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The Protection of *Anthrodia camphorata* against Acute Hepatotoxicity of Alcohol in Rats

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ABSTRACT

Anthrodia camphorata is a unique mushroom of Taiwan and has been used as a folk medicine for protection against liver damage induced by alcohol intoxication. However, no report has been presented in this respect. In this rat study, we examined whether the mycelium and sporocarp of *Anthrodia camphorata* protect against acute liver damage induced by ethanol (EtOH). Rats were orally administered with mycelium and sporocarp of *Anthrodia camphorata* for 9 days before EtOH challenge (5.5 g/kg body wt., i.p.). Rats were divided into eight groups (A-H) and except for groups A and H, all rats were injected with alcohol. A: Control; B: EtOH control; C: Silymarin (250 mg/kg bw., p.o.); D: 0.5 g mycelium/kg; E: 1.0 g mycelium/kg; F: 0.5 g sporocarp/kg; G: 1.0 g sporocarp/kg; and H: 1.0 g mycelium/kg. The results showed that EtOH administration markedly increased the activities of glutamate-pyruvate aminotransferase (GPT) and glutamate-oxaloacetate aminotransferase (GOT). Both mycelium and sporocarp of *Anthrodia camphorata* significantly decreased the activity of GOT and GPT, but the effects were not dose-dependent. Mycelium and sporocarp of *Anthrodia camphorata* also significantly and dose-dependently decreased lipid peroxidation (measured as TBARS) induced by EtOH. EtOH treatment significantly increased the activities of hepatic superoxide dismutase (SOD) and catalase, but did not significantly affect the activity of glutathione peroxidase. Pre-treatment with either the mycelium or the sporocarp completely prevented the rise in the activity of SOD and catalase. The histopathological examination revealed that both mycelium and sporocarp markedly protected against lipid vacuole accumulation and hydropic degeneration of hepatocytes induced by EtOH. Thus, the present results demonstrated that both mycelium and sporocarp of *Anthrodia camphorata* protect against acute liver damage induced by EtOH. In addition, rats fed 1.0 g mycelium without EtOH treatment produced no observable toxicity during the experimental period.

Key words: alcohol toxicity, *Anthrodia camphorata*, hepatoprotection, oxidative damage

INTRODUCTION

Liver is an important organ for detoxification and metabolism, and it has a good repairing capability. Common damage caused by non-persistent or mild toxicity can be repaired through certain mechanisms. However, liver damage induced by persistent alcohol overdose or virus attack may cause chronic hepatitis, cirrhosis, and even hepatoma to death. In recent years, the death rate of male hepatoma is the highest among the ten major causes of death by cancer in Taiwan⁽¹⁾. Reports have described that liver damage caused by alcohol increased with the dosage, and the severity of damage is different among gender, species, and genes. For example, about 50% of Orientals lack the important enzyme for alcohol metabolism, aldehyde dehydrogenase. Therefore, their tolerance of alcohol is poor and liver damage occurs easily⁽²⁻⁴⁾.

Anthrodia camphorate, named *A. cinnamomea* previously, is much different from the common *Ganoderma* species because it has a strong camphor-like aroma. Its shape is like a plate or a bell. It only grows on the inner walls of hollow *Cinnamomum kanehirae* hay woods in mountain areas between altitudes 200 to 2000 m. *Cinnamomum kanehirae* hay is an evergreen broadleaf

arbor with a heavy aroma and it could be used as a good anti-insect material. Fungi cannot grow on it except *Anthrodia camphorate* which is then treated as a treasure in the countryside. For a long time, the natives in Taiwan have found that liver damage caused by alcohol overdose can be cured by drinking *Anthrodia camphorate* tea or holding a small piece of it in the mouth. In addition, the capability of detoxification, anti-cancer, alcohol relief, and anti-inflammatory is often heard⁽⁷⁾. Therefore, it has become the most expensive wild fungus in the market, and it will be one of the most important health foods. Gao has reported studies of triterpene compound B isolated from *Anthrodia camphorate*⁽⁸⁾. It was found that such compound can reduce blood GPT level in mice which have acute liver abnormality induced by tetrachloride methane. Chen reported that the methanol extract from *Anthrodia camphorate* has 30% of triterpoids which is much higher than the extract from common *Ganoderma* species (3%)⁽⁹⁾. *Anthrodia camphorate* tastes more bitter than *Ganoderma*, possibly due to the richness of sterols and triterpoids of multiple oxidized types. According to a report by Huang and colleagues, the antioxidative capability of *Anthrodia camphorate* is as good as BHA (butylated hydroxyanisole)⁽¹⁰⁾. Therefore, it is important to study whether *Anthrodia camphorate* has the same liver protection capability

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ty. However, studies related to liver-protective effects of *Antrodia camphorata* against ethanol induced hepatitis are few. The purpose of this research was to study the protective effects of mycelium and sporocarp of *Antrodia camphorata* against acute liver damage induced by alcohol.

MATERIALS AND METHODS

I. Material

1. The mycelium was cultured from *Antrodia camphorata* CCRC 35398, which was purchased from the Food Industry Research and Development Institute, at Grape King Biotechnology Research Center using potato dextrose agar (PDA) followed by a deep fermentation method. The sporocarp of wild *Antrodia camphorata* collected from mountain areas in Taiwan was provided by Grape King Biotechnology Research Center. Its polysaccharide contents have been determined as 1.7% in mycelium and 6 to 8% in sporocarp. Both mycelium and sporocarp were grounded to powder and then prepared with saline to different concentrations of suspension for oral administration to rats.

2. Silymarin, the extract of *Silybum marianum* purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA), was used as the positive control in this research. Its main active ingredient is polyphenol silibinin, which is considered as an effective liver-protective agent because it has protective effect on liver damage induced by many drugs such as tetrachloride methane and acetaminophen. It has been used as a liver protecting agent in the US and Europe for 20 to 30 years. It is also often used as controls for comparisons in research. Therefore, silymarin was used as the drug control in our research. The dose for rats is usually 200 to 400 mg/Kg⁽¹¹⁻¹³⁾.

II. Experimental Design

1. Animals: Experimental animals in this research were S.D. white male rats purchased from the National Laboratory Animal Center weighing 180 to 200 g. The feeding condition was ad libitum using Lab 5001 standard diet (Purina Mills, St. Louis, MO) and deionized water. Room temperature was set at $22 \pm 2^\circ\text{C}$. Humidity was set at $65 \pm 5\%$. Light exposure and dark were both set at 12 hr.

2. In this research, high and low doses were used according to the instruction in the dried product of fermented mycelium (*Antrodia camphorata* King) produced by the Grape King Corporation. It is 3 to 5 pills (about 0.42 g per pill) each time and 3 times per day, that is, 9 to 15 pills daily. Therefore, 9 pills were used as the low dose and 15 pills as the high dose. Based on body surface areas (14), it is about 0.34 g/Kg (low dose) and 0.57 g/Kg (high dose) daily for rats. To calculate conveniently, about 1.5 to 1.7 times of dosage was used in this research, that is, 0.5 g/Kg (low dose) and 1.0 g/Kg (high dose).

3. Grouping: Rats were administered (p.o.) with mycelium or sporocarp of *Antrodia camphorata* for 9 consecutive days at 9 a.m. There were 8 groups with 7 rats in each group: Group A (control) was administered with the same amount of saline instead of *Antrodia camphorata*; Group B (liver damaged) was administered with the same amount of saline instead of *Antrodia camphorata*; Group C (positive control) was administered with silymarin (200 mg/Kg) to replace *Antrodia camphorata*; Group D was administered with low dose (0.5 g/Kg) of mycelium of *Antrodia camphorata*; Group E was administered with high dose (1 g/Kg) of mycelium of *Antrodia camphorata*; Group F was administered with low dose (0.5 g/Kg) of sporocarp of *Antrodia camphorata*; Group G was administered with high dose (1 g/Kg) of sporocarp of *Antrodia camphorata*; Group H was administered with high dose (1 g/Kg) of mycelium of *Antrodia camphorata*.

4. On the 9th day, 6 hr after administration (3 p.m.), ethanol was injected to the abdominal cavity of rats (Saline was injected instead of ethanol in Group A and H.). The design of ethanol injection such as dosage and timing was adapted from the method described by Zhang et al.⁽¹⁵⁾. That is, a 20% ethanol solution was used for injection (5.5 g/Kg). Eighteen hr after ethanol injection, rats were put unconscious using ethyl ether and blood samples for biochemical analysis were then taken from carotid. In addition, liver samples were taken for determination of lipid peroxidation, analysis of protein carbonyl groups, determination of antioxidant enzymes, and histopathological examination.

III. Analytical Methods

(I) The absolute and relative weight change of rat liver and kidney

Absolute weights are actual weights of rat liver and kidney. Relative weights are weights of rat liver and kidney relative to 100 g weight of rat.

(II) Determination of serum biochemical indexes

Blood samples taken from tail and neck of rats were centrifuged at $3000 \times g$ per minute for 10 min to obtain serum samples. The levels of GOT (glutamate-pyruvate aminotransferase), GPT (glutamate-oxaloacetate aminotransferase), and serum glucose were determined by automatic biochemical analyzer (Roche COBAS, Mira Plus). The standard method from IFCC^(16, 17) was used for determination.

(III) Determination of liver protein contents

Bio-Rad Protein Assay kits were used for this analysis. The Coomassie Brilliant blue reagent formed a blue complex with protein, then the absorbance at 590 nm was measured by a Hitachi U-2000 Spectrophotometer. Bovine

serum albumin was used as standards.

(IV) Determination of liver lipid peroxidation

According to the method by Buege and Aust⁽¹⁸⁾, a colorimetric method that measures the reaction product of thiobarbituric acid (TBA) with aldehydes such as malondialdehyde (MDA) formed by lipid peroxidation was used for this analysis. The value was named TBARS. Liver tissues were homogenized with 9 volumes of EDTA solution, and 10.5 μL of 50 mM BHT was added to 1 mL of this mixture to avoid the interference caused by MDA generated by strong acid and heat during this analytical process. For precipitating the protein, 2 mL of 7.5% TCA was added followed by ice bathing for 5 min. After centrifugation, 1 mL of 0.7% TBA was added to 2 mL of supernatant followed by heating in boiling water for 10 min. After cooling, equal amount of n-butanol was added to extract MDA followed by centrifugation at 10,000 $\times g$ for 10 min. Finally, a fluorescence spectrophotometer (Ex: 515 nm; Em: 555 nm) was used for the quantitative analysis. MDA generated by 1,1,3,3-tetramethoxypropane (TMP) with 1 N H_2SO_4 was used as standards.

(V) Analysis of liver protein carbonyl groups

According to the method by Reznick and colleagues⁽¹⁹⁾, DNPH was used as the reagent for this analysis. One hundred μL of homogenized solution from liver tissues was mixed with 0.5 mL of 10 mM DNPH (in 2 N HCl) then incubated at room temperature in dark for 1 hr with shaking once every 15 min. After adding 0.6 mL of 20% TCA, this mixture was centrifuged at 10,000 $\times g$ for 10 min to precipitate protein. After discarding the supernatant, the precipitate was washed 3 times with ethanol-ethyl acetate (1:1, v/v) to remove the residual DNPH. The precipitate was reconstituted with 1 mL of guanidine-HCl (pH 2.3) then incubated in water bath at 37°C for 1 hr. After centrifuged at 12,000 $\times g$ for 15 min, the absorbance at 370 nm was measured using a Hitachi U-2000 spectrophotometer. E_{370} (molar coefficient) = $2.2 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ was used to calculate the content of protein carbonyl groups. The result was expressed as nmoles carbonyl groups/g liver.

(VI) Determination of liver antioxidant enzymes

1. Catalase

According to the method by Cohen and colleagues⁽²⁰⁾, 25 μL of homogenized solution from liver tissues was mixed with 975 μL of 6 mM H_2O_2 /50mM potassium phosphate, pH 7.0. The decrease of $A_{240\text{nm}}$ in 2 min was recorded. The enzyme activity was calculated by $E_{240} = 43.6 \text{ M}^{-1}\text{cm}^{-1}$.

2. Superoxide dismutase (SOD)

According to the method by Marklund and Marklund⁽²¹⁾, 10 μL of homogenized solution from liver tissues was mixed with 965 μL of 100 mM Tris-HCl containing 2 mM diethylenetriaminepentaacetic acid, pH 8.2 and 25 μL of 8 mM pyrogallol (in 3 mM HCl). The change of $A_{412\text{nm}}$ in 3 min was recorded. Deionized water was used as blank. The activity of 1 unit SOD was defined as 50% of inhibited reaction.

3. GSH peroxidase (GSH-Px)

According to the method by Lawrence and Burk⁽²²⁾, 25 μL of homogenized solution from liver tissues was mixed with 925 μL of reaction solution (1 mM EDTA, 1 mM NaN_3 , 1 U/ml GSH-Rd, 1 mM GSH, and 100 mM potassium phosphate, pH 7.4) and 25 μL of 6 mM β -NADPH (in 0.5% NaHCO_3). Finally, 25 μL of 10 mM H_2O_2 was added to initiate the reaction. The change of $A_{340\text{nm}}$ in 3 min was measured at 37°C. The decrease of β -NADPH was used to calculate the activity of this enzyme ($E_{340} = 6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$).

(VII) Histopathological examination

A small piece of liver was cut from the same location on a liver lobe in 10% formalin then put in an embedding box. After dehydration overnight, this piece was embedded with paraffin at -20°C . A 5 μm thick section was sliced then put in an oven to dry and fix the paraffin. Finally, the section was stained with Haematoxylin-Eosin followed by microscopic examination.

(VIII) Statistical analysis

The experimental data were indicated by mean \pm S.D., and were analyzed by an analysis of variance using ANOVA (SAS). When the F value is significant, the significance of difference between groups was determined by Duncan's test. Significant difference was assumed for $p < 0.05$.

RESULTS AND DISCUSSION

I. The Absolute and Relative Weights of Rat Liver and Kidney

The increase of absolute and relative organ weights is a sensitive parameter for toxicity, but it is probably a reaction of organ to adapt to the toxic substance. Most substances that damage the liver will change the structure of smooth endoplasmic reticulum, decrease the content of cellular pigments, increase the content of saturated acyl side chains, and cause the hyperplasia and fibrosis of collagen. Finally, the weight increases⁽²³⁾. In this research, the absolute weights of liver and kidney tissues were not significantly different among all groups (Table 1). The relative

weights also were not significantly different. These results showed that no significant adaptation or damage occurred under the damage induced by acute ethanol intoxication.

II. Serum Biochemical Indexes

When the liver cell is damaged, the GOT and GPT in liver will be released to serum. Therefore, levels of GOT and GPT are the most commonly used biochemical indexes for evaluating the damage of liver⁽²⁴⁾. According to the biochemical indexes (Figure 1), the administration of ethanol caused the GOT and GPT of liver-damaged group (Group B) 233% and 370% higher than control group (Group A), respectively ($p < 0.01$). Compared with Group B, all experimental groups (Group C to G) had markedly decreased GOT and GPT ($p < 0.05$). However, there was no significant difference among Group C to G. On GOT, high dose of mycelium and sporocarp was better than low dose, but the difference was not significant. On GPT, there was no difference between high and low doses. GOT and GPT of the group administered only with high dose of mycelium of *Antrodia camphorate* (Group H) were not increased, indicating that the mycelium is not toxic to liver.

Insulin secretion induced by glucose is inhibited during the metabolism of ethanol, and subsequently causes the disorder of glucose metabolism such as decrease of glucose tolerance. To examine the effect of *Antrodia camphorate* against the disorder of glucose metabolism induced by ethanol, the serum glucose from all groups was measured. Results showed that serum glucose increased markedly in the ethanol injected group (Group B or liver damaged group). Both mycelium and sporocarp of *Antrodia camphorate* can significantly inhibit the increase of glucose, but there was no difference among doses. These results proved that ethanol causes the disorder of glucose metabolism, and demonstrated that low dose of mycelium and sporocarp of *Antrodia camphorate* can significantly prevent the disorder of glucose metabolism.

III. Liver Lipid Peroxidation

Many reports have described that lipid peroxidation of liver cell membrane caused by reactive oxygen species (ROS) is the main reason of liver damage induced by ethanol⁽²⁵⁻³⁴⁾. Tuma et al. have shown that rat liver produced large amounts of MDA and acetaldehyde after oral administration with ethanol⁽³⁵⁾. In this research, liver TBARS increased markedly after rats were injected with ethanol (Figure 3-1). However, orally administration with either high dose or low dose of mycelium and sporocarp of *Antrodia camphorate* can completely inhibit lipid peroxidation induced by ethanol. This effect may be due to the clearance of free radicals produced during the metabolism of ethanol by the antioxidative material in *Antrodia camphorate*. In addition, the liver TBARS of rats in Group H (the group administered only with high dose of mycelium of *Antrodia camphorate*) did not increase, indicating that

mycelium itself does not cause lipid peroxidation.

IV. Liver Protein Carbonyl Groups

In addition to lipid peroxidation, active oxygen

Table 1. Effects of mycelium and sporocarp of *Antrodia camphorate* on organ weights of rats injected with alcohol¹

Group	Organ absolute weights (g)	
	Liver	Kidney
A: control (normal saline)	10.5 ± 1.6	2.6 ± 0.24
B: alcohol control	10.6 ± 1.1	2.4 ± 0.21
C: silymarin (200mg/kg)	10.8 ± 0.8	2.4 ± 0.17
D: mycelium (0.5g/kg)	11.4 ± 1.2	2.5 ± 0.19
E: mycelium (1.0g/kg)	10.9 ± 1.4	2.5 ± 0.25
F: sporocarp(0.5g/kg)	11.7 ± 1.4	2.6 ± 0.25
G: sporocarp (1.0g/kg)	11.3 ± 1.3	2.5 ± 0.2
H: mycelium (1.0g/kg)	10.3 ± 1.6	2.4 ± 0.3

¹ Rats were orally administered with the mycelium or sporocarp of *Antrodia camphorate* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). Rats except groups A and H were injected with alcohol. Values are means ± SD of 7 rats. No significant differences in either liver or kidney weights existed among the various groups ($p < 0.05$).

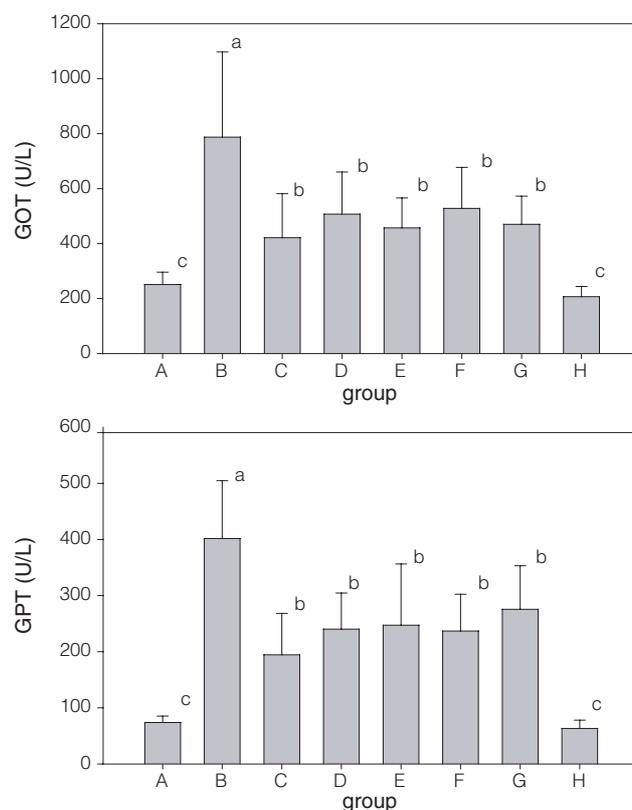


Figure 1. Serum levels of GOT and GPT in rats orally administered with the mycelium or sporocarp of *Antrodia camphorate* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). Rats except groups A and H were injected with alcohol. A: control (saline, p.o.); B: alcohol control; C: silymarin (200 mg/kg, p.o.); D: mycelium (0.5 g/kg); E: mycelium (1 g/kg); F: sporocarp (0.5 g/kg); G: sporocarp (1.0 g/kg); H: mycelium (1.0 g/kg) control. Values (means ± SD of 7 rats) not sharing a common superscript are significantly different ($p < 0.05$).

molecules induced by ethanol will cause protein oxidation⁽³⁶⁾. According to previous clinical reports, the contents of lipid peroxidation products (such as conjugated dienes, 4-hydroxynonenal, MDA, and F₂-isoprostanes) and protein carbonyl groups (an index of oxidative damage to protein) in the liver affected by alcoholic liver diseases are higher than normal individuals⁽³⁷⁻⁴⁰⁾. Additional reports indicated that the lipid peroxidation products, 4-hydroxynonenal and MDA, cause modifications to proteins and the production of protein adducts^(41, 42). Our research also showed that injection of ethanol to rats markedly increased protein carbonyl groups (Figure 3-2). Although carbonyl contents of Group C to G were all lower than Group B (the liver-damaged group), only those of the group administered with high dose of mycelium of *Antrodia camphorata* (Group E) were significantly different. There were no differences between control group (Group A) and the group administered with high dose of mycelium of *Antrodia camphorata* without injection of ethanol (Group H). These results show that mycelium of *Antrodia camphorata* has a better effect to inhibit protein damage induced by ethanol than sporocarp. Meanwhile, high doses of mycelium of *Antrodia camphorata* does not cause protein damage.

V. Changes of Liver Antioxidant Enzymes

Catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) are the major antioxidant enzymes in liver. Previous articles reported that alcoholic liver damage induces the expression of SOD^(35, 43). It may be a feedback reaction of liver cells under oxidative pressure. Our research also proved that the Cu/ZnSOD activity increased markedly in the liver damaged group (Group B) (Figure 4-

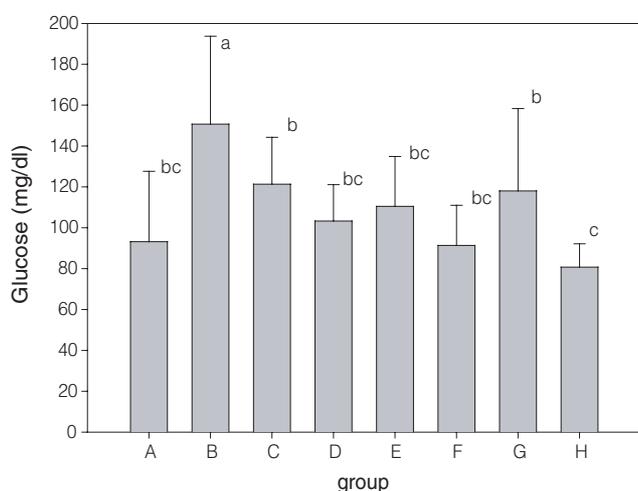


Figure 2. Serum levels of glucose in rats orally administered with the mycelium or sporocarp of *Antrodia camphorata* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). Rats except groups A and H were injected with alcohol. A: control (saline, p.o.); B: alcohol control; C: silymarin (200 mg/kg, p.o.); D: mycelium (0.5 g/kg); E: mycelium (1 g/kg); F: sporocarp (0.5 g/kg); G: sporocarp (1.0 g/kg); H: mycelium (1.0 g/kg) control. Values (means \pm SD of 7 rats) not sharing a common superscript are significantly different ($p < 0.05$).

1). Silymarin as well as mycelium and sporocarp of *Antrodia camphorata* resumed the SOD activity, that is, they completely inhibited the increase of SOD. Therefore, the antioxidative capability in all experimental groups completely inhibited the SOD induced by ethanol, which may be the reason why the inhibitory effect of mycelium and sporocarp of *Antrodia camphorata* was not dose-dependent.

When the SOD activity is actively expressed, superoxide anion will transform to H₂O₂ and additionally activate the expression of catalase activity. Our research also showed that ethanol injection increased the catalase activity markedly (Group B). Administration of silymarin as well as mycelium and sporocarp of *Antrodia camphorata* inhibited the increase of catalase activity, but only Group E and F were significantly different from Group B (the liver-damaged group). However, groups administered with high or low dose of mycelium and sporocarp were not significantly different from control group (Group A) (Figure 4-2), indicating that the antioxidative capability in all experimental groups has completely inhibited catalase activity

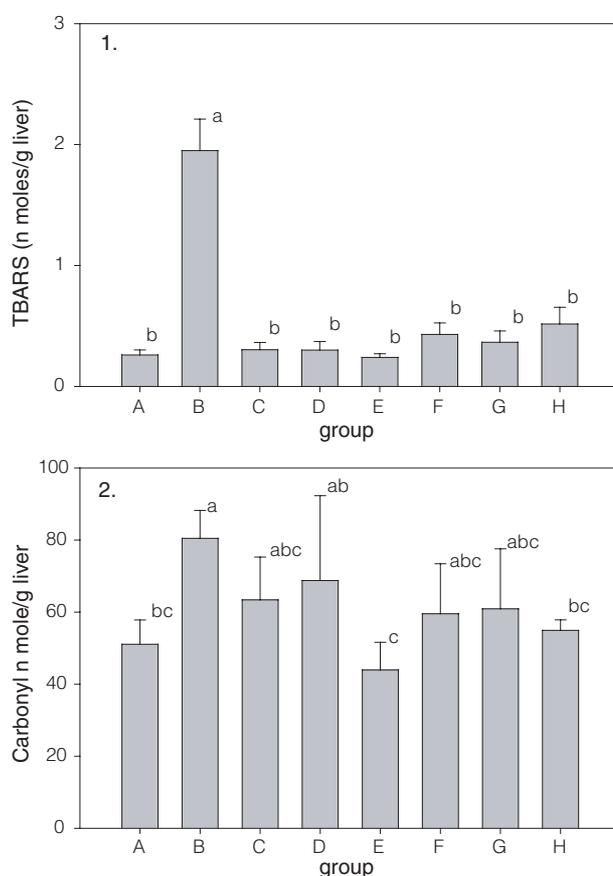


Figure 3. Hepatic levels of thiobarbituric acid-reactive substances and protein carbonyls in rats orally administered with the mycelium or sporocarp of *Antrodia camphorata* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). Rats except groups A and H were injected with alcohol. A: control (saline, p.o.); B: alcohol control; C: silymarin (200 mg/kg, p.o.); D: mycelium (0.5 g/kg); E: mycelium (1 g/kg); F: sporocarp (0.5 g/kg); G: sporocarp (1.0 g/kg); H: mycelium (1.0 g/kg) control. Values (means \pm SD of 7 rats) not sharing a common superscript are significantly different ($p < 0.05$).

induced by ethanol. On the activity of GSH-Px, the injection of ethanol significantly decreased the activity in Group B, but the effect was not significant (Figure 4-3). This result is consistent with the result reported by Jarvelainen et al.⁽⁴⁴⁾. The probable reason is that the H₂O₂ produced during ethanol metabolism is cleared by catalase, so that H₂O₂ has less effect on GSH-Px. This can also explain why the GSH-Px activity is not significantly different among all groups. Based on results from these antioxidant enzymes, *Antrodia camphorata* can inhibit SOD and catalase but not GSH-Px activities induced by ethanol.

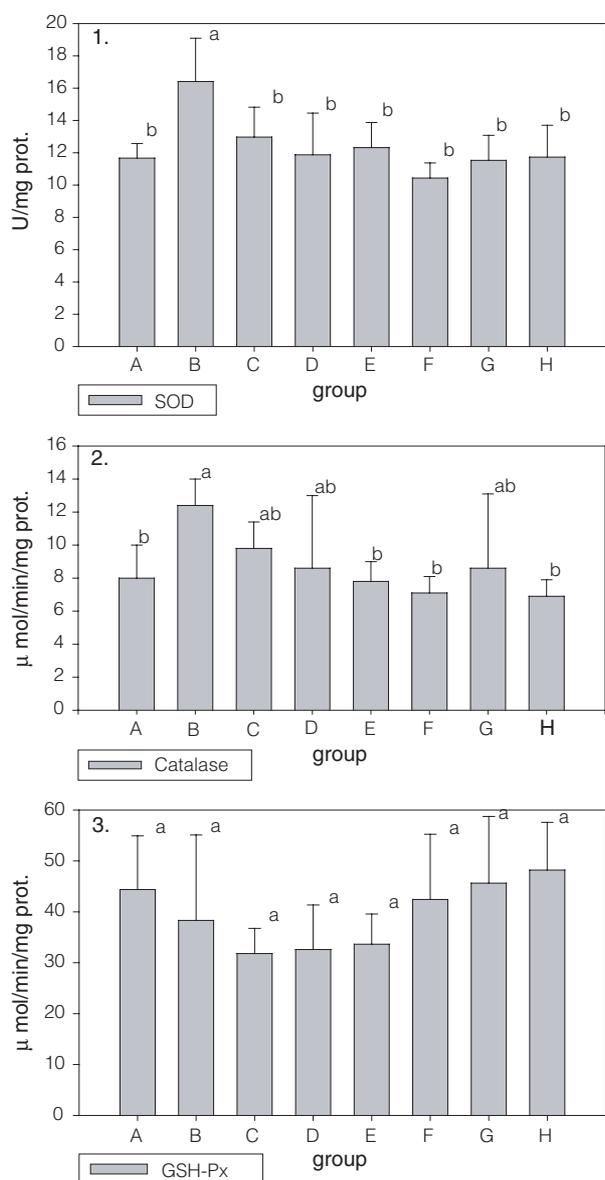


Figure 4. Hepatic levels of antioxidant enzymes (catalase, SOD, GSH-Px) in rats orally administered with the mycelium or sporocarp of *Antrodia camphorata* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). Rats except groups A and H were injected with alcohol. A: control (saline, p.o.); B: alcohol control; C: silymarin (200 mg/kg, p.o.); D: mycelium (0.5 g/kg); E: mycelium (1 g/kg); F: sporocarp (0.5 g/kg); G: sporocarp (1.0 g/kg); H: mycelium (1.0 g/kg) control. Values (means \pm SD of 7 rats) not sharing a common superscript are significantly different ($p < 0.05$).

VI. Histopathological Examination

As shown in Figure 5-1, the cell and cell plate from liver tissue sample of the control group (Group A) have intact structure, and the boundary between cells is clear. Structures inside the cells are clean without impurities and droplets. Both cell plate and sinusoid are centripetal from the central vein. Infiltration of inflammatory cells does not exist in the central venous area. However, the ethanol-damaged group (Group B) has obvious pathological structure changes. Figure 5-2 shows that cells near the central venous area are full of ballooning degeneration and fatty droplets, and look like shiny droplet. These results are consistent with findings reported by Korsrud et al.⁽⁴⁵⁾. In addition, most boundaries between cells are blurred and some even disappeared to become homogenized. Inflammatory reaction of lymphoid infiltration was observed in the central venous area. Hyperplasia of Kupffer cells, metaplasia of liver plate structures, and discontinuousness of sinusoid structures are also observed. Inner space is full of cell debris and waste. The nuclei of some liver cells are swelled. Multiple nuclei and over-stain are also observed. Some cells are necrotic (red area).

Significant liver-protective effect was found in the silymarin group (Group C, Figure 5-3). Its liver plate and cell structure were intact, and the boundary between cells was clear. The only pathological change was that shiny small fatty droplets were still visible in liver cells. The group administered with high dose of mycelium of *Antrodia camphorata* (Group E, Figure 5-4) also showed some recovery effects. The cell fusion condition was improved, and the amount of small fatty droplets decreased. The group administered with high doses of sporocarp of *Antrodia camphorata* (Group G, Figure 5-5) gave similar results. Although the recovery effects of all groups administered with *Antrodia camphorata* were not as good as the group administered with silymarin, the liver damage induced by ethanol improved markedly and the effect was dose-dependent (data not shown). The liver structure of the group administered only with high doses of mycelium (Group H, Figure 5-6) was normal, indicating that mycelium of *Antrodia camphorata* does not cause liver damage.

CONCLUSION

The results of serum GOT, GPT, and glucose, liver TBARS, SOD, catalase, and protein carbonyl groups, and histopathological examination illustrated that mycelium and sporocarp of *Antrodia camphorata* have good liver-protective effects under the mode of single ethanol injection. Presumably, these liver-protective effects are related to its antioxidative reactions, though the active ingredient has not been identified. Further research related to the effects of mycelium and sporocarp of *Antrodia camphorata* on chronic alcoholic liver damage will be continued, so that the application of *Antrodia camphorata* can be expanded.

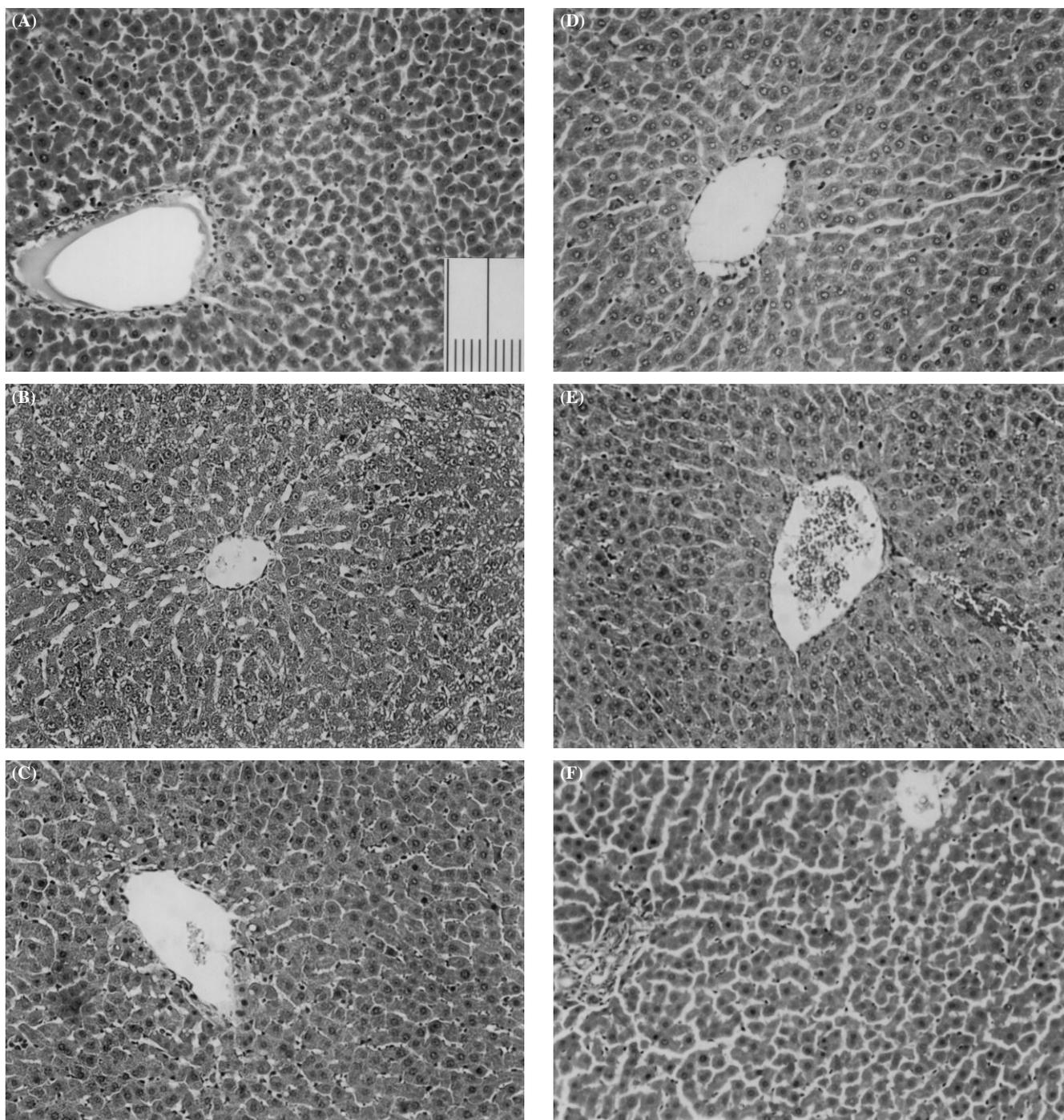


Figure 5. H & E stain under light microscope (200X) of liver tissues in rats orally administered with the mycelium or sporocarp of *Antrodia camphorata* for 9 days before injection (i.p.) with alcohol (5.5 g/kg body wt.). 1: Blank (saline, p.o., without alcohol, i.p.); 2: Alcohol control (saline, p.o., alcohol, i.p.); 3: Silymarin (200 mg/kg, p.o.) with alcohol, i.p.; 4: Mycelium (1 g/kg) with alcohol, i.p.; 5: Sporocarp (1.0 g/kg) with alcohol, i.p.; 6: Mycelium (1.0 g/kg, p.o.) without alcohol i.p.

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