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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Antrodia camphorata-fermented product cultured in deep ocean water has more liver protection against thioacetamide-induced fibrosis

Li-Chun Wang · Iau-Uen Kuo · Tsung-Yu Tsai · Chun-Lin Lee

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Abstract *Antrodia camphorata* is a unique fungus in Taiwan; the submerged fermentation product is used as the functional food for liver protection. Deep ocean water (DOW) containing rich metals and trace elements is proven to stimulate the production of functional metabolites and health function of functional fungus product in our previous study. Therefore, *A. camphorata*-fermented product cultured in DOW (DOW-AC) or reverse osmosis water (ROW-AC) as culture water was daily fed thioacetamide (TAA)-induced fibrosis rat for 8 weeks in order to investigate whether DOW promoted the effect of *A. camphorata*-fermented product on the prevention against TAA-induced liver damage and fibrosis. In the results, feeding one dose of DOW-AC prevented from TAA-induced weight loss and had more effect on inhibiting lipid peroxidation, reactive oxygen species, iNOS, and TNF-α expression than one dose of ROW-AC. Furthermore, DOW-AC also had more potent effect on protection against TAA-induced liver damage and fibrosis according to the results of H&E stain and collagen stain. However, higher liver protection of DOW-AC should be due to the fact that DOW not only increased the production of *A. camphorata*-fermented functional metabolites including triterpenoids, polysaccharides, flavonoids, and polyphenols but also contributed to protection against TAA-induced damage and fibrosis.

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Keywords *Antrodia camphorata* · Deep ocean water · Liver fibrosis · Thioacetamide

Introduction

Many chronic liver diseases including viral hepatitis (hepatitis B virus and hepatitis C virus), alcoholic liver disease, and biliary diseases finally lead to liver fibrosis (Beloborodova et al. 2010). However, the reversible liver fibrosis will become serious irreversible cirrhosis if the treatment is weak (Bataller and Brenner 2005). Liver injury is induced by oxidative stress (Jaeschke 2011; Tsukamoto et al. 1995), and the supplementation with antioxidants is effective for preventing the development of liver fibrogenesis (Gebhardt 2002; Wang et al. 2012a). However, developing a potent functional food for the prevention of liver fibrosis is important currently.

Antrodia camphorata, a medicinal mushroom, is a species of fungus unique to Taiwan, possessing numerous healthy characteristics and functional metabolites. The physiologically functional compounds of the *A. camphorata* are yet to be identified. The functional compounds found in *A. camphorata* include polysaccharide, ergosterol, and triterpenoids (Chen et al. 2008; Lee et al. 2002; Shen et al. 1997; Yu et al. 2009). Triterpenoids are considered as one of the most biologically functional compounds for antioxidative stress and anti-inflammatory response in recent years (Wang et al. 2012b). Polysaccharides of *A. camphorata* are also proven to perform immunomodulatory effect and anti-inflammatory effect (Kuo et al. 2008). Furthermore, more and more studies have indicated that *A. camphorata* is able to express protection against CCl_4 and ethanol-induced liver injury in cell model and animal tests (Lu et al. 2011).

Deep ocean water (DOW) generally means ocean water from a depth of more than 200 m. The character of DOW includes high purity, cold temperature, and abundant nutrients

and minerals (Fujita 2001; Othmer and Roels 1973). Currently, DOW has been applied to food, agriculture, cosmetic, and medical field in many countries such as Taiwan, Japan, Korea, and USA due to its rich minerals (Hataguchi et al. 2005; Hwang et al. 2009; Katsuda et al. 2008; Kimata et al. 2002; Kuwayama and Nagasaki 2008). Using DOW as the culture water was proven to stimulate the functional metabolite production of functional fungi and further strengthened the heath function (Lee et al. 2011). In that study, *Monascus*-fermented product cultured in DOW has greater effect on lowering serum total cholesterol, triglyceride, and low density lipoprotein cholesterol levels and raising high density lipoprotein cholesterol levels than that cultured in reverse osmosis water (ROW) (Lee et al. 2011). However, the application of DOW in the fermentation of functional microorganism and the effect of DOW on raising the functional effect of the fermented product is still rare. More evidences and studies related to other microorganisms should be invested before DOW is used as an industrial bioactive material for fermentation application or functional food production.

Therefore, *A. camphorata*-fermented product cultured in DOW (DOW-AC) or reverse osmosis water (ROW-AC) as culture water was daily fed thioacetamide (TAA)-induced cirrhosis rat for 10 weeks in order to investigate whether DOW promoted the effect of *A. camphorata*-fermented product on the prevention against TAA-induced liver damage and cirrhosis. In the evaluation of animal test, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured for the liver function. Collagen stain and hematoxylin and eosin (H&E) stain were measured for the evaluation of liver damage and cirrhosis. The protein expressions of liver fibrosis factors were also measured in this study.

Materials and methods

Chemicals

Potato dextrose agar (PDA) and potato dextrose broth (PDB) were purchased from Difco Co. (Detroit, MI, USA). Ethanol (95 %) was purchased from Taiwan Tobacco and Liquor Co. (Taipei, Taiwan), and silymarin and TAA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Deep ocean water was purchased from Taiwan Yes Deep Ocean Water Co. (Hualien, Taiwan). Folin–Ciocalteau agent and gallic acid were purchased from Panreac Quimina S.A. (Barcelona, Spain). Phenol, sulfuric acid, and sodium carbonate were purchased from Merck Co. (Darmstadt, Germany). Monoclonal α-smooth muscle actin (α-SMA) antibody and monoclonal inducible nitric oxide synthase (iNOS) antibody were purchased from Millipore, Inc. (Billerica, MA, USA). Monoclonal tumor necrosis factor α (TNF-α) antibody was purchased from Peprotech, Inc. (New Jersey, NJ, USA).

The source of DOW

The DOW purchased from Taiwan Yes Deep Ocean Water Co. (Hualien, Taiwan) was pumped from a depth of 670 m in the Pacific Ocean near the Eastern Taiwan and processed the electrodeionization. The concentrations of the trace elements and minerals in DOW including Cu, Zn, Ba, Cr, Se, Ca, Mg, K, Na, fluoride, nitrate, sulfate, chloramines, and chlorine have been measured and published in our previous study (Lee et al. 2011).

Microorganism and seed cultures

A. camphorata BCRC 35396 was purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). *A. camphorata* was maintained on PDA at 28 °C and transferred to fresh medium at 20-day intervals. Seed cultures were prepared by transferring a loopful of colony from the PDA agar slant into a 500-mL Hinton flask containing 100 mL medium (24 g/L PDB, 20 g/L glucose). The cultures were incubated at 28 °C and 100 rpm for 7 days. After that, inoculum at a size of 5 % was transferred to submerged cultured medium.

Fermentation of *A. camphorata* in DOW or ROW

A. camphorata was cultured in the 10-L glass bottle containing 0.7 L DOW or ROW including 24 g/L PDB and 20 g/L glucose with 1 v/v/m aeration, sterile at 28 °C, for 14 days. After submerging the culture, mycelium and filtrate were separated using a filter paper. For the animal test, the mycelium was dried by freeze dryer, and the filtrate was concentrated tenfold by rotary evaporators. The dried mycelium and concentrated filtrate were used as test substances. In addition, dried mycelium powder and fresh filtrate were analyzed for functional metabolites.

Total polysaccharide, total triterpenoids, and antioxidative substances analysis

The polysaccharide of fruiting body or mycelium was precipitated by 95 % ethanol at 4 °C for 24 h. The precipitated polysaccharides were collected by centrifugation (3,000×, 15 min) and dried. The polysaccharide was resolved to a suitable concentration by distilled water. The polysaccharide concentration was analyzed according to Dubois et al. (1951). Total triterpenoids were measured according to a previous study (Cui et al. 2006). The total phenol concentration of the extract was analyzed according to the Folin–Ciocalteu method as described by a previous study (Cliffe et al. 1994). In brief,

0.25 mL extract or standard (gallic acid) was added with 2.25 mL of distilled water, 0.5 mL of the Folin–Ciocalteu stock reagent, and 1.0 mL of Na_2CO_3 reagent (75 g/L) to the mixture at room temperature for 30 min. The absorbance was measured at 765 nm by ELISA reader. Total flavonoid concentration was analyzed according to Zhu et al. (2004) Half milliliter of extract or standard (rutin) was mixed with 2 mL of distilled water and 0.15 mL 5 % $NaNO_2$ for 6 min and then mixed with 0.15 mL 10 % $AlCl_3$ for 6 min. After adding 2 mL 4 % NaOH and 0.2 mL ddH_2O and mixing for 15 min, the mixture was measured at 510 nm.

Animal experiments

Male Sprague Dawley (SD) rats at 6–8 weeks of age were purchased from BioLasco Co. (Taipei, Taiwan). They were kept in a temperature-controlled room (23 °C) under a 12 L/12D cycle (light on at 6:00) and were given free access to food and water. In the experiment, 30 rats were randomly divided to five groups. The animal model of liver fibrosis was induced by TAA injection according to a previous study (Kwak et al. 2011). During 8 weeks, two groups of the rats were intraperitoneally (i.p.) injected with vehicle solution (NOR group) or TAA (100 mg/kg bw) (TAA group) three times per week as well as daily orally administrated with ROW. The other groups were i.p. injected with TAA (100 mg/kg bw) three times per week as well as daily orally administrated with silymarin (100 mg/kg/day) (SL group), tenfold concentrated DOW (1.138 mL/kg/day) (DOW group), onefold dosage of *A. camphorata* product (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate) fermented using ROW (ROW-AC-1X group), onefold dosage of *A. camphorata* product (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate) fermented using DOW (DOW-AC-1X group), and twofold dosage of *A. camphorata* product (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate) fermented using DOW. The dosage of *A. camphorata* is calculated in accordance with Boyd's formula of body surface area as recommended by the FDA (Food and Drug Administration) (Boyd 1935; Wang et al. 2012b). Feeding rat with *A. camphorata*-fermented product at a onefold dosage (63.22 mg/kg bw of mycelium powder and 1.138 mL/kg bw of tenfold concentrated filtrate) per day corresponds to daily supplementing with 1 g of dried mycelium powder and 180 mL nonconcentrated filtrate for an adult. Each sample was orally administrated to the rats by stomach tube in each group.

After 10 weeks, the rats were deprived of food for 16 h before being scarified by CO_2 asphyxiation. Blood samples were collected from the posterior vena cava and centrifuged at 700× for 10 min; the serum was stored at −20 °C until analysis. Liver tissues were removed and weighed. Portions of the biggest leaf of liver tissue were immersed in 10 % formaldehyde for histological inspection. Half of the other portion was ground in ice-cold phosphate-buffered saline and then centrifuged (8,000×, 15 min). The supernatant was collected and stored at −80 °C for the assay of glutathione reductase activity, glutathione peroxidase activity, catalase activity, reactive oxygen species (ROS), and thiobarbituric acid reactive substances (TBARS). The other tissue (100 mg) was homogenated in 1.0 mL of lysis buffer (1 % Triton X-100, 20 mM Tris, pH 7.5, 100 mM NaCl, 40 mM NaF, 0.2 % SDS, 0.5 % deoxycholate, 1 mM EDTA, 1 mM EGTA, and 1 mM Na_3VO_4) and subjected to brief sonication (10 s). The homogenate was centrifuged at 100,000×*g* for 30 min, and the supernatant was used for immunoblotting assay. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taitung University.

Biochemical analyses

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum and glutathione reductase (GRd) and glutathione peroxidase (GPx) activities in liver were measured using commercial kits (Randox Laboratories Ltd., Antrim, UK). The catalase activity assay was similar to the method of our previous studies. Catalase activity was monitored by the disappearance of 10 mM H_2O_2 by measuring the changes in absorbance at 240 nm for 3 min (Lee et al. 2007a). TBARS level was determined by the method of thiobarbituric acid (TBA) colorimetric analysis, and the optical density (OD) value was measured at 532 nm (Ohkawa et al. 1979). In the measurement of ROS, homogenates were added to 96-well plates, and NBT reduction was measured by absorbance at 550 nm in triplicate (Lee et al. 2007b).

Immunoblotting

Protein concentration was determined by bicinchoninic acid (BCA) method. A total of 40 μg of total protein from each sample was applied for Western blot representative of three independent experiments according to the previous studies (Bihaqi et al. 2012; Lee et al. 2010). The samples were separated on 10 % SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. After blocking in a gelatin-NET solution, blots were incubated with monoclonal α-SMA antibody (1:4,000), monoclonal TNF-α antibody (1:500), and monoclonal iNOS antibody (1:500) at room temperature for 1 h. Then, bands were incubated with specific horse radish peroxidase (HRP)-conjugated secondary antibodies (1:100,000) at room temperature for 1 h and visualized by enhanced chemiluminescence (ECL) substrate with UVP AutoChemi Image system (UVP Inc., Upland, CA, USA).

Protein loading was evaluated by anti-β-actin antibody (1:5,000).

Histological analysis and collagen staining

Liver tissue sections were cut at a thickness of 7 μm and mounted on silanized slides (Dako Japan, Tokyo, Japan). The sections were stained with H&E to observe the histological features of the livers. Collagen staining of liver tissue section was stained using the picro-sirius red solution (0.1 % sirius red in saturated picric acid). By this procedure collagen is stained red (Phadnis et al. 2011).

Statistical analysis

Data are expressed as means ± standard deviation. Analysis of variance by Duncan's test and Pearson's product–moment correlation coefficient test was determined using SPSS version 10.0 software (SPSS Institute, Inc., Chicago, IL, USA). Differences with $p<0.05$ were considered as statistically significant.

Results

Intracellular functional metabolites production of *A. camphorata*

This study employed the characteristics of DOW in promoting microorganism growth to examine its influence on the biomass of *A. camphorata*. As shown in Table 1, *A. camphorata* biomass cultured using DOW (DOW-AC) reached 3.949 g/L, whereas that cultured with ROW (ROW-AC) reached only 1.604 g/L, indicating that DOW can effectively increase *A. camphorata* biomass by 2.46-fold. The intracellular triterpenoids content of DOW-AC (11.43±0.69 mg/g) was greater than that of ROW-AC (10.54±0.59 mg/g). Furthermore, the intracellular polysaccharide content of DOW-AC (3.244±0.19 mg/g) was substantially greater than that of ROW-AC (0.7±0.04 mg/g). No significant difference was observed between the intracellular polyphenol yields of DOW-AC and ROW-AC ($p<0.05$). In addition, the intracellular flavonoid production of DOW-AC (24.85± 0.79 mg/g) was greater than that of ROW-AC (10.33± 0.41 mg/g) ($p<0.05$).

Extracellular functional metabolites production of *A. camphorata*

Table 2 shows the influence of DOW on the extracellular polysaccharide and polyphenol production amounts for *A. camphorata*-fermented filtrate. DOW effectively and significantly increases extracellular polysaccharides of *A. camphorata* from 274±21 mg/L to 468±50 mg/L, and DOW also increases polyphenol production of *A. camphorata* from 189.36±1.28 mg/L to 379.92±8.78 mg/L ($p<0.05$). However, DOW did not significantly raise flavonoid production of *A. camphorata* ($p<0.05$).

According to abovementioned, DOW was proven to promote the production of functional metabolites in *A. camphorata*-fermented product, which should indirectly increase the antioxidative and anti-inflammatory abilities. Furthermore, the fermented filtrate of DOW-AC also included the DOW which was also proven as the functional drink for liver production in a previous study. The two advantages should strengthen the health function of *A. camphorata*-fermented product. Therefore, the DOW-AC was used to evaluate liver protection against TAA-induced liver fibrosis and compared with ROW-AC and DOW in order to understand the possible mechanism.

Body weight gain and liver weight

Figure 1 shows the body weight change for rats with TAA-induced liver fibrosis fed with DOW-AC and ROW-AC. TAA injection (ip) caused a significant reduction in animal body weight ($p<0.05$), and the NOR and DOW-AC-2X groups exhibited considerable weight differences from that of the TAA group by week 2, indicating that TAA injections result in rat weight loss. The DOW-AC-2X group, which was provided a double dose of DOW-AC, showed effective increases in weight gain recovery ($p<0.05$), and although the results for the SL, DOW, ROW-AC-1X, and DOW-AC-1X groups exhibited no significant differences, some weight recovery was observed.

Table 1 The effect of DOW and ROW on the functional intracellular metabolites production of *A. camphorata*-fermented mycelium

Groups	Biomass (g/L)	Intracellular metabolites			
		Triterpenoids (mg/g)	Polysaccharides (mg/g)	Total polyphenols (μg/g)	Total flavonoids (μg/g)
ROW-AC	1.60±0.21[a]	10.54±0.59[a]	0.70±0.04[a]	1079±22[a]	10.33±0.41[a]
DOW-AC	3.95±0.54[b]	11.43±0.69[b]	3.24±0.19[b]	1060±10[a]	24.85±0.79[b]

Ventilation 1/v/v/m, 28 °C for 18 days. Data are presented as means ± SD ($n=3$). Data with various letters are significantly different ($p<0.05$)

Table 2 The effect of DOW and ROW on the functional extracellular metabolites production of *A. camphorata*-fermented filtrate

Groups	Extracellular metabolites		
	Polysaccharides (mg/L)	Total polyphenols (mg/L)	Total flavonoids (mg/L)
ROW-AC	275±21[a]	189.36±1.28[a]	0.75±0.03[a]
DOW-AC	468±50[b]	379.92±8.78[a]	0.93±0.06[b]

Ventilation 1 v/v/m, 28 °C for 18 days. Data are presented as means ± SD ($n=3$). Data with various letters are significantly different ($p<0.05$)

As shown in Table 3, the body weight gain for the NOR group was approximately 212.72±43.0 g. Because of hepatic cirrhosis, the body weight gain for the TAA group decreased to 69.3±17.0 g. No significant differences were noted between the body weight of the DOW and TAA groups, suggesting that DOW could not effectively inhibit the body weight loss caused by TAA. Body weight effectively increased in the SL, DOW-AC-1X, and DOW-AC-2X groups; however, no significant differences were observed ($p>0.05$). Providing a single dose of ROW-AC did not significantly improve the body weight reduction caused by TAA, which indicates that compared to ROW-AC, DOW-AC more effectively reduces body weight loss caused by liver fibrosis.

The liver weight for the TAA group was significantly greater than that of all other groups, implying hepatomegaly. Nevertheless, the DOW group could not reduce the TAA-increased liver weight. Therefore, the simple intake of DOW may not mitigate liver inflammation and enlargement and, consequently, cannot reduce liver weight. Regarding liver weight, significant differences were observed between the TAA group and all other groups ($p<0.05$), and liver weight for all groups approximated that of the NOR group. A single dose of DOW-AC demonstrated superior effects for reducing liver enlargement compared to ROW-AC.

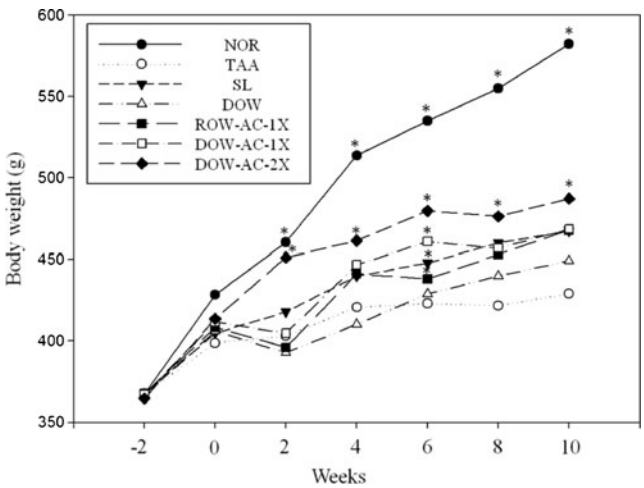

Fig. 1 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the body weight change of the TAA-induced liver fibrosis rats. *NOR* normal group, *TAA* TAA-induced fibrosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of 10-fold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate). Data are presented as means ± SD ($n=7$). (*$p<0.05$ versus TAA group)

AST and ALT activities

AST and ALT are enzymes that exist in liver cells and are released into the bloodstream upon liver cell damage or necrosis. Therefore, increased AST and ALT activity found in serum signifies liver damage. Numerous studies have indicated that oxidative stress increases when the liver metabolizes TAA, which leads to apoptosis (Toyama et al. 2004). In this

Table 3 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the body weight gain and liver weight of the TAA-induced fibrosis rats

Groups	Body weight gain (g)	Liver weight (g)
NOR	272.7±43.0c	16.08±2.91a
TAA	69.3±17.0a	19.21±0.79b
SL	105.6±10.7b	16.04±0.82a
DOW	72.3±21.1a	18.01±0.93a
ROW-AC-1X	86.6±14.8a	17.78±1.06ab
DOW-AC-1X	98.4±24.6a	16.16±1.42a
DOW-AC-2X	112.4±10.2a	16.19±1.34a

Data are presented as means ± SD ($n=7$) Data with various letters are significantly different ($p<0.05$)

NOR normal group, *TAA* TAA-induced cirrhosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate)

study, Table 4 shows the analysis results for rat AST and ALT activity. No significant differences in the ALT activity for the various groups were observed at weeks 0 and 10 ($p > 0.05$). However, compared to the NOR group, TAA caused AST activity to increase from 76.9±13.2 to 134.6±19.8 U/L. AST activity of the positive control group (the SL group) decreased to 94.2±13.5 U/L, and no significant differences were observed between the ROW-AC-1X and TAA groups, implying that a single dose of ROW-AC cannot effectively inhibit AST release in the bloodstream. Conversely, a single dose of DOW-AC and DOW can significantly reduce AST activity ($p < 0.05$) to 113.7±10.3 and 108.4±13.4 U/L, respectively, and superior results can be obtained by administering a double dose of DOW-AC. Thus, DOW is effective for mitigating the AST increase induced by TAA, and consequently DOW-AC demonstrates superior effectiveness.

According to cited results, the serum ALT activity is not significantly influenced by TAA injection, which is similar to the previous study. Although serum AST and ALT is not significantly increased, the results of studies confirm the occurrence of collagen fibrosis-related liver disease (Nakajima et al. 1998). This is because the AST and ALT produced by the liver injuries induced in animal models do not increase significantly, but hepatic cirrhosis is primarily caused by a substantial amount of collagen, which is released following the activation of oxidative stress in static HSCs. Although increases in AST and ALT levels indicate liver cell damage, they do not directly affect hepatic cirrhosis (Rockey et al. 1992; Wang et al. 2000).

Table 4 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on serum AST and ALT activities in the TAA-induced fibrosis rats

Groups	AST (U/L)		ALT (U/L)	
	0 week	10 week	0 week	10 week
NOR	98.6±20.8a	76.9±13.2a	41.6±10.0a	54.1±15.3a
TAA	109.8±18.8a	134.6±19.8e	44.6±7.0a	46.6±9.3a
SL	100.6±10.9a	94.2±13.5b	42.7±3.7a	57.1±6.4a
DOW	93.3±20.7a	108.4±13.4bc	39.7±7.1a	55.6±10.0a
ROW-AC-1X	102.4±18.0a	127.7±15.6de	41.0±6.7a	50.4±7.3a
DOW-AC-1X	109.3±14.3a	113.7±10.3cd	39.0±5.5a	55.0±10.1a
DOW-AC-2X	108.4±11.4a	98.3±14.6bc	39.6±5.7a	47.9±4.8a

Data are presented as means ± SD (n=7) Data with various letters are significantly different ($p < 0.05$)

NOR normal group, *TAA* TAA-induced cirrhosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate)

MDA and ROS concentrations

A greater MDA concentration caused by lipid oxidation is an important marker in the development of hepatic cirrhosis (Gebhardt 2002). Figure 2a shows that the TAA group demonstrated increased MDA concentration effects compared to the NOR group, indicating that continuous TAA injections raised the MDA production in rat livers. However, the SL group exhibited the ability to suppress MDA production.

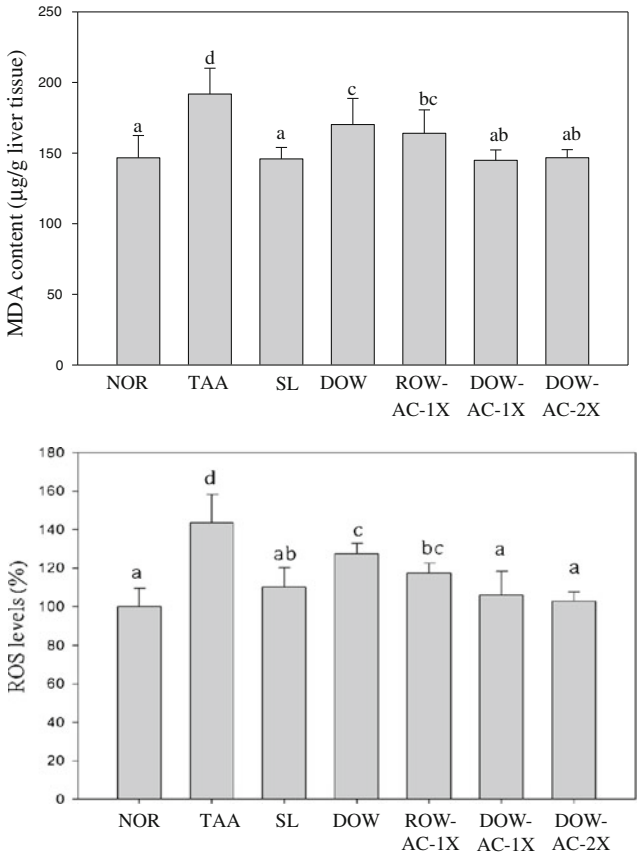

Fig. 2 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the TBARS (**a**) and ROS levels (**b**) in liver tissue of the TAA-induced fibrosis rats. *NOR* normal group, *TAA* TAA-induced fibrosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate). Data are presented as means ± SD ($n=7$). Data with various *letters* are significantly different ($p < 0.05$)

MDA concentrations were lower in the DOW group compared to the TAA group ($p<0.05$), indicating that DOW intake may mitigate TAA-induced MDA production, thereby restoring liver function and further decreasing MDA production. The MDA concentrations for the ROW-AC-1X, DOW-AC-1X, and DOW-AC-2X groups were significantly lower than that of the TAA group ($p<0.05$), and the DOW-AC-1X and ROW-AC-1X groups showed a greater trend of decreasing MDA concentrations.

Figure 2b indicates that TAA injections caused ROS increase in liver tissue. The increasing ROS level was successfully inhibited in the positive control (SL) group. Moreover, ROS levels for the ROW-AC-1X, DOW-AC1X, and DOW-AC-2X groups significantly decreased ($p<0.05$), and the ROS level of the DOW-AC-1X group was significantly lower than that of the ROW-AC-1X group. In addition, DOW also inhibits the TAA-induced ROS level in liver tissue, indicating that DOW contributes antioxidative and anti-inflammatory abilities to the *A. camphorata*-fermented product for inhibiting TAA-induced ROS levels.

GRd and GPx activities

The liver is affected by oxidative stress, which consequently causes enzyme content changes in the body's antioxidative system (Jaeschke 2011). The activity results in Table 5 for the antioxidative enzymes GRd and GPx indicate that GRd and GPx activities in the NOR group were low when liver injuries were not present, but it significantly increased following TAA injections. For the SL group, only GRd activity was increased, whereas only GPx activity was increased in the ROW-AC-1X group. GRd and GPx activities both significantly increased in the DOW-AC-1X and DOW-AC-2X groups compared to the ROW-AC-1X group ($p<0.05$). However, DOW could not increase GRd and GPx activity to prevent TAA-induced oxidative stress.

Table 5 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the liver glutathione reductase, glutathione peroxidase, and catalase in the TAA-induced fibrosis rats

Groups	Glutathione reductase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Catalase (U/mg protein)
NOR	50.47±4.91a	12.97±2.30a	140.64±11.22f
TAA	67.17±4.11b	17.63±0.89b	108.91±9.94e
SL	75.61±5.10c	12.45±1.12a	96.24±9.96d
DOW	55.17±9.56a	11.50±1.33a	89.92±12.22fd
ROW-AC-1X	58.32±5.34a	16.48±1.72b	66.64±4.56a
DOW-AC-1X	98.73±5.45d	22.39±2.02d	72.31±13.02ab
DOW-AC-2X	90.19±13.10e	19.57±2.51c	82.78±6.72bc

Data are presented as means ± SD ($n=7$) Data with various letters are significantly different ($p<0.05$)

NOR normal group, *TAA* TAA-induced cirrhosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate)

Histochemical stain

As shown in Fig. 3, the metabolism of liver lipid in the TAA group was impaired because of metabolic disorders caused by long-term injection with TAA. Furthermore, vacuoles formed, and cell integrity was poor. Normal liver cells could not be clearly identified, and the arrow in the figure shows the vacuolation and nucleus condensation or compression caused by incomplete metabolism, demonstrating that a damaged liver contains incomplete cells in which normal cells are difficult to identify. Normal liver cells were identified in the NOR group. The nuclear structure of normal liver cells is complete and easily identifiable and exhibits no vacuolation caused by abnormal cellular metabolism. Significant inhibition of vacuolation was observed in the SL group; therefore, silymarin is capable of repairing and restoring the TAA-induced liver damage. In addition, vacuoles caused by incomplete metabolism were significantly smaller in the DOW group compared to those in the TAA group. However, the DOW group exhibited a greater amount of vacuolation and no significant effects regarding liver improvement. Liver cells for the DOW-AC-1X group were relatively complete and possessed fewer vacuoles, and less vacuolation was present in the DOW-AC-1X group compared to the ROW-AC-1X group.

Collagen stain

Collagen formation and accumulation is proven to cause liver fibrosis and further results in the occurrence of hepatic cirrhosis (Beloborodova et al. 2010). Figure 4 shows collagen staining for the liver tissue sections, in which the collagen is the red plaque. The NOR group expressed normal liver tissue and the collagen content was minimal. The TAA group contained a greater amount of collagen, whereas the SL group exhibited a significant decrease in collagen formation. Although collagen formation in the DOW and ROW-AC-1X groups decreased, a minimal amount of accumulated collagen still remained. The DOW-AC-1X and DOW-AC-2X groups also exhibited lower collagen production. These test results indicated that the DOW-AC-1X group could more effectively inhibit collagen formation compared to the ROW-AC-1X group.

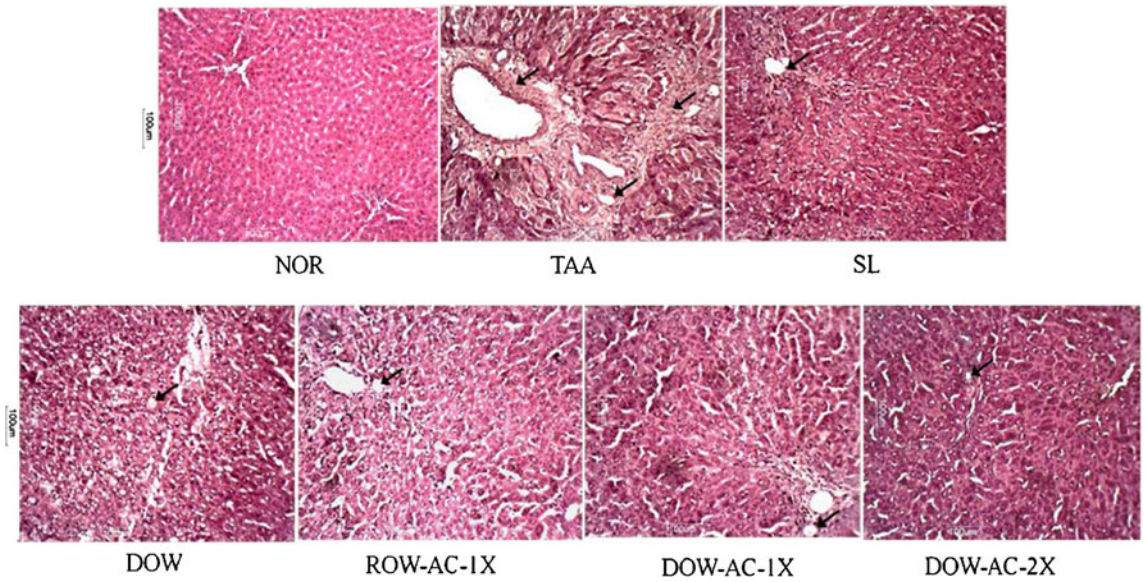

Fig. 3 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the pathological changes in liver tissue of the TAA-induced fibrosis rats. *Black arrow* indicated the fibrosis area. *NOR* normal group, *TAA* TAA-induced fibrosis rats (TAA 100 mg/kg/i.p, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate)

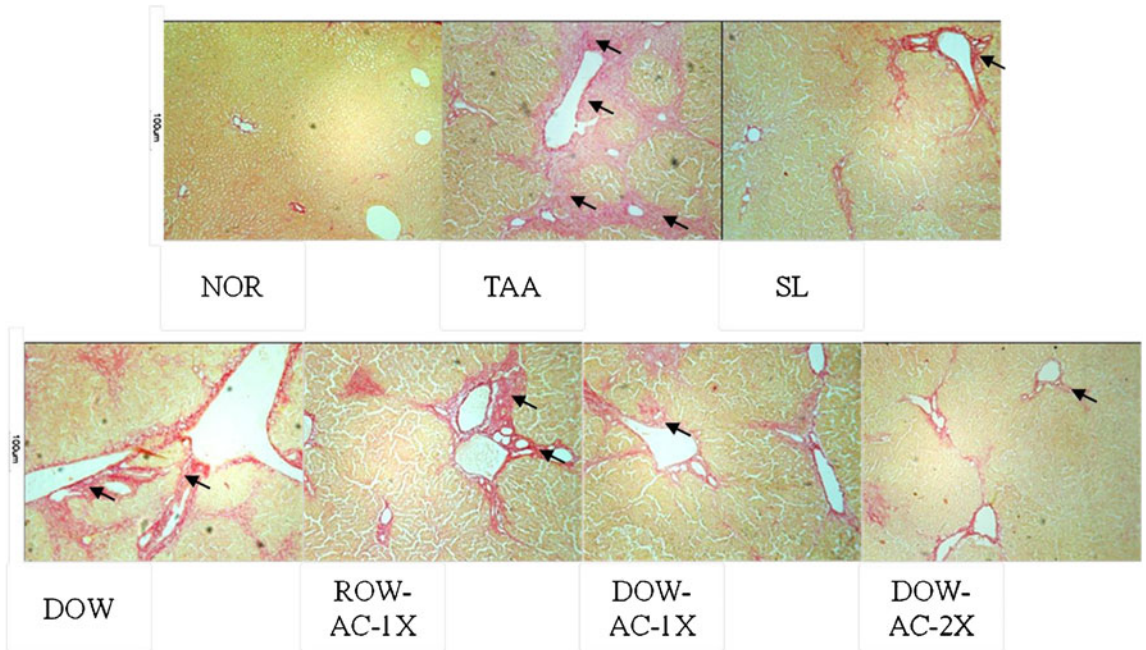

Fig. 4 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on the collagen accumulation in liver tissue of the TAA-induced fibrosis rats. The *red plaque* was the collagen accumulation (*black arrow*). *NOR* normal group, *TAA* TAA-induced fibrosis rats (TAA 100 mg/kg/ip, three times per week), *SL* TAA-induced liver fibrosis rats fed 100 mg/kg/day of silymarin, *DOW* TAA-induced liver fibrosis rats fed tenfold concentrated DOW (1.138 mL/kg/day), *ROW-AC-1X* onefold dosage of *A. camphorata* product fermented using ROW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-1X* onefold dosage of *A. camphorata* product fermented using DOW (63.22 mg/kg/day of mycelium and 1.138 mL/kg/day of tenfold concentrated filtrate), *DOW-AC-2X* twofold dosage of *A. camphorata* product fermented using DOW (126.44 mg/kg/day of mycelium and 2.276 mL/kg/day of tenfold concentrated filtrate)

TNF-α, iNOS, and α-SMA protein expression

TNF-α induces inflammatory responses, and greater expression denotes a more severe liver inflammatory response (Tsukamoto et al. 1995). As shown in Fig. 5a, b, the TNF-α protein expression for the TAA group was significantly higher than that for the NOR group ($p<0.05$). The SL group performed significantly lower TNF-α protein expression than the TAA group. In addition, the DOW-AC-1X and DOW-AC-2X groups experienced similar effects that were significantly greater than those of the ROW-AC-1X group. DOW inhibited TAA-induced TNF-α protein expression, indicating that it provided excellent anti-inflammatory abilities for DOW-AC.

A previous study asserted that iNOS is the primary proinflammatory factor during the process of hepatic cirrhosis (Mizumoto et al. 1997). The results in Fig. 5a, c show the influence of DOW-AC on the iNOS protein expression of the liver tissue for rats with TAA-induced hepatic cirrhosis. The SL group was capable of inhibiting iNOS protein expression ($p<0.05$), whereas no significant reduction effect was observed in the DOW group ($p>0.05$). The iNOS protein expression of the ROW-AC-1X and DOW-AC-1X groups was significantly lower than that of the TAA group, and no significant differences existed between the ROW-AC-1X and DOW-AC-1X groups, potentially because DOW contributes ineffective inhibition in iNOS protein expression.

Finally, liver HSCs are activated by TAA stimulation and express considerable α-SMA proteins. Activated HSCs secrete collagen, which accumulates in the liver and causes liver fibrosis. Therefore, the α-SMA protein is a key factor causing liver fibrosis (Ueno et al. 1997). As shown in Fig. 5a, d, α-SMA protein expression was only slightly reduced in the ROW-AC-1X group, whereas inhibitory effects in the DOW-AC-1X and DOW-AC-2X groups significantly increased. The DOW group also exhibited significant improvement, demonstrating that DOW is also a functional factor in the ability of DOW-AC to reduce α-SMA protein expression.

Discussion

Previous studies have investigated and discussed the hepatoprotective effect of a *A. camphorata*-fermented product (Ao et al. 2009; Lee et al. 2002; Lu et al. 2011). *A. camphorata* polysaccharides possess anti-hepatitis B activity and exhibit no cytotoxicity. In particular, *A. camphorata* B86 substantially inhibits the surface antigens of the hepatitis B virus at 50 μg/mL and demonstrates superior performance compared to 1,000 unit/mL of α-interferon (Lee et al. 2002). Previous studies have also contended that the ethanolic extract of *A. camphorata* may exert its hepatoprotective activity by up-regulating GSH-dependent enzymes and inhibiting free

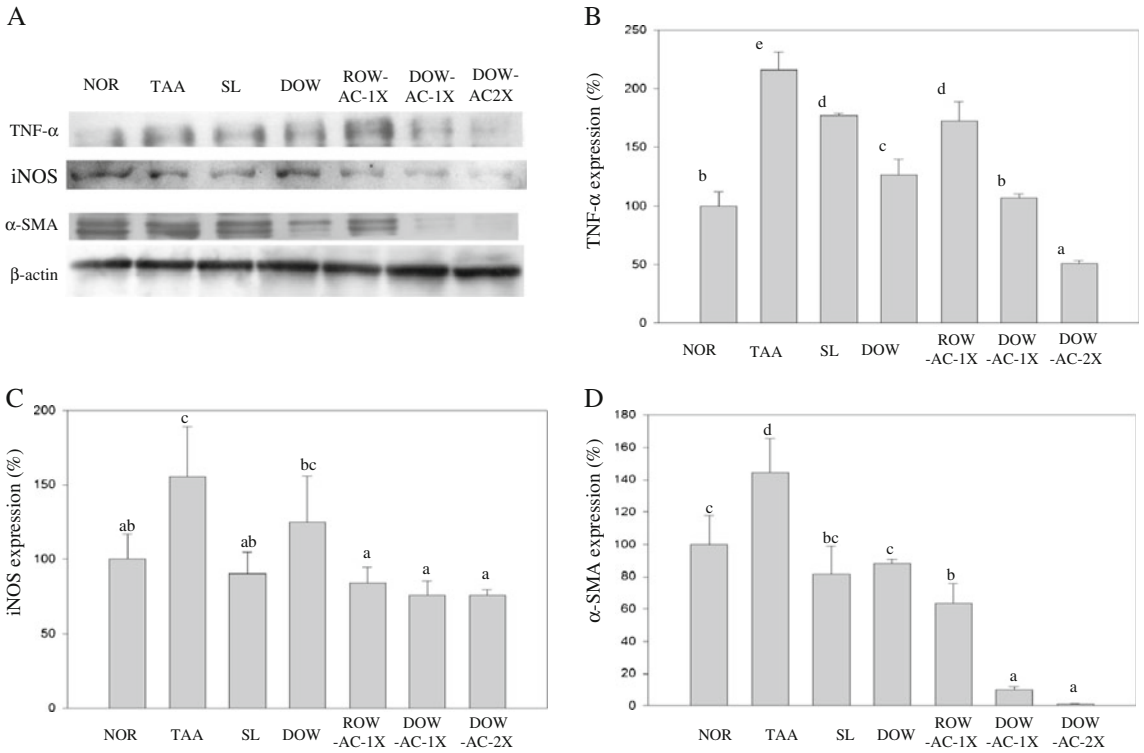

Fig. 5 The effects of *A. camphorata*-fermented product cultured with DOW or ROW on iNOS, TNF-α, and α-SMA protein expressions in liver tissue of the TAA-induced fibrosis rats. Target protein expressions were visualized using immunoblotting (**a**) and quantified using image J software (**b**–**d**). Mean values with different *letters* are with significant difference ($p<0.05$)

radical formation in the liver on ethanol-induced acute liver injury in rats (Lu et al. 2011). These studies have inferred that the *A. camphorata*-fermented product is effective in hepatitis prevention. However, few studies have focused on improving hepatic cirrhosis conditions. The results of this study indicate that for certain indicators, ROW-AC is effective for ameliorating hepatic cirrhosis (e.g., by significantly inhibiting TAA-induced MDA and ROS levels and iNOS expression). Nevertheless, ROW-AC exhibited weaker effectiveness for improving TAA-induced AST activity, SOD activity, Grd activity, TNF-α, and α-SMA, suggesting that although ROW-AC possesses a hepatoprotective effect, its ability to mitigate comparatively severe hepatic cirrhosis is non-significant.

This study used DOW as the only culture water during *A. camphorata* submerged fermentation. The results of this study indicate that DOW-AC significantly prevents TAA-induced hepatomegaly, reduces increased levels of AST activity, MDA levels, and ROS levels, and shows substantial improvement effects for the results of H&E and collagen stains. These data verify that DOW-AC reverses TAA-induced liver fibrosis. Regarding the effectiveness of DOW-AC and ROW-AC for preventing liver fibrosis, a single dose of DOW-AC more effectively reduces increased AST activity, MDA and ROS content, and TAA-induced hepatomegaly than an equivalent dose of ROW-AC. The test results of the H&E and collagen stains also indicated that one dose of DOW-AC is more effective than ROW-AC, which signifies that *A. camphorata*-fermented product cultured in DOW is more beneficial for liver fibrosis prevention.

In addition, silymarin has been verified in past studies to reduce the consumption of the antioxidant glutathione, decrease the O^{2-} and NO free radicals in rat livers produced by HSC activation, and inhibit TNF-α (Manna et al. 1999). Numerous studies have confirmed that silymarin can reduce CCl_4-induced liver injury (Shaker et al. 2011) and is capable of preventing and resisting liver fibrosis and hepatic cirrhosis (Sugiyama et al. 2009). The efficacy of DOW-AC and silymarin for improving liver fibrosis was compared in this study. The two demonstrated a similar efficacy in a multiple-indicator analysis, and both significantly improved TAA-induced liver fibrosis. However, DOW-AC still showed a superior effect for inhibiting α-SMA expression compared to silymarin.

Previous studies have asserted that cells that express α-SMA are also capable of secreting collagen (Rockey et al. 1992). Experiments using rats indicate that under normal conditions, static HSCs do not induce α-SMA expression; however, activated HSCs express α-SMA. Therefore, HSCs are activated when the liver is damaged, are subsequently transformed into liver myofibroblasts, and finally express α-SMA (Rockey et al. 1992; Ueno et al. 1997). In this study, TAA i.p. injection was used to induce liver fibrosis in rats. The liver injury mechanism induced by TAA is related to oxidative stress, which creates a complex that damages the liver. TAA is oxidized as TAA sulfoxide (TAASO) and TAA-S,S-dioxide ($TAASO_2$) through cytochrome P450 monooxygenases. This substance undergoes covalent binding with enzymes or macromolecules in cells, causing cell dysfunction (Dyroff and Neal 1983; Hunter et al. 1977). This eventually leads to apoptosis and necrosis, and oxidative stress. The influence of oxidative stress causes the HSC to transform into a myofibroblast-like cell, which secrets considerable collagen and extracellular matrix. Liver fibrosis is the scarring process of the liver caused by the accumulation of abundant extracellular matrix. Abnormal changes in the liver structure result in the development of hepatic cirrhosis (Bataller and Brenner 2005). Therefore, previous studies have proposed that a functional component against liver fibrosis should facilitate the reduction of HSC activation, α-SMA protein expression, and collagen accumulation (Rockey et al. 1992; Ueno et al. 1997).

The antioxidative system is also crucial for the hepatoprotective effect. Previous studies have stated that increased catalase activity may inhibit HSC activation (Toyama et al. 2004). TAA-induced ROS in liver can be transformed to H_2O_2 by SOD. Subsequently, H_2O_2 is transformed to nontoxic H_2O and O_2 with catalase and glutathione peroxidase. Scavenging of free radicals by the natural antioxidative system inhibits the HSC activity and prevents liver fibrosis development. Therefore, insufficient antioxidative enzyme content is default to prevent the generation of free radicals, thereby causing liver fibrosis (Toyama et al. 2004). Glutathione eliminates ROS and further reduce MDA production, thereby significantly decreasing iNOS and TNF-α expression in liver tissue (Kwak et al. 2011). Studies have shown that 2-week-old rats possess significantly greater amounts of SOD and glutathione and stronger protection against liver injury compared to 30-week-old rats (Sanz et al. 2002).

Based on the mentioned mechanism for liver fibrosis development, we compared the differences in molecular mechanisms for inhibiting liver fibrosis formation in *A. camphorata*-fermented products cultured using DOW and UPW. The results of this study indicated that consuming DOW-AC has superior effects for reducing ROS and MDA content compared to ROW-AC. DOW-AC demonstrated significantly superior performance to that of ROW-AC in increasing the activity of antioxidative enzymes GRd and GPx. This result verified that the liver tissue of rats fed with DOW-AC possessed a superior antioxidative prevention system for inhibiting and resisting TAA-induced oxidative stress. Furthermore, TNF-α, a key proinflammatory factor for HSC activation, is significantly mediated by DOW-AC. According to the results of Fig. 5, DOW-AC may inhibit TNF-α protein expression to further reduce downstream iNOS expression, thereby significantly decreasing TAA-induced oxidative stress and inflammatory responses, as well as α-SMA protein

expression. Comparing liver fibrosis-related protein expression for DOW-AC and ROW-AC, DOW-AC is significantly superior to ROW-AC in reducing TNF-α protein expression as well as α-SMA protein expression. However, these effects result in that DOW-AC more effectually and significantly inhibits collagen formation compared to ROW-AC. Thus, on a molecular level, DOW-AC is superior to ROW-AC in mitigating the risk factor expression of liver fibrosis.

Two possibilities could be connected to the reasons for DOW-AC exhibiting superiority in liver fibrosis prevention: (1) The production of functional metabolites in *A. camphorata*-fermented product can possibly be increased with the addition of DOW, and this *A. camphorata*-fermented product with greater functional components is superior for liver fibrosis prevention, and (2) DOW possesses a hepatoprotective effect. DOW is the primary water source for submerged fermentation; therefore, DOW is accumulated in *A. camphorata*-fermented mycelium and filtrate and may increase the preventive effects of *A. camphorata*-fermented product against liver fibrosis. The two possible reasons are explained in the following text.

First, according to the results of the metabolite component analysis, DOW significantly increases the contents of polysaccharides, β-1,3 glucan, total triterpenoids, total polyphenols, and total flavonoids in *A. camphorata*-fermented product. This confirms that DOW can significantly increase the functional components of *A. camphorata*. However, the enhanced components are mostly antioxidative and anti-inflammatory. Neutral polysaccharide isolated from the mycelium of *Antrodia cinnamomea* performed protective effects against *Propionibacterium acnes* and lipopolysaccharide (LPS)-induced hepatic injury in mice. The administration of neutral polysaccharide (0.4, 0.8 g/kg/d, p.o.), significantly prevented increases in serum AST and ALT activities in mice treated with *P. acnes*–LPS, indicating a hepatoprotective activity in vivo (Han et al. 2006). In addition, a beta-1,3-D-glucan isolated from *Euglena gracilis* Z was also proven to perform hepatoprotective effects against acute liver injury induced by carbon tetrachloride in rats. Pre-administration of beta-1,3-D-glucan reduced the liver apoptotic index. The treatment of beta-1,3-D-glucan recovered reductions of activity of hepatic superoxide dismutase, catalase, and glutathione peroxidase induced by CCl_4. These results demonstrate that beta-1,3-D-glucan exhibits a protective action on acute hepatic injury induced by CCl_4 via an antioxidative mechanism (Sugiyama et al. 2009). The triterpenoids antcin B and its ester derivative from *A. camphorata* induce apoptosis in hepatocellular carcinoma cells, which involves enhancing oxidative stress coincident with activation of intrinsic and extrinsic apoptotic pathway (Hsieh et al. 2011). Consequently, DOW increases the existing hepatoprotective effects of the functional components in a *A. camphorata*-fermented product. The majority of substances achieve a hepatoprotective effect through antioxidative and anti-inflammatory effects. ROW-AC demonstrated a weaker effect for improving liver fibrosis, whereas DOW-AC possessed greater functional metabolites and enabled *A. camphorata* to possess relatively significant efficacy for preventing liver fibrosis. Previous studies have maintained that DOW facilitates RMD in exhibiting a more significant hypolipidemic effect by increasing the RMD functional components monascin and ankaflavin. Hence, our results, in combination with those of our previous studies (Lee et al. 2011), verified that employing DOW to increase the functional components of functional microorganisms is a feasible method for enhancing protective effects.

The addition of DOW causes DOW accumulation in *A. camphorata* mycelium and fermentation broth during the fermentation process. The results of this study indicated that DOW is mildly effective in preventing liver fibrosis and is able to reduce AST activity, ROS, and MDA. Although not as effective as DOW-AC, DOW is significantly effective in decreasing these indicators compared to the TAA group. In addition, DOW significantly reduced TAA-induced TNF-α and α-SMA expression, and substantial improvement effects were verified by the histochemical and collagen staining tests. This indicates that DOW drinking can improve TAA-induced liver injury. Among studies on DOW, this study is the first to verify that DOW reduces α-SMA expression and collagen accumulation by inhibiting TNF-α expression.

Regarding the liver protection of DOW in the previous study, there is no study to investigate the effect of DOW drinking on the protection against TAA-induced liver injury. However, a relative study had proven that DOW was able to lower hepatic lipid accumulation and oxidation induced by a high-fat diet. Serum/liver lipids, liver sizes, liver MDA content, and serum AST and ALT activities of high-fat-diet hamsters were reduced by drinking DOW. DOW maintained higher liver glutathione and Trolox equivalent antioxidant capacity levels. Although hepatic sterol regulatory element-binding protein-1c, acetyl-CoA carboxylase, fatty acid synthase, and malic enzyme gene expression was not altered, DOW upregulated hepatic peroxisome proliferator-activated receptor-alpha, retinoid X receptor alpha, and uncoupling protein-2 gene expression in high-fat-diet hamsters (Chen et al. 2013). Furthermore, DOW is also found to perform anti-inflammatory effects in previous studies (Bak et al. 2012). Treatment atopic dermatitis mice with DOW inhibited up-regulation of IgE, histamine, and pro-inflammatory cytokines in the serum. Also, the CD4+/CD8+ ratio in spleen lymphocyte was down-regulated after treatment with DOW. Finally, cytokines, especially IL-4 and IL-10 which are important for Th2 cell development, were reduced (Bak et al. 2012). However, the ions of DOW may contribute the hepatoprotective effect. A previous study indicated that selenium and zinc concentrations were elevated in patients with

low transaminase levels, which were significantly different in comparison with patients with a high transaminase level ($p < 0.05$). Elevated levels of transaminase concentrations were independently associated with low zinc and selenium concentrations in chronic HBV patients. Serum zinc and selenium levels are associated with less hepatic damage in chronic HBV patients and might have a protective role during liver injury (Abediankenari et al. 2011). According to the aforementioned studies on the hepatoprotective and anti-inflammatory effects of DOW and the hepatoprotective effect of DOW demonstrated in this study, we infer that DOW trace elements accumulated in DOW-AC may enable a greater hepatoprotective effect through the use of DOW and may thereby achieve liver fibrosis prevention.

In conclusion, although ROW-AC possesses a hepatoprotective effect, its ability to mitigate comparatively severe hepatic cirrhosis is non-significant. DOW-AC prevented from TAA-induced weight loss and had more effect on inhibiting the lipid peroxidation, ROS, iNOS, TNF-α, and α-SMA expressions, and collagen formation than ROW-AC. DOW not only expressed hepatoprotective effect in inhibiting TNF-α, α-SMA expressions, and collagen formation but also stimulated the production of *A. camphorata*-fermented functional metabolites including triterpenoids, polysaccharides, flavonoids, and polyphenols. Therefore, DOW-cultured *A. camphorata*-fermented product can potentially be developed as a health care product for preventing hepatic cirrhosis.

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