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The Effect of Luteolin-7-*O*-β-D-Glucuronopyranoside on Gastritis and Esophagitis in Rats

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This study evaluated the inhibitory action of luteolin-7-*O*-β-D-glucuronopyranoside, luteolin which was isolated from *Salix gilgiana* leaves, and omeprazole on reflux esophagitis and gastritis in rats. Reflux esophagitis and gastritis were induced surgically and by the administration of indomethacin, respectively. The intraduodenal administration of luteolin-7-*O*-β-D-glucuronopyranoside decreased the ulcer index, injury area, gastric volume and acid output, and increased the gastric pH compared with luteolin. Luteolin-7-*O*-β-D-glucuronopyranoside significantly decreased the size of the gastric lesions that had been induced by exposing the gastric mucosa to indomethacin. The malondialdehyde content, which is the end product of lipid peroxidation, was increased significantly after inducing of reflux esophagitis. The malondialdehyde content was decreased by Luteolin-7-*O*-β-D-glucuronopyranoside but not luteolin or omeprazole. Luteolin-7-*O*-β-D-glucuronopyranoside has a more potent antioxidative effect than luteolin. Luteolin-7-*O*-β-D-glucuronopyranoside is a promising drug for the treatment of reflux esophagitis and gastritis.

Key words: Reflux esophagitis, Lipid peroxidation, Gastric secretion, Free radical, Omeprazole, Luteolin, Luteolin-7-*O*-β-D-glucuronopyranoside, Rat

INTRODUCTION

Flavonoids have anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. In addition, the anti-inflammatory activities of flavonoids and their potential utility as therapeutic agents have been reported (Lewis, 1989). Flavonoids are typical phenolic compounds that act as potent metal chelators and free radical scavengers (Middleton *et al.*, 2000).

Reflux esophagitis is a common disease in which gastric juice enters the esophagus *via* transient lower esophageal sphincter (LES) relaxation, the speed of esophageal clearance, mucosal resistance etc, and is often associated with a low LES pressure (Bell *et al.*, 1992). If left untreated, erosion and ulceration of the esophageal mucosa by gastric acid, chronic esophagitis, aspiration pneumonia, esophageal strictures and Barrett's esophagus (a premalignant condition) may result (Biancani *et al.*, 1997). However, the severity of reflux esophagitis cannot be predicted accurately based on the extent of acid exposure, which suggests the involvement of other factors or possibly impaired mucosal resistance.

Gastritis includes many disorders involving inflammatory changes in the gastric mucosa including erosive gastritis caused by a noxious irritant, reflux gastritis from exposure to bile and pancreatic fluids, hemorrhagic gastritis, infectious gastritis, and gastric mucosal atrophy. Stress, ethanol, bile, free radicals, oxidants, and non-steroidal anti-inflammatory drugs (NSAIDs) disrupt the gastric mucosal barrier, making it vulnerable to normal gastric secretion. Infections with *Helicobacter pylori* are the leading cause of peptic ulcers and are associated with virtually all ulcers not induced by NSAIDs.

Oxygen derived free radicals as a result of ischemia (Stein *et al.*, 1990), or ethanol (Pihan *et al.*, 1987) play an important part in the pathogenesis of injury to various tissues including the digestive system (Naya *et al.*, 1997), and can cause acute gastric and esophageal mucosal

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injury. Free radicals also act as carcinogens because they can cause DNA damage (Haegele *et al.*, 1994). Free radical damage to the gastric or esophageal mucosa can be prevented by the administration of free radical scavengers (Wetscher *et al.*, 1995).

This study examined the effects of luteolin-7-*O*-β-D-glucuronopyranoside (LGC), which was isolated from *Salix gilgiana* leaves, on the development of reflux esophagitis surgically induced in rats, gastritis induced by NSAIDs, gastric secretion, and lipid peroxidation, which indicates the level of oxidative stress, using omeprazole (OMP) as a reference drug.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats with a body weight of approximately 200-250 g were used for the experiments. The rats were starved for 24 h prior to the experiments, but were provided with drinking water *ad libitum*. All the animals were kept in raised mesh-bottom cages in order to prevent coprophagy.

Evaluation of reflux esophagitis

Under ether anesthesia, the abdomen was incised along the midline and both the pylorus and limiting ridge (transitional region between the forestomach and corpus) were then ligated simultaneously (Nakamura *et al.*, 1982). An approximately 1 cm long longitudinal cardiomyotomy across the gastroesophageal junction was performed to enhance reflux. Six hours later, the rats were sacrificed by a cervical dislocation, and the esophagus was harvested. The total area (mm^2) of the lesions that had developed in the esophagus was determined using a dissecting microscope (X10), and was graded as follows: 0, no visible lesions; 1, a few erosions; 2, total area of the lesions 30 mm^2; 3, total area of the lesions 30 mm^2; 4, perforation (Okabe *et al.*, 1995). LGC dissolved in 0.01% dimethyl sulfoxide (DMSO) were administered intraduodenally (i.d.) immediately after ligating the pylorus and limiting ridge. The volume of the drug or vehicle was 1 mL/kg of body weight. The drugs were freshly prepared each time.

Evaluation of indomethacin-induced gastritis

LGC, luteolin, or omeprazole were administered *per os* (p.o.) one hour before administering the indomethacin. The volume of the drug or vehicle was 2 mL/kg of body weight. The drugs were freshly prepared each time. The ulcers were induced by the p.o. administration of 50 mg/kg of indomethacin, suspended in a 3% $NaHCO_3$ solution. The animals were sacrificed 5 hours after administering the indomethacin. The stomach was excised, opened along the greater curvature and spread out on a corkboard with pins. The area (mm^2) of mucosal erosive lesions was measured using a dissecting microscope with a squared grid (X10; Olympus, Tokyo, Japan).

Study of gastric secretion

Six hours after the pylorus ligation, the rats were sacrificed by a cervical dislocation and the esophagus was clamped. Samples of gastric juice were collected in graduated conical centrifuge tubes and centrifuged at 3,000 *g* for 10 min at 4°C. After centrifugation, the volume (mL/rat), pH (Toledo 320, Mettler, Swiss) and acidity (mEq/L) of the supernatant was measured. The total acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (665 Dosimat, Metrohm, Swiss). The acid output is expressed as mEq/h (Okabe *et al.*, 1995).

TBARS Assay

The level of lipid peroxidation was determined using the method reported by Buege and Aust, in which the level of thiobarbituric acid-reactive substances (TBARS) was determined spectrophotometrically (Buege and Aust, 1978). The esophageal mucosa was harvested, and sonicated in 1 mL of Tris-HCl buffer (pH 7.0). After centrifugation at 600 *g* for 10 min at 4°C (Micro17TR, Hanil, Korea), 0.9 mL of 8% trichloroacetic acid (TCA) was added to 0.3 mL of the supernatant. After centrifugation at 10,000 *g* for 5 min at 4°C, 0.25 mL of TBA (1%) was added to 1 mL of the supernatant and the resulting solution was heated at 100°C for 20 min. The tubes were cooled, 2 mL of n-butanol was added, and each tubes was vortexed for 90 sec. After centrifugation at 3,000 *g* for 5 min at 4°C, 1 mL of the butanol phase was subjected to the TBARS assay at 532 nm (UV-160A, Shimadzu, Japan) against malonaldehyde bis (dimethyl acetal) standards. The results are expressed as ng/mg protein. The protein level was determined using the Bradford method (Bradford, 1976) with bovine serum albumin as the standard.

Drugs

The LGC was isolated and purified from *Salix gilgiana* leaves in the Department of Pharmaceutical Botany (Chung Ang Univ., Seoul, Korea). The purity of the LGC was approximately 96% according to HPLC. The luteolin, indomethacin, thiobarbituric acid, trichloroacetic acid, malonaldehyde bis (dimethyl acetal), bovine serum albumin, *o*-phthalaldehyde, diethylenetriaminepentaacetic acid, ascorbic acid, *N*-ethylmaleimide, and omeprazole were purchased from Sigma (St. Louis, MO, U.S.A.). The potassium phosphate (dibasic) and potassium dihydrogenphosphate were purchased from Showa (Tokyo, Japan). The protein assay kits were purchased from BioRad

(Richmond, CA, U.S.A.).

Analysis of data

The values are expressed as a mean ± SEM (standard error of means). The Student's *t*-test, ANOVA (analysis of variance) and Fischer's exact test were used to determine the statistical significance of the data. A P value < 0.05 was considered significant.

RESULTS

The preventive effect of LGC on indomethacin-induced gastritis

Indomethacin (50 mg/kg) induced ulceration of the gastric mucosa. However, LGC administered p.o. decreased the level of ulceration in a dose–dependent manner (Fig. 1), and more effective than same dose of OMP This suggests that LGC is an effective agent. These results show the preventive effect of glucuronopyranoside on gastritis in rats, as well as why LGC has a higher efficacy and potency. The mean lesion area in the control group (Compound dose 0 = sham operated rats.) was 4.5~0.5 cm^2 (N = 6-8). The LGC (0.001-0.1 mg/kg, p.o.) inhibited the formation of indomethacin-induced lesions in a dose dependent manner.

The effect of LGC, luteolin, or OMP on reflux esophagitis induced surgically in rats

Six hours after the pylorus ligation, severe ulcerations were observed in the esophagus of the control group. However, the LGC, luteolin, or OMP treated groups showed significantly less reflux esophagitis in a dose-dependent manner (Fig. 2). This suggests that LGC and luteolin have similar activity, and are more potent against surgically induced reflux esophagitis than luteolin. However, omeprazol has a significant effect. The mean lesion area in the control group was 4 (**0**, no lesions; **1**, thin of ulcer area; **2**, ulcer area 30 mm^2; **3**, ulcer area 30 mm^2; **4**, complete lesion of the mucosa). The areas of reflux esophagitis lesion was lower in the LGC treated groups.

The effect of LGC, luteolin, or omeprazol on gastric secretion in reflux esophagitis

LGC administered i.d. significantly decreased the gastric volume (Fig. 3) and increased the pH of the gastric content (Fig. 4). In addition, LGC or omeprazole administered i.d. decreased the gastric volume, respectively. However, luteolin did not affect the pH of the gastric content (Fig. 3, 4). LGC dose-dependently inhibited the gastric acid output (Fig. 5), which decreased the level of ulceration of the gastric and esophageal mucosa.

The inhibitory effect of LGC on lipid peroxidation

In the control group (Compound dose 0 = sham operated rats), the amount of malondialdehyde (MDA), which is the end product of lipid peroxidation, was significantly higher than in the normal group (Fig. 6). LGC significantly decreased the level of MDA formation, which

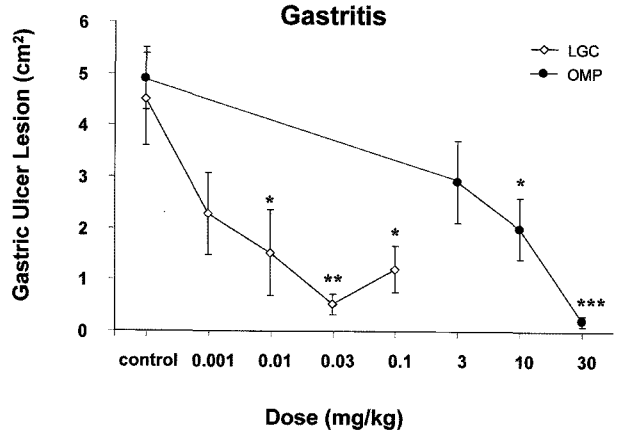

Fig. 1. The preventive effect of LGC on indomethacin-induced gastritis. The mean lesion area in the control group was 4.0 ± 0.7 cm^2. LGC (0.001 – 0.01 mg/kg, p.o.) inhibited the formation of indomethacin-induced lesion in a dose dependent manner. OMP (0, 3, 10, 30 ß²/ß³, p.o.) decreased the ulcer lesion in a dose dependent manner. LGC is more effective against indomethacin-induced gastritis than the same dose of OMP. The data is reported as a mean ± S.E.M. of 6-8 animals. *Significant differences from the corresponding control, respectively. * P < 0.05, ** P < 0.01, and *** P < 0.001vs sham (Compound dose 0 = sham operated rats).

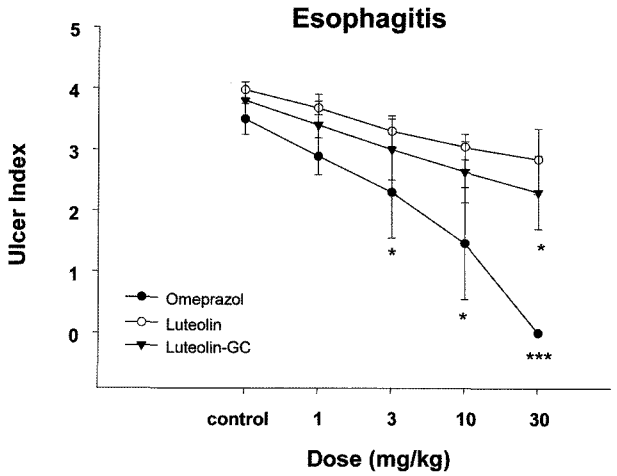

Fig. 2. The effect of LGC on reflux esophagitis induced surgically in rats. The mean lesion area in the control group was 4 (**0**, no lesions; **1**, thin of ulcer area; **2**, ulcer area < 30 mm^2; **3**, ulcer area > 30 mm^2; **4**, complete lesion of the mucosa). The LGC treated groups showed significantly less reflux esophagitis. The data is reported as a mean ± S.E.M. of 6 - 8 animals. *Significant differences from the corresponding control, respectively. * P < 0.05, ** P < 0.01, and *** P < 0.001 vs. the control.

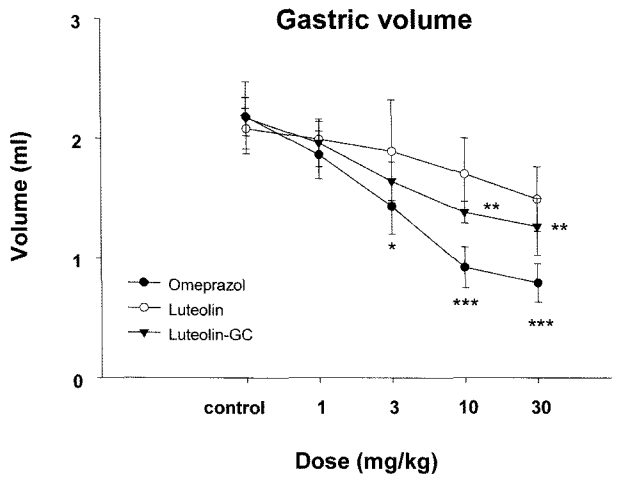

Fig. 3. The effect of LGC on gastric volume. The compounds decreased the gastric volume in a dose dependent manner. The data is reported as a mean ± S.E.M. *Significant differences from the corresponding control, respectively. * P < 0.05, ** P < 0.01, and *** P < 0.001 vs. the control (n = 6 – 8).

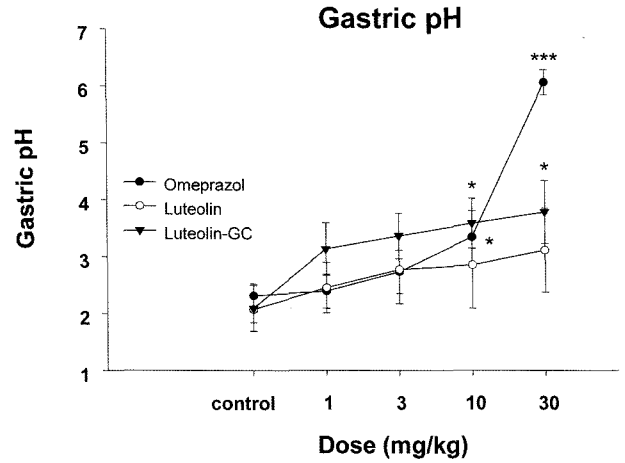

Fig. 4. The effects of LGC on gastric pH. The administration of compounds changed the pH slightly in the rats with surgically-induced reflux esophagitis . LGC significantly increased the pH compared with luteolin, but has a lower effect than omeprazol. The data is reported as a mean ± S.E.M. *Significant differences from the corresponding control, respectively. * P < 0.05; *** P <0.001 vs. the control (n=6 – 8).

showed the inhibition of gastritis-associated lipid peroxidation as a result of the antioxidant treatment (Fig. 6).

DISCUSSION

Gastro-esophageal reflux disease is a common condition with a complex pathophysiology. Despite the wide range of abnormalities, gastric acid plays a key role in mucosal damage, and the suppression of gastric acid secretion is the basis of many treatments (Bell and Hunt, 1992). There is increasing evidence showing that gastric acid pump inhibitors have more favorable effects on reflux

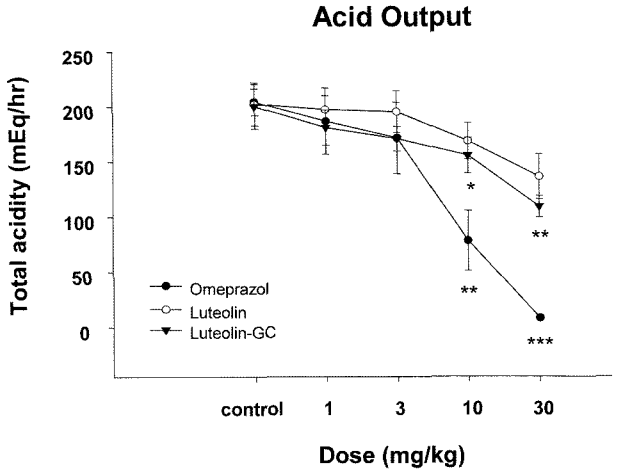

Fig. 5. The reducible effect of LGC on gastric acid output. An introduodenal injection of LGC decreased acid output in a dose dependent manner. Gastric acid is considered to be essential to esophageal mucosal damage. LGC significantly increased the pH compared with luteolin, but has a lower effect than omeprazol. The data is reported as a mean ± S.E.M. *Significant differences from the corresponding control, respectively. * P < 0.05, ** P < 0.01, and *** P <0.001 vs. the control (n = 6 - 8).

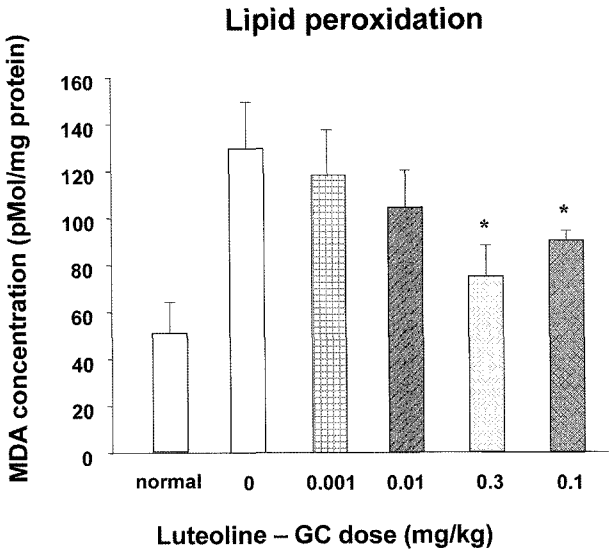

Fig. 6. The inhibitory effects of LGC on lipid peroxidation. In gastritis control, LGC decreased the level of lipid peroxidation compared with the normal group. LGC decreased the level of MDA formation, highlighting the inhibition of gastritis – associated lipid peroxidation by the antioxidant treatment. Each column represents the mean ± S.E.M. of 6 - 8 animals. * P <0.05 vs. sham (Compound dose 0 = sham operated rats).

esophagitis than H_2-blockers due to their profound and long-lasting antisecretory activity (Min *et al.*, 2005). In this study, a disease model was developed to examine the efficacy of different LGC concentrations against reflux esophagitis and gastritis induced by surgery and chemi-

cals, respectively, as well as its antioxidant effects.

The results showed that the protective effect of LGC could be attributed to the inhibition of gastric secretion and the prevention of oxidative stress. In addition, these results support the antiulcer, gastroprotective, gastric antisecretory activities of LGC and luteolin, in a similar manner to omeprazole. LGC, like omeprazole, contains a sulfinyl group in a bridge between the substituted benzimidazole and the pyridine ring and is an irreversible proton pump inhibitor. Flavonoids are naturally occurring plant polyphenols that are found in abundance in diets rich in fruit, vegetables and plant-derived beverages such as tea (Lewis, 1989).

Flavonoids also have gastric antisecretory activity (Alvarez *et al.*, 1999). It was reported that the pH-dependent pattern of a mucosal injury is best explained by the very low activity of gastric pepsin pH > 4.0 (Ito *et al.*, 1998). It was also reported that the duration of the suppression of intragastric acidity pH > 4.0 achieved by each drug regimen could be used to predict the effect of antisecretory therapy on reflux esophagitis (Bell and Hunt, 1992). This activity occurred through different mechanisms. For example, Parmar *et al.* reported that the gastric antisecretory activity is as effective in reducing the level of gastric acid secretion as cimetidine (Parmar and Hennings, 1984). It was reported that more than 40% inhibition of acid output is sufficient to prevent the development of esophagitis (Okabe *et al.*, 1995), and gastric acid is considered to be essential for preventing esophageal mucosal damage (Bell and Hunt, 1992). Flavonoids show antiulcer and gastroprotective activity, and have been shown to have antiulcerogenic properties in rats and guinea pigs. These properties are of particular interest with respect to the adverse effect of gastric ulceration, which commonly develops in subjects taking anti-inflammatory drugs (Gambhir *et al.*, 1987). Gerritsen *et al.* showed that apigenin blocked the cytokine-induced expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on human endothelial cells (Gerritsen *et al.*, 1995). Luteolin was also demonstrated to be an active anti-inflammatory agent in a rat paw carrageenan model and in a contact sensitivity test in mice. Panees *et al.* characterized the inhibitory effect of apigenin on the TNF-stimulated ICAM-1 expression in different rat tissues *in vivo* (Panes *et al.*, 1996).

In this study, the intraduodenal administration of LGC decreased the gastric volume significantly in a dose dependent manner. LGC inhibited the gastric acid output, which prevented the development of reflux esophagitis. These results show that LGC has inhibitory effects on reflux esophagitis and gastritis in rats. In addition, these experiments support the antiulcer, gastroprotective, and gastric antisecretory activities of LGC.

In addition, there was a significant decrease in the lipid peroxide levels. Some authors have implicated oxygen free radicals in the pathogenesis of indomethacin-induced mucosal damage (Kvietys *et al.*, 1990). This results show that LGC has an antioxidative effect and free radical scavenging activity. The MDA content was higher in the esophageal mucosa after inducing reflux esophagitis.

It was reported that reflux esophagitis in rats mediated by oxygen-derived free radicals or superoxide anions are the main source of free-radical damage observed with reflux esophagitis of rats (Wetscher *et al.*, 1995). Superoxide anions produced by inflammatory cells (namely, neutrophils, macrophages, and monocytes) play an important part in the pathogenesis of acid- and pepsin-induced esophagitis in rabbits (Naya *et al.*, 1997). Recently, the levels of free radical production and lipid peroxidation increased with increasing degree of esophagitis, and was reported to be highest in patients with Barrett's esophagus, a premalignant condition (Wetscher *et al.*, 1995). Therefore, this study examined the effects of the proton pump inhibitors on the level of lipid peroxidation. It was reported that omeprazole attenuates the oxygen-derived production of free radicals and lansoprazole inhibits the oxygen-derived production of free radicals from the neutrophils activated by *Helicobacter pylori*. However, it is unclear if LGC and omeprazole are involved in the antioxidant mechanism.

These results confirmed that LGC at a dose of 0.03 mg/kg has a protective effect against indomethacin-induced gastritis. LGC administered orally significantly reduced the level of gastric mucosal damage at all doses tested.

Overall, AGC might be promising drug for the treatment of reflux esophagitis and gastritis. LGC showed efficacy against ulcers through the absorption and the antioxidant effect of glucuronoside. Hollman and Katan reviewed the bioavailability and health effects of dietary flavonoids in humans (Hollman and Katan, 1998). They reported that quercetin glycosides from onions were more readily absorbed than pure aglycone. The quercetin absorbed was eliminated slowly from the blood, suggesting that the enterohepatic circulation may be responsible (Hollman *et al.*, 1997). In addition, it was suggested that glycosides might be absorbed *via* the intestinal sugar uptake route. Hollman *et al.* (1997) and Jung *et al.* (2001) examined an exercise training rat model and proposed that glucuronic acid reduces the level of tissue peroxidation as an anti-oxidative defense mechanism and promotes the recovery of muscle fatigue (Hollman *et al.*, 1997; Jung *et al.*, 2001). LGC reduced the ulcer index, the amounts of malondialdehyde (MDA= the end product of lipid peroxidation), the gastric volume and gastric acid output.

In conclusion, LGC has the preventive activity against

the development of gastritis and esophagitis in rat models, so that LGC may be promising drug for the treatment of reflux esophagitis and gastritis.

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