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Gastroprotective effects of the isopropanol extract of *Artemisia princeps* and its gastroretentive floating tablets on gastric mucosal injury

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In this study, we investigated the gastroprotective effect of an isopropanol extract from the aerial parts of *Artemisia princeps* (IPAP) and developed a gastroretentive floating tablet of IPAP (IPAP-FR) for maximized local gastroprotective effects. Pre-treatment with IPAP ameliorated the gastric mucosal hemorrhagic lesions in ethanol/HCl- or indomethacin-treated rats. IPAP decreased mucosal hemorrhage of gastric ulcers induced by ethanol or indomethacin plus pyloric ligation in rats. The optimized floating tablet, IPAP-FR, floated on medium surface with more sustained eupatilin release compared to the non-floating control tablet. X-ray photographs in beagle dogs showed that IPAP-FR was retained for > 2 h in the stomach. In the ethanol-induced gastric ulcer rat model, the gastric hemorrhagic lesion was improved more substantially with IPAP-FR compared to the non-floating control tablet. Based on these data, our data suggest that IPAP-FR has an improved therapeutic potential for the treatment of gastric ulcer.

Keywords: Artemisia princeps Pamp., antiulcer, eupatilin, floating drug delivery system, X-ray photographs

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Peptic ulcer is a disturbance in the lining of the esophagus, stomach, or small intestine, manifested by irritating symptoms such as burning pain, bloating, heartburn, nausea, or vomiting (1). It is a common and recurrent disease, affecting 10 % of the general population globally. It is caused by disturbances in the equilibrium between the gastric aggressive factors (acid, pepsin, and *Helicobacter pylori*) and the mucosal defensive factors (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of mucosal cells) (2). Drugs commonly used in the treatment of gastric ulcers are antacids, anticholinergics, proton pump inhibitors, and H_2-receptor antagonists. However, current therapy of peptic ulcers may fail due to inadequate effectiveness, hypersensitivity, or adverse effects such as gynecomastia, impotence, arrhythmia, and hematopoietic changes although rare (3). This has led to the need to develop new antiulcer drugs, so the search for novel molecules has been extended to medicinal plants, which can offer better protection and reduce relapses (4).

Drug delivery systems with prolonged gastric residence time (GRT) may provide an innovative and advanced therapeutic option for gastric ulcers by retaining the drug for a longer period of time in the stomach (5). Several strategies are used to extend GRT of dosage forms, such as, superporous hydrogel, high-density (sinking), mucoadhesive, expandable, bioadhesive, magnetic, or low density (floating) systems (6). Among them, floating drug delivery systems allow the formulation to remain buoyant in the stomach without affecting the gastric emptying rate and thereby enable prolonged release of the drug, making it potentially more effective to treat gastric ulcers compared to conventional dosage forms. Several floating system approaches have been designed and developed, including: the gas generating system, raft forming system, colloidal gel barrier system, microporous compartment system, floating microsphere system and the low density system (7).

Artemisia princeps Pampanini (Asteraceae), commonly known as 'Sajabalssuk' in Korea, is commonly used for the management of various diseases including colic pain, diarrhea, uterine metrorrhagia, metritis, vomiting, ulcers, dysmenorrhea, microbial infections, and cancer (8). It may be used alone for the management of mild diseases or in combination with other medicinal herbs and/or conventional evidence-based drug therapy for the management of moderate to severe diseases as a traditional medical treatment option in Asian countries (8). It is well known that *Artemisia* possesses many biological active phytochemicals such as flavonoids, alkaloids, phenols, steroids, and essential oils. Eupatilin (5,7-dihydroxy-3′,4′,6 trimethoxy flavone) is the main active compound isolated from *Artemisia* plants and has a variety of biological activities, such as anti-inflammatory, anti-oxidative, cytoprotective, and pro- or anti-apoptotic effects (9, 10). Many *Artemisia* species have biological activities, such as anti-inflammatory, anti-bacterial, anti-asthmatic, anti-cancerous, and neuroprotective activities. Ethanol extract of *A. asiatica* has been reported to possess anti-oxidative and anti-inflammatory effects on experimentally induced gastrointestinal damage as well as hepatic and pancreatic lesions (11, 12). Based on these pharmacological properties, the first antipeptic ulcer drug prepared from ethanol extracts of *A. asiatica*, Stillen™, was developed to treat patients with gastric mucosal ulcers and is commercially available in South Korea and other Asian countries (13). One of the problems associated with the ethanol extracts of *Artemisia* is the risk of coagulopathy due to dicoumarol in ethanol extracts (14). To minimize this risk, isopropanol extract was chosen in our current study because dicoumarol is not extracted if isopropanol is used as the extraction solvent. Furthermore, compared to ethanol extract, the isopropanol extract of *A. princeps* contains

more eupatilin and jaceosidin, the major active flavonoids in *Artemisia*. Therefore, the isopropanol extract of *A. princeps* is expected to have more potent pharmacological actions and fewer toxic or unwanted side effects. Although various biological activities of *A. princeps* have been demonstrated, including anti-cancer, immunomodulatory, anti-oxidant, anti-diabetic and antiatherosclerotic activities (15, 16), no report has been published on gastroprotective or anti-ulcer activities of isopropanol extracts from the aerial parts of *A. princeps* (IPAP). In this study, we investigated the gastroprotective potential of IPAP in rats with ethanol and HCl or indomethacin-induced gastric ulcer with or without pylorus ligation. In addition, we have applied floating drug delivery systems to IPAP to enhance its pharmacological potency by improving gastric retention.

EXPERIMETAL

Chemicals and reagents

Sodium bicarbonate was obtained from Dr. Paul Lohmann (Lüneburg, Germany). Hydroxypropylmethylcellulose (HPMC) was purchased from Kanto Chemical (Japan). HPLC-grade formic acid and acetonitrile were purchased from Wako (Japan) and from Fisher Scientific Korea (South Korea), respectively. Hydroxypropyl celluloses, hypromellose (Metolse 60 SH-10,000), Carbomer, calcium silicate (Florite-RE), lactose hydrate (Pharmatose 100M), croscarmellose sodium, microcrystalline cellulose (Comprecel D101), sodium carboxymethylcellulose (CMC-Na), ethanol, indomethacin, and sodium lauryl sulfate (SLS) were purchased from Sigma Chemical Co. (USA). Reference compounds of chrologenic acid (purity ≥ 98.0 %), luteolin (purity ≥ 97.0 %) and 3,5-di-caffeoylquinic acid (purity ≥ 95 %) were purchased from Sigma-Aldrich (USA). Reference compounds of jaceosidin (purity > 95 %) and eupatilin (purity > 98 %) were purchased from Chengdu Biopurify Phytochemicals Ltd (China).

Preparation of EAP and IPAP

Aerial parts of *Artemisia princeps* Pampanini were dried (2.0 kg), cut, and extracted in 20 volumes of isopropanol at room temperature for 48 h. Extracted solutions were filtered and the filtrates were evaporated at 60 °C. IPAP was prepared by concentrating the extracted solutions to one-twentieth (100 g, *m*/*m*) of the initial herbal weight. IPAP was then tested according to an in-house quality assurance procedure for physicochemical properties and microorganism and heavy metal contaminants. A standardized 95 % ethanol extract from the aerial parts of *A. princeps* (EAP) was obtained from Dongbang FTL (Seoul, Korea).

UPLC-PDA-ESI-MS analysis

A Waters Acquity^{TM} H-class ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, USA) equipped with a photo diode array (PDA) detector and a JMS-T100TD (AccuTOF) (JEOL Ltd., Tokyo, Japan) spectrometer with an electrospray ionization (ESI) source were used for chromatographic and mass spectrometric (MS) analyses. For the UPLC-MS analysis, IPAP (5 mg) was dissolved in methanol (1 mL). Reference

Table I. Compositions of the IPAP tablet and various floating tablets (F1-F4) consisting of different ingredients

Process	Object	Ingredient	IPAP tablet	Floating tablets			
				F1	F2	F3	F4
Granulation	active	IPAP (mg)	60 (17.9)[a]	90 (18.0)	90 (16.1)	90 (29.6)	90 (25.3)
	binder	hydroxypropyl cellulose (mg)				12 (3.9)	12 (3.4)
	filler	MicroceLac® 100 (mg)	246 (73.4)				
		lactose hydrate (mg)		195 (39.0)	195 (34.8)	48 (15.8)	48 (13.5)
		microcrystalline cellulose (mg)		150 (30.0)	150 (26.8)	54 (17.8)	54 (15.2)
	gas generating agent	sodium bicarbonate (mg)		60 (12.0)	60 (10.7)	60 (19.7)	60 (16.9)
	erosion polymer	Eudragit® E100 (mg)	7 (2.1)				
	disintegrating agent	croscarmellose sodium (mg)	12 (3.6)		60 (10.7)	20 (6.6)	20 (5.6)
	lubricant	calcium silicate				18 (5.9)	18 (5.1)
	glidant	Aerosil® 200 (mg)	5 (1.5)				
Mixing	controlled release agent	carbomer (mg)					30 (8.4)
		hypromellose (mg)					20 (5.6)
	lubricant	magnesium stearate (mg)	5 (1.5)	5 (1.0)	5 (0.9)	5 (1.6)	2 (0.6)
	glidant	talc (mg)					2 (0.6)
Total (mg)			335	500	560	304	356

[a] Numbers in parentheses indicate the content (%).

standard compounds (0.5 mg mL^{-1}) were dissolved separately in methanol and then mixed together into a cocktail solution, which was used as a reference standard solution. The sample solution and reference standard solution were filtered through a 0.2-μm PTFE syringe filter (Whatman, UK) before injection into the UPLC system.

Animals

Male Sprague-Dawley rats weighing 230-300 g and male beagle dogs weighing 9–10 kg were obtained from Orient Bio (Seoul, Korea) and maintained under constant laboratory conditions and (temperature: 20 ± 2 °C, humidity: 40–60 %, light/dark cycle: 12 h). All experiments were conducted in accordance with the Standard Operation Procedure for Animal Care and Experiments (SOP-ANC) of the Korea Animal Medical Science Institute. All animal experimental protocols were approved by the Animal Ethical Committee of Korea of the Animal Medical Science Institute (189, Donggureung-ro, Guri-si, Gyeonggi-do, Republic of Korea); Approval number: KAMSI IACUC 15-KE-048, KAMSI IACUC 15-KE-050, KAMSI IACUC 15-KE-132, KAMSI IACUC 15-KE-132).

Ethanol-induced ulcer in rats

IPAP and EAP were dissolved in 1 % HPMC solution in distilled water before use. IPAP (30, 60, or 90 mg kg^{-1}), EAP (60 mg kg^{-1}), or vehicle (1 % HPMC) was administered orally to rats (n = 10/group) after fasting for 24 h. One hour after the tested sample treatment, the rats received intragastrically 5 mL kg^{-1} of 60 % ethanol in 150 mM HCl *via* the intragastric route to induce acute gastric ulcer. One hour later, the rats were sacrificed by cervical dislocation; the stomachs were then removed and the area of the lesions was measured. Percent of lesion inhibition was estimated by:

$$\text{lesion inhibition (\%)} = (\text{lesion area of vehicle control group} - \text{lesion area of test group}) / (\text{lesion area of vehicle control group}) \times 100$$

Indomethacin-induced ulcer in rats

One hour after the tested sample treatment, rats received indomethacin in 0.5 % CMC-Na solution in distilled water (100 mg kg^{-1}, *p.o.*) to induce acute gastric ulcer. Six hours later, the rats were sacrificed by cervical dislocation, the stomachs were removed and then the area of the lesions was measured.

Ethanol plus pyloric ligation-induced ulcer rat model

Acute gastric damage was induced with 70 % ethanol administered *via* the intragastric route to rats after fasting for 24 h. Thirty min later, pyloric ligation was performed under anesthesia with intramuscular injection of 15 mg kg^{-1} tiletamine/zolazepam (Zoletil50®; Virbac Lab., France) and 9 mg kg^{-1} xylazine (Rompun®; Bayer, Germany). The abdomen was opened below the xiphoid process, and the pyloric end of the stomach was ligated avoiding injuries to the adjacent blood vessels. Afterwards, the ligated stomach was returned to its original position and the abdomen was sutured. After 30 min, IPAP (90 mg kg^{-1}) or rebamipide (100 mg kg^{-1}) was administered orally. After 10 min or 120 min, the rats were sacrificed by cervical dislocation, the stomachs were removed, and the area of the lesions was measured.

Indomethacin plus pyloric ligation-induced ulcer rat model

To induce gastric damage, 100 mg kg^{-1} indomethacin (in 0.5 % CMC-Na) was orally administered to the rats after fasting for 24 h. After 4 h, gastric pylorus was ligated under anesthesia using the same procedure as described above.

Preparation of IPAP or IPAP FR tablets

IPAP tablets were prepared by the conventional wet granulation method (17). IPAP (60 mg) and MicroceLac 100 (246 mg, as a filler) were kneaded with ethanol in a high-speed mixer and granulated. Granules were dried using a fluidized bed dryer (inlet temp. 50 ± 10 °C, product temp. 35 ± 5 °C, drying time 20 min). Dried granules were sieved using an oscillator (20 mesh). Moisture content was measured using a moisture analyzer (< 2.0 %). Granules were mixed with additives (7 mg of Eudragit® E100 as an erosion polymer, 12 mg of croscarmellose sodium as a disintegrating agent and 5 mg of silicon dioxide Aerosil® 200 as a glidant) and then pressed to form capsule-shaped tablets using a single-punch tablet pressing machine (Sejong, Korea). Tableting force was 300–500 kg/cm^2, tablet hardness was 5.0–10.0 kp and dimensions were 13.0 × 7.0 × 4.0–4.8 mm.

IPAP gastroretentive floating tablets were prepared so that IPAP, hydroxypropyl cellulose, lactose hydrate, microcrystalline cellulose, sodium bicarbonate, croscarmellose sodium and calcium silicate were kneaded with ethanol in a high-speed mixer and granulated. Granules were sieved using an oscillator (20 mesh) and were then dried using a fluidized bed dryer (inlet temp. 70 ± 10 °C, product temp. 40 ± 5 °C, drying time 20 min). Moisture content was measured using a moisture analyzer (< 2.0 %). Dried granules were sieved with the aid of an oscillator (30 mesh). Granules were mixed with carbomer, hypromellose, magnesium stearate or talc using a V-mixer, and then pressed to form tablets using the single-punch tablet pressing machine. The formulation of gastroretentive floating tablets (F1, F2, F3, F4) was optimized by adjusting the amounts of ingredients. The four formulations of gastroretentive floating tablets (F1, F2, F3, F4) were prepared using varied concentrations of ingredients, as shown in Table I.

Assay of eupatilin tablets in pH 1.2 dissolution media (Solubility test)

Each gastroretentive floating tablet (F1-F4) was put into a vial containing 20 mL of hydrochloric acid buffers (pH 1.2). Vials were kept at 37 °C and rotated at a speed of 40 rpm using a dialysis tester for 24 h. Solutions were then passed through a 0.45-μm filter membrane, and the amount of eupatilin (representative standard of tested tablets) was measured using high-performance liquid chromatography (HPLC). HPLC analysis of eupatilin released from the tablets in solutions was conducted using a Waters Alliance HPLC system (Massachusetts, USA), which consisted of an e2695 pump and 2498 UV/Vis detector. Calibration curve was constructed by plotting various concentrations of eupatilin (0.04–4 μg mL^{-1}) with a correlation coefficient of > 0.999.

Dissolution rate of tablets at pH 1.2 (Determination of disintegration time)

The release of eupatilin from IPAP gastroretentive floating (F1 to F4) or IPAP tablets was investigated using the USP paddle method. The tablet was added to the dissolution medium (900 mL of acidic buffer solution at pH 1.2 supplemented with 0.5 % SLS) without a sinker and rotated at a speed of 50 rpm at 37.0 ± 0.2 °C (n =12). Samples were collected at specific time intervals (5, 10, 15, 30, 45, and 60 min) to analyze the amount of eupatilin using HPLC.

In vivo *buoyancy study*

Six male beagle dogs (9–10 kg) were used to assess *in vivo* floating behavior. They were divided into two groups where one received IPAP tablets while the other received IPAP-FR tablets. Animals were fasted overnight and were only allowed free access to water. For the radiographic study, an IPAP tablet and IPAP-FR tablets containing 50 mg barium sulfate as a radio opaque agent were prepared. An abdominal radiograph in the standing position was taken every 5 min after administration of the tablet until the absence of radio-opaque material in the stomach using an X-ray machine (Arcadis Varis, Simens, USA).

Preparation of mini-IPAP FR or IPAP tablet formulae for the in vivo *test*

The same formula (Table I) was prepared, each tablet containing 22.5 mg of active ingredients (IPAP). Weights of mini-IPAP FR and mini-IPAP tablets were 89.0 mg and 125.6 mg, respectively. Simply, granules were pressed to produce tablets using the single-punch machine (2 mm diameter/tablet, Young Chang Punch Co., Ltd., Korea).

Therapeutic antiulcer activities of mini-IPAP-FR or IPAP tablets in ethanol-induced gastric ulcers in the rat model

Gastric damage was induced with 70 % ethanol administered *via* the intragastric route (18) to rats after fasting for 24 h. A mini-IPAP-FR or IPAP tablet was encapsulated in a size 9 gelatin capsule (Torpac, USA), and each capsule contained 22.5 mg of active ingredients (IPAP). A gelatin capsule containing IPAP-FR (90 mg kg^{-1}), IPAP tablet (90 mg kg^{-1}) or vehicle (only gelatin capsule) was administered orally to rats (n = 6/group) by oral gavage (Torpac, USA). After 2 h, rats were sacrificed by cervical dislocation, the stomachs were removed, and the area of the lesions was measured.

Statistical analysis

The results are presented as mean ± standard error of the mean (SEM) of animals per group. Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) followed by the post-hoc Dunn's test. P values lower than 0.05 were considered significant. All statistical tests were conducted using the Graph Pad Prism (California, USA).

RESULTS AND DISCUSSION

Phytochemical identification of IPAP by UPLC-PDA-ESI-MS

The UPLC chromatogram of IPAP monitored at 320 nm is shown in Fig. 1. Five peaks (chlorogenic acid, eupatilin, 3,5-dicaffeoylquinic acid, luteolin, and jaceosidin) were identified by direct comparison with the corresponding reference standards (Fig. 1). Detailed MS data and absorption maxima data of these peaks are listed in Table II. These compounds have been previously identified in *Artemisia princeps* (19, 20). The results show that the botanical origin of the raw material was re-confirmed by chemical fingerprinting, and the major components of the extract were anti-oxidant phenolic compounds such as eupatilin, jaceosidin, and 3,5-dicaffeoylquinic acid. IPAP was analyzed for major flavonoids, and

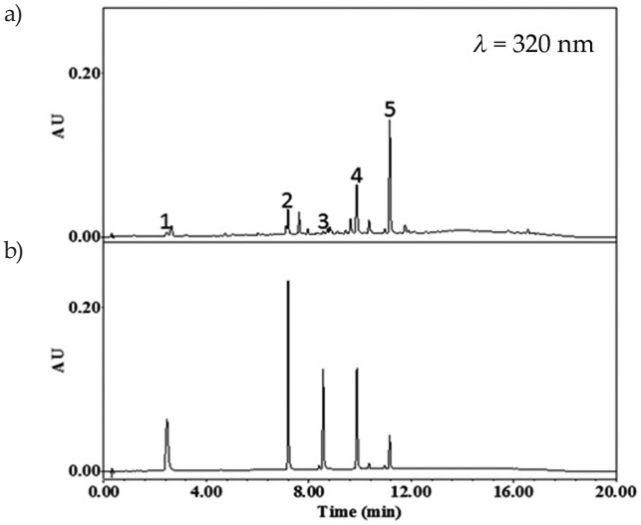

Fig. 1. UPLC chromatogram of: a) IPAP and b) reference standard solution.

eupatilin and jaceosidin were present at concentrations of 1.89 % (in-house quality assurance criterion: 0.8–2.4 %) and 0.72 % (in-house quality assurance criterion: 0.25–0.67 %), respectively, by HPLC (data not shown). Eupatilin and jaceocidin concentrations in EAP were 1.10 and 0.48 %, respectively.

Gastroprotective effect of orally administered IPAP in the ethanol/HCl- or indomethacin-induced rat ulcer models

The current study reports for the first time that IPAP had *in vivo* antiulcer activity in the ethanol/HCl- and indomethacin-induced gastric ulcer model (n = 10) in order to compare its pharmacological efficacy for EAP. Oral administration of ethanol accelerated gastric muco-

Table II. Retention time (R_t), precursor ion, molar mass, and UV maxima (λ_{max}) of identified peaks

Compound	R_t (min)	Precursor ion *(m/z)*	Molar mass (g mol^{-1})	λ_{max} (nm)
chlorogenic acid	2.45	355.10 $[M+H]^+$ 377.09 $[M+Na]^+$	354.10	219, 249, 326
3,5-dicaffeoylquinic acid	7.19	517.14 $[M+H]^+$ 539.12 $[M+Na]^+$	516.17	219, 245, 327
luteolin	8.57	287.06 $[M+H]^+$	286.05	253, 349
jaceosidin	9.88	331.08 $[M+H]^+$	330.07	212, 272, 345
eupatilin	11.17	345.10 $[M+H]^+$	344.09	214, 273, 343

Table III. Gastroprotective effects of IPAP on ethanol- or indomethacin-induced gastric ulcers in rats

Treatment (*p.o.*)		Dose (mg kg^{-1})	Hemorrhagic lesion (mm^2)			Inhibition (%)
Vehicle		0	0			–
IPAP		90	0			–
Ethanol/HCl	–	0	133.9	±	12.4	
	+ IPAP	30	46.9	±	7.9[a]	65.0
		60	15.0	±	4.3[a]	88.8
		90	9.4	±	3.2[a]	93.0
	+ EAP	60	28.1	±	5.0[a]	79.1
Indomethacin	–	0	31.3	±	7.4	0
	+ IPAP	30	14.4	±	3.7[a]	54.0
		60	10.3	±	1.7[a]	67.1
		90	15.0	±	3.5[a]	52.1
	+ EAP	60	20.0	±	6.0	36.1

Data are presented as mean ± SEM (n = 10/group).

[a] $p < 0.05$ *vs.* the ethanol/HCl-induced group or indomethacin-induced group.

sal necrosis and apoptosis due to damaged gastric mucosal defense system and, consequently, produced acute hemorrhagic gastric erosions (21). Thus, the ethanol-induced gastritis *in vivo* model is considered a reliable tool to evaluate gastritis pathogenesis (22). To investigate the *in vivo* ability of IPAP to prevent ethanol/HCl-induced acute gastric ulcer in rats, we measured the total mucosal hemorrhagic lesion area after oral administration of IPAP (30, 60 or 90 mg kg^{-1}) to rats. No mortality of rats at a high dose of 1 g kg^{-1} of body mass was observed in a toxicity test, indicating that IPAP was well tolerated by experimental rats. As shown in Table III, a single dose of 60 % ethanol in 150 mmol L^{-1} HCl (5 mL kg^{-1}, *p.o.*) severely increased mucosal hemorrhagic lesions to 133.9 ± 12.4 mm^2 compared to the vehicle-treated control group (0 mm^2), which is consistent with the results of a previous report (23). Pretreatment with IPAP (30, 60 or 90 mg kg^{-1}, *p.o.*) showed significant ($p < 0.05$) reduction in mucosal hemorrhagic lesions with inhibition of 68, 88.8, or 93 %, respectively, compared to the ethanol/HCl alone-treated group. Percent inhibition of mucosal hemorrhagic lesions was higher in rats pretreated with 60 mg kg^{-1} of IPAP (88.8 %) compared to those with EAP (79.1 % at 60 mg kg^{-1}), but statistical significance was not reached. Here, we have clearly demonstrated that the ethanol/HCl-induced group produced the expected characteristic intraluminal bleeding in the glandular portion of the stomach, while pretreatment with IPAP significantly protected rats with ulcers induced by ethanol and HCl from intraluminal bleeding. This result suggests that IPAP exerted an antiulcer effect associated with cytoprotective activity. To confirm the gastroprotective effect of IPAP, we evaluated its effect on the indomethacin-induced gastric ulcer model. Non-steroidal anti-inflammatory drugs (NSAIDs) cause gastric ulcers by oxidative damage and prostaglandin (PG) deficiency (24). According to a previous study (25), administration of indomethacin significantly induced ulcerative lesions; therefore, we used this method to induce ulcer lesions in rats. As shown in Table III, after

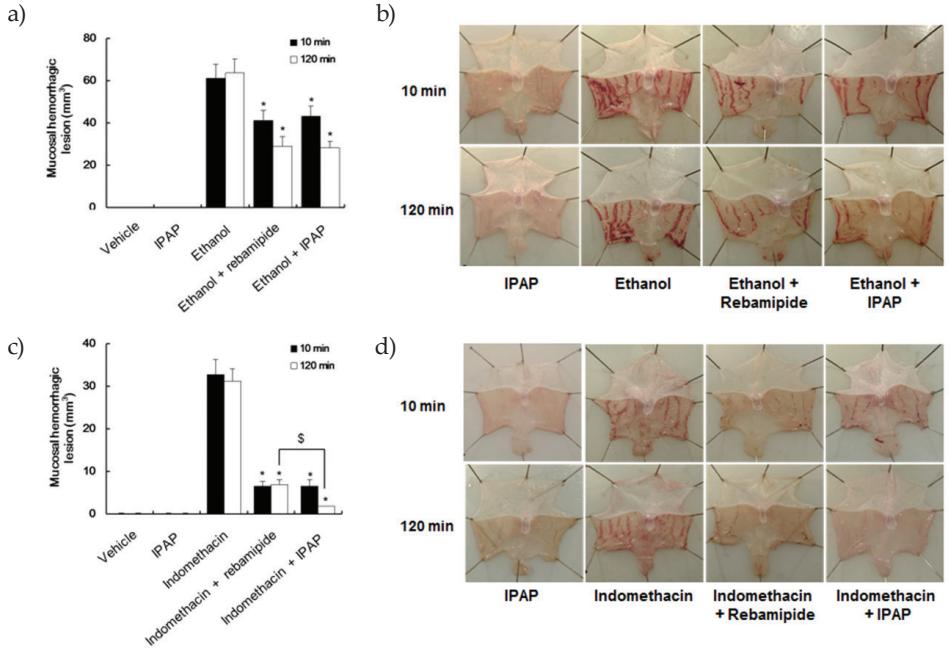

Fig. 2. Therapeutic effects of IPAP on the ethanol or indomethacin plus pyloric ligation-induced gastric ulcers in rats. Pyloric ligation was done 30 min after oral administration of 60 % ethanol (5 mL kg^{-1}, a and b) or 4 h after oral administration of indomethacin (100 mg kg^{-1}, *p.o.* c and d). After 30 min, rats were treated with IPAP (90 mg kg^{-1}, *p.o.*) or rebamipide (Reba, 100 mg kg^{-1}, *p.o.*, positive control) for 10 min or 120 min. Gastric lesions in the stomach were measured (a and c) and photographs of the lesions were taken (b and d). Data are presented as means ± SEM (n = 10/group). * $p < 0.05$ *vs.* the ethanol- or indomethacin-induced group. $^{\$}$ $p < 0.05$ *vs.* group treated with indomethacin plus rebamipide.

administration of indomethacin (100 mg kg^{-1}, *p.o.*), the area of observed ulcerative lesions was 31.3 ± 7.4 mm^2. IPAP (30, 60, or 90 mg kg^{-1}) or EAP (60 mg kg^{-1}, as a positive control) inhibited mucosal hemorrhagic lesions by 54, 67.1, 52.1, or 36.1 %, respectively, compared to their respective indomethacin-induced groups. This result indicates the gastroprotective potential of IPAP in the indomethacin-induced ulcer model. Pretreatment with EAP (60 mg kg^{-1}, *p.o.*) decreased the mucosal hemorrhagic lesions caused by indomethacin (20.0 ± 6.0 mm^2); however, the result lacked statistical significance. Therefore, the gastroprotective effect of IPAP was observed to be more potent than that of EAP at the same dose of 60 mg kg^{-1}. This result indicated that IPAP could be administered together with NSAIDs such as indomethacin in order to minimize adverse effects of NSAIDs on the gastric mucosa.

Therapeutic effects of IPAP on gastric ulcers induced by ethanol or indomethacin plus pyloric ligation in rats

Ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach and produces ulcers. These ulcers result from autodigestion of the gastric mucosa,

leading to a breakdown of the gastric mucosal barrier (26). In the antiulcer activity test using the ethanol plus pyloric ligation-induced ulcer rat model (Fig. 2a,b), the vehicle-treated control group and only IPAP-treated group (90 mg kg^{-1}, *p.o.*) did not show any significant gastric hemorrhagic lesions (0 mm^2). Intragastric administrations of ethanol plus pyloric ligation induced gastric ulcer with a significant increase in mucosal hemorrhagic lesions in rats (61.3 ± 6.4 mm^2; 10 min or 63.8 ± 6.6 mm^2; 120 min). After post-treatment with IPAP (90 mg kg^{-1}, *p.o.*) or rebamipide (100 mg kg^{-1}, *p.o.* as a positive control) for 10 or 120 min, mucosal hemorrhagic lesions were significantly suppressed compared to lesions without treatment in the ethanol-induced ulcer group ($p < 0.05$, Fig. 2a).

In the antiulcer activity test using the indomethacin plus pyloric ligation-induced ulcer rat model (Fig. 2c,d), the mucosal hemorrhagic areas of only indomethacin administered groups were 32.7 ± 3.6 mm^2 (10 min group) and 31.2 ± 2.9 mm^2 (120 min group), respectively. Post-treatment with IPAP (90 mg kg^{-1}, *p.o.*) or rebamipide (100 mg kg^{-1}, *p.o.*) significantly reduced the mucosal hemorrhagic lesions caused by indomethacin plus pyloric ligation in rats. Notably, the therapeutic effect of the 120-min IPAP-treated group was significant ($p < 0.05$) compared to the rebamipide-treated group in indomethacin plus pyloric ligation-induced ulcer rats. Macroscopically, severe visible lesions induced by ulcer to the gastric mucosa appeared as elongated bands of hemorrhage. However, the IPAP post-treatment group had considerably reduced gastric lesions in the mucosa compared to the ulcer-induced group without post-treatment. The vehicle-treated control rats or those given IPAP only (90 mg kg^{-1}, *p.o.*) did not develop any gastric hemorrhagic lesions (Fig. 2b,d). Our results confirm that IPAP has cytoprotective and therapeutic properties against various stimuli and might have anti-secretory properties that reduce secretion of gastric aggressive factors, such as acid and pepsin. However, further study will be needed to determine the mechanisms of IPAP action.

Dissolution rates of gastroretentive floating tablets of IPAP in pH 1.2 dissolution media

Use of a gastroretentive system that releases drugs in a sustained manner is likely to enhance the gastroprotective effects of IPAP. Therefore, we have formulated various gastroretentive floating tablets (F1-F4) of IPAP by using an effervescent floating matrix system, which was designed to cause the tablet to float in the gastric fluid and release eupatilin continuously. The drugs were formulated using sodium bicarbonate as a gas-generation agent in combination with acidic media of the stomach, carbomer and hypromellose as controlled release agents, and croscarmellose sodium as a disintegrating agent (27). Compositions of gastro-retentive floating tablets (F1-F4) are given in Table I. All gastro-retentive tablets F1-F4 contained 90 mg of IPAP. The non-floating IPAP tablet was prepared by the conventional wet granulation method without the previously mentioned specialty excipients. To optimize the tablet manufacturing process, we tested two methods: direct compression and wet granulation methods. Since the direct compression method was problematic due to inability of IPAP to mix homogeneously due to the low density of IPAP (< 0.7 g mL^{-1}), the wet granulation method was applied using ethanol and hydroxypropyl cellulose as the liquid binding solution.

Physicochemical characteristics of the IPAP tablet and gastroretentive floating tablets (F1-F4) are summarized in Table IV. F1 formulation was composed of granules, produced with IPAP as an active ingredient, microcrystalline cellulose and lactose hydrate as filler,

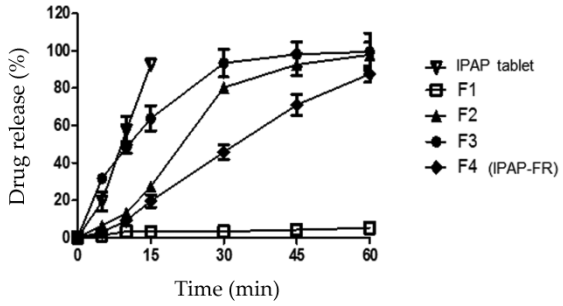

Fig. 3. Dissolution rates of eupatilin from gastoretentive floating tablets of IPAP compared to the IPAP tablet (n = 12) in pH 1.2 dissolution media. Release studies of the tablets were carried out using the USP paddle method, as described in the Method section. The formulations used are detailed in Table I.

and sodium bicarbonate as a gas-generating agent, but eupatilin was not released from the F1 tablet in the dissolution test due to lack of disintegration (Fig. 3). Therefore, croscarmellose sodium was added as a disintegrating agent to the F2 formulation. When disintegration continues and the tablets become smaller, the tablets are expected to float. The problem of F2 formulation was the inability of the tablet to float because of high density. F3 formulation was developed to reduce density by decreasing the amount of lactose hydrate and microcrystalline cellulose and to solubilize by adding calcium silicate as a lubricant. Microcrystalline cellulose was added to the F3 formulation to allow calcium silicate to adsorb the IPAP. F3 tablets showed a floating time of 10 min, but disintegrated within 30 min. For the purpose of delaying disintegration, 30 mg carbomer and 20 mg hypromellose were added as controlled release agents to formulation F3 to develop formulation F4. Tablet F4 floated for 12 min and disintegrated within 120 min, tablet F4 first swelled at the beginning of the dissolution test and then floated for 12 min. Solubility of

Table IV. The physicochemical properties of IPAP and F1-F4

	IPAP[a] tablet	F1	F2	F3	F4[a]
Mass (mg)	335	500	560	307	354
Friability (%)	0.5	0.2	0.8	1.2	0.3
Thickness (mm)	4.3	4.6	4.9	4.1	4.5
Content of eupatilin (%)	98.8	98.9	99.2	99.4	99.3
Disintegration time (min)	10	-	60	30	120
Floating time[b] (min)	-	-	45	10	12
Solubility (μg mL^{-1})	30.0	45.5	48.6	76.4	68.1

[a] IPAP-FR

[b] floating time – time for tablets to float above the surface

these tablets was studied in pH 1.2 USP buffer solutions using eupatilin as an active ingredient. Solubility of tablet F4 was 2.3 times (68.1 μg mL^{-1}) higher compared to the IPAP tablet (30.0 μg mL^{-1}).

Fig. 3 shows eupatilin release in the dissolution test of F1, F2, F3, F4, or the control IPAP tablet in pH 1.2 dissolution media. Addition of croscarmellose and/or hydropropyl cellulose into the matrix (F2, F3, and F4) increased the dissolution rate compared to sodium bicarbonate addition only (F1). Controlled release agents (carbomer and hypromellose) in formulation F4 markedly extended dissolution profiles compared to tablets prepared without these agents (F2 and F3). Disintegration time of tablet F4 was time-dependently sustained up to 60 min compared to those of IPAP (F1, 10 min), F2 (60 min) and F3 (30 min), whereas F1 did not show any integration up to 60 min (Fig. 3). Collectively, formulation F4 was expected to float in the stomach and show local effects on the gastric mucosa. Therefore, formulation F4 (IPAP-FR) was selected as the optimized gastroretentive floating tablet of IPAP and was expected to show more potent pharmacological actions.

X-ray photographs taken after administering the IPAP- FR tablet to a beagle dog

X-ray is currently a very popular evaluation method for floating dosage forms. It helps locate dosage forms in the gastrointestinal tract and enables one to predict and correlate the gastric emptying time and the passage of dosage forms through the gastrointestinal tract. Here, the inclusion of 50 mg of barium sulfate as a radio-opaque material into a tablet enabled it to be visualized by X-rays (28, 29). To examine whether the IPAP-FR could remain floating in the stomach, evaluation using an X-ray apparatus was conducted in six male beagle dogs in the fasting state. Fig. 4 shows the radiographic images taken at different time periods after administration of the barium sulfate-loaded IPAP-FR and IPAP tablets to beagle dogs. IPAP tablet was observed to be buoyant on the fluid in the stomach at 5 min and then

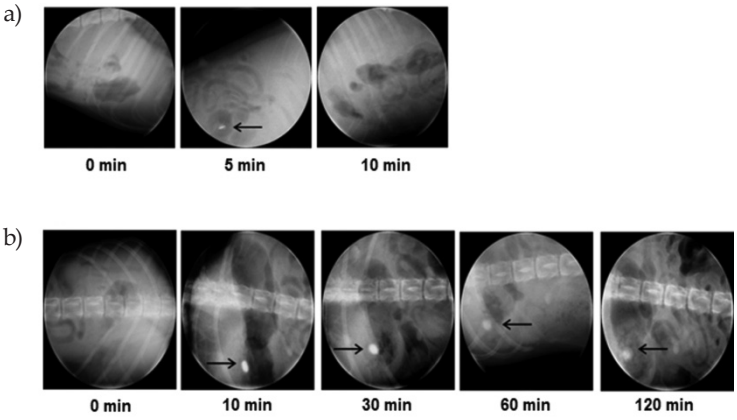

Fig. 4. Radiograph images of the: a) IPAP tablet (at 5 and 10 min) and b) IPAP-FR tablets (at 10, 30, 60, and 120 min) after oral administration to beagle dogs in the fasting state. Standing radiographs of the abdomen were taken 5 min after the ingestion of the tablet and then every 5 or 10 min, until the absence of the radio-opaque material in the stomach.

Table V. Therapeutic effects of the IPAP-FR tablet or IPAP tablet on 70 % ethanol-induced gastric ulcers in rats

Treatment (*p.o.*)		Dose (mg kg^{-1})	Hemorrhagic lesion (mm^2)			Inhibition (%)
Vehicle		0		0		–
Ethanol		0	44.8	±	3.3	0
IPAP tablet		90		0		–
IPAP- FR		90		0		–
Ethanol	+ IPAP-tablet	90	14.6	±	1.4[a]	67.4
	+ IPAP- FR	90	8.5	±	1.1[a,b]	81.0

Data are presented as means ± SEM (n = 5/group).

[a] $p < 0.05$ *vs.* the ethanol-induced group.

[b] $p < 0.05$ *vs.* the ethanol + IPAP tablet-treated group.

disappear 10 min after oral administration. However, IPAP-FR continued floating in the stomach for at least 2 h. Therefore, the IPAP-FR delivery system was suggested to be useful to attain longer sustained release of drugs in the fasting state. Since the gastric motility and stomach emptying of both dogs and humans show little difference (30), it could be deduced the IPAP-FR could show increased retention times in human stomach.

Therapeutic effects of IPAP-FR on the ethanol-induced rat model

Finally, we compared the therapeutic effects of IPAP-FR and IPAP tablets in the ethanol-induced ulcer rat model. To establish the ethanol-induced ulcer model, 70 % ethanol was administrated to fasting rats. As shown in Table V, ethanol markedly increased hemorrhagic lesion (44.8 ± 3.3 mm^2) in the stomach. As expected, post-treatment with the IPAP tablet or IPAP-FR (90 mg kg^{-1}, *p.o.*) significantly ameliorated the gastric hemorrhagic lesion (14.6 ± 1.4 and 8.5 ± 1.1 mm^2, respectively). More importantly, the IPAP-FR tablet (81.0 % inhibition) exhibited significantly higher healing effects than the IPAP tablet (67.4 % inhibition). These data suggest the superiority of the IPAP-FR tablet over the IPAP tablet owing to its prolonged therapeutic effect against gastric ulcer.

CONCLUSIONS

IPAP has favorable protective and therapeutic effects against ulcer damage in various gastric ulcer models. The IPAP-FR, a gastroretentive tablet of IPAP, was successfully developed by a floating system combination approach and showed improved properties in the ethanol--induced ulcer model. Both IPAP and IPAP-FR are potentially attractive therapeutic options for treating gastric ulcer.

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REFERENCES

1. A. F. Syam, M. Sadikin, S. I. Wanandi and A. A. Rani, Molecular mechanism on healing process of peptic ulcer, *Acta Med. Indones.* **41** (2009) 95–98.
2. J. H. Park, K. J. Jang, C. H. Kim, Y. H. Lee, S. J. Lee, B. H. Kim and H. M. Yoon, Ganoderma lucidum pharmacopuncture for the treatment of acute gastric ulcers in rats, *J. Pharmacopuncture* **17** (2014) 40–49; http://doi.org/10.3831/KPI.2014.17.025
3. A. A. Gohar and A. A. Zaki, Assessment of some herbal drugs for prophylaxis of peptic ulcer, *Iran J. Pharm. Res.* **13** (2014) 1081–1086.
4. V. Lakshmi, N. Singh, S. Shrivastva, S. K. Mishra, P. Dharmani, V. Mishra and G. Palit, Gedunin and photogedunin of *Xylocarpus granatum* show significant anti-secretory effects and protect the gastric mucosa of peptic ulcer in rats, *Phytomedicine* **17** (2010) 569–574; http://doi.org/10.1016/j.phymed.2009.10.016
5. S. S. Davis, Formulation strategies for absorption windows, *Drug Discov. Today* **10** (2005) 249–257; http://doi.org/10.1016/S1359-6446(04)03351-3
6. A. Streubel, J. Siepmann and R. Bodmeier, Gastroretentive drug delivery systems, *Expert. Opin. Drug Deliv.* **3** (2006) 217–233; http://doi.org/10.1517/17425247.3.2.217
7. P. L. Bardonnet, V. Faivre, W. J. Pugh, J. C. Piffaretti and F. Falson, Gastroretentive dosage forms: overview and special case of *Helicobacter pylori*, *J. Control. Release* **111** (2006) 1–18; http://doi.org/10.1016/j.jconrel.2005.10.031
8. H. T. Trinh, I. A. Lee, Y. J. Hyun and D. H. Kim, Artemisia princeps Pamp. Essential oil and its constituents eucalyptol and alpha-terpineol ameliorate bacterial vaginosis and vulvovaginal candidiasis in mice by inhibiting bacterial growth and NF-kappaB activation, *Planta Med.* **77** (2011) 1996–2002; http://doi.org/10.1055/s-0031-1280094
9. E. J. Choi, H. M. Oh, B. R. Na, T. P. Ramesh, H. J. Lee, C. S. Choi, S. C. Choi, T. Y. Oh, S. J. Choi, J. R. Chae, S. W. Kim and C. D. Jun, Eupatilin protects gastric epithelial cells from oxidative damage and down-regulates genes responsible for the cellular oxidative stress, *Pharm. Res.* **25** (2008) 1355–1364; http://doi.org/10.1007/s11095-008-9531-5
10. S. Lee, M. Lee and S. H. Kim, Eupatilin inhibits H(2)O(2)-induced apoptotic cell death through inhibition of mitogen-activated protein kinases and nuclear factor-kappaB, *Food Chem. Toxicol.* **46** (2008) 2865–2870; http://doi.org/10.1016/j.fct.2008.05.026
11. K. Huh, T. H. Kwon, U. S. Shin, W. B. Kim, B. O. Ahn, T. Y. Oh and J. A. Kim, Inhibitory effects of DA-9601 on ethanol-induced gastrohemorrhagic lesions and gastric xanthine oxidase activity in rats, *J. Ethnopharmacol.* **88** (2003) 269–273; http://doi.org/S0378874103002356
12. T. Y. Oh, J. S. Lee, B. O. Ahn, H. Cho, W. B. Kim, Y. B. Kim, Y. J. Surh, S. W. Cho, K. M. Lee and K. B. Hahm, Oxidative stress is more important than acid in the pathogenesis of reflux oesophagitis in rats, *Gut* **49** (2001) 364–371; http://doi.org/10.1136/gut.49.3.364
13. Y. D. Kim, S. C. Choi, T. Y. Oh, J. S. Chun and C. D. Jun, Eupatilin inhibits T-cell activation by modulation of intracellular calcium flux and NF-kappaB and NF-AT activity, *J. Cell Biochem.* **108** (2009) 225–236; http://doi.org/10.1002/jcb.22244
14. J. M. Park, Y. M. Han, J. S. Lee, K. H. Ko, S. P. Hong, E. H. Kim and K. B. Hahm, Nrf2-mediated mucoprotective and anti-inflammatory actions of Artemisia extracts led to attenuate stress related mucosal damages, *J. Clin. Biochem. Nutr.* **56** (2015) 132–142; http://doi.org/10.3164/jcbn.14-76
15. K. S. Chung, H. E. Choi, J. S. Shin, E. J. Cho, Y. W. Cho, J. H. Choi, N. I. Baek and K. T. Lee, Chemopreventive effects of standardized ethanol extract from the aerial parts of Artemisia princeps Pampanini cv. Sajabal via NF-kappaB inactivation on colitis-associated colon tumorigenesis in mice, *Food Chem. Toxicol.* **75** (2015) 14–23; http://doi.org/1016/j.fct.2014.11.007
16. T. H. Kim, S. J. Lee, H. K. Rim, J. S. Shin, J. Y. Jung, J. S. Heo, J. B. Kim, M. S. Lee and K. T. Lee, *In vitro* and *in vivo* immunostimulatory effects of hot water extracts from the leaves of Artemisia

princeps Pampanini cv. Sajabal, *J. Ethnopharmacol.* **149** (2013) 254–262; http://doi.org/10.1016/j.jep.2013.06.030

17. S. Shanmugam, Granulation techniques and technologies: recent progresses, *Bioimpacts* **5** (2015) 55–63; http://doi.org/10.15171/bi.2015.04
18. A. Robert, J. E. Nezamis, C. Lancaster and A. J. Hanchar, Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury, *Gastroenterology* **77** (1979) 433–443.
19. N. Yamamoto, M. Ueda, K. Kawabata, T. Sato, K. Kawasaki, T. Hashimoto and H. Ashida, Artemisia princeps extract promoted glucose uptake in cultured L6 muscle cells via glucose transporter 4 translocation, *Biosci. Biotechnol. Biochem.* **74** (2010) 2036–2042; http://doi.org/10.1271/bbb.100305
20. S. W. Min, N. J. Kim, N. I. Baek and D. H. Kim, Inhibitory effect of eupatilin and jaceosidin isolated from Artemisia princeps on carrageenan-induced inflammation in mice, *J. Ethnopharmacol.* **125** (2009) 497–500; http://doi.org/10.1016/j.jep.2009.06.001
21. J. K. Ko, C. H. Cho and S. K. Lam, Adaptive cytoprotection through modulation of nitric oxide in ethanol-evoked gastritis, *World J. Gastroenterol.* **10** (2004) 2503–2508; http://doi.org/10.3748/wjg.v10.i17.2503
22. Y. A. Sohn, S. A. Hwang, S. Y. Lee, I. Y. Hwang, S. W. Kim, S. Y. Kim, A. Moon, Y. S. Lee, Y. H. Kim, K. J. Kang and C. S. Jeong, Protective effect of liriodendrin isolated from *Kalopanax pictus* against gastric injury, *Biomol. Ther. (Seoul)* **23** (2015) 53–59; http://doi.org/10.4062/biomolther.2014.103
23. I. Szelenyi and K. Brune, Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats, *Dig. Dis. Sci.* **33** (1988) 865–871.
24. H. Matsui, O. Shimokawa, T. Kaneko, Y. Nagano, K. Rai and I. Hyodo, The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine, *J. Clin. Biochem. Nutr.* **48** (2011) 107–111; http://doi.org/10.3164/jcbn.10-79
25. T. Urushidani, S. Okabe, K. Takeuchi and K. Takagi, Effects of various amino acids on indomethacin-induced gastric ulcers in rats, *Jpn. J. Pharmacol.* **27** (1977) 316–319.
26. M. B. Adinortey, C. Ansah, I. Galyuon and A. Nyarko, In vivo models used for evaluation of potential antigastroduodenal ulcer agents, *Ulcers* **2013** (2013) 1–12; http://doi.org/dx.doi.org/10.1155/2013/796405
27. S. W. Jang, J. W. Lee, S. H. Park, J. H. Kim, M. Yoo, D. H. Na and K. C. Lee, Gastroretentive drug delivery system of DA-6034, a new flavonoid derivative, for the treatment of gastritis, *Int. J. Pharm.* **356** (2008) 88–94; http://doi.org/0.1016/j.ijpharm.2007.12.042
28. S. H. Shaha, J. K. Patel, K. Pundarikakshudu and N. V. Patel, An overview of a gastro-retentive floating drug delivery system, *Asian J. Pharm. Sci.* **4** (2009) 65–80.
29. H. Rathod, V. Patel and M. Modasia, Floating drug delivery system: innovative approach of gastroretention, *Int. J. Pharm. Sci. Rev. Res.* **4** (2010) 183–192.
30. A. E. Yassin, F. Alkhaled, S. al-Suwayeh and S. Elkheshen, Intra-gastric performance and bioavailability study of a new per-oral bioadhesive Verapamil HCl matrix tablet in dogs, *Boll. Chim. Farm.* **142** (2003) 285–289.