Protective Effect of Pinitol against D-Galactosamine-Induced Hepatotoxicity in Rats Fed on a High-Fat Diet

Yusi Zhou,1 Chung-Mu Park,1 Chung-Won Cho,2 and Young-Sun Song1,

1Department of Food and Nutrition, Food Science Institute and BPRC, Inje University, Obang-dong, Gimhae, Gyeongnam 621-749, Korea
2School of Biotechnology and Biomedical Science, Inje University, Obang-dong, Gimhae, Gyeongnam 621-749, Korea

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The protective effect of pinitol against D-galactosamine (GalN)-induced liver damage was examined. Forty male Sprague-Dawley rats were divided into normal control, GalN control, and pinitol groups (0.5%, 1%, and 2%). After 8 weeks of feeding, a single dose of GalN (650 mg/kg) was administered 24 h before their sacrifice. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and tumor necrosis factor-alpha (TNF-α) levels were significantly increased after an injection with GalN (P < 0.05), but pinitol supplementation at the level of 0.5% reversed these changes to normal levels. Significant decreases in serum triglyceride and cholesterol and increases in hepatic cholesterol were observed in GalN-intoxicated rats. However, supplementation with pinitol significantly attenuated these trends. In addition, pinitol elevated the Mn-superoxide dismutase, glutathione reductase, and catalase activities, prevented hepatic lipid peroxidation, and restored the hepatic GSH levels and catalase activities, preventing hepatic lipid peroxidation, and restored the hepatic GSH levels and catalase activities, which are essential materials for the biosynthesis of such macromolecules as nucleic acids and proteins. Consequently, organelle injury and necrosis of hepatocytes occur. Recent studies have also found that GalN directly activated mast cells to release histamine, which increases gut permeability and the release of bacterial products. Kupffer cells, which are resident macrophages in the liver, are activated to release tumor necrosis factor-alpha (TNF-α). TNF-α then leads to cell death in various ways, including the promotion of oxidative stress and the inflammatory process. This mechanism is thought to be central to the toxicity of GalN in vivo. GalN inhibits macro-molecule synthesis in hepatocytes, increasing the sensitivity to TNF-α-mediated events.

Pinitol (3-O-methyl-chiro-inositol) is a monomethylated form of D-chiro-inositol, which is one chiral form of cyclohexitol. It is a naturally occurring compound found in pine wood, alfalfa, and legumes, but is generally derived from soy or carob for manufacturing purposes. Pinitol has well-known insulin-like effects, driving creatine in addition to other nutrients into the muscle cells. Pinitol may also possess multifunctional properties. Shin et al. have shown that pinitol had a protective effect on fatty changes, infiltration of inflammation cells, and tissue necrosis of the liver induced by carbon tetrachloride in rats. Kim et al. have reported the anti-inflammatory activity of pinitol against carrageenan- and cotton pellet-induced acute and sub-acute inflammation in rats. Kim et al. have suggested that pinitol could prevent cardiovascular diseases, and Lee et al. have demonstrated that pinitol could inhibit ovalbumin-induced airway inflammation in rats. All of these functions are connected with the ability of pinitol to attenuate or suppress oxidative stress and the inflammatory process.

Key words: D-galactosamine; hepatotoxicity; tumor necrosis factor-alpha (TNF-α); pinitol; rat

Among the numerous models for experimental hepatitis, D-galactosamine (GalN)-induced liver damage is similar to human virtual hepatitis in its morphological and functional features. Early biochemical studies have postulated that GalN trapped uracil nucleotides by its toxic intermediary metabolites (UDP-galactosamine and UDP-glucose) in the liver, thus resulting in the depletion of hepatic uridine triphosphate, UDP-glucose, and UDP-galactose, which are essential materials for the biosynthesis of such macromolecules as nucleic acids and proteins. Consequently, organelle injury and necrosis of hepatocytes occur. Recent studies have also found that GalN directly activated mast cells to release histamine, which increases gut permeability and the release of bacterial products. Kupffer cells, which are resident macrophages in the liver, are activated to release tumor necrosis factor-alpha (TNF-α). TNF-α then leads to cell death in various ways, including the promotion of oxidative stress and the inflammatory process. This mechanism is thought to be central to the toxicity of GalN in vivo. GalN inhibits macromolecule synthesis in hepatocytes, increasing the sensitivity to TNF-α-mediated events.

Pinitol (3-O-methyl-chiro-inositol) is a monomethylated form of D-chiro-inositol, which is one chiral form of cyclohexitol. It is a naturally occurring compound found in pine wood, alfalfa, and legumes, but is generally derived from soy or carob for manufacturing purposes. Pinitol has well-known insulin-like effects, driving creatine in addition to other nutrients into the muscle cells. Pinitol may also possess multifunctional properties. Shin et al. have shown that pinitol had a protective effect on fatty changes, infiltration of inflammation cells, and tissue necrosis of the liver induced by carbon tetrachloride in rats. Kim et al. have reported the anti-inflammatory activity of pinitol against carrageenan- and cotton pellet-induced acute and sub-acute inflammation in rats. Kim et al. have suggested that pinitol could prevent cardiovascular diseases, and Lee et al. have demonstrated that pinitol could inhibit ovalbumin-induced airway inflammation in rats. All of these functions are connected with the ability of pinitol to attenuate or suppress oxidative stress and the inflammatory process.

1 To whom correspondence should be addressed. Tel: +82-55-320-3235; Fax: +82-55-321-0691; Email: fdsnsong@inje.ac.kr

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; Cu/Zn-SOD, copper/zinc-superoxide dismutase; CYP2E1, cytochrome P450 2E1; GalN, galactosamine; GPX, glutathione peroxidase; GR, glutathione reductase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HFD, high-fat diet; MDA, malondialdehyde; Mn-SOD, manganese-superoxide dismutase; NU, nitric unit; TBARS, thiobarbituric acid-reactive substances; TG, triglyceride; TNF-α, tumor necrosis factor-alpha
The promotion of oxidative stress and the inflammatory process are involved in GalN-induced liver damage. However, previous studies have not investigated the effect of pinitol on GalN-induced liver damage. We therefore examined whether pinitol would have a protective effect on the hepatotoxicity induced by GalN. The extent of liver damage caused by GalN intoxication and the protective effect of pinitol were evaluated by measuring the levels of serum transaminases, the lipid profile in the serum and liver, the level of serum TNF-α, liver lipid peroxidation, GSH content, antioxidative enzyme activities, and liver cytochrome P450 2E1 (CYP2E1) protein expression.

Materials and Methods

Reagents. Pinitol was obtained from Amicogen Co. (Jinju, Korea); alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), and cholesterol kits were purchased from YD diagnostics Co. (Yongin, Korea); and TNF-α kit was obtained from R&D Systems (Minneapolis, MN, USA). GalN-HCl, oxidized L-glutathione, reduced L-glutathione, glutathione reductase (GR), xanthine oxidase, and NADPH were obtained from Sigma (St. Louis, MO, USA). The Bradford protein assay reagent and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G secondary antibody were obtained from Bio-Rad (Hercules, CA, USA).

The anti-rabbit cytochrome P450 2E1 antibody and anti-rabbit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody were obtained from Abcam (Cambridge, UK). The SDS–PAGE standard, NuPAGE 4–12% Bis-Tris Gel, TRIZOL-reagent, and SuperScript™ III First-strand System were from Invitrogen (Carlsbad, CA, USA), PRO-PREP™ was from iNtRON (Sungnam, Korea), and the nitrocellulose membrane was from Schleicher and Schuell (Dassel, Germany). All other chemicals were of the highest commercial grade available.

Animals, diets, and treatment. This study was conducted in accordance with the Guidelines for Animal Experiments approved by Inje University, Gimhae, Korea. Male Sprague-Dawley rats (n = 40; 6–8 weeks old) purchased from Hyochang Science (Daegu, Korea) were individually housed in cages in a room with controlled temperature (22 ± 2 °C), relative humidity (60 ± 5%), and lighting (12-h light/dark cycle). The animals were allowed free access to tap water and a commercial stock diet for 7 d for acclimatization. After this acclimatization, the rats were randomly assigned to five groups. A high-fat diet (HFD) containing 20% lard was used to cause fat accumulation in the rats' livers.16–18) Such liver cells are more sensitive to endotoxin,19) which will show a transient increase in the portal 1 h after the intraperitoneal administration of GalN. As a result, the toxicity of GalN will be more effective.

The normal control (group I) and GalN control (group II; both n = 5) were fed with HFD, whereas groups III, IV, and V (n = 10) received 0.5%, 1%, and 2% pinitol, respectively, with HFD (Table 1). The body weight and food intake were measured every 3 d. After 8 weeks of feeding, all the groups, except group I, received a single dose of 2% GalN (650 mg/kg; i.p.) suspended in normal saline at 10 a.m., group I only receiving normal saline. After 24 h, all of the rats were anesthetized by using dry ice, before blood was collected from the posterior vena cava. To obtain serum, the blood was allowed to clot at room temperature for 30 min and then centrifuged at 3,000 rpm for 15 min. The whole liver was excised, weighed, and stored at −70 °C until needed. The adipose tissue (epidermyal fat pad and abdominal adipose tissue) was weighed.

**ALT and AST assay.** The serum ALT and AST activities were determined by using commercially available reagent kits (YD diagnostics Co., Yongin, Korea).

**Lipid profile assay.** The TG and total cholesterol concentrations in the serum were determined by using colorimetric kits (YD diagnostics Co., Yongin, Korea). Hepatic lipids were extracted by the method of Folch et al.20) Each liver sample was homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the liver sample (1 g in 20 ml of the solvent mixture). The homogenate was filtered, and the solvent was washed with a 0.88% potassium chloride solution. After mixing for several seconds, the mixture was placed in a separation funnel. The lower phase was collected and mixed with a chloroform/methanol/H2O

<table>
<thead>
<tr>
<th>Compound</th>
<th>Groups I and II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
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<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>40</td>
<td>40</td>
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<tr>
<td>Cornstarch</td>
<td>8</td>
<td>7.5</td>
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<td>Lard</td>
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<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cholesterol</td>
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<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium cholate</td>
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<td>α-Cellulose</td>
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<td>5</td>
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<td>5</td>
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<td>AIN76 vitamin mixture</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AIN76 mineral mixture</td>
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<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>DL-methionine</td>
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<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Choline</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>β-pinitol</td>
<td>—</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Group I, normal control; Group II, GalN control; Group III, 0.5% pinitol + GalN; Group IV, 1% pinitol + GalN; Group V, 2% pinitol + GalN.
Lipid peroxidation assay. Lipid peroxidation in the liver was quantified by measuring the levels of thiobarbituric acid-reactive substances (TBARS) as described by Fraga et al. Malondialdehyde (MDA) formed a 1:2 adduct with thiobarbituric acid and produced a compound which could be measured by fluorometry or spectrophotometry. Lipid peroxidation is expressed as nmol of MDA equivalents per gram of liver, using a standard curve for 1,1,3,3-tetraethoxypropane.

Glutathione content determination. GSH was measured as described by Summer et al. A liver sample was first deproteinized with a 5% 5-sulfosalicylic acid solution. Catalytic amounts of glutathione caused a continuous reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid to TNB. The product, TNB, was assayed colorimetrically at 412 nm. The GSH content was determined by comparing the measured absorbance to a standard curve generated by using known amounts of GSH.

Antioxidative enzyme activity assay. Preparation. The liver sample was homogenized in 20 parts (w/v) of a 50 mM sodium phosphate buffer (pH 7.4) at 4°C; the homogenate was centrifuged at 600 × g at 4°C for 10 min to remove the cell debris, and the resulting supernatant was further centrifuged at 10,000 × g for 20 min to separate the mitochondrial pellet and cytosolic fraction. The protein concentration of each fraction was measured by the Bradford assay, with bovine serum albumin used as the standard. The mitochondrial pellet was used to test the manganese-superoxide dismutase (Mn-SOD) activity, and the cytosolic fraction was used to test for the copper/zinc-superoxide dismutase (Cu/Zn-SOD), catalase (CAT), glutathione peroxidase (GPX), and GR activity.

Cu/Zn-SOD and Mn-SOD assay. The activity of the SOD isoenzymes just described was determined by Oyanagui’s method. The superoxide anion radical, produced in the reaction with xanthine with O$_2^-$ and catalyzed by xanthine oxidase, reacts with hydroxylamine, producing the nitric ion. This nitric ion combines with naphthalene diamine and sulfanilic acid to produce a colored product, the concentration of this mixture being proportional to the amount of O$_2^-$ produced. Enzymatic activity is expressed as the nitric unit (NU) per 1 mg of liver protein (NU/mg). The SOD isoenzymes, Mn-SOD and Cu/Zn-SOD, were assayed in the liver samples, using potassium cyanide as the inhibitor of Cu/Zn-SOD, by Oyanagui’s method.

CAT activity assay. CAT activity was analyzed with Abei’s kinetic method by following the decrease in absorbance of H$_2$O$_2$ at 240 nm. Standards containing 0, 0.2, 0.5, 1, and 2 mM of H$_2$O$_2$ were used to construct a standard curve.

GPX activity assay. GPX activity in the liver samples was assayed by the Paglia and Valentine kinetic method. GPX catalyzes the reaction between reduced glutathione and hydrogen peroxide. The product of this reaction, oxidized glutathione, was recovered as reduced glutathione by using the reduced form of NADPH catalyzed by GR. The decrease in absorbance was measured at 340 nm. One unit of GPX activity is defined as the amount of enzyme that oxidased one nmole of NADPH consumed per min.

GR activity assay. GR activity in the liver samples was also assayed by a kinetic method. The decrease in NADPH concentration after the reduction of oxidized glutathione back to reduced glutathione was measured. One unit of GR activity is defined as the amount of enzyme that catalysed the reduction of one nmole of NADPH per min.

Expression of CYP 2E1 protein. The expression of CYP2E1 protein was analyzed by western blotting. Liver proteins were extracted using PRO-PREP™. Electrophoretic separation of the proteins (200 μg) was performed in NuPAGE 4–12% Bis-Tris gel, using an SE260 Mini-vertical Gel Electrophoresis Unit (GE Healthcare, Chalfont St. Giles, UK). The separated proteins were electrotransferred to nitrocellulose membranes by using a Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). The separated proteins were electrotransferred to nitrocellulose membranes by using a Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). All signals were normalized to the level of the housekeeping gene, GAPDH, and are expressed as a ratio.

Statistical analysis. Data are expressed as the mean ± standard deviation. Statistical analyses were performed with SPSS software. Student’s t-test was used, with statistical significance at $p < 0.05$ or $p < 0.01$.

Results

Body weight gain, food intake, and tissue weight
There was no significant difference in body weight gain, food intake, relative adipose tissue weight, or relative liver weight between the groups fed on the experimental diets for 8 weeks (Table 2).

Serum ALT and AST activity
Such serum enzymes as ALT and AST have been used as biochemical markers of acute hepatic damage.
The serum ALT activity was significantly higher in the GalN-injected rats than in normal rats \((P < 0.01, \text{Fig. 1})\). However, the increase in the activity of these enzymes was markedly suppressed by supplementing pinitol \((P < 0.01, \text{Fig. 1})\) to the level of the normal control. However, the supplementation level of pinitol did not result in dose-dependency.

**Serum TNF-\(\alpha\) level**

The TNF-\(\alpha\) level in the GalN group was significantly higher than that in the normal control \((P < 0.05, \text{Fig. 2})\). However, the TNF-\(\alpha\) level in the pinitol-supplemented groups at the levels of 1% and 2% was significantly lower than the level in the GalN group \((P < 0.01, \text{Fig. 2})\) and similar to the level of the normal control. However, the supplementation level of pinitol did not significantly affect the TNF-\(\alpha\) level.

**Lipid profile**

The serum TG and cholesterol levels were significantly lowered by GalN injection \((P < 0.01, \text{Fig. 3A})\). However, pinitol supplementation significantly increased the serum cholesterol levels \((P < 0.05, \text{Fig. 3A})\) at low doses \((0.5\%\) and 1\%) and serum TG at a 2\% dose \((P < 0.01, \text{Fig. 3A})\), although the levels were still lower than those of normal control group \((P < 0.05, \text{Fig. 3})\). However, a comparison of serum lipid levels between the pinitol-supplemented groups showed no difference between groups. On the other hand, GalN injection significantly increased the hepatic cholesterol level \((P < 0.01, \text{Fig. 3B})\), while 1\% and 2\% pinitol supplementation significantly attenuated the levels that had been elevated by the GalN injection \((P < 0.01, \text{Fig. 3B})\), the levels being slightly higher than that of the normal control group \((P < 0.05, \text{Fig. 3B})\). Thus, the hepatic cholesterol-lowering effect was more evident in the 1\% and 2\% pinitol-supplemented rats than in the 0.5\% pinitol-supplemented rats \((P < 0.05, \text{Fig. 3B})\). However, the hepatic TG level was not significantly changed by the GalN treatment and pinitol supplementation \((\text{Fig. 3B})\) and thus maintained the level of the normal control group.

Table 2. Effect of Pinitol on the Body Weight Gain, Good Intake, and Tissue Weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g/day)</td>
<td>3.9 ± 0.41</td>
<td>3.6 ± 0.52</td>
<td>3.8 ± 0.44</td>
<td>3.7 ± 0.38</td>
<td>3.9 ± 0.16</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>20.2 ± 2.34</td>
<td>18.7 ± 1.71</td>
<td>18.2 ± 1.46</td>
<td>18.3 ± 2.01</td>
<td>19.3 ± 2.32</td>
</tr>
<tr>
<td>Relative adipose tissue weight*</td>
<td>4.6 ± 0.41</td>
<td>4.7 ± 0.60</td>
<td>4.8 ± 0.63</td>
<td>4.6 ± 0.55</td>
<td>4.6 ± 0.67</td>
</tr>
<tr>
<td>Relative liver weight*</td>
<td>5.9 ± 0.61</td>
<td>6.9 ± 0.84</td>
<td>6.7 ± 1.17</td>
<td>6.5 ± 0.53</td>
<td>6.7 ± 0.62</td>
</tr>
</tbody>
</table>

*Relative adipose tissue weight is expressed as grams of adipose tissue \(\times 100\) per gram of body weight.

Groups: I, normal control; II, GalN control; III, 0.5% pinitol + GalN; IV, 1% pinitol + GalN; V, 2% pinitol + GalN.

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Fig. 1. Effect of Pinitol on the Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels.

Each value is the mean ± standard deviation; letters indicate significant difference at \(p < 0.01\) \((**, p < 0.01 \text{ Group I vs. Group II; }***, p < 0.01 \text{ Group II vs. Groups III, IV and V})\). Group I, normal control; Group II, GalN control; Group III, 0.5% pinitol + GalN; Group IV, 1% pinitol + GalN; Group V, 2% pinitol + GalN.
Fig. 2. Effect of Pinitol on the Serum Tumor Necrosis Factor-alpha (TNF-α) Level.
Each value is the mean ± standard deviation; letters indicate significant difference at $p < 0.01$ or 0.05 ($^+$, $p < 0.05$ Group I vs. Group II; $^*$, $p < 0.01$ Group II vs. Groups IV and V). Group I, normal control; Group II, GalN control; Group III, 0.5% pinitol + GalN; Group IV, 1% pinitol + GalN; Group V, 2% pinitol + GalN.

Fig. 3. Effect of Pinitol on the Lipid Profile.
A. Effect of pinitol on the serum lipid profile; B. Effect of pinitol on the hepatic lipid profile. Each value is the mean ± standard deviation; letters indicate significant difference at $p < 0.01$ or 0.05 ($^+$, $p < 0.01$ Group I vs. Group II; $^*$, $p < 0.01$ and $^*$, $p < 0.05$ Group II vs. Groups III, IV and V). Group I, normal control; Group II, GalN control; Group III, 0.5% pinitol + GalN; Group IV, 1% pinitol + GalN; Group V, 2% pinitol + GalN.
Lipid peroxidation

Figure 4 shows the significant increase in MDA level as a result of lipid peroxidation in the GalN-intoxicated rats ($p < 0.01$). However, pinitol supplementation for 8 weeks significantly inhibited this lipid peroxidation when compared to the GalN control ($p < 0.01$) and returned it to the level of the normal control group. The MDA levels between the pinitol-supplemented groups were not significantly different.

Hepatic glutathione level

Figure 5 shows that the hepatic GSH level was markedly lower in the GalN-intoxicated rats ($P < 0.01$). However, pinitol supplementation restored the decreased level of hepatic GSH caused by GalN injection ($P < 0.01$) to the level of the normal control group. The GSH levels between the pinitol-supplemented groups were not significantly different.

Hepatic antioxidative enzyme activity

Significantly lower GR, catalase and Mn-SOD activities were observed in the GalN-intoxicated rats ($P < 0.01$). Pinitol supplementation significantly elevated these activities and almost returned them to the normal rats ($P < 0.05$, $P < 0.01$). However, these enzyme activities between the pinitol-supplemented groups were not significantly different. There were no significant changes of GPX and Cu/Zn-SOD activities by the GalN injection or pinitol supplementation (Table 3).
Fig. 6. Effect of Pinitol on the Hepatic Cytochrome 2E1 Protein Expression.

Each value is the mean ± standard deviation; letter indicates significant difference at \( p < 0.05 \) between Group II and Group V. Group I, normal control; Group II, GalN control; Group III, 0.5% pinitol + GalN; Group IV, 1% pinitol + GalN; Group V, 2% pinitol + GalN.

**Hepatic CYP 2E1 protein expression**

A slight decrease in CYP2E1 protein expression was found in the GalN-intoxicated rats (Fig. 6). In contrast, pinitol supplementation elevated this protein expression that had been suppressed by GalN in a dose-dependent manner. The supplementation with 2% pinitol almost restored the CYP2E1 protein expression to the level of the normal control group, this being significantly higher than the GalN-control, and 0.5% and 1% pinitol groups (\( P < 0.05 \)).

**Discussion**

GalN-induced hepatotoxicity is well established as morphologically and functionally similar to human viral hepatitis. The liver damage induced by GalN generally reflects a disturbance of liver cell metabolism, which leads to characteristic changes in serum enzyme activity. The increased levels of ALT and AST activity may be interpreted as a result of liver cell destruction or a change in membrane permeability. These enzymes are characteristic of liver damage; therefore, their release into the serum confirms GalN-induced liver damage. Significant increases in ALT and AST activity were observed in the GalN-intoxicated rats, consistent with previous reports. However, pinitol supplementation at the level of 0.5% markedly decreased the elevated enzyme activity induced by GalN to the level of the normal control group, but not in a dose-dependent manner. These trends were also found in the levels of TNF-\( \alpha \), TBARS and GSH, indicating that the level of 0.5% pinitol supplementation was enough to have a hepatoprotective effect against the liver damage induced by GalN in rats.

One of the functions of the liver is the packaging of TG, cholesterol, and apolipoprotein into tiny spheres called lipoproteins. These lipoproteins are released into circulation and delivered to the cells of the body. The cells remove TG and cholesterol from the lipoproteins as they are needed. In our study, significant decreases in serum TG and cholesterol were observed in the GalN-intoxicated rats, which means that lipid influx into the blood was inhibited by the GalN injection, possibly because of a malfunction of lipoprotein metabolism such as the biosynthesis of apolipoprotein and transportation. Thus, the hepatic lipid profile was affected, where the cholesterol level was significantly higher in the GalN control than in the normal control group, which means that GalN injection impeded hepatic cholesterol accu-
mulation. Although the mechanism by which GalN impaired lipid metabolism is not certain, several researchers have proposed that carbon tetrachloride (CCl₄), a xenobiotic which can induce free radical-mediated hepatitis in humans as well as in animals,³¹,³² impairs the secretion of TG as very-low-density lipoprotein from rat liver cells.³³ In addition, CCl₄ increases the synthesis of fatty acids, triglycerides and cholesterol from acetate and the rate of lipid esterification.³⁴ Meanwhile, pinitol supplementation with GalN intoxication slightly increased the serum cholesterol level in the 0.5% and 1% pinitol-supplemented groups (P < 0.05), and decreased the hepatic cholesterol level in the 1% and 2% pinitol-supplemented groups (P < 0.01), although the level was still higher than that of the normal control group (P < 0.05). This hepatic cholesterol-lowering effect was more evident in the 1% and 2% pinitol-supplemented rats than in the 0.5% pinitol-supplemented rats (P < 0.05). However, the serum and hepatic TG levels were not significantly changed by pinitol supplementation, although 2% pinitol supplementation decreased serum TG compared to the GalN control group (P < 0.01).

These data support the notion that the administration of GalN impaired lipid metabolism and that pinitol supplementation may alleviate the adverse effect of GalN. The mechanism by which pinitol maintained lipid metabolism at almost the normal level in the GalN-intoxicated animals is not certain. Nishida et al.³⁵ have suggested that the administration of γ-glutamylvcysteinyl-ethyl ester ameliorated the hepatic TG accumulation in mice treated with CCl₄ through maintenance of the hepatic GSH level, this also being found in our study. Based on this finding, we draw the conclusion that pinitol may act as an antioxidant or activate antioxidative enzyme systems and thus possibly protect against protein and membrane damage by suppressing the oxidative stress caused by the GalN treatment.

TNF-α is a pleiotropic inflammatory cytokine. It is produced by several types of cell, especially macrophages, and plays a key role in host defense responses against injury and infection. However, excessive, prolonged production of TNF-α is thought to contribute to the pathology of several diseases. The production of TNF-α from Kupffer cells, which are activated by endotoxin derived from the gut after GalN administration, is regarded as a significant event in the toxicity of GalN in vivo.³⁶-⁶ The increase in the serum TNF-α level induced by GalN was significantly reduced by pinitol supplementation, which might have been due to its anti-inflammatory effect,³⁷ and thus inhibited the production of TNF-α from Kupffer cells.

Oxidative stress has been reported as one of the major causes of GalN-induced liver damage.³⁶-⁸ Excessive production of free radicals resulting from oxidative stress can damage such macromolecules as lipids.³⁹ The GalN-intoxicated rats showed an increased MDA level, a typical parameter of lipid peroxidation. Glutathione is an important endogenous antioxidant that is found in a particularly high concentration in the liver and has key functions in protective processes. The reduced form of GSH becomes readily oxidized to GSSG when interacting with free radicals and can induce lipid peroxidation in vivo.³⁹ GR, which is an important antioxidative enzyme, reduces GSSG to GSH. In our study, GalN injection increased MDA, and decreased the GSH content and such antioxidative enzyme activities as GR, catalase and Mn-SOD, implying that the reduced GR activity by GalN depleted the GSH pool and aggravated lipid peroxidation. It is certain why the activities of Cu,Zn-SOD and GPX were not affected by the GalN injection. Selective inhibition of antioxidative enzymes by GalN might be justified by the suggestion of Dekker and Keppler that GalN can selectively block the transcription and indirectly block hepatic protein synthesis. However, supplementation with pinitol significantly decreased MDA, and elevated the levels of GSH and antioxidative enzyme activities that had been decreased by GalN. These results suggest that pinitol might have an antioxidative effect³⁰ or stimulative effect on the antioxidative enzyme system, which might contribute to its ability to maintain the hepatic GSH level and to suppress lipid peroxidation.

Such biotransformation enzymes as cytochrome P450 isoforms are essential for the detoxication of xenobiotics.⁴¹ A significant decrease in hepatic CYP 2E1 protein expression was found in the GalN-intoxicated rats. In contrast, 2% pinitol supplementation elevated this protein expression that had been decreased by GalN and almost restored to the normal level of CYP 2E1 protein expression. Although it is not known how pinitol affected the biosynthetic mechanism of CYP 2E1, the restoration of CYP 2E1 by pinitol supplementation implies that pinitol could have a remarkable hepatoprotective effect which led to rapid recovery from GalN-induced liver injury.

In summary, the results of this study show that GalN caused acute hepatitis, as was evident by the elevation of AST, ALT and TNF-α levels, and that supplementation with 0.5% pinitol for 8 weeks to rats offered significant protection from the hepatic damage by GalN and improved the liver function by lowering the levels of serum aminotransferases, inflammatory cytokines and oxidative stress. A dose-dependent effect was not found in the 1% and 2% pinitol-supplemented groups when compared to the 0.5% group. It is concluded from all these results that 0.5% pinitol supplementation would be enough to give a protective effect against GalN-induced hepatotoxicity, at least part of this effect being attributable to attenuation of the oxidative stress and inflammatory process promoted by GalN.

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References


