The gastroprotective effect of the foxtail millet and adlay processing product against stress-induced gastric mucosal lesions in rats

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A B S T R A C T  
Foxtail millet (Setaria italica (L) P. Beauv.) and adlay (Coix lachryma-jobi L var. ma-yuen Stapf.) seeds have substantial benefits possesses remarkable edible and nutritive values, and ease of processing and food manufacturing. They have nutraceutical properties in the form of antioxidants which prevent deterioration of human health and have long been used in traditional Chinese medicine as a remedy for many diseases. The present study is designed to investigate the gastroprotective effect of foxtail millet and adlay processing product (APP) diet on water immersion restraint stress (WIRS) induced ulceration in rats. We examined the effects of intake of AIN-93G diet containing either foxtail millet (10, 20 and 40%, 4 weeks) or APP (15 and 30, 5 weeks) on macroscopic ulcer index (UI), plasma calcium level, lipid per-oxidation products (estimated by the thiobarbituric acid reactive substances; TBARS), non-protein sulf-hydryl (NPSH), digestive enzyme activities, and histopathology were determined. The results showed that pretreatment with millet and adlay diets significantly prevented the gastric mucosal lesion development. In addition, ulcerated rats showed depletion of NPSH levels whereas treatment with millet and adlay reverted this decline in stress-induced rats. Histological studies confirmed the results. The finding suggests that millet and adlay diets promote ulcer protection by the decrease in ulcer index, TBARS values and increase NPSH concentrations. Millet and adlay diets retain the advantage of being a natural product which may protect the gastric mucosa against ulceration.

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1. Introduction

Peptic ulcer disease (PUD) is a common disorder of the gastrointestinal system also known as peptic ulcer or gastric ulcers, follows gastric mucosal injuries as a result of imbalance between the defensive and the aggressive factors affecting the mucosa.1 The leading cause of PUD include Helicobacter pylori infection,2 and the use of non-steroidal anti-inflammatory drugs (NSAIDs, such as aspirin, Advil or Motrin (ibuprofen), Aleve (naproxen), and others), are the major risk factors for PUD, and also the genetic, pepsin, smoking, alcohol, bile-acids, steroids and stress and comorbidity increase the risk of PUD occurrence.3 Stress is an acute (single or short exposure) threat to homeostasis that evokes an allostatic or adaptive response, affects the function of the gastrointestinal tract either in short-or-long term impacts.3

Typically, primary mucosal erosions are referred to as stress-related injury and, namely, stress ulcers represent focal deep mucosal damages with a high risk for bleeding. The pathophysiology of these disorders focus on the aggressive stress and gastric defense mechanism, such as hydrochloric acid (HCl) production,
mucus secretion, non-protein sulfhydryl (NPSH) groups from the stomach and liver, and blood flow. Insufficient blood microcirculation in the upper gastrointestinal tissues is considered as the major cause of mucosal defense reduction leading to the ulcer formation. Reactive oxygen species (ROS), such as superoxide anion (O2-), hydrogen peroxide (H2O2), and hydroxyl radical (OH•), accompany ischemic tissue and are suggested as mediators of gastrointestinal injuries of different etiology including stress-induced lesions. In addition, ROS trigger lipid peroxidation (LPO) with subsequent loss of membrane fluidity, weakened ion transport and membrane integrity, and finally cell death.

Several drug treatment approaches are available of PUD, but compliance is often poor and frequently associated with adverse effects thus limiting their use. Hence, the search for alternative products continues due to their perceived relative lower side effects, ease of accessibility and affordability, as well as natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives.

Foxtail millet (Setaria italica (L.) P. Beauv.) is one of the most important drought-resistant whole grain, has long been used to treat vacuity heat of spleen and stomach, stomach re ux vomiting, reduced food intake with abdominal distention, and diabetes mellitus in traditional Chinese medicine (TCM). In particular, millets have nutraceutical properties in the form of antioxidants which prevent deterioration of human health such as lowering blood pressure, risk of heart disease, prevention of cancer and cardiovascular diseases, diabetes, decreasing tumor cases etc. 7 Several drug treatment approaches are available of PUD, but compliance is often poor and frequently associated with adverse effects thus limiting their use. Hence, the search for alternative products continues due to their perceived relative lower side effects, ease of accessibility and affordability, as well as natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives.

In this preventive study, the protective effect of modi ed feeds containing foxtail millet and adlay processing product (APP, by using dehulled adlay as the main raw material with burdock, lico-rice root and ganoderma to become health food by extrusion) at least four weeks feeding was investigated, and ulceration progress in rats subjected to water immersion restraint as a stress conditions model.

2. Materials and methods

2.1. Materials

Millet employed in this study is the Taitung No.7 millet (Setaria italica (L.) P. Beauv.), a foxtail millet, which is bred by the Taitung District Agricultural Research and Extension Station (Taitung, Taiwan), while APP was kindly provided by Kuang Ta Foods Ltd. (Taichung, Taiwan). Corn starch, deextrinized corn starch, casein, alphacel non-nutritive bulk, AIN-93 M vitamin, AIN-93 M mineral, L-cysteine, maleic acid, iodoacetic acid, phenylmethylsulfonyl fluoride (PMSF), bovine serum albumin (BSA), D-(+)-maltose monohydrate, α-lactose monohydrate, sucrose, potassium chloride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), cimetidine, butylated hydroxytoluene (BHT), potassium chloride, ethylenediaminetetraacetic acid disodium salt (EDTA-Na2), trichloroacetic acid (TCA), 1,1,3,3-tetramethoxypropane (TEP), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), Tris base, L-cysteine, maleic acid, iodoacetic acid, phenylmethylsulfonyl fluoride (PMSF), bovine serum albumin (BSA), D-(+)-maltose monohydrate, α-lactose monohydrate, sucrose, potassium chloride, sodium phosphate monobasic, disodium phosphate dodecahydrate, potassium chloride, sodium hydroxide, sodium chloride, n-butanol, isobutanol, formaldehyde, hydrochloric acid, phosphoric acid, and sulfuric acid, etc. were from Sigma-aldrich (St. Louis, MO, US). Amylase (AY-891), lipase (LI-188), and leucine aminopeptidase (LA-561) activities were determined using commercially available kit (Randox Laboratories Ltd., Antrim, UK).

2.2. Feed preparation

This study designed the feed recipe with reference to the AIN-93G diet 24 and modified the ratio of protein, fat, and starch in accordance with the composition of the foxtail millet and APP. The AIN-93G feed was used as the base feed for the normal, negative control, and positive control groups. Foxtail millet-containing experimental diets contained ash (1.0%), crude protein (12.3%), crude fat (2.4%), total dietary fiber (3.9%), moisture content (8.0%) and nitrogen-free extract (72.4%) (as presented in Table 1), was divided into low, medium, and high dosages with 10%, 20%, and 40% of the corn starch, casein, and soybean oil replaced by foxtail millet flour. APP-containing experimental diets contained ash (2.0%), crude protein (13.5%), crude fat (2.9%), total dietary fiber (12.6%), moisture content (2.3%) and nitrogen-free extract (66.7%), was divided into low and high dosages with 15% and 30% of the corn starch, casein, and soybean oil replaced by the APP (Table 2).

2.3. Induction of gastric ulceration

The experimental protocols for the animal study were approved by the Institutional Animal Care and Use Committees of National Taiwan University (95-EL-104). Wistar rats (3 weeks old) from the Laboratory Animal Center of the College of Medicine of National Taiwan University were used. All experiments were carried out in accordance with the International Council for Laboratory Animal Science guidelines for the care and use of laboratory animals. They were given ad libitum access to food and water and allowed to adapt
for 24 h were restrained in (C2-mucosal lesions were examined under a dissecting microscope tissue were quickly removed. Gastric and the stomachs were quickly removed. Gastric and the relative humidity was set at 40 ± 20°C experiments. The room temperature was maintained at 24°C to laboratory conditions for one week prior to the beginning of Adlay processing product (APP)-containing experimental diets. Table 1 Foxtail millet-containing experimental diets.

| Diet constituents (%) | B       | NC      | L       | M       | H       | PC      |
|-----------------------|---------|---------|---------|---------|---------|---------|
| Corn starch           | 39.749  | 39.749  | 31.436  | 23.124  | 6.499   | 39.749  |
| Casein                | 20.000  | 20.000  | 18.588  | 17.176  | 14.351  | 20.000  |
| Dextrinized corn starch| 13.200  | 13.200  | 13.200  | 13.200  | 13.200  | 13.200  |
| Sucrose               | 10.000  | 10.000  | 10.000  | 10.000  | 10.000  | 10.000  |
| Soybean oil           | 7.000   | 7.000   | 7.242   | 6.449   | 5.898   | 7.000   |
| Alphacel              | 5.000   | 5.000   | 5.000   | 5.000   | 5.000   | 5.000   |
| AIN-93M-mineral mix   | 3.500   | 3.500   | 3.500   | 3.500   | 3.500   | 3.500   |
| L-Cystine             | 1.000   | 1.000   | 1.000   | 1.000   | 1.000   | 1.000   |
| AIN-93M-vitamin mix   | 0.300   | 0.300   | 0.300   | 0.300   | 0.300   | 0.300   |
| Choline bitartrate    | 0.250   | 0.250   | 0.250   | 0.250   | 0.250   | 0.250   |
| tert-Butyldihydroquinone | 0.001 | 0.001 | 0.001   | 0.001   | 0.001   | 0.001   |
| Foxtail millet        | —       | —       | 10.000  | 20.000  | 40.000  | —       |
| Total                 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 |

B, normal group; NC, negative control group; L, low-dose group; M, medium-dose group; H, high-dose group; and PC, positive control group.

Table 2 Adlay processing product (APP)-containing experimental diets.

| Diet constituents (%) | B       | NC      | L       | M       | H       | PC      |
|-----------------------|---------|---------|---------|---------|---------|---------|
| Corn starch           | 39.749  | 39.749  | 27.829  | 15.909  | 39.749  |
| Casein                | 20.000  | 20.000  | 17.467  | 14.933  | 20.000  |
| Dextrinized corn starch| 13.200  | 13.200  | 13.200  | 13.200  | 13.200  |
| Sucrose               | 10.000  | 10.000  | 10.000  | 10.000  | 10.000  |
| Soybean oil           | 7.000   | 7.000   | 5.906   | 6.449   | 7.000   |
| Alphacel              | 5.000   | 5.000   | 5.000   | 5.000   | 5.000   |
| AIN-93M-mineral mix   | 3.500   | 3.500   | 3.500   | 3.500   | 3.500   |
| L-Cystine             | 1.000   | 1.000   | 1.000   | 1.000   | 1.000   |
| AIN-93M-vitamin mix   | 0.300   | 0.300   | 0.300   | 0.300   | 0.300   |
| Choline bitartrate    | 0.250   | 0.250   | 0.250   | 0.250   | 0.250   |
| tert-Butyldihydroquinone | 0.001 | 0.001 | 0.001   | 0.001   | 0.001   |
| APP                   | —       | —       | 15.000  | 30.000  | —       |
| Total                 | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 |

B, normal group; NC, negative control group; L, low-dose group; H, high-dose group; and PC, positive control group.

to laboratory conditions for one week prior to the beginning of experiments. The room temperature was maintained at 24 ± 2°C and the relative humidity was set at 40–70%. An automatic timer was set to control the light cycle; a light period and dark period were each set to account for 12 h. During the feeding period, the amount of food intake and body weight (body-wt.) of the rats were measured twice a week. The animals were randomly assigned to one of the five or six groups of 10 animals each according to weight: normal (B, without the WIRS process); negative control (NC); low-dose (L); medium-dose (M); high-dose (H); and positive control (PC, with 100 mg/kg cimetidine medication process). Injuries (ulcers, erosion, and hemorrhages) in gastric mucosa were caused by water immersion restraint stress (WIRS). The rats that had fasted for 24 h were restrained in firmly fitted restraint cages (6 × 7 × 20 cm3) and vertically immersed in water maintained at 20 ± 1°C to the level of the xiphoid process for 4 h to induce gastric mucosal lesions.

2.4. Sample collection and biochemical assays

Two dietary feeding periods were used, foxtail millet (4 weeks) and APP (5 weeks), in this study. The 9-week-old rats were subjected to a 24-h fast the day before application of WIRS, and they were force-fed a saline solution or cimetidine 30 min before a 4-h induction of WIRS. Rats were sacrificed under CO2 anesthesia after treatment and the stomachs were quickly removed. Gastric mucosal lesions were examined under a dissecting microscope (× 10), and the values of the ulcer index (UI) was expressed by measuring the total length (mm) of all gastric mucosal lesions in the stomachs induced during the stress. Also, rats were eviscerated for collection of livers, and small intestines as well as blood samples were separated and stored at −80°C for later analyses. Histologic assessments were made with photomicroscope. Specimens from normal and abnormal gastric tissues were fixed in 10% formaldehyde, routinely dehydrated, cleaned, infiltrated with wax, embedded and made into serial 4-μm thick sections. The sections were dewaxed, stained with haematoxylin and eosin technique.

2.4.1. Appreciation of UI

Mucosal lesions were evaluated by macroscopic analysis and the score systems reported previously. Briefly, the stomach was dissected out and opened along the greater curvature and rinsed with 0.1 mol/L ice-cold phosphate-buffered saline. The stomach was then examined with a 10 × magnifier to observe erosions and make scores as 1–5: 1 point for small round hemorrhagic erosion, 2 points when the length of hemorrhagic erosion was less than 1 mm, 3 points when the length was 1–2 mm, 4 points when the length was 2–3 mm, 5 points when the length was longer than 4 mm. The score was multiplied by 2 when the width of erosion was larger than 1 mm.

2.4.2. Determination of plasma MDA concentration

The amount of produced malondialdehyde (MDA) was used as an index of lipid peroxidation, which was estimated by the thiobarbituric acid reactive substances assay (TBARS). To 20 μl of plasma was taken in centrifuge tube, 4 ml of N/12H2SO4 was added and the mixture was shaken gently. Then 0.5 ml of 10% phosphotungstic acid was added and mixed after standing at room temperature for 5 min. The mixture was centrifuged at 1570 × g for 10 min. The supernatant was discarded and the sediment was suspended in 4 ml double-distilled (dd) water and 1 ml of 0.67% TBA reagent was added. The reaction mixture was heated for 60 min at 95°C in boiling water bath. After cooling 5 ml of n-butanol was added and mixture was shaken vigorously. After the centrifugation at 1570 × g for 15 min. The supernatants (n-butanol layer) were measured by a fluorophotometer spectrometer (F-4500, Hitachi, Japan) (with excitation at 515 nm and emission at 553 nm), and the results were recorded as absorbance units (AU).

2.4.3. Determination of gastric mucosal MDA concentration

Gastric mucus homogenate (0.2 g) was mixed with 0.2 ml of 1.15% KCl/3 mM EDTA-Na2, 3 ml of 1% H3PO4, 0.3 ml of 0.3% BHT/EtOH, and 1 ml of a 0.6% TBA solution. Test samples were placed in a water bath at 100°C for 60 min after being vortex-mixed. Iso-butanol (2 ml) was added to cooled samples, and the absorbance of...
the BuOH fraction was measured at 532 nm. The calibration curve was established using various concentrations of TEP solutions. Results were recorded as μmol/g of tissue.22

2.4.4. Determination of gastric mucus and liver NPSH

A previously described method was used to examine the NPSH in the gastric mucus and liver.27 Brieﬂy, 1 ml of gastric mucus or liver homogenate was mixed with 0.8 ml of dd water and 0.5 ml of a 50% TCA solution. After vortex mixing for 2 min, the solutions were centrifuged at 1570 × g for 20 min. Supernatants (0.5 ml) were mixed with 1 ml of 0.4 M Tris-buffer (pH 8.9) and colored by 25 μl of a 0.01 M DTNB solution. The absorbance at 412 nm was recorded, and the results were presented as μg/g of tissue.22

2.4.5. Determination of digestive enzymes in small intestine

A segment of the small intestine was removed, washed in 0.9% NaCl solution, dried on filter paper, weighed, trimmed, and homogenized (1570 × g) with 0.9% NaCl containing protease inhibitors (1 μM PMSE and 2.2 mM iodoacetic acid) for 10 min at 4 °C. The supernatant was used for the measurement of in vitro maltase, sucrase, and lactase activities and protein determination. Maltase (EC 3.2.1.20), lactase (EC 3.2.1.23), and sucrase (EC 3.2.1.48) activities (in mmol/mg protein) were determined using a glucose digestion kit based on the glucose oxidase reagent by the method described in work.28 The protein content was determined by Lowry’s method with bovine serum albumin as the standard.29 The assays for amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), leucine aminopeptidase (LAP; EC 3.4.11.2) activity (in U/mg protein) have been described elsewhere with minor modification.30–32

2.5. Statistical analysis

The data are presented as the mean ± standard error of mean (SEM). Statistical analyses were performed using one-way ANOVA followed by the Duncan and dark red with elongated bands. The values of the UI increased dramatically after stress as compared with the normal group. Fig. 5 shows the inﬂuence on the UI of intake foxtail millet (Fig. 5a) and APP (Fig. 5b) in the stress-loaded rats. A gastroprotective effect was observed in rats administrated foxtail millet/APP after the development of stress-induced gastric ulcers, which exhibited a statistically signiﬁcant reduction in the severity and number of gastric lesions compared to the NC group (p < 0.05).

3.3. Changes in NPSH

The effects of foxtail millet and APP feeding followed by the onset of WIRS (4 h) and changes in the gastric mucosal NPSH concentrations in the rats are shown in Fig. 3. The gastric NPSH was significantly lower in the NC, medium-dose and high-dose group compared to the normal rats without WIRS (p < 0.05). The gastric NPSH showed signiﬁcant differences among the NC, low-dose and high-dose group compared to the normal group (p < 0.05, Fig. 3b). The hepatic NPSH decreased with administration of foxtail millet and APP diet, which was statistically suppressed compared to the normal group (p < 0.05, Fig. 4a and b).

3.4. Changes in UI

When rats were subjected to WIRS for 4 h, gastric mucosal lesions with a diameter of >2 mm were observed in the glandular regions of the stomach. These mucosal lesions appeared to be black and dark red with elongated bands. The values of the UI increased dramatically after stress as compared with the normal group. Fig. 5 shows the inﬂuence on the UI of intake foxtail millet (Fig. 5a) and APP (Fig. 5b) in the stress-loaded rats. A gastroprotective effect was observed in rats administrated foxtail millet/APP after the development of stress-induced gastric ulcers, which exhibited a statistically signiﬁcant reduction in the severity and number of gastric lesions compared to the NC group (p < 0.05).

3.5. Pathological changes in gastric mucosa

The pathological examination of gastric mucosa in our ulcer model indicated obvious ulcer injury. There was no gastric mucosal lesion in normal group. Scattered spot or lineal erosions, hemorrhage and ulcers were observed in gastric mucosa in stress group. These observed changes were consistent with the signiﬁcant elevation of the values of the ulcer index in NC rats subjected to WIRS (Fig. 6). Numbers of bleeding clots on the surface of the

Table 3a

| Group | Initial body-wt. (g/rat) | Final body-wt. (g/rat) | Food intake (g/day/rat) | Feed efficiency (%) |
|-------|-------------------------|------------------------|------------------------|---------------------|
| B     | 135 ± 15a               | 297 ± 30a              | 18.6 ± 1.3b            | 36.3 ± 4.0a         |
| NC    | 135 ± 11b               | 306 ± 16c              | 18.6 ± 0.9b            | 38.3 ± 2.1c         |
| L     | 136 ± 10e               | 320 ± 14d              | 20.1 ± 1.3c            | 38.2 ± 2.7d         |
| M     | 135 ± 11f               | 312 ± 27e              | 15.4 ± 1.1e            | 38.1 ± 5.0f         |
| H     | 134 ± 12g               | 309 ± 12f              | 18.9 ± 0.7g            | 38.6 ± 2.1g         |
| PC    | 136 ± 12h               | 304 ± 15g              | 18.9 ± 0.6h            | 36.9 ± 3.0h         |
stomach of the low-/medium-/high-dose foxtail millet (Fig. 6a) and low-/high-dose APP (Fig. 6b) groups were fewer than those of the NC group with marked blood coagulum, while no macroscopic lesion was observed in the PC group.

### Table 3b

| Group | Initial body-wt. (g/rat) | Final body-wt. (g/rat) | Food intake (g/day/rat) | Feed efficiency (%) |
|-------|--------------------------|------------------------|-------------------------|---------------------|
| B     | 93 ± 11a                 | 294 ± 44a              | 15.4 ± 2.1a             | 37.1 ± 3.4a         |
| NC    | 93 ± 9a                  | 292 ± 41a              | 15.3 ± 1.9a             | 37.0 ± 4.7a         |
| L     | 93 ± 8a                  | 323 ± 24a              | 16.5 ± 1.1a             | 40.0 ± 2.8a         |
| H     | 93 ± 8a                  | 316 ± 16a              | 15.9 ± 0.9a             | 40.0 ± 2.6a         |
| PC    | 94 ± 7a                  | 291 ± 51a              | 15.4 ± 2.0a             | 36.4 ± 6.7a         |

Results are expressed as mean ± SEM (n = 10).

Feed efficiency = (Body weight gained/Total feed intake) × 100.

Values in the same column sharing common superscript small letter showed no significant difference (ANOVA, p > 0.05).

B, normal group; NC, negative control group; L, low-dose group; H, high-dose group; and PC, positive control group.

#### Fig. 1.

Effect on the plasma TBARS of WIRS-treated rats administrated (a) foxtail millet and (b) APP. Each bar represents the mean ± SEM (n = 10). There is no significant difference between groups (p > 0.05). B, normal group; NC, negative control group; L, low-dose group; M, medium-dose group; H, high-dose group; and PC, positive control group.

#### Fig. 2.

Effect on the mucosal TBARS of WIRS-treated rats administrated (a) foxtail millet and (b) APP. Each bar represents the mean ± SEM (n = 10). *p < 0.1 compared to the normal group without the WIRS process, **p < 0.05 compared to the normal and negative control group. B, normal group; NC, negative control group; L, low-dose group; M, medium-dose group; H, high-dose group; and PC, positive control group.

3.6. **Histology**

Fig. 7 shows the histopathological observations of H&E stained sections in the stomach of rats. The results showed that the erosion was severe in the NC group, while it was inhibited when low-/
medium-dose foxtail millet (Fig. 7a) and low-/high-dose APP (Fig. 7b) were given. The present histopathological findings supported the protective effects of cimetidine and foxtail millet/APP diet and revealed relatively normal mucosa in rats pretreated with cimetidine and erosion and gastric healing in rats pretreated with foxtail millet/APP diet.

3.7. Changes in plasma calcium

Plasma calcium was measured using calorimetric assay by a Johnson & Johnson (Ortho-Clinical) Vitros 250 Chemistry Analyzer (Raritan, NJ, US). The plasma calcium concentrations in the rats with 4 h of WIRS were significantly lower than that in the normal rats without WIRS. No significant differences were observed between the treatment groups subjected to stress in the foxtail millet diet group.

In contrast to the plasma calcium concentrations in the APP diet group, low-dose APP elevated significantly higher than NC and PC group, but was no difference compared with those of the high-dose APP group. Moreover, there was no statistically significant difference in concentrations of plasma calcium between the NC group subjected to stress and the normal rats without WIRS.

3.8. Changes in small intestinal digestive enzyme activity

Table 4(a) shows that foxtail millet diet group had no statistical difference in the lipase, amylase, and ALP activities among the treatments. Maltase and sucrase in low-dose foxtail millet group were significantly higher than that in normal rats without WIRS, were similar to the positive control group. Both lactase and sucrase activities showed significantly higher increases than NC group subjected to stress fed with low-dose diet. Table 4(b) shows that APP diet group had no statistical difference in the lipase activity among the treatments. Both amylase and LAP activities in the rats
with 4 h of WIRS (NC group) were significantly lower than that in the normal rats without WIRS, while the amylase and LAP activities in low/high-dose APP group were slightly higher as compared to the negative controls.
In comparison to the disaccharidase activities in APP diet presented in Table 4(b), both lactase and sucrase activities in NC group were significantly lower than that in normal rats without WIRS. In contrast, the reverse was observed when assessing maltase and sucrase activities of the rats pretreated with cimetidine. Otherwise, maltase activity showed significantly higher increases than normal rats without WIRS and gave sucrase activity similar to that of the normal rats fed with high-dose APP diet.

WIRS mimics the clinical acute gastric ulcers caused by trauma, surgery or sepsis and has been widely accepted for studying stress ulcers. Previous studies revealed a positive correlation between free radical-induced oxidative stress both in gastric and duodenal ulceration, and excessive stress leads to consumption of the internal antioxidative barrier. Since an antioxidant defense mechanism may be critically important in protecting against the development of acute gastric mucosal injury, the anti-ulcer response and extensive antioxidant effect of foxtail millet and APP diet may be valuable in prevention, which possesses preventive and gastroprotective effects on experimental gastric mucosal lesions in rats. Consumption of whole grains has been associated with reduced risk of developing major chronic diseases. Millet and adlay diet retain the advantage of being a natural product with no reported side effects which may prove a promising protective role in gastric ulcer.

**Declaration of competing interest**

All authors declare no conflicts of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.01.003.

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