Note

Suppression of D-Galactosamine-induced Liver Injury by Mushrooms in Rats

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Six species of edible mushroom were found to suppress D-galactosamine-induced enhancement of plasma alanine and aspartate aminotransferase activities when powdered mushrooms were added to the diet (5%) and fed to rats for 2 wk. Grifola frondosa exhibited the most potent effect in a dose-dependent manner. A significant effect was observed only from the water-soluble low-molecular-weight fraction of G. frondosa. The results indicate that several mushrooms possess a protective effect against liver injury induced by D-galactosamine.

Key words: mushroom; Grifola frondosa; liver injury; D-galactosamine; rat

A number of studies so far reported have shown that various species of mushroom have a wide range of biological effects (see reviews1-2). In the course of studies on the protective effect of various types of food against liver injury, we found that D-galactosamine (GalN)-induced rat liver injury could be significantly suppressed by traditional beverages3-6 and by certain species of vegetables and fruits (unpublished data). Many species of mushroom are now frequently served at tables, and mushrooms are one of the important foodstuffs. However, little information is currently available concerning the hepatoprotective activity of mushrooms.

This report describes the effects of seven species of edible mushroom on GalN-induced liver injury, as assessed by the plasma enzyme activity, in rats. Since the Grifola frondosa mushroom exhibited the most potent effect, the effect of each fraction obtained from the mushroom by successive extraction with organic solvents was also investigated.

Seven species of mushroom, Lentinus edodes (Shiitake), Pleurotus ostreatus (Hiratake), Hipsizigus marmoreus (Bunahimeji), Fulmumina velutipes (Enokitake), Agaricus bisporus (Tsukuritake), Grifola frondosa (Maitake) and Auricularia auricula (Kikurage), which were all cultivated in Japan, were obtained from local supermarkets (Shizuoka, Japan). The fruiting bodies of each type of mushroom were lyophilized and powdered with a mixer. The powder of G. frondosa was fractionated into five fractions by successively extracting with organic solvents and hot water (Fig. 1). Five-wk-old male rats of the Wistar strain (Japan SLC, Hamamatsu, Japan) weighing 90–100 g were used as the experimental animals. The rats were individually housed in hanging stainless steel wire cages and kept in an isolated room at a controlled temperature (23–25°C) and ambient humidity (50–60%). Lighting was maintained on a 12-h light-dark cycle (lights on from 06:00 to 18:00 h). The animals were fed on a stock diet (type MF; Oriental Yeast, Tokyo, Japan) for 4 or 5 d, and they were then given free access to the experimental diets and water for 14 d. The composition of the control diet was as follows (g/100 g): casein, 25.0; corn starch, 40.1; sucrose, 20.0; corn oil, 5.0; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1.0; choline bitartrate, 0.4; and cellulose, 5.0. Supplements were added to the control diet at the expense of cellulose, since cellulose had no significant effect on GalN-induced liver injury.3-6 Four separate animal experiments were conducted in this study. In experiment 1, the effects of dietary supplementation (5%) with powder of each of seven spe-

Fig. 1. Procedure for Fractionating Grifola frondosa into Five Fractions (I to V) by Successive Extraction with Different Organic Solvents.

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cies of mushroom on GalN-induced liver injury were evaluated. In experiments 2 and 3, the dose-depen-
dent effect of dietary *G. frondosa* (1–5%) and the effects of five fractions (fractions I to V) from *G. frondosa* on GalN-induced liver injury were respectively investigated. In experiment 4, the effect of *G. frondosa* on CCl4-induced liver injury was investigat-
ed. After feeding the experimental diets for 14 d, GalN (350 mg/kg of body weight) or CCl4 (1 ml/kg of body weight; dissolved in an equal volume of olive oil) was injected intraperitoneally between 14:00 and 14:30 h. Untreated normal rats were fed on the control diet and injected with saline or olive oil alone. After 22 h, the rats were killed by decapitation be-
tween 12:00 and 12:30 h to obtain the blood and liver. The experimental plan for the present study was approved by the Laboratory Animal Care Com-
mittee of the Faculty of Agriculture at Shizuoka University. The activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were measured with kits (Transaminase C II-Test and LDH C II-Test, respectively; Wako Pure Chemicals, Osaka, Japan). The enzyme activity is expressed as I.U. (μmol/min/l of plasma at 25°C). Data were sub-
jected to an analysis of variance, and the difference be-
tween means was tested at *p* < 0.05 by Duncan’s mul-
tiple-range test when the *F* value was significant at
*p* < 0.05.

Table 1 summarizes the results of experiment 1. Several species of mushroom caused slight decreases in the growth and food intake of the animals. The relative liver weight was significantly higher in the *L. edodes-*fed rats and lower in the *A. auricula-*fed rats than in the GalN-treated control rats. The activities of plasma ALT and AST were dramatically increased by the injection of GalN. Most of the mushrooms used, except for *A. auricula*, significantly suppressed the increase in these enzyme activities, although the magnitude of the effect varied. In particular, the

effects of three species of mushroom, *L. edodes*, *F. velutipes* and *G. frondosa*, were relatively strong. *Gribofa frondosa*, which had the strongest effect, decreased the activities of ALT and AST to 1/8 and 1/9 of the levels of the GalN-injected control rats, respectively. When graded levels of powdered *G. frondosa* were added to the diet, the plasma ALT and AST activities were suppressed by the mushroom in a dose-dependent manner; the minimum dose to cause a significant effect was 1% or less (Fig. 2). Each frac-
tion prepared from *G. frondosa* was added to the diet (Fr. I, 0.17%; Fr. II, 1.69%; Fr. III, 0.44%; Fr. IV, 0.10%; Fr. V, 2.70%) to make it comparable to a 5% addition of powdered mushroom on the basis of the yield of each fraction. Unlike the powder of the mushroom, not all of the fractions affected the growth and food intake of the animals. Only fraction

![Fig. 2. Dose-dependent Effect of Dietary *Gribofa frondosa* on the α-Galactosamine-induced Enhancement of Plasma Alanine Aminotransferase (A) and Aspartate Aminotransferase (B) Activities in Rats.](image)

### Table 1. Body Weight Gain, Food Intake, Liver Weight, and Plasma Enzyme Activities of Rats Fed on the Control Diet or on Diets Supplemented with Different Species of Mushroom

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain (g/14 d)</th>
<th>Food intake (g/14 d)</th>
<th>Liver weight (% of body weight)</th>
<th>ALT (μmol/min/l)</th>
<th>AST (μmol/min/l)</th>
<th>LDH (μmol/min/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>74 ± 2d</td>
<td>193 ± 3e</td>
<td>23 ± 3f</td>
<td>1536 ± 89a</td>
<td>5511 ± 215a</td>
<td>0.7 ± 0.0f</td>
</tr>
<tr>
<td>Control</td>
<td>69 ± 1b</td>
<td>191 ± 2g</td>
<td>28 ± 1f</td>
<td>2222 ± 105b</td>
<td>2563 ± 228b</td>
<td>10.0 ± 1.3b</td>
</tr>
<tr>
<td>+ 5% <em>Lentinus edodes</em></td>
<td>67 ± 1c</td>
<td>176 ± 2g</td>
<td>23 ± 3e</td>
<td>1103 ± 95b</td>
<td>1586 ± 81b</td>
<td>3.0 ± 0.2g</td>
</tr>
<tr>
<td>+ 5% <em>Pleurotus ostreatus</em></td>
<td>65 ± 1d</td>
<td>162 ± 2g</td>
<td>26 ± 1f</td>
<td>3.61 ± 0.04d</td>
<td>4.62 ± 0.08d</td>
<td>2.2 ± 0.1d</td>
</tr>
<tr>
<td>+ 5% <em>Hispisigus marmoreus</em></td>
<td>73 ± 2e</td>
<td>180 ± 3e</td>
<td>38 ± 1f</td>
<td>1101 ± 65b</td>
<td>2563 ± 228b</td>
<td>6.0 ± 0.2f</td>
</tr>
<tr>
<td>+ 5% <em>Fulamunilina velutipes</em></td>
<td>55 ± 2f</td>
<td>150 ± 2e</td>
<td>34 ± 0.8f</td>
<td>441 ± 28d</td>
<td>1133 ± 58d</td>
<td>2.3 ± 0.2g</td>
</tr>
<tr>
<td>+ 5% <em>Agaricus bisporus</em></td>
<td>70 ± 2g</td>
<td>178 ± 2g</td>
<td>41 ± 1f</td>
<td>3.66 ± 0.07d</td>
<td>3.70 ± 0.06e</td>
<td>2.7 ± 0.1f</td>
</tr>
<tr>
<td>+ 5% <em>Gribofa frondosa</em></td>
<td>61 ± 2f</td>
<td>169 ± 2e</td>
<td>187 ± 30d</td>
<td>1211 ± 91b</td>
<td>4538 ± 156a</td>
<td>30.9 ± 2.5g</td>
</tr>
<tr>
<td>+ 5% <em>Auricularia auricula</em></td>
<td>69 ± 1b</td>
<td>183 ± 2d</td>
<td>187 ± 30d</td>
<td>1497 ± 71b</td>
<td>1211 ± 91b</td>
<td>30.9 ± 2.5g</td>
</tr>
</tbody>
</table>

1 Each value is the mean ± SEM for 5 (normal), 12 (control) and 8 (+ mushroom) rats. Values in a column with no common letters are significantly different at *p* < 0.05. The rats were injected with saline (normal) or α-galactosamine (control and + mushroom) on the 15th day of feeding, and blood was sampled after 22 h. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.
II significantly suppressed the GalN-induced enhancement of plasma ALT activity (Fig. 3) and AST activity (data not shown). On the other hand, *G. frondosa* had no suppressive effect on the CCl₄-induced enhancement of plasma enzyme activities when the powder of the mushroom was added to the diet at a 5% level (ALT activity: normal, 12 ± 1; control, 1003 ± 56; + mushroom, 991 ± 91; AST activity: normal, 54 ± 6; control, 2804 ± 170; + mushroom, 2943 ± 222).

The results of the present study clearly demonstrate that most of the species of mushroom tested had a suppressive effect on the GalN-induced enhancement of plasma ALT and AST activities. Since the extent of increase in these plasma enzyme activities is known to be parallel to that of liver injury, the results are taken to indicate that several species of mushroom can protect against a certain type of liver injury such as that induced by GalN in rats. *D*-Galactosamine is thought to induce hepatotoxicity by inhibiting the synthesis of RNA and protein through a decrease in cellular UTP concentration, which finally leads to the necrosis of liver cells. Although the symptoms of GalN-induced liver injury are regarded as being similar to those of viral hepatitis, it must be further validated whether mushrooms are effective in protecting against virus-induced hepatitis. *Grifola frondosa* was found to have the most potent protective effect against the hepatotoxicity of GalN among the seven species of mushroom tested. With regard to this, Kubo *et al.*⁹ have recently reported the effect of dietary supplementation with powder of *G. frondosa* or β-glucans on the autoimmune hepatitis induced by the injection of a liver-specific antigen in mice. They have shown that β-1,6-glucan, having α-1,4 branched glucan (X-fraction) caused a slight suppression of the hepatitis, although powder of the mushroom had no suppressive effect. In contrast, our results demonstrate that the liver injury-suppressing effect of *G. frondosa* could be solely ascribed to the constituents of low molecular weight, because only fraction II, which contained water-soluble compounds of low molecular weight, had a significant effect. On the other hand, *G. frondosa* failed to suppress the hepatotoxicity of carbon tetrachloride. Carbon tetrachloride is thought to elicit its hepatotoxicity by the trichloromethyl and trichloromethylperoxy radicals which are formed directly or indirectly by reductive dehalogenation catalyzed by microsomal cytochrome P450. In this respect, radical scavengers or inhibitors of cytochrome P450 have been to suppress the hepatotoxicity of carbon tetrachloride.¹¹,¹² It is likely that *G. frondosa* could not suppress the hepatotoxicity of carbon tetrachloride, probably because of the lack of radical scavenging or cytochrome P450 inhibitory activity under the experimental conditions used. Further studies on the isolation and identification of the active constituents of *G. frondosa* are now in progress.

References


