

Protective Effects of BK-1202 on the Indomethacin-induced Gastric Ulcer in Rats

Hae-Won Kwon, Dae-Jun Kim

Dept. of Internal Medicine, College of Oriental Medicine, Daegu Hanny University

Purpose: The object of this study is to observe the anti-ulcerative effects of BK-1202 (IGM), a mixed herbal formula consisting of 9 herbal drugs, which have been traditional Korean medicine for treating various digestive diseases, on indomethacin-induced gastric ulcer in rat.

Methods: Three different doses of IGM extract (200, 100 and 50 mg/kg) were orally administered once 30 min before indomethacin treatment. Six hours after indomethacin treatment, changes in the gross lesion scores, fundic histopathology, MPO activity and antioxidant activities were observed. The results were compared with two reference groups treated with omeprazole (10 mg/kg), antioxidant and proton pump inhibitor, and DA-9601 (100 mg/kg), a standardized extract of the herb *Artemisiaasiatica*.

Results: In all three doses of IGM extract, significantly decreased gastric damages were observed in the indomethacin-induced gastric ulcer rats, when compared with the indomethacin-treated control rats. IGM extracts also strengthened the antioxidative defense systems, decreasing the level of lipid peroxidation and catalase activity while increasing the superoxide dismutase and glutathione contents. IGM extracts showed similar anti-ulcerative effects to those shown by equal dose of DA-9601, and the effects of 50 mg/kg IGM extracts were comparable to those of 10 mg/kg omeprazole.

Conclusion: The results obtained in this study suggest that IGM extract has favorable effects on the indomethacin-induced gastric damages by strengthening the antioxidative defense systems and enhancing anti-inflammatory effects.

Key Words : BK-1202, Indomethacin, Gastric ulcer

Introduction

Gastritis and gastric ulcer are defined as pathological conditions developed by exposure of the gastric mucosa to endogenous and/or exogenous aggressive factors and subsequent disturbances in gastric mucosal defenses¹⁾. Gastric ulcers are ulcers occurring in the stomach that are manifested by acute erosive and superficial ulcerative lesions in the regions exposed to gastric juices. In the Korean herbal medicine, gastric ulcer is known to be associated with the symptoms related to epigastric

pain, heartburn, acid regurgitation, and dyspepsia²⁾. Since the recognition of the finding of a study that identified nonsteroidal anti-inflammatory drugs (NSAIDs) as one of the major causes of gastric ulcer³⁾, many anti-ulcerative drugs have been developed to address the related symptoms. However, such drugs have a wide range of side-effects, such as constipation, diarrhea, itching, spotty skin, inhibition of antifungal agents' metabolism mediated by proton pump inhibitor (PPI), headaches, anti-androgenic effects, dizziness, and misoprostol-induced stillbirth or discharge of blood in pregnant women⁴⁾.

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• Correspondence to : Dae-Jun Kim
#907-8 Daejam-dong, Nam-gu, Pohang, Gyung-sangbuk-do, South Korea
Tel : +82-54-281-0055, Fax : +82-54-281-7463, E-mail : emdkdj@naver.com

Ijintang-Gamibang (IG) recipe prescribes the herbal formula of Ijintang extract added with *Atractylodis rhizoma*, *Massa medicata fermentata*, *Hordei fructus germinatus*, and *Coptidis rhizoma*⁵⁾. BK-1202 is a new IG formula modified for the purpose of this study by adding *Ostrea gigas*⁶⁾ whose acid-removing effect was verified. To determine the protective effects of BK-1202 against indomethacin-induced gastric ulcer, BK-1202 at the doses of 200, 100, and 50 mg/kg was orally administered to rats 30 min prior to administering them indomethacin (25 mg/kg). Six hours after the medication, all animals were sacrificed and postmortem observations were made to check the changes in the surface area of a hemorrhagic gastric ulcerative lesion (lesion score; mm²/gastric mucosa), myeloperoxidase (MPO) content in the gastric mucosal tissue, antioxidant defense system, lipid peroxidation, glutathione (GSH), catalase, and superoxide dismutase (SOD) along with histomorphometric alterations. The experimental results were then compared with the results of the reference groups treated with 10mg/kg omeprazole and 100 mg/kg DA-9601, respectively.

Materials and Methods

1. Experimental drugs

The IG used in this study was purchased from a

local pharmaceutical company (Hyosung Pharmaceutical Co., Daegu, Korea) and screened for use through microscopic inspection. Table 1 presents the composition of a sachet of IG. The ingredients contained in a selected sachet (68 g) were dissolved in 2000 ml distilled water and heat-extracted, followed by suction filtration of the extracted liquid and the depressurization and concentration of the filtered extract using a rotary vacuum evaporator (N-N type; LAB Camp, Daejeon, Korea). The extract with viscous consistency thus acquired underwent freeze drying in a programmable freeze dryer (PVTFD10A; Ilshin Lab., Seoul, Korea), which yielded a total of 17.00 g (yield rate: 25.01%) dark brown fluid extract. BK-1202 was then produced by adding 4 g *Ostrea gigas* to the IG extract, which was then pulverized and stored in a freezer (-20°C) to be used for the experiments. The prepared BK-1202 powder dissolved well in distilled water up to the concentration of 40 mg/ml. Drugs for the experimental and reference groups, i.e. indomethacin, omeprazole, and DA-9601 were purchased from Sigma (MO, USA) and Dong-A Pharmaceutical Co. (Yongin, Korea).

2. Lab animals and their management

A total of 56 male Sprague-Dawley rats (6-week old upon receipt, SLC, Japan) were used for the

Table 1. Composition of Ijintanggamibang (IG) used in this study

Herbs	Scientific name	Korean name	Amounts (g)
Pinella Rhizoma	<i>Pinellia ternate</i> (THUNB.) BREIT.	[半夏]	16
Citri Pericarpium	<i>Citrus unshiu</i> MARKOVICH	[陳皮]	8
Holelen	<i>Poria cocos</i> WOLF	[茯苓]	8
Atractylodis Rhizoma	<i>Atractylodes japonica</i> KOIDZ	[蒼朮]	8
Massa Medicata Fermentata	<i>Hordeum vulgare</i> LINNE var.	[神曲]	8
Hordei Fructus Germinatus	<i>hexastichon</i> ASCHERS.	[麥芽]	8
Coptidis Rhizoma	<i>Coptis japonica</i> (THUNB.), MAKINO	[黃連]	8
Glycyrrhizae Radix	<i>Glycyrrhiza uralensis</i> FISCH	[甘草]	4
Total	8 types		68

experiments after a 7-day acclimation. Throughout the duration of acclimation and experiments, they were kept in polycarbonate boxes designed for rats, 5 rats per box, in constant temperature (20-25°C) and humidity (30-35%) conditions in a 12-h light:dark cycle, and were given ad libitum access to feed (Samyang, Korea) and water. Of 56 rats, 48 were used for developing indomethacin-induced gastric mucosal damage, and the remaining 8 were used as an intact control group. All animals were administered their respective drugs (except for the two control groups, which were administered sterilized distilled water) after 24-h feed deprivation (with free access to water), and gastric mucosal damage was induced by administering indomethacin 30 min after the initial treatment (except for the intact control group, which was administered sterilized distilled water). Individuals were identified by picric acid. All animals were treated in compliance with the "Guide for the Care and Use of Laboratory Animals"⁷⁾.

3. Experimental group formation and drug administration

Lab animals were divided in 7 groups, 8 individuals per group, as listed in Table 2: 1) Intact control group treated with sterilized distilled water after the initial administration of sterilized distilled water used as medium, 2) Indomethacin control group with indomethacin-induced gastric mucosal

damage after the administration of the medium sterilized distilled water, 3-7) Experimental groups with indomethacin-induced gastric mucosal damage after being administered omeprazole, DA-9601, and three different doses (200, 100, and 50 mg/kg) BK-1202, respectively (Table 2). BK-1202, omeprazole, and DA-9601 were dissolved in sterilized distilled water and orally administered to the animals using a 3-ml syringe with metallic probe at the ratio of 5 ml per 1 kg animal weight. The omeprazole and DA-9601 doses were set at 10 mg/kg⁸⁾ and 100 mg/kg⁹⁾, respectively, doses known to have sure efficacy in reducing indomethacin-induced gastric mucosal damage.

4. Induction of gastric mucosal damage

After 24-h feed deprivation of all animals, the two reference groups were administered with omeprazole and DA-9601, respectively, and the three experimental groups were administered with three different doses of BK-1202. After 30 min, a single dosage of 25 mg/kg indomethacin dissolved in sterilized distilled water was orally administered in accordance with the Guidobono method¹⁰⁾ to induce gastric mucosal damage. The two control groups were administered sterilized distilled water in the initial treatment. In the second treatment, the intact and indomethacin control groups were administered sterilized distilled water and 25 mg/kg indomethacin, respectively.

Table 2. Experimental design of this study

	Groups	Test article/Dose (mg/kg/day)
Control	Intact	Distilled water administered rats
	Indomethacin	Distilled water and indomethacin treated rats
Reference	Omeprazole	Omeprazole 10 mg/kg and indomethacin treated rats
	DA-9601	DA-9601 100 mg/kg and indomethacin treated rats
IGM	200	IGM 200 mg/kg and indomethacin treated rats
	100	IGM 100 mg/kg and indomethacin treated rats
	50	IGM 50 mg/kg and indomethacin treated rats

5. Measurement of visually inspected lesions

In accordance with the method proposed by Süleyman et al.¹¹⁾, all animals were sacrificed with cervical dislocation 6 h after being treated with indomethacin. From each animal, the stomach was harvested and, with the greater curvature cut open, fixed in 10% neutral buffered formalin for 24 h. The total surface area of the gastric mucosal lesions with hemorrhagic foci was estimated by the unit of mm² by overlapping a grid (area: 1 mm²) on the area of gastric mucosal damage.

6. Measurement of MPO activity

In accordance with the method proposed by Morai et al.¹²⁾, the gastric mucus layer was separated and homogenized in an ice-cold solution of 10 mL KCl (100 g/L; pH 7.4; Sigma, MO, USA), and the MPO content within the stomach tissue homogenate was measured using the method proposed by Bradley et al.¹³⁾ with a light modification as follows. The homogenate underwent 3 freeze-thaw cycles and centrifuged at 1500×g for 10 min at 4°C. Of its supernatant, 100 ml was harvested and added with 1 ml of 1.5 mM/L o-dianisidinehydrochloride (Sigma, MO, USA) containing 1.9 ml of 10 mM/l phosphate buffer (pH 6.0) and 0.0005% (w/v) hydrogen peroxide (Merck, CA, USA). Finally, PMO activity was estimated at the unit of $\mu\text{M}/\text{minute}/\text{mg}$ tissue by measuring absorbance at 450 nm with a UV-vis spectrophotometer (UV-3600, Shimadzu Scientific Instruments, CO, USA).

7. Measurement of lipid peroxidation

Lipid peroxidation was measured by the method of Ohkawa et al.¹⁴⁾, by measuring the content of malondialdehyde (MDA) in the prepared stomach tissue homogenate using thiobarbituric acid (Sigma, MO, USA). First, 0.5 ml homogenate was mixed with 0.2 ml solution containing 80 g/l sodium lauryl sulfate (Sigma, MO, USA), 1.5 ml 200 g/l acetic acid

(Merck, CA, USA), 1.5 ml 8 g/l 2-thiobarbiturate, and 0.3 ml sterilized distilled water. The mixed solution was heated to 98°C for 1 h and added with 5 ml n-butanol:pyridine (15:1) (Merck, CA, USA), which was mixed by shaking for 1 min. The solution was then centrifuged at 4000 rpm for 30 min, and the absorbance of the supernatant was measured at 532 nm. Finally, MDA was estimated at the unit of nM/g tissue by comparing the measured absorbance values with the standard curve predetermined using 1,1,3,3-tetramethoxypropane (Sigma, MO, USA).

8. Measurement of glutathione (GSH) content

Tissue GSH content was measured by the method of Sedlak and Lindsay¹⁵⁾. The harvested stomach was homogenized in 2 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 20 mM EDTA (Sigma, MO, USA) and 0.2 mM sucrose (Merck, CA, USA) and immersed in 0.1 ml 25% trichloroacetic acid (Merck, CA, USA). Precipitates were then removed by centrifuging at 4200 rpm for 40 min at 4°C. After taking its supernatant, absorbance was measured at 412 nm using 5,5'-dithiobis(2-nitrobenzoic acid) (Sigma, MO, USA). Finally, GSH content was measured at the unit of nM/mg tissue.

9. Measurement of catalase activity

Catalase activity was measured by measuring absorbance of hydrogen peroxide (H₂O₂) decomposition at 240 nm in the presence of catalase¹⁶⁾. Catalase activity was defined as the amount of enzyme necessary for decomposing 1 nM H₂O₂ in one min at pH 7.8 and 25°C, and catalase was estimated the unit of mM/min/mg tissue.

10. Measurement of superoxide dismutase (SOD) activity

SOD activity was estimated at the unit of mM/min/mg tissue using the method proposed by Sun et al.¹⁷⁾. The amount of superoxide radicals

produced was estimated by measuring absorbance at 560 nm with respect to the formazan dye formation from xanthine and xanthine oxidase reacted with nitrotetrazolium blue (Sigma, MO, USA).

11. Histomorphometric analysis

Gastric fundus tissue was harvested, cut into cell layers, fixed in 10% neutral-buffered formalin over 18 h, dehydrated, and embedded in paraffin wax. The paraffin block was then sliced into 4 μ m sections. These tissue sections were stained with hematoxylin & eosin (H&E). The tissue samples thus prepared were analyzed under an optical microscope. In the histomorphometry, tissue damage such as gastric mucosal damage, edema, and hyperemia was estimated by means of semiquantitative scoring on a 4-grade scale (0 = normal, 1 = slight: mucosal surface injury, 2 = moderate: moderate-to-severe mucosal injury and edema, 3= severe: total mucosal damage). Additionally, using a conventional method used by Ku et al¹⁸⁾, the invasion rate of gastric mucosal damage [(thickness of the damaged gastric mucosa / thickness of the total gastric mucosa) \times 100, %] and the average mucosa thickness around the lesion were measured using CCD image analyzer (DMI-300, DMI, Korea).

Invasive Percentages of Lesions (%)

$$= (\text{Length of lesions on the crossly trimmed fundic walls} / \text{total thickness of crossly trimmed fundic walls}) \times 100$$

12. Statistical analysis

All numerical values were expressed as mean \pm standard deviation. Statistical analysis was performed using multiple comparison testing, and the equality of variance was tested with Levene's test. In case of equal variance, one way ANOVA test was used, followed by post-hoc testing with the least-significant differences (LSD) test, to estimate the minimum

difference required for the significance of between groups. When variances were unequal, Kruskal-Wallis non-parametric H test was used, and if significance was established, Mann-Whitney U (MW) test was performed to test the significance between groups. All statistical analysis was performed using SPSS for Windows (Release 14.0K, SPSS Inc., USA), and significance was considered to be present at the 5% level ($p \leq 0.05$). Moreover, the degree of indomethacin-induced gastric mucosal damage was estimated by a percent change between the intact control group and indomethacin control group using Eq. [2]. For a more concrete demonstration of the anti-ulcerative drugs, a percent change was performed between treatment group and indomethacin control group using Eq. [3].

$$= ((\text{Data of indomethacin control} - \text{Data of intact control}) / \text{Data of intact control}) \times 100$$

$$= ((\text{Data of administered groups} - \text{Data of indomethacin control}) / \text{Data of indomethacin control}) \times 100$$

Results

1. Changes in visually inspected lesions

In all of the 7 indomethacin-induced gastric mucosal damage groups, hemorrhagic gastric ulcers were observed throughout the gastric mucus layer in broad distribution. While indomethacin control group showed significantly greater ($p < 0.01$) increase in visually inspected lesions than the intact control group, the two reference groups (omeprazole- and DA-9601-treated groups) and three experimental groups (BK-1202-treated groups of three different doses) demonstrated significant decrease ($p < 0.01$) of visually inspected lesions compared with the indomethacin control group (Figs. 1, 2).

The indomethacin control group exhibited changes in the area affected by gastric mucosal damage as high as 833.71% with respect to the intact control

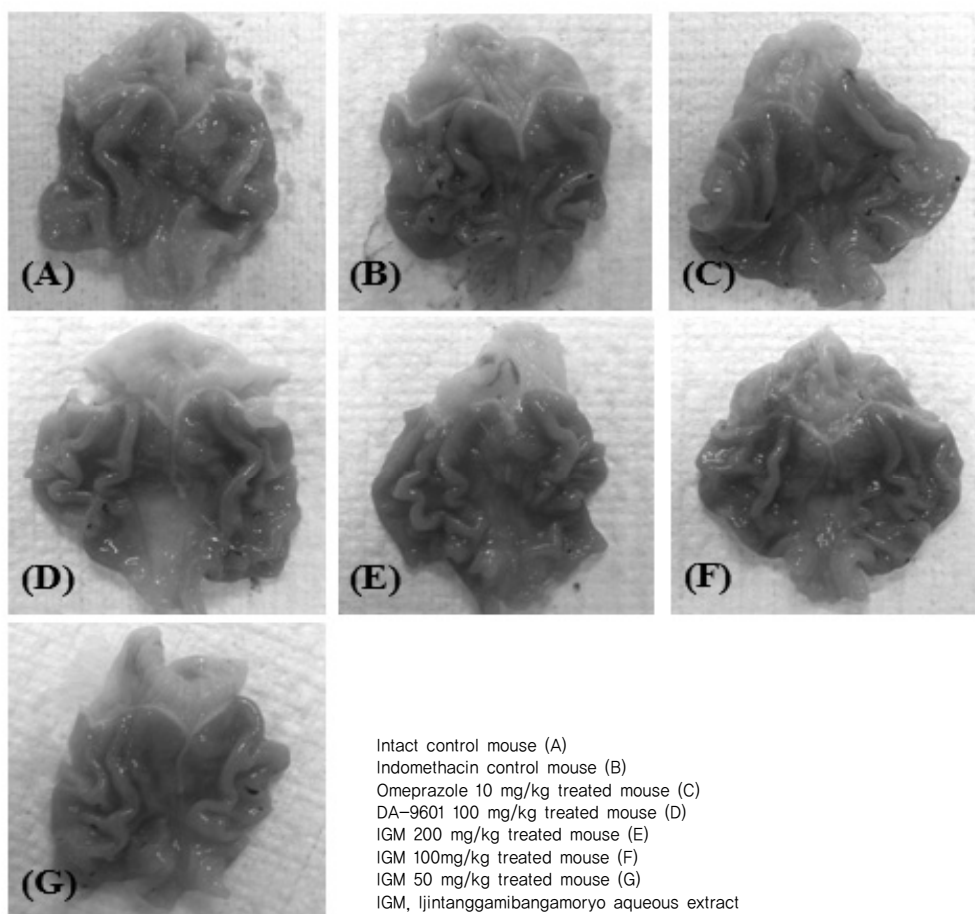


Fig. 1. Representative gross observations of the fundic mucosa

group. However, the groups treated with omeprazole, DA-9601, and 200, 100, and 50 mg/kg BK-1202 showed changes of -21.16, -35.88, -51.57, -36.40, and -22.09% with respect to the indomethacin control group.

2. Changes in MPO content

The indomethacin control group showed significantly greater ($p < 0.01$) increase in gastric MPO activity compared to the intact control group, with the amount of change of 302.09% with respect to the intact control group. In contrast, the two reference

groups (omeprazole- and DA-9601-treated groups) and three experimental groups (200, 100, and 50 mg/kg BK-1202-treated groups) demonstrated significant decrease ($p < 0.01$) in gastric MPO activity, -22.55, -31.06, -50.85, -33.02, and -33.91%, respectively, when compared with the indomethacin control group (Fig. 3).

3. Changes in lipid peroxidation

The indomethacin control group showed significantly greater ($p < 0.01$) increase in gastric MDA content, i.e. increase in lipid peroxidation, compared to the

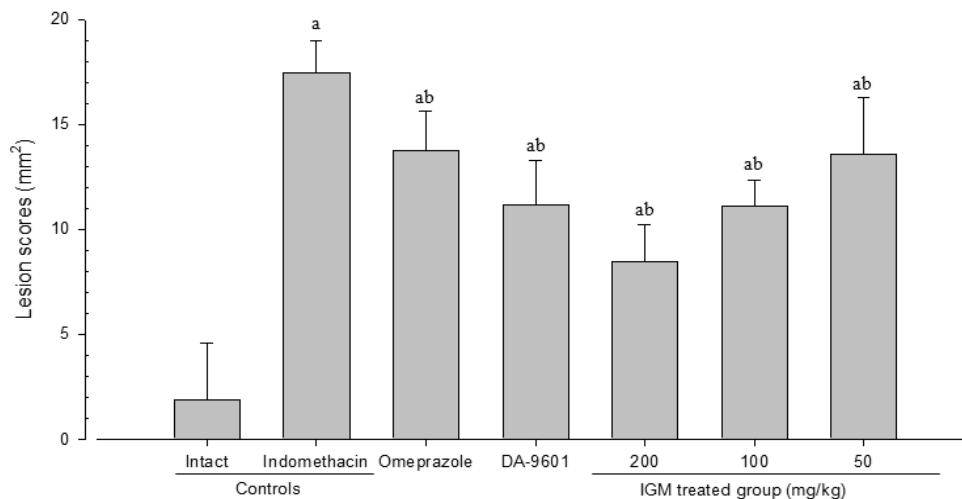


Fig. 2. Changes on the gastric lesion score
Values are expressed Mean±SD of eight rats
a p<0,01 as compared with intact control by LSD test
b p<0,01 as compared with indomethacin control by LSD test

intact control group, with the magnitude of change amounting to 570.04% with respect to the intact control group, whereas the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups demonstrated significant decrease ($p<0.01$) in gastric MDA content with changes of -21.51, -37.43, -48.98, -38.66, and -23.11%, respectively, when compared with the indomethacin control group (Table 3).

4. Changes in glutathione (GSH) content

The indomethacin control group showed significantly greater ($p<0.01$) decrease in gastric GSH content compared to the intact control group, exhibiting a change of -59.88% with respect to the intact control group. In contrast, the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups demonstrated significant increase ($p<0.01$ or $p<0.05$) in gastric GSH content, marking changes of 20.86, 33.98, 51.40, 34.70, and 22.15%, respectively, when compared with the indomethacin control group

(Table 3).

5. Changes in catalase activity

The indomethacin control group showed significantly greater ($p<0.01$) increase in gastric catalase activity compared to the intact control group, exhibiting 122.76% increase with respect to the intact control group. In contrast, the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups demonstrated significant decrease ($p<0.01$) in gastric catalase activity, exhibiting changes of -20.65, -32.49, -45.88, -34.04, and -22.27%, respectively, with respect to the indomethacin control group (Table 3).

6. Changes in superoxide dismutase (SOD) activity

The indomethacin control group showed significantly greater ($p<0.01$) decrease in gastric SOD activity compared to the intact control group, changing -42.73% with respect to the intact control group. In

Table 3. Changes on the anti-oxidative defense systems

Groups	Anti-oxidative defense systems			
	Lipid peroxidation (nM of MDA/g tissue)	Glutathione (nM/mg tissue)	Catalase (nM/min/mg tissue)	Superoxide dismutase (nM/min/mg tissue)
Controls				
Intact	2.37±0.58	4.35±1.07	79.63±13.92	138.38±18.73
Indomethacin	15.91±2.26 ^a	1.74±0.30 ^d	177.38±16.17 ^a	79.25±12.22 ^a
.....				
Omeprazole 10 mg/kg	12.48±1.42 ^{ab}	2.11±0.13 ^{de}	140.75±20.38 ^{ab}	97.63±4.34 ^{ac}
.....				
DA-9601 100 mg/kg	9.95±1.29 ^{ab}	2.34±0.36 ^{de}	119.75±20.67 ^{ab}	106.38±13.82 ^{ab}
.....				
IGM extracts				
200 mg/kg	8.12±1.86 ^{ab}	2.64±0.70 ^{de}	96.00±17.20 ^b	117.00±13.63 ^{ab}
100 mg/kg	9.76±1.84 ^{ab}	2.35±0.37 ^{de}	117.00±15.78 ^{ab}	108.13±14.51 ^{ab}
50 mg/kg	12.23±2.16 ^{ab}	2.13±0.18 ^{df}	137.88±17.82 ^{ab}	99.25±17.25 ^{ab}

Values are expressed as mean±S.D. of eight rats

IGM, Ijintangambangamoryo aqueous extract

^a p<0,01 as compared with intact control by LSD test

^b p<0,01 and ^c p<0,05 as compared with indomethacin control by LSD test

^d p<0,01 as compared with intact control by MW test

^e p<0,01 and ^f p<0,05 as compared with indomethacin control by MW test

contrast, the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups demonstrated significant increase ($p<0.01$ or $p<0.05$) in gastric SOD activity, marking changes of 23.19, 34.23, 47.63, 36.44, and 25.24%, respectively, when compared with the indomethacin control group (Table 3).

7. Histomorphometric changes

While the indomethacin control group exhibited typical manifestations of gastric ulcer, such as superficial desquamation, necrosis, and inflammatory cell infiltration, such symptoms were considerably suppressed in omeprazole-, DA-9601- and all BK-1202-treated groups (Fig 4). Moreover, while significant increases ($p<0.01$) were confirmed in the indomethacin control group in terms of the invasion rate of gastric mucosal damage, mean thickness of the gastric mucus layer around the lesion, and semiquantative score, significant decrease ($p<0.01$) in these pathological conditions were confirmed in the omeprazole-, DA-9601-, and all BK-1202-treated

groups compared to the indomethacin control group (Table 4).

The ratio of gastric mucosal damage in the indomethacin control group to that in the intact control group was as high as 5452.01%, whereas the same for the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups were -17.52, -53.92, -76.82, -55.81, and -18.85% with respect to the indomethacin control group.

The change in the gastric mucosa thickness in the indomethacin control group was 61.45% with respect to the intact control group, whereas the same for the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups were 33.33, 43.94, 67.68, 48.99, and 37.37% with respect to the indomethacin control group.

The change in the gastric mucosa thickness in the indomethacin control group was 61.45% with respect to the intact control group, whereas the same for the omeprazole- and DA-9601-treated reference groups

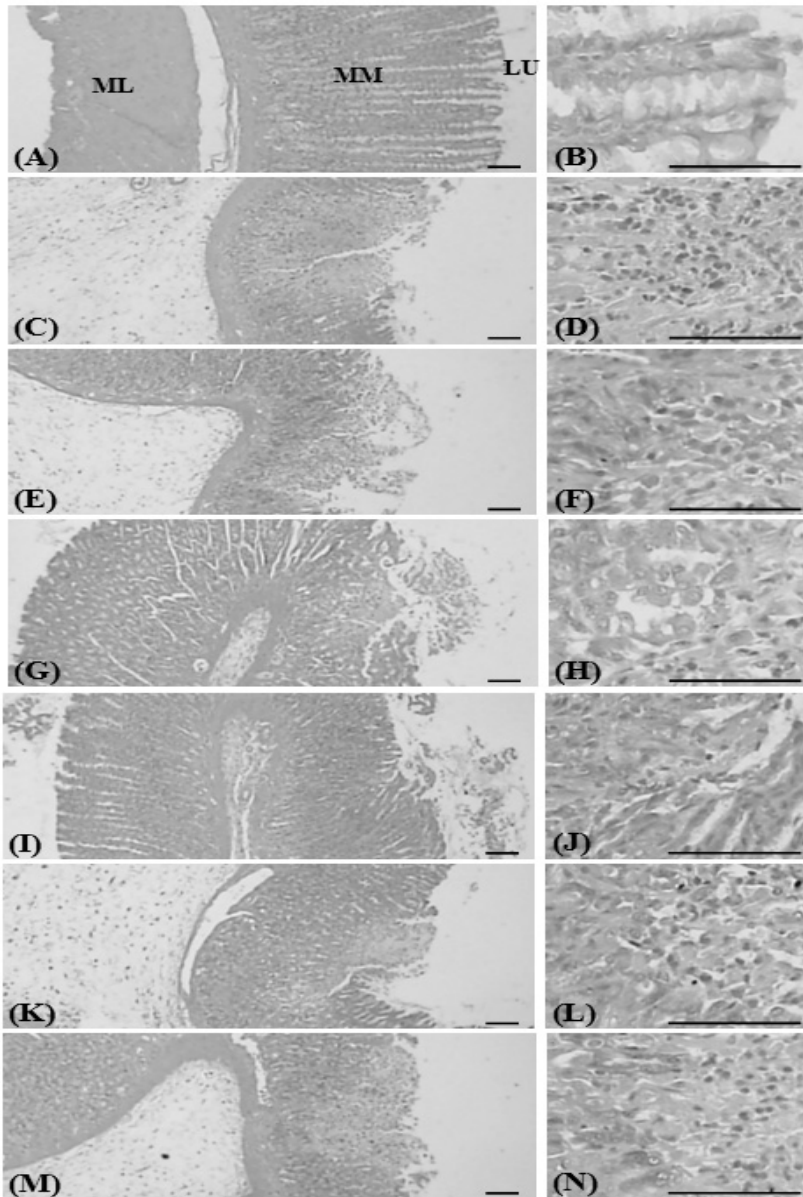


Fig 4. Microscopic appearance of fundic damages

Intact control rat (A, B)

Indomethacin control rat (C, D)

Omeprazole 10 mg/kg treated rat (E, F)

DA-9601 100 mg/kg treated rat (G, H)

IGM 200 mg/kg treated rat (I, J)

IGM 10 0mg/kg treated rat (K, L)

IGM 50 mg/kg treated rat (M, N)

LU, lumen; ML, mucosa layer; SL, submucosa layer; MM, muscle layer

All Hematoxylin-Eosin stain

Scale bars = 200 μ m

Table 4. Changes on the fundic histomorphometrical analyses

Groups	Fundic histomorphometrical measurement		
	Semiquantitative scores (Max = 3)	Invaded % of lesions into the gastric mucosa	Mean gastric mucosa thicknesses (mm)
Controls			
Intact	0.25±0.46	1.53±1.04	1.93±0.38
Indomethacin	2.75±0.46 ^a	84.74±5.56 ^d	0.74±0.10 ^d
Omeprazole 10mg/kg	2.00±0.53 ^{ac}	69.89±7.23 ^{de}	0.99±0.12 ^{de}
DA-9601 100mg/kg	1.13±0.35 ^{ac}	39.04±2.68 ^{de}	1.07±0.14 ^{de}
IGM extracts			
200 mg/kg	0.88±0.64 ^{bc}	19.64±6.00 ^{de}	1.25±0.20 ^{de}
100 mg/kg	1.00±0.53 ^{ac}	37.44±6.54 ^{de}	1.11±0.14 ^{de}
50 mg/kg	1.75±0.46 ^{ac}	68.77±6.82 ^{de}	1.02±0.12 ^{de}

Values are expressed as mean±S.D. of eight rat

IGM, Ijintanggamibangamoryoaqueousextract

^a p<0,01 and ^b p<0,05 as compared with intact control by LSD test

^c p<0,01 as compared with indomethacin control by LSD test

^d p<0,01 as compared with intact control by MW test

^e p<0,01 as compared with indomethacin control by MW test

and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups were 33.33, 43.94, 67.68, 48.99, and 37.37% with respect to the indomethacin control group.

The change in the semiquantitative score in the indomethacin control group with respect the intact control group was as high as 1000.00%. In contrast, the omeprazole- and DA-9601-treated reference groups and the 200, 100, and 50 mg/kg BK-1202-treated experimental groups showed semiquantitative score changes of -27.27, -59.09, -68.18, -63.64, and -36.36% with respect to the indomethacin control group.

Discussion

Nonsteroidal anti-inflammatory drugs (NSAIDs) enjoy increasing demands, not only for their effects as analgesics, but also for their preventive effects against malignant tumors, stroke, eclampsia, and Alzheimer's-type dementia¹⁹. However, 25% of all emergency gastric ulcer cases are known to be ascribable to NSAIDs²⁰, and various factors such as stress, fasting, and *Helicobacter pylori* infection

contribute to exacerbating the NSAID-associated gastric ulcers²¹. Gastric mucosa offering various defense systems are thereby impaired for a variety of reasons²², and oxygen-derived free radicals are known to play a very important role in inducing gastric mucosal damage²³. In fact, anti-oxidative agents are known to have either protective or therapeutic effects on various gastrointestinal damages²⁴. Indomethacin is used to induce gastric ulcer in lab animals by producing ROS²⁵, and such indomethacin-induced gastric ulcers can also be treated or preventive by various antioxidants²⁶⁻⁷. Anti-oxidative effects Original Ijintang and Ijintang -Gamibang have been studies²⁸⁻⁹, and *Coptidis rhizoma* contained in BK-1202 is also known to have anti-oxidative effects³⁰.

Assuming that BK-1202 would have superior effects on indomethacin-induced gastric ulcer owing to its antioxidative effects, we performed histomorphometric analyses of the area of visually inspected lesions, MPO activity, and lipid peroxidation as well as histomorphometric analysis of gastric mucosa, in order to determine the protective effect of BK-1202 on the indomethacin

-induced gastric mucosal damage by estimating GSH content, which is the anti-inflammatory protective and anti-oxidative defense system, catalase activity, and SOD activity. The analysis and measurement results were compared with those of the references groups treated with the PPI-based omeprazole (10 mg/kg) and DA-9601 (100 mg/kg).

Results of the observational examination of visually inspected changes in gastric mucosal lesions confirmed that indomethacin administration increased ulcerative lesions and substantial increase in hemorrhagic lesions compared with the intact control group. Change in the area of ulcerative lesions formed on the gastric mucosa is a marker for the direct efficacy of candidate drug^{8,31)}, whereby the smaller the ulcerative lesion area formed, the higher its efficacy. The greatest decrease in the visually inspected gastric mucosal lesions was demonstrated by 200 mg BK-1202, followed by 100 mg BK-1202, 100 mg DA-9601, 50 mg BK-1202, and 10 mg omeprazole.

In terms of change in MPO content, indomethacin induced significant increase in the gastric MPO activity, but it decreased by the effects of 200 mg BK-1202, 50 mg BK-1202, 100 mg DA-9601, 100 mg BK-1202, and omeprazole 10 mg in decreasing order of efficacy. MPO is a peroxidase enzyme secreted by the neutrophil, and increase in MPO activity in the gastric mucosal tissue is used as a marker for increased neutrophils in the gastric mucosal tissue in various gastric mucosal damage³²⁻³⁾. In NSAID-associated gastric mucosal damage as well, MPO activity is known to increase due to the infiltration of neutrophils in the induced ulcerative lesions³⁴⁾. The decrease in MPO activity mediated by BK-1202 is hence considered a direct proof of BK-1202's anti-inflammatory effect.

Changes in lipid peroxidation demonstrated the effects of the drugs under investigation in suppressing the increase in MDA content in the order of 200 mg BK-1202, 100mg BK-1202, 100 mgDA-9601, 50 mg

BK-1202, and 10 mg omeprazole, thus confirming superior anti-oxidative effect of BK-1202. Increase in MDA content, which is the product of lipid peroxidation, is known to be an important cause of gastric mucosal damage caused by NSAIDs including indomethacin³⁵⁾, whereby the toxic products of lipid peroxidation are known to be responsible for the oxidative destruction of the adjacent tissues³⁶⁻⁷⁾.

Indomethacin-induced gastric mucosal damage has been known to trigger substantial increase in catalase activity³⁸⁾, which is an important enzyme changing hydrogen peroxide into hydrogen³⁹⁾. In this experiment as well, indomethacin triggered significant increase in catalase activity in the gastric mucosal tissue, which was then suppressed by 200 mg BK-1202, 100 mg BK-1202, 100 mg DA-9601, 50 mg BK-1202, and 10 mg omeprazole in decreasing order of catalase inhibition effect. This result can serve as a proof of the anti-oxidative effect of BK-1202.

SOD activity and GSH content, which were reduced under the effect of indomethacin, increased under the effects of 200 mg BK-1202, 100 mg BK-1202, 100 mg DA-9601, 50 mg BK-1202, and 10 mg omeprazole in decreasing order of increasing SOD activity and GSH content. SOD is a highly efficient representative enzyme in the anti-oxidative defense system, and GSH is a representative endogenous antioxidant and is known to decrease substantially in the presence of NSAID-associated gastric mucosal damage^{31,39)}. Indomethacin-induced ROS formation leads to increase in SOD activity, representative endogenous antioxidant enzyme, and GSH is known to resist the ROS-induced gastric mucosal damage³⁵⁾, and secondary decrease in SOD activity and GSH depletion is known to trigger considerable gastric mucosal damage^{11,41)}.

Under the histomorphometric aspect, indomethacin triggers typical gastric ulcer symptoms concomitant with local superficial desquamation, necrosis, and inflammatory cell infiltration^{21,42)}, and the decrease in

the extent and number of histomorphometrically analyzed ulcerative lesions after the administration of the candidate drugs serves as a direct proof of their anti-ulcerative effects. In this study as well, the visually inspected gastric mucosal lesions were in good agreement with the histomorphometrically analyzed superficial desquamation, necrosis, and hyperemia. In the indomethacin control group, significant increase in lesion invasion rate and semiquantitative score was observed along with significant decrease in the mucosal thickness. However, such histomorphometrically analyzed ulcerative lesions substantially decreased under the effects of BK-1202.

Taking these results together, it can be concluded that 200 mg BK-1202 has an excellent effect in mitigating indomethacin-induced gastric ulcers by virtue of its anti-inflammatory and antioxidative effects, whereas 100 mg BK-1202 demonstrated similar level of anti-ulcerative effect to the same dose DA-9601, and 50 mg/kg BK-1202 was comparable to 10 mg/kg omeprazole.

Conclusion

The results of this study, which was conducted to evaluate the anti-ulcerative effects of BK-1202 using Indomethacin-induced gastric ulcer rat model, can be summarized as follows.

1. Changes in visually inspected gastric mucosal lesions: significant decrease in the area of gastric mucosal lesions through the effect of BK-1202 was confirmed.
2. Changes in MPO content: significant decrease in MPO content through the effect of BK-1202 was confirmed.
3. Changes in lipid peroxidation: significant decrease in lipid peroxidation through the effect of BK-1202 was confirmed.
4. Changes in GSH content: significant increase in GSH content through the effect of BK-1202 was confirmed.
5. Changes in catalase activity: significant increase in catalase activity through the effect of BK-1202 was confirmed.
6. Changes in SOD activity: significant increase in SPD activity through the effect of BK-1202 was confirmed.
7. Histomorphometric changes: histomorphometric ulcerative lesions decreased substantially through the effect of BK-1202.

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