

## Effect of ECQ on Iodoacetamide-Induced Chronic Gastritis in Rats

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This study investigated effect of extract containing quercetin-3-O- $\beta$ -D-glucuronopyranoside from *Rumex Aquaticus Herba* (ECQ) against chronic gastritis in rats. To produce chronic gastritis, the animals received a daily intra-gastric administration of 0.1 ml of 0.15% iodoacetamide (IA) solution for 7 days. Daily exposure of the gastric mucosa to IA induced both gastric lesions and significant reductions of body weight and food and water intake. These reductions recovered with treatment with ECQ for 7 days. ECQ significantly inhibited the elevation of the malondialdehyde levels and myeloperoxidase activity, which were used as indices of lipid peroxidation and neutrophil infiltration. ECQ recovered the level of glutathione, activity of superoxide dismutase (SOD), and expression of SOD-2. The increased levels of total NO concentration and iNOS expression in the IA-induced chronic gastritis were significantly reduced by treatment with ECQ. These results suggest that the ECQ has a therapeutic effect on chronic gastritis in rats by inhibitory actions on neutrophil infiltration, lipid peroxidation and various steps of reactive oxygen species (ROS) generation.

**Key Words:** Chronic gastritis, Iodoacetamide, QGC, Reactive oxygen species, Superoxide dismutase

### INTRODUCTION

Gastritis represents an inflammatory state of the stomach lining. It often includes certain symptoms resulting from stomach lining inflammation, burning sensation, or discomfort [1]. Chronic-type gastritis may be due to many pathological factors such as *Helicobacter pylori* infection, autoimmune disease, and lymphocytic and eosinophilic gastritis [2]. Experimental models of gastric mucosal damage have been developed and includes various categories such as stress-induced gastritis, due to either water immersion restraint stress or cold restraint stress; the use of NSAIDs; intra-gastric application of *Helicobacter pylori* itself or its lipopolysaccharide; and iodoacetamide (IA)-induced gastritis [3,4]. In humans, a significant number of cases of gastritis occur in the absence of any evidence of *H. pylori* infection. Therefore, inflammatory processes might also be involved in the secretory, motor and perception changes ob-

served in disorders. Chemical irritants of the gastric mucosa, such as aspirin, indomethacin or alcohol, which induce acute chemical injury have been used as a model of erosive gastritis and gastric ulceration [4,5]. However, these models do not mimic states of mild gastritis without macro or microscopic mucosal alterations. Recently, the sulfhydryl acetylating agent IA has been used to induce a mild sub-chronic gastritis in rats. Rats treated with IA developed a mild inflammatory reaction with significant changes in sensory and acid secretory functions [6-8].

IA is a sulfhydryl blocker that depletes sulfhydryl components in the gastric mucosa, leading to gastric damage [9]. Oxidative stress is defined as an imbalance between free-radical production and antioxidant defenses, leading to tissue injury and harmful cytotoxic effects [4,10,11]. Non-protein sulfhydryl compounds in the epithelium (reduced glutathione) may be important in maintaining mucosal integrity, they are capable of binding reactive free oxygen radicals [12]. Reactive oxygen species (ROS) and their metabolites are involved in gastric mucosal injury [13].

When ROS such as oxygen ions, free radicals and per-

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**ABBREVIATIONS:** CMC, Carboxymethylcellulose; ECQ, extract containing QGC; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; HETAB, hexadecyl trimethyl ammonium bromide; IA, iodoacetamide; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; MPO, myeloperoxidase; NSAIDs, non-steroidal anti-inflammatory drugs; PBS, phosphate-buffered saline; QGC, quercetin-3-O- $\beta$ -D-glucuronopyranoside; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance.

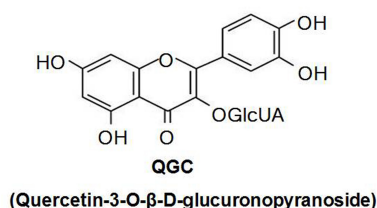
oxides attack cells including the membrane, mitochondria and DNA [14], the cells defend themselves using several enzymes and non-enzyme process such as superoxide dismutases (SOD) and GSH [15,16]. Decreasing of GSH level often means GSH depletion caused by oxidative stress or damage to scavenge ROS in tissues or cells. Therefore, anti-oxidation activity is very important for protection against gastric damage. The damage to membrane proteins decreases the activities of enzymes and receptors, and activation of cells. In addition, lipid peroxidation results in the production and release of substances that recruit and activate polymorphonuclear leukocytes [17]. Neutrophil infiltration into gastric mucosal tissues is related to the genesis of gastric lesions [18] and is also critical in the pathogenesis of a variety of gastric ulcers [19]. MPO activity, as an index of neutrophil infiltration into the gastric mucosal tissues, is widely used in various experimental gastric injuries.

Glutathione exists in a combination of its reduced form and oxidized dimer in almost all mammalian cells. GSH is a nucleophilic scavenger of superoxide and also acts as a cofactor in the GSH peroxidase mediated reduction of H<sub>2</sub>O<sub>2</sub> [20]. GSH has an important role in maintaining mucosal integrity in the gastrointestinal tracts. In rats, the sulfhydryl (SH) blocker, IA, induces gastritis and significant colonic injury, indicating the important contribution of SH compounds to the maintenance of gastrointestinal integrity [21].

NO has an important role in maintaining gastric mucosal integrity. The role of gastric NO formation in IA-induced gastric damage and its prevention by scavenging of free radicals was evaluated [9].

Flavonoids, which are secondary metabolites in the plants, are considered relatively non-toxic bioactive substances and have diverse biological effects, including anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic activities and major ability as free radical scavengers [22,23]. Of the many actions of flavonoids, antioxidant and anti-inflammatory effects stand out. The best described property of almost every group of flavonoids is their capacity of act as anti-oxidants [24].

*Rumex Aquaticus* is a family of polygonaceae, and this plant has been used to treat disinfestation, diarrhea, antipyretic drug, edema, jaundice and constipation in traditional oriental medicine. Its active ingredient is quercetin-3-O- $\beta$ -D-glucuronopyranoside (QGC), a quercetin derivative widely distributed in the plant kingdom. The chemical structure of QGC is shown in Fig. 1. Quercetin is a potent natural antioxidant that attenuates H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity [25] and prevents the gastric mucosal lesions



**Fig. 1.** Chemical structure of QGC (Quercetin-3-O- $\beta$ -D-glucuronopyranoside).

produced by ethanol [26]. QGC is more potent than quercetin at inhibiting experimental reflux esophagitis [27]. The extract including QGC (ECQ) was extracted from *Rumex Aquaticus* Herba by ethanol. In our previous study, ECQ was protective against indomethacin- and ethanol- induced acute gastric damage [28]. Therefore, the purpose of the present study was to investigate the gastro protective effect of ECQ against IA-induced chronic gastritis by determining of various biochemical parameters such as MDA level, MPO activity, mucosal GSH, activity and expression of SOD, and total NO concentration and iNOS expression.

## METHODS

### Materials

ECQ was extracted from *Rumex Aquaticus* with ethanol and subsequently partitioned between chloroform and water to provide a chloroform-soluble fraction and a water-soluble fraction. ECQ is a water-soluble fraction and we used ECQ containing 10.7% QGC [27,29]. Iodoacetamide (IA) and superoxide dismutase (SOD) assay kit were from Sigma Chemical Co. (St Louis, MO, USA). MDA assay kits were purchased from Bio-Rad (CELL BIOLABS, INC). NO assay kit from Assay designs (Ann Arbor, MI, USA). Carboxymethylcellulose (CMC), Hexadecyl trimethyl ammonium bromide (HETAB), EDTA, EGTA, dithiothreitol, phenylmethylsulphonyl fluoride, hydrogen peroxide and dimethyl-formamide were purchased from Sigma (St. Louis, MO, USA). Stillen<sup>®</sup> was kindly provided by Dong-A Pharmaceutical Co. Ltd. (Yong-In, Korea). Stillen<sup>®</sup> was dissolved in CMC for treatment. Superoxide dismutase-2, i-NOS, and GAPDH antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The protein assay kit was purchased from Bio-Rad (Richmond, CA, USA).

### Animals

Male Sprague-Dawley rats with body weights of about 220~230 g were used for the experiments. Animals were group-housed with direct bedding and had free access to food and tap water. The rats were starved for 24 h before the experiments, but were allowed to freely drink water. All animals were kept in raised mesh-bottom cages to prevent coprophagy. All animal experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University, in accordance with the guide for the Care and Use of Laboratory Animals in Seoul, Korea.

### Induction of gastritis

Gastric inflammation was induced with the sulfhydryl blocker iodoacetamide (IA), based on a previously described model in rats [6-8]. Normal group animals received a daily intra-gastric administration of 0.1 ml of 2.0% sucrose solution for 7 days, and 2% sucrose was added to the drinking water. IA treatment groups as the control groups received a daily intra-gastric administration of 0.1 ml of the IA solution for 7 days, and 0.15% IA and 2% sucrose were also added to the drinking water for them during the same period. The control groups received normal rat chow and tap water without IA for a week after 7-day administration of IA. Experimental groups of rats were then divided into four groups and administered drugs *per os* (p.o) as follow:

1 mg/kg, 3 mg/kg, and 10 mg/kg of ECQ dissolved in saline, and 20 mg/kg of Stillen<sup>®</sup> suspended in 0.5% CMC for a week after 7-day administration of IA. Stillen<sup>®</sup> was used as a positive control because it is a cytoprotective agent which has been clinically proven and already marketed for treatment and prevention of erosive gastritis. The volume of the extract or vehicle of drug was 2 ml/kg of body weight. All the extracts were prepared freshly each time.

#### ***Evaluation of Iodoacetamide-induced gastric lesion***

After 7-day administration of IA, the animals were sacrificed and the stomach was excised, opened along the greater curvature and spread out with pins on cork board. The area (mm<sup>2</sup>) of mucosal erosive lesions was measured under a dissecting microscope with a squared grid (×10; Olympus, Tokyo, Japan). The number of animals used in each experimental group is indicated in figure legends.

#### ***Food and water intake and body weight***

In each group of animals, changes in food and water intake and body weight were monitored daily during the period of IA or drug treatment. During the treatment days, animals were housed individually in cages with indirect bedding, with food and water (appropriately modified) ad libitum. Food intake, water intake and body weight were measured daily between 12:00 p.m. and 2:00 p.m.

#### ***Biochemical investigation of stomach tissues***

SOD activities, SOD expression, MPO and MDA levels in rat stomach tissues were determined. To prepare the tissue homogenates, stomach tissues were cut with iris scissors. The ground tissues were treated with 2.0 ml of phosphate or tris buffer. The mixtures were homogenized on ice using a homogenizer (TMZ-20DN, TAEMIN, Korea) for 30 sec. Homogenates were centrifuged by using a refrigerated centrifuge. These supernatants were used for the determination of the enzymatic activities. All assays were carried out at room temperature in duplicate.

#### ***Myeloperoxidase assay***

Neutrophil infiltration into the stomach was assessed indirectly using the myeloperoxidase (MPO) activity assay. The assay was performed as a previous method [30] and partly modified. One milliliter of the leukocyte suspension was centrifuged at 620× g at 4°C for 2 min. The precipitate was suspended in 1 ml of 80 mM sodium phosphate buffer, pH 5.4, containing 0.5% Hexadecyl trimethyl ammonium bromide (0.5% HETAB solution), sonicated and centrifuged at 1,400× g at 4°C for 5 min. Duplicate 30 μl samples of resulting supernatant were poured into 96-well microtiter plates. For the assay, 200 μl of a mixture containing 100 μl phosphate buffered saline, 85 μl 0.22 M sodium phosphate buffer, pH 5.4, and 15 μl of 0.017% hydrogen peroxide were added to the each well. The reaction was started by the addition of 20 μl of 18.4 mM TMB·HCl in 8% aqueous dimethylformamide. Plates were stirred and incubated at 37°C for 3 min and then placed on ice where the reaction was stopped by addition to each well of 30 μl of 1.46 M sodium acetate, pH 3.0. The MPO value was evaluated by measuring the absorbance of samples at 620 nm (OD value) using MPO standard.

#### ***TBARS assay (Malondialdehyde levels)***

TBARS (thiobarbituric acid reactive substance) is the most commonly used test for the quantification of the end-products of lipid peroxidation, to be specific, malondialdehyde (MDA). Lipid peroxidation was determined using Malondialdehyde (MDA) kits. Stomach tissue was homogenized on ice for 30 sec (TME-20DN, TAEMIN, Korea), and sonicated in 1 ml of 0.9% NaCl saline (pH 7.0). After centrifugation at 12,000 g for 15 min at 4°C, the supernatant was assayed according to the manufacturer's instruction. Reacted samples were replaced to 96-well microtiter plates and then this product measured spectrophotometrically at 532 nm using MDA (UV-160A, Shimadzu, Japan) standard. Results were expressed as nmole/mg.

#### ***Glutathione assay***

Glutathione (GSH) concentration of gastric tissue was determined according to Beutler and partial modified method was used [31]. Stomach tissue was homogenized on ice for 30 sec. The homogenates were centrifuged at 600 g for 10 min at 4°C and then the supernatant was sonicated for 45 sec, and re-centrifuged at 12,000 g for 15 min at 4°C using a refrigerated centrifuge to obtain a mitochondrial fraction. The obtained mitochondrial fraction was added to metaphosphoric acid for protein precipitation and stand for 5 min. Phosphate buffer and 5'-dithiobis-2-nitro-benzoic acid were added for color development. GSH was determined by spectrophotometrically at 415 nm using GSH (Sigma) standard.

#### ***Measurement of SOD activity***

Stomach tissue was homogenized on ice in 5 ml of 50 mM potassium phosphate buffer (pH 7.4, containing 1 mM EDTA) per gram tissue. After centrifugation at 10,000 g for 15 min at 4°C, the supernatant was used for SOD assays using SOD assay kit. Assays were performed according to the manufacturer's instruction.

#### ***Nitric Oxide assay***

Mucosal scrapings (100 mg wet wt) were homogenized for 30 seconds at 4°C in 0.3 ml of 50 mM TRIS HCl, pH 7.4 containing 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM dithiothreitol, and 1 mM phenylmethylsulphonyl fluoride. The homogenates were centrifuged at 20,000× g for 60 minutes at 4°C and the supernatant was used as the source of NOS. Total nitric oxide (NO) was determined using the Total Nitric Oxide Assay Kit. Assays were performed according to the manufacturer's instruction. Results were expressed as nmole/mg.

#### ***Protein determination***

The protein concentration of the supernatant in each reaction vial was measured spectrophotometrically using the Bio-Rad assay kit (Bio-Rad Chemical Division, Richmond, CA). Absorption was monitored at 595 nm.

#### ***Western blot analysis***

Equal amounts of protein from each sample were sub-

jected to electrophoresis on a 7% SDS-polyacrylamide gel and transferred to a nitrocellulose (NC) membrane, using power supply, Power Pac 1000 (Bio-Rad, Melville, NY). To block nonspecific binding, the NC membrane was incubated in 5% nonfat dry milk in PBS for 60 min followed by three rinses in milk-free PBS. The membranes were incubated for 1 h with shaking with primary antibodies raised against SOD-2, iNOS followed by three washes with PBS containing 0.05% Tween 20. This was followed by 60 min incubation in horseradish peroxidase-conjugated secondary antibody. Detection was with an enhanced chemiluminescence agent. Molecular masses were estimated by comparison with a prestained molecular mass. To confirm uniformity of protein loading, the same blots was subsequently stripped with western blot stripping buffer and reprobed with GAPDH antibody. Developed films were scanned and analyzed densitometrically using Scion Image. Percent of SOD-2 and iNOS expressions was calculated as the ratios of SOD-2 or iNOS to GAPDH, respectively.

**Statistical analysis**

All data are expressed as means±SEM of separate experiments and the statistical differences between means were determined by Student's *t*-test. Differences between multi-

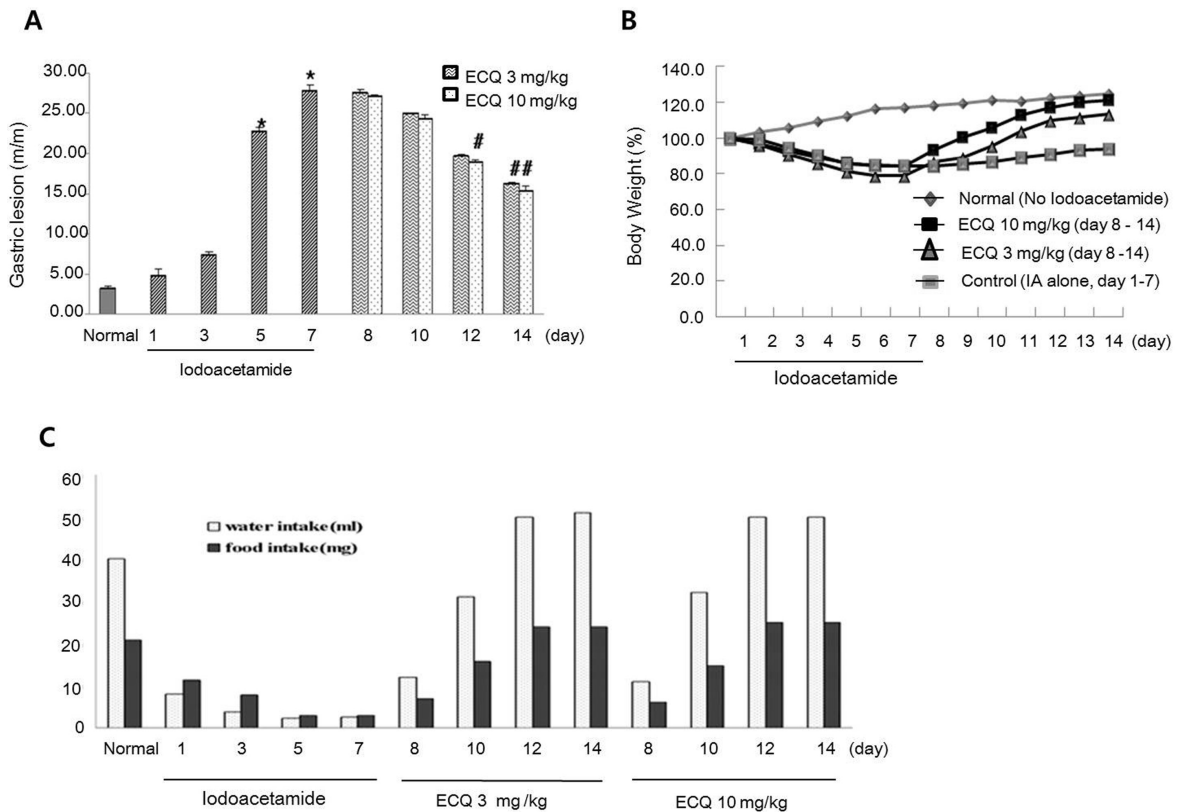
ple groups were tested using analysis of variance (ANOVA) for repeated measures. Null hypotheses of no difference were rejected if p-values were less than .05.

**RESULTS**

**Iodoacetamide-induced gastritis in rats**

The daily intra-gastric administration (0.1 ml) of 0.15% iodoacetamide (IA) solution, and the intake of drinking water containing 0.15% IA and 2.0% sucrose induced gastric mucosal damage. The damage was already apparent after 1 day and lasted for the entire 7 days of the experiment. Moderate gastric damage was visible on the surface of the gastric tissue. The IA-induced gastric lesion seemed to be recovered after treatment with ECQ.

The IA-induced gastric lesion was measured (Fig. 2A). Generation of gastric lesion increased to about 27.8±1.5 mm<sup>2</sup> after IA treatment for 7 days. Treatment with 3 mg/kg and 10 mg/kg of ECQ for 7 days significantly decreased the gastric lesion by 41% and 45%, respectively, compared with the lesion in rats treated with IA for 7 days. The preventive effect of gastric lesion of ECQ was dose dependant.



**Fig. 2.** (A) Measurement of the gastric mucosal lesions induced by Iodoacetamide (IA) and protective effect of ECQ on various days. (B) Changes in body weight during daily administration of IA for a week and 7-day treatment with ECQ. (C) Changes in amount of food intake and water intake during daily administration of IA and then treatment with ECQ. The animals were given intragastric administration of 0.1 ml IA solution daily for 7 days; in addition, 0.15% IA and 2.0% sucrose were added to the drinking water during the treatment period, and then ECQ was treated for a week. Normal group received 2.0% sucrose solution for a week. Values are presented as the mean from 10 rats in each group. \*p<0.01 vs Normal. #p<0.05 vs 7-day treatment with IA (control).

### Changes in body weight and food and water intake during iodoacetamide treatment

As shown in Fig. 2B, the normal group and the IA-treated groups had similar body weights at the beginning of the treatment period ( $229.68 \pm 4.73$  g), and body weight in the normal group did not change during the experiment. However, the body weights of the IA-treated groups gradually decreased over the duration of the IA treatment, and a significant effect was observed at day 7 of induction. The decrease of body weight was 15.8% from the first day. Lower food and water intake could explain the weight loss (Fig. 2C). After the 7-day administration of IA, the food intake was reduced about 90% ( $2.5 \pm 0.2$  ml) and water intake about 85% ( $3 \pm 0.1$  mg), compared with those ( $40 \pm 1.3$  ml,  $20.67 \pm 1$  mg) of the normal rats.

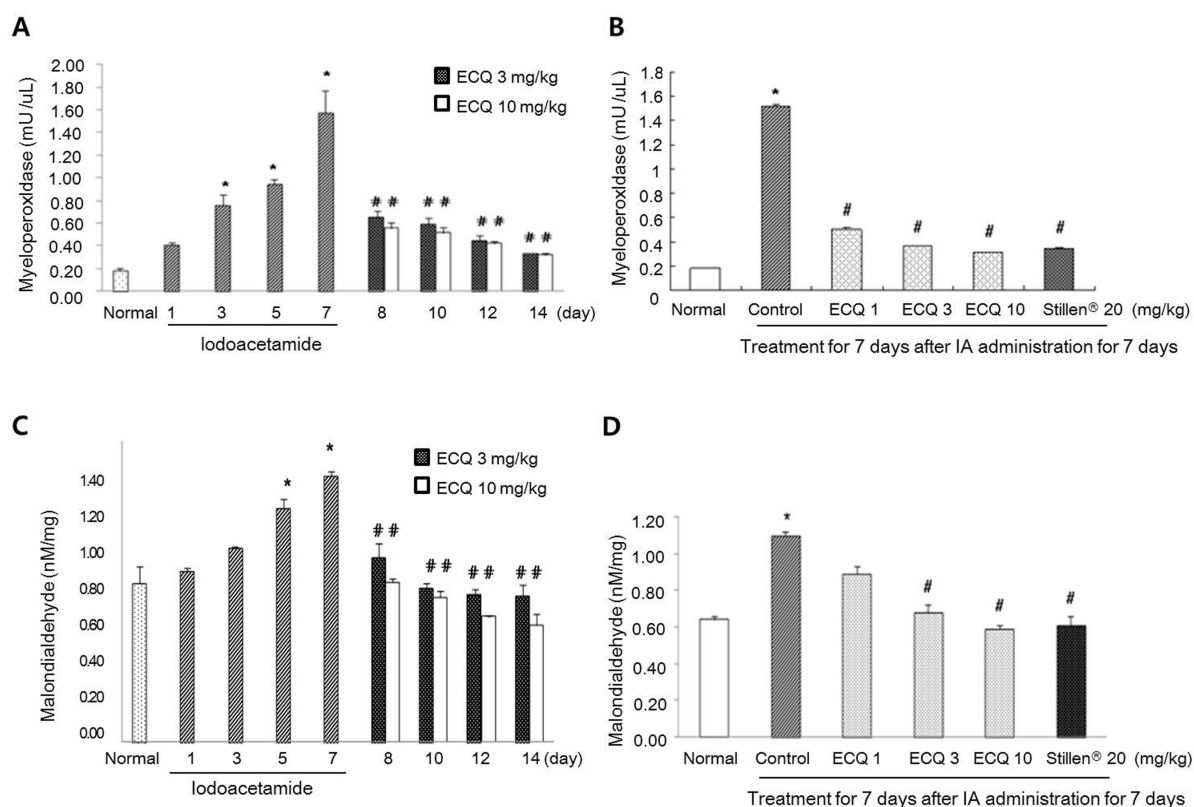
When the control group received normal rat chow and tap water without IA for 7 more days following the 7-day administration of IA, their body weight slightly increased to 9.8% from the 7th day of IA administration (Fig. 2B). A similar pattern was observed in the group of ECQ treatment for 7 days. Their body weight increased to 34.4% in the rat group treated with ECQ 3 mg/kg and 36.5% in those

treated with ECQ 10 mg/kg for 7 days, compared with those of the 7th day of IA administration. Food and water intake were reduced about 85% and 94% after 7-day administration of IA ( $3 \pm 0.1$  mg,  $2.5 \pm 0.2$  ml), compared with those ( $20.67 \pm 1$  mg,  $40 \pm 1.3$  ml) of the normal group (Fig. 2C). ECQ treatment for 7 days significantly increased the food and water intake to the normal level.

### Effect of ECQ on MPO activity on Iodoacetamide-induced chronic gastric damage

In the initial inflammatory process, tissue damage caused by ROS or other factors induces infiltration of neutrophil. MPO is considered as an index of neutrophil infiltration because MPO is abundantly expressed in neutrophil granulocytes. MPO activity was significantly increased by 170% after IA treatment for 7 days, compared with the normal. The treatment of ECQ decreased MPO activity gradually and dose dependently (Fig. 3A).

ECQ treatment at doses of 1 mg/kg, 3 mg/kg and 10 mg/kg for 7 days after the 7-day administration of IA significantly decreased MPO activities to 67%, 76% and 80%, respectively, compared with control group which received normal rat chow and tap water without IA for 7 more days



**Fig. 3.** (A) Effect of ECQ on IA-induced gastric MPO activity as an index of neutrophil infiltration. MPO activities were measured at day 1, 3, 5 and 7 during administration of IA and then at day 8, 10, 12 and 14 during treatment with ECQ. (B) Effects of ECQ and Stiller® on IA-induced gastric MPO activity. Experimental groups were treated with ECQ (1, 3, 10 mg/kg) or Stiller® (20 mg/kg) for a week after the induction of chronic gastritis by IA. (C) Effect of ECQ on IA-induced gastric MDA level increase as an index of lipid peroxidation. MDA levels were measured at day 1, 3, 5 and 7 during administration of IA and then at day 8, 10, 12 and 14 during treatment with ECQ. (D) Effects of ECQ and Stiller® on IA-induced gastric MDA level. ECQ (1,3,10 mg/kg) or Stiller® (20 mg/kg) were treated for a week after the induction of chronic gastritis by IA. Data are expressed as means $\pm$ SEM, n=5. \*p<0.01 vs Normal. #p<0.01 vs 7-day treatment with IA (control).

following the 7-day administration of IA. Treatment of Stillen<sup>®</sup> at a dose of 20 mg/kg for 7 days also decreased MPO activity to 78%, compared with the control (Fig. 3B).

**Effect of ECQ on MDA level in Iodoacetamide- induced chronic gastric damage**

MDA level was significantly increased when tissue was damaged by the administration of IA that causes generation of ROS in tissue. As an index of lipid peroxidation, MDA levels in gastric tissue are shown in Fig. 3C. IA administration markedly enhanced tissue MDA levels (1.24±0.03 nMmg, p<0.01) from the day 5. IA-induced increases of MDA levels were reduced gradually and dose dependently by ECQ treatment (Fig. 3C).

In the control group which received normal rat chow and tap water without IA for 7 more days after the 7-day administration of IA, tissue MDA concentration was 1.10±0.04

nM/mg (Fig. 3D). In the groups treated with 3 mg/kg and 10 mg/kg of ECQ for 7 days, IA- induced increases of MDA levels were significantly dropped to 39% and 47%, respectively, compared with the control. In addition, the treatment with Stillen<sup>®</sup> (20 mg/kg) for 7 days significantly decreased the MDA level to 45%, compared with the control.

**Effect of ECQ on GSH level on Iodoacetamide-induced chronic gastric damage**

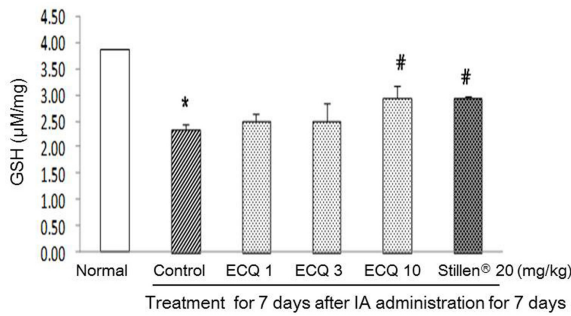
Treatment with ECQ 10 mg/kg or Stillen<sup>®</sup> 20 mg/kg for a week after the 7-day administration of IA significantly restored GSH level to 75% in the both groups, compared with the normal (Fig. 4).

**Effect of ECQ treatment on SOD activities and SOD-2 expression in iodoacetamide- induced chronic gastric rat model**

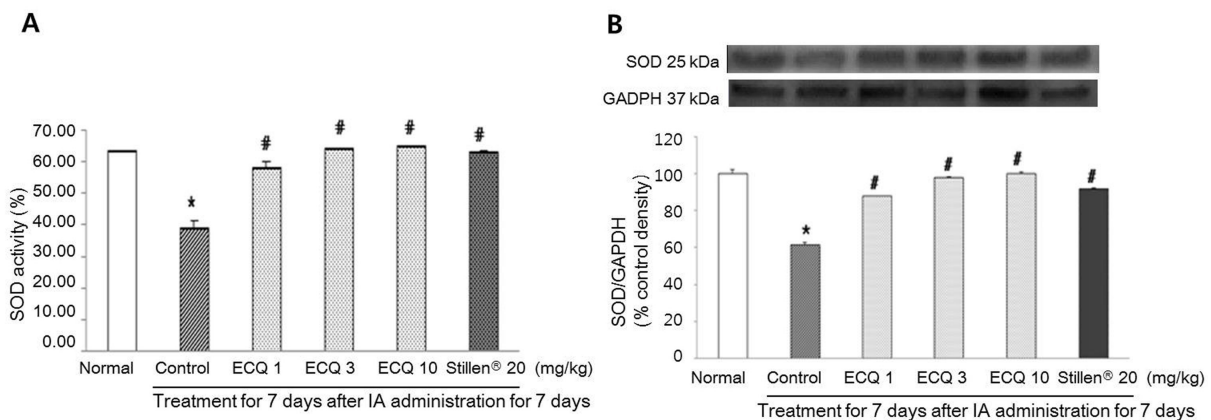
SOD activity in the gastric tissue of the control group was significantly decreased at day 14, compared with the normal group. However, it was significantly increased by treatment of ECQ and Stillen<sup>®</sup>, compared with the control group (Fig. 5A). In addition, expression of SOD-2 in gastric tissue of the control group was significantly decreased, compared with the normal. The expression of SOD-2 was increased dose-dependently by ECQ or Stillen<sup>®</sup> treatment (Fig. 5B).

**Effect of ECQ treatment on total NO concentration and iNOS expression in iodoacetamide-induced chronic gastric damage**

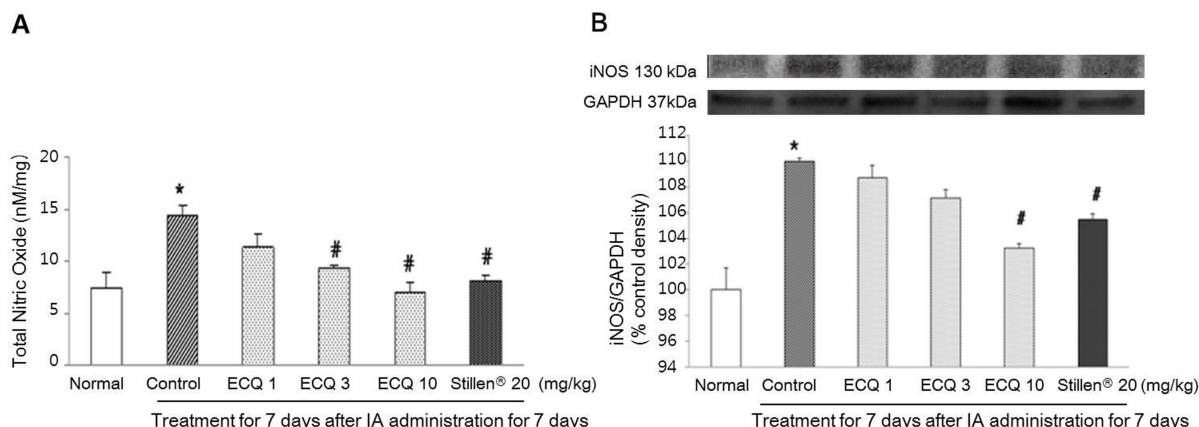
As shown in Fig. 6A, NO concentration in gastric mucosa of the control group was significantly increased to 14.32±1.03 nM/mg. NO level was decreased by treatments ECQ or Stillen<sup>®</sup> for a week. By treatment with ECQ 10 mg/kg or Stillen<sup>®</sup> 20 mg/kg, total NO concentration was decreased to 7.05±0.95 nM/mg and 8.16±0.42 nM/mg, respectively. Those levels were similar to the normal NO level (7.41±1.36



**Fig. 4.** Effect of ECQ and Stillen<sup>®</sup> on IA-induced depletion of GSH. The animals were given intragastric administration of 0.1 ml IA solution daily for 7 days; in addition, 0.15% IA and 2.0% sucrose were added to the drinking water during the treatment period, and then ECQ (1, 3, 10 mg/kg) or Stillen<sup>®</sup> (20 mg/kg) was treated for a week. Normal group received 2.0% sucrose solution for a week. Data are expressed as means±SEM, n=5. \*p<0.01 vs Normal. #p<0.05 vs 7-day treatment with IA (control).



**Fig. 5.** (A) Effect of ECQ and Stillen<sup>®</sup> on IA-induced SOD activity changes. (B) Effect of ECQ and Stillen<sup>®</sup> on IA-induced expression of SOD-2. The animals were given intragastric administration of 0.1 ml IA solution daily for 7 days. In addition, 0.15% IA and 2.0% sucrose were added to the drinking water during the treatment period, and then ECQ (1, 3, 10 mg/kg) or Stillen<sup>®</sup> (20 mg/kg) was treated for a week. Normal group received 2.0% sucrose solution for a week. Data are expressed as means±SEM, n=5. \*p<0.01 vs Normal. #p<0.05 vs 7-day treatment with IA (control).



**Fig. 6.** (A) Effect of ECQ and Stiller® on IA-induced total NO concentration. (B) Effect of ECQ and Stiller® on IA-induced expression of iNOS. The animals were given intragastric administration of 0.1 ml IA solution daily for 7 days. In addition, 0.15% IA and 2.0% sucrose were added to the drinking water during the treatment period, and then ECQ (1, 3, 10 mg/kg) or Stiller® (20 mg/kg) was treated for a week. Normal group received 2.0% sucrose solution for a week. Data are expressed as means±SEM, n=5. \*p<0.01 vs Normal. #p<0.05 vs 7-day treatment with IA (control).

nM/mg). Expression of iNOS was significantly increased in the control group, compared with the normal group (Fig. 6B). However, treatment with ECQ 10 mg/kg significantly decreased iNOS expression, compared with the control group.

## DISCUSSION

There are many experimental procedures for gastritis induction. Endogenous SH compounds are important in maintaining mucosal integrity in the gastrointestinal tract. SH blockers, Iodoacetamide (IA), added to the drinking water induce gastric mucosal injury [32]. Gastric damage induced by IA accompanied by inhibition of nitric oxide synthase activity [9]. These findings were confirmed by Barnett et al., who showed that the same treatment caused a 3-fold increase in gastric MPO activity and endogenous PGs derived from both COX-1 and COX-2 are involved in mucosal defense of the inflamed stomach after IA treatment [6,33]. The previous study noted that daily administration of 0.15% IA in drinking water produced gastric inflammation with few hemorrhagic lesions [6,33]. Further, IA treatment up-regulated the expression of iNOS mRNA and NO production in the stomach, implying that endogenous NO derived from iNOS contributes to mucosal defense of the inflamed stomach, partly by decreasing acid secretion, and to maintaining mucosal integrity [34].

Although many products are used to treat gastric ulcers, most of these drugs produce several adverse reactions. Plant extracts, however, are some of the most attractive sources of new drugs and produce promising results in the treatment of gastric ulcer [35]. Flavonoids exert beneficial effects in a multitude of disease states, including anti-inflammatory, anti-oxidant, anti-allergic, hepato-protective, anti-thrombotic, anti-viral, and anti-carcinogenic activities [36]. The biological actions of flavonoids are mostly through their antioxidant properties either to their reducing capacities or to their possible influences on redox status [37,38].

Quercetin is one of the most frequently researched natural flavonoids. It has active anti-inflammatory [39], an-

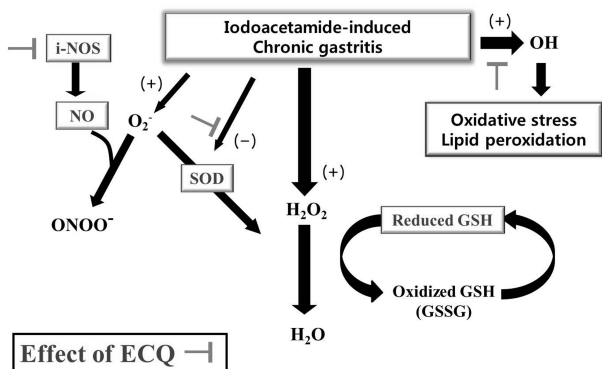
ti-thrombotic [40], anti-bacterial [41] and anti-tumoral [42,43] effects. In addition, Quercetin may be more efficacious than quercitrin as an anti-diabetic agent [44], and has been applied for clinical therapy because of its multiple pharmacological activities [45].

Quercetin-3-O- $\beta$ -D-glucuronopyranoside (QGC) is a quercetin derivative, extracted from *Rumex Aquaticus Herba* and purified through several steps. In our previous study, the oral administration of QGC significantly and dose-dependently reduced gastric content, and prevented the development of reflux esophagitis by inhibition of gastric acid output [27]. In cultured feline esophageal epithelial cells, QGC had protective effects on ethanol-induced cell damage by inhibiting of ROS generation and downstream activation of the ERK [46]. In addition, the protective effects of QGC against indomethacin-induced gastric damage are associated with increased gastric mucus secretion, inhibition of free radical production by activated neutrophils via ICAM-1, and pro-inflammatory cytokine downregulation [47].

For the present study, ECQ from *Rumex Aquaticus Herba* by simple ethanol-extracting method and ECQ contained QGC by 10.78% per gram of extract [29]. In the previous study, ECQ prevented indomethacin or ethanol-induced acute gastric damage [28].

In this study, gastric injury and MPO activity induced by IA increased with time for up to 7 days with mucosal surface erosions. The stimulated MPO activity may indicate the contribution of granulocytes to the inflammatory response. ECQ ameliorated the reduction in gastric mucosal injury and MPO activity. MPO uses the generated superoxides, leading to the formation of HOCl and other reactive oxidants that mediate generation of lipid peroxides and worsen gastric mucosal injury [48]. The present study suggests that ECQ has a gastroprotective effect in the chronic gastric mucosa induced by IA administration.

NO is involved in the modulation of gastric mucosal integrity and is important in the regulation of acid and alkaline secretion, mucus secretion and gastric mucosal blood flow [49]. The previous study showed that NO produced by iNOS is critical in mucosal defense mechanism of the inflamed stomach after subchronic irritation by IA [34]. Our



**Fig. 7.** Putative inhibitory pathways of ECQ-mediated anti-oxidant actions. In iodoacetamide-induced chronic gastritis model, the production of superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $-OH$ ), the major causes of oxidative stress, were increased. ECQ inhibited production of peroxynitrite ( $ONOO^-$ ) via suppressing i-NOS activity, and hydroxyl radical production. Impaired SOD activity was also recovered by ECQ.

result also shows that the total nitric oxide concentration increased by IA administration was significantly reduced by the treatment with ECQ. Increased NO concentration may be attributed to generated free radicals activating inflammatory cytokines production, in turn stimulating the expression of inducible nitric oxide synthase (iNOS) and leading to the formation of large fluxes of cytotoxic NO [50]. ECQ was also reduced the expression of iNOS induced by IA administration in rats. These results indicate that a protective effect of ECQ could be related to antioxidant properties by inhibiting NO formation.

Our results demonstrated that gastric mucosal GSH concentrations were depleted in IA-induced chronic gastritis. When ECQ was daily administered for a week, the mucosal GSH levels were normalized. GSH acts as a scavenger of free radicals and toxic substances ingested with foods or produced directly in the gastrointestinal tract [51] and as a major non-protein thiol in living organisms, is central in coordinating the body's antioxidant defense process [52].

In the present study, TBARS was increased in rat chronic gastritis induced by IA administration. Decreases in TBARS and increases in antioxidant levels such as GSH level and SOD activity in gastric tissues of rats treated with ECQ can be explained by the antioxidant activity of ECQ.

Taken together, ECQ markedly attenuated gastric lesions, enhanced MPO activity and lipid peroxidation, depletion of GSH levels, and reduction in SOD activity and SOD-2 expression, and up-regulated the expression of iNOS and NO production in chronic gastritis induced by IA treatment in rats. The therapeutic effect of ECQ at dose of 10 mg/kg was similar to Stillen<sup>®</sup> (20 mg/kg) on gastric lesion, MDA level, MPO activity, GSH depletion, SOD activity and total NO concentration. ECQ was also proven to be safe in our recent study which investigated the acute toxicity and the general pharmacological effects of the ECQ on general behavior, central nervous system, digestive system, smooth muscles, cardiovascular and respiratory systems to search for any side effects in rats, mice, guinea pigs, and cats [53]. As summarized in Fig. 7, ECQ could have antioxidant properties by suppressing lipid peroxidation and NO concentration and enhancing SOD activity and GSH

content, and will be further developed as an herbal remedy for preventive and/or curative purposes in gastrointestinal inflammatory diseases.

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