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RESEARCH NOTE

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# Hepatoprotective Effect of *Grifola frondosa* Water Extract on Carbon Tetrachloride-induced Liver Injury in Rats

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**Abstract** The present study aimed at assessing the protective effect of water extract from fruit body of the *Grifola frondosa* (GFW) on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity. Rats orally administered with GFW 0.5, 1.0, 2.0 g/kg for 14 days were treated with $CCl_4$ to induce hepatotoxicity. Pretreatment with GFW remarkably prevented the elevation of serum AST, ALT, ALP, LDH, γ-GTP, and liver lipid peroxides in $CCl_4$-treated rat and GFW administration in liver injured rats by $CCl_4$ showed significant ($p<0.05$) protection of liver as evidenced from normal serum enzymes and malondialdehyde (MDA) levels. In the ultrastructural changes, administration of $CCl_4$-induced damage of hepatocytes with vacuolation, a highly damaged endoplasmic reticulum, and degenerating nuclei. However, pre-administration with GFW preserved normal ultrastructure of hepatocytes. These results suggest that GFW had an effect to inhibit $CCl_4$-induced liver injury in rat, and that it could be used as an effective hepatoprotective agent against chemical-induced liver damage.

**Keywords:** *Grifola frondosa*, carbon tetrachloride ($CCl_4$), hepatoprotection, ultrastructural change, aspartate aminotransferase (AST)/alanine aminotransferase (ALT)

## Introduction

Mushrooms have been reported to exhibit various biological effects such as antiviral, antibiotic, anti-inflammatory, hypoglycemic, hypocholesterolemic, and hypotensive activities (1,2). *Grifola frondosa*, especially, is one of the most popular edible mushrooms, and has been also used as a medicine for centuries in China and Japan (3). The dried powder of *G. frondosa* fruit body has been shown a blood pressure lowering effect in the spontaneously hypertensive rat (4), and also been recommended as a remedy for palsy and neuralgia (3). The D-fraction, polysaccharides extracted from *G. frondosa*, has shown particular promise as immunomodulating agents, and as an adjunct to cancer and HIV therapy (5-11). The polysaccharides are also suggested to provide some benefit in the treatment of hyperlipidemia, hypertension, and hepatitis. Because of its amazing health benefits as well as its large fruiting body, it has come to be called 'the king of mushrooms'. However, in spite of many biological effects of *G. frondosa*, there is little evidence to address that *G. frondosa* has a hepatoprotective activity.

The liver is the main site of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds including clinically useful drugs can cause cellular damage through metabolic activation of the compound to highly reactive substances such as free radicals. Carbon tetrachloride ($CCl_4$) is one such environmental toxicant and is most widely used for the different animal models of liver damage including hepatic fibrosis and cirrhosis in case of long term administration in laboratory animals (12,13). Hepatotoxicity of $CCl_4$ is attributed to the formation of trichloromethyl and trichloromethyl peroxyl radicals initiating lipid peroxidation and resulting in fibrosis and cell necrosis (14,15). Liver injury by administration of $CCl_4$ is characterized by the presence of high level of liver enzymes in serum (16) and histological manifestations of inflammation and fibrosis (14,17). Furthermore, $CCl_4$-induced fibrosis in experimental animals is very similar to human fibrosis in the aspects of morphology and pathophysiology (13,18). In the present study, we investigated the hepatoprotective effect of water extract from *G. frondosa* fruit body (GFW) against $CCl_4$-induced liver toxicity in rats.

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## Materials and Methods

**Preparation of the GFW** Dried *Grifola frondosa* fruit body purchased from Chiba market (Tokyo, Japan) was extracted with 20-fold water for 24 hr at 40°C. The extract was filtered with Whatman filter paper No. 2. The filtrate was concentrated by a vacuum evaporator, freeze dried, and preserved at −20°C until use. About 49.2 g of dried water extract was obtained from 100 g dried *G. frondosa* fruit body. The extract was resuspended in distilled water, and used for experiments.

**Experimental design** Male Sprague-Dawley rats (5 weeks old) were acclimatized for 5 days under the experimental environment on a 12/12-day/night cycle, temperature at 25±2°C and relative humidity of 50±10%. They were provided with standard pellet diet (5L79 Lab diet; Purina Mills Co., St. Louis, MO, USA) and water *ad libitum*. Rats were divided into 5 groups with 7 rats in each group by GFW and $CCl_4$ treatment. Table 1 summarizes the experimental groups and treatments used in this study.

**Table 1. Experimental groups**

| Group[1)] | GFW dose (g/kg) | Treatments |
|---|---|---|
| CON | - | Basal diet |
| $CCl_4$ | - | Basal diet+$CCl_4$ |
| GFW I | 0.5 | Basal diet+GFW+$CCl_4$ |
| GFW II | 1 | Basal diet+GFW+$CCl_4$ |
| GFW III | 2 | Basal diet+GFW+$CCl_4$ |

[1)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

**Table 2. The changes of body weight of rats[1)]**

| Group[2)] | Initial weight | Final weight | Weight gain (g/day) |
|---|---|---|---|
| CON | 173.58±8.83[a] | 264.31±8.78[a] | 6.52±0.24[a] |
| $CCl_4$ | 173.03±6.83[a] | 249.91±5.28[b] | 5.49±0.65[c] |
| GFW I | 173.33±6.26[a] | 259.04±5.21[a] | 6.11±0.42[b] |
| GFW II | 172.69±5.53[a] | 258.57±4.60[a] | 6.13±0.47[ab] |
| GFW III | 172.49±7.54[a] | 259.34±5.20[a] | 6.20±0.26[ab] |

[1)]The values are mean±SD (n=7); the values followed by the same superscript letter in each column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

**Blood collection and measurement of internal organ weight** Blood was collected from the main abdominal artery of rats fasted for 12 hr prior to the administration of ether anesthesia. The blood was centrifuged at 2,250×g for 20 min at 4°C in order to obtain serum. Internal organs (liver, kidney, spleen, heart, and lung) were extracted after washing out the blood with ice cold 0.15 M KCl buffer solution, then, washed with physiological saline. The weight of the organs was expressed as gram per 100 g body weight.

**Biochemical analyses of serum** The marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP) were assayed in serum from kit (Asan Pharma. Co., Ltd., Hwasung, Korea). Also, components (albumin, total protein, high density lipoprotein (HDL)-cholesterol, triglyceride, and total cholesterol) of serum were determined by enzymatic assay using standard test kits (Asan Pharma. Co., Ltd.).

**Lipid peroxidation assay** The mean malondialdehyde (MDA) content (μmol/mg protein), a measure of lipid peroxidation, was assayed in the form of thibarbituric acid-reacting substances (TBARS) by the method of Ohkawa *et al.* (19).

**Electron microscopy of liver tissues** The liver was cut into 1 $mm^3$ cubes right after dissection and prefixed in 2.5 % glutaraldehyde solution in 0.1 M phosphate buffer (PBS, pH 7.4) for 2.5 hr. The prefixed tissues were washed 3 times at 4°C at 10 min interval with PBS, and then, fixed in 1% osmium tetraoxide in PBS for 2 hr. The fixed tissues were dehydrated by using ethanol with increasing concentrations from 50 to 100%, followed by finishing up with propylene oxide. These were then formatted with epoxy and cut into 1 μm semi-thin sections with an ultra microtome. The sections were observed after simple dyeing with 0.5% toluene blue in order to choose the sites for electron microscope. The selected sites were then cut into ultra thin sections, stained with double dye, uranyl acetate and lead citrate, and then, viewed under transmission electron microscopy (H-600; Hitachi Ltd., Tokyo, Japan).

**Statistical analysis** The data were expressed as mean± standard deviation (n=7). The statistical significance was tested by Duncan's multiple range test of one-way ANOVA with SPSS program for Windows Version 10.0.

## Results and Discussion

**Protective effects of GFW on the $CCl_4$-induced changes in the body and liver weight** Administration of $CCl_4$ significantly reduced the body weight of rats compared to the untreated control group, which was significantly inhibited by pre-administration with GFW for 2 weeks (Table 2). In contrast to the changes of body weight, the liver weight among internal organs tested was significantly increased in the group treated with $CCl_4$ specificity liver of rat (Table 3). Rajesh and Latha (20) investigated the anti-

**Table 3. The changes weight of internal organs of rats[1)]**

| Group[2)] | Relative weight (%) | | | | |
|---|---|---|---|---|---|
| | Liver | Kidney | Spleen | Lung | Heart |
| CON | 3.42±0.46[b] | 0.83±0.09[a] | 0.21±0.08[a] | 0.47±0.03[a] | 0.37±0.03[a] |
| $CCl_4$ | 4.32±0.79[a] | 0.93±0.03[a] | 0.25±0.05[a] | 0.48±0.04[a] | 0.38±0.02[a] |
| GFW I | 3.78±0.13[b] | 0.84±0.04[a] | 0.23±0.03[a] | 0.45±0.05[a] | 0.36±0.05[a] |
| GFW II | 3.73±0.26[b] | 0.88±0.13[a] | 0.23±0.02[a] | 0.48±0.05[a] | 0.37±0.03[a] |
| GFW III | 3.67±0.27[b] | 0.88±0.08[a] | 0.22±0.03[a] | 0.47±0.06[a] | 0.37±0.02[a] |

[1)]The values are mean±SD (n=7); the values followed by the same superscript letter in each column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW III (dose 2 g/kg) followed by $CCl_4$ treatment.

**Table 4. Effect of pretreatment with GFW on liver damage markers in the serum of rat treated with $CCl_4$[1)]**

| Group[2)] | AST (Karmen unit/mL) | ALT (Karmen unit/mL) | ALP (K-A unit) | LDH activity (Wroblewski unit) | γ-GTP activity (mU/mL) |
|---|---|---|---|---|---|
| CON | 134.71±27.21$^{d}$ | 59.80±14.05$^{d}$ | 26.31±7.81$^{d}$ | 360.21±19.52$^{c}$ | 5.78±2.42$^{c}$ |
| $CCl_4$ | 230.10±11.77$^{a}$ | 142.17±14.45$^{a}$ | 67.18±3.19$^{a}$ | 505.10±8.32$^{a}$ | 14.77±2.67$^{a}$ |
| GFW I | 188.53±8.88$^{b}$ | 100.41±5.19$^{b}$ | 52.82±5.62$^{b}$ | 457.27±40.63$^{ab}$ | 10.28±1.56$^{b}$ |
| GFW II | 184.45±5.73$^{bc}$ | 102.86±10.59$^{b}$ | 47.32±2.28$^{b}$ | 440.24±19.13$^{bc}$ | 7.71±2.28$^{c}$ |
| GFW II | 166.26±4.10$^{c}$ | 83.42±12.04$^{c}$ | 36.49±4.04$^{c}$ | 435.38±23.34$^{bc}$ | 8.08±3.34$^{c}$ |

[1)]The values are mean±SD (n=7); the values followed by the same superscript letter in each column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

hepatoxicity activity of polyherbal formulation in $CCl_4$-induced rats and reported that the polyherbal formulation significantly lowered the liver enzyme activities, which had been increased by $CCl_4$ injection, and restored the body weight to normal level.

**GFW prevents hepatic enzyme release by $CCl_4$** Protection of hepatic damage induced by $CCl_4$ administration was observed by evaluating serum LDH, AST, and ALT levels in treatment groups. Since these enzymes are cytoplasmic in nature, upon liver injury these enzymes enter in to the circulatory system due to altered permeability of membrane (21). Marked increased release of AST, ALT, and LDH indicates a severe damage to liver tissue membranes by $CCl_4$. As shown in Table 4, $CCl_4$ treatment to rat significantly increased the serum levels of AST and ALT from 134.71±27.21 and 59.80±14.05 to 230.10± 11.77 and 142.17±14.45, respectively. However, pretreatment with GFW before the injection of $CCl_4$ significantly prevented the elevation of the serum levels of AST and ALT ($p<0.05$). ALP, LDH, γ-GTP activities have also similar to those of AST and ALT. Jeong and Park (22) investeigated the hepatoprotective effect of *Chrysanthemum boreale* M. water extract against $CCl_4$-induced rats. And Jayakumar *et al.* (23) reported that the increased serum enzyme levels in $CCl_4$-induced rats decreased with post administration of *Pleurotus ostreaus* extract. These results suggest that GFW have a hepatoprotective effects against chemical compounds including $CCl_4$ (Table 5).

**Preventive effects of GFW on the total serum protein, albumin, and lipid profiles** Since all the serum proteins except gamma-globulin are synthesized in the liver, the decreased protein synthesis has also been reported in the damaged liver (24,25). Thus, reduced level of serum albumin, the major serum protein, and other proteins can reflect abnormal liver function (26). Albumin and total protein concentrations were decreased in the rats treated with $CCl_4$ compared to control group. However, pretreatment with GFW prevented $CCl_4$-altered decrease in total serum protein and albumin contents, maintaining at nearly the same level as in the control group (Table 5). And triglyceride and total cholesterol volume in the serum were increased in the rats treated with $CCl_4$ compared to control group, whereas a significant decrease was observed in GFW group (Table 6).

**Table 5. Effect of GFW on the serum albumin and total protein contents of rats[1)]** (unit: g/dL)

| Group[2)] | Albumin | Total protein |
|---|---|---|
| CON | 3.10±0.09$^{a}$ | 6.24±0.22$^{a}$ |
| $CCl_4$ | 2.05±0.97$^{b}$ | 5.33±0.24$^{c}$ |
| GFW I | 2.87±0.28$^{a}$ | 5.53±0.37$^{bc}$ |
| GFW II | 3.13±0.18$^{a}$ | 5.75±0.21$^{b}$ |
| GFW III | 3.14±0.32$^{a}$ | 5.85±0.17$^{b}$ |

[1)]The values are mean±SD (n=7); the values followed by the same superscript letter in each column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

**Table 6. Effect of GFW on the serum lipid profiles of rats[1)]** (unit: mg/dL)

| Group[2)] | Triglyceride | Total cholesterol | HDL-cholesterol |
|---|---|---|---|
| CON | 30.59±7.03$^{c}$ | 37.10±5.07$^{c}$ | 41.40±7.71$^{a}$ |
| $CCl_4$ | 50.39±13.69$^{a}$ | 58.19±8.03$^{a}$ | 21.40±5.96$^{c}$ |
| GFW I | 40.77±6.50$^{b}$ | 50.32±8.20$^{b}$ | 27.19±6.18$^{bc}$ |
| GFW II | 38.84±8.87$^{bc}$ | 50.19±4.79$^{b}$ | 29.77±4.81$^{b}$ |
| GFW III | 36.28±3.02$^{bc}$ | 46.62±3.81$^{b}$ | 28.87±5.84$^{b}$ |

[1)]The values are mean±SD (n=7); The values followed by the same superscript letter in each column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

**The protective effect of GFW on $CCl_4$-induced hepatic lipid peroxidation** In the present study, we also found that in the liver of $CCl_4$-treated rats the level of MDA, a major reactive aldehyde resulting from lipid peroxidation of biological membranes (27), was markedly increased compared to the untreated control group ($p<0.05$). The MDA concentration in the $CCl_4$-treated group was 15.25 ±3.77 nmol, while in the group adiminstrated with 0.5, 1.0, 2.0 g/kg/mL of GFW for 14 days, the MDA in their liver was lower by 41, 61, 51%, respectively. Jeon *et al.* (28)

**Table 7. Effect of GFW on malonaldehyde levels in the liver of rats[1)]**

| Group[2)] | MDA (nmole/g of tissue) |
|---|---|
| CON | 6.21±2.86[c] |
| $CCl_4$ | 15.25±3.77[a] |
| GFW I | 11.51±3.52[b] |
| GFW II | 9.72±3.70[bc] |
| GFW III | 9.45±1.01[bc] |

[1)]The values are mean±SD (n=7); the values followed by the same superscript letter in the column are not significantly different ($p<0.05$).
[2)]CON, Normal group; $CCl_4$, rats were treated with $CCl_4$ after 2 weeks of normal feeding; GFW I, rats were fed with basal diet containing GFW (dose 0.5 g/kg) followed by $CCl_4$ treatment; GFW II, rats were fed with basal diet containing GFW (dose 1 g/kg) followed by $CCl_4$ treatment; GFW III, rats were fed with basal diet containing GFW (dose 2 g/kg) followed by $CCl_4$ treatment.

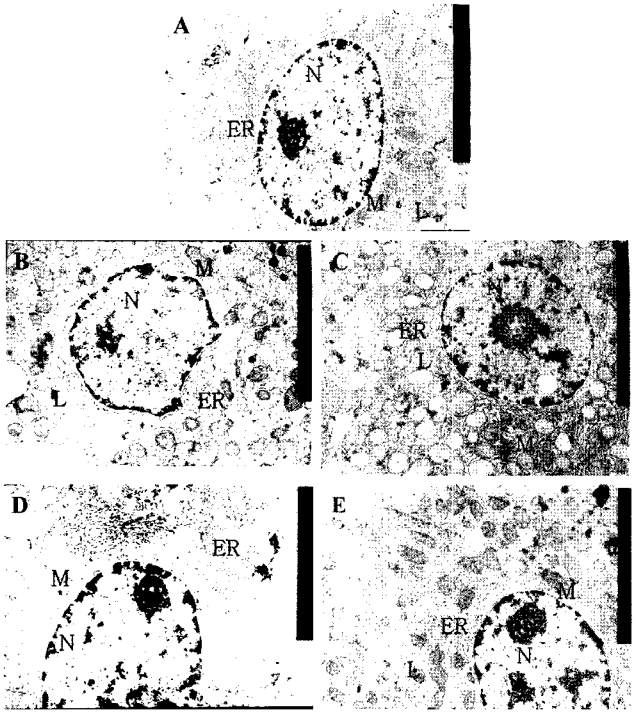


**Fig. 1. Inhibitory effect of GFW for $CCl_4$-induced ultrastructural changes in rat hepatocytes.** The rat hepatocytes were examined under the TEM of *5000; control (A), $CCl_4$-treated group (B), GFW I (dose 0.5 g/kg)+$CCl_4$ treat group (C), GFW II (dose 1.0 g/kg)+$CCl_4$ treat group (D), and GFW III (dose 2.0 g/kg)+ $CCl_4$ treat group (E). ER, L, M, and N represent endoplasmic reticulum, lipid droplets, mitochondrion, and nucleus, respectively.

observed that when an extract of the mushroom *Phellinus linteus* grown on germinated brown rice was administered, it slightly inhibited 10% compared to $CCl_4$-treated rats. Zhang *et al.* (29) investigated the hepato-protective effect of polysaccharides of *Ganoderma lucidum* against $CCl_4$-induced rats. These results suggest that GFW is potent antioxidant agent in protecting the liver from $CCl_4$-induced damages.

**GFW dose-dependently protects $CCl_4$-induced ultrastructural changes in rat hepatocytes** Several mushrooms as *Lentinus edodes*, *Tricholoma lobeyence*, and *Inonotus obliquus* have been shown to have protective effects against chemically induced (D-galactosamine and $CCl_4$) hepatic injury (30-34). The sections of untreated control liver showed normal hepatic cells with well-preserved cytoplasm, a prominent nucleus and organelles such as mitochondria, Golgi complex, endoplasmic reticulum, and ribosomes (Fig. 1A). As previously reported (13), $CCl_4$ administration induced damage of hepatocytes revealed as vacuolation, a highly damaged endoplasmic reticulum, and degenerating nuclei (Fig. 1B). However, transmission electron micrographs (TEM) examination of hepatocytes showed that GFW significantly prevented the damage induced by $CCl_4$ and was dose-dependent (Fig. 1C, 1D, and 1E). The results suggest that GFW has a protective effect against $CCl_4$-impaired ultrastructral changes. It has been shown that the principal cause of $CCl_4$-induced heaptotoxicity is peroxidation of hepatocyte membranes by free radicals generated from $CCl_4$ (14,35). The results of serum biochemical parameter, level of hepatic lipid peroxides, and ultrasturctural studies in the pre-treatment group support the potent hepatoprotective activity of GFW.

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