BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 23(5), 458-464 (2015)

Gastroprotective Activities of Sennoside A and Sennoside B via the Up-Regulation of Prostaglandin E_2 and the Inhibition of H^+/K^+-ATPase

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Abstract

Sennoside A (erythro) and sennoside B (threo) are dianthrone glycosides and diastereomers. We investigated their abilities to prevent the gastric lesions associated with diseases, such as, gastritis and gastric ulcer. To elucidate their gastroprotective effects, the inhibitions of HCl•EtOH-induced gastritis and indomethacin-induced gastric ulcers were assessed in rats. It was observed that both sennoside A and sennoside B increased prostaglandin E_2 (PGE_2) levels and inhibited H^+/K^+-ATPase (proton pump). In a rat model, both compounds reduced gastric juice, total acidity and increased pH, indicating that proton pump inhibition reduces gastric acid secretion. Furthermore, sennoside A and B increased PGE_2 in a concentration-dependent manner. In a gastric emptying and intestinal transporting rate experiment, both sennoside A and sennoside B accelerated motility. Our results thus suggest that sennoside A and sennoside B possess significant gastroprotective activities and they might be useful for the treatment of gastric disease.

Key Words: Sennoside A, Sennoside B, Prostaglandin E_2, H^+/K^+-ATPase, Gastric lesion

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as, aspirin, ibuprofen, and naproxen contribute to gastric epithelial cells damage and accelerate gastric mucosal damage by reducing endogenous prostaglandin (PG) by inhibiting systemic cyclo-oxygenase (COX) activity (Kimmey, 1992). In particular, PGE_2 regulates the secretions of pepsinogen and mucus and the motility of gastric smooth muscle (Ruppin *et al*., 1981; Araki *et al*., 2000). H^+/K^+-ATPase is the proton pump of the stomach and, as such, is primarily responsible for the acidification of stomach contents by gastric acid secreted parietal cells (Bandyopadhyay *et al*., 2002).

In this study, screening tests using animal models of HCl•EtOH-induced gastritis and indomethacin-induced gastric ulcer were performed to investigate gastric protection. To evaluate the gastroprotective effects, a quantitative analysis of PGE_2 was performed, and to evaluate inhibitory effects against aggressive factors, proton pump inhibitory activity and acid neutralizing capacity were investigated. Using an animal model, gastric-juice parameters, such as, gastric volume and pH were measured, after submitting rats to pylorus ligature with or without administering sennoside A or sennoside B intraduodenally. Most chronic gastritis patients experience symptoms of functional dyspepsia and early satiety. Because gastrointestinal tract motility is an important aspect of alleviating the symptoms of functional dyspepsia and increased gastric stasis time can act potently against gastric damage, gastric emptying rates were measured and an intestinal transporting rate experiment was performed.

Sennoside A and sennoside B are dianthrone glycosides found in *Rhei Rhizoma* and senna leaf. They have identical molecular weights and formulae, and are in fact diastereomers with the same substituent (H) located in opposite directions (Fig. 1). Sennosides were known as laxatives, cause purgative actions via biotransformation of rhein anthrone. About this purgative actions, regionally differential effects of sennoside A on spontaneous contractions of colon have been reported (Kobayashi *et al*., 2007). Sennoside B was reported that it has inhibitory effects against PDGF receptor signaling and cell proliferation induced by PDGF-BB in human osteosarcoma cells (Chen *et al*., 2009). The present work was conducted to

Open Access **http://dx.doi.org/10.4062/biomolther.2015.052**

Received May 4, 2015 **Revised** May 21, 2015 **Accepted** May 28, 2015
Published online Sep 1, 2015

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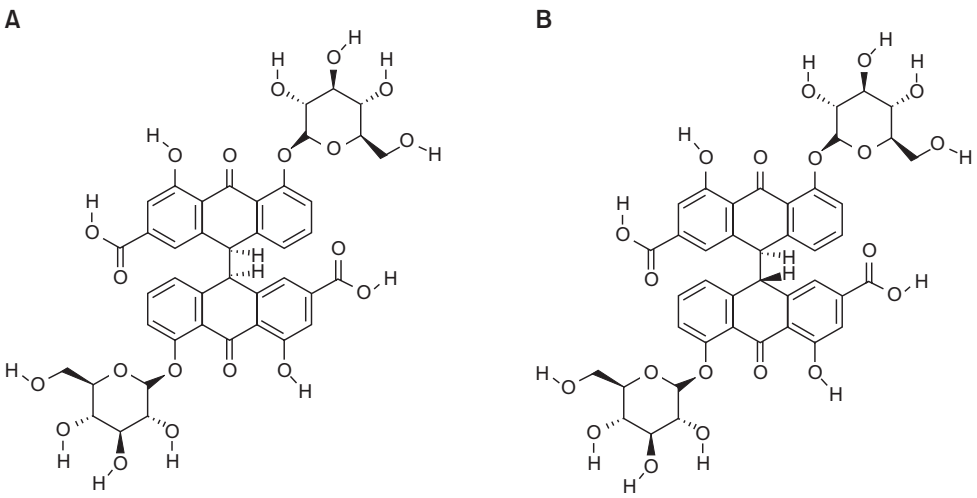

Fig. 1. Sennoside A and sennoside B. (A) Sennoside A; (B) Sennoside B.

investigate the evidence gastric protection by sennoside A and sennoside B against gastric damage.

MATERIALS AND METHODS

Reagents and laboratory equipments

Indomethacin, ATP, phenol red, carboxymethyl cellulose (CMC), and the positive controls, ascorbic acid, hydrotalcite, ampicillin, and cimetidine were purchased from Sigma (Sigma-Aldrich Inc., MO, USA). HCl, EtOH, and NaOH were purchased from the Duksan Pure Chemical Co. Ltd. (Kyunggi-do, Korea). All other reagents and solvents used were of pharmaceutical or analytical grade.

Animals

Male Sprague-Dawley rats, weighing 170 to 200 g and male ICR mice, weighing 25 to 30 g were purchased from Samtako, Kyunggi-do, Korea, and acclimatized to standard laboratory conditions (22 ± 2°C, 55 ± 5% relative humidity, and 12 h light/dark cycle) for 14 days in the animal facility at Duksung Women's University. All animal experimental procedures were conducted in accordance with the Guidelines of the Care and Use of Laboratory Animals issued by Duksung Women's University. The animals were allowed access to food (standard pellet diet) and water *ad libitum*.

HCl•EtOH-induced gastritis in rats

After a 24-hour fast with free access to water, sennoside A or B were administered orally to the rats, and 30 minutes later, 1 mL of HCl•EtOH solution (150 mM HCl in 60% EtOH) was administered orally. After 1 hour, animals were sacrificed by ether inhalation and stomachs were excised and fixed for 1 hour in 2% formalin. Stomachs were then incised along the greater curvature and the glandular portion was examined for hemorrhage. HCl•EtOH-induced gastric damage was observed in the gastric mucosa as elongated black-red lines parallel to the long axis of the stomach of the rats. The lesion index was based on the average erosion length per rat. Hydrotalcite and cimetidine were used as positive control drugs (Mizui and Dodeuchi, 1983).

Indomethacin-induced gastric ulcers in rats

Using the method reported by Kasuya *et al*., rats were fasted for 24 hours with free access to water (Kasuya *et al*., 1979). Sennoside A and B were dosed orally and 30 minutes later, indomethacin (35 mg/kg in 0.5% CMC) was injected subcutaneously in a volume of 0.5 mL per 100 g of body weight. Animals were sacrificed 7 hours after the indomethacin injection. The stomach was then incised along the greater curvature and the length (mm) of each mucosal ulcer developed in the glandular portion was measured. The sum of the length of each mucosal ulcer per rat was used as the ulcer index. Cimetidine was used as positive control drug.

Measurement of prostaglandin E_2 (PGE_2)

The production of PGE_2, a protective factor against gastric lesions, was measured using a commercially available assay (PGE_2: R&D Systems, KGE004, Wiesbaden, Germany). AGS cells were cultured in 96-well culture plates (1×10^4/well). As a blank, we measured cell culture medium from wells without cells that had been treated in the same way as the samples, according to the manufacturer's instructions. Briefly, The supernatant (100 μL) of cell culture that treated with sennoside A and sennoside B were added to the plate, and mixed with 50 μL of a primary mouse monoclonal PGE_2-antibody and PGE_2-conjugated with horseradish peroxidase (HRP). The cells were then incubated for 2 hours on a shaker at room temperature, the supernatant was discarded, and cells were washed 4 times. A mixture of hydrogen peroxide and chromagen (tetramethylbenzidine as substrate) was then added to terminate the reaction, and absorbance was read at 450 nm within 30 minutes.

H^+/K^+-ATPase activity

H^+/K^+-ATPase is called the proton pump which secretes gastric acid in the stomach wall cell. H^+/K^+-ATPase activity was assessed by a modification of the method of Saccomani *et al*. (1981) Gastric mucosal scrapings from rat stomach were homogeneously suspended in 20 mM Tris-HCl buffer (pH 7.4).

The protein from gastric mucosal scrapings was determined using the method of Bradford with bovine serum albumin as standard (Bradford, 1976). Briefly, protein (300 μg), reaction medium (300 μL) were mixed with sennoside A or sennoside B. The mixture was incubated at 37°C. After 30 minutes, assay medium (300 μL) was added to the mixture and centrifuged at 1050×*g* for 10 minutes. Absorbance was measured at 400 nm [Reaction mixture: 20 mM Tris-HCl, 20 mM $MgC1_2$, 20 mM ATP, and 100 mM KC1 (pH 7.4); Assay mixture: 30% trichloroacetic acid, 4.5% ammonium molybdate].

Gastric secretion

After a 24 hours fast with free access to water, rats were administered sennoside A or B intraduodenally (Shay *et al*., 1945). 4 hours after pyloric ligation, animals were sacrificed, stomach contents were collected, and centrifuged at 1050×*g* for 10 minutes. Total volumes of gastric juice and their pH values were measured, and acid output (mEq/4 hrs) was determined by titration versus 0.1 N NaOH using phenol red as an indicator.

Evaluation of gastric emptying

Using the method reported by Scarpignato *et al*., mice were fasted for 24 hours with free access to water (Scarpignato *et al*., 1980). Gastric emptying was performed by administering a 0.05% (w/v) phenol red solution (0.5 mL/mouse), 30 minutes after treatment with sennoside A or B. 20 minutes later, mice were sacrificed, stomachs were immediately removed, cut into several pieces in 5 mL of 0.01 N NaOH, and treated with 0.2 mL of 20% trichloroacetic acid per 1 mL of homogenate. Mixtures were centrifuged for 10 minutes at 1050×*g*, and the supernatants (0.05 mL) so obtained were added to 0.5 N NaOH (0.2 mL). The absorbances of these mixtures were measured using a spectrometer at 560 nm. The gastric emptying rate (%) was calculated using 100-(A/B)×100, where A is the amount of phenol red remaining in the stomach 20 min after administering phenol red solution, and B is the amount of phenol red in the stomach immediately after administering phenol red solution.

Evaluation of intestinal transport

Intestinal transport was evaluated using a modification of the method described by Takemori *et al*., 1969. Mice were fasted for 24 hours with free access to water and administered sennoside A or B orally. 30 minutes later, charcoal meal (3% in 0.5% CMC solution) was administered orally, and 20 minutes later, mice were sacrificed and gastrointestinal tracts immediately removed. Intestinal transport rate (%) was calculated using 100-(A/B)×100, where A is charcoal meal transporting length in the gastrointestinal tract 20 min after the administration of the charcoal meal, and B is charcoal meal transporting length in the gastrointestinal tract immediately after administering the charcoal meal.

Statistical analysis

Statistical analysis was performed using the Student *t*-test. Results were expressed as mean values ± standard error of the mean (S.E.M.). Results were considered significant if $p<0.05$.

Table 1. Effects of sennoside A and sennoside B on HCl•ethanol-induced gastritis in rats

Treatment	Dose (mg/kg)	Lesion index (mm)	Inhibition (%)
Control	-	101.0 ± 12.68	-
Sennoside A	100	57.5 ± 13.89*	43.1
Sennoside B	100	60.8 ± 18.41*	39.9
Hydrotalcite	100	70.1 ± 13.5*	30.6
Cimetidine	150	69.3 ± 9.5*	31.4

The values are mean±S.E.M. of 6 animals. Significant difference. *$p<0.01$ compared to the control group.

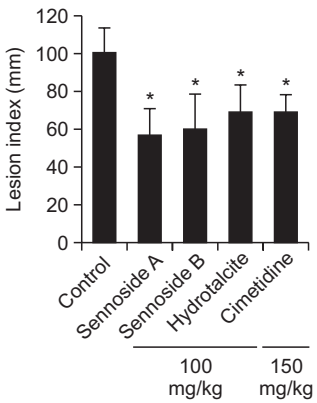

Fig. 2. Effects of sennoside A and sennoside B on HCl•ethanol-induced gastritis in rats. The values are mean ± S.E.M. of 6 animals. Significant difference *$p<0.01$ compared to thecontrol group.

RESULTS

HCl•EtOH-induced gastritis in rats

The effects of sennoside A and sennoside B on HCl•EtOH-induced gastritis were investigated (Table 1, Fig. 2). The lesion index in the control group was 101.0 ± 12.7 mm. Sennoside A and sennoside B at a dose of 100 mg/kg reduced lesion indices to 57.5 ± 13.9 mm and 60.8 ± 18.4 mm, respectively, or by 43.1% and 39.9%, respectively. These results were better than those of the hydrotalcite (100 mg/kg) and cimetidine (150 mg/kg) positive controls.

Indomethacin-induced gastric ulcer in rats

The effects of sennoside A and sennoside B on indomethacin-induced gastric ulcer were also investigated; results are shown in Table 2 and Fig. 3. Sennoside A and sennoside B were administered orally to examine their inhibitory effects on indomethacin-induced gastric lesions. The lesion index in the controls was 29.7 ± 3.1 mm, whereas in the sennoside A and B (100 mg/kg) treated groups lesion indices were 19.0 ± 14.1 mm and 11.0 ± 4.2 mm, that is, inhibitions of 36.0% and 62.9%, respectively. Furthermore, sennoside B was found to have a better effect than cimetidine (150 mg/kg; the positive control), which inhibited lesion development by 43.0%.

Table 2. Effects of sennoside A and sennoside B on indomethacin-induced gastric ulcer in rats

Treatment	Dose (mg/kg)	Lesion index (mm^2)	Inhibition (%)
Control	-	29.7 ± 3.1	-
Sennoside A	100	19.0 ± 14.1	36.0
Sennoside B	100	11.0 ± 4.2**	62.9
Cimetidine	150	16.9 ± 2.8*	43.0

The values are mean±S.E.M. of 6 animals. Significant difference. *p<0.05; **p<0.001 compared to the control group.

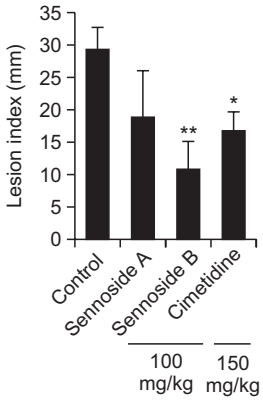

Fig. 3. Effects of sennoside A and sennoside B on indomethacin-induced gastric ulcer in rats. The values are mean ± S.E.M. of 6 animals. Significant difference *p<0.05; **p<0.001 compared to the control group.

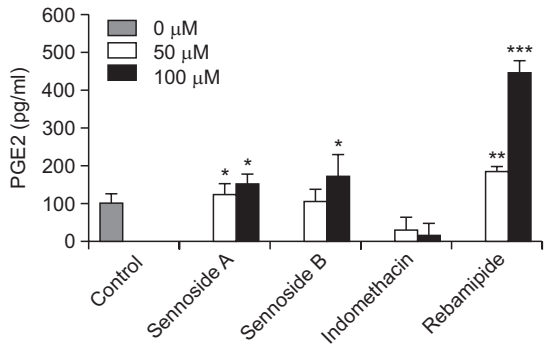

Fig. 4. Quantitation of sennoside A and B on PGE_2. The values are mean ± S.E.M. of 3 experiments. Significant difference *p<0.05; **p<0.01; ***p<0.001 compared to the control group.

Quantitative analysis of prostaglandin E_2

Quantitative analysis of PGE_2 was performed using AGS gastric cells treated with or without sennoside A or B (Fig. 4). Sennoside A significantly increased concentration of PGE_2 to 123.1 pg/mL and 151.4 pg/mL at doses of 50 μM and 100 μM, respectively. Similarly sennoside B increased concentration of PGE_2 to 105.4 pg/mL and 173.6 pg/mL at doses of 50 μM and 100 μM, respectively. One of the gastric mucosal protectors, rebamipide, increased PGE_2 levels to 185.1 pg/mL (50 μM) and 447.1 pg/mL (100 μM), whereas indomethacin, which inhibits

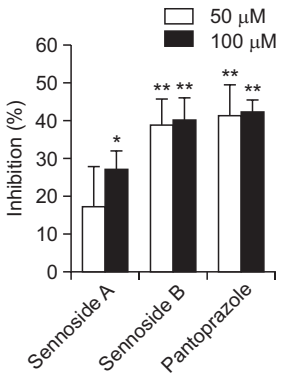

Fig. 5. H^+/K^+-ATPase inhibitory activities of sennoside A and sennoside B. The values are mean ± S.E.M. of 3 experiments. Significant difference *p<0.05; **p<0.01 compared to the control group.

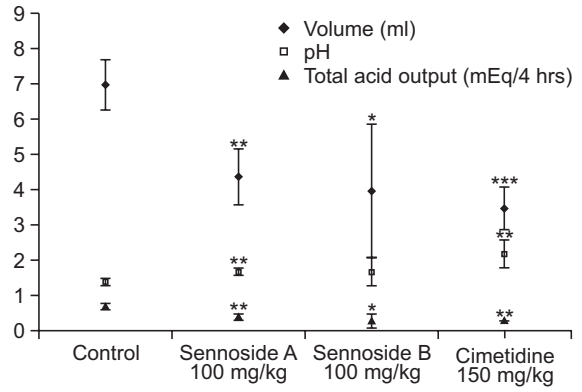

Fig. 6. Effects of sennoside A and sennoside B on gastric secretion in pylorus-ligated rats. The values are mean ± S.E.M. of 6 animals. Significant difference *p<0.05; **p<0.01; ***p<0.001 compared to the control group.

the synthesis of PGE_2 from arachidonic acid, decreased PGE_2 levels to 29.6 pg/mL (50 μM) and 14.3 pg/mL (100 μM). These results show that sennoside A and B increase concentration of PGE_2 in a dose-dependent manner, which implies that sennoside A and B have effective protective effects against gastric lesions.

H^+/K^+-ATPase activity

When we measured the H^+/K^+-ATPase activities of sennoside A and B, pantoprazole (the positive control) exhibited inhibitions of 41.1% and 42.4% at 50 μM and 100 μM, whereas sennoside A showed dose-dependent inhibitions of 17.3% and 27.1% at 50 μM and 100 μM. Similarly, sennoside B showed significant inhibition at 38.8% and 40.2% at 50 μM and 100 μM, respectively (Fig. 5).

Gastric secretion in pylorus-ligated rats

We measured gastric secretion parameters, such as, volume of gastric-juice and its pH, after submitting rats to pylorus ligature after administering or not administering sennoside A or B intraduodenally (Fig. 6). The volume of gastric secretion by sennoside B was approximately 4.0 mL, which was less than the 7.0 mL of the control, and sennoside A showed a

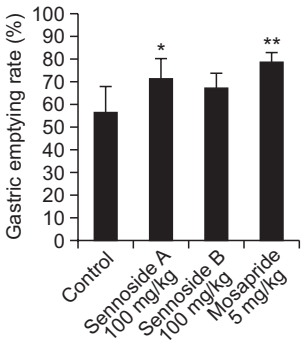

Fig. 7. Effects of sennoside A and sennoside B on gastric emptying. The values are mean ± S.E.M. of 6 animals. Significant difference **p*<0.05; ***p*<0.01 compared to the control group.

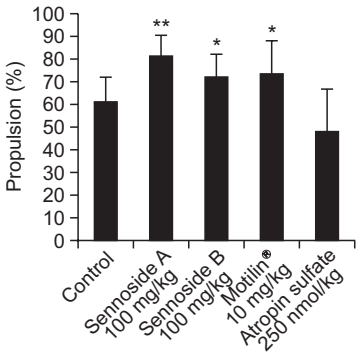

Fig. 8. Effects of sennoside A and sennoside B on intestinal transport. The values are mean±S.E.M. of 6 animals. Significant difference **p*<0.01; ***p*<0.001 compared to the control group.

similar volume of 4.4. Sennoside A and sennoside B increased gastric-juice pH to 1.7, as compared to the 1.4 of the control, and reduced total acid output to 0.4 mEq/4hrs and 0.3 mEq/4hrs, respectively, as compared with 0.7 mEq/4hrs for the control. Accordingly, sennoside A and B were found to inhibit the volume of gastric-juice, increase pH, and decrease total acid output, it was expected to suppress the aggressive factor through the reduction of gastric secretion.

Gastric emptying

To investigate the effects of sennoside A and B on gastric emptying rate, we measure the amount of phenol red (an indicator that is bright pink at pH values >8.2) remaining in stomachs 20 min after administration. As shown in Fig. 7, both sennoside A and B increased the gastric emptying rate by 71.1 ± 9.1%, 67.2 ± 6.4%, respectively, at a dose of 100 mg/kg as compared with 56.2 ± 11.7% for the control group.

Intestinal transport

To investigate the effects of sennoside A and B on intestinal peristalsis and segmentation movement, the transporting length of a 3% charcoal meal over 20 minutes was measured from pylorus to ileocecum. The control group showed a transportability of 61.2 ± 10.8%, hereas motilin® (250 nmol/kg; the positive control) increased transportability to 73.4 ± 14.7%. On the other hand, sennoside A and B increased transportability to 81.1 ± 9.3% and 72.2 ± 10.0%, respectively (Fig. 8).

DISCUSSION

The oral administration of EtOH interrupts the mucosal defense system, thereby aggravating mucosal damage possibly causing the necrosis or apoptosis of gastric mucosal cells (Lee *et al*., 2005) Furthermore, EtOH induces wide ulcers and petechial lesions within a relatively short time, which makes this technique suitable for investigations of anti-gastric lesion drugs (Seiki *et al*., 1990) NSAIDs, such as indomethacin and aspirin, cause gastric ulcers when taken inlarge dosages because they hinder the synthesis of prostaglandins. Furthermore, NSAIDs can cause hemorrhages and ulcers by stimulating the gastric mucosal barrier directly and cause a decrease in gastric mucosal blood flow (Ashley *et al*., 1985; Wallace and Granger, 1992; Karmeli *et al*., 1995). Indomethacin-induced gastric lesions are characterized by significant oxidative injury and reduced secretion of mucus/bicarbonate, the latter of which is mainly due to inhibition of PG secretion (Rao *et al*., 2004). PG, which is expressed abundantly in gastric mucous membranes, is important for the maintenance of gastric mucosal blood flow, repair, and the promotion mucus secretion. PG also importantly promotes the healing of mucosal damage by contracting villi after damage (Rovert, 1979; Rovert *et al*., 1979; Basson *et al*., 1992; Kim *et al*., 2004; Sekiguchi *et al*., 2007) Furthermore, epidermal growth factor (EGF) and PGE_2 have been reported to accelerate mucosal recovery from stress-induced gastric lesions by attenuating apoptosis via the up-regulation of bcl-2 in gastric mucosa (Konturek *et al*., 2001). In addition, it was recently reported that toll-like receptor 4 (TLR4) participates in gastric mucosal protection by activating COX-2 and PGE_2 (Zhang *et al*., 2010). Nandi *et al*. found that inhibition of the synthesis of PGE_2 derived from COX-1 increases acid secretion by modulating H^+/K^+-ATPase expression in parietal cells by enhancing expression and activation of the proton pump (Nandi *et al*., 2009). Moreover, Konturek *et al*. reported that oral PGE_2 has a protective action on gastric mucosa exposed to aspirin, and the rate of gastric bleeding and DNA loss (Konturek *et al*., 1983). Since omeprazole was introduced in 1988, several Proton pump inhibitors (PPIs), such as, omeprazole, pantoprazole, lansoprazole, rabeprazole, and esomeprazole have been used to treat dyspepsia, peptic ulcers, and gastroesophageal reflux disease (Raghunath *et al*., 2005; Watson *et al*., 2013). Moderate inhibition of gastric secretion is known to promote gastric lesion healing and to prevent the complications of gastric diseases (Walker *et al*., 2012). Orally administered PPIs are absorbed in the gastrointestinal tract and reach gastric parietal cells via the bloodstream. Consequentially, gastric acid secretion is inhibited by inhibiting H^+/K^+-ATPase activity. Ulcers may arise when there is an imbalance between aggressive and defensive factors that renders the mucosa susceptible to damage (Mcquaid and Isenberg, 1992). Pylorus ligation strongly stimulates the secretion of gastric acid, which acts as an aggressive factor during the early stage of gastric ulcer (Okabe *et al*., 1974). Functional dyspepsia is caused by delayed gastric emptying, gastric accommodation, and duodeno-jejunum motility and central nervous system disorders. Most chronic gastritis patients experience symptoms of func-

tional dyspepsia with early satiety, and such patients usually take medicines to inhibit gastric acid secretion in combination with a gastroprokinetic agent. Mosapride is a gastroprokinetic agent that acts as a selective $5HT_4$ agonist and accelerates gastric emptying throughout the entire gastrointestinal tract in humans (Kato *et al*., 1995; Odaka *et al*., 2006).

In this study, the gastroprotective activities of sennoside A and B were found to be due to the up-regulation of PGE_2 and the inhibitions of H^+/K^+-ATPase activity. In animal models, they both exhibited potent inhibitory activity against HCl•EtOH-induced gastritis and against indomethacin-induced gastric ulcer formation, and gastric secretion. Furthermore, they both accelerated gastrointestinal tract motility, which is known to alleviate the symptoms of functional dyspepsia. Therefore, both sennoside A and sennoside B are expected to have a protective effect against gastric lesions.

ACKNOWLEDGMENTS

This study was supported by Duksung Women's University Research Grants 2014.

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