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Hepatoprotective Effect of Myristicin from Nutmeg (*Myristica fragrans*) on Lipopolysaccharide/D-Galactosamine-Induced Liver Injury

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To evaluate the hepatoprotective activity of spices, 21 different spices were fed to rats with liver damage caused by lipopolysaccharide (LPS) plus D-galactosamine (D-GalN). As assessed by plasma aminotranferase activities, nutmeg showed the most potent hepatoprotective activity. Bioassay-guided isolation of the active compound from nutmeg was carried out in mice by a single oral administration of the respective fractions. Myristicin, one of the major essential oils of nutmeg, was found to possess extraordinarily potent hepatoprotective activity. Myristicin markedly suppressed LPS/D-GalN-induced enhancement of serum TNF-α concentrations and hepatic DNA fragmentation in mice. These findings suggest that the hepatoprotective activity of myristicin might be, at least in part, due to the inhibition of TNF-α release from macrophages. However, further studies are needed to elucidate the hepatoprotective mechanism(s) of myristicin.

KEYWORDS: Liver injury; hepatoprotective activity; myristicin; nutmeg; *Myristica fragrans*

INTRODUCTION

Spices have been used since ancient times not only for increasing the flavor of foods but also for their preservative and medicinal properties. Many spices have been reported to show antidiabetic (*1*), antimicrobial (*2*), anti-inflammatory (*3*), antioxidant (*4*), and anticarcinogenic (*5*) properties. Clearly, spices have a wide range of potential benefits for maintaining health. To our knowledge, however, except for an example of turmeric (*6*, *7*), limited information is available to systematically examine the effects of spices on liver injury. During screening for hepatoprotective effects of spices, we found potent activity of nutmeg, a dried kernel of *Myristica fragrans*, which is widely used for food products such as sauces and curry powders. In the present study, therefore, we attempted to isolate the active compound from nutmeg and to clarify its plausible action mechanism for the hepatoprotective activity.

MATERIALS AND METHODS

Materials. All of the spices including allspice (*Pimenta officinalis*), cardamom (*Elettaria cardamomum*), caraway (*Carum carvi*), gardenia (*Gardenia jasminoides*), cumin (*Cuminum cyninum*), clove (*Syzygium aromaticum*), laurel (*Laurus nobilis*), coriander (*Coriandrum sativum*), fenugreek (*Trigonella foenum-graecum*), cinnamon (*Cinnamomum zeylonicum*), sage (*Salvia officinale*), celery seed (*Apium graveolens*), thyme (*Thymus vulgaris*), chenpi (*Citrus unshiu*), nutmeg (*Myristica fragrans*), star anise (*Illicium verum*), paprika (*Capsicum frutescens*), fennel (*Foeniculum vulgare*), black pepper (*Piper nigrum*), Japanese mint (*Mentha arvensis*), and rosemary (*Rosmarinus officinalis*) were purchased from a local store (Shizuoka, Japan). Spices were dried and milled to pass 30 mesh (<244 μm in diameter) and used for feeding experiments and isolation of active compounds. Lipopolysaccharide from *Escherichia coli* 055 and d-galactosamine (D-GalN) were purchased from Wako Pure Chemicals (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. Silica gel for chromatography (kieselgel 60) and silica gel TLC (kieselgel 60 F254) were purchased from Merck (Darmstadt, Germany). Other chemicals were purchased from Wako Pure Chemicals.

General Procedures. NMR spectra were obtained on a JEOL lambda-500 spectrometer (JEOL, Tokyo, Japan). FAB-MS and EI-MS were recorded on JEOL DX-303HF and JEOL DX-302 spectrometers (JEOL, Tokyo, Japan), respectively. IR spectra were determined with a JASCO FT/IR-410 spectrometer (JASCO, Tokyo, Japan). Optical rotation was measured by a JASCO DIP-100 polarimeter. HPLC was carried out using a Gulliver HPLC system (JASCO).

Extraction and Isolation of Active Compounds from Nutmeg. Dried nutmeg powder was extracted with *n*-hexane by shaking at room temperature for 24 h. The resulting suspension was filtered, and the filtrate was concentrated and dried under reducing pressure (fraction I). Ethyl acetate was added to the residue, and the suspension was extracted by shaking at room temperature for 24 h. The suspension was filtered, and the filtrate was concentrated and dried under reducing pressure (fraction II). The residue was further extracted with 70% ethanol by shaking at room temperature for 24 h, and the filtrate was obtained and dried (fraction III). Fraction I was applied to a silica gel

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column (8 × 57.5 cm) and eluted successively with *n*-hexane/ethyl acetate (98:2, 95:5, 90:10, 80:20, and 60:40, v/v) and methanol, giving six fractions (fractions I-1−I-6). Fraction I-2 was further separated by HPLC using a diol column [column, Ultra Pack Diol-40D, ∅ 50 mm × 300 mm, Yamazen Corp.; solvent, *n*-hexane (100%); flow rate, 20 mL/min; detection, 220 nm], giving five fractions (fractions I-2-1−I-2-5). Finally, fraction I-2-5 was analyzed by HPLC using a silica gel column [column, Senshu Pak Aquasil SS-1251 (60), ∅ 4.6 mm × 250 mm, Senshu Scientific Co. Ltd.; solvent, *n*-hexane/ethyl acetate (95:5); flow rate, 0.5 mL/min; detection, 254 nm].

Animal Experiments. Six-week-old male rats of the Wistar strain weighing 120−135 g obtained from Japan SLC (Hamamatsu, Japan) were used for a spice feeding study in order to select the spice with hepatoprotective effect. Rats were housed individually in wire screen-bottomed stainless steel cages in a room with controlled temperature (23 ± 1 °C) and lighting (lights on from 8:00 a.m. to 8:00 p.m.). After adaptation to a control diet (casein, 250; cornstarch, 422.5; sucrose, 200; corn oil, 50; mineral mixture, 35; vitamin mixture, 10; choline bitartrate, 2.5; and cellulose, 30) for 3 days, rats were divided into groups on the basis of body weight and allowed free access to experimental diets and water. Each spice powder was added to the control diet at a 3% level by replacing an equal amount of cellulose.

Six-week-old male mice of the ddY strain obtained from Japan SLC were used for further evaluation of hepatoprotective fractions prepared from nutmeg. Mice were maintained in the same condition mentioned above but kept in a room isolated from rats. Mice were acclimated to the facility for 5−7 days and allowed free access to water and a commercial diet (MF chow diet, Oriental Yeast, Tokyo, Japan). The study was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals. In this study, seven separate experiments were conducted.

In experiments 1 and 2, 96 (experiment 1) or 88 rats (experiment 2) were divided into 12 or 11 groups of 8 rats and fed the control diet or diets supplemented with each spice powder including allspice, cardamom, caraway, gardenia, cumin, clove, laurel, coriander, fenugreek, cinnamon, sage (experiment 1), celery seed, thyme, chenpi, nutmeg, star anise, paprika, fennel, black pepper, Japanese mint, and rosemary (experiment 2). After 10 days of the experimental feeding, rats were administered intraperitoneally LPS (10 μg/kg) plus D-GalN (250 mg/kg) on day 11. Rats were not starved either before or after intoxication. At 8 h after intoxication, rats were killed by decapitation to obtain blood. Serum was separated from whole blood by centrifugation at 2000*g* for 20 min at 4 °C and was stored at −20 °C until analysis.

In experiments 3−7, mice weighing 30−36 g (10 mice per group except normal group = 5) received a single oral administration of one of the respective fractions prepared from nutmeg powder (experiments 3−5), myristicin (50, 100, or 200 mg/kg; experiments 6 and 7), or vehicle alone (olive oil) at 3 h before intravenous administration of LPS (10 μg/kg) plus D-GalN (700 mg/kg) or saline alone (as normal). At 8 h (experiments 3−6) or 1 h (experiment 7) after intoxication, mice were killed by decapitation to obtain blood and liver. The serum and liver obtained were stored at −20 °C until analysis.

In experiment 3, the administration dosage of each fraction (fractions I−III) was determined from the daily intake of nutmeg powder (∼1833 mg/kg of body weight) in the rat experiment and the distribution of each fraction. The distribution of the fractions in the nutmeg powder was 38.3% (fraction I), 2.2% (fraction II), and 7.0% (fraction III). As a consequence, the administration dosage of each fraction in the mouse experiment was as follows: fraction I, 702.0 mg/kg of body weight; fraction II, 40.3 mg/kg; and fraction III, 128.3 mg/kg. In experiment 4, the administration dosage of each fraction was based on the distribution of the fractions (fraction I-1, 0.32%; fraction I-2, 4.82%; fraction I-4, 18.85%; fraction I-5, 9.07%; and fraction I-6, 5.90%) and was designed to make it comparable to 6 times the dose of fraction I (702.0 mg/kg of body weight) to avoid dispersing activities due to fractionation. Accordingly, the administration dosage of each fraction was as follows: fraction I-1, 15.0 mg/kg of body weight; fraction I-2, 202.8 mg/kg; fraction I-4, 792.0 mg/kg; fraction I-5, 382.2 mg/kg; and fraction I-6, 248.4 mg/kg. In experiment 5, the administration dosage of each fraction was designed to make it comparable to the dose of fraction I-2 (202.8 mg/kg of body weight), based on the distribution of the fractions (fraction I-2-1, 1.45%; fraction I-2-2, 5.13%; fraction I-2-3, 13.3%; and fraction I-2-5, 35.6%). Accordingly, the administration dosage of each fraction was as follows: fraction I-2-1, 2.9 mg/kg of body weight; fraction I-2-2, 10.4 mg/kg; fraction I-2-3, 26.9 mg/kg; and fraction I-2-5, 72.2 mg/kg.

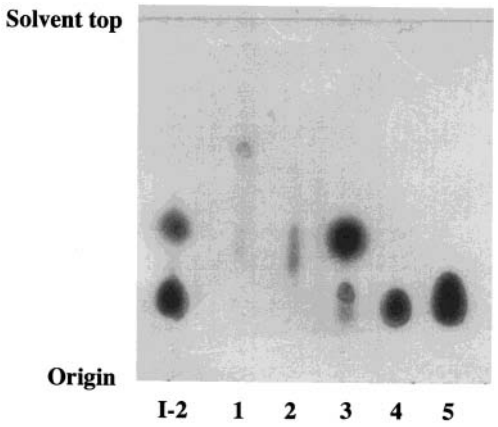

Figure 1. TLC chromatogram after the separation of fraction I-2 into five fractions. Fraction I-2 was separated on silica gel F_{254} using a solvent system of *n*-hexane/ethyl acetate (95:5, v/v). I-2, fraction I-2; 1, fraction I-2-1; 2, fraction I-2-2; 3, fraction I-2-3; 4, fraction I-2-4; 5, fraction I-2-5, respectively.

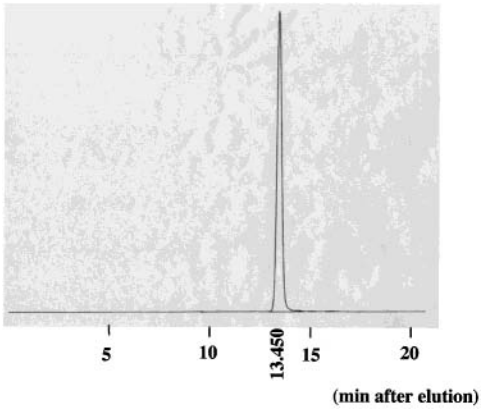

Figure 2. HPLC chromatogram of fraction I-2-5 analyzed by HPLC using a silica gel column.

Biochemical Analyses. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the marker enzymes of liver injury, were measured colorimetrically with a kit (Transaminase CII-Test Wako) after an appropriate dilution. The enzyme activity was expressed as micromoles per minute per liter at 25 °C. After an appropriate dilution, serum concentrations of TNF-α were measured by an ELISA assay kit (Mouse TNF-α ELISA kit, Cosmo Bio, Tokyo, Japan) with polyclonal antibody specific for mouse TNF-α and biotin-conjugated monoclonal antibody for mouse TNF-α. The assay procedure followed the manufacturer's recommendation. Hepatic DNA fragmentation was detected and quantified by 1.8% agarose gel electrophoresis and DNA ELISA, respectively, according to the methods of Leist et al. (*8*) and Hase et al. (*9*) with slight modifications. Briefly, frozen liver (∼1 g) was homogenized in 4 volumes (v/w) of ice-cold phosphate-buffered saline containing 10 mmol/L EDTA. The homogenate was centrifuged at 13000*g* for 20 min at 4 °C, and total DNA was prepared from the supernatant (0.3 mL) by extraction with an equal volume of phenol/chloroform (1:1, v/v), precipitation in ethanol, and subsequent treatment with 20 mg/L of ribonuclease A (Sigma-Aldrich) for 30 min at 37 °C. The total DNA was electrophoresed on 1.8% agarose gel. The supernatant of liver homogenate was diluted 300-fold and subjected to the direct analysis of oligonucleosome-bound

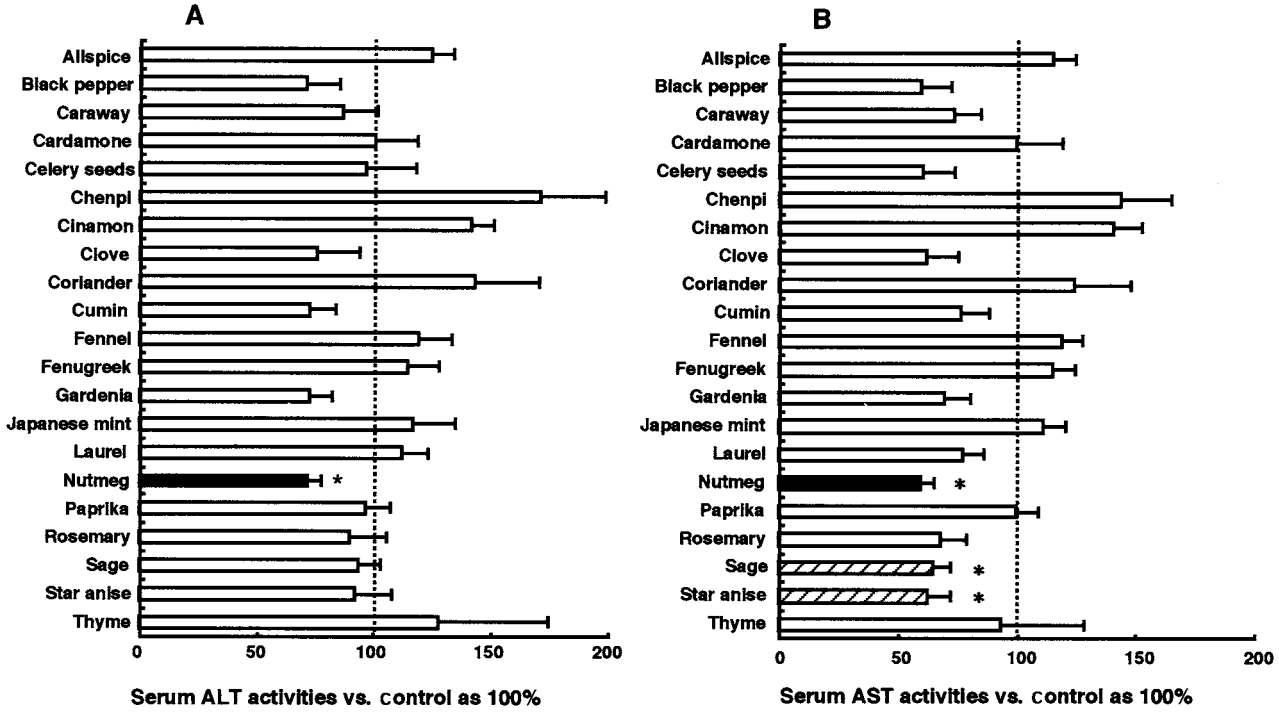

Figure 3. Effects of various spices on serum ALT and AST activities in rats with liver injury induced by LPS/D-GalN. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (*) are significantly different at $p < 0.05$.

DNA with an ELISA kit (Boehringer Mannheim, Mannheim, Germany). The assay procedure followed the manufacturer's recommendation.

Statistical Analysis. Results were expressed as mean ± standard error (SE). Analysis to determine differences between the control and experimental groups was done by unpaired *t* test, and a *p* value of <0.05 was considered to be significant.

RESULTS AND DISCUSSION

Extraction and Isolation of Active Compounds from Nutmeg. After successive extraction with *n*-hexane (fraction I), ethyl acetate (fraction II), and 70% ethanol (fraction III), the distribution of each fraction was 191.9 g (fraction I), 10.9 g (fraction II), and 34.8 g (fraction III) from 500 g of starting material (nutmeg powder). Fraction I was separated by silica gel column into six fractions, and the distribution of each fraction was 0.32 g (fraction I-1), 0.48 g (fraction I-2), 51.32 g (fraction I-3), 18.86 g (fraction I-4), 9.08 g (fraction I-5), and 5.90 g (fraction I-6) from 100 g of fraction I. Fraction I-2 was further separated by HPLC into five fractions, and the distribution of each fraction was 0.03 g (fraction I-2-1), 0.09 g (fraction I-2-2), 0.24 g (fraction I-2-3), 0.51 g (fraction I-2-4), and 0.64 g (fraction I-2-5) from 1.79 g of fraction I-2. Fraction I-2-5 showed a single spot on silica gel (**Figure 1**) and was identified as a known compound, myristicin 1-allyl-5-methoxy-3,4-methylenedioxybenzene by spectroscopic analyses. All of the spectroscopic data of the compound were identical with those of a commercially available authentic standard (myristicin, Sigma). Also, HPLC analysis of fraction I-2-5 showed a single peak (**Figure 2**).

Effects of Spice Feeding on the Level of Serum ALT and AST Activities (Experiments 1 and 2). In experiments 1 and 2, 21 spices were evaluated for the protective effects on LPS/D-GalN-induced liver injury in rats. Initial body weight of rats ranged from 140 to 146 g in both experiments. Generally, control rats daily consumed 9−12 g of the diet and gained 3−5 g of body weight per day. Rats well accustomed to the diets were supplemented with each spice at a 3% dietary level. There

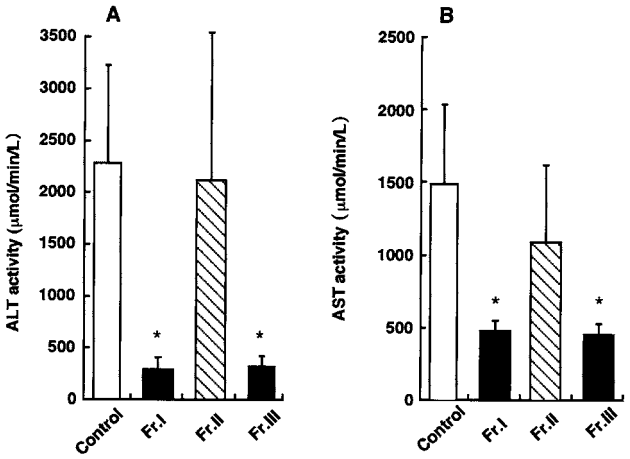

Figure 4. Effects of fractions I, II, and III on serum ALT and AST activities in mice with liver injury induced by LPS/D-GalN. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (*) are significantly different at $p < 0.05$.

were no significant differences in food intake and body weight gain between the control and spice-supplemented groups (data not shown).

After the experimental feeding for 10 days, rats were administered LPS/D-GalN, and the serum ALT and AST activities were determined at 8 h after intoxication. **Figure 3** shows combined data for experiments 1 and 2, and the data represent the relative activities of serum ALT and AST when the mean value of the control group was regarded as 100% in each experiment. The absolute values of ALT and AST activities (μmol/min·L) in the control groups were as follows: ALT, 2103 ± 287, and AST, 5497 ± 908, for experiment 1; ALT, 2298 ± 453, and AST, 6459 ± 1354, for experiment 2. AST activities in rats fed the diet supplemented with nutmeg, star anise, and sage were significantly lower than in those fed the control diet. ALT activities in rats fed the nutmeg-supplemented diet were also significantly lower than in those fed the control diet, but

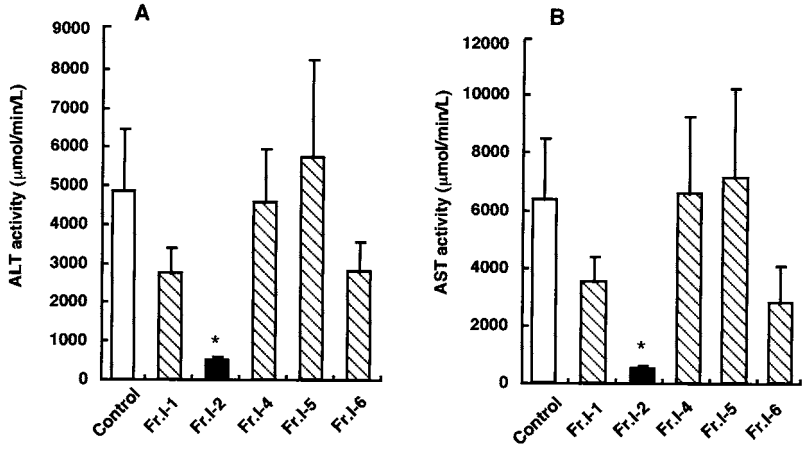

Figure 5. Effects of fractions I-1, -2, -4, -5, and -6 on serum ALT and AST activities in mice with liver injury induced by LPS/D-GalN. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (*) are significantly different at $p < 0.05$.

no significant differences were observed between the control and other spice groups. Thus, nutmeg was found to be the most potent hepatoprotective spice of all of the spices tested. Accordingly, we attempted to isolate the active principle(s) from nutmeg.

Bioassay-Guided Isolation of Hepatoprotective Fractions from Nutmeg (Experiments 3—5). After fractionations, hepatoprotective activities were evaluated in mice by a single oral administration of each fraction. First, nutmeg powder was fractionated into three fractions. As shown in **Figure 4**, both fractions I and III exhibited significant suppression in the serum ALT and AST activities (experiment 3). The hepatoprotective potency of fraction III was comparable to that of fraction I. Therefore, both fractions I and III may contribute to the hepatoprotective activity of nutmeg powder. However, we chose fraction I for further fractionations, because fraction I accounted for the major part (38.3%) of the nutmeg extract. Also, from our experiences in isolating an active compound, an *n*-hexane extract (fraction I) is easier to manipulate when compared with a 70% ethanol extract (fraction III). Second, fraction I was separated into six fractions. **Figure 5** shows that only fraction I-2 significantly prohibited the elevations of serum ALT and AST activities (experiment 4). Fraction I-3 was almost pure triglycerides and was omitted from the evaluation. Finally, fraction I-2 was separated into five fractions. All of the fractions tended to suppress the elevations of serum ALT and AST activities, but significant suppression was solely achieved with fraction I-2-5 (**Figure 6**) (experiment 5). Fraction I-2-4 was omitted because TLC analysis showed that compounds in fraction I-2-4 were composed of a mixture of those from fractions I-2-3 and I-2-5, and most of them were derived from fraction I-2-5.

Hepatoprotective Effect of Myristicin (Experiments 6 and 7). Because further analyses showed that fraction I-2-5 was composed of a single compound, which turned out to be myristicin (**Figure 7**), one of the major essential oils of nutmeg, we further evaluated the hepatoprotective effect of this compound. **Figure 8** demonstrated that a single oral administration of myristicin at a dose of 50, 100, or 200 mg/kg dose-dependently prohibited elevations of serum ALT and AST activities in mice with liver injury induced by intravenous administration of LPS/D-GalN (experiment 6). In this experiment, DNA ladder and DNA fragmentation were also measured as markers of the apoptosis of liver cells. The administration of LPS/D-GalN showed a DNA fragmentation on agarose gel electrophoresis in the control group, but this fragmentation was

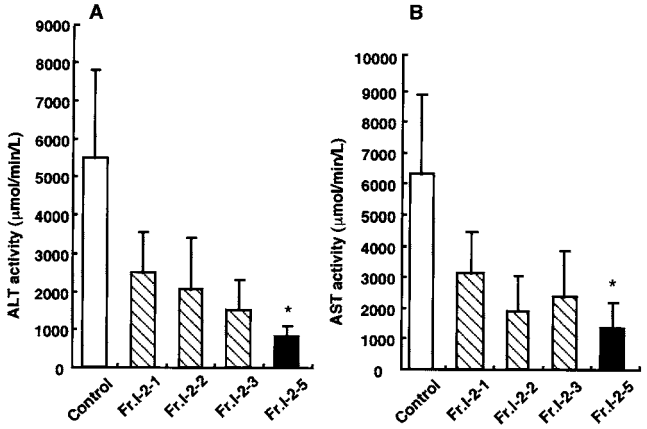

Figure 6. Effects of fractions I-2-1, -2, -3, and -5 on serum ALT and AST activities in mice with liver injury induced by LPS/D-GalN. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (*) are significantly different at $p < 0.05$.

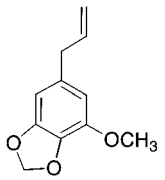

Figure 7. Structure of myristicin.

effectively suppressed by a single oral administration of myristicin at the dose of 200 mg/kg (**Figure 9**). These were confirmed by the quantitative determination of DNA fragmentation (**Figure 10**) (experiment 6).

To get more insight into the mechanism by which myristicin suppresses liver cell apoptosis, serum concentrations of TNF-α were measured at 1 h after intravenous administration of LPS/D-GalN. Our previous study showed that peak concentrations of TNF-α were reached 1 h after intoxication (*10*). As shown in **Figure 11**, pretreatment of the mice with myristicin at the dose of 200 mg/kg markedly inhibited the elevation of serum TNF-α concentrations (experiments 7).

The present study clearly demonstrates that some spices, especially nutmeg, have protective effects on LPS/D-GalN-induced enhancement of serum ALT and AST activities and that myristicin, one of the major essential oils of nutmeg, possesses a potent hepatoprotective activity. Myristicin has been reported to be an inducer of glutathione *S*-transferase (*11*) and

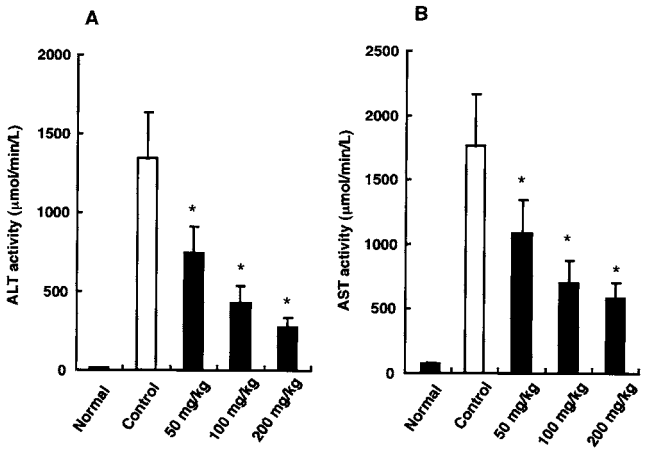

Figure 8. Dose-dependent effects of myristicin on serum ALT and AST activities in mice with liver injury induced by LPS/D-GalN. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (∗) are significantly different at $p < 0.05$.

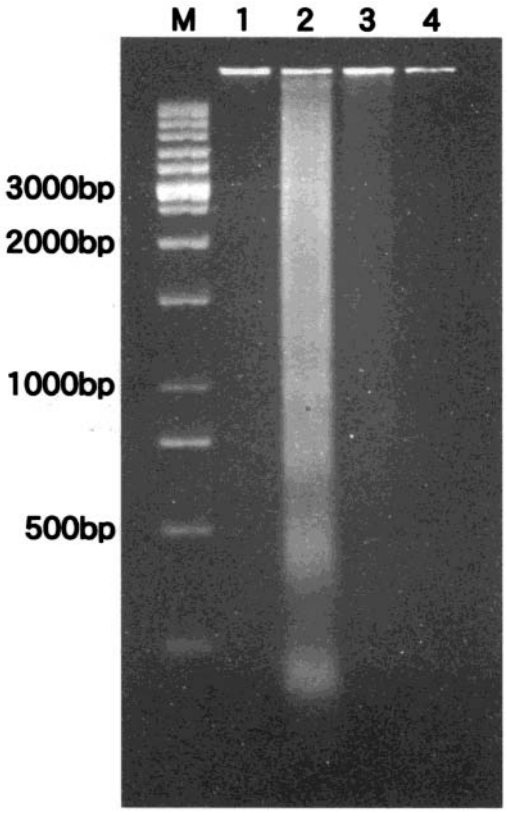

Figure 9. Agarose gel electrophoresis of cytosolic oligonucleosomal DNA from the livers of mice with liver injury induced by LPS/D-GalN: lane M, DNA ladder marker; lane 1, normal; lane 2, control; lane 3, 100 mg/kg of myristicin; lane 4, 200 mg/kg of myristicin. Each sample was pooled from 5 or 10 mice.

an inhibitor of benzo[*a*]pyrene-induced tumorigenesis in mouse tissues (*12*). However, we believe that this is the first time that the hepatoprotective effect of myristicin has been demonstrated in murine models.

It has been well established in murine models that administration of LPS in combination with D-GalN causes apoptosis of liver cells through the action of TNF-α released from macrophages in response to LPS (*8*). Although the apoptosis per se is not a direct cause of liver injury, excessive apoptosis of liver cells represents an important signal for the transmigration of

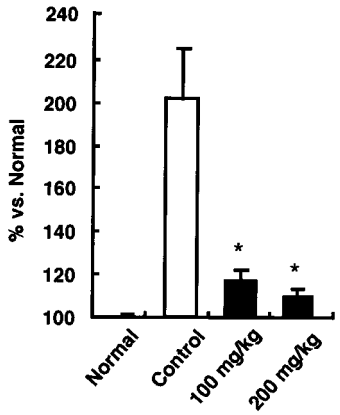

Figure 10. Effect of myristicin on LPS/D-GalN-induced enhancement of DNA fragmentation in mouse liver. Data represent the relative percentage when mean value of the normal group was regarded as 100%. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (*) are significantly different at $p < 0.05$.

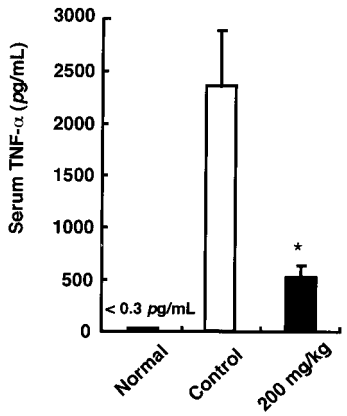

Figure 11. Effect of myristicin on LPS/D-GalN-induced enhancement of serum TNF-α concentrations in mice. The column and its bar represent the mean value ± SE, respectively. Values with an asterisk (∗) are significantly different at $p < 0.05$.

the primed neutrophils sequestered in sinusoids during LPS/D-GalN-induced liver injury (*8*). In the present study, we observed that myristicin markedly suppressed the elevation of serum TNF-α and the DNA fragmentation of the liver in rats treated with LPS/D-GalN. However, as shown in **Figure 9**, the fragmentation pattern in the control group resembled a smear rather than a ladder, characteristic of apoptosis. DNA from necrotic cells is normally shown as a smear, and in this context, LPS/D-GalN administration may have ended up with necrosis as a result of the sustained hepatic damage. Therefore, from only the present study, it cannot be ruled out that myristicin exerts the hepatoprotective activity through the suppression of apoptosis. Further studies are needed to clarify whether the apoptosis suppression is a direct cause for the hepatoprotective mechanism of myristicin.

In humans, liver injury is caused by viruses, chemicals, alcohol, and autoimmune diseases. It has been demonstrated that TNF-α also participates in liver injury by virus (*13*), alcohol (*14*), and immune disease (*15*). Therefore, the present findings obtained with myristicin may offer useful information about means for protecting against human liver injury, although LPS/D-GalN induced liver injury in rodents does not rigorously correspond to human liver injuries.

In the present study, myristicin was isolated as a major active compound in the *n*-hexane extract (fraction I) of nutmeg.

However, the 70% ethanolic extract (fraction III) also exhibited potent activity comparable to that of the *n*-hexane extract (**Figure 4**). Accordingly, further isolation and structure determination of the active compounds in ethanolic extract are now in progress.

ABBREVIATIONS USED

LPS, lipopolysaccharide; D-GalN, D-galactosamine; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

LITERATURE CITED

(1) Broadhurst, C. L.; Polamsky, M. M.; Anderson, R. A. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J. Agric. Food Chem.* **2000**, *48*, 849−852.

(2) De, A. M.; De, A. K.; Banerjee, A. B. Antimicrobial screening of some Indian spice. *Phytother. Res.* **1999**, *13*, 616−618.

(3) Reddy, A. C.; Lokesh, B. R. Studies on anti-inflammatory activity of spice principles and dietary *n*−3 polyunsaturated fatty acids on carrageenan-induced inflammation in rats. *Ann. Nutr. Metab.* **1994**, *38*, 349−358.

(4) Nakatani, N. Phenolic antioxidants from herbs and spices. *Biofactors* **2000**, *13*, 141−146.

(5) Surh, Y. J. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food Chem. Toxicol.* **2002**, *40*, 1091−1097.

(6) Park, E. J.; Jeon, C. H.; Ko, G.; Kim, J.; Sohn, D. H. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.* **2000**, *52*, 437−440.

(7) Matsuda, H.; Ninomiya, K.; Morikawa, T.: Yoshikawa, M. Inhibitory effect and action mechanism of sesquiterpenes from Zedoariae Rhizoma on D-galactosamine/lipopolysaccharide-induced liver injury. *Bioorg. Med. Chem. Lett.* **1998,** *8*, 339−344.

(8) Leist, M.; Gantner, F.; Bohlinger, I.; Tiegs, G.; Germann, P. G.; Wendel, A. Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. *Am. J. Physiol.* **1995**, *146*, 1220−1234.

(9) Hase, K.; Xiong, Q.; Basnet, P.; Namba, T.; Kadota, S. Inhibitory effect of tetrahydroswertianolin on tumor necrosis factor-α-dependent hepatic apoptosis in mice. *Biochem. Pharmacol.* **1999**, *57*, 1431−1437.

(10) He, P.; Noda, Y.; Sugiyama, K. Green tea suppresses lipopolysaccharide-induced liver injury in D-galactosamine-sensitized rats. *J. Nutr.* **2001**, *131*, 1560−1567.

(11) Ahmad, H.; Tijerina, M. T.; Tobola, A. S. Preferential overexpression of a class MU glutathione *S*-transferase subunit in mouse liver by myristicin. *Biochem. Biophys. Res. Commun.* **1997**, *236*, 825−828.

(12) Zheng, G.; Kenny, P. M.; Lam, L. K. T. Inhibition of benzo[*a*]pyrene-induced tumorigenesis by myristicin, a volatile aroma constituent of parsley leaf oil. *Carcinogenesis* **1992**, *13*, 1921−1923.

(13) Ando, K.; Hiroshi, K.; Kaneko, T.; Moriyama, T.; Muto, Y.; Kayagaki, N.; Yagita, H.; Okumura, K.; Imawari, M. Perfolin, Fas/Fas ligand, and TNF-α pathways as specific and bystander killing mechanism of hepatic C virus-specific human CTL. *J. Immunol.* **1997**, *158*, 5283−5291.

(14) Yin, M.; Wheeler, M. D.; Kono, H.; Bradford, B. U.; Gallucci, R. M.; Luster, M. I.; Thurman, R. G. Essential role of tumor necrosis factor α in alcohol-induced liver injury in mice. *Gastroenterology* **1999**, *117*, 942−952.

(15) Gantner, F.; Leist, M.; Lohse, A. W.; Germann, P. G.; Tiegs, G. Concanavalin A-induced T cell-mediated hepatic injury in mice: the role of tumor necrosis factor. *Hepatology* **1995**, *21*, 190−198.

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