Red Potato Extract Protects from D-Galactosamine-induced Liver Injury in Rats

Kyu-Ho Han,1 Naoto Hashimoto,2 Makoto Hashimoto,1 Takahiro Noda,2 Ken-ichiro Shimada,1 Chi-Ho Lee,3 Mitsuo Sekikawa,1 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 Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea

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The protective effects of red potato extract (RPE) as to liver damage were determined in d-galactosamine (GalN)-intoxicated rats. Increases in serum aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase activities, all of which were induced by GalN injection, decreased in RPE administered rats, suggesting that RPE acts as a functional food showing anti-hepatotoxicity.

Key words: colored potato; anthocyanin; d-galactosamine; hepatotoxicity; rat

It is well-known that polyphenols originating from various fruits and vegetables have a wide range of biological effects such as antioxidant, anti-inflammatory, and anti-carcinogenic activities.1,2) Generally, potato has not been regarded as a food rich in antioxidants, but several researchers have found that depending on the genetic variety, some potato contain considerable amounts of polyphenols, flavonoids, and anthocyanins.3,4) Furthermore, recently new varieties of potato have been developed around the world to find new uses, as these are rich in antioxidants. Among new potatoes, the colored potato especially has attracted special interest in many countries due to its color appeal, such as yellow, purple, or red. The pigments of colored potatoes have been identified as anthocyanin derivatives.5–6) Like natural antioxidants from other plants, anthocyanins from potato are believed to behave as an important functional food,3,5) but little information is available. Recently, we found that purple potato extract (PPE, Hokkai no.91) attenuates the liver damage induced by d-galactosamine (GalN) in rats,7) but it has not yet been reported that different colored potato extracts have analogous health benefits. Hence in this study we investigated the hepatoprotective effects of red potato extract (RPE, Hokkai no.91) against GalN-induced liver injury in rats.

RPE was prepared according to our previous report.7) Briefly, pared potato was homogenized with 5% formic acid and centrifuged at 2,000 × g for 20 min at 4 ℃. Finally, the supernatant fraction was lyophilized and dissolved in 70% ethanol or distilled water (RPE). The total polyphenol content in the ethanol solution was determined by the Folin-Ciocalteu method.8) Absorbance was measured at 750 nm using a spectrophotometer (Shimadzu 1600-UV, Kyoto, Japan). The flavonoid content in the ethanol solution was determined according to the report of Jia et al.,9) using standards based on the known (+)-catechin concentration. Briefly, 200 μl of a known concentration of extract was mixed with 1.25 ml of distilled water and 75 μl of 5% NaNO2, and then 150 μl of 10% AlCl3 was added 6 min later. Next, 500 μl of 1 N NaOH was added, and the total was made up to 2.5 ml with distilled water. Absorbance was measured at 510 nm using a spectrophotometer. The monomeric anthocyanin content in aqueous solution was measured by a pH differential method using a spectrophotometer.10) It was calculated using the molar extinction coefficient of pelargonidin-3-glucoside (31,6001 cm−1 mg−1), a molecular weight of 433.2 g mol−1, and absorbance A = [(A510 − A700)441.0 − (A510 − A700)444.5]. Next, RPE was applied into a Diaion HP-20 resin (Nippon Rensui, Tokyo, Japan) to purify anthocyanins. Polyphenols containing anthocyanins were eluted with 70% ethanol, and then ethanol was removed with a rotary evaporator. Finally the residue was lyophilized and dissolved in water. HPLC analysis of the dissolved sample was carried out using an LC-10AD pump system (Shimadzu, Kyoto, Japan) and a
TSKgel ODS-80Ts column (4.6 mm × 250 mm, Tosoh, Tokyo, Japan) with a flow rate of 1.0 ml/min monitoring at 360 and 520 nm. The solvent system was composed with water, methanol, and trifluoroacetic acid (70:30:0.1, by volume ratio). The molecular weights of the major peaks were determined by LC/MS (Waters ZQ 2000, Milford, MA, USA) using the same HPLC conditions as above. The major peaks on HPLC that were collected by preparative HPLC used 1H and 13C NMR analyses (JEOL ECA-500, Tokyo, Japan) for determination of chemical structures. The antioxidant activity of the purified extract from RPE in vitro was followed by a modification of the method of Mitsuda et al.,11) which is based on linoleic acid oxidation. In brief, 4 ml of 0.03% extract was mixed with 36 ml of 30 mM linoleic acid solution containing 0.1 M phosphate buffer (pH 7.0) and ethanol (4:1, v/v), and incubated at 40 °C for 0, 1, 2, 4, or 5 d. Control groups were treated with 4 ml of distilled water or 0.03% trolox solution. Two-milliliter fractions of the supernatant were obtained and mixed with 1 ml of 20% trichloroacetic acid and 2 ml of 0.67% thiobarbituric acid in acetic acid. The mixtures were incubated in a boiling water bath for 15 min. After cooling, 2 ml of chloroform was added and the samples were centrifuged at 2,000 x g for 10 min. Absorbance of the supernatant was measured at 532 nm using a spectrophotometer. Male F344/DuCrj rats (7 weeks old) were purchased from Charles River Japan (Yokohama, Japan). The animal facility was maintained at approximately 23 ± 1 °C and 60 ± 5% relative humidity and 12 h light/dark cycle. The composition of the experimental diet was as according to the AIN-76 diet.12) Animals were assigned randomly into three groups according to the type of treatment (five animals/group). Potato extract-treated rats were administered at a dose of 400 mg RPE/0.5 ml for 8 d by intragastral gavage. RPE contained approximately 2.8% total polyphenols, 0.6% flavonoids, and 0.8% anthocyanins. The control animals (n = 10) were given orally 0.5 ml of distilled water for 8 d by intragastral gavage. GalN was injected intraperitoneally at a dose of 250 mg/kg of body weight in control and RPE-treated rats. GalN-un-treated rats were injected with distilled water. Twenty-two hours after being injected with GalN, the rats were anesthetized with pentobarbital, and the liver and blood were obtained. All animal procedures conformed to the principles in the “Guide for the Care and Use of Laboratory Animals.”13) Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities were determined enzymatically using commercially available reagent kits ( assay kits for the TDX system; Abbott Laboratory, Irving, TX, USA). Lipid peroxidation in the hepatic homogenate was assessed by the thiobarbituric acid-reactive substance (TBARS) method.14) The hepatic glutathione (GSH) level was determined by the method of Cohn and Lye.15) Data are presented as means and standard deviations. The significance of differences among groups was determined by ANOVA with Duncan’s multiple-range test (SAS Institute, Cary, NC) when the F value was significant at p < 0.05.

Several researchers have found that colored potatoes have various kinds of anthocyanins depending on the genetic varieties, such as malvidin, peonidin, pelargonidin, and petunidin.4–6) Lewis et al. characterized the major pigments in the red potato as pelargonidin together with peonidin,4) while Rodriguez-Saona et al. reported that red potato contains only traces of peonidin derivatives.6) In this study, the pigment in RPE was identified as mostly pelargonidin-3-O-[6-O-(4-O-E-p-coumaroyl-O-α-thamopyranosyl)-β-glucopyranoside]-5-O-β-glucopyranoside by comparison with a previous report.16) Some reports have shown that anthocyanins have remarkable antioxidant and oxygen radical scavenging activities in vivo and in vitro.17,18) In the present study, the purified extract from RPE showed an antioxidant property against linoleic acid oxidation (Fig. 1). Recently, Igarashi et al. reported that GalN-induced liver injury can be suppressed by dietary supplementation with boysenberry anthocyanins.19) Previously, we reported that polyphenol-rich PPE attenuates the serum aminotransferases and LDH activities of GalN-induced hepatotoxicity in rats.7) In the present study, we found that serum ALT, AST, and LDH activities in the RPE + GalN group decreased dramatically more than those in the control + GalN group.

![Fig. 1. Antioxidant Effect of Purified Extract of Red Potato Extract (RPE) on Linoleic Acid Oxidation.](image-url)

Four-milliliters of 0.03% potato extract (●) was mixed with 36 ml of 30 mM linoleic acid solution containing 0.1 M phosphate buffer (pH 7.0) and ethanol (4:1, v/v), and incubated at 40 °C for 0, 1, 2, 4, or 5 d (n = 3). Control groups were treated with 4 ml of distilled water (■) or 0.03% trolox solution (●).
(Table 1). In view of these data, RPE might have preventive effects as to GalN liver injury in rats. On the other hand, it has been reported that GalN causes a decrease in liver weight as a result of atrophy followed by necrosis in rats.20,21) This phenomenon might originate in hepatocellular injury secondary to the atrophy of liver produced by GalN treatment, because GalN reduces the intracellular pool of uracil nucleotide in hepatocytes, thus inhibiting RNA and protein synthesis.22) As Table 1 shows, liver weight in the GalN-treated groups was significantly (p < 0.05) lower than in the control group. Nevertheless, the RPE + GalN group tended to increase in liver weight more than the control + GalN group (Table 1). It might be that RPE inhibits hepatocellular injury secondary to the atrophy of liver produced by GalN injection. Several reports have shown an increase in lipid peroxidation and a decrease in GSH level in damaged liver treated with GalN in animal experiments.21,23,24) Furthermore, it has been suggested that GalN induces serious oxidative stress in the rat liver, leading to apoptosis and necrosis.25) In a previous study, we found that PPE hinders the development of GalN-induced hepatic damage by inhibiting alterations in liver TBARS and GSH levels.7) In the present study, although liver TBARS levels between the GalN-treated groups were not found to be significantly different, RPE tended to lower the increased TBARS level in the liver homogenate (Table 1). Furthermore, the hepatic GSH level in the RPE + GalN group significantly (p < 0.05) increased over that in the control + GalN group (Table 1). These data are similar to those of a previous report.7)

In conclusion, this study indicates that RPE had a protective effect against GalN-induced hepatotoxicity, acting by inhibiting intracellular GSH depletion in rats. However, since the anthocyanin concentration of RPE might not have been enough to protect against liver injury in the present study, further work is necessary to clarify which of the compounds in RPE affects the protective activity in rats.

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