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Research Article

Gastroprotective Effects of Plants Extracts on Gastric Mucosal Injury in Experimental Sprague-Dawley Rats

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Rubus crataegifolius (black raspberry, RF), *Ulmus macrocarpa* (elm, UL), and *Gardenia jasminoides* (cape jasmine, GJ) are well known for hundreds of years as folk medicines in China and Korea to treat various gastrointestinal disturbance. The present study evaluated the gastroprotective effects of these plants either single or in combination against HCl/EtOH-induced gastritis and indomethacin-induced ulcer in rat model. Stomach ulcer was induced by oral ingestions of HCl/EtOH or indomethacin. Treatment with RF, UL, and GJ separately or in combination was done 1 h before ulcer induction. On HCl/EtOH-induced gastritis RF, UL, and GJ at a dose of 150 mg/kg showed comparable antigastritis effect (less than 50% inhibition) with lesion index of 94.97±8.05, 108.48±11.51, and 79.10±9.77 mm compared to cimetidine (45.33±23.73 mm). However, the combination of RF, UL, and GJ at a dose of 150 mg/kg with a ratio of 50:50:50 showed remarkable antigastritis effect with 77% inhibition. The observed lesion index at a ratio of 50:50:50 was 23.34±9.11 mm similar to cimetidine (18.88±19.88 mm). On indomethacin-induced ulcer, RF and GJ showed 38.28% and 51.8% inhibition whereas UL showed around 17.73% inhibition at 150 mg/kg. Combination of RF, UL, and GJ at 150 mg/kg showed strong antigastritis effect with 83.71% inhibition. These findings suggest strong gastroprotective effect of combined extract. In addition, these plants showed significant antioxidant activity in DPPH scavenging assay and antilipid peroxidation activity. Combination of black raspberry, elm, and cape jasmine might be a significant systemic gastroprotective agent that could be utilized for the treatment and/or protection of gastritis and gastric ulcer.

1. Introduction

The incidence of gastritis and gastric ulcer is the most prevalent gastrointestinal disorder and the complications greatly increased during the last decades with major cause of morbidity and mortality worldwide [1]. A variety of pathogenic mechanisms may contribute to the formation of ulcer characterized by an imbalance between aggressive factors such as acid and pepsin and the maintenance of mucosal integrity through endogenous defense mechanisms [2]. Currently, treating gastritis and ulcer requires a combination of drugs, such as proton pump inhibitors, anticholinergic, histamine receptor antagonists and antibiotics [3]. Although effectiveness can be obtained with these clinical drugs, their potential side effects, limited efficacy, and drug interactions represent a major problem in therapy [4, 5]. Hence, owing to safety concern there is a need for more effective and less toxic gastroprotective agent.

Since the ancient time nature provides several herbal phytochemicals for the benefit of humans. Most recent studies on the treatment of gastrointestinal disorder have focused on the potential role of natural medicine due to their availability, better protection, lower cost, and lower toxicity [6, 7]. Based on ethnopharmacological information, our previous hepatoprotective experiment, and reported gastroprotective and anti-inflammatory effects, we selected three Korean folkloric plants *Rubus crataegifolius* (RF) [8, 9], *Ulmus macrocarpa* (UL) [10], and *Gardenia jasminoides* (GJ)

[7, 11] for gastroprotective evaluation. In some study rubus showed the antigastric inflammation and anti-*Helicobacter pylori* effect [12, 13]. While in a study *Ulmus macrocarpa* showed the protective effect against colitis [14]. And *Gardenia jasminoides* showed the preventive effect against gastric injury [15]. Since stomach has many core values of disease like gastritis, gastric ulcer, or GERD, we try to use combined plant extracts which cure many problems in the stomach.

In this study, we analyzed HPLC chromatogram for standardization of RF (ellagic acid), UL (catechin), and GJ (geniposide), in order to compare gastroprotective and acid neutralizing effect of the selected plant extracts and their mixture using male Sprague-Dawley rats. We also report antioxidant activities of the extracts using DPPH radical scavenging and antilipid peroxidation activities of the combined plant extract. We also check HCl/EtOH-induced gastric damage, indomethacin-induced gastric ulcer and stomach tissues for histological analysis.

2. Material and Methods

2.1. Ethical Statement. All the procedures were performed in compliance with the regulations and guiding principles in the care of animals, Animal Welfare Committee and Ethics Committee of Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

2.2. Plant Materials and Preparation of Extracts. The unripened fruit of RF, stem bark of UL, and ripened fruit of GJ were purchased from Kyung Dong Medicinal Herb Market at Seoul, Korea. The plant samples were authenticated by taxonomist (Dr. Kwak sang soo) in Plant Resource Centre, Korea Research Institute of Bioscience and Biotechnology (KRIBB) and were kept in the herbarium of KRIBB. The experimental extracts of RF, UL, and GJ were prepared using extraction, concentration, and spray drying in Sam Woo-Dayeon Company (Chung Nam province, Korea). The extracts were dissolved in 20% DMSO and were kept at −20°C for further use.

2.3. HPLC Chromatogram for Standardization of RF, UL, and GJ. The unripened fruit of RF, the stem bark of UL, and ripened fruit of GJ were extracted separately with ethanol or hot water in round bottom flasks. The extracts were filtered (No. 1, Whatmann, USA) and concentrated under vaccum condition, and samples were dried. Each 50 mg of the extracted powder samples were dissolved in water and sonicated for 30 min. After sample was filtered through a 0.22 um PTFE syringe filter, the filtrate was injected for the HPLC analysis.

The quantification of RF, GJ, and UL was carried out using a high performance liquid chromatography analysis system (Agilent technologies 1260 infinity) equipped with auto sampler (G1329B) and UV (G1316A) detector. Chromatographic separation was achieved at 35°C on a reversed-phase C18 (4.6 × 150 mm, 5 μm) column. The flow rate was 0.8 mL/min, and the injection volume was 10 μL. The wavelengths used for detection were 254 nm for the ellagic acid and geniposide analysis and 280 nm for the catechin-7-O-ß-D-apiofuranoside analysis. Mobile phase was prepared not only A (0.1% aqueous TFA) and B (acetonitrile) for ellagic acid and geniposide analysis but also A (water/acetonitrile 95:5) and B (water/acetonitrile 5:95) for catechin-7-O-ß-D-apiofuranoside analysis. Two different HPLC gradients were used as described follows.

Use ellagic acid and geniposide: 0-10 min, 5-10% B, 10-20 min, 10-17% B, 20-35min, 17-35% B, 35-36 min, 35-70% B and keep for 4 min. The column was equilibrated for 10 min. Use catechin-7-O-ß-D-apiofuranoside: 0-30 min, 0-15% B, 30-21min, 15-50% B and keep for 4 min. The column was equilibrated for 10 min. The contents of ellagic acid, geniposide, and catechin-7-O-ß-D-apiofuranoside were calculated for standardization. The contents were 14.2 mg/g, 15.6 mg/g, and 30.5 mg/g, respectively. The HPLC analysis of ellagic acid, geniposide, and catechin-7-O-ß-D-apiofuranoside was shown in Figure 1, and the standard samples were shown in Figures 1(a), 1(c) and 1(e), while experiment samples used in study were shown in Figures 1(b), 1(d) and 1(f).

2.4. Animals. Male Sprague-Dawley rats, weighing 200-250 g, were purchased from Orient Bio Animal Laboratories, Kyunggi-do, Korea, and were acclimatized to standard laboratory conditions (24 ± 2°C, 45 ± 5% humidity and 12 h light/dark cycle) for 7 days. Fasting was used prior to all assays because vehicle, standard drugs, and samples were administered orally (by gavage). The animals were kept in cages with raised wide mesh floors to prevent coprophagy.

2.5. HCl/EtOH-Induced Gastric Damage. The rats were randomly divided into the following 9 groups with 7 rats in each group: HCl/EtOH-induced control group, cimetidine (dose 100 mg/kg) group, single extract group for RF, UL and GJ (dose 150 and 300 mg/kg) and combined extract group for RF plus UL (dose 75+75 mg/kg), and RF plus GJ (dose 75+75 mg/kg) and RF plus UL plus GJ (dose 50+50+50 mg/kg and 75+25+50 mg/kg). After testing dose-dependent gastroprotection effect in both HCl/EtOH, indomethacin-induced gastric ulcer, we selected the potent concentration of each plants for animal experiment. These doses were selected on the basis of our previous reports on the hepatoprotective effects of the plants [8, 11].

After 24 h fasting with free access to water prior to the experiment, the administration of extracts and standard cimetidine was carried out intragastrically (i.g.) using gavages. Thirty minutes later, 1 mL of HCl/EtOH solution (150 mM HCl in 65% ethanol) was administered orally. Each animal was sacrificed by ether after 1 h fasting and the stomach was fixed for 2 h in 2% formalin solution. After opening along the greater curvature, 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, the sum of erosion length per rat, was determined using UTHSCSA Image Tool 3.00 [16].

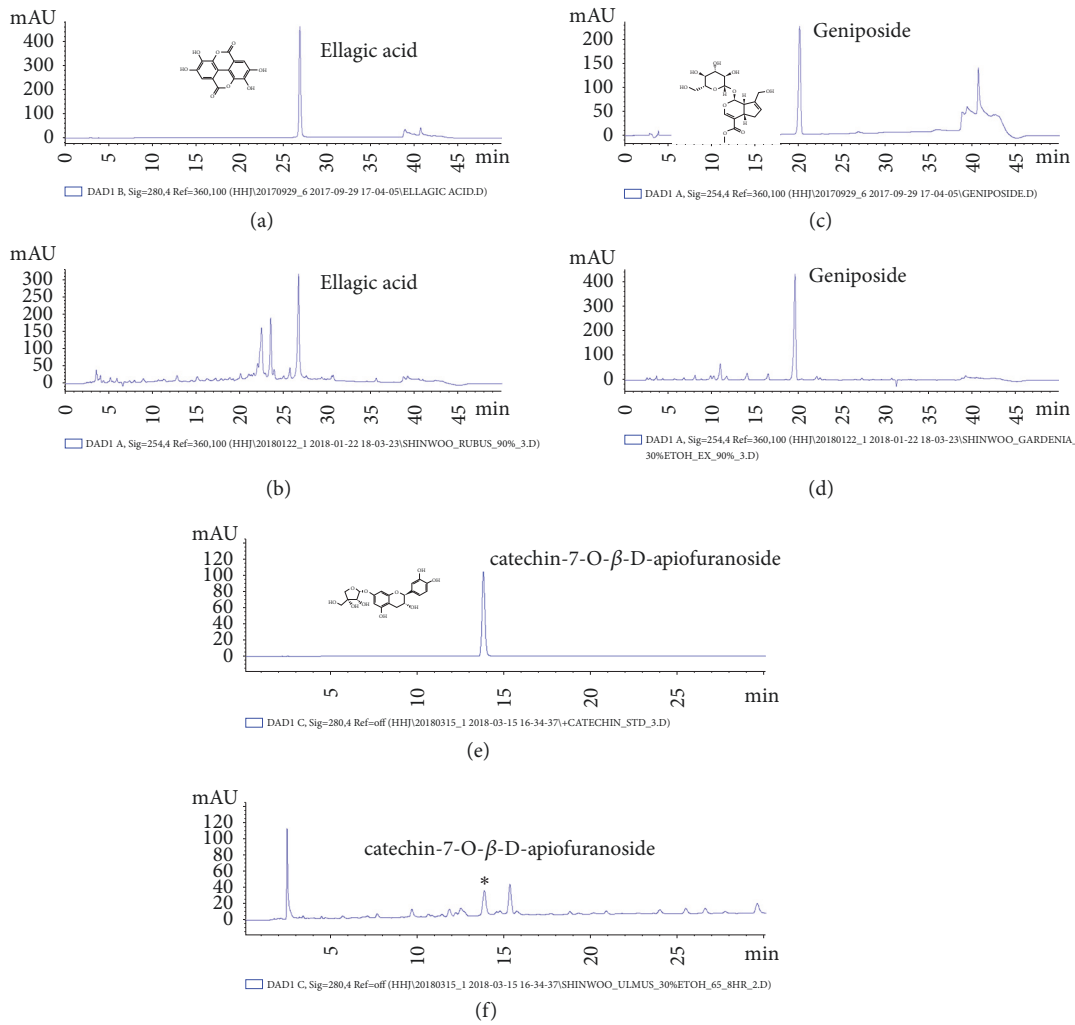

Figure 1: HPLC-DAD chromatogram for standardization of sample. (a) ellagic acid standard at 254 nm, (b) *Rubus crataegifolius* extracts, (c) geniposide standard at 254 nm, (d) *Gardenia jasminoides* extracts, (e) catechin-7-O-ß-D-apiofuranoside at 280 nm, and (f) *Ulmus macrocarpa* extracts. The contents were standardized to contain 14.2 mg/g, 15.6 mg/g, and 30.5 mg/g, respectively.

2.6. Indomethacin-Induced Gastric Ulcer. Gastric hemorrhagic lesions in rats were induced by intragastric administration of 30 mg/kg of indomethacin according to the procedure described by Sayanti et al. [17]. Briefly, rats were randomly divided into the following groups with 7 rats in each group: indomethacin treated group, cimetidine (100 mg/kg) group, and extract group receiving either RF, UL, or GJ (150 and 300 mg/kg) and combined extract group for RF plus UL (dose 75+75mg/kg) and RF plus GJ (dose 75+75mg/kg) and RF plus UL plus GJ (dose 50+50+50mg/kg and 75+25+50 mg/kg). Drugs were administered 1 h before indomethacin application. Four hours after indomethacin administration, the animals were killed by cervical dislocation, and the stomachs were removed, inflated with 1 mL of 1% paraformaldehyde overnight to fix the tissue walls, and opened along the greater curvature. The lesion index, the sum of erosion length per rat, was determined using UTHSCSA Image Tool 3.00.

2.7. Histological Analysis. The stomach was exposed following a middle laparotomy and opened with an incision along the greater curvature. It was pinned over a plexiglass cylinder. Stomach tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 μm thick sections were prepared and stained with haematoxylin and eosin using standard procedures.

2.8. Lipid Peroxidation. In the in vitro model of lipid peroxidation, the supernatant fraction of a stomach homogenate from male adult SD rats was prepared. Stomach tissues were homogenized in homogenizer with 5 vols of ice-cold 1.15% NaCl solution. Five ul of stomach homogenate were added to 5 μl of test compound and 50 μl of $FeSO_4$ and incubated at 37°C for 1 h. The reaction was stopped by adding 200 μl of 35% $HClO_4$ and centrifuged at 3,000 rpm for 10 min. 500 μl supernatant was heated with 500 μl of TBA, 750 μl of

phosphate buffer, and 100 μl of SDS for 60 min at 100°C. After being cooled, equivalent amount of n-butanol was added and centrifuged 10 min at 4000 rpm. Optical density was measured spectrophotometrically at 532 nm.

In the ethanol-induced gastric-ulcer model, rats were pretreated with plant extract for 1 h before ethanol treatment. At 1 h after the application of ethanol, the animal was killed, and a glandular segment from each stomach was homogenized in 5 vol. of ice-cold 1.15% sodium chloride solution. The remaining protocol was followed as describe above.

2.9. Antioxidant Activity. Antioxidant effect of RF, UL, GJ, and standard ascorbic acid on DPPH radicals was observed according to the method described by Sanchez-Moreno et al. [18]. 2.4 mL of 0.1 mM DPPH in ethanol was mixed with 1.6 ml of extracts at different concentrations (0-200 μg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured by spectrophotometer (NJ, USA) at 517 nm. Percentage of DPPH radical scavenging activity was calculated by the following equation: scavenging activity = $\{(A_0\text{-}A_1)/A_0\} \times 100$, where A_0 is the absorbance of blank and A_1 is the absorbance of the sample. Percent of inhibition was plotted against concentration and from the graph IC_{50} was calculated.

2.10. Statistical Analysis. All experiments were performed in triplicate, repeated at least twice, and were expressed as the mean ± standard deviation. Data expressed as means were compared by the analysis of variance followed by unpaired Student's t test. The differences were considered to be statistically significant when $P < 0.05$.

3. Results

In the present study, we examined gastroprotective effect of plants extract, RUG-com, on gastric mucosal injury in experimental Sprague-Dawley rats. As described in Materials and Methods the quantity of RF, GJ, and UL used in the experiment was standardized by determining the amounts of ellagic acid, geniposide, and catechin 7-o-B-D-apiofuranoside using HPLC chromatography (Figure 1).

3.1. Effects of Plants Extract on HCl/EtOH-Induced Gastritis. The effects of plants extract and combined extracts on HCl/EtOH-induced gastric lesions are shown in Figures 2 and 3, respectively. Intragastric administration of HCl/EtOH caused multiple band-like lesions in the gastric mucosa (Figures 2(a) and 3(a)). Administration of RF, UL, and GJ showed decreased lesion index (94.97±8.05, 108.48±11.51 and 79.10±9.77 mm, respectively) compared to control and cimetidine (134.32±16.94 and 45.33±23.73 mm, respectively). The activity order was cimetidine>GJ>RF>UL. Based on the experiment, RF and GJ showed significant antigastritis effect (inhibition percentage more than 50% at 300 mg/kg compared to the cimetidine 66.04%) than UL (37.59%). RF in combination with UL or GJ at two different doses (Figures 3(c)-3(d)) did not show remarkable changes. However, RF combined with UL and GJ at a dose of 150 mg/kg with ratio 50:50:50 showed strong antigastritis effect (Figure 3(e)) with lesion index 23.34±9.11 compared to cimetidine (18.88±19.88 mm) and control (105.37±6.77 mm). Inhibition percentage (78-81%) was similar to that of cimetidine (82%). These results demonstrated strong antigastritis activity of combined plants and the activity was dose dependent.

3.2. Effect of Plant Extract on Indomethacin-Induced Gastric Ulcer. The effects of RF, UL, and GJ on the ulcer index and % inhibition against ulcer are shown in Figure 4. Indomethacin caused a significant increase in the degree of ulceration (ulcer index) in the rats. A significant improvement in the level of inhibition against ulceration was observed in the extracts-treated animals. The RF and GJ at a dose of 150 mg/kg offered protection (38 and 51.8% inhibition each) against ulceration with ulcer index of 76.69±2.37 and 64.36±7.73 mm, respectively, when compared to control (124.26±7.97 mm) and cimetidine (21.58±3.92 mm). UL was less effective (17.63% inhibition with lesion index of 102.35±2.16 mm) than the two extract. However, combination of RF, UL, and GJ at a dose of 150 mg/kg with 50:50:50 ratios showed strong antiulcer effect (79.16% inhibition) (Figure 5). The data represents that the healing of ulcer is suggestive of gastroprotective attributes of the combined plants.

3.3. Effect of Combined Plants Extracts on Histological Evaluation in HCl/Ethanol-Induced and Indomethacin-Induced Ulcer Model. Histological examinations of gastric damage were observed in rats receiving HCl/EtOH or indometacin for 1 hour in gastric tissues as compared with untreated normal rats. Pretreatment with combined plant extracts (RF+UL+GJ at a dose of 150 mg/kg and a ratio of 50:50:50) prevented HCl/EtOH-induced or indomethacin-induced histological changes in the superficial layer of the gastric mucosa with congestion by H&E-staining (Figures 6(a) and 6(b)).

Compared with the sham operating group, HCl/EtOH or indomethacin administration induced a disruption of the superficial region of gastric gland with epithelial cell loss, while plant extracts (RF+UL+GJ) prevented this damage (Figures 6(a)(C) and 6(b)(C)). Evidence of gastric damage attenuation in stomach tissue pretreated with three mixed extracts demonstrated the strong effectiveness of combined plant extract.

3.4. Antioxidant Activity of the Extracts. Gastric ulcer is well accepted oxidative stress-induced stomach disease and the mechanism of ulcer induction is mediated by reactive oxygen species (ROS). For these reasons, antioxidant activities of RF, UL, and GJ extract were measured.

In DPPH free radical scavenging activity RF demonstrated highest activity (IC_{50} 62.9±1.02 μg/ml) followed by UL (71.7±1.57 μg/ml) and GJ (79.24±0.978 μg/ml). However, these activities were less than that of standard ascorbic acid (17.24±0.425 μg/ml) shown in Table 1. Fe^{3+} reducing assay

Table 1: Antioxidant activities plant extracts.

Sample	DPPH radical		Reducing power	
	IC_{50} (μg/ml)[a]	Scavenging activity (%) at 50 μg/ml	EC_{50} (μg/ml)[b]	Absorbance[c]
RF	62.9±1.02	48.37±1.3	15.1±0.07	1.92±0.156
UL	71.7±1.57	46.02±1.4	27.72±0.05	1.25±0.126
GJ	79.24±0.978	38.59±1.9	30.32±0.4	1.012±0.29
AA	17.24±0.425	79.01±2.9	7.01±0.2	2.012±0.12

Each value is expressed as mean ± standard deviation (n = 3), [a]IC_{50} (μg/ml): the concentration at which 50% is inhibited; [b]EC_{50} (μg/ml): effective concentration at which the absorbance is 0.5; [c]absorbance at 100 μg/ml.

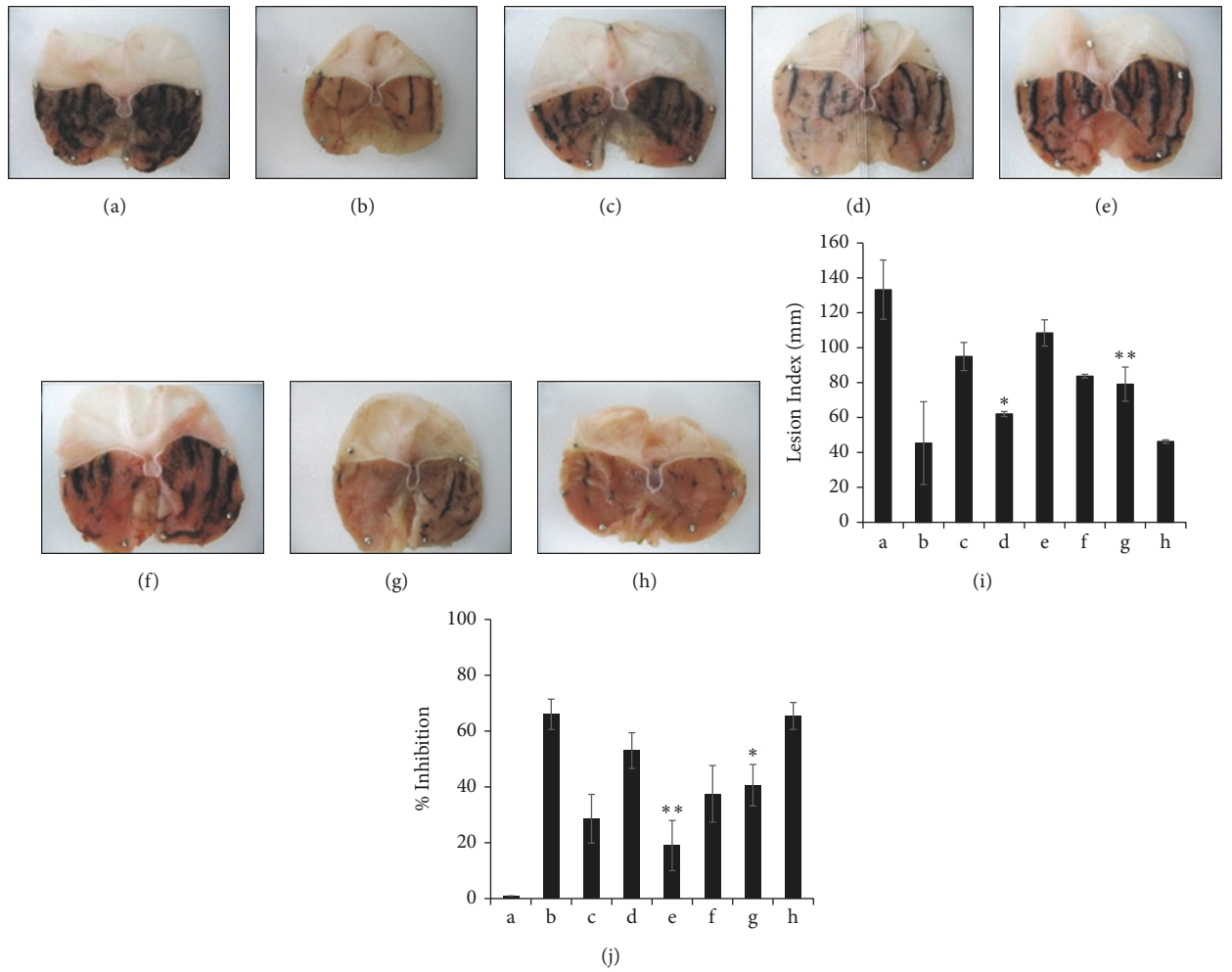

Figure 2: Inhibitory effects of plants extract on HCl-EtOH-induced gastritis. (a) HCl-EtOH; (b) Cimetidine (150 mg/kg); (c) RF (150 mg/kg); (d) RF (300 mg/kg); (e) UL (150 mg/kg); (f) UL (300 mg/kg); (g) GJ (150 mg/kg); (h) GJ (300 mg/kg). n=7 for each group. The sum of erosion length (lesion index) per rat was determined using UTHSCSA Image Tool 3.00. Each value represents the mean ±SEM. $*$ $p<0.05$, $**$ $p<0.01$ vs. control rat. % inhibition of gastritis formation of each sample was determined.

measures the ability of extracts to reduce ferric ion to ferrous ion. The results of the Fe^{3+} reducing activities are presented in Table 1. Reducing activity of the standard ascorbic acid was found to be highest with observed EC_{50} (effective concentration at which the absorbance is 0.5) which was 7.01±0.2 μg/ml. Among the three extracts, significant reducing activity was observed for RF followed by UL and GJ with EC_{50} 15.1±0.07, 27.72±0.05, and 30.32±0.4 μg/ml, respectively. All the extracts showed a dose-dependent reduction of Fe^{3+} ion indicating the significant antioxidant potentials of the extracts.

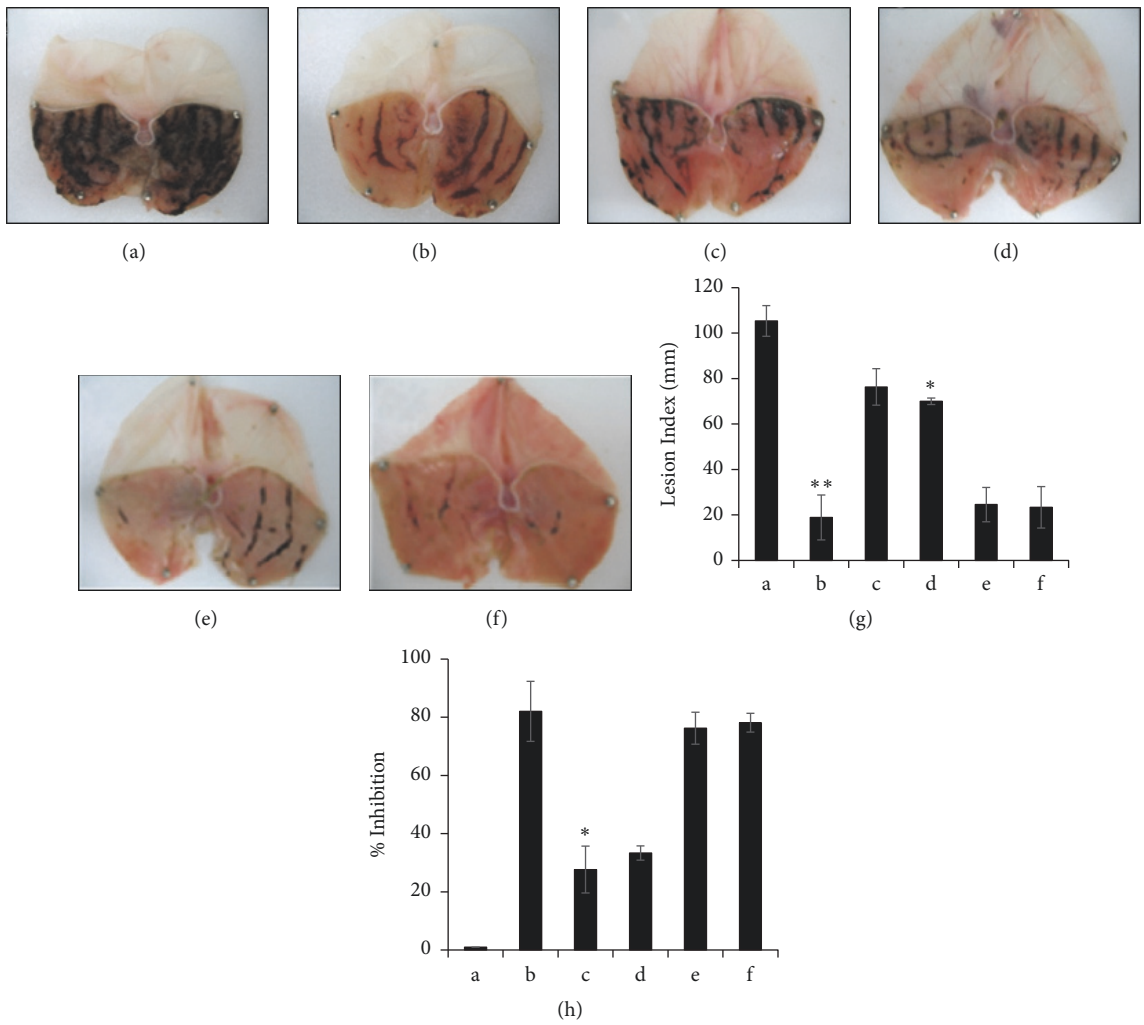

Figure 3: Inhibitory effect of combined plant extracts on HCl-EtOH-induced gastritis. (a) HCl-EtOH, (b) Cimetidine (100 mg/kg), (c) RF combined UL (75+75 mg/kg), (d) RF combined GJ (75+75 mg/kg); (e) RF combined UL and GJ (50+50+50 mg/kg), and (f) RF combined UL and GJ (75+25+50 mg/kg). n=7 for each group. Gastric index was determined using UTHSCSA Image Tool 3.00. Each value represents the mean ±SEM. $*$ $p<0.05$, $**$ $p<0.01$ vs. control rat. % inhibition of gastritis formation of the combined samples was determined.

3.5. Effect of Combined Plant Extract on Lipid Peroxidation. In vitro lipid peroxidation level in the mice stomach homogenate was measured as TBARS used as an index of lipid peroxidation. The TBARS level increased after 1 h incubation with the $FeSO_4$ at 37°C. RF-UL-GJ plant complex extract at the ratio of 1:1:1 significantly inhibits the production of the TBARS level in a concentration-dependent manner in vitro (Figure 7(a)). The antioxidant trolox (10μM) also inhibited the production of TBARS in vitro. Next, we investigated the antioxidative effects of RF-UL-GJ plant complex extract in the model of EtOH/HCl induced gastric ulcer. Ethanol enhanced the TBARS level compared to the value seen in the control group, pretreatment of animals with plants complex (30, 90, 150, and 300 in the ratio of 1:1:1 of RF, UL, and GJ each) resulted in mark suppression of ethanol-induced TBARS (Figure 7(b)).

4. Discussion

Ulcer, a common disorder of the gastrointestinal system, is characterized by inflamed lesions or excavations of the mucosa and tissue due to imbalance between aggressive factors like acid, pepsin, *H. pylori,* and defensive factors such as gastric mucus, bicarbonate ions, and prostaglandins along with innate resistance of mucosal cells [19]. Higher incidence of gastric ulcer and gastritis usually occurs in people who smoke, use nonsteroidal anti-inflammatory drugs (NSAIDs), or consume alcohol [20]. In Korea, the annual medical costs for gastric ulcer range from $959.6 to $2553.10 and are increasing each year [21]. Although conventional treatments are effective, both clinical and experimental studies have demonstrated that traditional herbal medicines exhibit therapeutic benefit for gastric ulcer [22]. Korea has a long

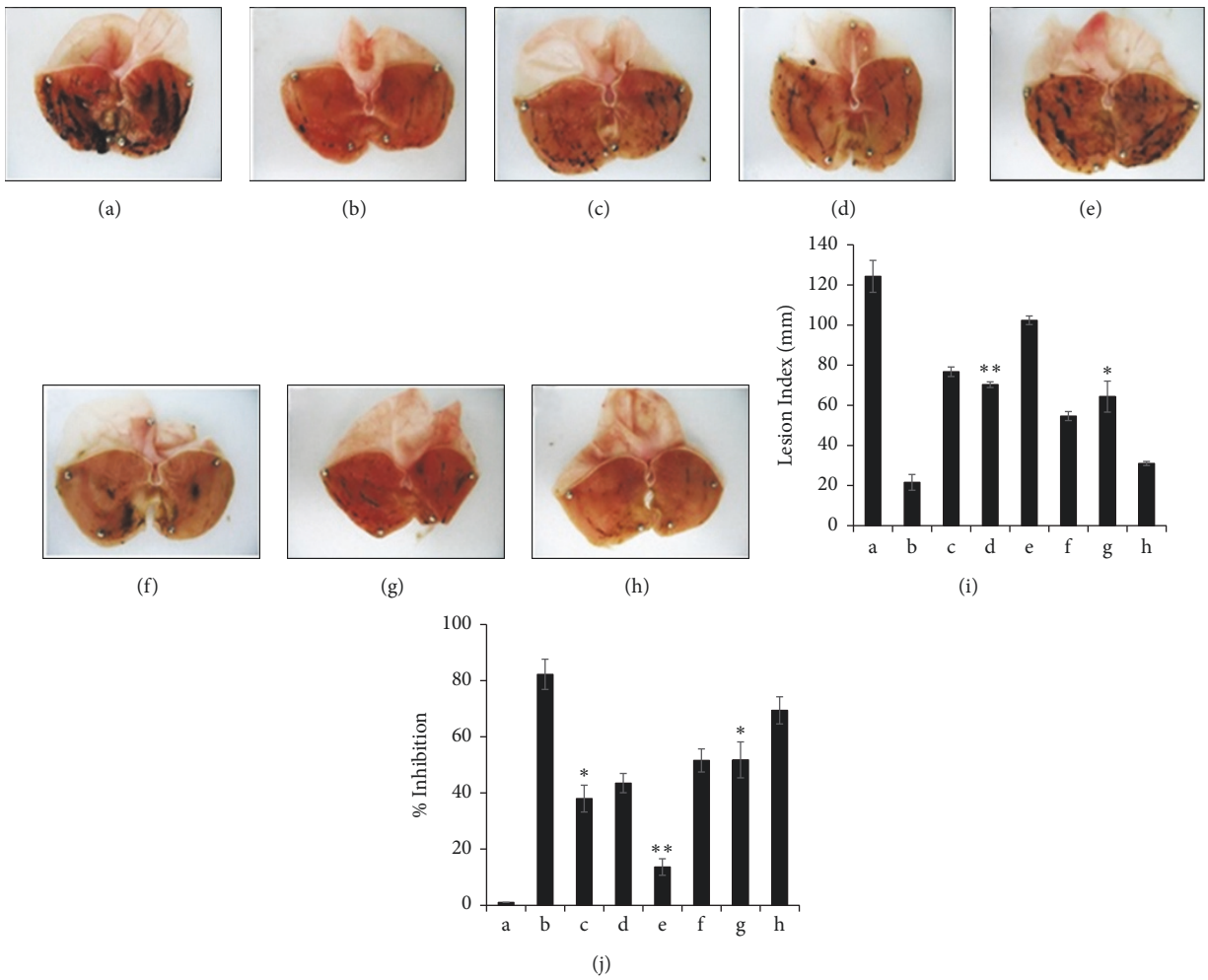

Figure 4: Inhibitory effects of plants extract on indomethacin-induced gastric ulcer. (a) Indomethacin treated group, (b) Cimetidine (100 mg/kg), (c) RF (150 mg/kg), (d) RF (300 mg/kg), (e) UL (150 mg/kg), (f) UL (300 mg/kg), (g) GJ (150 mg/kg), and (h) GJ (300 mg/kg). n=7 for each group. Gastric lesion index was determined using UTHSCSA Image Tool 3.00. Each value represents the mean ±SEM. $*$ $p<0.05$, $**$ $p<0.01$ vs. control rat. % inhibition of gastric value formation of each sample was determined.

history of using herbal medicine from the ancient time and 69% of the Korean present population has experienced traditional medicine for the treatment of several diseases [9, 11, 23, 24]. *Rubus crataegifolius* [8, 9], *Ulmus macrocarpa* [10], and *Gardenia jasminoides* [7, 11] are three well-known Korean as well as Chinese medicinal plants used to treat a variety of disorders including gastritis and ulcer. Since studies suggested that combination of herbal medicine achieved superior efficacy to synthetic drug in treating gastric and duodenal ulcers and gastritis [25], we selected these three plants to evaluate the inhibitory activities on gastritis and gastric ulcer using combination therapy. We already finished all samples in simple clinical trial. In simple clinical trial we applied samples on 15 persons in each group (control and examine); in simple clinical trials we found out that there is no side effect occuring after treatment of these three combined plant extracts.

HCl/EtOH-induced gastritis is considered as a reliable tool of the pathogenesis of gastritis since HCl and EtOH both stimulate acid secretion and accelerate gastric mucosal necrosis and apoptosis by damaging gastric mucosal defense system [26, 27]. In present study, multiple band-like lesions in the gastric mucosa by HCl/EtOH were inhibited by cimetidine (82%). Though the extracts have fewer effects on inhibition when treated separately, combination showed superior inhibition (84.76%) of gastric lesion, similar to cimetidine. In histological examination the depth and severity of HCl/EtOH-induced gastric damage were almost cured by combined plant at 150 mg/kg (RF: UL: GJ 50:50:50); hence, the study suggests there might be strong synergistic effects to use the combined plants for the treatment of gastritis.

NSAIDs are important anti-inflammatory group which have been used widely to establish animal models of gastric

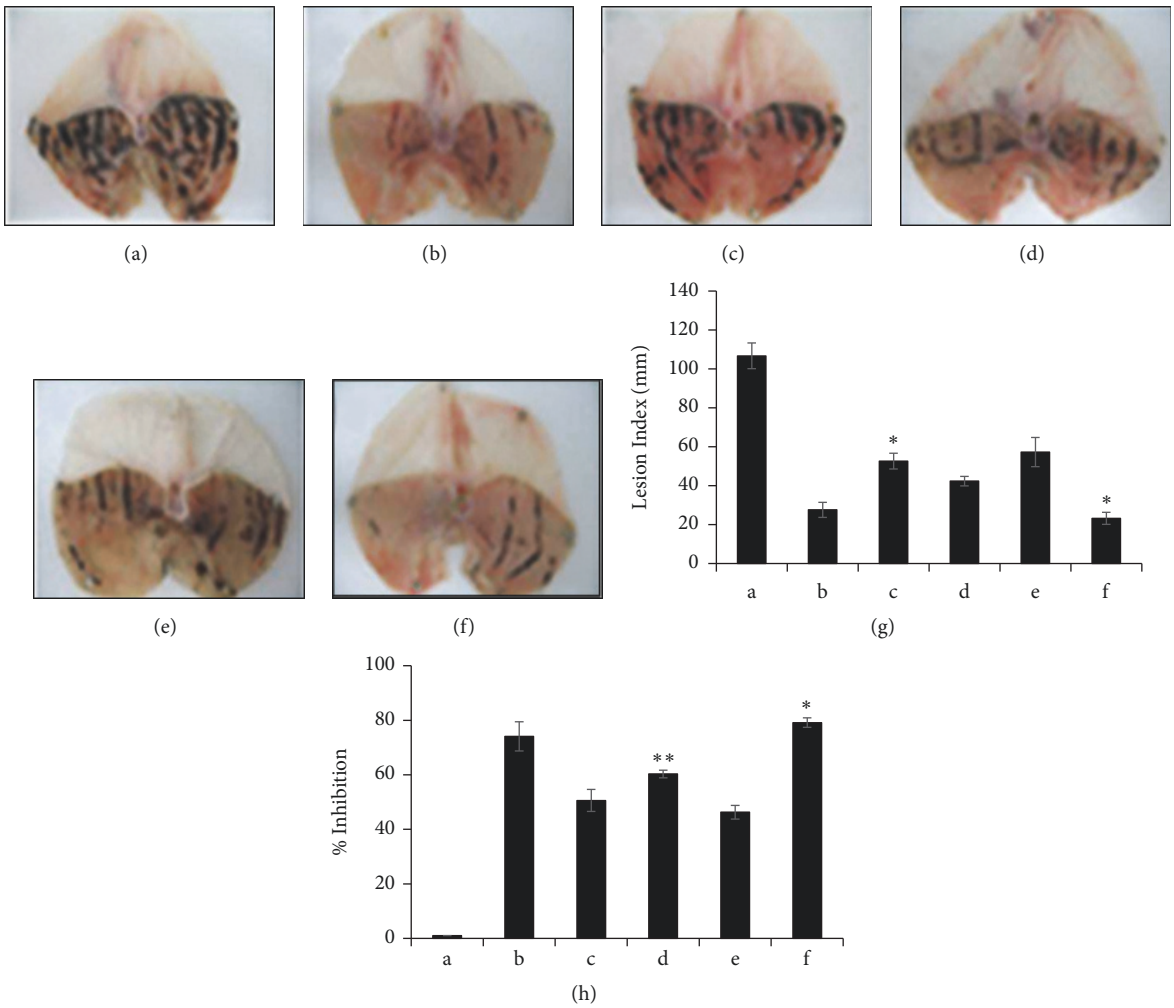

Figure 5: Inhibitory effect of combined plant extracts on indomethacin-induced gastric ulcer. (a) Indomethacin, (b) Cimetidine (100 mg/kg), (c) RF combined UL (75+75 mg/kg), (d) RF combined GJ (75+75 mg/kg), (e) RF combined UL and GJ (50+50+50 mg/kg), and (f) RF combined UL and GJ (75+25+50 mg/kg). n=7 for each group. Gastric index was determined using UTHSCSA Image Tool 3.00. Each value represents the mean ±SEM. $*$ $p<0.05$, $**$ $p<0.01$ vs. control rat.

ulcer [28]. In our study we selected indomethacin because it induces gastric ulcer-like damage in rats and has a higher ulcerogenic potential than other NSAIDs [29]. According to our observation, *Rubus crataegifolius* and *G. jasminoides* at 300 mg/kg showed strong ulcer healing effect with 32.28% and 51.80% inhibition, respectively, compared to cimetidine (100 mg/kg, 82.63%). There was no visible sign of ulceration or perforation observed in combined plant (RF+ UL+ GJ) *G. jasminoides* treated stomach when compared with the control. The results are similar to previously reported data on these plants [7, 30], suggesting the combined plants RF, UL, and GJ at ratio (50:50:50) could be useful in treating NSAID-induced gastric ulcer.

Oxidative stress is believed to initiate and aggravate many digestive system diseases including stomach ulcer and gastric carcinoma. Especially, ethanol-induced gastric damage has been suggested to be mediated by the generation of free radical [31]. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals.

Recent study showed that antioxidants prompt gastroprotective and healing effects by increasing the amount of gastric mucus glycoprotein and inhibition of prostaglandin production [32]. ROS are important factors in ethanol-induced and NSAIDs related mucosal damage [33]. Antioxidants can scavenge ROS and are expected to heal or prevent gastric ulcers. In our experiment we found significant scavenging action of free radicals generated by DPPH suggesting that the extracts would have significant antioxidant action which was subsequently confirmed by its Fe^{3+} reducing power activity. Therefore, the significant antigastritis and antiulcer activity of the extracts could be due in part to their reducing and antioxidant properties.

As reported, oral administration of HCl/EtOH significantly increases the lipid peroxidation compared with the

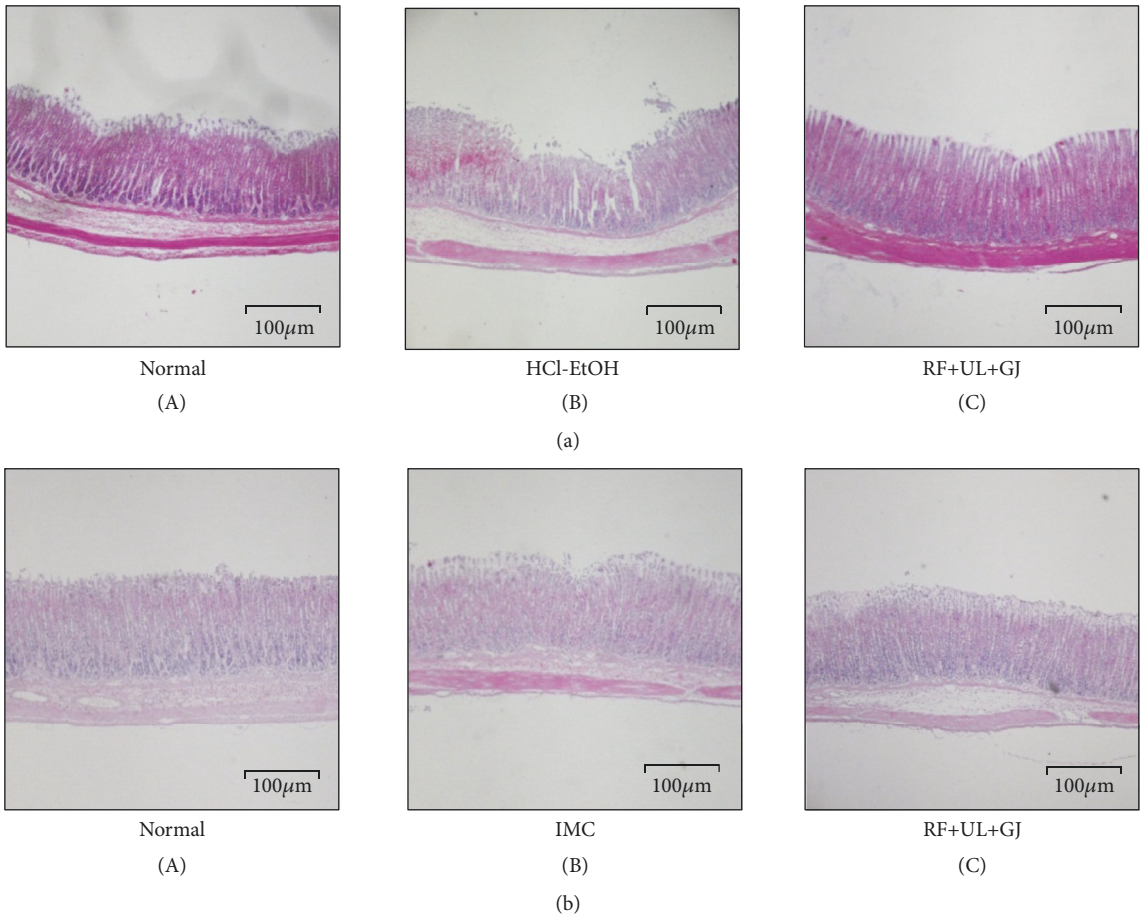

Figure 6: Effect of RF+UL+GJ plants extract on histological evaluation in HCl/ethanol and indomethacin-induced ulcer model. Section of hematoxylin and eosin (HE) staining of HCl/ethanol 5(a) and IMC-induced 5(b) gastric mucosa. Microscopic appearance of lesions induced by HCl/ethanol 5(a)(B) and IMC 5(b)(B) and lesion pretreated with 150 mg/kg of RUG-complex 5(a)(C) and 5(b)(C). Pathophysiological examination of the tissue sections was performed under light microscopy with 200x magnification.

sham treating group, and the RF+ UL+ GJ plant complex significantly reduced the production of TBARS in a concentration-dependent manner. The gastroprotective effect of herbal preparations has been attributed to three main functions including antisecretory, cytoprotective, and antioxidant properties [34]. Our results showed that the pretreatment with single as well as combined plants protected the rat's gastric mucosa against HCl/EtOH-induced ulcer, suggesting that the extracts exhibit efficient cytoprotective activity, since it inhibits the gastric lesion formation. Additionally, all the extracts significantly reduced mucosal damage in the NSAID-induced gastric ulcer and possess antioxidant properties and antilipid peroxidation properties demonstrating gastroprotective effects and suggesting the possible involvement of prostaglandins (data not shown) and/or mucus production in antiulcer activity. Though all three plants showed similar gastroprotective effect, we found out that the Gardenia treated group showed strong gastroprotective effect among three plants.

5. Conclusion

In conclusion, our results demonstrate the synergistic gastroprotective effects of combination of RF, UL, and GJ. RF+ UL+ GJ complex showed protective effect on HCl/EtOH and indomethacin-induced gastric mucosa injury in a dose-dependent manner. These results lead us to believe that RF+ UL+ GJ complex is powerful remedy for gastric mucosa lesions by inhibiting oxidative activity including lipid peroxidation inhibitions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

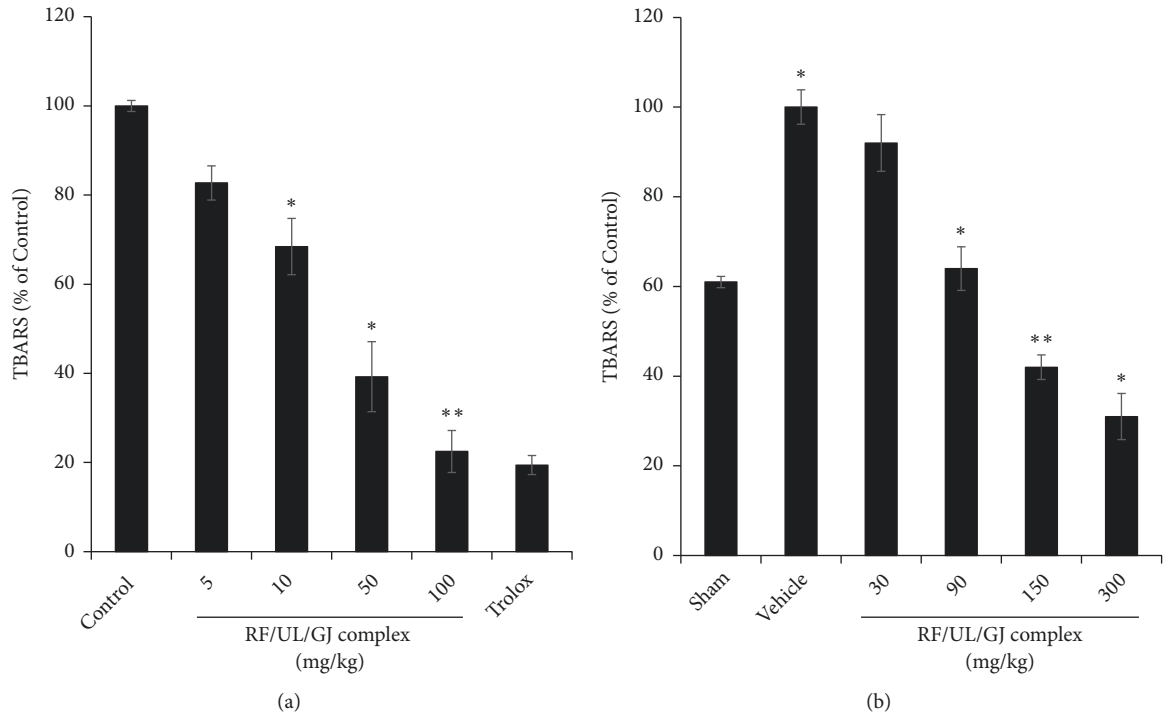

Figure 7: Effect of RF+UL+GJ plants extract on lipid peroxidations. Effect of RF+UL+GJ complex on TBARS content on in vitro mouse stomach homogenate (Figure 6(a)). n=7 for each group. Each value represents the mean ±SEM. ∗ $p<0.05$, ∗∗ $p<0.01$ vs. control (Dunnett's multirange test). Effect of RF+UL+GJ complex in mice on HCl/ethanol-induced gastric damage (Figure 6(b)). n=7 for each group. 30, 90, 150, 300 mg/kg of the plants complex (at a dose of 1:1:1 ratio of RF, UL, and GJ) were used. Each value represents the mean ±SEM. ∗ $p<0.05$, ∗∗ $p<0.01$ vs. control (Dunnett's multirange test).

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