Hepatoprotective Effects of Novel Cultivated *Antrodia cinnamomea*

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***Abstract*—*Antrodia cinnamomea* (AC) is a basidiomycete native only to Taiwan and the relatively rare natural source in the forest makes this fungus very expensive. In order to provide alternative sources of AC, a patent pending novel method for AC cultivation and a novel bioactive component extraction protocol were introduced in the present study. The hepatoprotective activity was tested to confirm the functionality of this novel cultivated AC. The results showed that the extract prepared from this AC could effectively protect against the liver damage caused by CCl_4.**

***Index Terms*—hepatoprotective effects, liver injury, Carbon tetrachloride, *Antrodia cinnamomea*, novel cultivation protocol**

I. Introduction

Antrodia cinnamomea (AC) is a basidiomycete native only to Taiwan and originally found growing in the rotting empty truck of *Cinnamomum kanehirai* Hay [1]. Being a local species, AC was historically used in Taiwan by the aborigines as a traditional prescription for the discomforts caused by alcohol drinking or exhaustion [2]-[4]. Since the fruiting body of this fungus is reported to be a remedy for alcohol-induced liver diseases, the relatively rare natural source in the forest makes this fungus very expensive. Therefore, various preparations including extraction solutions or dried powders from fruiting bodies and/or mycelium have been used for the demonstration of prevention/treatment of numerous diseases including liver diseases, food and drug intoxication, diarrhea, abdominal pain, hypertension, itchy skin and tumorigenic diseases based on the theory of antioxidant capability or the presence of bioactive compounds [5]-[9].

The major bioactive components in the fruiting bodies of AC are polysaccharides, steroids, triterpenoids and sesquiterpene lactone [2], [10]. However, due to the very slow growing rate in the nature environment, the availability of wild AC fruiting body is very limited.

In order to provide alternative sources of AC, many artificial cultivation methods including log-wood cultivation, submerged liquid culture and solid-state culture have been developed [11]-[15]. Culture conditions such as cultivation medium composition, temperature, pH, and time can significantly affect the concentrations of bioactive components. The resulting phytochemicals can also lead to different outcomes in beneficial or toxicological studies. Compared to the fruiting body of wild AC, the log-wood cultivation results in similar bioactive components but all other artificially cultivated methods exert marked difference. However, log-wood cultivation is usually takes about 2 to 3 years, while the cultivation periods for submerged liquid culture and solid-state culture are 7-14 days and 3-7 months, respectively. Novel cultivation methods which can produce AC with similar bioactive compound composition in a relatively short period time are urgently needed. In the present study, a patent pending novel method for *A. cinnamomea* cultivation and a novel bioactive component extraction protocol were introduced. Since the hepatoprotative capability is the most important functionality of all AC related products, the preliminary hepatoprotective activity was investigated to demonstrate the efficacy of this newly developed AC preparation.

II. Materials and Methods

A. Cultivation of Antrodia Cinnamomea

The cultivation medium consisted of corn core meal, rice bran, wheat bran and corn flour at ratio of 15.8: 2.6: 2.6: 1 (w/w). Magnesium sulfate (0.1%) and zinc sulfate (0.1%) were also included. Distilled water was mixed with the above mentioned materials to reach a moisture content of 53.94%. The mixture was packed into polypropylene bags (1.3 kg per bag). After sealed, polypropylene bags were sterilized at 120 ℃ for 2 h and then cooled to room temperature before inoculation.

The culture of *A. cinnamomea* (AC) was homogenized with sterile saline and inoculated into PP bags. The bags were incubated at 25℃ in the dark for three months. The plastic sheet of PP bag was removed and the surface was kept moist to facilitate the growth of fruiting body in the following 3-6 months until the fruiting body spread over the whole surface of growth medium. The fruiting body was scraped from the medium surface and the mycelium was grounded, dried to moisture content of 5%, and stored at 4℃ until further treatment. The fruiting body was homogenized with sterile normal saline and then sprayed onto the surface of *Cinnamomum kanehirai* log-wood. The log-wood was kept in dark with high relative humidity achieved by spraying sterile reverse osmosis

Manuscript received September 16, 2018; revised February 23, 2019.

doi: 10.18178/ijpmbs.8.2.65-70

water occasionally. After incubated for 3-6 months, the fruiting body of AC was scraped from the log-wood surface and dried to moisture content of 5%.

B. Preparation of Antrodia cinnamomea Extract

Dried mixture (mycelium and fruiting body) and 55% ethanol (1/10, w/w) were heated at 50℃ for 18 h with thoroughly mixing. The filtrate was concentrated at 45-55℃ under reduced pressure to obtain triterpenoid fraction. The residue and distilled water (1/10, w/w) were heated at 90℃ for 18 hr with stirring. The filtrate was concentrated at 45-55℃ under reduced pressure to obtain neutral fraction. The residue was further mixed with ten time of distilled water (w/w) and heated at 120℃ for 18 h. After filtration, the filtrate was concentrated under reduced pressure to obtain polysaccharide fraction. Triterpenoid fraction, neutral fraction and polysaccharide fraction were individually spray-dried. The spray-dried fractions were combined, ground, and sieved through 100 mesh screen to obtain AC preparation for further use.

C. Treatment of Animals for Hepatoprotective Effect Assay

Eighty seven-week-old male Sprague-Dawley rats (BioLASCO, Taiwan) were quarantined for 2 weeks and acclimated in polyethylene cages for at least 5 days prior to being randomly assigned to the control and treatment groups of 10 rats in each group. The animals had free access to standard rodent diet and sterile reverse osmosis water, and were maintained at controlled temperature (20-23℃), relative humidity (40-70%) and light cycle (12 h light/12 h dark). Body weight of all rats was measured prior to the administration of test sample. To induce liver damage, rats were gavaged with 20% CCl_4/olive oil (2 mL/kg BW) twice a week for eight consecutive weeks. To test the hepatoprotective effect of AC extract, the AC extract at specified dosage (206.7, 413.4 and 1033.5 mg/kg BW) was given once in each day for the eight consecutive weeks (Table I).

During the test period, the body weight of animal and the average feed intake were recorded weekly. Tail vein blood was sampled in weeks 1, 3 and 6. At the end of experiment, all animals were euthanatized with carbon dioxide and the blood sample was taken directly from animal heart. The serum from all blood samples was prepared by centrifugation (3000 x g, 4℃ and 15 min).

D. Hematological and Histological Analyses

The serum samples were analyzed by using an automatic serum analyzer (ADVIA 1800, Siemens). Aspartate aminotransferase and alanine aminotransferase of all blood samples was analyzed. For the blood sample collected when animals were sacrificed, serum triglyceride, cholesterol, total protein, albumin, and globulin were also analyzed.

E. The Biochemical Determinations of Liver Tissue

After sacrificed, the liver and spleen were taken, weighted and examined. The preparation of liver tissue for the following test was conducted according to the method described by [16]. The liver tissue (0.5 g) and 1.15% KCl (5 mL) were homogenized. An aliquot of homogenized mixture (1 mL) was mixed with 1 mL trichloroacetic acid (10%) and then centrifuged (3000 x g, 15 min, 4℃).

For the determination of liver total glutathione content, supernatant (0.01 mL), phosphate-EDTA (0.18 mL), fresh prepared ó-phthalaldehyde (1 mg/1 mL methanol, 0.01 mL) were thoroughly mixed and the fluorescence was detected with exciting wavelength 350 nm and emission wavelength 420 nm. The results were expressed as ìmol/mg tissue.

The above mentioned supernatant from homogenized liver tissue and a commercial test kit (Ransod, RANDOX Lab. Ltd., UK) were used to determine superoxide dismutase (SOD) activity. The SOD activity (U) was defined as the amount of enzyme needed to inhibit the 50% reducing rate of 2-(-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride. The results were expressed as U/mg protein.

TABLE I. TREATMENTS OF ANIMAL GROUPS IN HEPATOPROTECTIVE TEST

Group	Inducing agent	Protective agent	Dose per day	Volume given	AC concentration
Normal control	olive oil	Distilled water	−	10 mL/kg	0 mg/mL
Negative control	20% CCl_4/olive oil	Distilled water	−	10 mL/kg	0 mg/mL
Positive control	20% CCl_4/olive oil	Silymarin	200.0 mg/kg	10 mL/kg	20.00 mg/mL
Low dose	20% CCl_4/olive oil	AC extract	206.7 mg/kg	10 mL/kg	20.67 mg/mL
Medium dose	20% CCl_4/olive oil	AC extract	413.4 mg/kg	10 mL/kg	41.34 mg/mL
High dose	20% CCl_4/olive oil	AC extract	1033.5 mg/kg	10 mL/kg	103.35 mg/mL

The catalase activity was determined according to the method reported by [17]. The first degree reaction coefficient (K) was defined as a unit (U) which was expressed as nmol/min/mg protein. The K value was calculated by following equation:

$$K = (2.3/t_2\text{-}t_1)\ (\log A_1/A_2)$$

where A_1 was the absorbance at t_1=0 and A_2 was the absorbance at t_2=25 sec.

The liver supernatant and a commercial test kit (Ransel, RANDOX Lab. Ltd., UK) were used for the determination of glutathione peroxidase (GPx) activity. The GPx activity (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 mol nicotinamide adenine dinucleotide phosphate (NADPH) per minute. The results were expressed as mU/mg protein. The glutathione reductase (GRd) activity was determined by using the liver supernatant and the commercial test kit (Ransel, RANDOX Lab. Ltd., UK). The GPd activity (U) was defined as the amount of enzyme that catalyzes the reduction of 1 mol nicotinamide adenine dinucleotide

phosphate (NADPH) per minute. The results were expressed as mU/mg protein.

The determination of hydroxyproline in liver tissue was conducted according to the method described by [18]. Weighted liver tissue was cut into thin strips, placed on aluminum foil, and dried at 80℃ for 20-22 hr. The dried liver tissue was digested with 6 M HCl at 100℃ for 24 hr. After cooled to room temperature, digested mixture was centrifuged and the supernatant was analyzed. The supernatant or hydoroxyproline standard was mixed with sodium hydroxide and distilled water. And then, 1 mL 0.01 M coppe sulfate, 1 mL 0.25 N sodium hydroxide and 1 mL 6% hydrogen peroxide were added into the mixture. After thoroughly mixing and heated at 80℃, 2 mL 5% 4-(dimethylamino)benzaldehyde and 4 mL 3 N sulfuric acid were added and heated at 70℃for 3 min. The absorbance at 540 nm was measured after cooled in ice water. The results was expressed asìg/100g tissue.

F. Pathological Examination of Liver Tissue

Liver was soaked in 10% formalin to fix tissue. Histological sample (5 ìm) was prepared by dehydration, wax infiltration, slicing (Leica RM 2145, Nussloch, Germany), and staining (Hematoxylin & Eosin, H&E; Sirius red). Pathological changes of liver tissue were observed with light microscope (Opticphot-2, Nikon, Tokyo, Japan).

The same location in left liver was used to examine inflammation, vacuolation, cell necrosis, and bile duct hyperplasia [19]. The above pathological changes were evaluated by a 4-point semi-quantification scale with 0 (or –), 1, 2, 3, and 4 representing no change, slight change, mild change, moderate change and severe change, respectively.

Semi-quantitative analysis of hepatic fibrosis was evaluated according to [20] by differentiating liver fibrosis into 0–4 levels. Level 0: normal liver tissue without any detectable fibrosis; level 1: collagen hyperplasia without septum formation (radial fibroplasia in the central vein or portal area); level 2: incomplete septum formation between the central vein and the portal area (with no rendezvous with each other); level 3: complete septum formed in the middle of each other and the hepatic parenchyma divided into many nodules, but this septum still very thin; and level 4: complete septum formed and the thickened septum to indicate a complete cirrhosis, which can be stained by Sirius Red to clearly showing red collagen tissue.

G. Statistical Analysis

Statistical analyses were performed using SPSS (Version 19) package (SPSS, Inc., Chicago, IL, USA). Data are presented as mean±S.D. of at least triplicate samples. One-way analysis of variance (ANOVA) and Duncan's multiple range analysis were used to compare the data with $p < .05$ regarded as statistically significant.

III. Results and Discussion

The body weight of animals during the eight weeks of experiment is shown in Table II. The body weight of animals in negative control group (received CCl_4 without protective agent) was significantly lower than that of normal control group (the only group without receiving CCl_4) from week 2 to week 8. At the end of experiment, the average body weight of normal control group was significantly higher than all other 5 groups ($p<0.05$). Throughout the eight weeks of experiment, the average feed intake per week of negative control group was significantly lower than that of normal control group ($p<0.05$) as shown in Table III. At week 1, 2, 3 and 8 week, the average feed intake of normal control group was significantly higher than those of negative control group and all three groups receiving AC extract ($p<0.05$).

The results of aspartate aminotransferase (ALT) and alanine aminotransferase (AST) for the six different groups at the end of 8-week experiment are listed in Table IV. The administration of CCl_4 in negative control group significantly ($p<0.05$) increased the ALT and AST as compared to the normal control group (without CCl_4 and hepatoprotective agents). The administration of Silymarin (positive control group) significantly lowered the liver ALT and AST with the reduction levels of 30.8% and 26.7%, respectively. The results also showed that administration of AC extract at all three doses could reduce the ALT and AST. The reduction levels of ALT for low, medium and high AC dose groups were 15.5%, 25.2% and 29.0 %, respectively; while the reduction levels of AST for low, medium and high AC dose groups were 9.5 %, 20.5 and 26.9 %, respectively. With the exception of low dose for AST, all other ALT and AST levels of AC extract treatment groups were significantly different from that of negative control group.

TABLE II. Average Body Weight (g) of Animals during the Hepatoprotective Test Period

Week	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
Initial	235.7 ± 7.7a	235.4 ± 7.1a	236.0 ± 10.4a	232.5 ± 11.3a	239.7 ± 10.2a	238.4 ± 11.5a
Week 1	245.5 ± 10.2c	236.6 ± 11.0abc	233.3 ± 11.8ab	230.5 ± 12.4a	241.5 ± 10.4abc	244.2 ± 12.4bc
Week 2	300.1 ± 16.3c	258.8 ± 19.1a	252.4 ± 16.0a	263.8 ± 17.9ab	279.7 ± 17.8b	277.2 ± 19.0b
Week 3	314.4 ± 18.6d	276.3 ± 24.0a	277.9 ± 15.6ab	278.0 ± 18.3ab	300.1 ± 15.6cd	295.1 ± 18.7bc
Week 4	368.7 ± 17.5b	316.5 ± 36.2a	326.5 ± 16.5a	319.8 ± 29.3a	340.3 ± 17.1a	340.8 ± 26.4a
Week 5	395.5 ± 19.0c	338.1 ± 39.7a	351.1 ± 18.9ab	347.4 ± 30.6ab	369.4 ± 27.2b	366.8 ± 24.5b
Week 6	406.9 ± 19.9b	344.6 ± 43.0a	354.1 ± 24.0a	346.8 ± 42.8a	367.7 ± 28.6a	369.9 ± 28.3a
Week 7	424.7 ± 21.1b	359.8 ± 47.3a	377.0 ± 25.6a	361.2 ± 46.8a	374.8 ± 39.0a	371.9 ± 29.5a
Week 8	436.6 ± 23.3b	364.8 ± 54.2a	377.8 ± 27.6a	365.2 ± 49.2a	381.2 ± 29.5a	384.7 ± 26.3a

Body weight (g) is expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different.

TABLE III. THE AVERAGE FEED INTAKE PER WEEK DURING THE HEPATOPROTECTIVE TEST

Week	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
Week 1	141.3 ± 5.2d	111.3 ± 3.9a	123.3 ± 5.9b	122.2 ± 4.0b	129.3 ± 7.9c	129.8 ± 9.1c
Week 2	181.0 ± 10.0d	138.5 ± 6.2ab	131.7 ± 11.6a	141.7 ± 11.3b	158.5 ± 8.1c	157.6 ± 9.9c
Week 3	178.5 ± 6.7c	149.4 ± 7.9a	164.2 ± 9.7b	149.1 ± 5.1a	164.4 ± 4.6b	166.7 ± 16.7b
Week 4	194.4 ± 5.4c	162.2 ± 17.0a	180.1 ± 10.7b	175.5 ± 13.5b	184.1 ± 9.9bc	183.0 ± 17.2bc
Week 5	195.4 ± 6.7d	143.7 ± 13.4a	171.4 ± 12.4b	176.4 ± 14.2bc	186.2 ± 13.5cd	187.0 ± 5.8cd
Week 6	179.8 ± 6.1d	139.0 ± 16.0a	153.4 ± 12.0bc	145.7 ± 22.9ab	166.5 ± 18.5cd	167.4 ± 7.0cd
Week 7	179.1 ± 5.2c	145.1 ± 9.4a	146.4 ± 14.9a	155.2 ± 21.6ab	155.9 ± 15.7ab	167.4 ± 8.6bc
Week 8	203.7 ± 3.6b	157.7 ± 20.5a	157.0 ± 15.4a	167.0 ± 25.3a	156.2 ± 23.2a	171.3 ± 11.1a

Body weight (g) is expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different.

TABLE IV. ASPARTATE AMINOTRANSFERASE (ALT) AND ALANINE AMINOTRANSFERASE (AST), AND SERUM TRIGLYCERIDE, TOTAL CHOLESTEROL, TOTAL PROTEIN, ALBUMIN, AND GLOBULIN CONTENTS OF TEST ANIMAL

	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
ALT	55.2 ± 5.9a	1193 ± 257d	826.7 ± 154b	1009.1 ± 122c	892.5 ± 120bc	847.4 ± 196.0b
AST	118.2 ± 21.8a	1491 ± 328d	1092 ± 152b	1349 ± 294cd	1185 ± 264bc	1090 ± 252b
Total protein (g/dL)	5.92 ± 0.48b	5.60 ± 0.18ab	5.50 ± 0.22a	5.66 ± 0.40ab	5.61 ± 0.34ab	5.75 ± 0.40ab
Albumin (g/dL)	3.69 ± 0.07a	3.81 ± 0.19a	3.74 ± 0.09a	3.80 ± 0.20a	3.76 ± 0.17a	3.65 ± 0.29a
Globulin (g/dL)	2.24 ± 0.51b	1.80 ± 0.32a	1.76 ± 0.21a	1.86 ± 0.38a	1.84 ± 0.29a	2.10 ± 0.35ab
Triglyceride (mg/dL)	58.5 ± 6.2a	58.8 ± 17.6a	65.8 ± 10.8a	66.2 ± 11.5a	62.3 ± 14.1a	58.6 ± 7.5a
Total cholesterol (mg/dL)	51.7 ± 9.1a	52.8 ± 13.3a	49.6 ± 8.5a	51.1 ± 14.1a	54.1 ± 6.3a	52.1 ± 15.5a

Data are expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different.

TABLE V. WEIGHT AND RELATIVE WEIGHT OF LIVER AND SPLEEN OF TEST ANIMALS

	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
Liver wt (g)	10.4 ± 1.0a	13.8 ± 2.5c	11.5 ± 1.0ab	12.1 ± 1.6b	12.7 ± 2.2bc	11.5 ± 1.4ab
Relative liver wt	2.52 ± 0.21a	4.02 ± 0.87d	3.20 ± 0.28bc	3.48 ± 0.44c	3.42 ± 0.42bc	3.01 ± 0.21b
Spleen wt (g)	0.75 ± 0.14a	1.31 ± 0.26c	1.11 ± 0.24b	1.06 ± 0.22b	1.05 ± 0.30b	0.91 ± 0.14ab
Relative spleen wt	0.18 ± 0.03a	0.38 ± 0.10c	0.31 ± 0.05b	0.30 ± 0.06b	0.29 ± 0.09b	0.24 ± 0.05b

Relative organ weight is defined as g/100 g B.W.

Data are expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different

Serum triglyceride, total cholesterol, total protein, albumin, and globulin contents are shown in Table IV. With the exception of globulin content, the results showed that triglyceride, total cholesterol, total protein, and albumin contents were not significantly different among all six animal groups ($p>0.05$). As for serum globulin, normal control group exerted significantly higher content than all other 5 groups.

The liver weight (g), relative liver weight (g/100 g B.W.), spleen weight (g) and relative spleen weight (g/100 g B.W.) of test animals are listed in Table V. All four parameters of negative control group were significantly higher than those of normal control group ($p<0.05$). With the exception of medium dose group for liver weight, all four parameters of positive control, low dose, medium and high dose groups were significant lower than those of the negative control group ($p<0.05$).

The activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GPd), and the contents of glutathione and hydroxyproline are listed in Table VI. The SOD activities of negative control group was significantly lower than that of normal control group ($p<0.05$). Although not restoring the level found in normal control group, positive control, medium dose and high dose groups exerted significantly higher SOD activity than negative control group ($p<0.05$). Compared with normal control group, significant lower catalase activity was observed in negative control group ($p<0.05$). On the other hand, catalase activity of positive control and high dose groups were significantly higher than that of negative control group ($p<0.05$). Glutathione peroxidase (GPx) activity of negative control group was significantly reduced as compared with normal control group ($p<0.05$). While the administration of Silymarin (positive control group) did not significantly increase the GPx activity, high dose group did exert significantly higher GPx activity when compared with the negative control group. Glutathione reducatase (GRd) activity of negative control group was significantly lower than that of normal control group ($p<0.05$). Positive control and high dose groups expressed significantly higher GRd activity than that of negative control group. Glutathione (GSH) content in liver of negative control group was significantly lower than that of normal control group

($p<0.05$). Positive control, medium dose and high dose groups showed significantly higher GSH content that negative control group. Hydroxyproline content in liver of negative control group was significantly higher than that of normal control group ($p<0.05$). Positive control, medium dose and high dose groups had significantly lower hydroxyroline content than negative control group. The pathological assessment of rat livers for vacuoles, necrosis, and fibrosis are listed in Table VII. The vacuoles and necrosis can be found in the negative control group, and the damages can not be recovered by treatment of AC extract ($p>0.05$). However, the degree of fibrosis can be decreased after treatment of high dose of AC extract when compared with the negative control group ($p<0.05$). All above results indicate that AC extract could improve the liver damage by CCl_4.

TABLE VI. THE ANTIOXIDATION ENZYME ACTIVITIES AND HYDROXYLPROLINE CONTENT OF LIVER TISSUE IN HEPATOPROTECTIVE TEST

	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
SOD (U/mg protein)	212.6 ± 7.8d	140.9 ± 26.2a	168.6 ± 15.6c	143.1 ± 17.5ab	159.5 ± 24.6bc	177.7 ± 19.9c
Catalase (nmol/min/mg protein)	1542.1 ± 132.4d	896.5 ± 257.7a	1225.3 ± 269.1bc	992.2 ± 278.7ab	1051 ± 387abc	1297.4 ± 348.0cd
GPx (mU/mg protein)	1610.7 ± 165.8c	893.2 ± 263.6a	1155.7 ± 243.4ab	986.7 ± 267.5ab	1082.7 ± 401.6ab	1234.2 ± 348.6b
GRd (mU/mg protein)	17.6 ± 1.9d	12.9 ± 2.6ab	15.7 ± 3.7cd	12.2 ± 2.3a	14.8 ± 3.5bc	16.4 ± 1.8cd
GSH (μmol/mg tissue)	16.2 ± 1.9c	5.1 ± 1.8a	8.4 ± 2.1b	5.1 ± 3.0a	7.7 ± 2.4b	8.6 ± 2.4b
Hydroxyproline (μg/100g tissue)	19.6 ± 5.8a	79.1 ± 15.9d	52.8 ± 12.5b	72.7 ± 18.4cd	64.7 ± 16.2bc	56.2 ± 9.3b

Data are expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different.

TABLE VII. PATHOLOGICAL ASSESSMENT OF RAT LIVERS

	Normal control	Negative control	Positive control	Low dose	Medium dose	High dose
Vacuoles	0.0 ± 0.0a	2.1 ± 1.0bc	2.4 ± 0.7c	1.6 ± 0.5b	1.7 ± 0.7b	2.2 ± 0.8bc
Necrosis	0.0 ± 0.0a	2.0 ± 0.5b	1.6 ± 0.7b	1.8 ± 0.6b	1.6 ± 0.5b	1.7 ± 0.5b
Fibrosis	0.0 ± 0.0a	3.6 ± 0.7d	2.4 ± 1.1bc	3.4 ± 0.8d	3.1 ± 0.7cd	2.3 ± 0.9b

Data are expressed as mean ± SD, n = 10.

Data in the same row with different lowercase letters are significantly different

IV. CONCLUSIONS

To shorten the cultivation time while maintaining the contents of bioactive components in AC, a patent pending novel cultivation method was developed. The hepatoprotective activity was tested to confirm the functionality of this novel cultivated AC. The results showed that the extract prepared from this AC could effectively protect against the liver damage caused by CCl_4.

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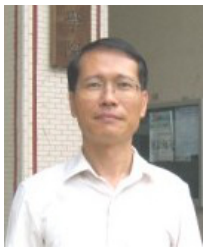

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