INTRODUCTION

Functional dyspepsia (FD) is a clinical syndrome characterized by a diverse range of persistent or recurrent upper abdominal symptoms, with no demonstrable organic or structural lesions and no specific underlying etiology. This heterogeneous symptom complex includes epigastric pain/discomfort, bloating, early satiation, postprandial heaviness/fullness, anorexia, and belching. There are no specific diagnostic biomarkers for FD. It is diagnosed symptomatically after the exclusion of organic diseases like peptic ulcers, gastroesophageal reflux disease, and malignancies (Stanghellini et al., 2003; Halder and Talley, 2007; Piessevaux et al., 2009). The Rome criteria III, an international system that attempts to categorize functional gastrointestinal (GI) disorders, provided diagnostic criteria for FD whereby patients must have had one or more of the following symptoms for the past 3 months, with symptom onset at least 6 months prior to diagnosis: postprandial fullness, early satiety, epigastric pain or burning, and no evidence of structural disease that could explain the symptoms (including any condition detected by upper endoscopy) (Thompson, 2006). FD is further subclassified as: (a) postprandial distress syndrome (PDS) characterized by meal-induced fullness and satiety; or (b) epigastric pain syndrome (EPS), characterized by epigastric pain or burning. These criteria, however, do not include nausea and vomiting as cardinal FD symptoms (Suzuki et al., 2006; Tack et al., 2006).

Several pathophysiological mechanisms have been proposed to contribute to FD, including abnormal gastrointestinal motility, central and autonomic nervous system dysregulation, neuro-hormonal dysfunction, infection by H. pylori and other GI organisms, psycho-somatic morbidities, visceral hypersensitivity, and genetic susceptibility, although the specific etiology remains to be elucidated (Mizuta et al., 2006). These
mechanisms, either alone or in combination, might be responsible for heterogeneous and multifactorial dyspeptic symptoms, which has made it difficult to establish an optimum and uniform therapeutic strategy for FD. However, a major patient subgroup shows symptoms associated with GI sensori-motor abnormalities such as delayed gastric emptying, impaired gastric accommodation or gastric misdirection of ingested material, and abnormal gastroduodenal motility and reflux. These findings have led to studies of the efficacy of prokinetic drugs such as D₂ receptor antagonists, 5-HT₄ agonists and CCK receptor antagonists in FD; in addition, acid suppressant drugs including H₂ receptor antagonists and proton-pump inhibitors have been employed, along with anti-H. pylori agents, anticholinergics, and laxatives for symptom relief.

Although a plethora of agents have been evaluated, no medication is currently approved in the US, Canada, or the European Union for the treatment of FD. Prokinetic compounds and other drugs affecting GI motility have exhibited relatively modest success in several randomized controlled trials and are thus prescribed empirically. These prokinetic drugs improve FD symptoms by reducing gastroesophageal reflux, promoting fundus relaxation and gastric emptying, and improving gastric regulation. However, these agents come with their own disadvantages, mostly arising from their limited clinical effects and unwanted side-effects. For example, despite earlier promising results, itopride produced only slightly clinical effects and unwanted side-effects. For example, despite its long-standing use for dyspeptic symptoms, no animal studies have been performed to investigate the potential mechanisms underlying the beneficial effects of BF.

The present study aimed to examine the pharmacological effects of BF on GI motor functions relevant to FD therapy. In particular, the effects of BF on gastric emptying, GI transit, gastric accommodation, and bile secretion in rat models were performed to investigate the potential mechanisms underlying the beneficial effects of BF.

**MATERIALS AND METHODS**

**Materials**

Cisplatin, morphine, apomorphine, phenol red, and hydroxypropylmethyl cellulose (HPMC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fluorescein isothiocyanate (FITC) and dextran were purchased from Choongwae Pharma (Seoul, South Korea) and Fluka (Tokyo, Japan), respectively. IB (Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany; lot No. 431254; expiration date: 2016/09/07), BF (Dong-A Pharmaceutical Co. Ltd., Seoul, South Korea; lot No. 1403001; expiration date: 2016/03/02), and WM (Dong-Wha Pharmaceutical Co. Ltd., Seoul, South Korea; lot No. D067; expiration date: 2016/05/15) were used as received after vacuum concentration to the desired volume and concentration.

**Animals and experimental procedure**

Male Sprague-Dawley rats (200-220 g) were purchased from Central Laboratory Animal Inc. (Seoul, South Korea). The rats were individually housed in single, air-conditioned boxes under adequate temperature and humidity control with a 12-h light-dark cycle and access to rat food pellets. All animal care and experimental procedures were conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council (http://www.nsa.edu/nrc), and approved by the Institutional Animal Ethics Committee, Yeungnam University, South Korea (approved protocol number: 2014-006). All rats were used after a week of acclimatization and were fasted for 12 h with free access to water prior to the experiments. Animals were orally administered 100 mg/kg body weight of BF, IB, or WM in a volume of 1 ml, or 3% (w/v) HPMC (control).

**Gastric emptying**

Gastric emptying was measured according to the method described by Ozaki and Sukamoto (Ozaki and Sukamoto, 1999) with some modifications. Each group of 6 rats were given 2 ml of a semi-solid meal 50 min after drug administration, and simultaneously injected subcutaneously with either morphine (0.05 mg/kg) or apomorphine (0.05 mg/kg). After 50 min, the animals were sacrificed, and the weights of their stomachs and stomach contents were measured in order to determine gastric emptying using the equation shown below.

\[
\text{Gastric emptying (\%)} = \frac{1 - \text{weight of test stomach/weight of 0 time control stomach}}{100}.
\]

Alternatively, the animals were given a 2 ml liquid meal containing 0.05% phenol red 60 min after drug administration, and simultaneously injected intraperitoneally with 10 mg/kg cisplatin. The amount of phenol red remaining in the stomach 20 min later was measured (optical density [OD] at 560 nm) and gastric emptying was calculated using the method described by Yoshida et al. (1989):

\[
\text{Gastric emptying (\%)} = \frac{1 - \text{OD 560 nm of test stomach/ OD of 0 time control stomach}}{100} [\text{0 time control}] .
\]
Gastric accommodation was de...overnight fast, the gastric volume was recorded via the intra...ed operating pressure and...ed to maintain an open airway. A mid-line incision and cannulated with a polyethylene cannula at the sphincter of Oddi. Bile secretions (combined bile-pancreatic secretions) were collected from each rat into a control group (n=7) received saline only, before being anesthetized. After 30 min, the bile duct was surgically exposed by a midline incision and cannulated with a polyethylene cannula inserted at the sphincter of Oddi. Bile secretions (combined bile-pancreatic secretions) were collected from each rat into pre-weighed vials at 15 min intervals for a period of 120 min.

Data analysis
Results were expressed as mean ± standard deviation (SD). Differences in the data were evaluated by Student’s t-test and p-values <0.05 were considered to indicate a statistically significant difference between the study groups.

RESULTS
Effects of BF on gastric emptying
Opioids such as morphine delay gastric emptying via interacting with central or peripheral opiate receptors. Fig. 1A shows the effect of the tested herbal preparations on morphine-delayed gastric emptying in rats. Morphine significantly delayed gastric emptying observed in the untreated normal group (66.6 ± 1.8%) to 26.6 ± 2.2% in the control group. This delay was reversed by BF to 40.0 ± 4.0%. WM and IB also significantly improved gastric emptying to 36.9 ± 5.5% and 57.7 ± 2.8%, respectively. The effects of BF were comparable to those of WM, but lower than those of IB. Apomorphine is a non-selective dopamine agonist that can decrease gastric tone and delay gastric emptying by acting both centrally and peripherally. The effects of the tested herbal preparations on apomorphine-induced delay of gastric emptying are shown in Fig. 1B. Apomorphine significantly decreased gastric emptying in the normal group (74.3 ± 4.5%) to 38.5 ± 5.2% in the control group. BF and IB significantly ameliorated this delay (63.6 ± 5.2% and 60.9 ± 4.4%, respectively), but
gression (BF, Benachio-F®; WM, Whalmyungsu®; IB, Iberogast®) or control treatment (3% HPMC) was administered orally 60 min before meal (FITC-dextran) administration. The mean geometric center of distribution was calculated as described in Materials and methods. Values shown are the mean ± SD; **p<0.01 vs. control, Student’s t-test.

Fig. 4. Bile flow in rats. Each herbal preparation (BF, Benachio-F®; WM, Whalmyungsu®; IB, Iberogast®) or control treatment (3% HPMC) was administered orally 30 min before meal administration. Results shown are means ± standard error of the mean (SEM). *p<0.05 vs. control, Student’s t-test.

DISCUSSION

Dyspepsia is one of the most routine presentations in many healthcare facilities, with an estimated prevalence of 20-40% in some countries; the majority of these cases are believed to have FD (Camilleri et al., 2005; Piessevaux et al., 2009). FD is known to significantly reduce the quality of life of the affected individuals and places a marked economic burden on healthcare facilities, because patients with FD can have complex treatment needs (Aro et al., 2011). Although the underlying pathophysiological mechanisms are not yet fully established, the available studies suggest interconnected and multifactorial causes that may include psychosocial factors, H. pylori infection, environment, diet, and genetics; these contribute to the manifestation of GI system changes including abnormal motility, visceral hypersensitivity, excess secretion of gastric acid,
and duodenal acidity (Mizuta et al., 2006). Considering the large number of factors involved, no single medicine is likely to be effective for all patients with FD. In this context, multidrug and multi-targeted phytotherapy might prove advantageous. Mono- and poly-herbal preparations have been used for the treatment of dyspeptic complaints since long before recorded history (Madsich et al., 2004; Allescher, 2006; Rösch et al., 2006). A herbal medicinal preparation derived from several plant extracts contains a large number of secondary phytochemical compounds such as essential oils; these are known to possess carminative, stomachic, spasmyloitic, and local anesthetic actions. The Corydalis Tuber is used in Chinese traditional medicine for its analgesic and anti-ulcer effects. Extracts of this root have been reported to have some biological activities including anti-spasmodic effects on the GI tract and analgesic activity (Ma et al., 2000). These preparations have been reported to enhance upper GI motility and GI transit in models of laparotomy-induced delay (Lee et al., 2008). Fruits of Foeniculum vulgare Miller (fennel) is used as a laxative and for the treatment of mild digestive disorders because it stimulates GI motility and shows anti-spasmodic activity at higher concentrations. Fennel seeds also act as a laxative, by providing roughage and stimulating peristaltic motion, thereby promoting production of gastric juices and bile and facilitating excretion (Klein et al., 1998). Fennel is also commonly found in medicines used to treat abdominal pain, diarrhea, irritable bowel syndrome, and other intestinal issues. Extracts of Cinnamon species (cinnamon) is a carminative agent, helping to reduce flatulence, and is traditionally used to combat diarrhea and morning sickness. Cinnamon was shown to reduce secretion of GI fluids in an animal model of diarrhea (Rao et al., 2008). The rhizomes of Atractylodes lancea DC (Compositae) are used to treat GI diseases including nausea, gastroparesis, and gastric atony in China and Japan (Chang, 2004). This extract may stimulate gastric emptying and increase small intestinal motility by inhibiting the dopamine D₃ receptor and the 5-HT₁ receptor (Kimura and Sumiyoshi, 2012). Powdered rhizomes of Zingiber officinale Roscoe (ginger) has long been used in traditional treatments for GI illnesses (Chopra et al., 1956). Recently, an acetone extract of ginger and its constituents were shown to enhance gastric emptying of a charcoal meal in mice. Ginger preparations also significantly reversed cisplatin-induced delayed gastric emptying (Yamahara et al., 1990). The roots and rhizomes of licorice (Glycyrrhiza glabra Linn) have been reported to possess anti-Helicobacter pylori activity, along with gastric mucus secretion enhancing activity (Raveendra et al., 2012). Aqueous extracts of Citri Unshiu Pericarpium significantly increased the intestinal transit rate in normal mice and rats with GI motility dysfunctions (Lyu and Lee, 2013).

Several mono- and poly-herbal medicinal products have undergone randomized controlled clinical trials, with varying degree of success. For example, IB contains extracts from 9 plants and has shown promising results in clinical and observational studies (Heinle et al., 2006; Rösch et al., 2006). IB exhibited dual motility-modulating effects; relaxing spastic intestine (Wegenera and Wagner, 2006), and improving atomic intestine (Ammon et al., 2006). Its effects were also region-specific; relaxing the gastric corpus and fundus and increasing tone in the antrum (Schemann et al., 2006). This region-specific effect has also been confirmed by clinical pharmacological data (Thompson and Ernst, 2002; Pilichiewicz et al., 2006). The mode of action of IB and its individual components on gastric motility include relaxation of the proximal stomach and increased antral motility (Sharma and Gupta, 1998).

Some components of BF have been linked to adverse effects when administered at very high doses for prolonged periods. The most prominent effects were reported for Corydalis Tuber (alkaloid: bulbocapnine) and Glycyrrhizae Radix (triterpenoid saponin: glycyrrhizine). Bulbocapnine has been reported to induce dose dependent catalepsis and convulsions, while glycyrrhizine has been associated with hypertension, hypokalemia, hypernatremia and some very rare cases of pseudoaldosteronism (Loizzo et al., 1971; Robles et al., 2013). The most commonly reported side-effect was allergic reaction in sensitive patients. However, the doses of these components used in BF therapy are much lower than the levels reported to cause these adverse reactions. For example, the single oral ethanolic extract dose that caused 50% mortality (LD₅₀) in mice was reported to be 100 ± 4.58 g/kg, which is more than 250 times the content found in BF (Koo et al., 2010). Glycyrrhizae Radix is Generally Recognized as Safe (GRAS) for use in foods by the U.S. FDA (21 CFR 184.1408).

After the ingestion of a solid or liquid meal, bolus passage through the esophagus normally causes an initial receptive relaxation, followed by adaptive relaxation of the proximal stomach to provide a reservoir for the food (gastric accommodation). The meal is then transferred to the antrum of the distal stomach where it is ground and mixed with the gastric contents prior to delivery to the duodenum via the pylorus (gastric emptying). In healthy humans, more than 90% of ingested food is emptied from the stomach within 4 h (Tougas et al., 2000). In this study, we observed that BF had beneficial effects on GI motor activity, which included acceleration of gastric emptying and meal transit, as well as enhancement of gastric accommodation. Morphine, amorphine, and cisplatin inhibit gastric emptying via effects on opiate, dopamine, and 5-HT₁ receptors, respectively (Blancquaert et al., 1982; Tyers, 1991; Ozaki and Sukamoto, 1999). BF significantly ameliorated the delayed gastric emptying induced by these agents, suggesting that it could accelerate gastric emptying under these conditions. Delayed gastric emptying is also a feature of gasteroparesis and impaired gastric accommodation. Gastric accommodation is a motor function reflex of the gastric corpus-fundus which enables it to house a high volume (of food) with a minimal rise in intragastric pressure, reducing discomfort. It has been reported that gastric accommodation is impaired in approximately 40% of FD patients (Mizuta et al., 2006). It has been suggested that the pre- and post-prandial bloating and sensation of fullness experienced by dyspeptic patients could result from impairments of gastric accommodation and intragastric distribution of the meal between the proximal and distal parts of the stomach. This has also been associated with early satiety and weight loss. In a subset of dyspeptic patients with visceral hypersensitivity to gastric distention, impaired gastric accommodation may lead to post-prandial pain, belching, and weight loss. It was found that the intragastric pressure-related symptom cluster in dyspeptic patients closely resembled that of vagotomized patients, suggesting that impaired gastric accommodation and antral hypomotility may be due to vagal dysfunction. Efferent vagal dysfunction was also implicated in patients with abnormal motility-related FD. In the present...
study, a miniature barostat attached to a small balloon via a polyethylene cannula was used to examine gastric relaxation in rats administered the test preparations. BF induced superior gastric relaxation.

POI often occurs after abdominal surgery and is characterized by a transient hypomotility of the GI tract; this prolongs hospitalization, raises medical costs, and increases morbidity (Holte and Kehlet, 2000). Inflammatory responses and inhibited neuronal reflex pathways have been implicated in the pathogenesis of POI (Zittel et al., 2001; Kreiss et al., 2003). Surgical stress stimulates the release of endogenous opioids, which can impair GI function after surgery. In the present study, a laparotomy-induced delay in GI transit was significantly reduced by BF treatment. Based on these results, BF may prove to be superior to conventional therapeutics, particularly for the improvement of GI transit and gastric accommodation.

We also found that BF significantly stimulated biliary flow in rats. Bile production represents an important function of the liver and changes in bile flow rates or alterations in biliary constituents have important impacts on health (Hofmann, 1999). Based on these results, BF would be predicted to enhance digestion and GI function by stimulating bile secretion. Taken together, the findings of the present study indicate that BF enhanced gastric emptying and intestinal transit, increased gastric accommodation by inducing gastric relaxation, and thus increased gastric compliance in rats. BF also stimulated bile flow, which may further enhance digestion.

In summary, the pharmacological effects of BF, a proprietary liquid formulation containing 7 herbal extracts that could provide multi-target phytotherapy for FD, were evaluated in rat models relating to the symptoms of this condition. BF significantly reversed the delayed gastric emptying caused by morphine, apomorphine, and cisplatin. In addition, it markedly increased gastric accommodation and showed higher gastric volumes, as compared with control rats. Furthermore, it exhibited significant stimulatory effects on bile flow. Its effects on these GI sensorimotor mechanisms were comparable to, or even better than, other similar herbal preparations. Taken together, BF might have great potential as an effective prokinetic agent capable of reducing GI symptoms and increasing quality of life in FD patients.

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DISCLOSURES

The authors report no conflicts of interest about this work.

REFERENCES


Poudel et al. The Pharmacological Effects of Benachfo-F