 as described in Materials and Methods. Data are representative of the three independent experiments.

lucidum and *P. linteus* extracts (20 μg/ml) on the activities of antioxidant enzymes, SOD, catalase, and GPx. As shown in Fig. 1, preincubation of the enzyme, purified from liver and brain with *G. lucidum* extracts, resulted in significantly increased enzymatic activity of catalase. However, SOD and GPx activities were not influenced by the pretreatment of the enzymes with *G. lucidum* extracts. The catalase activity was also enhanced significantly by treatment with *P. linteus* extracts (Fig. 1). Activities of catalase purified from liver were observed to be higher than that of catalase from brain in the presence of *G. lucidum* and *P. linteus* extracts. The dose-dependent effect of extracts on the catalase activity was investigated by preincubating the enzyme with various concentrations of *G. lucidum* and *P. linteus* extracts. The catalase activity was increased by the treatment of enzymes with extracts in a dose-dependent manner (Fig. 2). Furthermore, when enzyme mixtures were treated with 20 μg/ml *G. lucidum* and *P. linteus* extracts, respectively, the enzymatic activities increased as a function of time and reached maximum in 15 min (data not shown).

Changes of catalase activity and free radical level by the treatment of *G. lucidum* and *P. linteus* extracts *in vivo*

Changes in antioxidant enzyme activities and free radical levels with the aging process in the liver of ICR mice (3, 6, 9 and 12 months) were investigated. Hepatic levels of antioxidant enzyme activities and free radical with aging are shown in Fig. 3. The enzyme activity of SOD and catalase were increased until 9 months. In 12 month-old ICR mice, the enzyme activities were decreased, whereas the free radical levels were significantly increased.

In order to determine the effect of extracts on the antioxidant enzyme activities *in vivo*, the *G. lucidum* and *P. linteus* extracts were administrated into 12 month-old ICR mice for two months. Then the activities of antioxidant enzymes in the liver and brain of mice treated with extracts were determined (Fig. 4). The liver catalase activity after administration of *P. linteus* extracts increased two fold compared to that of the control. In *G. lucidum* treated groups, catalase activity was slightly increased. However, SOD and GPx activities were unchanged under the same experimental conditions (data not shown).

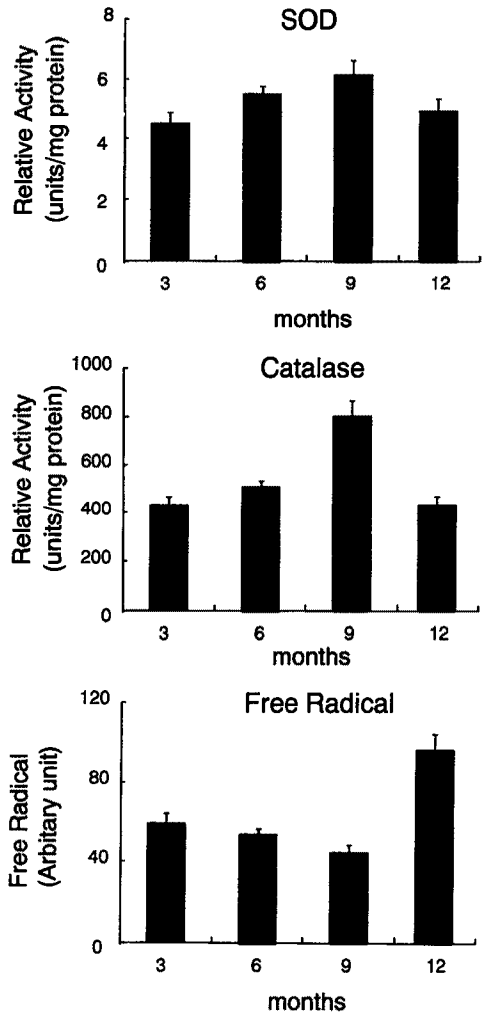

Fig. 3. Changes of antioxidant enzyme activities and the free radical level in the liver of ICR mice with aging process. The homogenates from liver of the different aged mice were prepared and assayed for antioxidant enzyme activities and free radical level as described in Materials and Methods. Data are representative of the three independent experiments.

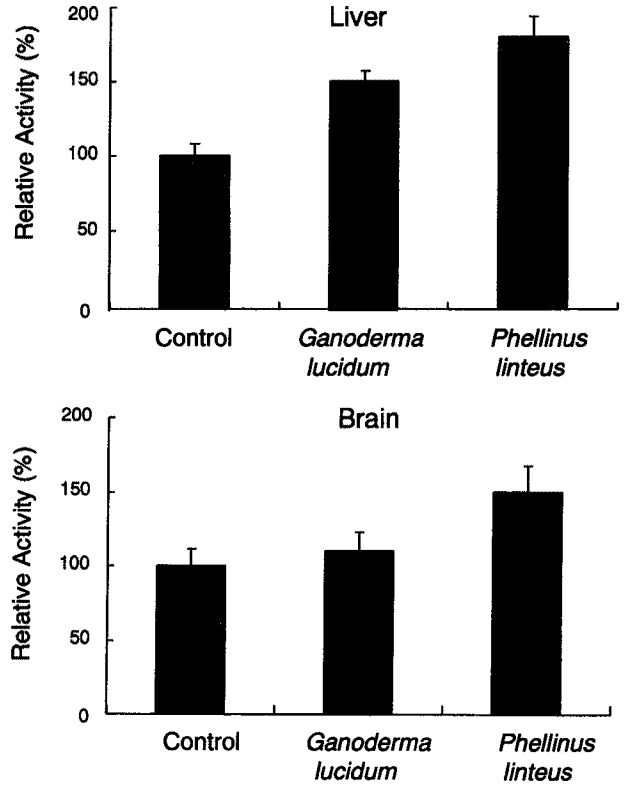

Fig. 4. Effect of *G. lucidum* and *P. linteus* extracts on the catalase activity *in vivo*. *G. lucidum* and *P. linteus* extracts were daily supplied to mice at a dose of 2.5 g/kg/day for 2 months. The homogenates from the liver and brain of mice were prepared and assayed for catalase activity using H_2O_2 as described in Materials and Methods. Data are representative of the three independent experiments.

Enhancement of the expression level of catalase by the treatment of *G. lucidum* and *P. linteus* extracts *in vivo* We next addressed the possibility that *G. lucidum* and *P. linteus* extracts may affect the expression level of catalase and SOD. The levels of catalase and SOD expression in liver of different aged mice (3, 6, 9 and 12 months), or 12 month-old mice treated with *G. lucidum* and *P. linteus* extracts for 2 months, were analyzed by Western blot analysis using anti-catalase and anti-SOD monoclonal antibodies, respectively (Fig. 5). In the liver of the control ICR mice that were not treated with extracts, the expression level of catalase was maximum at 6 months, and then declined significantly after 9 months as judged from the catalase band with the molecular weight of 60 kDa, while the level of SOD was not significantly increased over the aging process (lane 1, 2, 3 and 4 in Fig. 5). However, in the liver of 12 month-old mice treated with *G. lucidum* and *P. linteus* extracts, the level of catalase was observed to be dramatically increased, while the level of SOD was unchanged (Fig. 5). On the other hand, another natural product, total ginsenosides, did not elevate the expression levels of catalase and SOD under our experimental conditions.

Discussion

Ganoderma lucidum and *Phellinus linteus* are thought to have an efficacy in treating various diseases, including cancers in China and other oriental countries for hundreds of years. However, the effective components of these materials and/or detailed action mechanisms of those components are largely unknown. In this study, the effects of two mushroom extracts on the activity of antioxidant enzymes were investigated.

Activity of catalase purified from bovine liver and brain was increased by pretreatment with *G. lucidum* and *P. linteus* extracts in a dose- and time-dependent manner *in vitro* (Fig. 1 and 2). In addition, the catalase activity of the liver was also activated by administration of *G. lucidum* and *P. linteus* extracts for 2 months into the ICR mice, as shown in Fig. 4. On the other hand, the extracts had no effect on the other

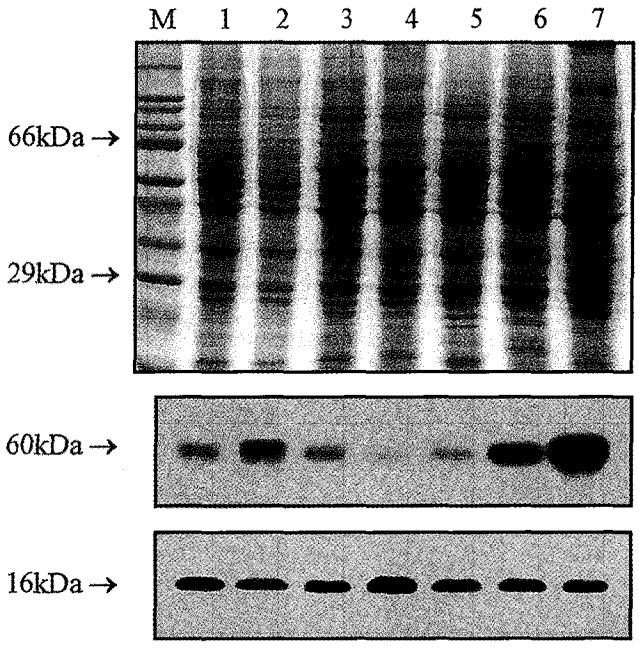

Fig. 5. Immunoblot analysis of total proteins of the ICR mice liver homogenate probed with anti-catalase mAb and anti-SOD mAb. *G. lucidum* and *P. linteus* extracts were daily supplied to mice at a dose of 2.5 g/kg/day for 2 months. Then the liver homogenates from mice were prepared and proteins were analyzed by a 12% SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue. Then the proteins separated by 12% SDS-PAGE were transferred to a nitrocellulose membrane. Membranes were incubated with anti-catalase mAb (60 kDa band) and anti-SOD mAb (16 kDa band) as the first antibody, then the peroxidase conjugated goat-anti-mouse IgG as the secondary antibody. M; molecular weight markers, lane 1; 3 month-old, lane 2; 6 month-old, lane 3; 9 month-old, lane 4; 12 month-old, lane 5; 12 month-old mice treated with total ginsenoside, lane 6; 12 month-old mice treated with *G. lucidum*, lane 7; 12 month-old mice treated with *P. linteus* extracts.

antioxidant enzymes including SOD, GPx. These results suggest that *G. lucidum* and *P. linteus* extracts can affect the activities of catalase *in vitro* and *in vivo*. A previous study reported that the water extracts of *G. lucidum* have free radical scavenging activities (Lin *et al.*, 1995). Therefore, the stimulatory effect of the extracts on catalase *in vivo* and *in vitro* suggests that the activation of catalase decreases H_2O_2 levels in the liver, and results in antihepatotoxic activity against oxidative stresses.

As shown in Fig. 3., the enzyme activity of SOD and catalase increased with age and exhibited maximum levels at 9 month, but decreased thereafter. These results suggest that the reduction of antioxidant enzyme activities may be involved in the aging process. The expression of catalase in various aged mice revealed different patterns with the aging process as shown in Fig. 5. The expression level of catalase reached a maximum peak at 6 month, and declined significantly until 12 months. Interestingly, the levels of catalase expression in 12 month old mice, treated with *G. lucidum* and *P. linteus* extracts for 2 months, were significantly elevated compared with that of the untreated control mice, as demonstrated by a Western blot analysis using anti-catalase. This result suggests that *G. lucidum* and *P. linteus* extracts may be able to enhance the expression level of catalase at the transcriptional or translational levels. This regulation is relevant in explaining the previous results that showed the liver-protective effects of *G. lucidum* in rats (Lin *et al.*, 1993).

Although the detailed action mechanisms of *G. lucidum* and *P. linteus* extracts on the activities and expression level of catalase are unclear, our results implicate that they can exert their effects on the catalase itself, as well as the expression of catalase. We reported that the total ginsenoside extracts stimulated the catalase *in vitro* and this stimulation effect is due to the conformational changes of the enzyme by ginsenoside (Choi *et al.*, 1999). Therefore, this kind of action mechanism would be relevant for the effect of *G. lucidum* and *P. linteus* extracts on catalase. Moreover, as shown in Fig. 5, in 12 month-old mice treated with *G. lucidum* and *P. linteus* extracts, the level of catalase expression was significantly increased compared to that of the control mice. The strong elevation of the catalase expression by *P. linteus* extracts suggests that the components of extracts may influence the catalase expression on the transcriptional or translational level. Therefore, the fractionation and purification of the active pure compound, which is responsible for stimulation of catalase from both mushroom extracts, will be undertaken.

To our knowledge, the present article is the first report that shows the activation of the antioxidant enzyme catalase by *G. lucidum* and *P. linteus* extracts. Further examination of the biological effects, such as the reduction of H_2O_2 levels by the extracts in liver, may provide insights into the effect of *G. lucidum* and *P. linteus*. And the elucidation of effective components and their action mechanisms provides a scientific basis for the pharmacological application of *G. lucidum* and *P. linteus* as effective antioxidant materials.

Acknowledgments The authors wish to acknowledge the financial support of the Korea Research Foundation from the program of the University Research Institute Fund in 1997, and also the support of the Industry-University-Research Institute Consortium grant from the Korean Ministry of Small and Medium Industry.

References

Agar, N. S., Sadrzadeh, S. M., Hallay, P. E. and Eaton, J. W. (1986) Erythrocyte catalase. A somatic oxidant defense? *J. Clin. Invest.* **77**, 319-321.

Auclair, C. and Voisin, E. (1984) Nitroblue tetrazolium reaction; in *CRC Handbook of Methods for Oxygen Radical Research*, pp. 123-132, CRC Press Inc., Boca Raten, Florida.

Beauchamp, C. and Fridovich, I. (1970) A mechanism for the production of ethylene from methional; the generation of

hydroxyl radicals by xanthine oxidase. *J. Biol. Chem.* **245**, 4641-4646.

Bradford, M. A. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.

Browett, W. R. and Stillman, M. J. (1979) Magnetic circular dichroism studies of bovine liver catalase. *Biochim. Biophys. Acta* **577**, 291-306.

Chance, B., Sies, H. and Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**, 527-604.

Cho, Y.-W., Park, E.-H. and Lim, C.-J. (2000) Catalase, glutathione S-transferase and thioltransferase respond differentially to oxidative stress in *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.* **33**, 344-348.

Choi, K. S., Lee, B. R., Sun, H. J., Kwon, H. Y., Park, J., Lee, K. S., Cho, J. H. and Choi, S. Y. (1999) Ginsenosides activate catalase from bovine liver. *Kor. J. Gerontol.* **9**, 1-6.

Cohen, G., Dembiec, D. and Marcus, J. (1970) Measurement of catalase activity in tissue extracts. *Anal. Biochem.* **34**, 30-38.

Dawson, J. H. (1988) Probing structure-function relations in heme-containing oxigenases and peroxidases. *Science* **240**, 433-439.

Deisseroth, A. and Dounce, A. L. (1970) Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. *Physiol. Rev.* **50**, 319-375.

Eglinton, D. G., Gadsby, P. M. A., Sievers, G., Peterson, J. and Thomson, A. J. (1983) A comparative study of the low-temperature magnetic circular dichroism spectra of horse heart metmyoglobin and bovine liver catalase derivatives. *Biochim. Biophys. Acta* **742**, 648-658.

El-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T. and Otake, T. (1998) Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. *Phytochemistry* **49**, 1651-1657.

Eo, S., Kim, Y., Lee, C. and Han, S. (2000) Possible mode of antiviral activity of acidic protein bound polysaccharide isolated from ganoderma lucidum on herpes simplex viruses. *J. Ethnopharmacol.* **72**, 475-481.

Eum, W. S., Kwon, O. B. and Kang, J. H. (1998) Hydroxyl radical-generating function of horseradish Cu,Zn-superoxide dismutase. *J. Biochem. Mol. Biol.* **31**, 492-497.

Fita, I. and Rossmann, M. G. (1985) The active center of catalase. *J. Mol. Biol.* **185**, 21-37.

Fridovich, I. (1978) The biology of oxygen radicals. *Science* **201**, 875-880.

Ikeda-Saito, M., Argade, P. V. and Rousseau, D. L. (1985) Resonance Raman evidence of chloride binding to the heme iron in myeloperoxidase. *FEBS Lett.* **184**, 52-55.

Jones, G. M., Sanford, K. K., Parshad, R., Gantt, R., Price, F. M. and Tarone, R. E. (1985) Influence of added catalase on chromosome stability and neoplastic ansformation of mouse cells in culture. *Br. J. Cancer* **52**, 583-590.

Lee, I.-H., Kim, I.-S. and Lee, S.-Y. (2000) Expression of α_1-acid glycoprotein and inflammatory cytokines during differentiation of HL-60 cells. *J. Biochem. Mol. Biol.* **33**, 402-406.

Lewis, W. (1985) Establishment of mouse cells lines homozygous for temperature- sensitive mutation in catalastic gene. *Somatic Cell Mol. Genet.* **11**, 319-324.

Lin, J. M., Lin, C. C., Chen, M. F., Ujiie, T. and Takada, A. (1995) Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo-japonicum*. *J. Ethnopharmacol.* **47**, 33-41.

Lin, J. M., Lin, C. C., Chiu, H. F., Yang, J. J. and Lee, S. G. (1993) Evaluation of the anti-inflammatory and liver-protective effects of *anoectochilus formosanus*, *ganoderma lucidum* and *gynostemma pentaphyllum* in rats. *Am. J. Chin. Med.* **21**, 59-69.

Mason, R. P. and Chignell, C. F. (1983) Free radicals in pharmacology and toxicology. *Pharmacol. Rev.* **33**, 189-211.

McCord, J. M. and Fridovich, I. (1969) Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**, 6049-6055.

Samejima, T. and Yang, J. T. (1963) Reconstitution of acid-denatured catalase. *J. Biol. Chem.* **238**, 3256-3261.

Shaffer, J. B., Sutton, R. B. and Bewley, G. C. (1987) Isolation of a cDNA clone for murine catalase and analysis of an acatalasemic mutant. *J. Biol. Chem.* **262**, 12908-12911.

Stults, F. H., Forstrom, J. W., Chiu, D. T. and Tappel, A. L. (1977) Rat liver glutathione peroxidase; purification and study of multiple forms. *Arch. Biochem. Biophys.* **183**, 490-497.

Towbin, H., Staehelim, T. and Gordon, T. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets; procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354.

Wang, S. Y., Hsu, M. L., Hsu, H. C., Tzeng, C. H., Lee, S. S., Shiao, M. S. and Ho, C. K. (1997) The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *Int. J. Cancer* **70**, 699-705.

Yoon, S. Y., Eo, S. K., Kim, Y. S., Lee, C. K. and Han, S. S. (1994) Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. *Arch. Pharm. Res.* **17**, 438-442.