



Protective Effects of *Antrodia Cinnamomea* Against Liver Injury

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Abstract

Chinese herbal medicine (中草藥) attracts much attention in the treatment of liver injuries. Numerous studies have revealed various biological activities of medicinal mushrooms such as *Antrodia Cinnamomea* (牛樟芝). Although *A. cinnamomea* is rare in the wild, recent developments in fermentation and cultivation technologies make the mycelia and fruiting bodies of this valuable medicinal mushroom readily available. Liver diseases such as fatty liver, hepatitis, hepatic fibrosis, and liver cancer are complicated processes of liver injuries that have tremendous impact on human society. In this article, we reviewed studies about the hepatoprotective effects of the fruiting bodies and mycelia of *A. cinnamomea* performed in different experimental models. The results of those studies suggest the potential application of *A. cinnamomea* in preventing and treating liver diseases and its potential to be developed into health foods or new drugs.

Key words: *Antrodia Cinnamomea*, Fruiting bodies, Mycelia, Liver injury

Introduction

Several medicinal mushrooms are known to be valuable for their effectiveness in the treatment of diseases. Among the popular medicinal mushrooms such as *Antrodia Cinnamomea* (Figure 1), *Ganoderma lucidum*, *Cordyceps sinensis*, and *Phellinus linteus*, *A. cinnamomea* can be found only in Taiwan. *Antrodia Cinnamomea* is used to treat liver diseases, hypertension, abdominal pain, diarrhea, and other diseases (Ao et al., 2009). Historically, indigenous Taiwanese people have used *A. cinnamomea* to ameliorate liver disorders from excessive alcohol consumption. However, it is now becoming more difficult to collect wild *A. cinnamomea* fruiting bodies.

With the development of fermentation technology, the mycelia of many medicinal mushrooms can now



Figure 1. *Antrodia Cinnamomea* fruiting bodies grown on wood.

be obtained through submerged fermentation. This technology can effectively manage the identity and purity of medicinal mushrooms without contamination. The process of substrate bag culture for growing

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fruiting bodies also uses liquid fermentation to provide the seed medium (Sharma et al., 2005). However, the medicinal effectiveness and applicability of the different compounds contained in fruiting bodies and mycelia remain debatable.

In this review, we discuss the hepatoprotective activities of *A. cinnamomea* in animal models of carbon tetrachloride (CCl₄)- and alcohol-induced liver injuries, as well as its *in vitro* anti-liver cancer activity.

The liver is the largest organ responsible for a spectrum of functions, including the uptake, metabolism, conjugation, and excretion of various

endogenous and foreign substances (Hoekstra et al., 2012). Chronic hepatitis or toxification leads to severe liver injury. The damaged hepatocytes are initially denatured and then undergo fibrosis and necrosis. This process eventually leads to hepatoma (Friedman, 1997).

Antrodia Cinnamomea, a species of the genus *Antrodia* (Polyporaceae), is a parasitic fungus that lives in the inner cavity of *Cinnamomum kanehirai*, which is endemic to Taiwan. *Antrodia Cinnamomea* is believed to be one of the most potent liver-protecting herbs in Taiwan (Ao et al., 2009).

Numerous reports have been published on the

Table 1. Reported bioactivities of *Antrodia Cinnamomea*.

Bioactivities	Sources	References
Antiadenocarcinoma	Ethanol extract of fruiting bodies	Chen et al., 2012a Chen et al., 2012b
Antiallergy	Polysaccharide from solid-state-cultured mycelia	Liu et al., 2010
Anti-breast cancer	Submerged fermentation culture	Lee et al., 2012
	Synthesized camphorataimide B (originally isolated from mycelia)	Lin et al., 2012
Anti-colon cancer	4,7-Dimethoxy-5-methyl-1,3-benzodioxole (SY-1) isolated from fruiting bodies	Tu et al., 2012
	SY-1 derivatives	Lien et al., 2011
Antihypertension	Hot water extract of mycelia	Wang et al., 2003
Antileukemia	Dehydroeburicoic acid isolated from the triterpenoid-rich fraction of the ethanolic extracts of fruiting bodies	Du et al., 2012
	Methanol extract of mycelia MMH01	Wen et al., 2012
	(24-methylenelanosta-7,9(11)-diene-3 β 15 α -diol-21-oic acid) isolated from fruiting bodies	Chen et al., 2009
Anti-liver cancer	Fruiting bodies	Hsu et al., 2007 Hsieh et al., 2010 Hsieh et al., 2011
	Mycelia	Lin et al., 2010 Lin et al., 2011 Song et al., 2005a Song et al., 2005b
	Antroquinonol (originally isolated from solid-state-cultured mycelia)	Chiang et al., 2010
	Alcohol extract of fruiting bodies	Chiou et al., 2011
	Alcohol extract of mycelia	Chan et al., 2010
Anti-non-small cell lung cancer	Mycelium polysaccharides	Chen et al., 2007
	Methanolic extract of fruiting bodies	Liu et al., 2007
	Compounds isolated from fruiting bodies	Shen et al., 2007
	Compounds isolated from fruiting bodies	Chien et al., 2008
	Fermentation filtrate	Hseu et al., 2010
	Ergostatrien-3-ol (ST1) isolated from submerged fermentation whole broth	Huang et al., 2010a
Antioxidant	Aqueous extract of mycelia	Hseu et al., 2008
Hepatoprotection against CCl ₄ -induced liver injury	Aqueous extract of fruiting bodies	Hsiao et al., 2003
	Fermentation filtrate	Song and Yen, 2003 Lin et al., 2006
	Mycelia	Lu et al., 2007
Hepatoprotection against ethanol-induced liver injury	Ethanolic extract of mycelia	Lu et al., 2011
	Ethanolic extract of mycelia	Kumar et al., 2011
	Fruiting bodies	Wu et al., 2011
	Methanolic extract of fruiting bodies	Huang et al., 2010a
Neuroprotection	Compounds isolated from the methanolic extract of fruiting bodies	Chen et al., 2006
	Fruiting bodies	Wang et al., 2012
Immunomodulation	Hot water extract of mycelia	Kuo et al., 2008

chemical components of *A. cinnamomea*. Compounds isolated from this mushroom, such as benzenoids, diterpenes, triterpenoids, steroids, maleic/succinic acid derivatives, and polysaccharides, have been reported to exhibit some biological activities.

Table 1 summarizes the reported biological activities of *A. cinnamomea*, including anti-allergy, anticancer, antihypertension, anti-inflammation, antioxidation, hepatoprotection, neuroprotection, and immunomodulation.

***Antrodia Cinnamomea* and Alcohol-induced Liver Injury**

Alcohol liver disease (ALD) is a kind of liver injury induced by alcohol drinking, which remains one of the most common causes of chronic liver disease worldwide. ALD includes steatosis (fatty liver), steatohepatitis (alcoholic hepatitis), and cirrhosis (Day and Yeaman, 1994). Steatosis is the earliest response of the liver to excessive alcohol use and is characterized by the accumulation of fat in hepatocytes. Steatohepatitis is characterized by infiltration of inflammatory cells into hepatocytes and hepatocellular injury (Gao and Bataller, 2011). Alcohol consumption suppresses the antifibrotic effects of natural killer cells and interferon- γ , and therefore enhances the progression of liver fibrosis (Jeong et al., 2008).

Alcohol promotes the accumulation of fat in the liver mainly by the substitution of ethanol for fatty acids as the major hepatic fuel (Baraona and Lieber, 1979). Ethanol increases the fatty acid synthesis by acetaldehyde through the upregulation of sterol regulatory element-binding protein 1c (SREBP-1c). AMP-activated protein kinase (AMPK) (You et al., 2004), sirtuin 1 (You et al., 2008), adiponectin (You, 2009), and signal transducer and activator of transcription 3 (STAT3) (Horiguchi et al., 2008) were reported to be downregulated by alcohol. The DNA-binding and transcriptional activation activities of peroxisome proliferator-activated receptor- α , a nuclear hormone receptor, in hepatocytes are directly inhibited by acetaldehyde (Galli et al., 2001).

Three main pathways are involved in the metabolism of ethanol: the alcohol dehydrogenase (ADH) pathway in the cytosol, the microsomal ethanol-oxidizing system (MEOS) in the endoplasmic reticulum, and the catalase pathway in peroxisomes (Jiménez-López et al., 2002). The ADH pathway is the major metabolic pathway during the early stage of chronic alcohol liver

injury. During the ADH-mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor nicotinamide adenine dinucleotide (NAD), resulting in excess conversion to its reduced form (NADH) with the production of acetaldehyde (Cronholm et al., 1988). Excess NADH changes the redox state and leads to metabolic irregularity in the liver. The increase in α -glycerophosphate level and the suppression of the citric acid cycle are due to the elevated NADH-to-NAD ratio, thus favoring the accumulation of hepatic triglycerides in the liver (Ao et al., 2009). In the early 1960s, Lieber et al. (1963) formulated a liquid ethanol diet and used experimental models to study ethanol-induced hepatotoxicity. Therefore, the hepatoprotective effects of herbs, natural products, and chemicals have been widely investigated using these models.

In Taiwan, *A. cinnamomea* is believed to effectively ameliorate liver disorders induced by excessive alcohol consumption. However, the effects of *A. cinnamomea*, either its fruiting bodies or mycelia, on alcohol-induced liver injuries have only recently been reported (Huang et al., 2010a; Lu et al., 2007, 2011; Kumar et al., 2011; Wu et al. 2011).

Hepatoprotective Effect of *A. cinnamomea* Fruiting Bodies Against Alcohol-induced Liver Injury

The effects of *A. cinnamomea* fruiting bodies on alcohol-induced liver damage have been reported. Huang et al. (2010a) investigated the effects of *A. cinnamomea* fruiting bodies in a chronic alcohol consumption model. Alcoholic fatty liver disease was induced by adding 20% (w/w) alcohol to the drinking water of experimental rats. *Antrodia Cinnamomea* fruiting body (0.1 g per kilogram body weight per day [$\text{g}\cdot\text{kg BW}^{-1}\cdot\text{day}^{-1}$]) orally administered to the chronic alcohol consumption group for 4 weeks increased the levels of fecal cholesterol and bile acid. The gene expression of 3-hydroxy-3-methoxyglutaryl-CoA reductase, SREBP-1c, acetyl-CoA carboxylase, fatty acid synthase, and malic enzyme was downregulated by *A. cinnamomea* fruiting body. The results of histological examination revealed that the alcohol-induced liver injuries, i.e., hepatocyte necrosis and inflammatory cell infiltration, were prevented by *A. cinnamomea* fruiting body (0.1 $\text{g}\cdot\text{kg BW}^{-1}\cdot\text{day}^{-1}$) and that its effect was even better than that of silymarin (0.25 $\text{g}\cdot\text{kg BW}^{-1}\cdot\text{day}^{-1}$).

Wu et al. (2011) investigated the beneficial effects of *A. cinnamomea* fruiting bodies on alcohol-induced liver

fibrosis. The expression of hepatic mRNAs, i.e., matrix metalloproteinase (MMP)-9, tumor necrosis factor (TNF)- α , Kruppel-like factor (KLF)-6, and transforming growth factor (TGF)- β 1, were downregulated by orally administered *A. cinnamomea* fruiting bodies (0.025 g/kg BW, once a day). The hepatoprotective effect of *A. cinnamomea* fruiting bodies (0.025 g/kg BW, once a day) was comparable to that of silymarin (0.25 g/kg BW, once a day). This protective effect might account for the acceleration of alcohol clearance and the suppression of ethanol-induced elevation of MMP-9, TNF- α , KLF-6, and TGF- β 1 levels.

Hepatoprotective Effect of *A. cinnamomea* Mycelia Against Alcohol-induced Liver Injury

Lu et al. (2007) reported the effects of *A. cinnamomea* mycelia against ethanol-induced liver injury. Male Sprague-Dawley (SD) rats were orally administered with 0.5 and 1.0 g/kg BW of *A. cinnamomea* mycelia for 10 days. At the end of 10th day, rats were administered with ethanol (5.0 g/kg BW) by gavage to induce acute hepatic injury. The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin in rats given *A. cinnamomea* were comparable with the silymarin positive group (0.1 g/kg BW). Thus, *A. cinnamomea* showed hepatoprotective activity in this acute ethanol-induced liver injury rat model. Lu et al. (2011) further reported that a

triterpenoid-enriched fraction of the ethanolic extract of *A. cinnamomea* mycelia showed the greatest effectiveness in preventing ethanol-induced acute liver injury and free radical generation in rats.

Kumar et al. (2011) evaluated the effect of the ethanolic extract of *A. cinnamomea* in ethanol-induced acute hepatotoxicity. Ethanolic extracts of *A. cinnamomea* mycelia (0.25, 0.5, and 1 g/kg BW, once a day) were orally administered to rats by gavage for 10 days. Silymarin (0.2 g/kg BW) was used as a positive control. After the final administration, hepatotoxicity was induced by administering ethanol (5 g/kg BW) through oral gavage. The results showed that the serum levels of ALT and AST in the group given the ethanolic extract of *A. cinnamomea* (1 g/kg BW) were comparable to those in the silymarin positive group (0.2 g/kg BW). The levels of protein expression of hemeoxygenase-1 (HO-1) and NF-E2 related factor-2 (Nrf-2) were increased in groups orally administered with *A. cinnamomea* ethanolic extract and silymarin. The transcription factor Nrf-2 can induce the expression of a variety of cytoprotective and detoxifying genes (e.g., *HO-1*). This study suggested that the hepatoprotective effects of *A. cinnamomea* ethanolic extract might be through a mechanism that involves Nrf-2 activation and upregulation of the expression of the downstream antioxidant gene.

Figure 2 shows the hepatoprotective mechanisms of *A. cinnamomea* against ethanol-induced liver injuries according to the above results.

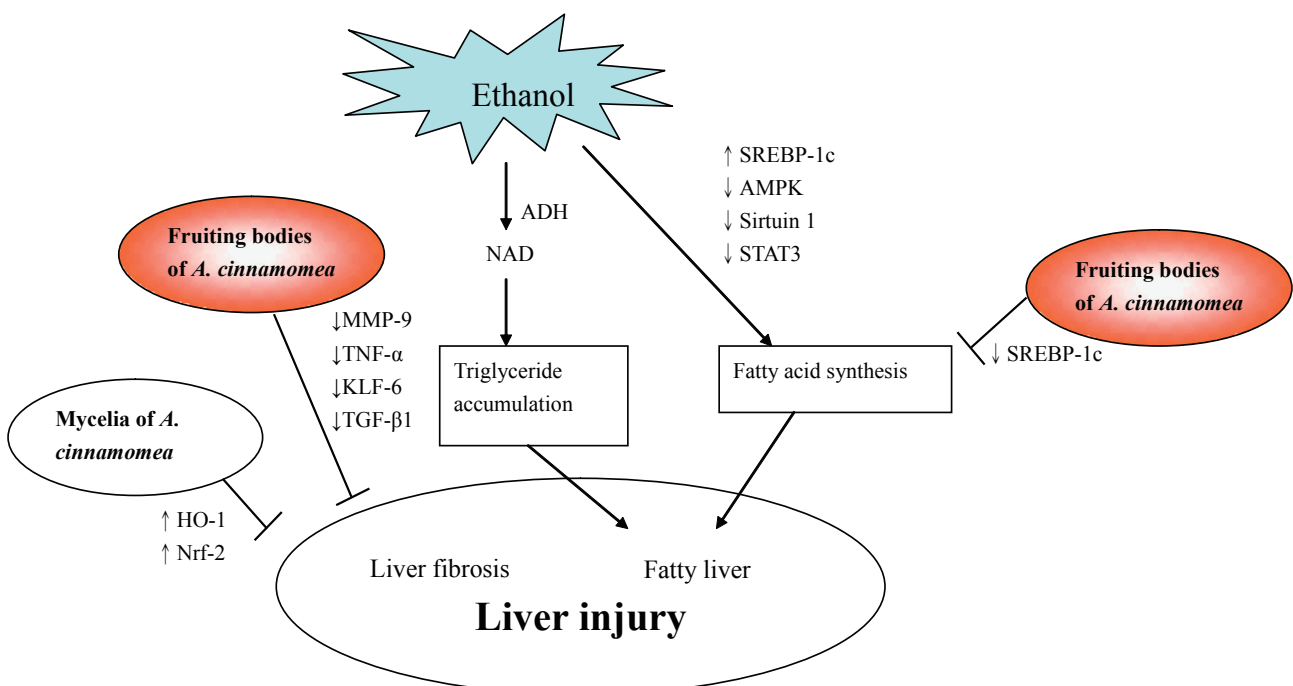


Figure 2. Mechanisms of fruiting bodies and mycelia of *Antrodia Cinnamomea* against ethanol-induced liver injuries.

***Antrodia Cinnamomea* and CCl₄-induced Niver Injury**

Chronic CCl₄ treatment causes liver injury, oxidative stress, and nitrosative stress. Collagen accumulation, which causes hepatic fibrosis, was also found to be a result of chronic CCl₄ treatment (Tipoe *et al.*, 2010). The hepatotoxicity of CCl₄ consists of 2 steps. The first step involves the production of free radicals (CCl₃• and CCl₃OO•) through hepatic metabolism mediated by the cytochrome P450 system. The second phase involves the activation of Kupffer cells, which is accompanied by the production of inflammatory and profibrogenic mediators. Cytochrome P450 2E1 (CYP2E1) knockout mice (*cyp2e1*^{-/-}) were used to investigate the involvement of CYP2E1 in the development of CCl₄-induced hepatotoxicity, and CYP2E1 was found to be a major factor in CCl₄-induced hepatotoxicity in mice (Wong *et al.*, 1998).

The enzymatic activation of CCl₄, leading to the generation of CCl₃ radicals, also disrupts the structure and function of lipids and proteins in the membrane and cell organelles (Xiao *et al.*, 2012). The injured hepatic tissue is thus degraded by MMPs, while tissue inhibitor of MMPs (TIMPs) act as regulators of MMPs, preventing further degradation and damage to the newly synthesized collagen and unaffected tissue (Hemmann *et al.*, 2007). CCl₄ intoxication increased the expression of mRNAs, including MMP-2, MMP-9, TIMP-1, TIMP-2, TGF-β1, α-smooth muscle actin (SMA), and procollagen (Knittel *et al.*, 2000). The synthesis of

collagen leads to liver fibrosis. CCl₄-induced liver injury involves the increased expression of TGF-β1 mRNA and hydroxyproline (the unique component of collagen) content, as observed in liver fibrosis.

Heptaprotective Gffect of *A. cinnamomea* Fruiting Bodies Cgainsnt CCl₄-induced INiver Injury

Hsiao *et al.* (2003) investigated the hepatoprotective activity of the water extract of *A. cinnamomea* fruiting bodies against CCl₄-induced liver injury in ICR mice. Mice were subcutaneously injected with CCl₄ (40% CCl₄ diluted with olive oil, 0.1 mL/10 g BW) twice a week for 8 weeks to induce chronic chemical liver injury. Water extracts of *A. cinnamomea* fruiting bodies (0.25, 0.75, 1.25 mg/kg BW, day, 4 days a week) were orally administered for 8 weeks. The levels of plasma transaminases, i.e., AST and ALT, were significantly lower in the group of mice orally administered with the water extract of *A. cinnamomea* (1250 mg/mL) than in the corn oil control group, and were comparable to the silymarin positive group (100 mg/kg BW). The activities of hepatic superoxide dismutase and catalase were dose-dependently increased by treatment with the water extract of *A. cinnamomea*. The water extract of *A. cinnamomea* fruiting bodies showed an ability to scavenge 1,1-diphenyl-2-picrylhydrazyl radicals in an *in vitro* study (Hseu *et al.*, 2008). Thus, the water extract of *A. cinnamomea* fruiting bodies could effectively prevent CCl₄-induced hepatotoxicity by scavenging the

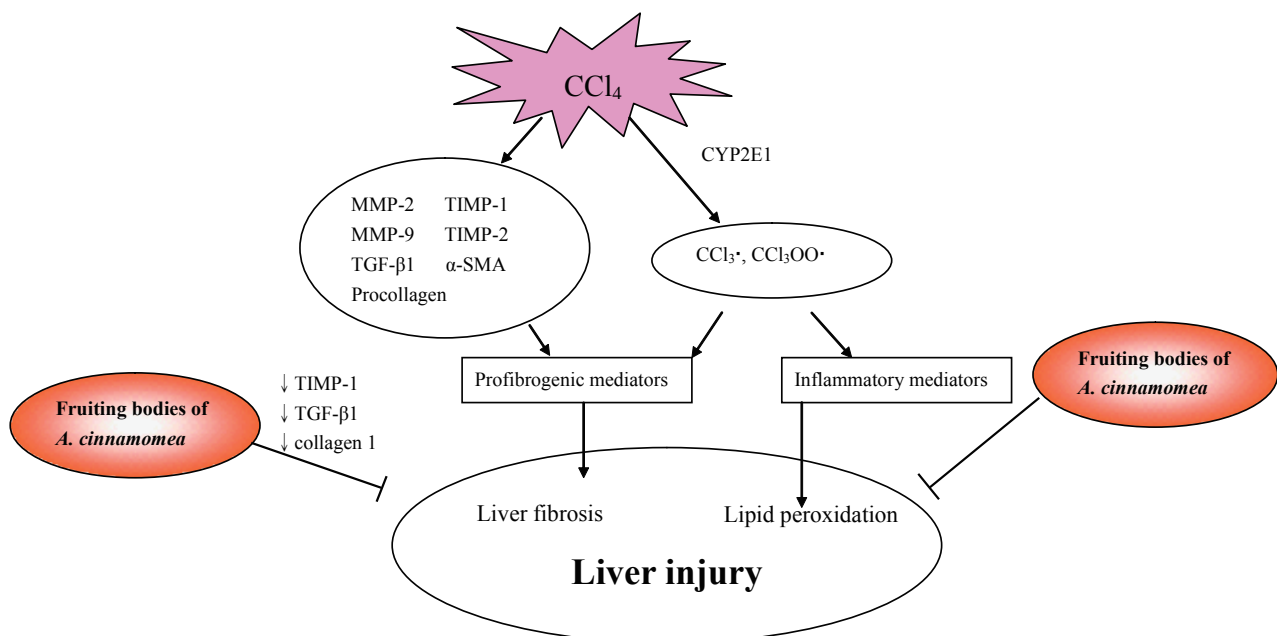


Figure 3. Mechanisms of fruiting bodies of *Antrodia Cinnamomea* against CCl₄-induced liver injuries.

free radical formation or by inhibiting the inflammatory mediators in CCl₄-mediated lipid peroxidation. The protective mechanisms of the fruiting bodies of *A. cinnamomea* against CCl₄-induced liver injuries, liver fibrosis, and lipid peroxidation are shown in Figure 3.

***Antrodia Cinnamomea* and Liver Cancer**

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies worldwide, especially in Taiwan, China, Korea, and Sub-Saharan Africa (Marrero, 2006). When cancer cells become invasive and metastatic, the excess breakdown of the extracellular matrix (ECM) occurs (Christofori *et al.*, 2006). MMP-2 and MMP-9, members of the MMP family that degrade the ECM during tissue remodeling, are believed to play important roles in the invasion and metastasis of liver cancers (Hofmann *et al.*, 2005).

Protective Effect of *A. cinnamomea* Fruiting Bodies Against Liver Cancer

Although the hepatoprotective effects of *A. cinnamomea* fruiting bodies and mycelia have been reported, studies on the effect of *A. cinnamomea* fruiting bodies in liver cancer are few. Hsu *et al.* (2005) reported the apoptotic effects of the ethyl acetate extract of *A. cinnamomea* fruiting bodies in human HCC cell lines (i.e., HepG2 and PLC/PRF/5). They found that the extract inhibited cell survival signaling by increasing the expression of I κ B α in the cytosol and decreasing the amount of NF- κ B in the nucleus, leading to the increased expression of Bcl-X_L in both HepG2 and PLC/PRF/5 cells. Hsu *et al.* (2007) further reported the anti-invasion potential of the ethyl acetate extract of *A. cinnamomea* fruiting bodies in PLC/PRF/5 cells. This extract decreased the expression of MMP-2, MMP-9, membrane type 1-MMP, and vascular endothelial growth factor, which resulted in the anti-invasion effects on PLC/PRF/5 cells. Hsieh *et al.* (2010) isolated 3 triterpenoid compounds, antcin A, antcin C, and methyl antcinate A, from the fruiting bodies and investigated the growth-inhibitory effects of these 3 compounds in human liver cancer cells (i.e., HepG2, Hep3B, and Huh7). Methyl antcinate A showed more inhibitory activity than the other 2 compounds and was most potent in Huh7 cells. Methyl antcinate A decreased the levels of antiapoptotic proteins (i.e., Bcl-2 and Bcl-X_L), increased the cytochrome *c* release from mitochondria to the cytosol, increased the mitochondrial translocation of cofilin and Bax, and enhanced NADPH activity in cells, suggesting that the apoptotic

effects of methyl antcinate A were partially through a mitochondrial signaling pathway. These results indicated that methyl antcinate A isolated from *A. cinnamomea* fruiting bodies is a potential candidate in the treatment of HCC. Hsieh *et al.* (2011) further reported the apoptotic activities of antcin B and methylantcinate B, 2 triterpenoids isolated from *A. cinnamomea* fruiting bodies. Antcin B and methylantcinate B both showed cytotoxicity in 3 HCC cell lines, i.e., HepG2, Hep3B, and Huh7. HepG2 showed more sensitivity to antcin B and methylantcinate B than the other cell lines and was further used for a mechanism study. Internucleosomal DNA fragmentation and subdiploid peak formation were observed in both antcin B and methylantcinate B-treated HepG2 cells, which suggested that both compounds triggered apoptotic cell death. The caspase activation and mitochondria-dependent pathways were both involved in the apoptosis induced by antcin B and methylantcinate B. The results indicated that antcin B and methylantcinate B induced NADPH oxidase-provoked oxidative stress and extrinsic and intrinsic apoptosis, as a critical mechanism of causing cell death in HCC cells.

Protective Effect of *A. cinnamomea* Mycelia Against Liver Cancer

Not only the fruiting bodies but also the fermented mycelia of *A. cinnamomea* were investigated for their anticancer activities. Song *et al.* (2005a) reported the effect of the methanolic extract of mycelia (MEM) from a submerged culture of *A. cinnamomea* on the inhibition of cell viability, as well as the mechanism of this effect. *Antrodia Cinnamomea* MEM inhibited the viability of liver cancer cells, i.e., HepG2 and Hep3B, in a dose-dependent manner. HepG2 cells showed more susceptibility to MEM than Hep3B cells. Furthermore, MEM had no significant inhibitory effect on normal primary rat hepatocytes. MEM induced DNA fragmentation in both HepG2 and Hep3B cells. However, G₀/G₁ cell cycle arrest was only observed in MEM-treated HepG2 cells. MEM resulted in the increase of caspase-3 and caspase-8 proteolytic activities and induced the proteolysis of pro-caspase-8 and procaspase-3. The above results indicated that MEM induced HepG2 apoptosis through the activation of caspase-3 and caspase-8 cascades and the regulation of cell cycle progression. Furthermore, Song *et al.* (2005b) reported the effects of MEM on the Fas and Bcl-2 pathways. Since Fas belongs to

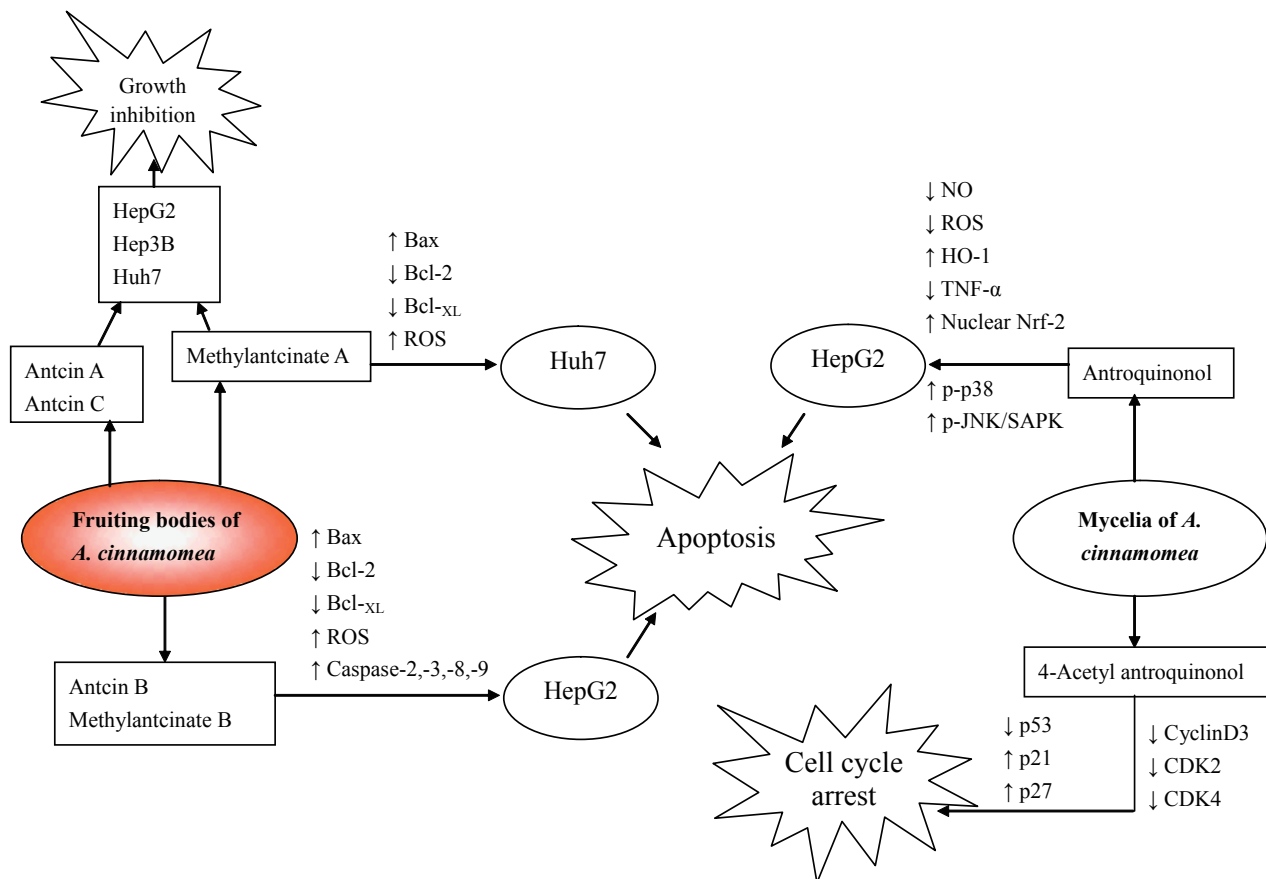


Figure 4. Mechanisms of fruiting bodies, mycelia, and active compounds of *Antrodia cinnamomea* against liver cancer cells.

the TNF receptor (TNFR) superfamily, Fas ligands are known to induce apoptosis through Fas-induced caspase cascade activation, first caspase-8 followed by caspase-3 (Yoon et al., 2002). The expression of Fas was significantly increased in MEM-treated HepG2 cells (Song et al., 2005b). The expression of death receptors (DR), including DR3, DR4, TNFR1, and TNFR2, were decreased by MEM in both a dose- and time-dependent manner. When HepG2 was treated with MEM, the expression of the antiapoptotic protein Bcl-2 was decreased but that of the proapoptotic protein Bcl-XL increased. The MEM-induced cell apoptosis possibly involves the upregulation of Fas expression, which promotes Fas and FasL ligation and then passes the death message to cytosolic messengers. Therefore, procaspase-8 is activated to caspase-8, thus triggering the caspase activation cascade. The studies of Song et al. (2005a,b) did not identify the active compounds that contribute to the anti-HCC effects of *A. cinnamomea* mycelia extracts. Lin et al. (2010) reported 4-acetylanthroquinonol B isolated from the ethanolic extract of *A. cinnamomea* mycelia to be the major antihepatoma constituent. The authors

used antiproliferative activity-guided isolation to trace the active compound and identify its structure. 4-Acetylanthroquinonol B was purified and identified as the most potent compound to inhibit the proliferation of HepG2 cells. The authors also reported that the antiproliferation mechanism of 4-acetylanthroquinonol B (Lin and Chiang, 2011) in HepG2 cells was through a dose-dependent cell cycle arrest at the G phase. In the G1 phase, cyclin D, cyclin E, CDK2, and CDK4/6 work together to promote cell cycle progression. In 4-acetylanthroquinonol B-treated HepG2 cells, the protein expression of CDK2 and CDK4 was slightly decreased. p27, a cell-cycle regulator, was reported to be inactivated in HCC and considered a suppressor of liver cancer cells. The expression level of p27 in HepG2 cells was increased by 4-acetylanthroquinonol B. Thus, the growth-inhibitory effect of 4-acetylanthroquinonol B was mostly mediated by cell cycle arrest through the decrease in CDK2 and CDK4 and increase in p27. Figure 4 shows the protective mechanisms of *A. cinnamomea* against liver cancer cells, including growth inhibition, apoptosis, and cell cycle arrest.

Active Compounds Against Liver Injury in the Fruiting Bodies and Mycelia of *A. cinnamomea*

Antrodia Cinnamomea fruiting bodies were reported to be rich in flavonoids, terpenoids, polyphenols, and polysaccharides (Chiang *et al.*, 2010). In the study of Huang *et al.* (2010a), the authors used high-performance liquid chromatography to analyze the components of the methanolic extract of *A. cinnamomea* fruiting bodies. Ten compounds were run under the same condition as standards to identify the chemical components in the sample, including ascorbic acid, 2,3-dimethoxy-5-methyl[1,4] benzoquinone, 2,4,5-trimethoxybenzaldehyde (COX-2 inhibition, Chen *et al.*, 2007), 4,7-dimethoxy-5-methyl-1,3-benzodioxide (antitumor, Tu *et al.*, 2012), *R,S*-zhankuic acid C, dehydrosulphurenic acid (anti-inflammatory activity, Shen *et al.*, 2007), *R,S*-zhankuic acid A, zhankuic acid B (anti-inflammatory activity, Shen *et al.*, 2004), dehydroeburicoic acid (antileukemia, Du *et al.*, 2012), and ergostatrien-3 β -ol (anti-inflammation, Huang *et al.*, 2010b). All 10 compounds were detected in the methanolic extract of the fruiting bodies. Wu *et al.* (2011) also analyzed the chemical contents of the *A. cinnamomea* fruiting body. According to their data, the fruiting bodies contain 0.24% polysaccharides, 12.95% triterpenoids, 0.3% total polyphenols, 0.05% flavonoids, and 0.015% condensed tannins.

Submerged fermentation or solid-state culture is a process extensively used for obtaining mycelia. Mycelial components such as maleic and succinic acid derivatives and triterpenoids were also investigated (Lu *et al.*, 2011; Nakamura *et al.*, 2004; Shao *et al.*, 2008). Nakamura *et al.* (2004) reported 5 new maleic and succinic acid derivatives together with ergosterol peroxide from *A. cinnamomea* mycelia. Shao *et al.* (2008) identified 12 compounds from the methanolic extract of *A. cinnamomea* mycelia. Among them are 4 new compounds: 10-hydroxy- γ -dodecalactone, 11-hydroxy- γ -dodecalactone, 2-(2-hydroxyethyl)phenol, and 12-hydroxydodecanoic acid methyl ester; the other 8 compounds were ergostatrien-3 β -ol (ST-1), ergosterol peroxide, methyl (4-hydroxyphenyl) acetate, vanillin, 4-hydroxybenzaldehyde, hexadecanoic acid, 5-methoxymethylfuran-2-carbaldehyde, and 5-hydroxymethylfuran-2-carbaldehyde.

Lu *et al.* (2011) investigated the hepatoprotective effect of the fractions from ethanolic extracts of *A. cinnamomea* mycelia and analyzed the composition of fractions (Fr-) I to III. Fr-I is a triterpenoid-

enriched fraction making up 359.8 mg per gram of mycelia. Fr-II is the polysaccharide content (483.1 mg/g), which consists of rhamnose, arabinose, xylose, mannose, glucose, and galactose in a ratio of 0.11:0.23:0.06:0.77:0.29:1.00. Finally, Fr-III is a polyphenol-enriched fraction. The triterpenoid-enriched fraction, Fr-I, showed the best hepatoprotective activity against ethanol-induced acute liver injury in SD rats. However, the active compounds contributing to these hepatoprotective effects have not been reported up to now.

The active compounds isolated from *A. cinnamomea* shown to exhibit anti-liver cancer activities are listed in Table 2. Anticin A, anticin B, anticin C, and their derivatives methylantcinate A and methylantcinate B belong to the ergostane-type triterpenoids. Antroquinonol and 4-acetylanthroquinonol B are ubiquinones. These compounds were reported to have anti-liver cancer activities in human hepatoma cell lines such as HepG2, Hep3B, and Huh7. As shown in Figure 4, anticin B, methylantcinate A, methylantcinate B, and antroquinonol exerted apoptosis-inducing effects through the mitochondrial- or ROS-mediated pathways, whereas 4-acetylanthroquinonol inhibited cell growth by activating cell cycle arrest.

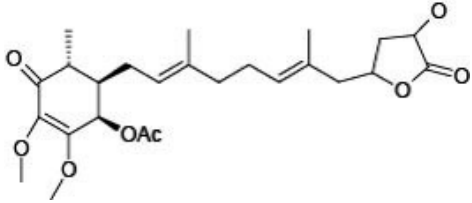
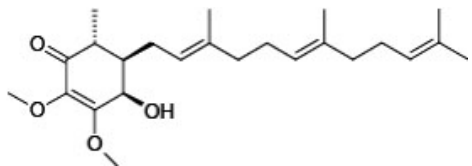
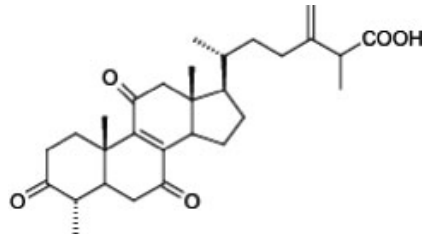
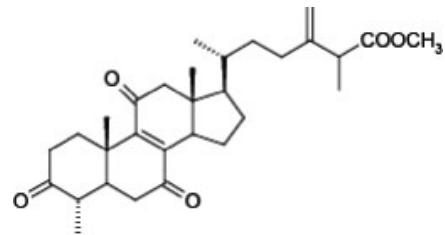
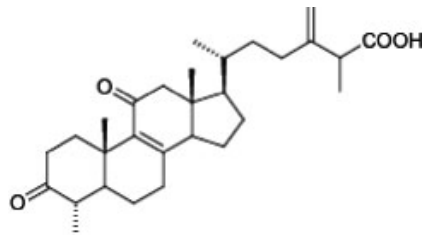
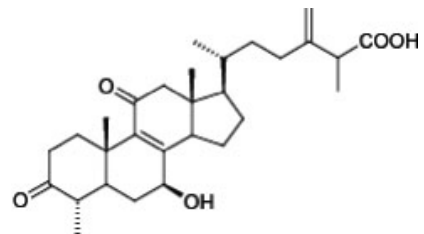
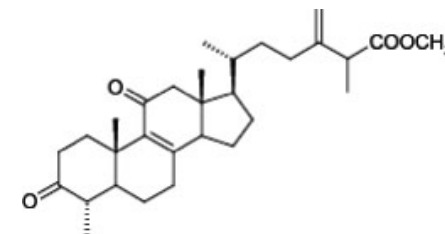
Conclusion

Antrodia Cinnamomea attracts much attention for its effectiveness in treating liver injuries, as shown in numerous published articles. The hepatoprotective effects of the fruiting bodies and mycelia of this mushroom have been investigated in animal models of ethanol- and CCl₄-induced liver injury. The extracts of *A. cinnamomea* fruiting bodies and mycelia have also been investigated for their anti-liver cancer activities. Several active compounds were identified to have anti-cancer activity *in vitro*.

The protective mechanisms of *A. cinnamomea* against ethanol-induced liver injuries were found to be through the inhibition of fatty acid synthesis and liver fibrosis (Figure 2). In addition, *A. cinnamomea* inhibited lipid peroxidation and liver fibrosis in CCl₄-intoxicated animals (Figure 3). As for the anti-liver cancer effects, 7 compounds from *A. cinnamomea* were found to inhibit cell growth by the activating apoptosis or cell cycle arrest in human hepatoma cells (Figure 4).

The present report provides scientific evidences for the use of *A. cinnamomea* in ameliorating liver diseases. However, human clinical trials for studying the

Table 2. Compounds isolated from *Antrodia Cinnamomea* exhibiting anti-liver cancer activity.

Compounds	Chemical structures	References
4-Acetylanthroquinol B		Lin and Chiang, 2011
Antroquinolol		Kumar et al., 2011
Antcin B		Hsieh et al., 2011
Methylantcinate B		Hsieh et al., 2011
Antcin A		Hsieh et al., 2010
Antcin C		Hsieh et al., 2010
Methylantcinate A		Hsieh et al., 2010

effectiveness of *A. cinnamomea* are still ongoing (<http://clinicaltrials.gov/ct2/show/results/NCT01287286>). The results of the studies described here suggest the potential of *A. cinnamomea* to prevent and treat liver diseases, as well as its potential to be developed into health foods or new drugs.

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