Protective Effects of Dietary Nasunin on Paraquat-induced Oxidative Stress in Rats

Yuriko Kimura, Yumi Araki, Asako Takenaka, and Kiharu Igarashi

Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka-shi, Yamagata 997-0037, Japan

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The preventive effects of nasunin (delphinidin-3-[4-p-coumaroyl]-rhamnosyl(1â†’6)glucoside]-5-glucoside) on paraquat-induced oxidative stress were determined in rats. Decreased food intake and body weight gain and increased lung weight by feeding the rats a diet containing paraquat were clearly suppressed by supplementing nasunin to the paraquat diet. Paraquat feeding increased the concentration of thiobarbituric acid-reactive substances (TBARS) in liver lipids and the atherogenic index, and decreased the liver triacylglycerol level. These effects were also suppressed by supplementing nasunin to the paraquat diet. In addition, catalase activity in the liver mitochondrial fraction was markedly decreased by feeding the paraquat diet, this decrease being partially suppressed by supplementing the paraquat diet with nasunin. These results suggest that nasunin acted preventively against the oxidative stress in vivo that may have been due to active oxygen species formed through the action of paraquat.

Key words: nasunin; oxidative stress; paraquat; anthocyanin

The physiological functions of polyphenols are receiving great attention because French paradox is closely related to the large amount of polyphenols in red wine. Anthocyanins are contained in many fruits, vegetables and cereals with red skins, belonging to polyphenols, and are also contained in red wine. Accordingly, the antioxidative and physiological functions of anthocyanins have been focused on by many researchers. However, the preventive effects of anthocyanins against oxidative stress in vivo have not been examined. We have previously found malvin, rubrobrassicin, and nasunin to be the major anthocyanins in wild grapes, atsumi-kabu (a variety of red turnip), and eggplant. These anthocyanins had antioxidative activity toward the autoxidation of linoleic acid and the oxidation of linoleic acid in a linoleic acid-â-carotene-lipoxygenase system. Of these three anthocyanins, nasunin showed the strongest activity. The strong antioxidative activity of nasunin suggests that it may also have a strong preventive effect against oxidative stress in vivo. On the other hand, it is also known that some anthocyanins have a cholesterol-lowering effect, anti-mutagenic and anti-tumor effects, an inhibitory effect on platelet aggregation, anti-ulcer effect, and a promoting effect on rhodopsin synthesis.

In this study, the preventive effects of nasunin against the oxidative stress induced by paraquat, which is known to produce active oxygen species in vivo, were determined in rats.

Materials and Methods

Preparation of nasunin. Nasunin was prepared as described in a previous paper. Nasunin was extracted from the peel of eggplant (chouja-nasu: Solanum melongena L. 'Chouja') with a 3% trifluoro-acetic acid solution at 5°C, and then filtered. The extract was passed through a column of XAD-7. After successively washing the column with H2O and 30% MeOH, nasunin was eluted with MeOH, and the eluate was evaporated. The resulting residue was dissolved in MeOH-AcOH-H2O (10:1:9, v/v) and chromatographed in a Sephadex LH-20 column, using the same solvent. The evaporated eluates corresponding to a major reddish purple band in the column, was dissolved in a small amount of 0.01% HCl-MeOH, and nasunin was precipitated by adding diethyl ether. The purity level of the isolated nasunin was more than 95% when analyzed by HPLC in an ODS (HG-5) column (4 mm × 150 mm, Nomura Chemical Co., Nagoya, Japan), using a linear gradient of 0–100% solvent B (88% HCOOH-AcOH-H2O-MeOH = 1:1:6:3, v/v) in solvent A (88% HCOOH–AcOH-H2O = 1:1:9, v/v). Nasunin was dried in vacuo for use in the subsequent animal experiment.

Animals and diets. Four-week-old male weanling Wistar-strain rats, each weighing about 50 g, were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and fed on a commercial diet (F-2, Funahashi Farm, Funahashi, Japan) for 2 d before use in the experiment, before being randomly divided into four groups of 6 rats each. The rats were individually housed in stainless-steel cages with screen bottoms, and kept under controlled conditions with a 12-h light-dark cycle (06:00–18:00 light), a temperature range of 22–24°C, and a relative humidity of about 55%.

The compositions of the experimental diets are given in Table 1. The basal diet contained 20% casein without either paraquat (PQ) or nasunin. The PQ diet was prepared by adding PQ (methyl viologen, Sigma Chemical Co., St. Louis, U.S.A.) to the basal diet at the 0.02% level. The nasunin (Nas) diet without PQ was prepared by adding nasunin to the basal diet at the 0.15% level.

* To whom correspondence should be addressed. Fax: +81-235-28-2812; E-mail: igarashi@tds1.tr.yamagata-u.ac.jp
The PQ + Nas diet was prepared by adding both PQ and nasunin (at the 0.02% and 0.15% levels) to the basal diet. The total amounts of the PQ, Nas and PQ + Nas diets were made up to 100% by adjusting the amount of the mixture of α-corn starch and sucrose (2:1), which was added to the basal diet. The amount of PQ added to the basal diet was selected to be less than that defined as the maximum non-toxic level to rats (250 mg per kg of diet) by a committee from FAO and WHO. Food and water were provided ad libitum for 11 d.

Collection and preparation of blood and liver. Blood was collected by cardiac puncture from rats that had been anesthetized with Nembutal (50 mg/ml; Dainippon Pharmaceutical Co., Osaka, Japan) after 10 h of starvation (from 03:00-07:00 to 13:00-17:00) at the end of the feeding period. A 0.1-ml aliquot of the blood was added to 1.9 ml of physiological saline while gently shaking, and the mixture was centrifuged at 1000 x g for 10 min to obtain serum for measuring the serum TBARS concentration and to provide erythrocytes. The erythrocytes were lysed with H₂O for measuring the CuZn-superoxide dismutase (CuZn-SOD), catalase, and glutathione peroxidase (GSH-Px) activities. A section from the right lobe of the liver, which had been excised from each of the rats, was used to measure the liver activities of CuZn- and Mn-SOD, GSH-Px, catalase, and glutathione reductase (GSSG-R) activities. The other parts of the liver were stored at −30°C until needed for measuring the lipid and TBARS concentrations. Centrifuging the blood at 1000 x g for 15 min separated the serum for measuring the lipids.

Preparation of the subcellular fraction. The supernatant for measuring the antioxidative enzyme activities in the liver was prepared according the method of Del Boccio et al. One g of the liver right lobe was homogenized in 5 ml of an ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA with a teflon pestle, and the homogenate was mixed with 2 volumes of 2.3% KCl, before being centrifuged at 600 x g for 3 min at 4°C to remove the fibrous material. The supernatant that had been obtained was further centrifuged at 14000 x g for 10 min at 4°C to remove the cellular debris. An aliquot of this supernatant was subsequently sonicated at 30 W for 2 min (4 times for 30 sec each) in a Branson 1200 sonicator (Yamato Co., Tokyo, Japan). It was then centrifuged at 10,000 x g for 30 min at 4°C to obtain the mitochondrial fraction as a precipitate. This precipitate was sonicated in a 0.1 M phosphate buffer (pH 7.8), and the SOD and catalase activities were then measured. An aliquot of the supernatant which had been separated from the mitochondrial fraction was centrifuged at 105,000 x g for 60 min at 4°C to obtain the cytosolic fraction for measuring the SOD, catalase, GSH-Px and GSSG-R activities.

SOD activity was measured by the xanthine-xanthine oxidase-nitroblue tetrazolium (NBT) system as described by Imanari et al., and catalase activity was measured by the spectrophotometric method to monitor the decrease in absorbance at 240 nm at 25°C due to hydrogen peroxide decomposition as described by Tomita et al. GSH-Px activity was measured by monitoring the decrease in absorbance of NADPH at 340 nm, using t-butyl hydroperoxide as a substrate. GSSG-R activity was also measured by monitoring the decrease in NADPH, but using oxidized glutathione as a substrate. The definition of one unit of SOD and catalase activity is the amount of enzyme required to inhibit the rate of dimofemaran formation from NBT by 50% per mg of hemoglobin or protein, and the amount of enzyme to decompose 1 μmol of H₂O₂ per min per mg of hemoglobin or protein, respectively. The definition of one unit of GSH-Px and GSSG-R activity is the amount of enzyme that is required to oxidize 1 μmol of NADPH per min per mg of hemoglobin or protein.

The hemoglobin and protein contents were measured by the method of Ohshiro et al. with a commercial hemoglobin test kit (Wako Pure Chemical Ind., Osaka, Japan), and by the method of Lowry et al., using bovine serum albumin as the standard, respectively.

Measurement of TBARS. The serum TBARS concentration was determined by the method of Yagi and is expressed as mmol of malondialdehyde per l of blood. The liver TBARS concentration was measured by the method of Uchiyama and Miura, using the homogenate which had been obtained by homogenizing 1 g of frozen rat liver (a section from the main lobe) with 9 ml of 1.15% KCl.

Lipid analyses. Serum total cholesterol was determined by the cholesterol oxidase method with a cholesterol E-test kit (Wako Pure Chemical Ind.), triacylglycerol by the glycerol-3-phosphate oxidase method with a triglyceride E-test kit (Wako Pure Chemical Ind.), and phospholipid by the choline oxidase method with a phospholipid B-test kit (Wako Pure Chemical Ind.). Serum HDL-cholesterol was enzymatically measured in the supernatant obtained after heparin-Mn precipitation of the other lipoproteins.

The lipids of the frozen livers were extracted and purified by the method of Folch et al. The lipids in the extract were measured by the same methods as those used for the serum, with the exception that total cholesterol was determined by the choline oxidase method with a Mono-test cholesterol kit (Boehringer...
Manheim Yamanouchi Co., Tokyo, Japan). The atherogenic index is expressed as \((\text{total cholesterol-HDL-cholesterol})/\text{HDL-cholesterol}\).

Statistical analyses. Data from 6 rats in each of the basal and nasunin diet groups, and from 5 rats in each of the PQ and PQ+Nas diet groups were used for statistical analyses, because one rat in each of the PQ and PQ+Nas diet groups died during the feeding period. The data for each of the 4 groups were statistically analyzed by Duncan’s multiple-range test after a two-way analysis of variance (ANOVA), with the exception that the food intake and body weight were compared by applying the Mann-Whitney test for nonparametric data. Significant differences in the means were inspected at \(P<0.05\).

Results

Food intake, and body and organ weights

As shown in Fig. 1, the food intake and body weight of the rats fed on the PQ diet (PQ group) underwent a statistically significant decrease after 9 d as compared with the figure for the control rats (basal diet-fed rats). Both the lung weight and relative weight of the lung were increased by PQ. The liver weight decreased in the PQ-fed rats compared with that of the control rats, but the relative weight of the liver did not (Table 2). The supplement of nasunin to the PQ diet relieved the decreased food intake, body weight and liver weight, and the increased lung weight. An interaction between PQ and nasunin was noted in the food intake, body weight gain, and in the liver and lung weights (Table 2).

TBARS and antioxidative enzyme activities

As shown in Table 2, the serum TBARS concentration did not differ among the 4 groups. The TBARS concentration in the liver lipids of the PQ-fed rats was significantly higher than that of the control rats, but the liver TBARS concentration expressed as nmol/g of liver did not differ among the 4 groups. The addition of nasunin to the PQ diet significantly relieved this increase, and an interaction between PQ and nasunin was apparent.

Table 2 also shows that the catalase activity in the erythrocytes was decreased by adding PQ to the basal diet, but was returned to the control level by supplementing the PQ diet with nasunin. Catalase activity in the erythrocytes showed the interaction between PQ and nasunin. Catalase activity in the liver mitochondrial fraction was markedly lower in the PQ-fed rats, but was partially recovered by supplementing the PQ diet with nasunin. The GSH-Px activity in the erythrocytes and liver cytosol fraction and the GSSG-R activity in liver cytosol fraction did not show any statistically significant differences among the 4 groups.

Lipid levels

Table 3 shows that the atherogenic index was increased to a statistically significant level in the PQ-fed rats when compared to that in the control rats. The supplement of nasunin to the PQ diet significantly suppressed this increase in the atherogenic index. The concentrations of serum total cholesterol and triacylglycerol were not affected by dietary manipulation.

The concentration of liver triacylglycerol was significantly decreased in the PQ-fed rats, compared to that in the control rats, and was almost returned to the level of the control rats by supplementing nasunin to the PQ diet. The other liver lipid concentrations were no different among the four dietary groups.

Discussion

The protection by nasunin from decreased food intake and body weight after the feeding period for 8 d, and from increased lung weight and from decreased liver weight at the end of the feeding period indicates the possibility that nasunin could act preventively against PQ toxicity which is known to induce oxidative damage to such organs as the lungs, liver, kidneys and heart.\(^{30,31}\) The decreased food intake by the PQ-fed rats after 8 d may indicate that biochemical markers such as antioxidative enzyme activities may have begun to change on day
Table 2. Effects of Nasunin on the Organ Weights, Erythrocytes and Liver Antioxidative Enzyme Activities, and on the Serum and Liver TBARS Levels of Rats Fed on a Diet with or without Paraquat

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Basal</th>
<th>PQ</th>
<th>Nas</th>
<th>PQ+Nas</th>
<th>ANOVA (P value)</th>
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<td>n</td>
<td></td>
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<tr>
<td>Initial body weight (g)</td>
<td>58.8 ± 1.3</td>
<td>58.8 ± 0.8</td>
<td>58.8 ± 0.8</td>
<td>58.8 ± 0.9</td>
<td>0.002 0.002 0.002</td>
</tr>
<tr>
<td>Food intake (g/11 d)</td>
<td>113 ± 1²</td>
<td>79.6 ± 3.3³</td>
<td>114 ± 0.4³</td>
<td>111 ± 4³</td>
<td>0.002 0.002 0.002</td>
</tr>
<tr>
<td>Body weight gain (g/11 d)</td>
<td>41.9 ± 1.0⁴</td>
<td>8.05 ± 3.0⁰</td>
<td>40.0 ± 0.5⁵</td>
<td>35.1 ± 7³</td>
<td>0.01 0.05 0.01</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>3.94 ± 0.08⁸</td>
<td>2.88 ± 0.01³</td>
<td>3.79 ± 0.1³</td>
<td>3.56 ± 0.1³</td>
<td>0.002 0.002 0.002</td>
</tr>
<tr>
<td>Liver weight (% of body weight)</td>
<td>3.92 ± 0.06</td>
<td>4.04 ± 0.10</td>
<td>3.84 ± 0.05</td>
<td>3.78 ± 0.18</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>0.632 ± 0.01³</td>
<td>0.974 ± 0.07³</td>
<td>0.613 ± 0.08³</td>
<td>0.691 ± 0.03³</td>
<td>0.002 0.002 0.002</td>
</tr>
<tr>
<td>Lung weight (% of body weight)</td>
<td>0.628 ± 0.01³</td>
<td>1.49 ± 0.1³</td>
<td>0.621 ± 0.00³</td>
<td>0.767 ± 0.02³</td>
<td>0.002 0.002 0.002</td>
</tr>
<tr>
<td>In erythrocytes</td>
<td>SOD (U/mg of Hb)</td>
<td>2.09 ± 0.05</td>
<td>2.06 ± 0.02</td>
<td>2.18 ± 0.07</td>
<td>2.01 ± 0.02</td>
</tr>
<tr>
<td>Catalase (U/mg of Hb)</td>
<td>90.5 ± 5.4³</td>
<td>70.7 ± 5.0³</td>
<td>83.4 ± 2.5³</td>
<td>86.5 ± 3.4³</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>GSH-Px (U/mg of Hb)</td>
<td>0.645 ± 0.23³</td>
<td>1.32 ± 0.37</td>
<td>1.16 ± 0.2</td>
<td>1.49 ± 0.25</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>In liver cytosol fraction</td>
<td>SOD (U/mg of protein)</td>
<td>2.65 ± 0.13</td>
<td>2.45 ± 0.06</td>
<td>2.57 ± 0.06</td>
<td>2.79 ± 0.1</td>
</tr>
<tr>
<td>Catalase (U/mg of protein)</td>
<td>372 ± 17</td>
<td>307 ± 29</td>
<td>342 ± 22</td>
<td>349 ± 15</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>GSH-Px (U/mg of protein)</td>
<td>0.211 ± 0.03¹</td>
<td>0.176 ± 0.01³</td>
<td>0.189 ± 0.02³</td>
<td>0.154 ± 0.02²</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>GS-SR (U/mg of protein)</td>
<td>0.0626 ± 0.0036</td>
<td>0.0595 ± 0.0032</td>
<td>0.0622 ± 0.0037</td>
<td>0.0667 ± 0.0027</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>In liver mitochondrial fraction</td>
<td>SOD (U/mg of protein)</td>
<td>3.43 ± 0.20³</td>
<td>2.46 ± 0.28³</td>
<td>4.01 ± 0.38³</td>
<td>3.31 ± 0.22³</td>
</tr>
<tr>
<td>Catalase (U/mg of protein)</td>
<td>205 ± 16³</td>
<td>67.8 ± 6.4³</td>
<td>229 ± 21³</td>
<td>145 ± 10³</td>
<td>0.002 0.004 NS</td>
</tr>
<tr>
<td>Serum TBARS (nmol/l of blood)</td>
<td>1.61 ± 0.12³</td>
<td>1.73 ± 0.10³</td>
<td>1.63 ± 0.08³</td>
<td>1.66 ± 0.15³</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Liver TBARS (nmol/g of liver)</td>
<td>26.5 ± 0.9</td>
<td>29.3 ± 0.2</td>
<td>28.5 ± 1.0</td>
<td>27.3 ± 2.1</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Liver TBARS (nmol/g of total lipids³)</td>
<td>0.584 ± 0.02³</td>
<td>0.870 ± 0.03³</td>
<td>0.684 ± 0.04³</td>
<td>0.676 ± 0.05³</td>
<td>0.002 NS NS</td>
</tr>
</tbody>
</table>

Each result is expressed as the mean±SE of 5 or 6 rats per group. Values within the same row and not sharing a common superscript letter are significantly different at p<0.05.

¹ Effect of paraquat (PQ) in the diets.
² Effect of nasunin (Nas) in the diets.
³ Interaction between PQ and Nas.

* Total lipids were extracted with chloroform-methanol (2:1) by the method of Folch et al.³⁹
SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GS-SR, glutathione reductase.

Table 3. Effects of Nasunin on the Serum and Liver Lipid Concentrations of Rats Fed on a Diet with or without Paraquat

<table>
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<tr>
<td>Serum lipids</td>
<td>Total cholesterol (mmol/l)</td>
<td>1.94 ± 0.03</td>
<td>2.15 ± 0.1</td>
<td>1.88 ± 0.04</td>
<td>1.84 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol (mmol/l)</td>
<td>1.61 ± 0.73</td>
<td>1.48 ± 0.18</td>
<td>1.55 ± 0.06</td>
<td>1.56 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Triacylglycerol (mmol/l)</td>
<td>0.443 ± 0.02³</td>
<td>0.318 ± 0.09³</td>
<td>0.409 ± 0.02³</td>
<td>0.369 ± 0.03³</td>
</tr>
<tr>
<td></td>
<td>Phospholipid (mmol/l)</td>
<td>1.67 ± 0.04³</td>
<td>1.80 ± 0.11³</td>
<td>1.68 ± 0.06³</td>
<td>1.52 ± 0.09³</td>
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<td>Atherogenic index³</td>
<td>0.216 ± 0.05³</td>
<td>0.510 ± 0.12³</td>
<td>0.221 ± 0.04³</td>
<td>0.186 ± 0.05³</td>
</tr>
<tr>
<td>Liver lipids</td>
<td>Total cholesterol (μmol/g of liver)</td>
<td>4.43 ± 0.15³</td>
<td>3.86 ± 0.16³</td>
<td>4.07 ± 0.16³</td>
<td>4.19 ± 0.33³</td>
</tr>
<tr>
<td></td>
<td>Triacylglycerol (μmol/g of liver)</td>
<td>11.7 ± 1.0³</td>
<td>1.71 ± 0.16³</td>
<td>12.1 ± 0.1³</td>
<td>7.98 ± 1.1³³</td>
</tr>
<tr>
<td></td>
<td>Phospholipid (μmol/g of liver)</td>
<td>17.6 ± 5.³</td>
<td>15.9 ± 1.0³</td>
<td>16.8 ± 0.5³</td>
<td>18.1 ± 0.8³</td>
</tr>
</tbody>
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Each result is expressed as the mean±SE of 5 or 6 rats per group. Values within the same row and not sharing a common superscript letter are significantly different at p<0.05.

¹ Effect of paraquat (PQ) in the diet.
² Effect of nasunin (Nas) in the diet.
³ Interaction between PQ and Nas.
⁴ (Total cholesterol: HDL cholesterol)/HDLC: cholesterol.

8. However, it is necessary to further examine the time-course for the changes in biochemical markers by administering PQ.

The serum TBARS concentration was not affected by feeding the PQ diet or by supplementing nasunin to the PQ diet. However, the concentration of TBARS in the liver lipids, which was increased by the PQ diet, was decreased by supplementing nasunin to the PQ diet. These results indicate the possibility that nasunin can suppress the increase in liver lipid peroxidation due to PQ. When the concentration of liver TBARS is expressed as nmol/g of liver, the value for the PQ-fed rats
did not differ from that of the rats fed on the PQ diet supplemented with nasunin. This result indicates that it may be necessary to investigate the protective effects of nasunin against oxidative stress by measuring another indicator for oxidative stress such as 8-hydroxydeoxyguanosine.\textsuperscript{32} However, the suppression by nasunin of decreased body weight gain and liver weight, and from increased lung weight and TBARS in the liver lipids which were induced by PQ may support the possibility that nasunin could act antioxidatively \textit{in vivo}.

The SOD and GSH-Px activities in the erythrocytes and in the liver cytosol fraction, and the GSSG-R activity in cytosol fraction were not affected by either PQ or nasunin. However, we found that the catalase activities in the erythrocytes and in the mitochondrial fraction were decreased in the PQ-fed rats when compared to those in the control rats. Catalase appears to be the enzyme susceptible to PQ-induced oxidative stress. The decrease in catalase activities in the erythrocytes and liver mitochondrial fraction by administering PQ had not previously been reported. Our results in this experiment provide the first evidence suggesting that severe oxidative stress by PQ over a long period may decrease the erythrocytes and liver mitochondrial catalase activities. This finding is of importance and suggests a new mechanism for PQ toxicity, because suppression of the catalase activities by PQ would further aggravate the oxidative damage by PQ. Although it may be necessary to investigate by the pair-feeding method whether the decreased liver catalase activity by PQ is due to PQ toxicity, the decreased catalase activity in the rats with decreased body weight by PQ feeding may have been due to PQ toxicity, because it has been reported that liver catalase activity instead increased in rats with decreased body weight by feeding on a restricted diet when compared to that in rats fed on an \textit{ad libitum} diet.\textsuperscript{33} The suppressed decrease in catalase activity by supplementing nasunin to the PQ diet may suggest that nasunin can prevent damage to the catalase which may be caused by active oxygen species produced by the action of PQ \textit{in vivo}.

The suppression by nasunin of an increase in atherogenic index due to PQ suggests that nasunin may be effective in preventing the development of atherosclerosis due to PQ. Although it has been reported that severe oxidative stress by PQ induced an increase in the serum cholesterol level,\textsuperscript{34} there are no reports dealing with the relationship between an increase in the atherogenic index and oxidative stress by PQ. The increase in atherogenic index in the PQ-fed rats may have been due to an increase in total cholesterol and a decrease in HDL cholesterol by PQ. However, the precise mechanism for increasing the atherogenic index by PQ remains to be further clarified. One reason for the decrease in liver triacylglycerol in the PQ-fed rats may have been due to its use as an energy source by the animals which could not retain their body weight after 8 d of the feeding period.

It has been reported that nasunin showed antioxidative activity \textit{in vitro},\textsuperscript{33} and that some anthocyanins could prevent the oxidation of LDL \textit{in vitro}.\textsuperscript{34} However, the antioxidative activity of nasunin \textit{in vivo} has not been studied before. The results of this study suggest that nasunin could act antioxidatively and prevent oxidative stress \textit{in vivo}. However, since the absorption of anthocyanins, including nasunin, from the digestive tract has not been fully determined, it is uncertain whether nasunin could be absorbed from the digestive tract and whether the absorbed nasunin itself acted antioxidatively against PQ-induced oxidative stress \textit{in vivo}. As the absorption of such anthocyanins as cyanidin-3-O-glucoside and cyanin from the digestive tract is low,\textsuperscript{30} the amount of nasunin which can be absorbed may also be low. It is necessary to determine the absorbability of nasunin and its degradation products \textit{in vivo}, and further to determine the relationship between the chemical structures of the degradation compounds and their preventive effects on oxidative stress.

The amount of nasunin fed per day to a rat weighing about 100 g was 18 mg, assuming the rat consumed 12 g of the diet per day. When converted to human dietary conditions, this figure may be high, since the nasunin content of chouija-nasu (about 25 g) is only about 12–15 mg. However, the ingestion of nasunin over a long period may be useful to prevent oxidative stress, even if the amount of nasunin ingested per day is small.

**Acknowledgments**

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