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Antihepatotoxic and Antioxidant Activities of Polysaccharide Obtained from Cultured Mycelia of *Ganoderma lucidum*

†June-Woo Lee

Associate Professor, Dept. of Hotel Culinary Art and Bakery, Kyungbuk College, Yeongju 36133, Korea

Abstract

The purpose of this study was to observe the effects of the polysaccharide (GLP) obtained from the liquid cultured *Ganoderma lucidum* on the lipidperoxidation in a rat liver microsome and hepatotoxicity in the primary cultured rat hepatocytes. It is well known that the polysaccharide of *Ganoderma lucidum* exhibits hepatoprotective activity, antitumor activity etc., which many suggest a relationship to lipidperoxidation. The effect of GLP on CCl_4- and galactosamine-intoxicated cytotoxicity in the primary cultured rat hepatocytes were reduced the GPT value. In order to the estimate the effects of anti-lipidperoxidation of the polysaccharide, enzymatic and nonenzymatic reaction assays were performed, *in vitro*, in the rat liver microsome. An enzymatic lipidperoxidation reaction by ADP+$FeCl_3$+NADPH and CCl_4+NADPH, GLP (1 mg/mL) inhibited 77.4% and 39.4%, respectively, and the nonenzymatic reaction displayed a 97.4% strongly inhibition. In the enzymatic and nonenzymatic inducers treated with GLP, the formation of malondialdehyde (MDA) progressively decreased by raising the GLP concentration. These results suggest that the anti-lipidperoxidation and radical scavenging activity of GLP may play an important part in the liver protection action.

Key words: *Ganoderma lucidum*, hepatoprotective activity, anti-lipidperoxidation, polysaccharide, microsomes.

Introduction

Ganoderma lucidum is a fungus belonging to Ganodermaceae Basidiomycetes, and the fruit bodies have been used folk medicine in China, Japan and Korea. It has been known to posses an activities as hepatoprotective activity, antihypertensive (Kanmatsuuse et al. 1985), anticancer (Sone et al. 1985), inhibition of platelet aggregation (Shimizu et al. 1985), hyperlipidemic effect (Chang et al. 2012), inhibition of lipidperoxidation (Oh & Lee 2005), antihypoglycemia (Hikino et al. 1989), inhibition of histamine release from rat mast cell (Kohda et al. 1985) and cytotoxicity of hepatoma cells *in vitro* (Toth et al. 1983).

Various components of *Ganoderma lucidum* have proved to be effective for the treatment of hepatitis, and it was reported that a extract of *Ganoderma lucidum* reduced the serum glutamate pyruvate transferase activity using CCl_4-intoxicated in a mouse (Liu et al. 1979). The various hydroxy radical scavengers are used to study the role of hydroxy radicals in biological systems (Halliwell et al. 1987). A number of hepatoprotective compounds are known to the natural products (Kiso et al. 1984), bacteria (Itoh et al. 1991), yeast (Williams et al. 1980) and basidiomycetes (Lee et al. 1992; Park et al. 1993; Ha et al. 2010; Jeong et al. 2014) have been reported. Some of these compounds, such as glycyrrhizin, gomisin and silymarin are used clinically for the treatment of chronic hepatitis. Glycyrrhizin and gomisin have been reported to exhibit antihepatotoxic activity by CCl_4-induced primary cultured rat liver cell and the inhibition of lipidperoxidation in rat microsome have been shown (Heo et al. 2006; Kleiner et al. 2016). According to other reports, lipid peroxides of the higher animals are known to injure the liver, kidney and blood vessels (Comporti 1993). Other reporters suggest that the possible relationship between the hepatoprotective effect and the lipidperoxidation inhibition, it is of the importance to investigatethe mechanism of the antihepatotoxic activity (Kiso et al. 1984;

† Corresponding author: June-Woo Lee, Associate Professor, Dept. of Hotel Culinary Art and Bakery, Kyungbuk College, Yeongju 36133, Korea. Tel: +82-54-630-5185, Fax: +82-54-630-5304, E-mail: jangsan60@naver.com

Sreenivasan et al. 2010).

In this report, for the investigation of the mechanism of antihepatotoxic activity on GLP, we have examined the antihepatotoxic activity in CCl_4- and galactosamine-intoxicated cytotoxity using primary cultured rat hepatocytes and anti-lipid peroxidative activity.

Material and Method

1. Materials

Glycyrrhizin, 18α-glycyrrhetinic acid (18α-GA) and 18β-glycyrrhetinic acid (18β-GA), known for its hepatoprotective effect, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). William's eagle medium (WEM), fatal bovine serum (FBS), and 100 μnit/mL penicillin-stretptomycin used for hepatocytes culture were purchased from Gibco Co. (Lafayette, CO, USA). Butylated hydroxytoluene (BHT) and vitamin E were used as reagents to measure antioxidant activity from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used were of the highest purity available from Sigma-Aldrich Co. (St. Louis, MO, USA).

2. Fermentation

The mycelium of *Ganoderma lucidum,* which was isolated from the cultured fruiting body was preserved on the agar slant and the seed medium used was potato dextrose broth (PDB; Difco Co.). A 500 mL erlenmeyer flask containing 100 mL of the medium was inoculated from an agar slant culture of *G. lucidum*. The flasks were grown on a rotary shaker (120 rpm) at 27.5℃ for 5 days to produce a second seed culture. Vegetative growth of the seed culture was transferred, at 10% (v/v), to 5 L jar-fermentor containing 3 L of a production medium. The production medium was composed of soluble starch 4%, CSL 3%, 0.087% KH_2PO_4, 0.05% $MgSO_4$, 0.05% $FeCl_2 \cdot 6H_2O$, 0.036% $MnCl_2.4H_2O$, 0.03% $ZnCl_2$, and 0.05% $CuSO_4 \cdot 5H_2O$; presterile pH 4.5.

Three liters of the second seed culture was transferred into 50-liter fermentor containing 27 liters of a production medium. Fermentation was carried out under aeration 0.5 vvm at 27.5℃ for 6 days and agitation of 200 rpm.

For large scale fermentations, a 400-liter fermentor was charged with 280 liters of the production medium followed by a supplement of 0.03% (v/v) silicon. After steam-sterilization at 27.5℃ for 5 days with an agitation of 150 rpm in the early stage and 200 rpm after 2 days.

3. Preparation of GLP

After cultivation for 5 days, the culture broth was extracted with aqueous 2 N NaOH for 1 day at room temperature. The extract was neutralized with acetic acid (glacial). The supernatant was collected by centrifugation at 3,200×g for 15 minutes, and the solution was added ethanol of 3 times for overnight. The precipitate was collected by centrifugation and washed with ethanol and then dialyzed against running water for 24 hours. The non-dialyzable fraction was concentrated and lyophilized (GLP).

4. Isolation of hepatocytes

Male Sprague-Dawley rats (200~250 g) were used and hepatic cells were prepared by a modification of the method of Seglen PO (1973). The rat was anesthetized with pentobarbital (45 mg/kg), the abdomen was opened, and the portal vein cannulated with a 18 gague angiocatheter (Desert Medical Inc.). The liver was perfused with Ca^{2+}, Mg^{2+}- free HBSS buffer (pH 7.4) for 5 minutes (flow rate: 30 mL/min) at 37℃. The thoracic portion of the inferior vena cava was cannulated via the right atrium. The inferior vena cava was ligated just above the level of the renal veins. The liver was then perfused with Ca^{2+}, Mg^{2+}-free HBSS buffer (pH 7.4) containing 0.06% collagenase and 0.25 mM $CaCl_2$ at 37℃ for 12 minutes. The liver was removed from the body, transferred to a beaker containing HBSS buffer, and gently broken up with a spatula. The isolated cell suspension was filtered through a nylon mesh and centrifuged at 50×g for 2 minutes. The cell was washed three times in William's E medium (WEM), and the cell viability was determined by trypan blue exclusion and the cells were used when their viability exceeded 90%.

5. Preparation of the microsomes

The rat microsomes were prepared by the method of Kiso et al. (1984). Male Sprague-Dawley rats weighing 200~250 g were used. The rat was anesthetized with pentobarbital (45 mg/kg, *ip*), the abdomen was opened, and the liver was perfused with 15 mL of 150 mM KCl－50 mM Tris-Cl buffer. The liver was immediately removed and was homogenized in 2 volumes (w/v, %) of ice-cold 150 mM KCl－50 mM Tris-Cl buffer (pH 7.4) by homogenizers (Polytron, Switzerland). The whole homogenates were centrifuged at 8,000×g for 20 minutes in a Hitachi

SCR 20 BB centrifuge. The supernatant was again centrifuged at 105,000×g for 60 minutes in a Beckman ultracentrifuge. The pellets were resuspended in 1.5 volume of KCl-Tris buffer and used as a microsomal fraction. The microsomes were adjusted to the concentration of protein (20 mg/mL) and stored at −20℃ until used. All operations were done at 4℃. The protein content was determined by the Smith et al. method (1985) using a BCA protein assay kit (Pierce Co).

6. Hepaprotective effect

The washed cells were diluted into WEM containing 10% FBS, 100 U/mL penicilline, 100 μg/mL streptomycine. The cells were adjusted to a concentration of 5×10^5 cells/mL and plated into 24 well plate (1 mL/each well) purchased from Corning Co. (USA). Cells were incubated at 37℃, in an atmosphere of a mixture of 95:5 air: CO_2 with WEM for 1.5 hours.

After preincubation for 1.5 hours, the medium was exchanged for a fresh medium and incubated for 18 hours. Hereafter the medium was decant and hepatocytes were treated with CCl_4 and materials in the medium. Sixty minutes after incubation, GPT in the medium was measured as the Karmen method using a UV-spectrophotometer (Shimadzu UV-160A). The hepatoprotective activity expressed as the percentage, The percentage of sample GPT values divided by control GPT values.

7. Measurement of lipidperoxidation

In order to estimate the *in vitro* antilipidperoxidative effect of GLP, the Ohkawa et al. (1979) were employed with some modifications. lipidperoxidation was induced at 37℃ for 20 minutes in 1 mL incubations consisting of 83.5 mM KCl and 37.2 mM Tris-HCl buffer (pH 8.0) contained 2 mg of microsomal protein with 0.1 mL of a sample in DMSO or DW (0.1 mL), 0.2 mM ascorbic acid and 10 μM $FeSO_4$.

To determine the inhibitory effect of GLP on an ADP+ $FeCl_3$-induced lipidperoxidation, lipidperoxidation was induced at 37℃ for 20 minutes in 1 mL incubations consisting of 83.5 mM KCl and 37.2 mM Tris-HCl buffer (pH 8.0). The incubations contained 2 mg of microsomal protein with 0.1 mL of a sample in DMSO or DW, 1 mM ADP, 10 μM $FeCl_3$ and 0.2 mM NADPH.

To determine the inhibitory effect of GLP on an CCl_4+ NADPH-induced lipidperoxidation, lipidperoxidation was induced at 37℃ for 20 minutes in 1 mL incubations consisting of 83.5 mM KCl and 37.2 mM Tris-HCl buffer (pH 8.0). The incubations contained 2 mg of microsomal protein with 0.1 mL of a sample in DMSO or DW (0.1 mL), 10 mM CCl_4 and 2 mM NADPH.

The degree of lipid peroxides was estimated by the method of Ohkawa et al. (1979). The amounts of formed thiobarbituric acid-reactive material was expressed as malondialdehyde equivalents using 1,1,3,3-tetramethoxypropane as the standard. The inhibition effects of lipidperoxidation of the sample was expressed in terms of percent inhibition. The calculation was based on the following equation:

$$\text{Inhibition}(\%) = \frac{\text{MDA value of control} - \text{MDA value of sample}}{\text{MDA value of control} - \text{MDA value of normal}} \times 100(\%)$$

8. Hydroxy radical scavenging activity

The hydroxy radical was measured by the Gutteridge method as modified by Halliwell et al. (1987). Briefly, the reaction mixtures (1 mL) containing 20 mM KH_2PO_4-KOH buffer (pH 7.4), deoxyribose 3.25 mM, ferric chloride 100 μM, EDTA 100 μM, ascorbic acid 100 μM and H_2O_2 1 mM were incubated at 37℃ for 1 hour, and the reaction was stopped with 5.0% TCA. The Fe^{3+} and ascorbic acid solutions were made up immediately before use. For the color developed, formed MDA was added to 1 mL of TBA 0.67%, and the reactants boiled at 96℃ for 30 minutes. The amount of thiobarbituric acid-reactive material formed was expressed as malondialdehyde equivalents using 1,1,3,3-tetramethoxypropane (TMP) as standard. The scavenging activity of the hydroxy radical of the sample was expressed in terms of percent inhibition. The calculation was as derscribed above.

9. Statistical analysis

Data were analysed by the *t*-test using the Pharmacologic Calculation System software (R. J. Tallarida and R. B. Murray, Springer-verlag, New York, 1986).

Results

1. Hepatoprotective activity of GLP

The effect of GLP on CCl_4- and galactosamine-intoxicated cytotoxicity in the primary cultured rat hepatocytes are shown in Table 1. At the concentration of 0.1 mg and 1.0 mg/mL of GLP, glycyrrhizin and its analogues reduced the leakage of

Table 1. The effect of GLP, glycyrrhizin and its analogues on CCl_4- and galactosamine-intoxicated cytotoxicity in primary cultured rat hepatocytes

Samples	Concentration (mg/mL)	GPT(%)	
		CCl_4[1)]	Galactosamine[2)]
GLP	0.1	88.3	90.1*
	1.0	70.7*	82.3*
Glycyrrhizin	0.1	80.6	88.2
	1.0	57.3**	38.7**
18α-Glycyrrhetinic acid	0.1	90.5	59.4
	1.0	75.8	88.7
18β-Glycyrrhetinic acid	0.1	91.5	59.0
	1.0	58.3	24.0

1) After preincubation for 1.5 hours, the medium was exchanged for a fresh medium (1 mL) and incubated for 18 hours. Hereafter the medium was decant and hepatocytes were treated with CCl_4 and materials in the medium. Sixty minutes after incubation, GPT in the medium was measured as the Karmen method using a UV-spectrophotometer (Shimadzu UV-160A). Control value were 21.6±0.9. Each value was average of the three plates. Means±S.D. Significantly different from the control, * $p<0.05$.

2) After preincubation for 2 hours, the medium was exchanged for a fresh medium (1 mL). Hereafter the medium was decant and hepatocytes were treated with galactosamine and materials in the medium. Twenty hours after incubation, GPT in the medium was measured as the Karmen method using a UV-spectrophotometer (Shimadzu UV-160A). Control value were 88.6±0.3. Each value was average of the three plates. Means±S.D. Significantly different from the control, * $p<0.05$, ** $p<0.01$.

transaminase. Among the four substances, 18β-glycyrrhetinic acid has strong antihepatotoxic activity in CCl_4- and galactosamine-induced cytotoxic in primary cultured rat hepatocytes.

2. Effect of GLP on ascorbate and $FeSO_4$-induced LPO

Microsomes were treated with ascorbic acid/$FeSO_4$ in the absence or presence of GLP, and the produced lipidperoxides were measured. Table 2 shows the inhibition of lipidperoxidation by GLP, BHT, Vit. E, glycyrrhizin, 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid, at concentration of 1.0 mg/mL, 97.7, 105.7, 97.8, 10.7, −3.7 and 57.0 %, respectively. GLP significantly inhibited peroxide formation by ascorbate + $FeSO_4$ the potency of which was similar to that of Vit. E. While glycyrrhizin and 18α-glycyrrhetinic acid did not affect the generation of lipidperoxidation the microsome at the concentration of 1 mg/mL. The MDA normal value was 4.6±0.4 nM/mL at 20 minutes after incubation, and the MDA value of the control was 34.4±0.5 nM/mL.

Fig. 1. shows that the concentration of 50 μg/mL of GLP progressively decreased, and the concentration from 10 μg/mL to 400 μg/mL linearly decreased to the formation of MDA.

3. Effect of ADP + $FeCl_3$-induced LPO on GLP

As shown in Table 3 at concentration of 0.5 and 1.0 mg/mL GLP inhibited LPO by about 19.2%, 77.4%, respectively. BHT and Vit E showed a significant inhibition of lipidperoxida-

Table 2. The effect of GLP and various material on an ascorbate/Fe^{2+}-induced lipidperoxidation in rat liver microsomes

Sample	Concentration (mg/mL)	Malondialdehyde (MDA, nM/mL)	Percent inhibition (P.I., %)
Normal	-	4.6±0.4	-
Control	-	34.4±0.5	-
GLP	0.5	7.1±0.2*	91.6
	1.0	5.3±0.2*	97.7
BHT	0.5	4.7±0.2*	99.7
	1.0	2.9±0.1*	105.7
Vitamin E	0.5	7.3±0.3*	90.9
	1.0	5.3±0.2*	97.8
Glycyrrhizin	1.0	31.2±0.5*	10.7
18α-Glycyrrhetinic acid	1.0	35.5±0.3*	−3.7
18β-Glycyrrhetinic acid	1.0	17.4±0.5*	57.0

The incubation mixture consisting of 1.0 mL was composed of a sample in DW, 83.5 mM KCl, 37.2 mM Tris-HCl buffer (pH 8.0), 0.2 mM ascorbate, 10 μM $FeSO_4 \cdot H_2O$, Microsomal suspension (protein, 2 mg). After incubation at 37℃ for 20 minutes. with ascorbate-Fe^{2+}, the lipid peroxide was measured by the method of Ohkawa et al. (1979). Results are expressed as the mean±S.D. of three experiments. * Significantly different from the control, $p<0.01$.

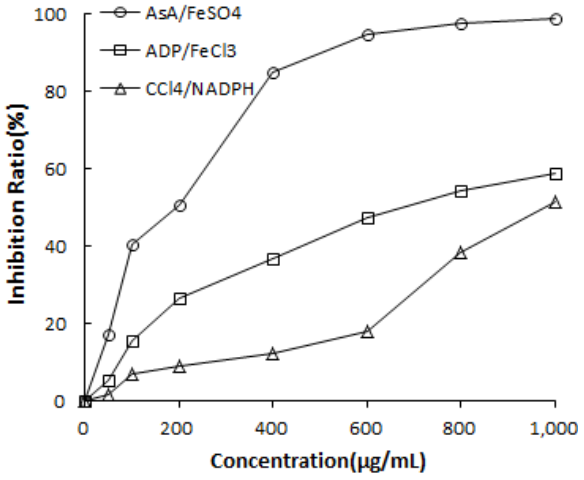

Fig. 1. The effect of the concentration of GLP on an ascorbate/Fe^{2+}-, ADP/Fe^{3+}- and CCl_4/NADPH-induced lipidperoxidation in rat liver microsomal suspension.

tion. We also examined the inhibition of lipidperoxidation of glycyrrhizin, 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid. These results show that, 18β-glycyrrhetinic acid inhibits the formation lipidperoxides at concentration of 1.0 mg/mL, but glycyrrhizin and 18α-glycyrrhetinic acid had no effect on the inhibition of lipidperoxidation.

Fig. 1 shows when the microsome treated with ADP + $FeCl_3$ + NADPH and GLP, the formation of MDA was progressively decreased to a greater exert by raising the GLP concentration.

4. Effect of CCl_4/NADPH-induced LPO on GLP

The effect of lipidperoxidation by the rat liver microsome on GLP was showned in Table 4. This result at a dose concentration of 0.5 mg/mL and 1.0 mg/mL, was that the lipidperoxidation was inhibited 11.1%, 39.4%, respectively. BHT significantly inhibited peroxide formation to a greater exert than Vit E. Also 18β-glycyrrhetinic acid inhibited the lipidperoxidation to the extent of 36.5% of the control, while 18α-glycyrrhetinic acid showed minus level of inhibition.

The dose-response of the inhibition of lipidperoxidation on GLP is shown in Fig. 1. The MDA formation of GLP was progressively decreased by raising the GLP concentration.

5. Hydroxy radical scavenging activity

Table 5 demonstrates that GLP significantly reduced the degree of 2-deoxy-ribose degradation in a dose dependent manner. This result, it was scavenged the ·OH radicals generated from attack the 2-deoxy-ribose by inducers and prevented from a series of reactions that eventually result in the reduction of the formation of MDA. This result concerned that exihibited was not only hydroxy radical scavenging by GLP, also it may be the formation of Fe-GLP complex inhibited the Fenton reaction.

Discussion

The present study has demonstrated that GLP effectively inhibited lipidperoxidation in the microsome and reduced the

Table 3. The effect of GLP and various material on an ADP/Fe^{3+}-induced lipidperoxidation in rat liver microsomes

Sample	Concentration (mg/mL)	Malondialdehyde (MDA, nM/mL)	Percent inhibition (P.I., %)
Normal	-	4.9±0.4	-
Control	-	48.7±0.6	-
GLP	0.5	40.3±0.2*	19.2
	1.0	14.8±0.1	77.4
BHT	0.5	6.6±0.3*	96.1
	1.0	5.3±0.1	99.1
Vitamin E	0.5	10.5±0.4	87.2
	1.0	7.5±0.2*	94.1
Glycyrrhizin	1.0	55.8±0.6*	−16.2
18α-Glycyrrhetinic acid	1.0	55.6±1.2**	−11.2
18β-Glycyrrhetinic acid	1.0	37.8±0.5**	24.9

The incubation mixture consisting of 1.0 mL was composed of a sample in DW, 83.5 mM KCl, 37.2 mM Tris-HCl buffer (pH 8.0), 1 mM ADP, 10 μM $FeCl_3 \cdot 7H_2O$, 0.2 mM NADPH, Microsomal suspension (protein, 2 mg). After incubation at 37℃ for 20 minutes with ADP/Fe^{3+}, the lipid peroxide was measured by the method of Ohkawa et al. (1979). Results are expressed as the mean±S.D. of three experiments. * Significantly different from the control, $p<0.01$; ** $p<0.05$.

Table 4. The effect of GLP and various material on an CCl_4/NADPH-induced lipidperoxidation in rat liver microsomes

Sample	Concentration (mg/mL)	Malondialdehyde (MDA, nM/mL)	Percent inhibition (P.I., %)
Normal	-	4.3±0.2	-
Control	-	25.1±0.5	-
GLP	0.4	22.8±0.2	11.1
	1.0	16.9±0.3**	39.4
BHT	1.0	14.0±0.4*	53.4
Vitamin E	1.0	18.6±0.7	31.3
Glycyrrhizin	1.0	22.6±0.4**	12.0
18α-Glycyrrhetinic acid	1.0	27.9±0.6	−13.5
18β-Glycyrrhetinic acid	1.0	17.5±0.4**	36.5

The incubation mixture consisting of 1.0 mL was composed of a sample in DW, 83.5 mM KCl, 37.2 mM Tris-HCl buffer (pH 8.0), 1 mM ADP, 10 μM $FeCl_3 \cdot 7H_2O$, 0.2 mM NADPH, Microsomal suspension (protein, 2 mg). After incubation at 37℃ for 20 minutes with CCl_4/NADPH, the lipid peroxide was measured by the method of Ohkawa et al. (1979). Results are expressed as the mean±S.D. of three experiments. * Significantly different from the control, $p<0.01$; ** $p<0.05$.

Table 5. The hydroxy radical scavenging activity of GLP

Sample	Concentration (μg/mL)	Malondialdehyde (MDA, nM/mL)	Percent inhibition (P.I., %)
Normal	-	8.6±0.6	-
Control	-	38.4±0.8	-
GLP	50	30.1±1.3**	27.9
	200	27.0±0.3*	28.3
	600	22.6±0.6*	53.0
	1,000	19.0±2.4*	66.0
Mannitol	1,000	19.3±0.2*	64.1
Glutathione	1,000	23.4±1.1*	50.3

The reaction mixtures contained 20 mM KH_2PO_4-KOH buffer (pH 7.4), deoxyribose 3.25 mM, ferric chloride 100 μM, EDTA 100 μM, ascorbic acid 100 μM and H_2O_2 1 mM were incubated at 37℃ for 1 hour, and the reaction was then stopped with 5.0% TCA. The amounts of thiobarbituric acid-reactive material formed was expressed as malondialdehyde equivalents using 1,1,3,3-tetramethoxy-propane (TMP) as standard. Results are expressed as the mean±S.D. of three experiments.
* Significantly different from the control, $p<0.01$; ** $p<0.05$.

cytotoxicity of rat primary cultured hepatocytes. Kiso et al. indicated that ascorbate + Fe^{2+}, ADP + Fe^{3+} and CCl_4 + NADPH caused elevation of the lipid peroxide level in a rat liver microsome. lipidperoxidation by liver microsome can be classified into enzymatic reaction by NADPH-cytochrome P-450 reductase and NADPH (ADP + Fe^{3+}) and non-enzymatic reaction catalyzed by ascorbate + Fe^{2+}. lipidperoxidation damage is involved in aging, and pathological disorders. Some aspects of atherosclerosis, neuronal ceroid lipofuscinosis, intermittent claudication, oxygen toxicity, and liver injury caused by orotic acid, ethanol, phosphorus, or chlorinated hydrocarbons have been discussed in relation to lipidperoxidation. In an enzymatic reaction, at a dose of 1 mg/mL GLP exihibited 77.4% and 39.4% anti lipidperoxidation effects, and in a non-enzymatic reaction exihibited 97.7% inhibition. And GLP caused a concentration dependent inhibition of the lipidperoxide formation. Although GLP was not that much more potent than BHT, the anti-lipidperoxidation effects of GLP were similiar to those of vitamin E. These studies demonstrate that GLP obtained from the mycelia of liquid-cultured *Ganoderma lucidum* can be inhibited the formation of lipidperoxidation induced by an enzymatic and nonenzymatic reaction. The lipidperoxidation of glycyrrhizin and its partly hydrolysates were examined in the presence of liver microsomes. These results showed that glycyrrhizin has no affect on anti lipidperoxidation, but 18β-glycyrrhetinic acid exhibited a significant inhibition. Kiso et al. (1984) demonstrated the relationship between the bio-

logical action and conformation of structure. That the structure and component of GLP was important as an expression of anti-lipidperoxidation activity was demonstrated by these results.

The results suggest that the action of the cytochrome P-450 enzyme system may be expressed differently by GLP. Further studies on the enzyme system would clarify this hypothesis.

It is well known that CCl_4-induced cytotoxicity is thought to stem from the conversion of CCl_4 to CCl_3 radicals ($\cdot CCl_3$, $\cdot OOCl_3$) by the cytochrome P-450 mixed function oxidase system in the smooth endoplasmic reticulum (Slater & Sayer 1971; Recknagel RO 1973; Albino et al. 1982).

The antioxidants BHT and the polysaccharide GLP were found to have a lower protective effect on lipidperoxidation induced by CCl_4/NADPH than in the ascorbate/Fe^{2+} and ADP/Fe^{3+} experimental groups. According to Zhang et al. (2002) and Huang et al. (2016), polysaccharides obtained from mushroom were found to have high liver protection against CCl_4-induced hepatic injury in mice. These results are considered to be the result of one difference between rat liver microsomal suspension and metabolism *in vivo*.

Galactosamine-intoxicated cytotoxicity inhibits the synthesis of DNA, RNA and finally results in the death of the hepatocytes.

Based on the above reports and experimental results, the hepatoprotective effect on GLP using primary cultured hepatocytes induced by CCl_4 and galactosamine was examined. GLP effectively protected the functional disorder of the primary cultured rat hepacytes by CCl_4 and galactosamine-intoxicated. Also, the hydroxy radical scavenger suggested that a number of therapeutically used, such as allopurinol, amygdalin and antiinflamatory drugs, may exert the scavenging activity of hydroxy radicals. The increase of hydroxy radicals by inducers was markedly reduced by the pretreatment with GLP and exhibited preventive activity from hydroxy radicals.

In conclusion, the results of the present investigation suggest that a radical scavenging action or a prevention of lipidperoxidation may be related to the liver protective effect of GLP.

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