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Original Research Article

Efficacy of Traditional Herbal Medicine, Bojungikki-Tang (Hochuekkito, Bu-Zhong-Yi-Qi-Tang), in Irritable Bowel Syndrome: Mechanisms Involving TRP and NaV Channels

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Abstract

Background: Irritable bowel syndrome (IBS) is a common functional disorder of the gastrointestinal (GI) tract, typically presenting with abdominal discomfort, distension, and irregular bowel movements. In IBS, the underlying mechanisms are thought to include visceral hypersensitivity and altered regulation of ion channels, where both transient receptor potential (TRP) channels and voltage-gated sodium (NaV) channels are considered key contributors.
Objectives: While Bojungikki-Tang (BJIKT), a traditional herbal medicine, has been used for GI disorders, its efficacy and mechanisms in IBS remain unclear.
Materials and Methods: A zymosan-induced IBS animal model was used to evaluate the effects of BJIKT on colon length, weight, mucosa thickness, body weight, and inflammatory markers. BJIKT-induced changes in TRP and NaV channel activity were examined through electrophysiological methods.
Results: BJIKT depolarized pacemaker potentials in interstitial cells of Cajal (ICC) *in vitro*. BJIKT restored shortened colon length, normalized increased colon weight, and reduced mucosal thickness and tumor necrosis factor-alpha (TNF-α) levels in the IBS animal model. BJIKT also prevented body weight loss. Electrophysiological studies revealed that BJIKT enhanced TRPV1 and TRPV4 currents, suppressed TRPA1 currents, and suppressed NaV1.5 and NaV1.7 channel currents in a dose-dependent fashion.
Conclusion: BJIKT alleviates IBS symptoms by regulating TRP and NaV ion channels, reducing inflammation, and restoring GI function. These findings provide a scientific basis for the therapeutic potential of BJIKT in IBS management.

Keywords

Irritable bowel syndrome, Bojungikki-Tang, gastrointestinal disorder, tumor necrosis factor-alpha channels, voltage-gated sodium channels

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Introduction

Irritable bowel syndrome (IBS) is a prevalent functional disorder of the gastrointestinal (GI) tract, typically presenting with persistent abdominal pain, abdominal distension, and irregular bowel patterns (Hung et al., 2023). Although IBS is highly prevalent, its underlying mechanisms are not yet fully clarified, which hampers the development of effective

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therapeutic strategies. Several studies have suggested that visceral hypersensitivity and dysregulated ion channel activity play pivotal roles in the development and persistence of IBS symptoms (Beyder et al., 2014; Du et al., 2022; Enck et al., 2016; Fuentes & Christianson, 2016).

Ion channels, such as transient receptor potential (TRP) and voltage-gated sodium channels (NaV), have emerged as critical modulators in the context of GI sensory and motor functions (Beyder et al., 2014; Du et al., 2022; Enck et al., 2016; Fuentes & Christianson, 2016). TRP channels, such as TRPA1, TRPV1, and TRPV4, are activated by various physical, chemical, and inflammatory stimuli, contributing to visceral hypersensitivity, a hallmark of IBS (Du et al., 2022). Their dysregulation leads to abnormal pain perception, altered gut motility, and heightened responses to mechanical and chemical stimuli, all of which are characteristic features of IBS (Du et al., 2022). These channels represent promising targets for therapeutic interventions aimed at alleviating IBS symptoms. Similarly, NaV1.5 channel is essential for the proper regulation of the enteric nervous system (ENS), which governs GI motility (Holm et al., 2002; Osorio et al., 2014; Strege et al., 2007). By modulating electrical signaling in enteric neurons, smooth muscle cells, and interstitial cells of Cajal (ICC), NaV1.5 contributes to the coordination of gut peristalsis and overall GI motility (Holm et al., 2002; Osorio et al., 2014; Strege et al., 2007). Dysfunction of NaV1.5 has been linked to motility disorders, highlighting its importance in maintaining normal digestive function. On the other hand, NaV1.7 is involved in the transmission of nociceptive signals and has been linked to heightened pain perception in IBS (Jiang et al., 2021).

Traditional herbal medicines, such as Bojungikki-Tang (BJIKT), have been used for centuries to manage GI disorders (Elmaghraby et al., 2023). BJIKT is composed of a blend of herbal ingredients thought to possess immunomodulatory and motility-modulating characteristics (Cho et al., 2024; Kwon et al., 2021; Lee et al., 2012; Yoo et al., 2018). Nevertheless, little research has addressed the therapeutic potential of BJIKT in IBS or clarified its mechanistic basis.

The present study is designed to assess the efficacy of BJIKT in alleviating IBS symptoms and to explore its modulatory effects on TRP and NaV ion channels. By clarifying these mechanisms, the research aims to establish a scientific rationale for the use of BJIKT in IBS therapy and enhance understanding of ion channel abnormalities in GI disorders.

Materials and Methods

Instruments and Reagents

A Waters ACQUITY™ ultra performance liquid chromatography (UPLC) instrument (USA) equipped with a photodiode array detector and a BEH C18 column (1.7 μm, 2.1 × 100 mm) was used to perform the quantitative analysis. Reagents included HPLC-grade methanol (Junsei), acetonitrile (JT-Baker), and tertiary distilled water. Standard compounds were obtained from the following sources: 5-hydroxymethyl-2-furfural, norcimifugin, prime-*O*-glucosylcimifugin, saikosaponin A, and calycosin-7-*O*-β-D-glucoside (ChemFaces, China); l-tryptophan, hesperidin, decursin, nodakenin, and ginsenoside Rg1 (Sigma–Aldrich, St. Louis, MO, USA); isoliquiritin (Wako, Japan).

Preparation of the Standard Solution

Each standard compound (5-hydroxymethyl-2-furfural, l-tryptophan, norcimifugin, prime-*O*-glucosylcimifugin, saikosaponin A, isoliquiritin, hesperidin, calycosin-7-*O*-β-D-glucoside, decursin, nodakenin, ginsenoside Rg1) was accurately weighed, dissolved in methanol, and prepared as a stock solution at 1 μg/μL. The calibration curves for all compounds had coefficients of determination (R^2) above 0.999.

Preparation of the Test Liquid for Quantitative Analysis

A 1 g portion of the BJIKT sample was accurately weighed, combined with 10 mL of 70% methanol, and subjected to microwave-assisted extraction for 1 h. The extract was filtered through a 0.22 μm membrane filter for analysis.

Quantitation of the Sample

Chromatographic analysis via UPLC was carried out at ambient temperature using the Waters ACQUITY™ BEH C18 column. PDA detection wavelengths were set as follows: 5-hydroxymethyl-2-furfural: 280 nm, l-tryptophan, norcimifugin, calycosin-7-*O*-β-D-glucoside: 230 nm, saikosaponin A, ginsenoside Rg1: 203 nm, prime-*O*-glucosylcimifugin, decursin, nodakenin: 330 nm, isoliquiritin, hesperidin: 254 nm. Retention times were used for qualitative identification, and quantification was based on peak area (Tables 1 and 2).

Table 1. The Analysis Condition of 5-Hydroxymethyl-2-Furfural, l-Tryptophan, Norcimifugin, Saikosaponin A, Prime-*O*-Glucosylcimifugin, Isoliquiritin, Hesperidin, Calycosin-7-*O*-β-D-glucoside, Decursin, and Nodakenin.

Time (min)	0.1% FA/ Water (%)	0.1% FA/ Acetonitrile (%)	Flow Rate (mL/min)
0	98	2	0.40
2.0	98	2	0.40
4.0	90	10	0.40
5.0	70	30	0.40
7.0	70	30	0.40
8.0	60	40	0.40
10.0	40	60	0.40
13.0	2	98	0.40
14.0	98	2	0.40
16.0	98	2	0.40

Table 2. The Analysis Condition of Ginsenoside Rg1.

Time (min)	Water (%)	Acetonitrile (%)	Flow Rate (mL/min)
0	85	15	0.40
1.0	85	15	0.40
14.0	70	30	0.40
15.0	68	32	0.40
16.0	60	40	0.40
17.0	45	55	0.40
19.0	45	55	0.40
21.0	10	90	0.40
22.0	10	90	0.40
23.0	85	15	0.40

Preparation of ICC Cultures

The large intestines of male and female ICR mice aged 3–6 days were harvested. After carefully dissecting and removing the mucosal layer, the remaining tissue was subjected to enzymatic digestion using a collagenase solution to isolate the smooth muscle layers. The resulting single cells were then cultured in smooth muscle growth medium (Clonetics Corp., San Diego, CA, USA) at 37°C. All experiments involving ICC were conducted after a 12-h incubation period.

Animal and Induction of IBS Using Zymosan

Male C57/BL6 mice (20–25 g) were used. IBS was induced by rectal administration of zymosan (Sigma–Aldrich, St. Louis, MO, USA) using a soft catheter, followed by holding mice head-down for 1–2 min to prevent leakage. Mice were divided into seven groups: Naive, Zymosan, BJIKT (100, 250, 500 mg/kg), sulfasalazine (30 mg/kg; Sigma–Aldrich, St. Louis, MO, USA), and amitriptyline (30 mg/kg; Sigma–Aldrich, St. Louis, MO, USA).

Measurement of Colonic Weight and Length

On the 4th day, the entire colon was carefully excised from the cecum to the rectum and immediately removed fecal contents. The weight of the colon was measured using a digital scale. Also, the colon was laid flat on a silicone-coated dish without applying excessive tension. Using a ruler, the total length of the colon was measured from the cecum to the rectum.

Assessment of Body Weight and Cumulative Food Intake

Mice were individually weighed on days 1, 4, 9, and 12 using a digital scale. Body weights were recorded at the same time each day to minimize variability. Daily food intake values were recorded and summed across the 12 days to determine the total amount of food consumed over the experimental period.

Measurement of Mucosal Thickness via Hematoxylin & Eosin (H&E) Staining

On day 12, the colon was carefully excised and immediately rinsed with ice-cold phosphate-buffered saline (PBS) to remove fecal contents. A segment of the colon was collected and fixed for 24 h at room temperature. After H&E staining, the tissue sections were examined using a light microscope (Nikon, Tokyo, Japan). Mucosal thickness was measured at multiple points, and measurements were taken from the basal lamina of the mucosa to the luminal surface of the epithelial layer.

Enzyme-linked Immunosorbent Assay (ELISA)-based Measurement of Tumor Necrosis Factor-alpha (TNF-α)

On day 12, an ELISA kit (BD Biosciences, San Diego, CA, USA) was used to measure TNF-α in the samples. The experiment was conducted according to the instructions provided in the kit.

Electrophysiological Measurement of ICC, TRPV1, TRPV4, TRPA1, NaV1.5, and NaV1.7 Channels

Whole-cell electrophysiological recordings were conducted to evaluate the pacemaker potentials of ICC in current-clamp mode. HEK293T cells were transfected with plasmids encoding TRPV1, TRPV4, TRPA1, NaV1.5, or NaV1.7 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Mock-transfected cells (no plasmid) were used as negative controls. An Axon 200B amplifier (Molecular Devices, San Jose, CA, USA) with glass pipettes (resistance 3–5 MΩ) was used. The pipette and bath solutions were prepared as previously described. For TRP channels, current–voltage (I–V) relationships were obtained using ramp pulses from −100 mV to +100 mV. I–V relationships for TRP channels were recorded using ramp pulses spanning −100 mV to +100 mV. For NaV channels, depolarizing pulses were delivered from a holding potential of −120 mV to test potentials between −120 and 0 mV. Data acquisition was performed with pCLAMP 10.7 (Molecular Devices), and IC_{50} values were calculated by fitting the results to the Hill equation.

Statistical Analysis

The results were presented as means ± SEMs, and Tukey's multiple comparison test was used for the analysis of variance (ANOVA) using GraphPad Prism 6. Statistical significance was set at p values <.05.

Results

Analysis of BJIKT

Contents of 5-hydroxymethyl-2-furfural, l-tryptophan, norcimifugin, prime-*O*-glucosylcimifugin, saikosaponin A, isoliquiritin, hesperidin, calycosin-7-*O*-β-D-glucoside, decursin, nodakenin, and ginsenoside Rg1 were analyzed by UPLC. Concentrations of the 11 compounds were determined based on calibration curves constructed from standard compounds (Table 3 and Figure 1).

Effects of BJIKT on the Pacemaker Potentials of ICC from Murine Large Intestines

Using a whole-cell patch-clamp technique, we evaluated pacemaker potentials of ICC in the murine large intestine. Under current-clamp mode ($I = 0$), ICC generated pacemaker potentials, which were depolarized by BJIKT (1–10 mg/mL) (Figure 2A–2D). These results suggest that BJIKT modulates intestinal motility by depolarizing pacemaker potentials.

Effects of BJIKT on the Colon in an IBS Animal Model

In the IBS animal model, BJIKT restored the colon length shortened by zymosan and normalized the increased colon weight. BJIKT effects were dose-dependent and comparable to those of the positive control's AMT and SSZ (Figure 2E and 2F). These findings suggest BJIKT contributes to restoring normal colon function in IBS.

Effects of BJIKT on Body Weight and Food Intake in an IBS Animal Model

In the zymosan-induced IBS animal model, body weight was reduced on days 4, 9, and 12 compared to the normal group, but

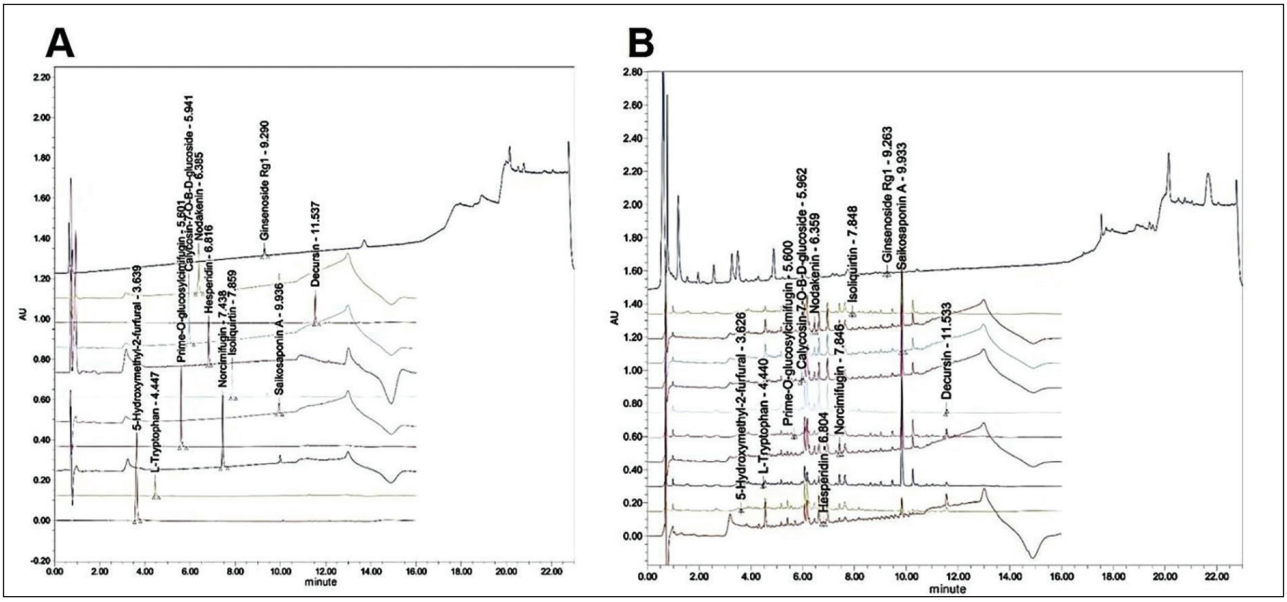


Figure 1. Ultra Performance Liquid Chromatography (UPLC) Profiles of 11 Major Compounds Identified in Bojungikki-Tang (BJIKT). (A) UPLC Profile of the Commercial Standard Compounds. (B) UPLC Profile of 11 Major Compounds in BJIKT.

Table 3. Retention Time-based Qualitative Analysis and Quantification by Peak Area Method.

Bojungikki-Tang (Unit: mg/kg)		
Atractylodes Rhizome White	5-Hydroxymethyl-2-furfural (5H2F)	0.515 ± 0.023
	l-Tryptophan	4.694 ± 0.214
Cimicifuga Rhizome	Norcimifugin,	8.078 ± 0.851
	Prime-*O*-glucosylcimifugin,	0.996 ± 0.162
Ginseng Radix	Ginsenoside Rg1,	13.714 ± 0.152
Angelicae Gigantis Radix	Decursin	4.051 ± 0.058
	Nodakenin	12.583 ± 0.192
Citri Unshius Pericarpium	Hesperidin	5.918 ± 0.288
Bupleurum falcatum Linné	Saikosaponin A	205.79 ± 13.76
Astragali Radix	Calycosin-7-*O*-β-D-glucoside	1.495 ± 0.238
Glycyrrhizae Radix et Rhizoma	Isoliquiritin	2.061 ± 0.154

it was restored by BJIKT [on day 4, 24.77 ± 1.00 g at naive, 23.37 ± 0.45 g ($p < .01$) at zymosan, 24.09 ± 0.95 g at BJIKT 100 mg/kg, 24.79 ± 0.33 g ($p < .01$) at BJIKT 250 mg/kg, 24.45 ± 0.34 g ($p < .05$) at BJIKT 500 mg/kg, 24.12 ± 0.56 g at SSZ, and 23.88 ± 0.62 g at AMT; on day 9, 27.10 ± 0.04 g at naive, 24.61 ± 0.81 g ($p < .01$) at zymosan, 25.55 ± 0.99 g ($p < .05$) at BJIKT 100 mg/kg, 26.03 ± 0.11 g ($p < .05$) at BJIKT 250 mg/kg, 26.14 ± 0.76 g ($p < .01$) at BJIKT 250 mg/kg, 25.32 ± 0.44 g at SSZ, and 25.44 ± 0.74 g at AMT; and on day 12, 27.44 ± 0.45 g at naive, 25.15 ± 0.58 g ($p < .01$) at zymosan, 26.03 ± 1.03 g at BJIKT 100 mg/kg, 26.77 ± 0.16 g ($p < .01$) at BJIKT 250 mg/kg, 26.66 ± 1.05 g at BJIKT 500 mg/kg, 25.91 ± 0.39 g at SSZ, and 26.26 ± 0.66 g at AMT; Figure 2G]. There were no significant changes in food intake (Figure 2H). These findings suggest that BJIKT prevents body weight loss in the zymosan-induced IBS animal model.

Effects of BJIKT on the Mucosa Thickness and the TNF-α in an IBS Animal Model

The IBS animal causes inflammation in the colon, leading to thickened colonic mucosa. Thickened mucosa was confirmed through H&E staining, and BJIKT reduced the thickened mucosa to normal levels (Figure 2I and 2J). Additionally, TNF-α, a potent pro-inflammatory cytokine that triggers various biological responses, was increased by zymosan but normalized by BJIKT (Figure 2K). These findings demonstrate that BJIKT regulates inflammatory responses in the zymosan-induced IBS animal model.

Effects of BJIKT on TRPV1, TRPV4, and TRPA1 Currents

First, the efficacy of BJIKT on TRPV1 was evaluated. I–V curves were obtained using ramp pulses, and TRPV1 activation was induced by capsaicin (Zhang et al., 2020). In HEK293T cells overexpressing mock TRPV1, no response to capsaicin was observed (Figure 3A). However, in HEK293T cells overexpressing TRPV1, TRPV1 was activated by capsaicin, and BJIKT further increased TRPV1 currents (Figure 3B). Figure 3C shows the relative TRPV1 current amplitude for each BJIKT concentration. Next, the efficacy of BJIKT on TRPV4 was assessed. TRPV4 activation was induced by GSK101A (Jin et al., 2011). In HEK293T cells overexpressing mock TRPV4, no response to GSK101A was observed (Figure 3D). However, in HEK293T cells overexpressing TRPV4, TRPV4 was activated by GSK101A, and BJIKT further increased TRPV4 currents (Figure 3E).

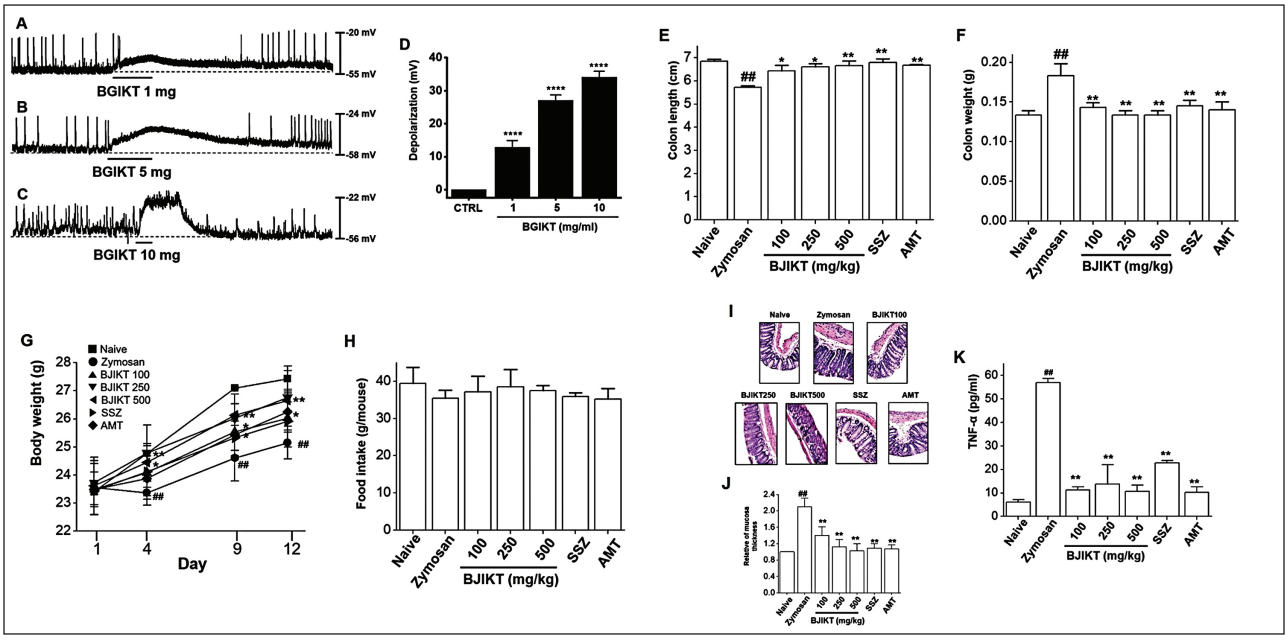


Figure 2. Bojungikki-Tang (BJIKT) Modulates Pacemaker Potentials of Colonic Interstitial Cells of Cajal (ICC) and Alleviates Colonic Pathophysiology in a Zymosan-induced Irritable Bowel Syndrome (IBS) Model. (A–C) BJIKT Induced Dose-dependent Depolarization of Pacemaker Potentials in Colonic ICC. (D) Quantified Analysis of the Frequency of Pacemaker Potentials Following BJIKT Application. (E, F) Zymosan Significantly Reduced Colon Length and Increased Colon Weight; These Changes Were Restored to Near-normal Levels by BJIKT Treatment, Comparable to the Effects of Positive Controls SSZ and AMT. (G, H) BJIKT Prevented Body Weight Loss Induced by Zymosan on Days 4, 9, and 12, while Food Intake Remained Unaffected Among Groups. (I–K) Zymosan Markedly Increased Mucosal Thickness and Tumor Necrosis Factor-alpha (TNF-α) Levels in Colonic Tissue; Both Parameters Were Normalized by BJIKT Treatment in a Dose-dependent Manner, Similar to SSZ and AMT. Data are Presented as mean ± SEM. ****$p < .0001$ versus Control; ##$p < .01$ versus Naive; *$p < .05$, **$p < .01$ versus Zymosan.

Figure 3F shows the relative TRPV4 current amplitude at −100 mV for each BJIKT concentration. Finally, the efficacy of BJIKT on TRPA1 was examined. TRPA1 activation was induced by AITC (Sandor et al., 2016). In HEK293T cells overexpressing mock TRPA1, no response to AITC was observed (Figure 3G). However, in HEK293T cells overexpressing TRPA1, TRPA1 was activated by AITC, and BJIKT suppressed TRPA1 currents (Figure 3H). Figure 3I shows the relative TRPA1 current amplitude for each BJIKT concentration. These studies suggest that TRPA1 is a key mediator of visceral hypersensitivity in the context of IBS.

Effects of BJIKT on NaV1.5 and NaV1.7 Currents

BJIKT suppressed the NaV1.5 current in a concentration-dependent manner, with inhibition rates of 87.97% ± 10.52% at 10 mg/mL, 63.20% ± 15.29% at 30 mg/mL ($p < .01$), and 38.53% ± 5.70% at 50 mg/mL ($p < .01$). The IC_{50} was determined to be 39.1 mg/mL (Figure 4A–4F). Similarly, BJIKT inhibited the NaV1.7 current in a concentration-dependent manner, with inhibition rates of 74.33% ± 13.20% at 10 mg/mL, 25.67% ± 10.60% at 30 mg/mL ($p < .01$), and 7.01% ± 7.55% at 50 mg/mL ($p < .01$). The IC_{50} for NaV1.7 was determined to be 17.0 mg/mL (Figure 4G–4L). The results indicate that NaV1.5 and NaV1.7 channels contribute to the modulatory effects of BJIKT on IBS.

Discussion

This study demonstrates that BJIKT exhibits significant therapeutic effects in IBS model, primarily through its modulation of TRP and NaV ion channels, as well as its anti-inflammatory properties. These results provide a new understanding of the mechanisms underlying BJIKT and its prospective use in IBS therapy. As a common GI disorder, IBS is characterized by ongoing abdominal pain, bloating, and variations in bowel habits. Despite its high prevalence, effective treatments remain limited, largely due to the complex and multifactorial nature of its pathophysiology. The results of this study suggest that BJIKT could serve as a valuable therapeutic approach by targeting multiple mechanisms involved in IBS.

The impact of BJIKT on pacemaker potentials in ICC provides evidence that it may modulate GI motility by affecting rhythmic contractions of the intestine. As shown in Figure 2A–2D, BJIKT suggested the potential role in managing dysregulated gut motility commonly associated with IBS. The IBS model mirrors key features of IBS, such as shortened colon length, increased colon weight, and thickened mucosa, all of which are indicative of inflammation and impaired GI function. BJIKT treatment successfully restored the shortened colon length (Figure 2E), normalized colon weight (Figure 2F), and reduced mucosa thickness to levels comparable to those of standard treatments, such as AMT and SSZ (Figure 2I and 2J). These effects demonstrate that BJIKT not only alleviates the physical manifestations of IBS but also promotes the restoration of normal GI structure and function. Furthermore, BJIKT's ability to prevent inflammation is particularly notable. IBS is often associated with low-grade inflammation, as reflected by elevated levels of pro-inflammatory cytokines like TNF-α. BJIKT effectively suppressed TNF-α levels (Figure 2K), suggesting that its

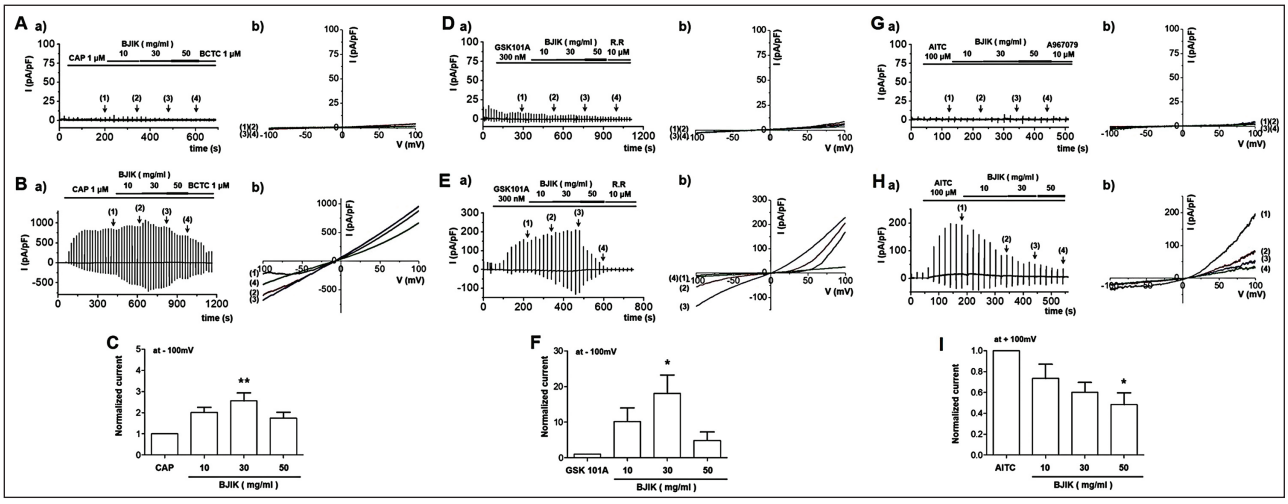


Figure 3. Modulatory Effects of Bojungikki-Tang (BJIKT) on Transient Receptor Potential (TRP) Channel Activity in HEK293T Cells. (A–C) In TRPV1-overexpressing Cells, Capsaicin-induced Currents Were Significantly Enhanced by BJIKT in a Concentration-dependent Manner, Whereas Mock-transfected Cells Showed No Response. (D–F) Similarly, GSK101A-induced TRPV4 Currents Were Further Potentiated By BJIKT, with No Response Observed in Mock-transfected Cells. (G–I) In Contrast, BJIKT Dose-dependently Inhibited AITC-induced TRPA1 Currents in TRPA1-overexpressing Cells, while Mock-transfected Cells Showed No Activation. Current Amplitudes Were Measured at −100 mV. Data are Presented as Mean ± SEM. $^{**}p < .01$ versus Capsaicin; $^{*}p < .05$ versus GSK101A or AITC.

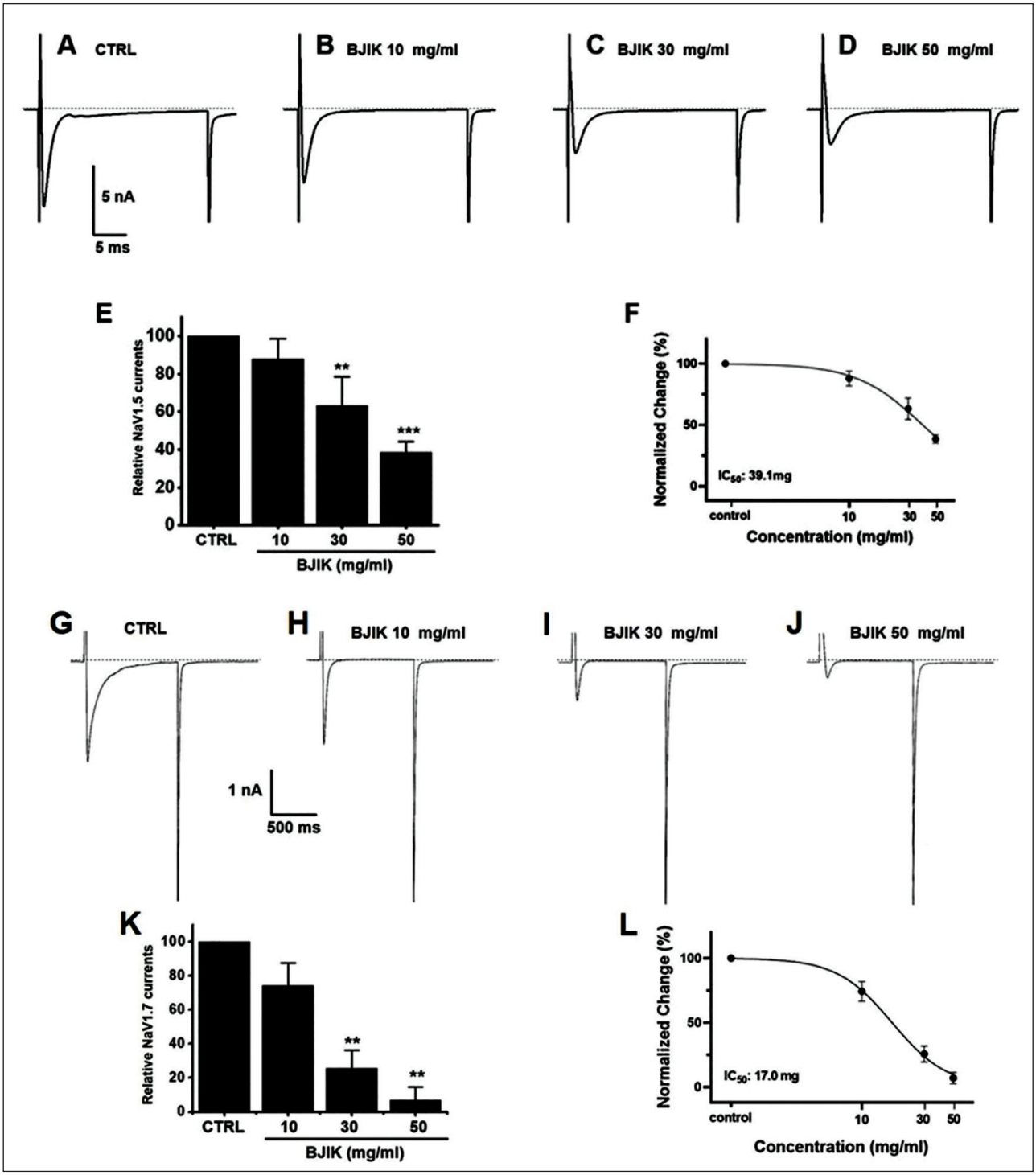


Figure 4. Inhibitory Effects of Bojungikki-Tang (BJIKT) on NaV1.5 and NaV1.7 Channel Activity in HEK293T Cells. (A–D) In NaV1.5-overexpressing HEK293T Cells, BJIKT Treatment at 10, 30, and 50 mg/mL Progressively Inhibited NaV1.5 Currents. (E) Quantified Data Showing a Dose-dependent Reduction in NaV1.5 Current Amplitude. (F) The Estimated IC_{50} for NaV1.5 Inhibition was 39.1 mg/mL. (G–J) In NaV1.7-overexpressing Cells, BJIKT Also Reduced Current Amplitude in a Concentration-dependent Manner. (K) Quantified Current Amplitudes Showing Dose-dependent Inhibition of NaV1.7. (L) The Estimated IC_{50} for NaV1.7 Inhibition was 17.0 mg/mL. Data are Presented as Mean ± SEM. $^{**}p < .01$, $^{***}p < .001$ versus Control.

therapeutic benefits extend to the regulation of inflammatory responses within the GI tract. This anti-inflammatory effect likely contributes to the improvement of IBS symptoms.

Visceral hypersensitivity, which is central to IBS, is strongly linked to the dysregulation of TRP and NaV ion channels (Beyder et al., 2014; Du et al., 2022; Fuentes & Christianson, 2016; Osorio et al., 2014). TRP channels, including TRPA1, TRPV1, and TRPV4, are integral to the sensory and motor functions (Du et al., 2022; Fuentes & Christianson, 2016; Yu et al., 2016). TRPV1, known as the capsaicin receptor, is activated by noxious heat, acid, and other irritants, playing a significant role in pain perception and heightened sensory responses in IBS (Holzer, 2008; Shuba, 2021; Zhang et al., 2020). TRPV4 is involved in mechanosensation and inflammation, mediating gut motility and hypersensitivity to mechanical stimuli (Cheng et al., 2022; Jin et al., 2011). TRPA1, activated by irritants, oxidative stress, and inflammatory mediators, amplifies visceral pain and hypersensitivity, contributing to the discomfort associated with IBS (Cheng et al., 2022; Sandor et al., 2016). All aforementioned channels have been linked to the mechanisms driving IBS, making them promising therapeutic targets. In this study, BJIKT enhanced TRPV1 (Figure 3A–3C) and TRPV4 (Figure 3D–3F) currents, which may help in regulating pain perception and gut motility. Notably, BJIKT also suppressed TRPA1 currents (Figure 3G–3I), suggesting that it may reduce visceral hypersensitivity and inflammation, thereby alleviating abdominal pain and discomfort in IBS patients. In addition to its effects on TRP channels, BJIKT modulates NaV channels, specifically NaV1.5 and NaV1.7, both of which play crucial roles in IBS (Beyder et al., 2014; Fuentes & Christianson, 2016; Holm et al., 2002; Jiang et al., 2021; Osorio et al., 2014; Strege et al., 2007). NaV1.5 plays a key role in the regulation of GI motility (Holm et al., 2002; Osorio et al., 2014; Strege et al., 2007). Expressed predominantly in the ENS, NaV1.5 influences electrical signaling in enteric neurons, smooth muscle cells, and ICC, which coordinate smooth muscle contractions and peristalsis in the intestines (Holm et al., 2002; Osorio et al., 2014; Strege et al., 2007). Dysregulation of NaV1.5 can lead to abnormal GI motility, which is commonly observed in IBS patients, manifesting as symptoms such as constipation or diarrhea. In this study, BJIKT inhibited NaV1.5 currents, suggesting that it may help to restore normal gut motility in IBS (Figure 4A–4F). NaV1.7 plays a key role in transmitting nociceptive signals and is essential for the manifestation of visceral hypersensitivity in IBS (Jiang et al., 2021). Heightened pain perception in IBS arises, at least partially, from the increased transmission of sensory signals by NaV1.7 expressed in primary sensory neurons. Activation of NaV1.7 results in the depolarization of these neurons, propagating pain signals to the brain (Jiang et al., 2021). Dysregulation of NaV1.7 has been associated with increased pain sensitivity, suggesting that targeting this channel could offer a novel approach to managing pain in IBS patients. In the current study, BJIKT inhibited NaV1.7 currents, indicating its potential to regulate pain-related signaling in IBS (Figure 4G–4L). Together, both NaV1.5 and NaV1.7 channels highlight BJIKT's potential to address the key aspects of IBS, including pain and motility disturbances.

These findings suggest that BJIKT may serve as an effective multi-target treatment for IBS. By modulating ion channels involved in visceral hypersensitivity and GI function, as well as exerting anti-inflammatory effects, BJIKT offers a comprehensive approach to alleviating the symptoms of IBS. This multi-faceted mechanism of action may make BJIKT an attractive alternative or adjunct to current IBS treatments, which often focus on a single aspect of the disorder.

Future research should prioritize validating these findings through clinical studies to establish the efficacy of BJIKT in human IBS patients. Clinical trials are essential to confirm the therapeutic potential of BJIKT and determine its optimal dosage and treatment duration. Moreover, the individual components of BJIKT should be investigated to identify the specific bioactive compounds responsible for its effects. This could provide valuable insights into how BJIKT exerts its therapeutic actions and guide the development of more targeted treatments. Future studies should examine the long-term effects of BJIKT, with a particular focus on its influence on the gut microbiota, which is a key factor in IBS pathophysiology, and its composition can influence disease severity and treatment outcomes. Exploring the potential interactions between BJIKT and the gut microbiota could open up new avenues for the management of IBS and improve our understanding of the underlying mechanisms of the disease.

Conclusion

Taken together, this study provides compelling evidence for the therapeutic potential of BJIKT in IBS management. By targeting key ion channels and modulating inflammatory responses, BJIKT addresses several critical aspects of IBS pathophysiology. Continued research in this area could lead to the development of more effective, multi-target therapies for IBS.

Abbreviations

IBS: Irritable bowel syndrome; ICC: Interstitial cells of Cajal; GI: Gastrointestinal; TRP: Transient receptor potential; BJIKT: Bojungikki-Tang; NaV: Voltage-gated sodium; UPLC: Ultra performance liquid chromatography; H&E: Hematoxylin & eosin; ELISA: Enzyme-linked immunosorbent assay; TNF-α: Tumor necrosis factor-alpha; I–V: Current–voltage.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

The experimental procedures involving animals were approved by the IACUC at Pusan National University (Busan, Korea; approval no. PNU-2022-0266).

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