Hepatoprotective Effects of Purple Potato Extract against D-Galactosamine-Induced Liver Injury in Rats

Kyu-Ho Han,1 Naoto Hashimoto,2 Ken-ichiro Shimada,1 Mitsuo Sekikawa,1 Takahiro Noda,2 Hiroaki Yamauchi,2 Makoto Hashimoto,1 Hideyuki Chii,3 David L. Topping,4 and Michihiro Fukushima1,†

1Department of Agriculture and Life Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
2Department of Upland Agriculture, National Agricultural Research Center for the Hokkaido Region, Memuro, Hokkaido 082-0071, Japan
3Department of Food Science and Human Nutrition, Faculty of Human Ecology, Fuji Women’s College, Ishikari, Hokkaido 061-3204, Japan
4CSIRO Division of Health Sciences and Nutrition, PO Box 10041, Kintore Avenue, Adelaide BC 5000, Australia

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We investigated the hepatoprotective effect of purple potato extract (PPE) against D-galactosamine (GalN)-induced liver injury in rats. PPE (400 mg) was administered once daily for 8 d, and then GalN (250 mg/kg of body weight) was injected at 22 h before the rats were killed. Serum tumor necrosis factor alpha (TNF-α), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels increased significantly after injection of GalN, but PPE inhibited GalN-induced alterations in serum TNF-α, LDH, ALT, and AST levels. Hepatic lipid peroxide and glutathione levels in the control + GalN group were higher and lower respectively than those in the control group, and those in the PPE + GalN group did not differ from that in the control group. The lipid peroxide level in hepatic microsomes treated with 2,2′-azobis (2-amidinopropane) dihydrochloride in the PPE group was significantly lower than that in the control group. This suggests that PPE has hepatoprotective effects against GalN-induced hepatotoxicity via inhibition lipid peroxidation and/or inflammation in rats.

Key words: purple potato extract; anthocyanin; D-galactosamine; hepatotoxicity; rat

Liver damage arises via direct injurious attack by a wide variety of primary hepatotoxins including alcohol, aflatoxin, heavy metals, and drugs.1 Among these, D-galactosamine (GalN) is well-established as a suitable experimental model of liver injury. The mechanism of GalN-induced hepatotoxicity appears to inhibit the synthesis of RNA and protein through a decrease in the cellular UTP concentration.2,3 Hepatic apoptosis and necrosis induced by GalN intoxication are involved in a free-radical dependent fashion in rat hepatocytes.4 Several researchers have reported that polyphenols originating from grapes, red cabbage, black rice, and strawberries have a wide range of biological effects such as anti-hepatotoxic,5 anti-inflammatory,6 and anti-carcinogenic7 activities. Furthermore, it has been reported that natural compounds can protect liver from GalN-induced toxicity.8–10 The positive effects of polyphenols from various colored fruits, vegetables, and cereals may be related to their potent antioxidant activities, demonstrated in various studies.6,11

Potato cultivars (Solanum tuberosum L.) are one of the longest and most widely cultivated high carbohydrate crops in the world. Generally, the potato has not been regarded as a food rich in antioxidants. However, depending on the genetic variety, the potato has extensive amounts of polyphenols.12 Recently, new varieties of potato have been also developed for new uses. Among these, the colored potato has become an object of increasing interest in many countries,13 due to its color appeal and excellent taste.14 The pigments in colored potato have been identified as anthocyanin derivatives.12 In view of the multiple health benefits of polyphenols, the bioavailability of potato polyphenols

† To whom correspondence should be addressed. Tel: +81-155-49-5557; Fax: +81-155-49-5577; E-mail: fukushima@obihiro.ac.jp

Abbreviations: GalN, D-galactosamine; AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; PPE, purple potato extract; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor alpha; ELAIS, enzyme-linked immunosolvent assay; TBARS, thiobarbituric acid-reactive substance; GSH, glutathione
might be important. Less well appreciated, however, is the bioavailability of polyphenols found in the colored potato, which can act as antioxidants in the human diet.

Hence in this study we investigated the hepatoprotective effects of purple potato extract containing high levels of anthocyanins against GalN-induced liver injury in rats.

Materials and Methods

Chemicals. Bovine serum albumin, Griess reagent, and thiobarbituric acid (TBA) were purchased from the Sigma Chemical (St. Louis, MO). 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Pure Chemicals Industries (Osaka).

Preparation of purple potato extract and determination of total polyphenols. Purple potato tubers (Hokkai no. 92) were supplied by the National Agricultural Research Center for the Hokkaido Region of Japan. Preparation and analysis of purple potato extract (PPE) was as follows: Potatoes were thoroughly washed with water and air dried on filter paper. The pared potatoes were homogenized with 5% formic acid and then centrifuged at 3,000 rpm for 20 min at 4°C. Next the supernatant fraction was lyophilized. The yield of PPE was approximately 0.3% based on weight. Finally the extract was dissolved in distilled water (800 mg/ml).

Total polyphenol concentration in PPE was determined by the Folin-Ciocalteu method.15) Briefly, 200 µl of diluted extracts was mixed with 2 ml of 2% Na2CO3 and then shaken. After 5 min, 200 µl of 1 N Folin-Ciocalteu’s reagent was added to the mixture, and then the solution was vigorously shaken. After 30 min, absorbance was measured at 750 nm using a spectrophotometer. Gallic acid was used as the standard. The total polyphenol concentration in PPE was approximately 4.6%, and PPE was the polyphenol rich-fraction.

Animals and diets. Male F344/DuCrj rats (7 weeks old) were purchased from Charles River Japan (Yokohama). The animal facility was maintained at 23 ± 1°C and 60 ± 5% relative humidity under a 12 h light/dark cycle. The animals were acclimated to the facility for 6 d and given free access to water and a commercial stock diet (Oriental Yeast, Tokyo). There was no significant difference in body weight between the groups at the start of the experiment. The composition of the experimental diet was as follows: casein, 25%; cornstarch, 15%; corn oil, 5%; cellulose powder, 5%; mineral mixture, 3.5% (AIN-76); vitamin mixture, 1% (AIN-76); choline chloride, 0.2%; and sucrose to 100%. This diet composition was used in our previous study.16) Animals were assigned randomly into four groups according to the type of treatment (five animals/group). PPE-treated rats were administered at a dose of 400 mg extract (18.4 mg as phenolic concentration)/0.5 ml for 8 d by intragastral gavage. The control animals were given 0.5 ml of distilled water orally for 8 d by intragastral gavage. On the 8th d, blood samples (1 ml) were collected from the jugular vein of each fasting rat in all groups for examination of the hepatotoxicity of PPE. After confirming no significant differences in serum alanine aminotransferase (ALT: control, 314 ± 38 IU/l; PPE, 311 ± 71 IU/l), aspartate aminotransferase (AST: control, 49 ± 3 IU/l; PPE, 53 ± 6 IU/l), or lactate dehydrogenase (LDH: control, 7638 ± 1093 IU/l; PPE, 7248 ± 1836 IU/l) levels among rats, GalN was injected intraperitoneally at a dose of 250 mg/kg of body weight in the control and PPE groups. GalN-untreated rats were injected intraperitoneally with distilled water. Twenty-two hours after injection with GalN, the rats were anesthetized with Nembutal, and liver and blood were obtained. Blood samples were taken into tubes without an anticoagulant. The samples stood at room temperature for 2 h, and then serum was prepared by centrifugation at 6,000 rpm for 20 min. The liver was washed with cold saline, dehydrated on filter paper, and weighed before freezing for storage. The Animal Experimental Committee of the Obihiro University of Agriculture and Veterinary Medicine approved this experimental design. All animal procedures conformed to the standard principles described in the Guide for the Care and Use of Laboratory Animals.17)

Biochemical analysis in serum. ALT, AST, and LDH activities were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory, Irving, TX).

The tumor necrosis factor alpha (TNF-α) level was measured with enzyme-linked immunosorbent assay (ELISA) according to the report of Suzuki et al.18) In the assay, monoclonal rat antimonoclonal antibody (BD Biosciences, San Jose, CA) and polyclonal rabbit antirat TNF-α primary antibody (Pierce Biotechnology, Rockford, IL) were used to detect rat TNF-α forms. Recombinant mouse TNF-α (R&D Systems, Minneapolis, MN) was used as the standard. Production of nitric oxide was measured by quantification of nitrate/nitrite using Griess reagent.19) In brief, the serum was diluted with distilled water and incubated with 0.3 M phosphate buffer containing 0.86 mM NADPH, 0.11 mM FAD, and 1.0 U/ml nitrate reductase for 1 h at room temperature. After Griess reagent was added to the buffer solutions, the mixture was incubated for 15 min at room temperature. The nitric oxide concentration was measured at 550 nm in an ELISA reader. Sodium nitrate (µM) was used as the standard.

Hepatic microsomal fraction and homogenate. The livers in the control and PPE groups were homogenized in 2 volumes of cold medium containing 50 mmol/l KCl, 2 mmol/l MgCl2, 20 mmol/l Tris–HCl (pH 7.6), and 250 mmol/l sucrose with a Potter-Elvehjem-type homogenizer. After homogenization with only four
washed by centrifugation at 12,000 × g for 10 min, and the supernatant was then centrifuged at 12,000 × g for 15 min. The supernatant from this centrifugation was further fractionated by centrifugation at 105,000 × g for 60 min, and the resulting pellet was called the microsomal fraction. This fraction was washed by centrifugation at 12,000 × g for 15 min and then at 105,000 × g for 60 min in the suspension medium, and finally suspended in 150 mmol/l KCl buffer, and finally suspended in 150 mmol/l KCl medium, and finally suspended in 150 mmol/l KCl medium, and then the microsomal fraction was exposed to 18 mmoi/l AAPH at 37°C for 0 to 120 min.

To prepare a 10% homogenate, the livers of all groups were weighed and homogenized in cold 1.15% KCl with a Potter-Elvehjem-type homogenizer.

Lipid peroxidation measurement. Lipid peroxidation in the hepatic homogenate and microsomal fractions was assessed by the thiobarbituric acid-reactive substance (TBARS) method. The reaction mixture contained 0.2 ml of homogenate or microsomal fractions, 0.2 ml of 0.8% SDS, 1.5 ml of 20% acetic acid solution (pH 3.5), and 1.5 ml of 0.5% aqueous solution of TBA. The mixture was heated for 60 min in a boiling water bath. After cooling, 4 ml of 1-butanol was added and mixed vigorously. The organic phases were separated by centrifugation, and absorbance was measured by spectrophotometer at 532 nm. Protein content was determined as described by Lowry et al.

Glutathione (GSH) concentration measurement. Hepatic GSH concentration was determined by the method of Cohn and Lye. In brief, liver was homogenized in 10 mm EDTA containing 5% trichloroacetic acid. The homogenates, consisting of 200 mg liver in 2 ml of the buffer, were centrifuged at 3,000 rpm for 10 min to remove proteins, and then the supernatant was stored at −80°C until use. Four milliliters of 0.4 M Tris–HCl buffer (pH 8.9) containing 10 mM EDTA and 250 μl of o-phthalaldehyde in methanol (1 mg/ml, w/v), was added to 200 μl of the sample supernatant. After a 5-min reaction period, the GSH concentration was determined with fluorometer.

Statistics. Data are presented as means and standard deviations. They were analyzed by two-way ANOVA to determine the effects of PPE and GalN. The significance of differences among all groups was determined by the Tukey-Kramer test (SAS Institute, Cary, NC), and the significance of differences of hepatic microsomal TBARS level between the control and PPE groups was determined by Student’s t-test.

Results

Food intake, rat growth, and liver weight
Table 1 shows the body weight, food intake, and liver weight of rats fed PPE by intragastric gavage. No differences were observed in the body weight or food intake among the groups. But food intake was lowered by PPE injections, which might have affected the final body weight in the PPE treated groups. The liver weight of rats fed PPE by intragastric gavage. No differences were observed in the body weight or food intake among the groups. But food intake was lowered by PPE injections, which might have affected the final body weight in the PPE treated groups. The liver weight in the control + GalN group was significantly lower (p < 0.05) than those of the control and PPE groups, and was not significantly different from that of the PPE + GalN group.

Liver damage indexes
Table 2 shows serum ALT, AST, and LDH activities in rats fed PPE by intragastric gavage after administration of GalN. ALT, AST, and LDH activities in the control + GalN group were significantly (p < 0.05) higher than in the control and PPE groups, but those levels in the PPE + GalN group were significantly lower (p < 0.05) than in the control + GalN group.

Serum TNF-α and NO levels
Table 3 shows serum TNF-α and nitric oxide levels in rats fed PPE by intragastric gavage after administration of GalN. The TNF-α level in the control + GalN group was significantly higher (p < 0.05) than those in the control and PPE groups, but that level in the PPE + GalN group was significantly lower (p < 0.05) than in the control + GalN group. Nitric oxide levels in all groups were not significantly different.
Table 3. Serum TNF-α and NO Levels, and Liver GSH and TBARS Concentrations of Rats Fed Purple Potato Extract

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>TNF-α (pg/ml)</th>
<th>NO (µM)</th>
<th>GSH (µmol/g liver)</th>
<th>TBARS (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.9 ± 6.3a</td>
<td>25.3 ± 9.6c</td>
<td>4.51 ± 0.31a</td>
<td>1.09 ± 0.08b</td>
</tr>
<tr>
<td>PPE</td>
<td>33.6 ± 7.4c</td>
<td>19.4 ± 14.5b</td>
<td>4.26 ± 0.40b</td>
<td>1.10 ± 0.09b</td>
</tr>
<tr>
<td>Control + GalN</td>
<td>69.9 ± 15.1b</td>
<td>31.3 ± 19.4b</td>
<td>3.13 ± 0.43b</td>
<td>1.63 ± 0.25b</td>
</tr>
<tr>
<td>PPE + GalN</td>
<td>51.1 ± 7.1a</td>
<td>37.4 ± 27.1a</td>
<td>5.01 ± 0.52a</td>
<td>1.26 ± 0.30b</td>
</tr>
</tbody>
</table>

P-value (2-way ANOVA)

| PPE       | 0.425b      | 0.0004     | 0.0005            | 0.0693                |
| GalN      | <0.0001    | 0.1176     | 0.1186            | 0.0017                |
| PPE X GalN| <0.0028    | 0.0464     | <0.0001           | 0.0606                |

Values are expressed as means ± SD for five rats. Means within the same column bearing different superscript roman letters are significantly different (p < 0.05), as determined by analysis of variance by the Tukey-Kramer test. (NO, Nitric Oxide. See Table 1 for abbreviations.

**Liver TBARS and GSH levels**

Table 3 also shows hepatic TBARS and GSH levels in rats fed PPE by intragastral gavage after administration of GalN. The TBARS level of liver homogenate in the control + GalN group was significantly higher (p < 0.05) than those in the control and PPE groups, but that level in the PPE + GalN group was not significantly different from the control + GalN groups. The GSH level in the control + GalN group was significantly lower (p < 0.05) than those in the control and PPE groups, but that level in the PPE + GalN group was significantly higher (p < 0.05) than in the control + GalN groups.

Figure 1 shows hepatic TBARS levels of the microsomal fraction in the control and PPE groups. At 40 min and 120 min incubation with 18 mM AAPH, TBARS levels of microsomal fraction in PPE group were significantly lower (p < 0.01 and p < 0.05 respectively) than those in the control group.

**Discussion**

In this study, serum ALT, AST, and LDH activities that were increased by GalN treatment were significantly decreased by pretreated PPE. Furthermore, although there was no significant difference in liver weight between the two GalN-treated groups, the PPE + GalN group tended to increase in liver weight more than the control + GalN group. Based on these data, PPE might have hepatoprotective activity against liver injury induced by GalN in rats.

Although the detailed mechanisms of the hepatotoxicity by GalN have not been clarified, the mechanism might have to do with the immune system and/or oxidative stress. Muntané et al. reported that liver injury induced by GalN is associated with an increase in serum TNF-α, but is not with serum nitrite/nitrate concentration. Itokazu et al. reported that dose-dependent administration of GalN (0.75–3.0 g/kg body weight) increases serum TNF-α and hepatic TNF-α mRNA expression in mice. Quintero et al. reported that intracellular free radical production gradually increases with GalN concentration in rat hepatocytes. In the present study, we examined the hepatoprotective effect of PPE against liver damage induced by GalN in rats. Administration of GalN causes an increase in the level of serum transaminases and hepatic necrosis and coma in rats. Some investigators have also observed that liver weight decreases 24 h after GalN injection as a result of atrophy followed by necrosis. In this study, serum ALT, AST, and LDH activities that were increased by GalN treatment were significantly decreased by pretreated PPE. Furthermore, although there was no significant difference in liver weight between the two GalN-treated groups, the PPE + GalN group tended to increase in liver weight more than the control + GalN group. Based on these data, PPE might have hepatoprotective activity against liver injury induced by GalN in rats.
different among groups, which shows that nitric oxide was not involved in GalN-induced liver damage. This finding agrees with the previous result.23) Our findings indicate that dietary PPE probably attenuates the development of GalN-induced hepatic damage by suppressing oxidative stress and/or inflammation.

Some investigators have suggested that GalN-induced liver injury can be suppressed by dietary supplementation with plant extracts and their constituents, such as catechin, glycoside, oligosaccharide, and soluble dietary fiber.8–10,27) Ramirez-Tortosa et al. reported that anthocyanin-rich extract decreases hepatic lipid peroxidation in vitamin E-depleted rats.30) In this study, increasing TBARS levels in the liver homogenate were found in GalN-intoxicated rats, consistent with previous results.8,9,27) In contrast, PPE treatment tends to attenuate the increased TBARS value in the liver homogenate. Furthermore, when the hepatic microsomal fraction from GalN-untreated rats was incubated with AAPH at 37 °C, TBARS levels of the microsomal fraction in the PPE group were significantly lower than those in the control group at 40 and 120 min. Miyazawa et al. reported that anthocyanins as forms of methylated derivatives were observed in the liver by direct intestinal absorption of red fruit anthocyanins into rats.31) Sarma and Sharma suggested that formation of anthocyanin-DNA copigmentation complex protects against oxidative damage.32) Therefore, it might be that polyphenols accumulate in the liver by the action of PPE, scavenge the free radicals derived from GalN in the aqueous phase efficiently before the radicals attack the liver cells to inhibit the chain initiation of lipid peroxidation, and thereby protect the liver from oxidative damage. However, since tissue concentrations of polyphenols in PPE were not measured in the present study, it is unclear which of the compounds in PPE had the most marked antioxidant effect in rats.

In conclusion, the results of this study indicate that PPE has hepatoprotective effects against GalN-induced hepatotoxicity in rats through inhibition of lipid peroxidation and/or inflammation. But further work is necessary to clarify the hepatoprotective mechanisms of colored potato against GalN-induced liver injury.

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Purple Potato and d-Galactosamine Liver Injury

1437


