



Ethnopharmacological communication

Bojanggalunbi-tang, a traditional Korean herbal prescription, ameliorates colonic inflammation induced by dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid in mice

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ABSTRACT

Ethnopharmacological relevance: In traditional Korean medicine, *Bojanggalunbi-tang* (BGT), which consists of 16 herbs, is one of the most frequently used herbal prescriptions in South Korea for treating intestinal disorders such as colitis.

Aim of the study: This study investigated whether BGT could show a protective action on 2 different mice models of experimental colitis induced by dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS), which have been popularly used as inflammatory bowel disease models.

Materials and methods: Colitis was induced by DSS and TNBS in institute of cancer research mice. BGT at doses of 50, 150, or 450 mg/kg were orally administered twice a day for 7 d in the DSS model and for 3 d in the TNBS model. The body weight of the mice was measured daily. Colon length and histological damages were assessed on day 7 in the DSS model and on day 3 in the TNBS model.

Results: BGT showed protective effects in both types of experimental colitis. In the DSS model, BGT dose dependently inhibited weight loss, shortening of colon length, and histological damages of the colon. In the TNBS model, BGT inhibited shortening of colon length and improved the survival rate of mice; however, it did not inhibit weight loss.

Conclusion: The current results indicate that BGT ameliorates both DSS- and TNBS-induced colitis in mice. Further investigations to unveil the exact mechanisms are needed.

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1. Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronically relapsing inflammatory disorders of the intestine (Lee et al., 2010a). Recently, the prevalence rate of IBD is increasing in the Asia-Pacific region (Chung et al., 2007). Currently, non-steroidal anti-inflammatory drugs and immunosuppressive agents are the main therapeutic agents available for IBD (Fujisawa et al., 2005). Although these drugs alleviate the symptoms, they do not cure the disease and have some limitations owing to their severe side effects such as allergy, nausea, lymphoma and etc. (Lee et al., 2009; Siegal, 2011). Thus, many researchers have shown an increasing interest in natural products and herbs as therapeutic agents for IBD (Lee et al., 2009).

In traditional Korean medicine, various herbs or herbal prescriptions have long been used for treatment of intestinal inflammation, including colitis, and some have shown significant effects on animal models of IBD (Kim et al., 2008; Lee et al., 2009). *Bojanggalunbi-tang* (BGT) is a combination of two famous herbal prescriptions, '*Daehwajungeum*' and '*Sambaek-tang*', in ancient Chinese medical literatures and a well known herbal prescription for IBD and colitis in South Korea (Joun et al., 1994). BGT consists of 16 herbs that have been used for the treatment of diarrhea and other gastrointestinal diseases in traditional Korean medicine (Joun et al., 1994). BGT has been prescribed to more than 310,000 IBD and other colitis patients at the Oriental Medical Center of Kyung Hee University from 1994 to 2010. Seo et al. (2004) recently reported clinical improvements in patients with CD who received BGT. Experimentally, it has been reported that BGT had gastro-protective and anti-diarrheal effects in murine models (Joun et al., 1994). Despite BGT's wide clinical usage and pharmacological reports aforementioned, there are few scientific evidences available for its effect on IBD.

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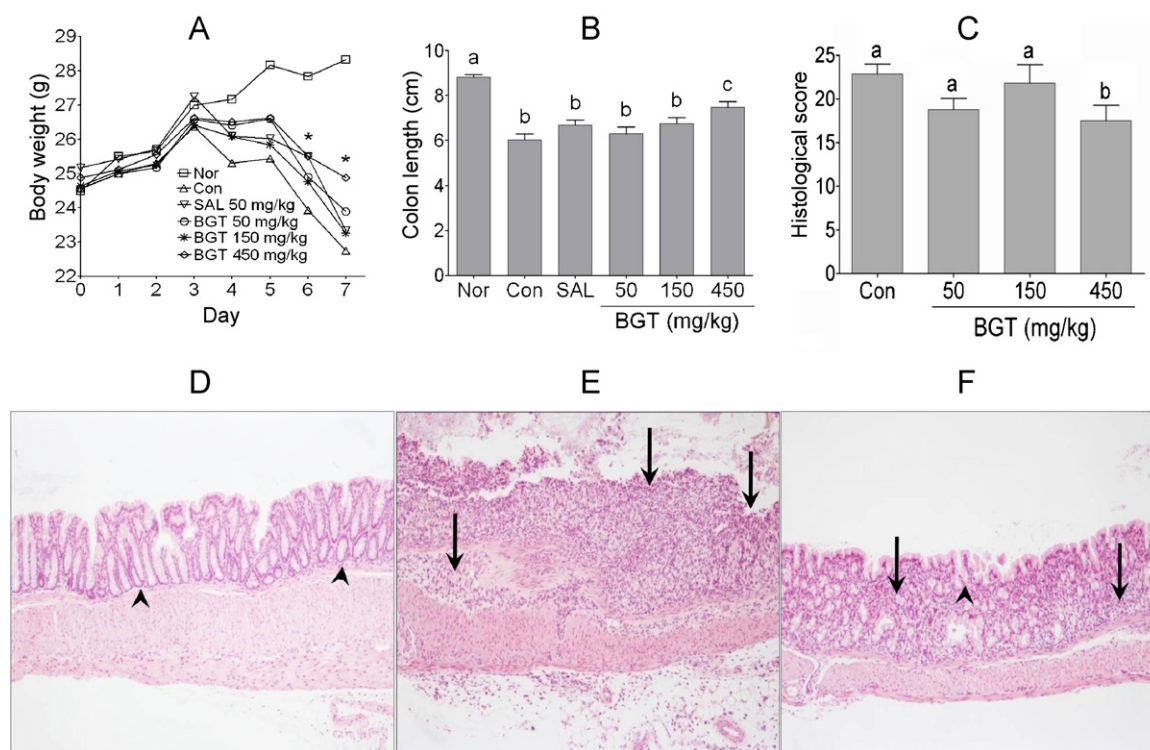


Fig. 1. Changes in mice weight, colon length, and histological scores in DSS-induced colitis mice treated with BGT. (A) Changes in mice weight. * $P < 0.05$ compared to control group. (B) Comparison of colon lengths at day 7. (C) Comparison of histological scores at day 7. (B) and (C) were analyzed by one-way ANOVA followed by the Tukey test. Data are expressed as mean \pm SEM (standard error of mean). SAL indicates sulfasalazine 50 mg/kg-treated group. (D)–(F) indicate representative histological photos of the normal group (D), control group (E) and BGT 450 mg/kg-treated group (F). Arrowheads indicate intact cryptal glands and surface epithelium. Arrows indicate damaged surface epithelium and infiltration of inflammatory cells (100 \times).

We hypothesized that BGT might show protective effects in the 2 IBD mice models: dextran sulfate sodium (DSS) induced colitis (corresponding to human UC) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis (corresponding to human CD) (Maharshak et al., 2010). Therefore, we investigated the effects of BGT by measuring body weight, colon length, histological score, and survival rate after DSS and TNBS treatment in mice.

2. Materials and methods

2.1. Animals

Male institute of cancer research (ICR) mice (7 wks, 23 ± 2 g) were supplied by Daehan Bio Link (Seoul, Korea). The mice were housed at $20\text{--}22^\circ\text{C}$ and in $50 \pm 10\%$ humidity and were provided food and water *ad libitum*. All procedures were in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication # 85-23, revised 1985) and Kyung Hee University guidelines, 2006 (Animal ethical permission number; KHUASP(SE)-09-036).

2.2. Sample preparation and grouping

Each crude herb of BGT was purchased from Kyung Hee Hanyak Co. (Seoul, Korea). Each daily dose of BGT in humans consists of flowers of *Lonicera japonica* Thunb. (Caprifoliaceae, PSS01, 40 g), roots of *Atractylodes macrocephala* Koidz. (Compositae, PSS02, 16 g), roots of *Paeonia lactiflora* Pall. (Ranunculaceae, PSS03, 16 g), seeds of *Dolichos lablab* L. (Leguminosae, PSS04, 16 g), roots of *Dioscorea japonica* Thunb. (Doiscoreaceae, PSS05, 16 g), fruits of *Crataegus pinnatifida* Bunge var. *typica* Schneider (Rosaceae, PSS06, 16 g), *Poria cocos* Wolf (Polyporaceae, PSS07, 16 g), stem bark of *Magnolia officinalis* Rehder and Wilson (Magnoliaceae, PSS08, 12 g),

cortex of *Citrus unshiu* Marcovich (Rutaceae, PSS09, 12 g), roots of *Alisma orientalis* (SAM.) Juzep. (Alismataceae, PSS10, 12 g), *Massa medicata* Fermentata (PSS11, 8 g), seeds of *Hordeum vulgare* Linne (Gramineae, PSS12, 8 g), roots of *Zingiber officinale* Roscoe (Zingiberaceae, PSS13, 8 g), roots of *Aucklandia lappa* Decne. (Compositae, PSS14, 6 g), fruits of *Amomum villosum* Lour. (Zingiberaceae, PSS15, 6 g), root of *Glycyrrhiza uralensis* Fisch. (Leguminosae, PSS16, 4 g) (Pharmacy, 2008). The herbal mixture (totally 216 g) was extracted with hot water (100°C) for 2 h, filtered, and freeze-dried to a powdered form (PS003, 14.3 g). Each ingredient and extract specimens of BGT were deposited in the herbal laboratory of the college of Oriental Medicine, Kyung Hee University. Mice were divided into normal, control, sulfasalazine-treated (positive control, 50 mg/kg per day, p.o.) and BGT-treated (50, 150, or 450 mg/kg twice a day, p.o.) groups ($n = 8$, each).

2.3. Induction of DSS colitis

Colitis was induced by DSS as previously described (Lee et al., 2010a). In brief, the mice were provided with drinking water containing 5% DSS *ad libitum* (MP Biomedicals, Japan) for 7 d. The mice were sacrificed on day 7 of the experiment.

2.4. Induction of TNBS colitis

Colitis was induced by TNBS as previously described (Lee et al., 2010a). In brief, mice were anesthetized by isoflurane (2%), and later 2.5% TNBS (Sigma–Aldrich, USA) in 50% ethanol solution (control) was injected intrarectally through a 3.5-cm flexible catheter. Three days after the TNBS injection, mice were sacrificed.

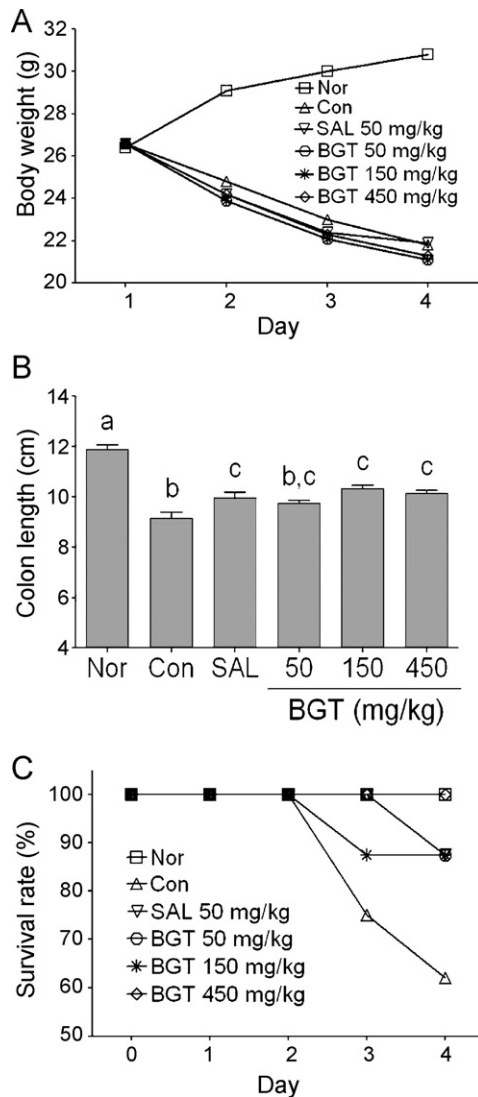


Fig. 2. Changes in mice weight, colon length, and survival rates in TNBS-induced colitis group treated with BGT. (A) Changes in mice weight. (B) Comparison of colon lengths at day 4 by one-way ANOVA followed by the Tukey test. Data are expressed as mean \pm SEM (standard error of mean). (C) Comparison of survival rates. SAL indicates sulfasalazine 50 mg/kg-treated group.

2.5. Assessment of body weight and colon length

In all mice, the body weight was monitored at 9 a.m. daily, and the whole colonic length was measured immediately after sacrifice.

2.6. Histological analysis

Histological sections of mice colons cut from paraffin blocks were stained with hematoxylin and eosin. In a blind fashion, the histological lesions were scored (modified scoring system) by Kitajima et al. (2000) and scoring system was divided into 2 categories, inflammatory cell infiltration and ulceration. The inflammatory cell infiltration of surface epithelium, cryptal gland, stroma, submucosa, the layer was graded on a scale of 0–3 (0 = none, 1 = mild, 2 = moderate, 3 = severe). The severity of ulceration was histologically graded on a scale of 0–4 (0 = none, 1 = mild and focal surface, 2 = mucosal, 3 = submucosa, 4 = transmural). We established a 0–19 point system after computing the sum of the 2 scores.

2.7. Statistical analysis

All results are expressed as mean \pm SEM (standard error of mean). Data were analyzed statistically using one-way ANOVA followed by the Tukey test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. The effects of BGT on DSS-induced colitis

The weight of mice in all groups increased until day 3, and then decreased until day 7. Only BGT 450 mg/kg-treated group showed a significant protective effect against weight loss caused by DSS on days 6 and 7 ($P < 0.05$). Shortening of colon length caused by DSS was dose dependently inhibited in the BGT groups, especially in BGT 450 mg/kg-treated group that showed a significant inhibitory effect ($P < 0.001$). A positive control, sulfasalazine seemed to inhibit colon shortening (17% compared with control), however it did not show any significance (Fig. 1).

3.2. The effects of BGT on TNBS-induced colitis

After TNBS injection, all mice lost weight without any significant differences among the groups. However, all BGT- and sulfasalazine-treated groups significantly exhibited protective effects on TNBS-induced colon shortening ($P < 0.05$). BGT and sulfasalazine also improved the survival rate of mice as compared with the control (Fig. 2).

3.3. Histological analysis

The normal group showed intact surface epithelium, cryptal gland, stroma, and submucosa (arrows in Fig. 1D), while the control group showed severe damage (arrowheads in Fig. 1E). The BGT 450 mg/kg-treated group showed more intact surface epithelium and cryptal glands than those in the case of the control mice ($P < 0.05$) (Figs. 1E and F).

4. Discussion and conclusion

In the current study, we demonstrated that BGT had protective effects against DSS- and TNBS-induced colitis models.

In the DSS model, which is widely used in colitis experiments (Maharshak et al., 2010), BGT inhibited the shortening of colon length and weight loss. Particularly, BGT 450 mg/kg-treated group showed more protective effects than sulfasalazine 50 mg/kg-treated group in weight loss and colon length. The administration of DSS for 7 d is known to be toxic to gut epithelium, affect the integrity of the mucosal barrier, induce acute colitis in mice and cause weight loss, shortening of colon length, diarrhea, bloody stool, and ulceration of colonic mucosa, similar to the manifestations of human UC (Egger et al., 2000). The DSS concentration is an important factor in deciding the severity of colitis (Egger et al., 2000). For our preliminary experiment, we selected 5% DSS as the most appropriate concentration (data not shown). The most effective dosage of 450 mg/kg twice a day, which translates as 22.5 g/day twice a day dosage in a human weighing 50 kg, is similar to the clinical dosage (18 g/day twice a day).

DSS-induced colitis exhibited specific histological findings characterized by severe intestinal damage, a massive inflammatory cell infiltration, ulceration, and muscle thickening (Kitajima et al., 2000). BGT exhibited protective effects against these histological changes. By multiplex array, BGT also dose-dependently inhibited expression of tumor necrosis factor alpha and interleukin-1 beta,

representative inflammatory cytokines, in inflammatory colonic epithelium (data not shown). Thus, these results indicate that BGT, a traditional Korean medicine, may be a potent and effective therapeutic agent for acute conditions of IBD such as UC. In the current results, a positive control, sulfasalazine did not show the significance. It has been reported that sulfasalazine has effects on IBD and used in IBD animal experiments as positive control (Lee et al., 2010b; Zhao et al., 2010). We thought the reason why sulfasalazine showed no significant effect in DSS model may be come from different mice strain, dosage, DSS concentration and period of observation (Singh et al., 2009; Lee et al., 2010b).

BGT also inhibited the shortening of colon length and improved the survival rate in TNBS-induced colitis model. This is a well-characterized colitis model, which shows a T helper 1 immune response analogous to the inflammatory course observed in CD because TNBS is a haptenizing substance in ethanol and cause autologous or microbial proteins to be immunogenic to the host (Maharshak et al., 2010). All BGT concentrations were found to produce a significant inhibitory effect on the shortening of colon length, moreover BGT 450 mg/kg-treated group showed the maximal survival rate. These results indicate the usefulness of BGT in the treatment of IBD such as CD, and that BGT 450 mg/kg may be the most beneficial therapeutic dosage to treat IBD.

However, there were no significant differences in the mice weight among the groups. This may be because of various factors including severity of colitis and mice species (te Velde et al., 2006). Another study has also shown an improvement in TNBS-induced colitis in terms of changes in colon length and macroscopic findings, but not in mice weight (Joh et al., 2010), and these are in agreement with our results. BGT 150 mg/kg-treated group showed more protective effects than sulfasalazine 50 mg/kg-treated group in colon length. The histological and clinical findings in the 2 colitic models showed that BGT attenuated the DSS- and TNBS-induced colitis and exhibited a protective action against intestinal damages. In the current study, the effect of BGT on colitis may have arisen from the combined effects of the key herbs of BGT, such as *Lonicera japonica*, *Crataegus pinnatifida*, and *Glycyrrhiza uralensis* (Chung et al., 2007; Xu et al., 2007; Kwon et al., 2008).

In conclusion, the current results revealed that BGT showed protective effects on both human UC-like DSS model and CD-like TNBS model. Further investigations for possible mechanisms and isolation of active components will be needed.

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