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Administration of *Wasabia koreana* Ameliorates Irritable Bowel Syndrome-Like Symptoms in a Zymosan-Induced Mouse Model

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ABSTRACT Irritable bowel syndrome (IBS) is a functional gastrointestinal disease with complex pathophysiology involving the brain-gut axis. To assess the effects of *Wasabia koreana* (WK) on IBS, we employed a mouse model of colonic zymosan injection presenting with diarrhea-predominant IBS-like symptoms. Oral WK administration significantly diminished stool score, suppressed colon length and weight change, and minimized body weight loss without affecting food intake. In WK-treated mice, the submucosal thickening and epithelial lining of the colon were inhibited and were similar to those of naïve mice. Infiltration of mast cells into the colon and serum tumor necrosis factor-α levels were markedly suppressed. These effects were comparable to those of sulfasalazine, an anti-inflammatory drug. Furthermore, the number of visceral pain-related behaviors was significantly decreased, and locomotion activities measured in the elevated plus maze and open field tests were significantly increased by WK in a dose-dependent manner compared with amitriptyline, an antidepressant. These changes were accompanied by reduced FosB2 expression in the brain. Taken together, these data suggest that WK may have potential as a medicinal food for IBS by acting on inflammatory diarrhea and neural activity.

KEYWORDS: • ***animal model*** • ***anxiety*** • ***colonic inflammation*** • ***diarrhea-predominant irritable bowel syndrome*** • ***pain*** • ***wasabi*** • ***zymosan***

INTRODUCTION

IRRITABLE BOWEL SYNDROME (IBS) is a chronic functional bowel disorder characterized by abdominal pain and bowel habit changes including diarrhea, constipation, or an alternating pattern between the two.[1] Although the pathophysiology of IBS is not completely understood, one widely accepted explanation is brain-gut axis dysfunction. The abdominal pain associated with IBS is thought to involve both the central nervous system and gastrointestinal tract. IBS patients show visceral hypersensitivity, characterized by sensitive recognition of local stimuli in the intestinal walls compared with healthy subjects; this phenomenon contributes to peripheral pain.[2] Low-grade inflammation, such as mast cell activation in the gastrointestinal tract, has been proposed as one factor involved in the hypersensitivity in postinfectious IBS,[3] which often develops after acute gastroenteritis.[4] The mediators from mast cells activate sensory nerves leading to increased abdominal pain frequency and severity.[5] Indeed, IBS patients have considerably more mast cells in the colonic mucosa than healthy subjects.[6] Furthermore, peripheral hypersensitivity enhances primary sensory afferent inputs to the central nervous system, which triggers long-term changes in the brain.[7–9] In brain imaging studies of animals and humans, IBS subjects exhibited neuronal activity in brain areas including the prefrontal cortex, anterior cingulate cortex, and amygdala, which are important for processing pain, discomfort, and emotional responses.[10–12] Indeed, IBS patients often exhibit severe anxiety, stress, and depression,[10–13] which may in turn negatively affect gastrointestinal function.

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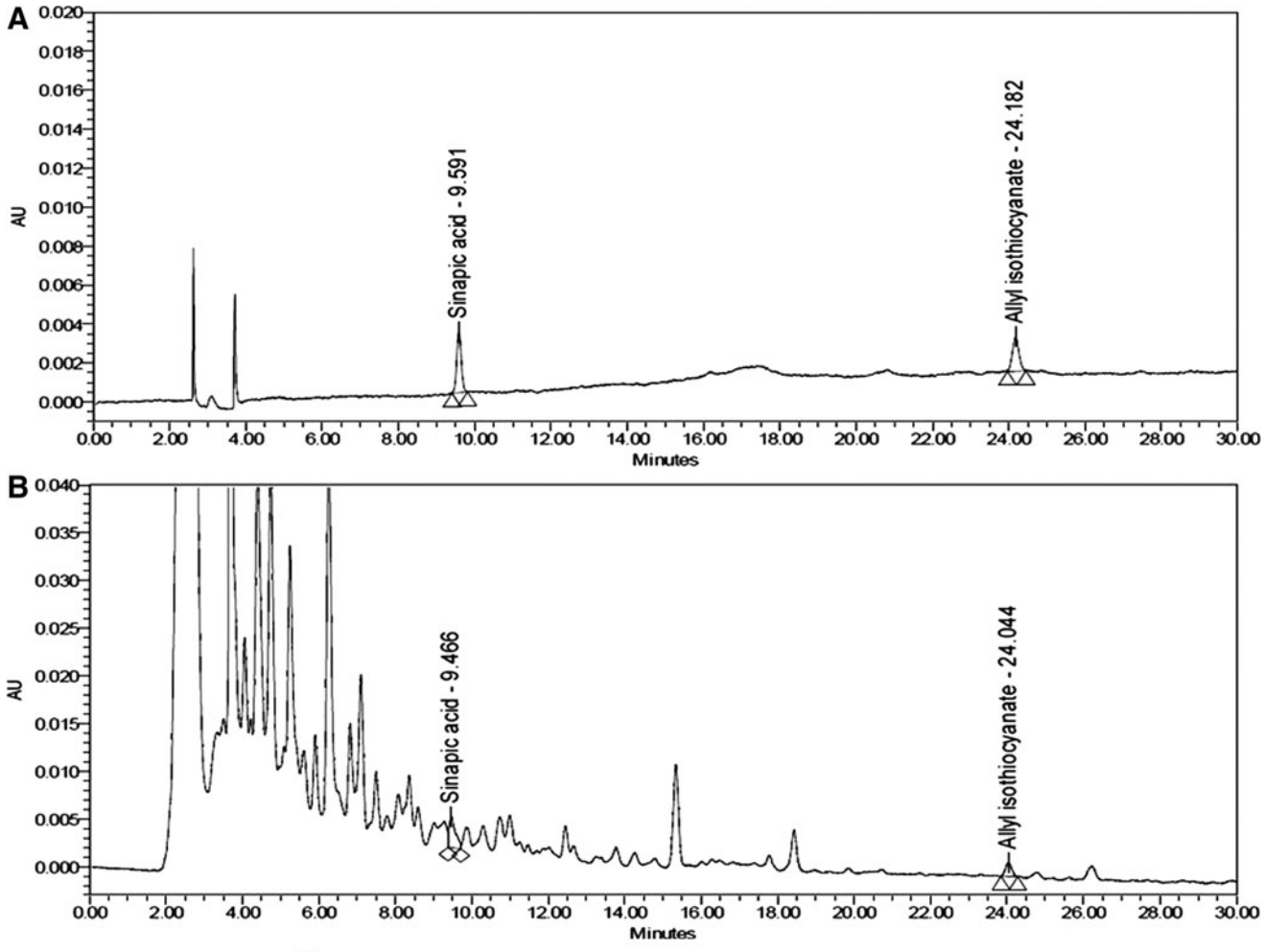
An animal model of IBS induced by zymosan injection into the colon has been suggested to mimic human diarrhea-predominant IBS.[14,15] Mast cell infiltration in the intestine is apparent, and the animals present not only spontaneous pain-related behaviors and discomfort but also behavioral anxiety; and are accompanied by increased expression of Fos protein,[16,17] a marker of neural activity, in the brain.

Wasabia koreana (WK), scientific name *Cardamine pseudowasabi* H. Shin & D. Kim, has been widely used as a spice, particularly to accompany raw fish,[18–20] and as a traditional medicine for gastrointestinal disorders such as indigestion and abdominal pain in Korea.[18] However, no study has investigated its medicinal use for functional gastrointestinal disorders. In this study, we analyzed the efficacy of a standardized extract of *W. koreana* for both inflammatory diarrhea and neural activity in a zymosan-induced IBS mouse model.

MATERIALS AND METHODS

Preparation of W. koreana

W. koreana (for more information see www.plantlist.org) was purchased from Semtong Farm (Cheorlwon, South Korea). Dried and powdered *W. koreana* (1.0 kg) was extracted with 50% ethanol under sonication for 1 h. The *W. koreana* extract was then filtered through a Whatman No. 2 filter paper (Maidstone, United Kingdom) and concentrated under vacuum. The yield of dried extract was ~9.5%. The extract was stored at −80°C and dissolved in phosphate-buffered saline (PBS) before use.



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	R^2	Amount (μg/g)
Sinapic acid	0.9976	11.555
Allyl isothiocyanate	0.9963	4.165

FIG. 1. HPLC chromatogram for the determination of sinapic acid and allyl isothiocyanate levels in WK. HPLC chromatogram of **(A)** standard mixture and **(B)** WK at 254 nm. **(C)** Identification of useful components from WK. HPLC, high-performance liquid chromatography; WK, *Wasabia koreana* extracts.

Standardization of W. koreana

High-performance liquid chromatography analysis of *W. koreana* was performed on a Waters system (Waters Corp., Milford, MA). Ultraviolet absorbance was monitored at 200–400 nm. Sinapic acid and allyl isothiocyanate were quantified by integration of the peak areas at 240 nm. A 10-μL volume was injected into the column (Triatt-C18, 250×4.6 mm; particle size, 5 μm; YMC Co. Ltd., Japan) that was maintained at 30°C. The mobile phase was composed of water containing 0.1% phosphoric acid (solvent A) and acetonitrile (solvent B). The flow rate was 1.0 mL/min. The gradient was 0.0 min, 20% B; 30 min, 70% B. The re-equilibration time between runs was 10 min. Chromatograms were obtained from a standard mixture and from the *W. koreana* extracts. The linearity detection of each compound was calculated from three (different) concentrations. The content of each surrogate compound in WK and retention time are indicated in Figure 1.

Animal experiments

Seven-week-old male C57/BL6 mice were purchased from Dae Han Biolink, Inc. (Chungbuk, Korea). Animal experiments were performed in accordance with the guidelines of the Daejeon University Animal Care and Use Committee (written approval number DJUARB2015-004). The mice were acclimated for 1 week, and to induce colitis, 0.1 mL zymosan suspension (30 mg/mL in PBS; Sigma-Aldrich, St. Louis, MO, USA) was administered trans-anally via a 22-gauge long stainless steel feeding needle into the colons of mice over a period of 2 min under anesthesia. Zymosan or PBS was administered daily for three consecutive days.[21] The zymosan-injected mice were divided into

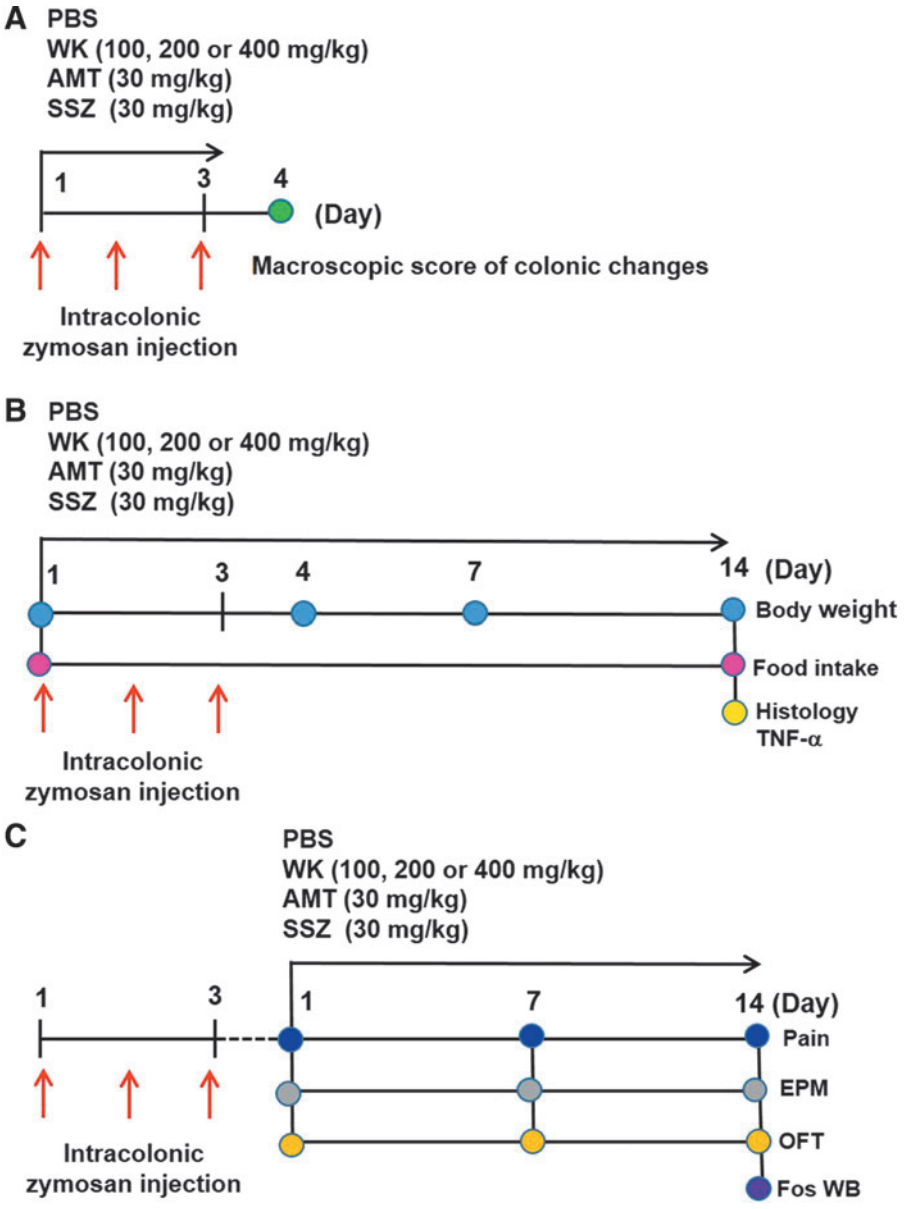


FIG. 2. Experimental schemes. Mice were orally administered with vehicle (PBS), WK (100, 200, or 400 mg/kg), amitriptyline (AMT, 30 mg/kg), or sulfasalazine (SSZ, 30 mg/kg) for the indicated durations daily base. Zymosan injection and oral administration schedules for **(A)** macroscopic score of colonic changes **(B)** body weight, food intake and histological examination, and TNF-α **(C)** pain and anxiety behavior experiments were presented. Naïve mice were injected and administrated with PBS. EPM, elevated plus maze test; OFT, open field test; PBS, phosphate-buffered saline; TNF, tumor necrosis factor; WB, western blotting. Color images available online at www.liebertpub.com/jmf

six groups ($n=8$ per group) and orally treated with PBS (control), amitriptyline (AMT, 30 mg/kg; Sigma-Aldrich), sulfasalazine (SSZ, 30 mg/kg; Sigma-Aldrich), or *W. koreana* extract (WK; 100, 200, or 400 mg/kg). The naïve (PBS-injected) mice were administered oral doses of PBS. The experimental schemes, including zymosan injection and administration schedule, are presented in Figure 2.

Macroscopic scoring of zymosan-induced colon changes

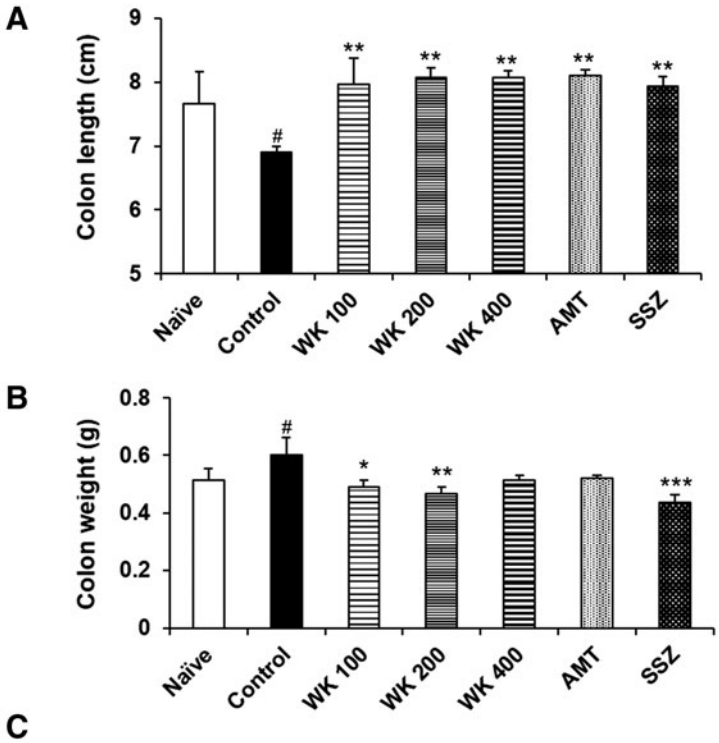
Colon weights were measured after removing fecal contents, and colon length from the aboral end of the cecum to the anus was determined. Stool condition was scored by three researchers in a blind manner. The individual scores were graded as follows: colon weight and colon length (score 0, <5%; 1, 5–14%; 2, 15–24%; 3, 25–35%; and 4, >35% vs. control), and stool score (0, normal; 1, loose/moist; 2, amorphous/sticky; and 3, diarrhea). A total macroscopic score index of the severity of colonic changes was defined as the sum of the individual macroscopic score indexes for each colon, with 0 representing normal and 11 being maximally affected, as described by Kimball *et al.*[22]

Body weight changes and food intake

Body weight was measured on days 1, 3, 7, and 14. Food intake was estimated as the difference between the amounts of food remaining in the feeder on day 14 from that given on day 1.

Histological examination

Biopsies were obtained from the large intestine of mice in each group ($n=8$). The sections were fixed and embedded in paraffin, cut to a thickness of 4 μm, and stained with hematoxylin and eosin (H&E) or toluidine blue for the detection of infiltrating inflammatory cells and mast cells, respectively. The cells were observed under a visible-light microscope at a magnification of 200× (Nikon, Japan).



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Group	Length score	Weight score	Stool score	Macroscopic score
Naïve	0.33 ± 0.58	0.33 ± 0.58	0.08 ± 0.10	0.75 ± 1.09
Control	1.00 ± 0.00	2.00 ± 1.00 ##	1.67 ± 0.60###	4.67 ± 1.53 ###
WK 100	0.00 ± 0.00 **	0.00 ± 0.00 **	0.50 ± 0.60 **	0.50 ± 0.43 ***
WK 200	0.00 ± 0.00 **	0.00 ± 0.00 **	0.42 ± 0.60 **	0.42 ± 0.14 ***
WK 400	0.00 ± 0.00 **	0.00 ± 0.00 **	0.50 ± 0.60 **	0.50 ± 0.25 ***
AMT	0.00 ± 0.00 **	0.00 ± 0.00 **	0.50 ± 0.00 **	0.50 ± 0.00 ***
SSZ	0.00 ± 0.00 **	0.00 ± 0.00 **	0.58 ± 0.10 **	0.58 ± 0.14 ***

FIG. 3. Effects of WK on macroscopic score of colonic changes induced by zymosan injection. **(A)** The colon length, **(B)** colon weight, and **(C)** macroscopic score indexes were analyzed as described in the Materials and Methods section. Data are mean ± SD values ($n=5$, one-way ANOVA; $^{\#}P<.05$, $^{\#\#}P<.01$, $^{\#\#\#}P<.001$ vs. naïve; $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ vs. control). ANOVA, analysis of variance; SD, standard deviation.

Enzyme-linked immunosorbent assay

Plasma levels of mouse tumor necrosis factor (TNF)-α were determined using enzyme-linked immunosorbent assay kits (BD Biosciences, San Diego, CA, USA).

Pain-related behavior test

Counts of visceral pain-related behaviors were measured according to the methods described by Laird *et al.*[23] The behaviors included licking of the abdomen in the absence of other grooming behavior, whole-body stretching, flattening the abdomen against the floor, or contracting the abdominal wall such that an arched posture is adopted for 1–2 sec (abdominal retractions). The total number of visceral pain-related behaviors was recorded over a 10-min period by two investigators unaware of the group identities.

Anxiety-related behavior test

For the elevated plus maze (EPM) test, the apparatus consisted of an elevated plus-shaped plastic maze (50 cm from the floor), with two open and two closed arms (1 m × 10 cm × 40 cm walls). Each mouse was placed in the center of the EPM and performance was recorded throughout a 5-min session. The number of entries into the open and closed arms of the maze was counted. The recordings were analyzed using video tracking software (SMART 3.0; Panlab S.I., Barcelona, Spain). For the open field test (OFT), the open field arena (30 × 30 cm) was constructed from acrylic sheets, and each mouse was placed in the center of the field. The mice were individually transferred to the test field, and their behaviors were recorded for 30 min. The recordings were analyzed using the same software as in the EPM, as described by Zhang *et al.*[21]

Western blots

Brain samples were homogenized in 1 mL of lysis buffer (Pro-Prep™; Intron Biotechnology, Korea) containing 100 nM phenylmethylsulfonyl fluoride (PMSF) and 5 μg/mL of protease inhibitor mixture. Equal amounts (40 μg) of proteins were separated using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA), which were blocked with 5% skim milk in TBS/T buffer (Tris-buffered saline with 0.1% Tween 20) for 1 h. The membranes were probed overnight with FosB- and c-Fos-specific antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 2 h at room temperature. HRP was detected using a chemiluminescent detection reagent (Amersham Biosciences). Band densities were compared with β-actin and measured using an Image-Lab densitometer (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data are expressed as mean ± standard deviation, and each figure is representative of three independent experiments. One-way analysis of variance (ANOVA) was performed using

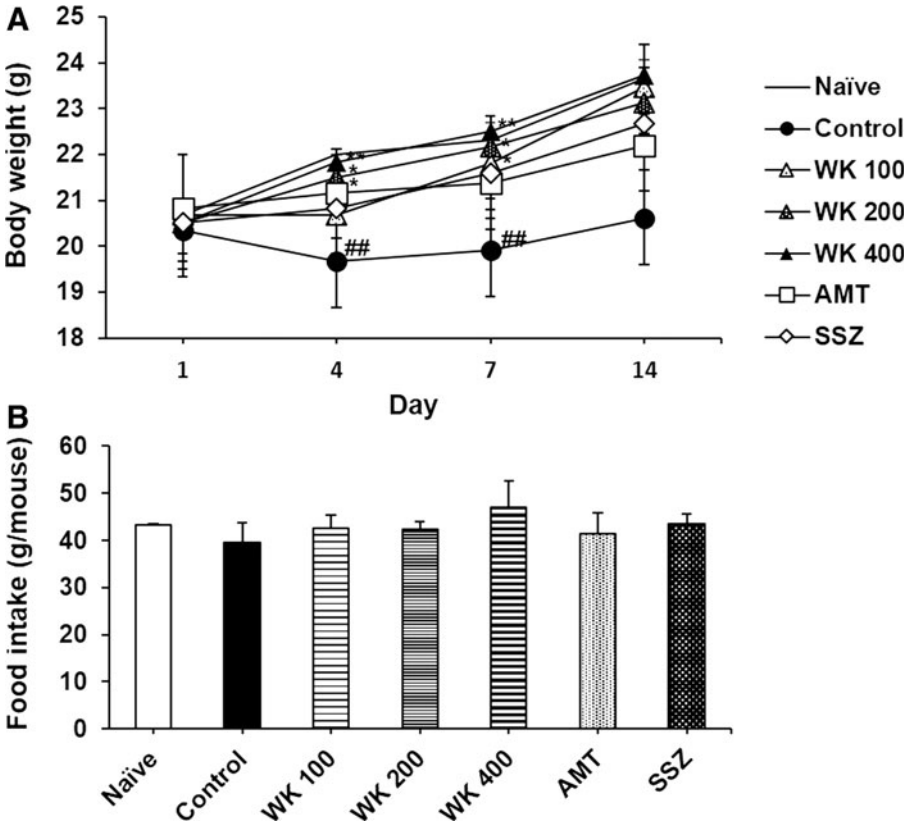

FIG. 4. Effects of WK on body weight and food intake in zymosan-induced IBS mice. **(A)** Body weight and **(B)** food intake were measured on the indicated days. Data are mean ± SD values ($n = 5$, one-way ANOVA; $^{\#\#}P < .01$ vs. naïve; $^{*}P < .05$, $^{**}P < .01$ vs. control). IBS, irritable bowel syndrome.

SPSS software (IBM-SPSS, Inc., Chicago, IL, USA) to assess between-group differences. Multiple group comparisons were performed using one-way ANOVA, followed by *post hoc* Tukey tests. Differences with $P < .05$ were considered statistically significant.

RESULTS

Standardization of WK

To standardize the WK, sinapic acid and allyl isothiocyanate were used as surrogate markers and quantified. A chromatogram of the markers and WK were obtained. The linearity of the method was determined using five different concentrations (0.1, 0.3, 0.5, 0.7, and 1.0 μg/mL) of the surrogate compound. The surrogate compounds in WK and retention times are shown in Figure 1.

Effects of WK on macroscopic score of zymosan-induced colon changes

We examined macroscopic changes to zymosan-injected colons by measuring colon length, weight, and stool condition after WK administration. Two drugs currently used to treat IBS patients, the antidepressant AMT and anti-inflammatory

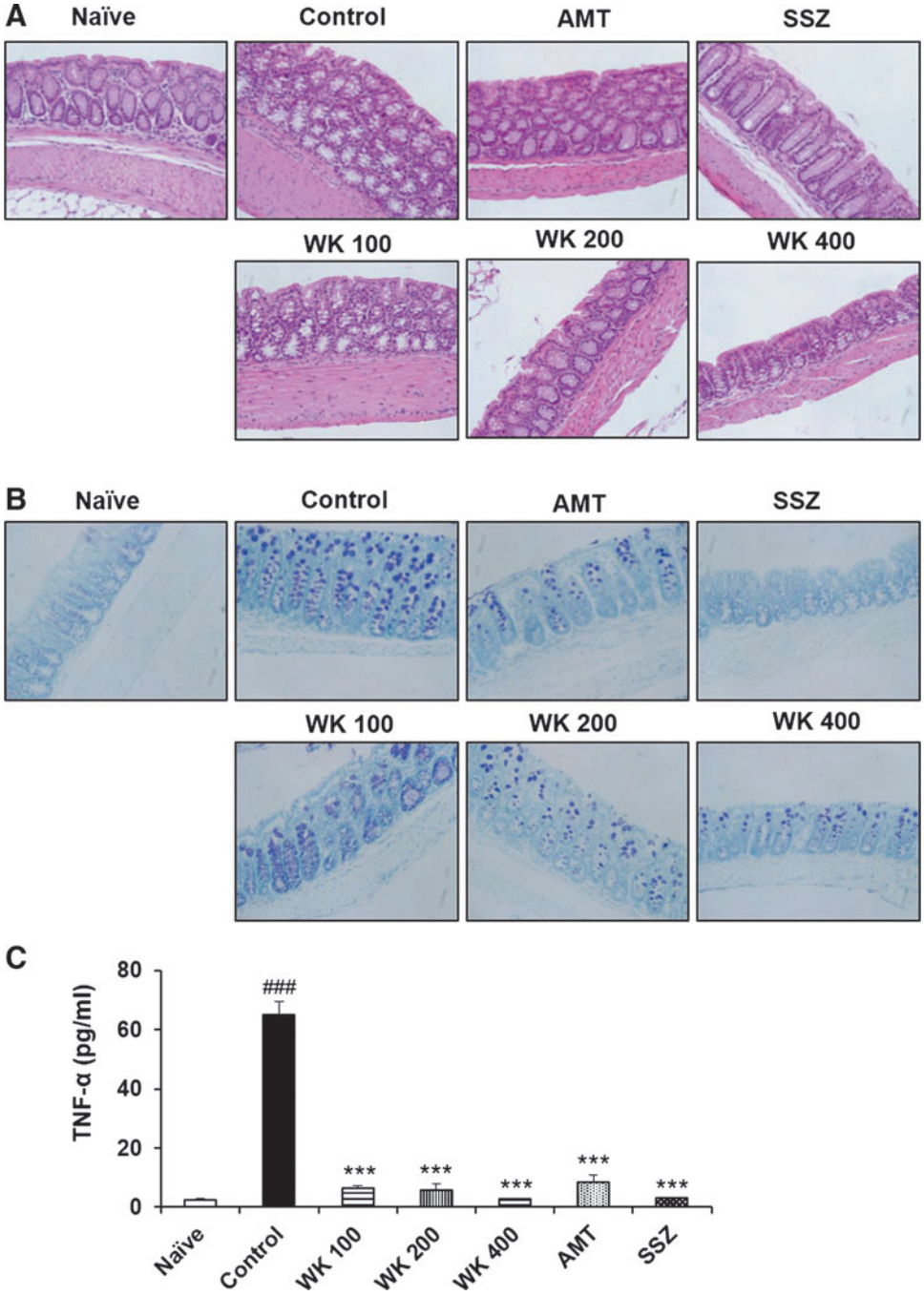

FIG. 5. Effects of WK on colonic inflammation and serum levels of TNF-α in zymosan-induced IBS mice. Formalin-fixed colons were stained with **(A)** hematoxylin and eosin or **(B)** toluidine blue. Cells in the colon were visualized under a visible-light microscope at a magnification of 200×. **(C)** The serum level of TNF-α was determined by enzyme-linked immunosorbent assay. The data represent the mean ± SD of triplicate determinations ($n = 8$, one-way ANOVA; $^{###}P < .001$ vs. naïve; $^{***}P < .001$ vs. control). Color images available online at www.liebertpub.com/jmf

SSZ, were used as positive controls.[24,25] Zymosan injection induced distal colon shortening compared with naïve mice, indicating colitis; however, oral WK administration increased colon length compared with the control zymosan-injected mice (WK 100: 7.97 ± 0.4 cm, WK 200: 8.07 ± 0.15 cm, and WK 400: 8.07 ± 0.12 cm; all $P < .01$; Fig. 3A). Furthermore, colon weight was significantly increased in control mice compared with naïve mice, indicating colitis, but WK significantly decreased colon weight (WK 200: 0.43 ± 0.03 g, $P < .001$ and WK 400: 0.49 ± 0.02 g, $P < .05$; Fig. 3B) to approach naïve levels. SSZ also suppressed the colon weight increase. The feces were dark brown, rigid masses in naïve mice; whereas zymosan-injected mice had watery and sticky masses of bright brown or yellow color, indicating moderate diarrhea. As expected, the stool score increased in zymosan-treated mice compared with naïve mice, but WK administration significantly reduced the stool score compared to control mice (WK 100: 0.5 ± 0.43; WK 200: 0.42 ± 0.14, and WK 400: 0.5 ± 0.25; all $P < .01$; Fig. 3C). The macroscopic score (sum of colon weight, length, and stool score) was significantly increased by zymosan injection and indicated low-to-moderate colitis and diarrhea. Oral administration of WK, SSZ, and AMT all decreased the macroscopic score compared to control mice, suggesting that WK might suppress zymosan-induced mild colitis and diarrhea.

Effects of WK on body weight changes and food intake

Mean body weights were initially comparable across the seven groups. After zymosan injection, control mice demonstrated significantly decreased body weight on day 4 and 7 compared to naïve mice; however, oral WK administration suppressed the body weight decrease on days 4 (WK 200: 21.5 ± 0.5 g, $P < .05$ and WK 400: 21.83 ± 0.29 g, $P < .01$) and 7 (WK 100: 21.8 ± 0.3 g, WK 200: 22.17 ± 0.96 g, both $P < .05$ and WK 400: 22.5 ± 0.35 g, $P < .01$; Fig. 4A). AMT administration (30 mg/kg) significantly improved body weight on day 4 (AMT: 21.17 ± 0.58 g, $P < .05$; Fig. 4A), but SSZ (30 mg/kg) did not attenuate the weight loss (Fig. 4A). No difference was observed in food intake among the groups (Fig. 4B). These results suggest that WK might protect against weight loss without affecting food intake in zymosan-induced IBS mice.

Effects of WK on zymosan-induced inflammation

Histological changes in the colon were examined by H&E and toluidine blue staining. Compared with naïve mice, the colons of zymosan-injected control mice showed low- to moderate-grade inflammation, as previously reported,[14] including decreased mucosal epithelium thickness and submucosal inflammatory cell infiltration. However, in WK-treated mice, H&E staining indicated significant, dose-dependent decreases in inflammatory cell infiltration and colon wall thickness (Fig. 5A). Toluidine blue staining revealed mast cells were associated with inflammation foci in control mice but not naïve mice. The number of infiltrating mast cells was markedly diminished in WK-treated mice, similar to SSZ-treated mice (Fig. 5B). Serum levels of TNF-α, an indicator of systemic inflammation, were significantly increased in zymosan-treated mice, but oral WK administration suppressed this increase, leading to near-normal TNF-α levels (Fig. 5C). AMT and SSZ also suppressed TNF-α production. These data suggest that WK might have inhibitory effects on gastrointestinal and systemic inflammation in zymosan-induced IBS mice.

Effects of WK on visceral pain-related behaviors

On day 1, we did not observe any significant pain-related behaviors in the zymosan-treated mice compared with naïve mice (Fig. 6A). However, on day 7, control mice frequently presented pain-related behaviors including licking of the abdomen, flattening the abdomen against the floor, whole-body

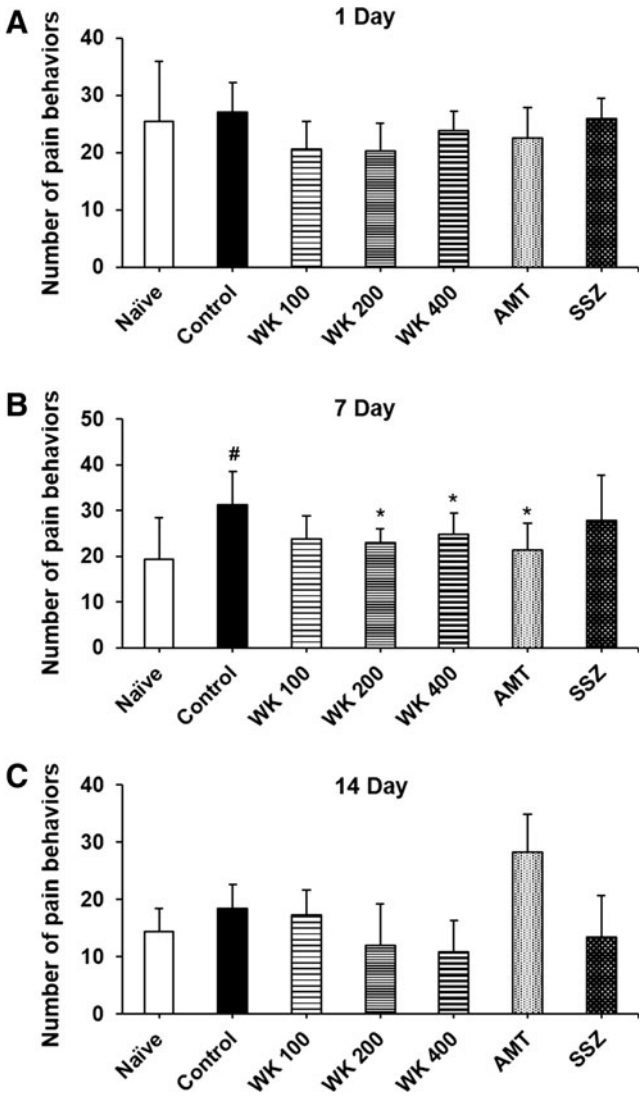

FIG. 6. Effects of WK on visceral pain-related behaviors in zymosan-induced IBS mice. The number of visceral pain-related behaviors was counted on days **(A)** 1, **(B)** 7, and **(C)** 14 according to methods described in the Materials and Methods section. Data are mean ± SD values ($n = 8$, one-way ANOVA; $^{\#}P < .05$ vs. naïve; $^{*}P < .05$ vs. control).

stretching, and abdominal retraction (F [6, 49] = 3.106, $P < .05$). Administration of WK or AMT (WK 200: 23 ± 2.92, WK 400: 24.8 ± 4.6, AMT: 21.4 ± 5.73; all $P < .05$; Fig. 6B), but not SSZ, lowered the frequency and intensity of pain-related behaviors on day 7. The behaviors did not persist until day 14 (Fig. 6C). These results suggest that WK might suppress visceral pain in zymosan-induced IBS mice.

Effects of WK on anxiety-like behaviors

In the EPM test, we measured the number of entries and duration of stay in open arms on days 1, 7, and 14. As expected, locomotion significantly diminished in control mice compared with that in naïve mice (F [6, 49] = 27.0, $P < .001$; Fig. 7A). The dramatic reductions were observed on day 7 and 14 (demonstrating low entry number [3.5 ± 1.29] and [1.5 ± 0.58], respectively). In WK-treated mice, the number of entries into open arms markedly increased compared with control mice on day 14 (WK 100: 5.25 ± 0.96, WK 200: 6.75 ± 0.96, WK 400: 7.75 ± 0.5; all $P < .001$; Fig. 7A), and AMT treatment significantly increased the number of entries on days 7 (AMT: 7.0 ± 1.41, $P < .05$) and 14 (AMT: 6.75 ± 0.96, $P < .001$), with values similar to those of WK 400. SSZ demonstrated a significant effect on day 14. Similarly, the duration of stay in open arms markedly increased in WK-treated mice compared to

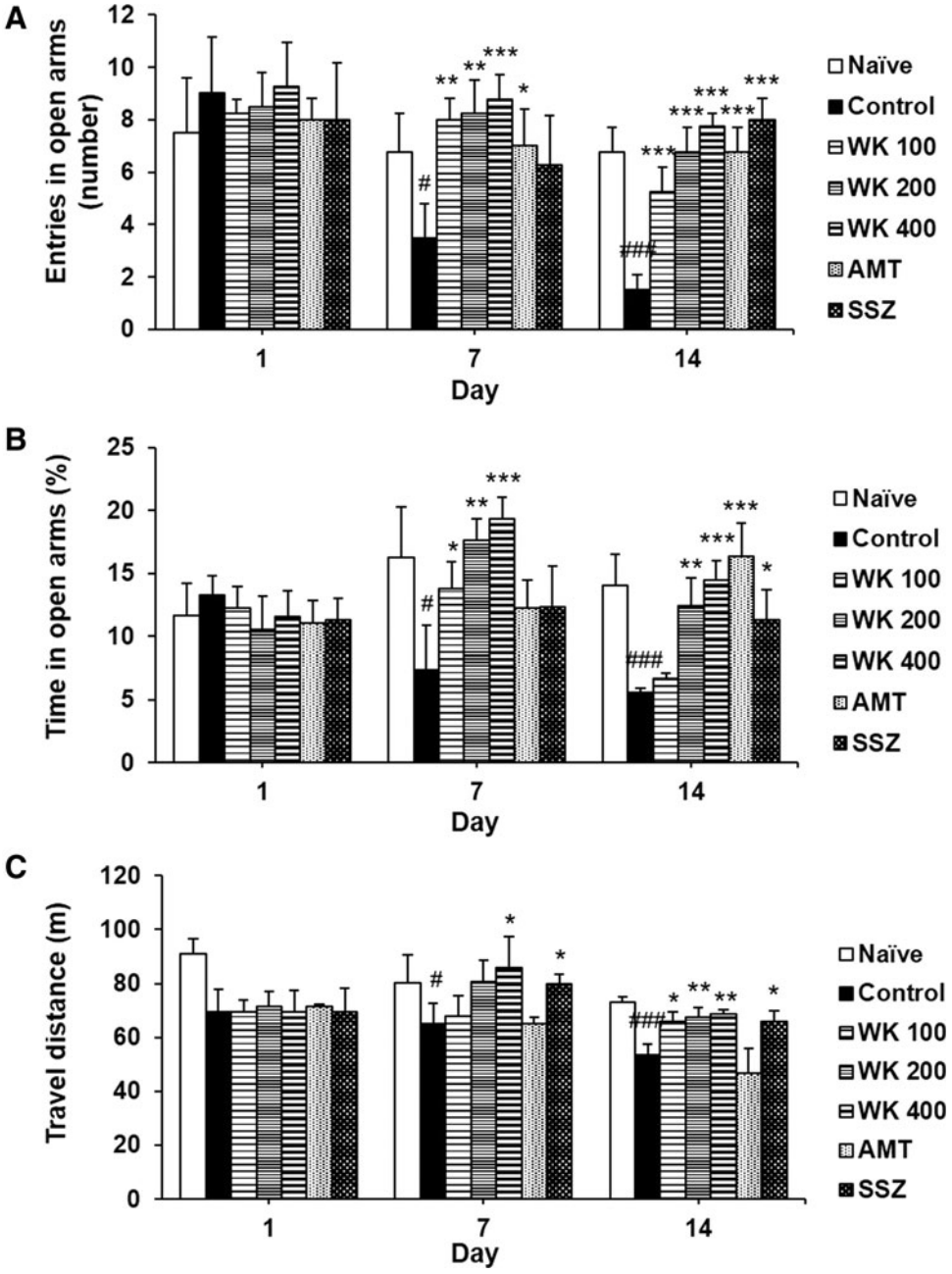

FIG. 7. Effects of WK on anxiety-related behaviors in zymosan-induced IBS mice. **(A)** The number of entries and **(B)** relative time spent in open arms in the elevated plus maze and **(C)** the travel distance in the OFT were measured on days 1, 7, and 14. Data are mean ± SD ($n = 8$, one-way ANOVA; $^{\#}P < .05$, $^{\#\#\#}P < .001$ vs. naïve; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs. control).

control mice on days 7 and 14 (Fig. 7B). In the OFT, the travel distance increased in WK-treated mice on day 7 (WK 400: 85.95 ± 11.37 m, $P < .05$) and day 14 (WK 100: 65.93 ± 3.54 m, $P < .05$; WK 200: 67.56 ± 3.27 m, WK 400: 68.44 ± 1.72 m, both $P < .01$) compared to control mice (Fig. 7C). SSZ showed similar effects to WK, but AMT was not effective in this test. These results suggest that WK might control anxiety like behaviors in zymosan-induced IBS mice.

Effects of WK on Fos expression in the brain

To understand the effects of WK on IBS-like symptoms at the molecular level, we measured FosB2 and c-Fos expressions[26] in the brain was examined by western blot analysis. FosB2 levels dramatically increased in control mice compared with naïve mice, indicating entry of noxious stimuli into the brain; however, WK treatment significantly reduced FosB2 levels in a dose-dependent manner (Fig. 8). AMT reduced the level of FosB2, whereas SSZ increased FosB2 in the brain. The level of c-Fos also increased in the brains of control mice and markedly decreased in WK (400 mg/kg) and AMT-treated mice, as previously reported.[27] These results suggest that WK might affect neuronal activity in the brains of zymosan-induced IBS mice.

DISCUSSION

Multiple drugs have been prescribed for IBS patients because multiple factors are known to complicate this gut disorder, including altered gut motility and visceral hypersensitivity involving dysfunction of the brain-gut axis, which are associated with the central, autonomic, and enteric nervous systems.[28] Intestinal inflammation and abnormal immune function have also been proposed as etiological factors in IBS.[29] Loperamide and 5-HT_3 antagonists for diarrhea, antispasmodics for abdominal pain, antidepressants for anxiety and depression, and antibiotics along with anti-inflammatory drugs such as mesalamine have been used to treat IBS. However, current medicines do not seem to provide sufficient benefit to IBS patients and there are also safety concerns about using most drugs.[30]

In this study, we used a mouse model of zymosan-induced IBS to investigate the traditional medicine WK as a potential therapy for IBS. Zymosan injection induced diarrhea and low-to-moderate-grade colon inflammation, which somewhat resembles human postinfectious IBS,[14] in addition to IBS-like behaviors including pain and anxiety, which appear to mimic human IBS.[21] Oral WK administration suppressed acute colon changes, such as colitis and diarrhea, and significantly decreased submucosal thickening and mast cell

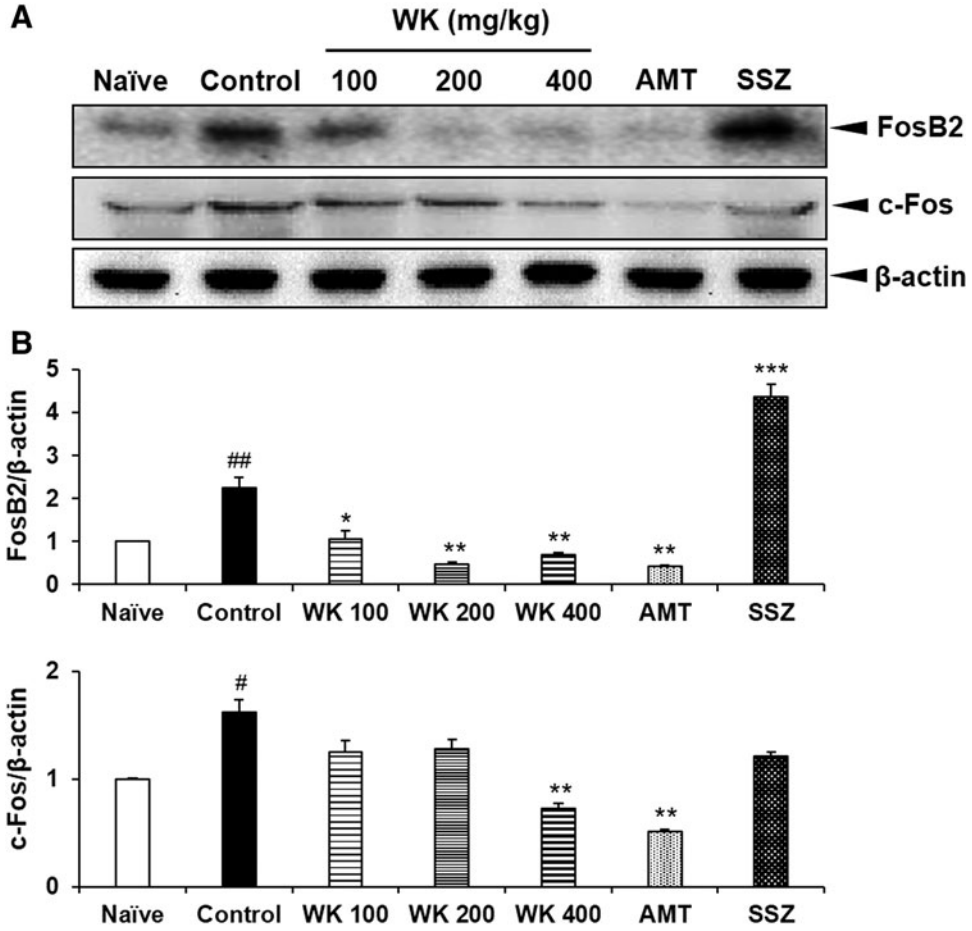

FIG. 8. Effects of WK on Fos expression in the brain of zymosan-induced IBS mice. **(A)** Isolated whole-brain lysates were analyzed by western blotting using FosB and c-Fos antibodies. **(B)** Band density was analyzed with a densitometer. β-actin was used as the loading control. The presented data are representative of three independent experiments. Data are mean ± SD ($n = 3$, one-way ANOVA; $^{\#}P < .05$, $^{\#\#}P < .01$ vs. naïve; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs. control).

infiltration to levels comparable to naïve mice. Body weight loss was reduced without affecting food intake. WK also ameliorated both pain-related and anxiety-like behaviors. Further, to provide molecular evidence for the effects of WK, we analyzed neuronal FosB2 and c-Fos expression, which are widely used as an indicator of neuronal activity. FosB2 and c-Fos are related to chronic and acute stress, respectively,[31] and are known to be induced by noxious stimuli, including formalin[27] and zymosan injection.[32] WK administration significantly suppressed the increase in FosB2 expression, in contrast to AMT, which primarily suppressed neural activity but not colonic inflammation, and SSZ, which mainly inhibited colon inflammation rather than pain or FosB expression.

The brain and gut closely interact via the brain-gut axis. There have been many reports that vagus nerve stimulation can activate FosB in the brain[33] and *vice versa*.[34] On the basis of our data, it is possible that active component(s) of WK not only directly enter the brain and improve brain activities, resulting in controlling gut function, but also influence the vagus nerve and/or inflammation in the colon, resulting in changes in FosB expression in the brain. Allyl isothiocyante, a hydrophobic small molecule, which is a WK component, may enter the brain through the blood-brain barrier and then affect Fos expression. On the other hand, this molecule may affect neurons[35] and inflammation[36] in the colon, leading to increased Fos expression in the brain. Sinapic acid, which is also a component of WK, may be unable to enter the brain as it is hydrophilic, but it may affect Fos expression by controlling colonic inflammation.[37] Taken together, we suggest that inhibition of colonic inflammation and/or neuronal activities by WK may contribute to suppressing noxious stimuli entering the brain, resulting in pain reduction and amelioration of anxiety-like mood disorders, or that WK may directly inhibit brain activity, which subsequently affects colon inflammation.

Further studies are warranted to fully understand the mechanisms of action of WK, including efficacy studies using various IBS animal models and *in vitro* mechanism studies using brain cells and mast cells. Studies are also needed to determine the active compounds of WK for IBS.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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