Gastroprotective Effects of Glutinous Rice Extract against Ethanol-, Indomethacin-, and Stress-induced Ulcers in Rats

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This study was designed to evaluate the efficacy of an orally administered aqueous extract of glutinous rice (GRE) to protect against acute gastric mucosal lesions induced by ethanol, indomethacin, and water immersion restraint stress in rats and to characterize the active substances responsible for the protection. GRE was shown to dose-dependently prevent the gastric lesions induced by the above ulcerogenic treatments at doses of 30 to 300 mg/kg. GRE treatment increased the gastric mucin content and partially blocked the ethanol-induced depletion of the gastric mucus layer. Also, it increased the nonprotein sulfhydryl concentration in the gastric mucosa. The gastroprotective action of GRE was markedly enhanced by co-treatment with 4-8 mg/kg tea extracts. The activity of GRE was completely lost by heat treatment at 80°C for 3 min or treatment with 0.01% pepsin at 37°C for 1 h. Protein extraction studies indicated that prolamins are involved in the gastroprotective activity of GRE. Our results suggest that glutinous rice proteins are useful for the prevention and treatment of gastritis and peptic ulcer.

Key Words: Glutinous rice; Gastroprotection; Ulcer; Proteins; Prolamins

INTRODUCTION

Rice is a staple food in many countries. In addition, rice, particularly glutinous rice, has been used for the treatment of gastrointestinal disorders in folk medicines in Korea and other countries. Recently, rice extract was reported to protect the gastric mucosa from toxic agents. These reports included the following findings: i) Rice extract protected the gastric mucosa from ethanol-induced injury in rats. ii) Rice extract prevented NaCl-induced gastric mucosal damage and cell proliferation in rats. iii) Rice extract attenuated the inflammatory changes of the gastric mucosa induced by Helicobacter pylori infection in Mongolian gerbils. The rice extract used in the above studies was prepared by saccharization of rice with Aspergillus oryzae followed by fermentation with Saccharomyces cerevisiae. The extract contained high concentrations of saccharides but no protein and no lipid.

Peptic ulcers are believed to result from the imbalance between protective and aggressive factors. The gastroprotective factors include mucus, mucosal antioxidants, prostaglandins and growth factors, and gastric blood flow. The aggressive factors, such as increased gastric secretion, increased generation of reactive oxygen species and pro-inflammatory cytokines, and infection with Helicobacter pylori, can cause mucosal damage. Researchers have developed many in vivo gastric ulcer models with different gastropathic mechanisms. Among them, the ethanol-, indomethacin-, and water immersion restraint stress (WIRS)-induced models have been used in many studies to screen antiulcer substances, because alcohol, nonsteroidal anti-inflammatory drugs, and stress are major risk factors in human life for peptic ulcers and other gastrointestinal diseases.

We have observed that the aqueous extract of glutinous rice (GRE) very effectively protects the gastric mucosa from injury induced by ethanol, indomethacin, and WIRS treatments in rats. Also, we have found that the active substances of GRE responsible for the protection differ from those of the rice extract used in earlier studies. The present study assessed the gastroprotective activity of GRE as...
exerted in the above-mentioned ulcer models and presents some characteristics of the gastroprotective activity and related GRE substances.

MATERIALS AND METHODS

1. Materials

Three cultivars each of glutinous (Dongjinchal, Baikokchhal, and Sinsunchal) and nonglutinous (Hopum, Sindongjin, and Saenuri) rice were supplied from National Agricultural Products Quality Management Service (NAQS) of Korea. Tea extracts were prepared by extracting green and black teas (Amorepacific, Seoul, Korea) with 100 volumes of boiling distilled water for 10 min and filtering and freeze-drying the resultant solutions. Ethanol was purchased from Merck KGaA (Darmstadt, Germany) and indomethacin was from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents were of the analytical grade commercially available.

2. Animals

Male Sprague-Dawley rats (230-250 g) were housed in cages with mesh bottoms to prevent coprophagia in an air-conditioned room with a 12-h dark-light cycle and were allowed free access to water and food. Prior to the experiments, the animals were fasted, but allowed water, for 36 h. This study was performed in accordance with the Institutional Guidelines for Experimental Animal Care and Use of Chonnam National University Medical School.

3. Preparation of rice extracts and administration to animals

Rice was ground to pass through a 0.18-mm screen (Daehwa Precision, Cheonan, Korea). Rice flour was added with 10 volumes of distilled water and agitated vigorously for 30 s with a vortex mixer 3 times. After agitation, the mixture was centrifuged at 1,000×g for 10 min to obtain a supernatant. The supernatant was then freeze-dried and eluted with the above Tris-Triton buffer. The hexose content was measured by the Alcian blue method with galactose as the standard, and the void volume fraction was collected as mucin. Mucin content was expressed as hexose content per stomach.

4. Induction and determination of gastric mucosal lesions

1) Ethanol-induced lesion: Thirty minutes after administration of the rice extract, rats were orally administered 4 mL/kg ethanol to induce gastric lesions. One hour after the ethanol treatment, the animals were killed and their stomachs were removed and opened. Mucosa in the glandular stomach was removed by scraping with a blunt knife. Mucosal preparations from three animals were pooled, lyophilized, and powdered for extraction of mucin as described by Ichikawa et al. The sample was homogenized in 50 mM Tris-HCl buffer, pH 7.2, containing 2% Triton X-100 and incubated at 37°C for 1 h. After centrifugation at 8,000×g for 30 min at 4°C, an aliquot of the supernatant was applied to a Bio-Gel A-1.5m column (Bio-Rad, Hercules, CA, USA) and eluted with the above Tris-Triton buffer. The hexose content in the eluates was measured by the phenol-sulfuric acid method with galactose as the standard, and the void volume fraction was collected as mucin. Mucin content was expressed as hexose content per stomach.

2) Gastric mucus content: After the rats were treated with GRE and ethanol, they were sacrificed and their gastric mucus content was measured by the Alcian blue method. The glandular portion of the stomach was immersed in 10 mL of 0.1% Alcian blue containing 0.16 M sucrose and 50 mM sodium acetate, pH 5.8, for 2 h and rinsed twice with 10 mL of 0.25 M sucrose for 20 min. The dye complexed with the gastric mucus was then extracted with 0.5 M MgCl₂ for 2 h. An aliquot of the blue extract was vortex-mixed vigorously with an equal volume of diethyl ether and the mixture was centrifuged at 1,500×g for 10 min. The absorbance of the aqueous layer was read at 580 nm.

5. Determination of gastric mucin content

1) Total mucin content in gastric mucosa: One hour after the GRE treatment, the rats were killed and their stomachs were removed and opened. Mucosa in the glandular stomach was removed by scraping with a blunt knife. Mucosal preparations from three animals were pooled, lyophilized, and powdered for extraction of mucin as described by Ichikawa et al. The sample was homogenized in 50 mM Tris-HCl buffer, pH 7.2, containing 2% Triton X-100 and incubated at 37°C for 1 h. After centrifugation at 8,000×g for 30 min at 4°C, an aliquot of the supernatant was applied to a Bio-Gel A-1.5m column (Bio-Rad, Hercules, CA, USA) and eluted with the above Tris-Triton buffer. The hexose content in the eluates was measured by the phenol-sulfuric acid method with galactose as the standard, and the void volume fraction was collected as mucin. Mucin content was expressed as hexose content per stomach.

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6. Determination of nonprotein sulphydryl (NP-SH) concentration in gastric mucosa

This was done as described by Szabo et al. One hour after the GRE treatment, gastric mucosa was obtained from rats as described above. The mucosa was homogenized in 40 volumes of 20 mM EDTA and the NP-SH concentration in the homogenate was measured with Ellman’s reagent as described by Sedlak and Lindsay.

7. Separation of the particulate (GREP) and soluble (GRES) fractions of GRE

GRE was allowed to stand overnight at room temper-
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at ure until it became aggregated and formed a precipitate. After centrifugation at 1,000×g for 10 min, the precipitate was used as GREP after freeze-drying and the soluble part was referred to as GRES.

8. Isolation of proteins from glutinous rice flour by the Osbourne method

Different kinds of proteins were extracted from glutinous rice flour on the basis of their solubility at room temperature in water, 5% NaCl, 1% lactic acid, and 70% ethanol by the procedure described by Ju et al.17 with minor modifications. Glutinous rice flour (50 g) was defatted with 200 mL hexane and dried in a fume hood at room temperature for 24 h. The defatted flour was then extracted by shaking with 200 mL distilled water for 4 h and centrifuged at 3,000×g for 30 min to isolate the soluble albumin fraction. After water extraction, the flour was similarly extracted with 200 mL of 5% NaCl to isolate the globulin fraction. The flour was then extracted with 200 mL of 1% lactic acid for 1 h to isolate the glutelin fraction, followed by extraction with 200 mL of 70% ethanol for 4 h to isolate the prolamin fraction. Each extraction step was repeated twice. The protein fractions were then dialyzed against distilled water, lyophilized, and stored at 4°C.

9. Statistical analysis

The results are presented as means± standard errors (SEs). The significance of differences of all results was analyzed by one-way analysis of variance (ANOVA) followed by the Scheffe’s test.

RESULTS

1. Gastroprotective effects of GRE in different gastric ulcer models

Hemorrhagic lesions of a linear type were observed in gastric mucosa after rats were treated with ethanol, indomethacin, or WIRS. The lesion indices were much higher in the ethanol-treated rats than in the indomethacin- and WIRS-treated rats (controls in Fig. 1). When the rats were pretreated with GRE prepared from the cultivar Dongjin-chal, the gastric lesions were obviously reduced in all the ulcer models. As shown in Fig. 1, GRE dose-dependently blocked the gastric lesions induced by ethanol, indomethacin, and WIRS treatments up to more than 70%. The efficacy of GRE, however, differed among the ulcer models. GRE could significantly block the ethanol-induced gastric lesion at the dose of 30 mg/kg, whereas it could significantly block the lesions induced by indomethacin and WIRS at the dose of 100 mg/kg.

**FIG. 1.** Effects of glutinous rice extract (GRE) on the formation of gastric lesions induced by ethanol (A), indomethacin (B), and water-immersion restraint stress (C) in rats. The indicated doses of GRE were administered to rats 30 min before ulcerogenic treatments. Each column represents the mean±SE (N=8). *p<0.05, †p<0.01 compared with the control (zero) group.
2. Comparison of gastroprotective effects of glutinous (GRE) and nonglutinous (NGRE) rice extracts

Rice extracts were prepared from three cultivars of glutinous and nonglutinous rice, respectively, and their gastroprotective effects were compared at 100 mg/kg in the ethanol- and indomethacin ulcer models. As shown in Table 1, all GREs exerted much stronger protective effects against ethanol- and indomethacin-induced gastric lesions than did the nonglutinous rice extracts (NGREs) tested, although the NGREs also showed considerable degrees of protection against both lesions. In the following experiments of this study, the cultivar Dongjinchal was used for the preparation of GREs.

3. Mechanisms for the gastroprotective action of GRE

Because GRE showed effective protection against ethanol-, indomethacin-, and WIRS-induced gastric ulcers, we examined the effects of GRE on the levels of two important protective factors in gastric mucosa: mucin and nonprotein sulfhydryls (NP-SHs).

1) Effect of GRE on the total mucin content in gastric mucosa: To study the effect of GRE on the synthesis of gastric mucin, the GRE-induced change in the total gastric mucin content was determined. The dose-related changes in the gastric mucin content following GRE administration to rats are shown in Fig. 2A. The average mucin content of the control was 0.86 mg hexose per stomach. The mucin content significantly increased to 148%, 167%, and 163% of the control at 30, 100, and 300 mg/kg, respectively.

2) Effect of GRE on the gastric mucus content: To study the effect of GRE on the gastric mucin release, GRE- and ethanol-induced changes in the content of the mucus layer lining the surface of the gastric mucosa were determined on the basis of Alcian blue binding ability (Fig. 2B). GRE administration at 100 mg/kg alone did not induce a significant change in the mucus content. Ethanol treatment (4 mL/kg) reduced the mucus content to 58.9% of the control. The mucus content significantly increased to 148%, 167%, and 163% of the control with GRE treatment at 30, 100, and 300 mg/kg, respectively.

3) Effect of GRE on the NP-SH concentration in gastric mucosa: The dose-related changes in the gastric mucosal NP-SH concentration following GRE administration to rats are shown in Fig. 2C. Average NP-SH concentration of the control was 0.68 μmol/g tissue. The NP-SH concentration significantly increased to 132% and 137% of the control with GRE treatment at 100 and 300 mg/kg, respectively.

4. Identification of gastroprotective substances in GRE

1) Distribution of gastroprotective activity in the particulate (GREP) and soluble (GRES) fractions of GRE: For this study, GRE of 25 mg/mL was allowed to stand overnight at room temperature and centrifuged at 1,000×g for 10 min. The resultant soluble fraction was used as GRES, and the precipitate was suspended in the starting volume of distilled water to use as GREP. To make the GREP plus GRES sample, the precipitate was suspended in the starting volume of GRES. The GRE, GRES, GREP, and GRES plus GREP samples thus obtained were administered to rats at a dose of 4 mL/kg and their protective effects against ethanol-induced ulcers were determined. The resulting lesion indices for the different experimental groups were as follows (N=8): distilled water control, 23.7±3.9%; GRE, 5.1±2.1%; GREP, 12.3±3.0%; GRES, 21.9±3.5%; and GREP plus GRES, 5.9±1.8%. GREP showed a significant gastroprotective activity, although the activity was far weaker than that of GRE. GRES did not show a significant gastroprotective activity. However, it could enhance the activity of GREP when the two fractions were combined. This result suggests that active substances responsible for the gastroprotective effect of GRE are present in GREP and that the active substances may need some soluble activators for full activity.

2) Enhancement of the gastroprotective activities of GRE and GREP by tea extracts: We have observed that tea extracts can enhance the gastroprotective activities of GRE and GREP at such low doses that alone they do not exert a significant gastroprotective effect. As shown in Table 2, the activities of GRE and GREP were markedly enhanced by co-treatment with 8 mg/kg green tea or black tea.

| Rice extract | Rice cultivar | Ethanol-induced ulcer | Indomethacin-induced ulcer |
|--------------|--------------|-----------------------|---------------------------|
| Distilled water |                | 22.3±4.3              | 7.4±2.3                   |
| GRE | Dongjinchal | 4.5±2.6† (79.8) | 1.2±0.6† (83.8) |
|       | Baikokchal | 7.0±2.8† (68.6) | 1.7±0.6† (77.0) |
|       | Sinsunchal | 5.1±2.4† (77.1) | 2.2±0.7† (70.3) |
| NGRE | Hopum | 11.3±3.1* (49.3) | 3.5±1.2* (52.7) |
|       | Sindongjin | 15.7±3.8 (29.6) | 5.5±2.0 (25.7) |
|       | Saenuri | 17.0±3.7 (23.8) | 5.4±1.8 (27.0) |

Glutinous (GRE) or nonglutinous (NGRE) rice extract was administered to rats in a dose of 100 mg/kg 30 min before ulcerogenic treatments. *p < 0.05, †p < 0.01 compared with the distilled water control (N=8).
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Fig. 2. Effects of glutinous rice extract (GRE) on the gastric mucin content (A and B) and gastric mucosal nonprotein sulfhydryl (NP-SH) concentration (C) in rats. (A) Change in the total gastric mucin content by GRE treatment. One hour after the indicated doses of GRE were administered to rats, the gastric mucin content was determined (N=3). *p < 0.05 compared with the control (zero) group. (B) Changes in the gastric mucus content by GRE and ethanol treatments. Rats were pretreated with the indicated doses of GRE, and 30 min later, they were treated with 4 mL/kg ethanol or distilled water. One hour after the ethanol treatment, the gastric mucus content was determined (N=8). *p < 0.05, †p < 0.01 compared with the untreated control, and ‡p < 0.05 compared with the ethanol-treated control. (C) One hour after the indicated doses of GRE were administered to rats, the gastric mucosal NP-SH concentration was determined (N=8). *p < 0.05 compared with the control (zero) group.

As shown in Fig. 3, the black tea extract dose-dependently increased the gastroprotective activities of GRE and GREP at doses of 0 to 8 mg/kg. Green tea extract also dose-dependently increased the activities of GRE and GREP under the condition of Fig. 3 (data not shown). Of the tea constituents, epicatechin and tannic acid showed significant enhancing effects for the gastroprotective activity of GREP at 40 μmol/kg, whereas epigallocatechin gallate, gallic acid, ascorbic acid, and caffeine did not (Table 3).

3) Effects of heat and pepsin treatments and solvent extraction on the gastroprotective activity of GREP: The gastroprotective activity of GREP was found to be very labile to heat or pepsin treatment (Table 4). When GREP was heat-treated at 80°C for 3 min, its activity was totally lost. GREP also lost its gastroprotective activity by incubation with 0.01% pepsin at 37°C for 1 h. These results strongly suggest that the active substances in GREP are proteins. In an effort to explore which proteins, if any, are involved in the gastroprotective activity of GREP, GREP was extracted with different solvents to remove different proteins: 5% NaCl, 1% lactic acid, and 70% ethanol were used as solvents to remove globulins, glutelins, and prolamins, respectively. As shown in Table 4, extraction with 5% NaCl and 1% lactic acid did not cause a significant change in the remaining gastroprotective activity of GREP. With 70% ethanol extraction, however, GREP markedly lost its gastroprotective activity. These results suggest that prolamins are involved in the gastroprotective activity of GREP.

4) Gastroprotective activity of the prolamin fraction isolated from glutinous rice flour: To study further the gastroprotective activities of glutinous rice proteins, the albumin, globulin, glutelin, and prolamin fractions were isolated sequentially from glutinous rice flour by the Osbourne method and their gastroprotective activities were determined (Fig. 4). From 50 g of glutinous rice flour, 0.28, 0.32, 1.09, and 0.24 g of albumin, globulin, glutelin, and pro-
Glutinous rice extract (GRE, 30 mg/kg) or its particulate fraction GREP (10 mg/kg) was administered to rats with or without 8 mg/kg black tea extract (BTE) as indicated 30 min before ethanol treatment. *p < 0.05, †p < 0.01 compared with the control, ‡p < 0.05 compared with the GRE group, and §p < 0.05 compared with the GREP group (N=8).

| Sample               | Lesion index (%) | Inhibition rate (%) |
|----------------------|------------------|---------------------|
| Distilled water (control) | 24.0±3.5        | 48.3                |
| GRE                  | 12.4±2.9*        | 87.1                |
| GRE+GTE              | 3.1±1.3†, ††     | 87.9                |
| GRE+BTE              | 2.9±1.0†, ††     | 87.9                |
| GREP                 | 13.7±2.4*        | 42.9                |
| GREP+GTE             | 4.9±1.5†, ††     | 79.6                |
| GREP+BTE             | 4.1±2.1†, ††     | 82.9                |
| GTE                  | 20.9±3.2         | 12.9                |
| BTE                  | 19.7±2.8         | 17.9                |

The particulate fraction of glutinous rice extract (GREP, 10 mg/kg) was administered to rats with tea constituents (40 μmol/kg) as indicated 30 min before ethanol treatment. *p < 0.05, †p < 0.01 compared with the distilled water group, and ‡p < 0.01 compared with the native GREP group (N=6).

| Sample               | Lesion index (%) | Inhibition rate (%) |
|----------------------|------------------|---------------------|
| Distilled water      | 21.7±3.6         | 41.0                |
| GREP                 | 12.8±2.7*        | 78.3                |
| GREP+epicatechin     | 4.7±2.8†, ††     | 82.5                |
| GREP+tannic acid     | 3.8±2.3†, ††     | 47.5                |
| GREP+epigallocatechin galate | 11.4±2.4* | 87.1                |

The particulate fraction of glutinous rice extract (GREP, 10 mg/kg) was administered to rats with different solvents on the gastroprotective activity of GREP as measured in the ethanol-induced ulcer model.

| Sample               | Lesion index (%) | Inhibition rate (%) |
|----------------------|------------------|---------------------|
| Grease               | 20.5±3.4         | 70.2                |
| Grep                 | 6.1±2.3†         | 21.0                |
| Pepsin-treated       | 22.3±3.1†        | 8.8                 |
| 5% NaCl-extracted    | 6.5±1.9†         | 68.3                |
| 1% Lactic acid-extracted | 8.0±2.6*     | 61.0                |
| 70% Ethanol-extracted | 16.6±3.8§       | 19.0                |

The particulate fraction of glutinous rice extract (GREP) was undergone various physical and chemical treatments to inactivate proteins or to remove different groups of proteins as follows: *GREP (3 mg/mL) was incubated at 80°C for 3 min. †GREP (3 mg/mL) was incubated with 0.01% pepsin and 1% lactic acid at 37°C for 1 h and neutralized with 2 N NaOH. ‡Sixty milligrams of GREP was suspended in 20 mL 5% NaCl, 1% lactic acid, and 70% ethanol and incubated at room temperature for 4, 1, and 4 h, respectively. GREP was then isolated by centrifugation and re-suspended in 20 mL distilled water. After the above treatments, GREP preparations were added with 2 mg/mL black tea extract and administered to rats in a dose of 0.4 mL/kg 30 min before ethanol treatment. *p < 0.05, †p < 0.01 compared with the distilled water control, and ‡p < 0.05, §p < 0.01 compared with the native GREP group (N=8).

**DISCUSSION**

In this study, GRE effectively protected the gastric mucosa from injury induced by ethanol, indomethacin, and WIRS. GRE exerted significant effects dose-dependently at doses of 30 to 300 mg/kg against ethanol-induced ulcers and at doses of 100 to 300 mg/kg against indomethacin- and WIRS-induced ulcers (Fig. 1). When rice extracts prepared from different cultivars of glutinous and nonglutinous rice were compared for their gastroprotective effects in the ethanol and indomethacin ulcer models, GREs showed much stronger effects than NGREs, although NGREs also...
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FIG. 4. Effects of the albumin, globulin, glutelin, and prolamin fractions isolated from glutinous rice flour on the gastric lesion formation induced by ethanol in rats. (A) The albumin (Alb), globulin (Glo), glutelin (Glu), and prolamin (Pro) fractions suspended in distilled water (DW) were administered to rats in a dose of 10 mg/kg with 8 mg/kg black tea extract 30 min before ethanol treatment. (B) The indicated doses of prolamin fraction were administered to rats with 8 mg/kg black tea extract 30 min before ethanol treatment. Each column represents the mean±SE (N=8). *p < 0.05, †p < 0.01 compared with the control.

showed considerable effects (Table 1). Considering that alcohol, nonsteroidal anti-inflammatory drugs, and stress are major risk factors for peptic ulcer and other gastrointestinal diseases, our results indicate that GRE may be useful for the prevention and treatment of gastritis and peptic ulcer.

It is well known that gastric mucin is depleted in animals treated with ethanol, indomethacin, and WIRS. Because the gastric mucin is important to protect the mucosa from physical and chemical stimuli and back diffusion of hydrogen ions and pepsin, depletion of mucin may result in gastropathy. Our results (Fig. 2A and B) showed that the GRE treatment increased the total gastric mucin content but did not alone cause a significant change in the content of the mucus layer lining the gastric mucosal surface. These results suggest that GRE stimulates mucin synthesis in the gastric mucosa but not its secretion into gastric lumen. However, pretreatment with GRE before ethanol treatment could partially block the ethanol-induced depletion of gastric mucus (Fig. 2B). This effect might be due to the fact that the intramucosal mucin content was increased by GRE treatment. Accordingly, more mucin might be secreted from the mucosa by the subsequent ethanol stimulation in the GRE-pretreated rats than in the control rats without GRE pretreatment. The exact mechanisms for the changes in gastric mucin content by GRE treatment remain to be clarified.

Free radicals are also involved as a major aggressive factor in the pathogenesis of gastric ulcers induced by ethanol, indomethacin, and WIRS, which is supported by the facts that lipid peroxide levels are increased and the major antioxidants (including reduced glutathione; vitamins A, C, and E; and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase) are depleted in the insulted gastric tissues. Among the antioxidants, NP-SH compounds play a pivotal role in gastrointestinal diseases. NP-SHs react with reactive oxygen species to transform them to inert products and also keep sulphydryl groups of proteins in reduced form. They also help in recycling endogenous antioxidant vitamins, thereby preventing lipid peroxidation. In addition, NP-SHs scavenge superoxide anions, thus preventing their interaction with nitric oxide to form peroxynitrite. Nitric oxide thus preserved can improve tissue perfusion and attenuate neutrophil infiltration and free radical generation. The facts that NP-SH concentration in the gastric mucosa is relatively high and treatment of animals with sulphydryl blockers results in induction of gastric mucosal injury suggest the importance of NP-SH in gastroprotection and maintenance of gastric mucosal integrity. In the present study, GRE treatment significantly increased the NP-SH concentration in gastric mucosa, which suggests that the gastroprotective effect of GRE may involve the mechanisms by which it enhances the NP-SH concentration in gastric mucosa.

In this study, the gastroprotective activity of GREP was totally destroyed by heat treatment at 80°C for 3 min or treatment with 0.01% pepsin at 37°C for 1 h, and the activity of GREP was decreased markedly by extraction with 70% ethanol (Table 4). In addition, the prolamin fraction isolated from glutinous rice flour by the Osbourne method exhibited a remarkable gastroprotective activity in the presence of black tea extract as an activator (Fig. 4). These results suggest that prolamins are responsible, at least in part, for the gastroprotective activity of GRE. This article is the first to describe the gastroprotective activity of prolamin. According to Jayaraj et al., fresh rice bran and its oil showed a gastroprotective effect but stored rice bran and its oil were ulcerogenic in pylorus ligated rats. Those
authors suggested that unsaturated fatty acids play a critical role in the above actions of rice bran and its oil. Matsushashi et al.\textsuperscript{1} reported that rice extract, which was prepared through saccharization of rice with \textit{Aspergillus oryzae} and fermentation with \textit{Saccharomyces cerevisiae}, protected rat gastric mucosa from ethanol-induced injury, and the protective activity was stable to heat treatment at 80°C for 3 min. The rice extract was reported to contain mainly saccharides but no protein and no lipid.\textsuperscript{9} Therefore, it seems that our GRE preparation is a novel rice extract with gastroprotective activity whose active compounds differ from those of the rice preparations used by the above investigators.

The gastroprotective action of GRE was markedly enhanced by co-treatment with tea extracts. It is uncertain how the tea extracts enhance the GRE action. We observed that tea constituents such as epicatechin and tannic acid showed significant enhancing effects for the GREP action that tea constituents such as epicatechin and tannic acid showed significant enhancing effects for the GREP action. We observed that tea constituents such as epicatechin and tannic acid showed significant enhancing effects for the GREP action.

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\textbf{CONFLICT OF INTEREST STATEMENT}

None declared.

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