In acute hepatic injury tests, an adzuki bean extract decreased d-galactosamine (GalN)-induced alterations in the serum alanine aminotransferase and aspartate aminotransferase activities to about 37% and 25%, respectively, although there were no significant differences in these activities between the GalN-treated group with the adzuki bean extract and the GalN-treated group without the adzuki bean extract. Furthermore, the hepatic glutathione peroxidase, glutathione reductase, and Mn– and Cu,Zn–superoxide dismutase mRNA levels in the GalN-treated group with the adzuki bean extract were higher than those in the control group and GalN-treated group without the adzuki bean extract.

Key words: adzuki bean extract; d-galactosamine; glutathione peroxidase mRNA; glutathione reductase mRNA; superoxide dismutase mRNA

It is known that liver injury occurs via direct injurious attack by a wide variety of primary hepatotoxins, including alcohol, aflatoxin, heavy metals and drugs. Among these, d-galactosamine (GalN) is similar to human viral hepatitis in its morphological and functional features. It has been reported that GalN induced hepatotoxicity by inhibiting the synthesis of RNA and protein through a decrease in the cellular UTP concentration.

Several researchers have recently shown that natural antioxidants and components of plant origin can prevent free radical-mediated hepatotoxicity. In epidemiological studies, the consumption of anthocyanins through fruit- and vegetable-based intake has been shown to be connected to improved health. The positive effects of these pigments could be related to their potent antioxidative activities demonstrated in various studies. Wu et al. have recently shown that a water-soluble extract of the adzuki bean could inhibit acetaminophen-induced liver damage. Han et al. have reported the protective action of an adzuki extract against acetaminophen-induced hepatotoxicity via a hepatic GSH-mediated antioxidation/detoxification system in rat liver after 4 wk of feeding. However, at present, the mechanism for protection by an adzuki bean extract against d-galactosamine-induced liver injury remains unclear. We therefore investigated in this study the therapeutic efficacy of an adzuki bean extract containing anthocyanins in an experimental model of liver injury induced by GalN in rats.

The adzuki bean extract was prepared according to the method of Igarashi et al. The yield of adzuki bean extract powder was approximately 0.5% based on weight, and the anthocyanin concentration of the powder was 40% by the method of Torre and Barritt. The powder was dissolved in distilled water. Male F344/DuCrj rats (7 wk old) were purchased from Charles River Japan (Yokohama, Japan). The animal facility was maintained at 23 ± 1°C and 60 ± 5% relative humidity with a 12-h light/dark cycle. The composition of the experimental diet was according to the AIN-93G diet. The animals were randomly assigned to 3 groups according to the type of treatment (5 animals/group). The control rats and adzuki bean extract-treated rats were respectively administered with 1 ml of distilled water and 40 mg of adzuki bean extract powder/1 ml of distilled water orally by forced feeding for 7 days. After feeding with the experimental diets for 7 days, blood samples (1 ml) were collected from the jugular vein of each fasting rat and GalN was injected intraperitoneally at a dose of 250 mg/kg of body weight to the control and
GalN reduces the intracellular pool of uracil nucleotide manual as described previously.17) The amounts of (Nippongene, Tokyo, Japan) according to the user’s U.S.A.). Total RNA in the liver was isolated by Isogen TDX system; Abbott Laboratory Co., Irving, TX, commercially available reagent kits (assay kits for the activities were determined enzymatically by using ferase (ALT) and aspartate aminotransferase (AST) shown in Fig. 1, the mean values for the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities that had been dramatically increased by in hepatocytes, thus inhibiting RNA and protein synthesis.1) No differences were observed in the body weight and food intake among the groups in this study (Table 1). The liver weights of the GalN-treated group without the adzuki bean extract and of the GalN-treated group with the adzuki bean extract were significantly higher than that of the control group (Table 1). As shown in Fig. 1, the mean values for the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities that had been dramatically increased by the GalN treatment were decreased to about 37% and 25%, respectively by the adzuki bean extract, although there were no significant differences in these activities between the GalN-treated group with the adzuki bean extract and the GalN-treated group without the adzuki bean extract. This indicates that the adzuki bean extract had hepatoprotective activity against the liver injury induced by GalN in rats, these results agreeing with previous results.12) Some investigators have reported that GalN-mediated hepatotoxicity may be prevented by

Table 1. Body Weight, Food Intake, and Liver Weight of Rats Fed with the Adzuki Bean Extract

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Liver weight (g/100 g b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>157 ± 1.9</td>
<td>180 ± 5.8</td>
<td>17 ± 0.5</td>
<td>3.1 ± 0.04^</td>
</tr>
<tr>
<td>G-BD</td>
<td>158 ± 1.8</td>
<td>178 ± 2.7</td>
<td>17 ± 0.4</td>
<td>3.4 ± 0.07^</td>
</tr>
<tr>
<td>G-AK</td>
<td>160 ± 3.6</td>
<td>179 ± 3.2</td>
<td>17 ± 0.7</td>
<td>3.4 ± 0.06^</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM for five rats. ^Means within the same column bearing different superscripted roman letters are significantly different (P < 0.05).

BD, control group; G-BD, GalN-treated group; G-AK, GalN-treated group with the adzuki bean extract. GalN, d-galactosamine.

Fig. 1. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Activities in Rats Fed with the Adzuki Bean Extract before or after Administering d-Galactosamine.

Each value is expressed as the mean ± SEM for five rats. BD, control group; G-BD, GalN-treated group; G-AK, GalN-treated group with the adzuki bean extract. GalN, d-galactosamine.

Adzuki bean extract groups. GalN-untreated rats were injected with distilled water. Twenty-two hours after being injected with GalN, the rats were anesthetized by pentobarbital, and the liver and blood were obtained. All animal procedures described conformed to standard principles in the Guide for the Care and Use of Laboratory Animals.16) The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined enzymatically by using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX, U.S.A.). Total RNA in the liver was isolated by Isogen (Nippongene, Tokyo, Japan) according to the user’s manual as described previously.17) The amounts of mRNA encoding glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R), Mn–superoxide dismutase (Mn–SOD), Cu,Zn–superoxide dismutase (Cu,Zn–SOD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, as an internal standard) were estimated by semi-quantitative RT-PCR and a subsequent Southern blot analysis.17) Each data value is presented as the mean and SEM. The significance of differences among all groups was determined by ANOVA with Duncan’s multiple-range test (SAS Institute, Cary, NC, U.S.A.).

Han et al. have reported that the water extract from adzuki bean hulls lowered the serum AST activity and elevated the hepatic GSH reductase activity of acetaminophen-induced damage to rat liver after 4 wk of feeding.12) In the present study, we examined the hepatoprotective effect of the adzuki bean extract against liver damage induced by GalN in rats. It is well established that GalN is a suitable experimental model of liver injury11 that is similar to viral hepatitis, and that GalN reduces the intracellular pool of uracil nucleotide

Table 1. Body Weight, Food Intake, and Liver Weight of Rats Fed with the Adzuki Bean Extract

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Liver weight (g/100 g b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>157 ± 1.9</td>
<td>180 ± 5.8</td>
<td>17 ± 0.5</td>
<td>3.1 ± 0.04^</td>
</tr>
<tr>
<td>G-BD</td>
<td>158 ± 1.8</td>
<td>178 ± 2.7</td>
<td>17 ± 0.4</td>
<td>3.4 ± 0.07^</td>
</tr>
<tr>
<td>G-AK</td>
<td>160 ± 3.6</td>
<td>179 ± 3.2</td>
<td>17 ± 0.7</td>
<td>3.4 ± 0.06^</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM for five rats. ^Means within the same column bearing different superscripted roman letters are significantly different (P < 0.05).

BD, control group; G-BD, GalN-treated group; G-AK, GalN-treated group with the adzuki bean extract. GalN, d-galactosamine.

Fig. 1. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Activities in Rats Fed with the Adzuki Bean Extract before or after Administering d-Galactosamine.

Each value is expressed as the mean ± SEM for five rats. BD, control group; G-BD, GalN-treated group; G-AK, GalN-treated group with the adzuki bean extract. GalN, d-galactosamine.
plant anthocyanins. Anthocyanins are known to be effective quenchers of ROS, and the anthocyanin content has a high correlation with the oxygen radical-absorbing capacity. It has recently been suggested that reactive oxygen species (ROS: OH*, O2•−, RO•, ROO•, and 1O2) produced by activated hepatic macrophages might be the primary cause of GalN-induced liver damage. In the present study, the serum AST and ALT activities were respectively about 6- and 26-fold higher in the GalN-treated group than in the control group. However, the hepatic anti-oxidant enzyme (GSH-Px, GSH-R, Mn–SOD and Cu,Zn–SOD) mRNA levels in the GalN-treated group with the adzuki bean extract were significantly higher, being 75%, 177%, 58% and 68%, respectively, as compared with the GalN-treated group without the adzuki bean extract (Fig. 2), the being set to 100. The top panel illustrates the representative southern hybridization of PCR-amplified GSH-Px cDNA, GSH-R cDNA, Mn–SOD cDNA and Cu,Zn–SOD cDNA of hepatic RNA.

Acknowledgments

This research was supported by a grant from the 21st Century COE Program (A-1) of Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Research and Development Program for New Bio-industry Initiatives of the Bio-oriented Technology Research Advancement Institution.

References

14) Torre, L. C., and Barratt, B. H., Quantitative evaluation of Rubus fruit anthocyanin pigments. J. Food Sci., 42,


