Evaluation of the Hepatic and Renal-protective Effects of *Ganoderma lucidum* in Mice

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Abstract: The antioxidative effect of hot water extract of the mushroom *Ganoderma lucidum* on ethanol-induced free radical generation had been studied. In order to further investigate the hepatic and renal protective mechanism of *Ganoderma lucidum*, rates of lipid peroxidation were determined. The hot water extract of *Ganoderma lucidum* dose-dependently exhibited antioxidative effect on mouse liver and kidney lipid peroxidation; our results indicated that hepatic and renal homogenates have a higher malonic dialdehyde level in an ethanol administered group than in the *Ganoderma lucidum* treated group. It was concluded that the hepatic and renal protective mechanism of *Ganoderma lucidum*, might be due at least in part to its prominent superoxide scavenging effect. *Ganoderma extract* could protect the liver and kidney from superoxide induced hepatic and renal damages.

The mechanism contributing to alcohol-induced hepatic and renal damage remains unclear. Increasing evidence indicates that alcohol toxicity is associated with increased oxidative stress and free radical-associated injury (Nanji et al., 1994; Manso, 1997). Several investigations indicated that the generation of oxygen metabolites, such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (·OH), is believed to be important in the pathogenesis of alcoholic liver (Nordmann et al., 1996) and renal injury (Kera et al., 1985; Paller et al., 1988).
Ganoderma lucidum is a white rot basidiomycete widely distributed worldwide; its fruit bodies have been used for the prevention and treatment of various diseases in Asia. Its antitumor, immune enhancing and non-cytotoxic properties raise the possibility that it could be effective in preventing oxidative damage related disease (Kim, et al., 1999).

In China, Ganoderma lucidum has been prescribed in Chinese Medicine for hundreds of years as a tonic and sedative, and has been used for treatment of hypertension (Kabir, et al., 1988), carcinoma (Kim, et al., 1999), inflammation and liver disease (Lin, et al., 1993). Ganoderma lucidum has also been reported to have excellent antioxidative activities in a dose dependent manner, and its terpene fraction was found to possess the most effective constituents. Chemical isolation of the terpene fraction resulted in the detection of ganodermic acids A, B, C and D, lucidenic acid B and ganoderma notriol as major ingredients (Zhu, M. et al., 1999).

The aims of the present study were to investigate the hepatic and renal protective effect and antioxidative effect of this crude drug. The active oxygen scavenging activity of Ganoderma lucidum was also examined by lipid peroxidation. TBA-MDA adduct was used as a free radical index.

Materials and Methods

Animals and Grouping

Male ICR mice weighing about 20–25g were purchased from the Animal Center, College of Medicine, National Yang-Ming University. They were kept at least one week on commercial diets (Fu-So Co., Taipei) under environmentally controlled conditions (25 ± 1°C, 55 ± 5% humidity) with free access to food and water. A 12-hr light/dark schedule was maintained and hard wood chips were used as bedding.

ICR mice were divided into eight groups with 10 animals each. Group 1 (control, untreated mice) received saline (10 ml/kg). Group 2 received 95% ethanol (0.1 ml, p.o.). Group 3–5 received the hot water extract of Ganoderma lucidum at the doses of 10, 25, and 50 mg/kg p.o. respectively. Groups 6–8 received the hot water extract of Ganoderma lucidum at the doses of 10, 25 and 50 mg/kg p.o. respectively, 30 min before oral administration of 0.1 ml 95% ethanol. The animals were killed 1 hr after 0.1 ml 95% ethanol administration. The above procedure was performed according to the method described by Zhang et al. (1995).

Drugs and Chemicals

95% ethanol, thiobarbituric acid (TBA), sodium dodecyl sulfate, ferric chloride, n-butanol were all purchased from Sigma Chemical Company (St. Louis, MO 63178, USA). Acetic acid was obtained from a local company in Taipei, Taiwan. Ganoderma lucidum was a gift of Kuun Yung, Co., Ltd., Taipei, Taiwan.

Preparation of Crude Extract

Ganoderma lucidum crude extract was prepared as follows: 100 gm of the dried, crushed Ganoderma lucidum was decocted in a Chinese herb boiler with one liter of distilled water. After filtration, the residues were decocted again in the same manner. Two fractions of fil-
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Ganoderma lucidum extract was combined and then concentrated into 1 gm/ml (w/v, based on the weight of the dried crude drug). In order to obtain the exact dosage of crude extract, this concentrated solution was freeze-dried into powder by a freeze-drier (Tokyo Rikakikai Co., Ltd.), then stored in an auto-drying container until use.

Preparation of Liver and Kidney Homogenate

After sacrificing the animals, the liver and kidney were removed and cut into slices respectively. The liver or kidney slices were homogenized with 1.15% KCl solution according to the method described by Mihara *et al.* (1978).

Determination of Lipid Peroxidation by Measuring Thiobarbituric Acid Reactive Substance

The protective effect of the hot water extract of *Ganoderma lucidum* on the liver and kidney lipid peroxidation was determined by the MDA-TBA adduct according to the modified method described by Yuda *et al.* (1991). Briefly, 0.5 ml of prepared liver or kidney homogenate were placed in an incubator with 0.1 ml of Tris-HCl buffer (pH 7.2) for 1 hr at 37 °C. After incubation, 9 ml of distilled water and 2 ml of 0.6% Thiobarbituric Acid (TBA) were added to 0.5 ml of the incubation solution and were shaken vigorously. The mixture was heated for 30 min in a boiling water bath. After cooling naturally at room temperature, 5 ml of n-BuOH was added and the mixture was again shaken vigorously. The n-BuOH layer was separated by centrifugation at 1000 gr. for 10 min, and the malonic dialdehyde (MDA) production amount was measured at 532 nm (Wong *et al.* 1987).

Statistical Analysis

All data were shown as mean ± S.E. (n=10). Statistical significance was assessed by one-way analysis of variance coupled with Dunnett’s test. The level of significance was chosen as p<0.05.

Results

Ganoderma lucidum Effect on Mice Without Ethanol Treatment

*Ganoderma lucidum* (GL) (10, 25 and 50 mg/kg) significantly improved the normal hepatic and renal function of groups 3, 4, 5 mice. *Ganoderma lucidum* dose-dependently inhibited the lipid peroxidation-induced MDA formation, the end product of lipid peroxidation (Tables 1, 3, respectively) in mice hepatic and renal homogenate.

Free Radical Scavenging Assessment of Ganoderma lucidum on Ethanol-induced Acute Toxicity

As shown in Tables 2 and 4, 95% ethanol treatment increased lipid peroxidation in the kidney and liver homogenates, respectively. Various concentrations (10, 25 and 50 mg/kg) of *Ganoderma lucidum* dose-dependently inhibited the ethanol-induced lipid peroxidation in the mice renal homogenate (Table 2) and liver homogenate (Table 4).
Table 1. Effect of Hot Water Extract of Ganoderma lucidum (GL) on Lipid Peroxidation in the Mice (Without Ethanol Treatment) Kidney Homogenates

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmole/mg protein)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>0.162 ± 0.006</td>
<td>–</td>
</tr>
<tr>
<td>GL (10 mg/kg)</td>
<td>0.129 ± 0.02</td>
<td>20.37</td>
</tr>
<tr>
<td>GL (25 mg/kg)</td>
<td>0.125 ± 0.0005*</td>
<td>22.84</td>
</tr>
<tr>
<td>GL (50 mg/kg)</td>
<td>0.121 ± 0.008*</td>
<td>25.31</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. (n=10)
*: p < 0.001, significantly different from normal control group
#: p < 0.05, significantly different from normal control group
One-way analysis of variance coupled with Dunnett’s test.
P value less than 0.05 was taken as significant.

Table 2. Inhibitory Effect of Hot Water Extract of Ganoderma lucidum (GL) on Ethanol-induced Lipid Peroxidation in the Mice Kidney Homogenate

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmole/mg protein)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>0.046 ± 0.01</td>
<td>–</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml)</td>
<td>0.095 ± 0.01*</td>
<td>–</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (10 mg/kg)</td>
<td>0.058 ± 0.03</td>
<td>38.9</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (25 mg/kg)</td>
<td>0.048 ± 0.02</td>
<td>49.5</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (50 mg/kg)</td>
<td>0.045 ± 0.01*</td>
<td>52.6</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. (n=10)
*: p < 0.05, significantly different from normal control group
#: p < 0.05, significantly different from ethanol group
One-way analysis of variance coupled with Dunnett’s test.
P value less than 0.05 was taken as significant.

Discussion

The aim of this study was to investigate the antioxidative effect of Ganoderma lucidum on ethanol-induced hepatic and renal injury. It has been hypothesized that one of the principal causes of ethanol-induced hepatic and renal injury is due to free radical induced lipid peroxidation, whereas free radicals could be generated largely by a long period of alcohol consumption (Meagher et al., 1999; Bautista et al., 1999; Jaya et al., 1993; Rodrigo et al., 1998). In recent years, the biological and pharmacological properties of Ganoderma lucidum have become a promising and important research. It was confirmed that Ganoderma
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Table 3. Effect of Hot Water Extract of Ganoderma lucidum (GL) on Lipid Peroxidation in the Mice (Without Ethanol Treatment) Liver Homogenates

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmole/mg protein)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>0.213 ± 0.007</td>
<td>-</td>
</tr>
<tr>
<td>GL (10 mg/kg)</td>
<td>0.178 ± 0.005*</td>
<td>16.43</td>
</tr>
<tr>
<td>GL (25 mg/kg)</td>
<td>0.170 ± 0.002**</td>
<td>20.19</td>
</tr>
<tr>
<td>GL (50 mg/kg)</td>
<td>0.165 ± 0.006*</td>
<td>22.54</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. (n=10).
* : p < 0.05, significantly different from normal control group
**: p < 0.001, significantly different from normal control group
One-way analysis of variance coupled with Dunnett’s test.
P value less than 0.05 was taken as significant.

Table 4. Inhibitory Effect of Hot Water Extract of Ganoderma lucidum (GL) on Ethanol-induced Lipid Peroxidation in the Mice Liver Homogenate

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmole/mg protein)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>0.08 ± 0.019</td>
<td>-</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml)</td>
<td>0.199 ± 0.02*</td>
<td>-</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (10 mg/kg)</td>
<td>0.113 ± 0.01*</td>
<td>43.2</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (25 mg/kg)</td>
<td>0.107 ± 0.008*</td>
<td>46.2</td>
</tr>
<tr>
<td>95% ethanol (0.1 ml) + GL (50 mg/kg)</td>
<td>0.05 ± 0.009*</td>
<td>74.9</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. (n=10)
* : p < 0.05, significantly different from normal control group
*: p < 0.05, significantly different from ethanol group
One-way analysis of variance coupled with Dunnett’s test.
P value less than 0.05 was taken as significant.

Ganoderma lucidum could exhibit a broad spectrum of activities, including hypotensive action (Lee et al., 1990), anti-tumor activity (Wang et al., 1997), antinociceptive effect (Koyama et al., 1997), anti-platelet aggregation (Tao et al., 1990), hepatoprotective and anti-inflammatory activities (Kim et al., 1999).

In the present study, the protective effect of Ganoderma lucidum and its action mechanism on ethanol-induced hepatic and renal injury were investigated in ICR mice. Superoxide scavenging activity was used as an indicator of hepatic and renal protective effect.

Results of this study showed that Ganoderma lucidum could inhibit lipid peroxidation, hence decreasing significantly the malonic dialdehyde (MDA) formation in the control
mice (without ethanol treatment) kidney and liver homogenates (See Tables 1, 3). It was reported that ethanol could stimulate lipid peroxidation in the kidney (Kera et al., 1985) and liver (Baustista et al., Meagher et al., 1999). Our results confirmed these findings (see Tables 2,4). We also found that Ganoderma lucidum had dose-dependently inhibited 95% ethanol-induced lipid peroxidation. These findings indicated that the preventive effect of GL on hepatic and renal injuries induced by ethanol is due, at least in part, to the decrease in MDA formation.

In conclusion, results of the present study suggested that production of free radicals may be involved in the pathogenesis of hepatic and renal injuries induced by ethanol, and that Ganoderma lucidum extract dose-dependently inhibited the ethanol-induced hepatic and renal injury significantly. These effects may be due, in part, to its inhibitory activity on membrane lipid peroxidation and free radical formation, or free radical scavenging ability. Clinical application of Ganoderma lucidum to treat ethanol-induced hepatic and renal damage warrants further study.

Reference


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