Antrodia Cinnamomea Reduces Carbon Tetrachloride-induced Hepatotoxicity In Male Wister Rats

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Abstract. Background/Aim: Antrodia cinnamomea is found with polysaccharides, lipids, vitamins, fibers and ash (minerals) and is well known in Taiwan as a traditional Chinese medicine. Its biological activities have been reported to have anti-inflammatory, anti-fatigue, anti-tumor and immunomodulatory effects, but its protective effects on liver function are still unclear. Materials and Methods: We determined if Antrodia cinnamomea was hepatoprotective against carbon tetrachloride (CCl4) toxicity in Wistar rats. Six groups were used in the study: 1) control (no induction by CCl4); 2) negative control (CCl4-induction and no treatment); 3) positive control (silymarin treatment); 4) groups 4-6 were treated with CCl4 and different concentrations (350 mg/kg, 1,400 mg/kg, 3,150 mg/kg) of Antrodia cinnamomea. Blood and liver samples of rats were harvested and then detected by biochemical and tissue histochemical analysis. Activity of the antioxidative enzymes glutathione peroxidase, superoxide dismutase and catalase in the liver were also monitored. Results: Only the high-dose treatment was able to decrease serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels and improve liver function. High and medium doses increased total liver protein and reduced hydroxyproline. It was also observed that the high dose treatment reduced lipid peroxidation. Liver sections of CCl4 treated animals receiving Antrodia cinnamomea showed less fibrosis compared to the CCl4 control group. Conclusion: This finding suggested that Antrodia cinnamomea can either enhance liver recovering from CCl4 damage or attenuate CCl4 toxicity in rats.

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Key Words: Antrodia cinnamomea, carbon tetrachloride, hepatotoxicity, Wistar rats, hepatic fibrosis.
health needs, and most of these therapies involve herbal extracts (1, 2). Although scientific studies have shown the uncertainty of CAM benefits and the lack of well controlled studies involving this type of medicine, cancer patients have increasingly selected complementary and alternative medicines for treatment (3). Recently, up to 50.4% of adults and 84.6 % of females in Taiwan use CAM to treat multiple cancers (4).

Mushrooms have been used in food since ancient times and with the oldest archaeological record regarding their profound flavor and distinct taste. Mushrooms contribute various nutrients in the human diet and are used as medicines in the oriental tradition. The use of mushrooms and/or their extracts has increased and many mushroom species are diminutive pharmaceutical factories with biological effects such as anti-oxidants, antitumor, anticarcinogenic, antiviral, anti-inflammatory, prebiotic, hypoglycemic, immunomodulating, anti-microbial, anti-diabetic and anti-hypertensive functions (5-9).

Antrodia camphorata (Syn. Antrodia cinnamomea), a popular medical mushroom well-known in Taiwan, is a natural fungal parasite on the inner trunk cavity of the endemic species Cinnamomum kanehirae (Bull camphor tree) Hayata (Lauraceae). The host plant is a large evergreen broad-leaf tree, that only grows in the central and northern parts of Taiwan, and is distributed over broad-leaf forests on hillsides at an altitude between 200 and 2,000 m (10).

Antrodia cinnamomea (AC) extract has been found to have a complex mixture of bioactive ingredients, such as triterpenoids, steroids, polysaccharides, and phenyl and biphenyl compounds (11) and possess health benefits (antioxidant, anti-itching and hepatoprotective effects) (12, 13). A previous study has demonstrated its potential use as complementary and alternative therapeutic agent for the treatment of various cancers (14). Maleimide derivative isolated from AC suppresses breast cancer cell migration and invasion (15). In addition, AC fruiting body extract has cytotoxic effects on hepatoma HepG2 and PLC/PRF/5 cells (16). AC extract combined with anti-tumor agents has also antiproliferative effects on hepatoma cells in vitro and in vivo by inhibiting multi-drug resistance (MDR) gene expressions and COX-2-dependent phospho-AKT (p-AKT) signaling (17). In addition to its anticancer properties, it is also thought to be efficacious for musculoskeletal disorders, psychiatric conditions, influenza, cold, headache, fever and other conditions. AC has attracted great attention, and the available information show that it is able to reduce chronic CCl4-induced hepatic fibrosis resulting from chronic damage to the liver in conjunction with the progressive accumulation of fibrillar extracellular matrix protein (18). The main causes of hepatic fibrosis in humans include infection by hepatitis B and C, alcohol abuse and non-alcohol steatohepatitis; and experimentally, liver cirrhosis can be induced by carbon tetrachloride (CCl4), (19) which has been used widely to induce liver injury in animal models (20). In the current study, we aimed to increase the understanding of the effects of A. cinnamomea on liver damage and determine if AC would reduce chronic CCl4-induced liver injury in rats.

Materials and Methods

Reagents. CCl4, olive oil and other reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Alanine aminotransferase (ALT) kit, aspartate aminotransferase (AST) kit and albumin were purchased from Roche Cobas Mira (Roche Diagnostic Systems, Montclair, New Jersey, USA).

Preparation of AC test solution. AC and 2 ml distilled water were mixed thoroughly to provide a final solution with a concentration of 350, 1,400 and 3,150 mg/kg. AC was obtained from Chang Gung Biotechnology Corporation, Ltd. (Taipei, Taiwan).

Experimental animals. Male Wistar rats, specific pathogen-free, 6 weeks old and weighing 250-300 g, were obtained from the Animal Medicine Center, College of Medicine, National Taiwan University and were used for the chronic CCl4-induced liver injury model in all experiments. The rats were housed in plastic cages and maintained under standard conditions of controlled room temperature at 20±2˚C and relative humidity of 75±15% with a 12 h light/night cycle with free access to standard laboratory diet and water, and filtered laminar air flow controlled room in the animal facility of the Animal Medicine Center, College of Medicine, National Taiwan University, Taipei, Taiwan. The use of experimental animals in this study was conducted under the guidance of the basic standards in the care and use of laboratory animals, which has been prepared and published by the National Institutes of Health. The study protocol has been approved by the Research Ethics Committee, the Institutional Animal Care and are in agreement with the Helsinki declaration.

Experimental design and protocol. After 1 week of acclimatization, a total of sixty rats were randomly divided into six groups (each group consisted of 10 rats) with healthy rats (Only H2O, on-induction group; normal control), cirrhotic rat group with vehicle (olive oil) treatment only (CCl4 + H2O, negative control; non-treatment group), cirrhotic rat group with silymarin treatment (CCl4 + silymarin, positive treatment control), and cirrhotic rat groups with three different doses of AC treatment (experiment groups). As a non-induction group, 10 rats without CCl4 induction were fed a regular diet and double-distilled water. The other 50 rats treated with CCl4 were further divided into non-treatment group (n=10), silymarin treatment group (n=10) and three AC treatment groups (n=30). In these 50 rats, animals were fed 20% CCl4 in a 1:4 mixture with olive oil at a dose of 2 ml/kg, twice a week for 8 weeks to induce liver damage. During the 8-week induction period, negative control rats were treated only by vehicle and positive control rats were treated with silymarin. Rats in the three AC groups received AC at a low dose (350 mg/kg/day), medium dose (1400 mg/kg/day) or high dose (3150 mg/kg/day) daily for 8 weeks. The animals’ weights were monitored prior to the start of experiment and then checked 2 times per week over the 8 week period. After the last dose, blood from all of the rats was drawn and collected by SST tube via caudal artery under mild ether anesthesia. Blood was
allowed to clot at room temperature and the serum was separated by centrifuging at 4000 rpm for 15 min and kept at \(-20^\circ\text{C}\) for further biochemical analysis. Rats were sacrificed, and the liver and spleen were dissected and used for histopathological (formalin fixed) and biochemical (frozen \(-80^\circ\text{C}\)) studies.

**Analysis of plasma transaminase activities and albumin level in serum.** Serum samples were prepared at the end of 8 weeks and were assayed for GOT, GPT activity and albumin concentration according to the manufacturer’s protocol for an estimation of liver function using a DxC 800 clinical chemistry analyzer with reagents (GOT lot number: M307050; GPT lot number: M312240; Albumin lot number: M310159) purchased from Beckman Coulter (Brea, CA, USA). These three tests were performed by the Animal Medicine Center, College of Medicine, National Taiwan University.

**Assays for Superoxide Dismutase Assay (SOD), Glutathione Peroxidase (GSH-Px) and Catalase.** Ten percent of homogenates of liver tissues were prepared separately in 100 mM KH₂PO₄ buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 \(\times\) g for 30 min at 4\(^\circ\)C. The supernatant was collected and used for the following experiments as described below.

In this method NADH (RANSOD manufactured by RANDOX Ltd. UK) was used as the substrate (21). Reaction mixture of this method contained 0.1 mL of phenazine methosulphate (186 \(\mu\)M), 1.2 mL of sodium pyrophosphate buffer (0.052 mM; pH 7.0), and 0.3 mL of supernatant after centrifugation (1500 \(\times\) g for 10 min followed by 10000 \(\times\) g for 15 min) of tissue homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 mL of NADH (780 \(\mu\)M) and stopped after 1 min by adding 1 mL of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

Glutathione peroxidase activity was assayed by the method of Mohandas et al. (22). Liver was homogenized with GSHPx cold buffer (50 mM Tris-HCl containing 5 mM EDTA and 1 mM diithiothreitol (DTT), pH 7.5). GSHPx activity was measured using a GSHPx assay kit. The reaction was initiated by the mixing glutathione, glutathione reductase, NADPH with cumene (isopropylbenzene) hydroperoxide, and then detecting conversion of NADPH to NADP with a spectrophotometer at 340 nm and 25\(^\circ\)C for 5 min. The specific enzyme activity was measured as nanomoles of NADPH oxidized to NADP per minute per milligram protein (oxidized/min/mg protein).

CAT activities were determined according to manufacturer’s protocols of corresponding kits. Using \(H_2O_2\) as a substrate (23), 0.1 mL of the supernatant was mixed with 2.5 mL of 50 mM phosphate buffer (pH 5.0) and 0.4 mL of 5.9 mM \(H_2O_2\), and change in absorbance was recorded at 240 nm after one min. One unit of CAT activity was defined as an absorbance change of 0.01 units/min.

**Hepatic protein, malondialdehyde (lipid peroxidation) and hydroxyproline assays.** Total liver protein concentration in each sample was measured using Coomassie blue assay reagent (KENLOR Industries Inc., CA, USA) based on an absorbance at 540 nm. Bovine serum albumin was used as a standard. The amount of total protein was expressed as mg/g tissue. Lipid peroxidation was measured using the method of Ohkawa et al. (24) and 2-thiobarbituric acid. Lipid peroxidation was expressed as the amount of malondialdehyde/mg protein. Hydroxyproline determination used procedures reported previously (25). After hydrolysis, dried liver tissue was oxidized by \(H_2O_2\) and colored by p-dimethylaminobenzaldehyde (Sigma Chemical Co. St Louis, Mo., USA). Absorbance was determined at 540 nm, and the amount of hydroxyproline was expressed as \(\mu\)g/g tissue.

**Histopathology.** For liver histology, liver tissue was immediately fixed in 10% buffered formaldehyde and the blocks were incubated twice with 10% neutral buffered formalin at room temperature for 0.5 h each, 75% alcohol at room temperature for 1 h, 85% alcohol at room temperature for 1 h, 95% alcohol at room temperature for 1 h, twice; 100% alcohol at 40˚C for 1 h, twice; xylene at 40˚C for 1 h, twice; and in molten wax at 60˚C for 0.5 h and repeated 4 times. Blocks were then embedded in paraffin. Some paraffin sections were stained using Haematoxylin-Eosin stain solution (Muto Pure Chemicals, Co., Ltd., Tokyo, Japan). Other paraffin sections were stained using Sirius red stain (Direct Red 80) (Sigma Chemical Co. St Louis, Mo., USA). The stained sections were examined under a microscope for histopathological changes in liver architecture and photomicrographs taken.

**Results**

**Analysis of GOT, GPT and Albumin in serum.** Table I shows that administration of CCl₄ caused an increase in glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) serum concentrations, that are markers of liver injury and dysfunction as compared with a non-treated control group. We also found that CCl₄ was associated with low albumin levels. The high dose AC administration reduced effects of CCl₄ on GOT and GPT levels but a high-dose treatment could not return serum markers to normal levels (Table I). AC treatment at a high dose inhibited effects of CCl₄ on albumin levels. Liver function was not significantly altered by silymarin which was considered a positive control treatment.

**SOD, Catalase and GSH-PX levels in CCl₄ and AC treated rats.** To determine if AC could inhibit effects of CCl₄ induced liver damage, we analyzed SOD, catalase and GSHPx levels. It can be seen in Table II that CCl₄ reduced SOD, catalase and GSHPx activities. Generally, AC did not counteract effects of CCl₄ on activity of the three proteins. An exception was that the high AC dose reduced the effects of CCl₄ on catalase activity (Table II).

**Hepatic protein, malondialdehyde and hydroxyproline levels.** A possible molecular mechanism involved in CCl₄ hepatotoxicity is the disruption of the delicate oxidant/antioxidant balance, leading to liver injury via oxidative damage (24). Moreover, CCl₄ is a prototypical lipid peroxidant that induces early lipid peroxidation in the liver (24). As a marker of lipid peroxidation, malondialdehyde (MDA) concentration was measured in all animal groups.

CCl₄-induced liver fibrosis in rats resulted in a significant decrease in hepatic protein content (\(p<0.001\)) and
Table I. The Effect of Antrodia cinnamomea on GOT, GPT and albumin in CCl₄-treated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GOT (U/L)</th>
<th>GPT (U/L)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only H₂O</td>
<td>68.8±6.8</td>
<td>36.6±7.9</td>
<td>3.61±0.14</td>
</tr>
<tr>
<td>CCl₄ + H₂O</td>
<td>1722.0±1005.4###</td>
<td>1576.0±802.9###</td>
<td>3.11±0.28###</td>
</tr>
<tr>
<td>CCl₄ +low dose</td>
<td>1534.5±810.5</td>
<td>1488.0±755.8</td>
<td>3.21±0.29</td>
</tr>
<tr>
<td>CCl₄ +medium dose</td>
<td>1454.5±820.5</td>
<td>1350.0±750.8</td>
<td>3.31±0.30</td>
</tr>
<tr>
<td>CCl₄ +high dose</td>
<td>709.5±365.9**</td>
<td>782.0±349.2*</td>
<td>3.39±0.09*</td>
</tr>
<tr>
<td>CCl₄ + silymarin$</td>
<td>1644.0±854.7</td>
<td>1506.5±705.0</td>
<td>3.19±0.26</td>
</tr>
</tbody>
</table>

All values are means±S.D. (n=10). ###p<0.001 compared to control group; *p<0.05; **p<0.01 compared to CCl₄ + H₂O group. $200 mg/kg.

Table II. The Effect of Antrodia cinnamomea on SOD, catalase and GSH-Px activities.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>GSH-Px (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only H₂O</td>
<td>13.5±2.5</td>
<td>19.1±3.2</td>
<td>1028.6±252.1</td>
</tr>
<tr>
<td>CCl₄ + H₂O</td>
<td>10.0±0.9###</td>
<td>12.5±4.0###</td>
<td>587.3±90.6###</td>
</tr>
<tr>
<td>CCl₄ +low dose</td>
<td>10.3±2.5</td>
<td>12.7±2.7</td>
<td>578.7±131.5</td>
</tr>
<tr>
<td>CCl₄ +medium dose</td>
<td>10.9±1.5</td>
<td>12.9±3.9</td>
<td>549.3±72.0</td>
</tr>
<tr>
<td>CCl₄ +high dose</td>
<td>11.3±0.7</td>
<td>15.0±1.9***</td>
<td>596.6±80.6</td>
</tr>
<tr>
<td>CCl₄ + silymarin$</td>
<td>10.4±1.2</td>
<td>15.2±3.7***</td>
<td>474.4±146.1</td>
</tr>
</tbody>
</table>

All values are means±S.D. (n=10). ###p<0.001 compared to control group; *p<0.05; **p<0.01; ***p<0.001 compared to CCl₄ + H₂O group. $200 mg/kg.

Table III. Effect of Antrodia cinnamomea on hepatic protein, Lipid Peroxidation and hydroxyproline content in CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/g tissue)</th>
<th>Lipid Peroxidation (nmol MDA/mg protein)</th>
<th>Hydroxyproline (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only H₂O</td>
<td>174.6±18.0</td>
<td>1.0±0.2</td>
<td>494.4±62.8</td>
</tr>
<tr>
<td>CCl₄ + H₂O</td>
<td>150.9±8.3###</td>
<td>1.5±0.4###</td>
<td>955.8±130.0###</td>
</tr>
<tr>
<td>CCl₄ +low dose</td>
<td>160.9±9.2</td>
<td>1.4±0.4</td>
<td>806.9±163.3</td>
</tr>
<tr>
<td>CCl₄ +medium dose</td>
<td>168.3±7.3*</td>
<td>1.2±0.1</td>
<td>791.2±89.6*</td>
</tr>
<tr>
<td>CCl₄ +high dose</td>
<td>177.7±19.0***</td>
<td>1.1±0.1**</td>
<td>732.1±78.7***</td>
</tr>
<tr>
<td>CCl₄ + silymarin$</td>
<td>146.5±13.3</td>
<td>1.5±0.3</td>
<td>828.3±211.3</td>
</tr>
</tbody>
</table>

All values are means±S.D. (n=10). ###p<0.001 compared to control group; *p<0.05; **p<0.01; ***p<0.001 compared to CCl₄+ H₂O group. $200 mg/kg.

accompanied by a marked elevation of malondialdehyde (p<0.001) and hydroxyproline (p<0.001) compared to the control group. Medium or high-dose treatment of Antrodia cinnamomea attenuated the decrease of hepatic protein level induced by CCl₄ but silymarin did not. Antrodia cinnamomea could not lower the elevation in malondialdehyde except with a high dose treatment. Only medium or high-dose treatment of Antrodia cinnamomea could lower the increase in hepatic hydroxyproline content (Table III).

Effects of AC on liver pathology. The pathological changes of liver tissues by HE staining were observed. In the negative control group, liver tissues were radiated around the central veins, and liver cells were arranged neatly without degeneration, steatosis, cavitation, fibrosis or necrosis. Negative control tissue sections exhibited no apparent pathological changes (Figure 1a). In contrast, Figure 1b shows that in the model group, sections from CCl₄-only treated rats displayed cavitations in broad areas and fatty changes like ballooning of cells, inflammatory cell infiltration, and dilation of central vein, cellular hypertrophy, necrosis, and degeneration of the lobular architecture. AC treatment attenuated CCl₄-induced liver pathology as seen in Figure 1c Silymarin was not effective in reducing effects.
Figure 1. Microphotograph of rat liver (H&E stain). (a) Representative section of liver from the control group showing normal histology. Normal hepatic cells are characterized by well-defined cell linings, prominent nucleus, and prominent central vein surrounded by reticular fibers. (b) Massive necrosis formation, hepatocytes ballooning, distortion of hepatocytes, shrinkage of nucleus, clear cell foci formation, loss of cellular boundaries, and reticular fibers were observed in CCl₄-intoxicated rat liver section thus indicative of extensive liver injuries. (c) High-dose treatment of Antrodia cinnamomea partly prevented hepatoprotective activity. The histopathological changes such as necrosis, ballooning, clear cell foci formation, and structural loss of hepatic lobules were moderate recovery. (d) However, the histological architecture of liver sections of the rats treated with silymarin still showed some cavities and necrosis.

Figure 2. Sirius red staining of rat liver sections. a: Control; b: CCl₄ + H₂O, showing micronodular formation and complete septa interconnection with each other; c: CCl₄ + high-dose treatment of Antrodia cinnamomea; d: CCl₄ + silymarin.
of CCl$_4$ on liver injury (Figure 1d). Figure 2 shows that CCl$_4$ induced liver lesions in rats. Sirius red stain indicated obvious nodular fibrosis (Figure 2b). AC at a high dose reduced CCl$_4$ induced-collagen accumulation in contrast to silymarin (Figure 2c and d).

Discussion

Medicinal plant extracts and their bioactive metabolites have been shown to induce the prevention of oxidative damages which especially from CCl$_4$ induced hepatic injuries animals studies (26). *Antrodia*, one of the widely known medicinal mushrooms, belongs to the family *Fomitopsidaceae* which is valued in Taiwan for its medicinal effects. Yang *et al.* described some of his early experimental findings that the fermented culture broth of *Antrodia camphorata* was associated with inducing cell cycle arrest and apoptosis of human estrogen-non-responsive breast cancer (MDA-MB-231) cells and also demonstrated that non-cytotoxic concentrations (20-80 μg/ml) of *Antrodia camphorata* markedly invaded the invasion/migration via inhibition of the MAPK signaling pathway (27). *Antroquinonol*, a ubiquinone derivative isolated from *Antrodia camphorata*, was capable of inducing anti-cancer activity involving G1 arrest of the cell cycle and subsequent apoptosis in human pancreatic cancers through asuppression of PI3-kinase/Akt/mTOR pathways. Moreover, antroquinonol induced the down-regulation of several cell cycle regulators and mitochondrial anti-apoptotic proteins but caused the up-regulation of p21(Waf1/Cip1) and K-ras (28). Peng *et al.* demonstrated that treatment with *Antrodia camphorata* crude extract inhibited both the superficial cancer cell line RT4 through overexpression of p21 and the metastatic cell lines (TSGH-8301 and T24) via down-regulation of Cdc2 and Cyclin B1 (29). Hseu *et al.* reported that *Antrodia camphorata* induced apoptosis and suppressed cyclooxygenase-2 (COX-2) in MDA-MB-231 cancer cells (30). Hseu *et al.* also demonstrated that cell-cycle arrest was related to a decrease in cyclin D1, cyclin E, CDK4 and cyclin A levels, and increased CDK inhibitor p27/KIP and p21 in *Antrodia camphorata*-treated MDA-MB-231 cells (31).

Our results demonstrated that treatment with CCl$_4$ promoted a marked increase in serum GPT and GOT activities that were due to liver damage in rats. The concentrations of GPT and GOT in the pathological model group significantly rose compared with the control group, indicating that the model is successful.

High-dose treatment with *Antrodia camphorata* protected the liver from damage by CCl$_4$. Albumin produced by liver was also ameliorated only with a high dose treatment which means that liver function was improved only by high-dose treatment. We may conclude that Rats treated with high dose *Antrodia cinnamomea* showed a protection against CCl$_4$-induced hepatotoxicity, with the levels of both plasma GOT and GPT being reduced. CCl$_4$ treatment caused alteration in cholesterol profile. In this present investigation, we did not analyze the concentration of cholesterol.

*Antrodia camphorata* did not alter CCl$_4$-induced effects on SOD and GPX, but at a high concentration inhibited the reduction in catalase. Catalase significantly rose compared with the model control group. The liver synthesizes not only the protein it needs, but also produces numerous export proteins including albumin, globulin, fibrinogen, clotting factors and regulatory proteins (32). We found that CCl$_4$ induced liver fibrosis in rats and it appeared to damage liver function and cause a decrease in both hepatic protein and serum albumin levels. High-dose treatment of *Antrodia camphorata* increased protein content in the lives (Table III) and albumin content in the serum (Table I). These results provide further support that there is a hepatoprotective effect.

Both free radical production and lipid peroxidation have been reported previously as major cellular mechanisms involved in CCl$_4$ hepatotoxicity (33). Furthermore, a dependent relationship has been proposed between lipid peroxidation and fibrogenesis in rats, in which fibrosis was induced by CCl$_4$ administration (34). We also showed that liver lipid peroxidation was associated with increased hepatic fibrogenesis. Moreover, we observed that *Antrodia camphorata* inhibited CCl$_4$-induced liver lipid peroxidation. CCl$_4$ treatment significantly increased MDA levels, whereas pre-treatment with AC eliminated CCl$_4$-induced MDA upregulation, suggesting that AC itself and/or AC-induced gene products may have antioxidant properties against reactive oxygen species (ROS) and free radical scavenging abilities.

Increased collagen synthesis contributes to liver fibrosis (35). Hydroxyproline is the characteristic component in collagen. The amount of collagen can be predicted by detecting hydroxyproline amounts and can be used to estimate the degree of liver fibrosis (36). We found that liver fibrosis was associated with hydroxyproline levels. *Antrodia camphorata* at medium or high doses was able to decrease hydroxyproline levels, indicating that it could lessen the actions of hepatic fibrosis caused by CCl$_4$. In our CCl$_4$-induced chronic liver injury model, we observed that livers of *Antrodia camphorata*-treated rats displayed less pathology as compared with the CCl$_4$-only treated group. An overriding conclusion of this study is that *Antrodia camphorata* has recovery/reparative effects in liver injury induced by CCl$_4$.

In conclusion, we demonstrated that treatment with *Antrodia cinnamomea* suppresses CCl$_4$-induced anorexia and hepatic injuries and also proved that *Antrodia cinnamomea* has the ability to recover the metabolic enzymatic activities and repair cellular injuries, thus providing scientific evidence in favor of its pharmacological use in hepatic dysfunction. We hypothesize that the hepatoprotective effect of *Antrodia cinnamomea* is attributed to its antioxidant role. To our knowledge, this is the first evidence suggesting that *Antrodia*
Antrodia cinnamomea protects against CCl₄ induced acute hepatotoxicity. Although further investigation is needed to clarify the active component within Antrodia cinnamomea, these findings are expected to improve our understanding of the protective effect of this herbal medicine against CCl₄ induced organ injury and disease.

Conflicts of Interest

The Authors declare that there is no conflict of interest to disclose.

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