**Hepatoprotective Effect of Tectoridin and Tectorigenin on tert-Butyl Hydroperoxide-Induced Liver Injury**

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**Abstract.** To clarify the hepatoprotective effects of tectoridin and tectorigenin from *Puerariae Flos*, their effects on tert-butyl hydroperoxide (t-BHP)-injured HepG2 cells and mice were investigated. When tectorigenin at a dose of 50 mg/kg was intraperitoneally administered to mice injured by t-BHP, it significantly inhibited the increase of plasma ALT and AST by 39% and 41%, respectively, in the t-BHP-treated group. The inhibitory effect of tectorigenin is much more potent than that of a commercially available dimethyl diphenyl bicarboxylate. Orally administered tectoridin showed hepatoprotective activity. However, when tectoridin was intraperitoneally administrated to mice, no hepatoprotective activity was observed. Tectorigenin also protected against the cytotoxicity of HepG2 cells induced by t-BHP. This protection may have originated from the inhibition of apoptosis. Tectorigenin may be hepatoprotective and tectoridin should be a prodrug that is transformed to tectorigenin.

**Keywords:** tectorigenin, HepG2, hepatic injury

*t*-Butyl hydroperoxide (t-BHP) can be metabolized to free radical intermediates by cytochrome P450 (hepatocytes) or hemoglobin (erythrocytes), which can subsequently initiate lipid peroxidation (1), affect cell integrity, and form covalent bonds with cellular molecules, resulting in cell injury (2). t-BHP is known to cause lactate dehydrogenase and alanine transferase (ALT) leakage in hepatocyte cells (3, 4). Therefore, t-BHP has been used as a chemical inducer for the preparation of a liver injured animal model (5, 6).

In traditional Chinese medicine, *Puerariae Flos* has been used in therapy to counteract the problems associated with alcohol drinking and liver injury (7). Niiho et al. (8, 9) reported that the isoflavonoid fraction of *Puerariae Flos* suppressed the increase in the concentration of blood ethanol, acetaldehyde, and ketones induced by ethanol administration and that its isoflavonoid and triterpenoid saponin fractions improved both the abnormal metabolism induced by ethanol and hepatic injuries induced by carbon tetrachloride or high-fat food. Jang et al. (10) reported that *Puerariae Flos* protected against ethanol-induced apoptosis in the human neuroblastoma cell line SK-N-MC. Lee et al. (11) reported that tectorigenin isolated from *Puerariae Flos* as an inhibitor of β-glucuronidase showed hepatoprotective activity on the CCl₄-induced hepatotoxicity in mice.

To clarify the hepatoprotective effects of isoflavones tectorigenin and tectoridin (Fig. 1) from *Puerariae Flos*, tectoridin was isolated from *Puerariae Flos* and its metabolite tectorigenin from human fecal microflora, and then we investigated their effects in t-BHP-injured HepG2 cells and mice.

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**Fig. 1.** Structures of tectoridin (R = β-D-glucosyl) and tectorigenin (R = H).
Tectoridin and tectorigenin were isolated according to the previously described methods (11–13). The flowers of *Pueraria thunbergiana* (500 g), produced in Korea, were extracted with 2.5 L of boiling water, concentrated in a rotary evaporator, extracted 3 times with ethyl acetate, and evaporated. The resulting extract (28 g) was loaded onto a silica-gel flash column and eluted with CHCl₃:MeOH (20:1→4:1), and tectoridin was isolated. The tectoridin was incubated with human fetal microflora, from which tectorigenin was isolated (13).

*t-BHP* was purchased from Sigma Co. (St. Louis, MO, USA). The mice (ICR, male, 20–25 g) were supplied from the Orient Charles Liver Co., Ltd. (Seoul, Korea) and maintained on pellet food (Samyang Co., Seoul, Korea) and tap water. Five mice in each group were orally or intraperitoneally administered with tectoridin, tectorigenin, or dimethyl diphenyl bicarboxylate (DDB), which was kindly donated by Dr. N.J. Kim at Kyung Hee University, suspended in 1% CMC-Na. The control group was administered vehicle alone (0.1 mL/20 g) instead of the sample compounds. Orally administered samples were treated three times (once per day) and those administered intraperitoneally were treated once. Animals were intraperitoneally treated with 1.5 mmol *t-BHP*/kg 24 h after the final sample administration. Blood samples were collected 18 h after *t-BHP* administration by cardiac puncture under ether anesthesia and then serum was obtained by centrifugation (1000 × g, 15 min).

The activities of ALT and aspartate transferase (AST) in the serum were analyzed by using the diagnostic kits of Asan Pharmaceutical Co., Ltd. (Soul, Korea) (14). HepG2 cells (hepatocellular carcinoma cell line), donated from the Korean Cell Bank (Seoul, Korea), were cultured in MEM containing 10% FBS, 1% anti-biotic-antimycological solution, and 1.5 g/L sodium bicarbonate, in a 5% CO₂ atmosphere at 37°C. The protective effect of tectoridin or tectorigenin on HepG2 cells injured by *t-BHP* was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma) assay (15). Briefly, HepG2 cells were dispensed into 96 well plates at a concentration of 1 × 10⁴ cells per well. The test compounds were added to the HepG2 cells and preincubated for 2 h. Then the cultured media were placed in media containing *t-BHP* (100 µM), incubated for 3 h, and then rinsed with phosphate-buffered saline. MTT reagent (0.25 mg/mL) was added into the cells, incubated for 1 h, and 100 µL dimethyl sulfoxide added. The absorbance at 540 nm was measured to estimate the cell survival.

All the data were expressed as the mean ± S.D., and statistical significance was analyzed by one way ANOVA followed by the Student-Newman-Keuls test.

The hepatoprotective effect of tectoridin was investigated in *t-BHP*-injured mice (Table 1A). When mice were orally treated with *t-BHP*, the serum ALT and AST levels were significantly increased, compared to those of the normal control group. The reference agent DDB (200 mg/kg) inhibited the increased serum ALT and AST levels by 18% and 32%, respectively, of those of the normal control group. The control group treated with *t-BHP* alone. Orally administered tectoridin potently inhibited the AST levels by 18% and 32%, respectively, of those of the normal control group. The reference agent DDB (200 mg/kg) inhibited the increased serum ALT and AST levels by 18% and 32%, respectively, of those of the normal control group. The control group treated with *t-BHP* alone. Orally administered tectoridin potently inhibited the increases in the serum ALT and AST levels induced by *t-BHP* treatment. However, intraperitoneally administered tectoridin at a dosage of 50 mg/kg did not. Intraperitoneally administered tectoridin at a dosage of 50 mg/kg did not. Intraperitoneally administered tectoridin at a dosage of 50 mg/kg did not.

### Table 1. Preventive effect of orally (A) or intraperitoneally (B) administered tectoridin and tectorigenin on *t-BHP*-induced hepatotoxicity in mice

<table>
<thead>
<tr>
<th>Group</th>
<th><em>t-BHP</em></th>
<th>Dose (mg/kg)</th>
<th>Administered route</th>
<th>AST (Karmen unit)</th>
<th>ALT (Karmen unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Orally administered</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>81.8 ± 31.95*</td>
<td>21.0 ± 2.45*</td>
</tr>
<tr>
<td><em>t-BHP</em> control</td>
<td>+</td>
<td>0</td>
<td>–</td>
<td>174.33 ± 31.18*</td>
<td>53.5 ± 8.66*</td>
</tr>
<tr>
<td>DDB</td>
<td>+</td>
<td>200</td>
<td>p.o.</td>
<td>145.67 ± 11.06bc</td>
<td>36.5 ± 18.88c</td>
</tr>
<tr>
<td>Tectoridin</td>
<td>+</td>
<td>100</td>
<td>p.o.</td>
<td>125.67 ± 11.55ad</td>
<td>33.25 ± 6.18a</td>
</tr>
<tr>
<td><strong>B. Intraperitoneally administered</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>49.0 ± 11.36e</td>
<td>32.3 ± 2.89e</td>
</tr>
<tr>
<td><em>t-BHP</em> control</td>
<td>+</td>
<td>0</td>
<td>–</td>
<td>146.33 ± 20.82b</td>
<td>76.33 ± 8.62e</td>
</tr>
<tr>
<td>Tectoridin</td>
<td>+</td>
<td>50</td>
<td>i.p.</td>
<td>143.67 ± 8.08b</td>
<td>59.67 ± 9.07b</td>
</tr>
<tr>
<td>Tectorigenin</td>
<td>+</td>
<td>25</td>
<td>i.p.</td>
<td>113.0 ± 11.53e</td>
<td>49.7 ± 15.31c</td>
</tr>
<tr>
<td>+</td>
<td>50</td>
<td>i.p.</td>
<td></td>
<td>105.0 ± 8.89d</td>
<td>39.67 ± 15.94c</td>
</tr>
</tbody>
</table>

abcde Those with the same letter in each column of A or B are not significantly different at *P* < 0.05.
To understand the hepatoprotective effect of tectoridin, its metabolite tectorigenin by human intestinal microflora was isolated, and its protective effects against the cytotoxicity in HepG2 cells induced by t-BHP were investigated (Fig. 2). When t-BHP only was used to treat HepG2 cells, the cell viability was significantly decreased. When t-BHP, at a dose of 100 μM, was used to treat HepG2 cells, their viability was decreased to 54.4% of the normal control group. The pretreatment of HepG2 cells with tectorigenin inhibited the t-BHP-induced cytotoxicity. Tectorigenin at a dose of 0.1 μM protected against the t-BHP-induced cytotoxicity of HepG2 to 75% of that in the control group. The protective effect of tectorigenin was stronger than that of silybin, a commercial agent (Carl Roth, Karlsruhe, Germany), protecting against the t-BHP-induced cytotoxicity of HepG2 by 68% compared to that of the control group at a dose of 1 μM. However, tectorigenin was not effective against t-BHP-induced cytotoxicity at the concentration of 10 μM. Tectorigenin and tectoridin showed no cytotoxicity at concentrations of less than 10 μM.

Most traditional medicinal herbs are orally administered, with their components inevitably coming into contact with intestinal microflora in the alimentary tract. The chemical components of herbs can be transformed by intestinal bacteria before being absorbed from the gastrointestinal tract. Park et al. (16) reported that when tectoridin was administered to rats, tectorigenin, but not tectoridin, was detected in the urine. However, tectoridin did not hydrolyze the tectorigenin from a rat liver homogenate. Bae et al. (12) reported that when tectoridin was incubated with human intestinal microflora, tectoridin was metabolized to tectorigenin. These results suggest that tectoridin can be easily transformed into tectorigenin by intestinal microflora before being absorbed from the gastrointestinal tract.

Therefore, to find the active compound against liver injury, the hepatoprotective activities of tectoridin and tectorigenin on the liver-injury induced in mice by t-BHP were investigated. t-BHP as well as carbon tetrachloride have been used as a chemical inducer for the preparation of liver injured animal models (5, 6). Therefore, in the present study, hepatotoxicity was induced using t-BHP. Orally administered tectoridin potently protected against the hepatotoxicity. However, when tectoridin was intraperitoneally administered, no hepatoprotective effect was observed, but tectorigenin exhibited a hepatoprotective effect. These results support the previous report that intraperitoneally and orally administered tectorigenin, which is a potent β-glucuronidase inhibitor, potently exhibited a hepatoprotective effect in a carbon tetrachloride-induced mouse model (11).

Therefore, to compare the hepatoprotective effects of tectoridin and tectorigenin, the cytotoxicity of HepG2 cell injured by t-BHP was investigated. The t-BHP showed potent cytotoxicity against HepG2 cells. However, the pretreatment with tectorigenin potently inhibited this cytotoxicity. The protective effect of tectorigenin was stronger than those of silybin, a commercially available agent. However, tectorigenin, at the high dosage of 10 μM, decreased the protective effect, compared with that at a concentration of 1 μM. In addition, Bae et al. (12) reported that tectorigenin exhibited cytotoxicity. The decrease in the hepato-protective effect of tectorigenin at the high dosage may be due to its cytotoxicity.

To investigate the protective mechanism of tectorigenin against liver injury, the antioxidant activity of tectoridin was measured. However, these compounds...
exhibited no antioxidant activities, such as radical scavenging and xanthine oxidase-inhibitory activities, as in the previous report (11). When \( t\)-BHP was used to treat HepG2 cells, procaspase 3 activation and DNA fragmentation were observed, as previously reported (6). The procaspase 3 activation and DNA laddering induced by \( t\)-BHP was inhibited by tectorigenin (data not shown). Based on these findings, tectorigenin inhibited \( \beta\)-glucuronidase (11) as well as apoptosis.

Finally, tectoridin may be metabolized to tectorigenin in human intestine, when \textit{Puerariae Flos} extracts are orally administered, and the biotransformed tectorigenin can protect against liver injury.

Acknowledgment

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References