

Article

Inhibitory Effects of Jakyakgamcho-Tang (*Glycyrrhiza uralensis* and *Paeonia lactiflora*) on the Pacemaker Potential of the Interstitial Cells of Cajal in the Murine Small Intestine

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Abstract: Jakyakgamcho-tang (JYGCT) has been used to treat various diseases. The interstitial cells of Cajal (ICC) regulate gastrointestinal (GI) motility as pacemaker cells. Here, we examined the effects of JYGCT on the pacemaker potential of the ICC in the small intestine. We observed that JYGCT inhibited the pacemaker potential in a dose-dependent manner. Glibenclamide did not affect the pacemaker potential and on these conditions, JYGCT also had no effect on the pacemaker potential. Pretreatment with capsazepine or SB452533 blocked the JYGCT-induced effects. In the presence of SQ-22536, JYGCT did not inhibit the pacemaker potential. Additionally, JYGCT inhibited spontaneous $[Ca^{2+}]_i$ oscillations and JYGCT-induced ITR increase was associated with TMEM16A, motilin and substance P activation. Moreover, JYGCT was effective in alleviating the symptoms of irritable bowel syndrome. Our results suggest that JYGCT inhibited the pacemaker potential of the ICC via KATP, the TRPV1 or the cyclic AMP pathway, and intracellular Ca^{2+} regulation, indicating that JYGCT can affect ICC and thus have the function of regulating GI motility. Therefore, JYGCT may be used as a GI motility disorder regulator or disease prevention agent.

Keywords: Jakyakgamcho-tang; gastrointestinal motility; interstitial cells of Cajal; pacemaker potential; prokinetic agent



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1. Introduction

Jakyakgamcho-tang (JYGCT; *Glycyrrhiza uralensis* and *Paeonia lactiflora*) is a traditional herbal medicine and has been used to treat various disease conditions, including muscle pain and acute abdominal pain [1–10]. JYGCT shows excellent efficacy in enhancing spleen function and relieving liver function and is used clinically in the treatment of various indications, such as muscle and abdominal pain, and bronchial asthma [2–4]. JYGCT has the function of decreasing the paclitaxel-induced painful neuropathy, such as allodynia and hyperalgesia [6] and reduces muscle cramps without any side effects [7]. It shows an anti-inflammatory effect and an antispasmodic effect on skeletal muscle [1,8]. It also reduces the serum uric acid levels and increases the sympathetic activities in hyperuricemic vegetarians [9]. In addition, JYGCT also affects the gastrointestinal (GI) tract. It is effective in relieving abdominal pain in acute GI inflammation [11], and it inhibits the transient receptor potential (TRPV) 1 channel and suppresses serotonin production, thereby alleviating the visceral hypersensitivity [12].

GI motility is a highly regulated process and an essential factor determining the quality of life [13]. The interstitial cells of Cajal (ICC) are essential in GI motility and are considered the pacemakers of the GI tract [14–16]. Injury of ICC leads to various diseases related to GI motility [17]. Therefore, research on these cells is important to understand the regulation of GI motility. However, the question remains as to whether JYGCT exerts regulatory

effects on ICC or GI motility and whether the underlying mechanisms require investigation. Therefore, we investigated the regulatory effect of JYGCT on ICC physiological signaling pathways and GI motility in mice.

2. Materials and Methods

2.1. Preparation of JYGCT

JYGCT extract was obtained using distilled water, as described previously [5]. The Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine have the voucher specimens (2012-KE42-1 and 2012-KE42-2). Gallic acid, oxypaeoniflorin, (+)-catechin, albiflorin, paeoniflorin, liquiritin, benzoic acid and so on, were detected by high-performance liquid chromatography (HPLC) [5].

2.2. Preparation of Cell Cultures

The Animal Experiment Ethics Committee of Pusan National University (no. PNU-2020-2831) guidelines were followed. Institute of Cancer Research (ICR) mice were sacrificed by cervical dislocation and then the small intestines were isolated and the mucous membranes were removed. Cells were isolated using collagenase and cultured in smooth muscle growth medium (SMGM; Clonetics, San Diego, CA, USA) in a CO₂ incubator at 37 °C.

2.3. Patch Clamp Experiments

We used the patch clamp technique to record ICC pacemaker potential. A Na⁺-Tyrode bath and KCl (140 mM) pipette solution were used. Electrophysiological techniques were conducted and results were analyzed using pClamp and Origin software (version 6.0, Microcal, Northampton, MA, USA).

2.4. Intracellular Free Calcium Ion Concentration [Ca²⁺]_i

The cultured ICC were loaded with fura-2 AM and measured [Ca²⁺]_i with a PTI Delta scan illuminator (Photon Technology International Inc., Birmingham, NJ, USA).

2.5. ITR Measurement

After intragastric administration of JYGCT to ICR mice, Evans blue (5%, *w/v*; 0.1 mL/kg) was intragastrically administered 30 min later. ITR was measured 30 min after the Evans blue administration.

2.6. GI Motility Dysfunction (GMD) Model Mice

We made the GMD mouse models by using the acetic acid. AA was injected intraperitoneally and allowed to rest for 30 min. ITR was measured 30 min after the Evans blue administration.

2.7. Gut Hormone Levels

After JYGCT (0.5 g/kg) was fed once a day for 5 days, MTL, substance P (SP), somatostatin (SS), and vasoactive intestinal polypeptide (VIP) hormone levels were detected using commercial kits.

2.8. Western Blotting

After feeding SM (0.5 g/kg) for 5 days, anti-transmembrane protein 16A (TMEM16A; Abcam, Cambridge, UK), anti-c-kit (Cell Signaling Technology, Denver, MA, USA), anti-transient receptor potential melastatin 7 (TRPM7; Abcam, Cambridge, UK), and anti-β-actin (Santa Cruz Biotechnology, Dallas, TX, USA) antibodies were used in the small intestine. Other experimental methods were carried out according to the general methods [18].

2.9. Irritable Bowel Syndrome (IBS) Experiments

C57/BL6 mice (6 weeks) induced colitis by 0.1 mL zymosan suspension (30 mg/mL) which was administered trans-anally via a feeding needle into the colons of mice. Zymosan, PBS or JYGCT were administered daily for 3 consecutive days. After 3 days of administration, the experiment was conducted on the 4th day. The zymosan-injected mice were divided into three groups ($n = 9$ per group) and treated with PBS (control), zymosan or JYGCT (500 mg/kg).

2.10. Macroscopic Scoring

The weight of the large intestine was measured after removing the stool, and the length of the large intestine was measured from the cecum to the anus. The individual scores were graded: stool score (0, normal; 1, loose/moist; 2, amorphous/sticky; and 3, diarrhea). The stool status was measured by three researchers using the blind method.

2.11. Drugs

The drugs used in the experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.12. Statistical Analysis

Results are represented as the mean \pm standard error (SE). The results were analyzed using Prism 6.0 (La Jolla, CA, USA) and Origin version 8.0 (OriginLab Corporation, Northampton, MA, USA). One-way analysis of variance (ANOVA) or Student's *t*-test for unpaired data were used to compare control and experimental groups. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. JYGCT Inhibits the Pacemaker Potential of the ICC in the Murine Small Intestine

We performed whole-cell patch clamp experiments using the cultured ICC to examine the pacemaker potential of these cells. We noted that, under these conditions, the ICC spontaneously generated a pacemaker potential with a mean frequency of 11.3 ± 0.9 cycles/min and a mean amplitude of 24.8 ± 1.3 mV. When JYGCT (1–10 mg/mL) was applied, the pacemaker potential decreased (Figure 1A). The mean frequency was 9.3 ± 0.5 cycles/min ($p < 0.01$) at 1 mg/mL, 5.6 ± 0.5 cycles/min ($p < 0.0001$) at 5 mg/mL, and 1.5 ± 0.4 cycles/min ($p < 0.0001$) at 10 mg/mL (Figure 1E); the mean amplitude was 23.9 ± 1.0 mV, 2.4 ± 0.5 mV ($p < 0.0001$), and 2.1 ± 0.8 mV ($p < 0.0001$) (Figure 1F). Thus, these results suggest that JYGCT inhibits the pacemaker potential of the ICC.

3.2. ATP-Sensitive K⁺ Channels Are Involved in the JYGCT-Induced Pacemaker Potential Inhibition in the ICC

To investigate the involvement of the K⁺ channels, we used several K⁺ channel blockers. In the presence of TEA, JYGCT inhibited the pacemaker potential in the ICC (Figure 2A). In addition, JYGCT inhibited the pacemaker potential when co-treated with 4-aminopyridine or apamin (Figure 2B,C). Moreover, glibenclamide (an ATP-sensitive K⁺ (KATP) channel blocker) did not affect the pacemaker potential and, under these conditions, JYGCT also showed no effect on the pacemaker potential (Figure 2D); further, glibenclamide reversed the effects of JYGCT (Figure 2E). In the presence of 5 mg/mL JYGCT, the mean frequency and amplitude were 5.6 ± 0.6 cycles/min and 2.6 ± 0.4 mV for TEA, 5.3 ± 0.5 cycles/min and 2.6 ± 0.4 mV for 4-aminopyridine, 5.5 ± 0.6 cycles/min and 2.5 ± 0.4 mV for apamin, and 12.1 ± 0.9 cycles/min ($p < 0.0001$) and 22.7 ± 1.0 mV ($p < 0.0001$) for glibenclamide (Figure 2F,G). These results suggest that JYGCT activates the KATP channels in the ICC.

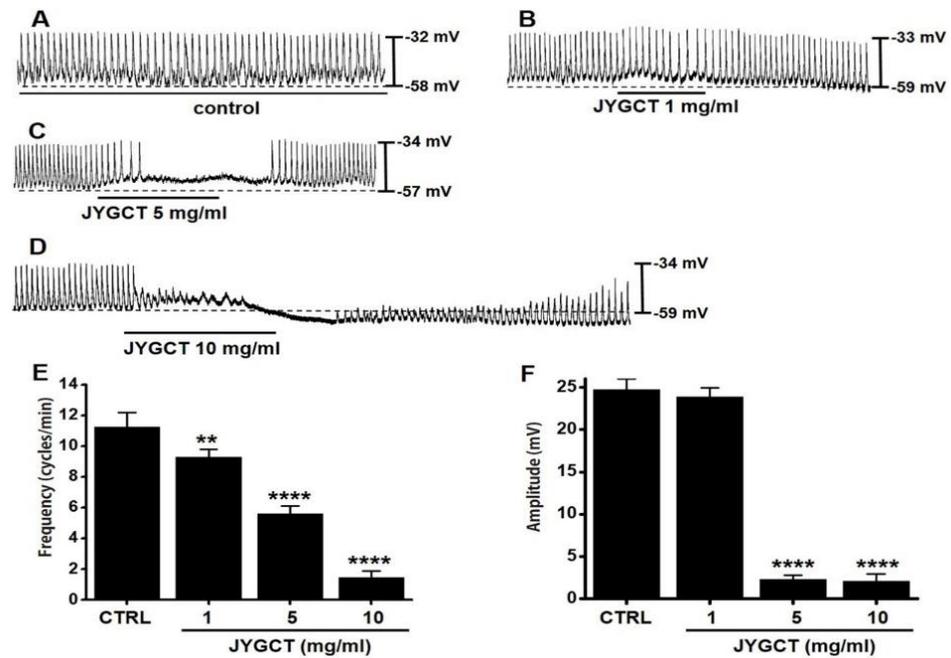


Figure 1. Effect of JYGCT on the pacemaker potential in the ICC. (A–D) JYGCT inhibited the pacemaker potential; (E,F) Summary of the inhibitory frequency and amplitude effects of JYGCT on the pacemaker potential in the ICC. Bars represents the mean \pm SE. ** $p < 0.01$. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; CTRL: control.

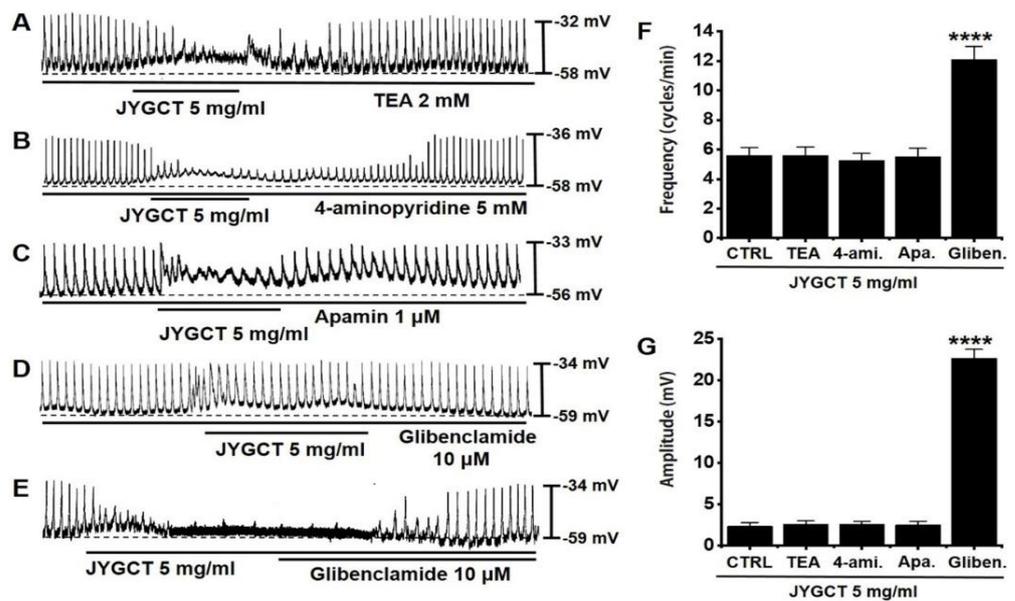


Figure 2. Effect of various K⁺ channel blockers on the inhibition of pacemaker potential by JYGCT in the ICC. (A) Pretreatment with TEA had no effect; (B) 4-aminopyridine did not affect the inhibition by JYGCT; (C) Apamin did not affect the pacemaker potential inhibition induced by JYGCT; (D) Glibenclamide blocked the pacemaker potential inhibition by JYGCT; (E) The suppression of pacemaker potential by JYGCT was restored by glibenclamide; (F,G) Summary of the inhibitory frequency and amplitude effects of pretreatment with TEA, 4-aminopyridine, apamin or glibenclamide. Bars represents the mean \pm SE. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; CTRL: control; TEA: tetraethylammonium; 4-amino: 4-aminopyridine; Apa: apamin; Gliben: glibenclamide.

3.3. Transient Receptor Potential Vanilloid 1 (TRPV1) Channels Are Involved in the JYGCT-Induced Pacemaker Potential Inhibition in the ICC

Many studies show that TRPV1 is involved in the physiological functions of the GI tract [19–21]; therefore, we investigated the relevance of the TRPV1 channel. We incubated cells with capsazepine or SB452533, a TRPV1 receptor antagonist, for 5 min. Pretreatment with capsazepine or SB452533 blocked the JYGCT-induced effects (Figure 3A,B). The frequency of the pacemaker potential with JYGCT and capsazepine or SB452533 was 11.4 ± 0.9 cycles/min ($p < 0.0001$) or 11.9 ± 1.0 cycles/min ($p < 0.0001$), with a corresponding amplitude of 22.7 ± 1.1 mV ($p < 0.0001$) or 22.8 ± 1.7 mV ($p < 0.0001$) (Figure 3C,D). Thus, these results suggest that the TRPV1 channels regulate the JYGCT-induced responses.

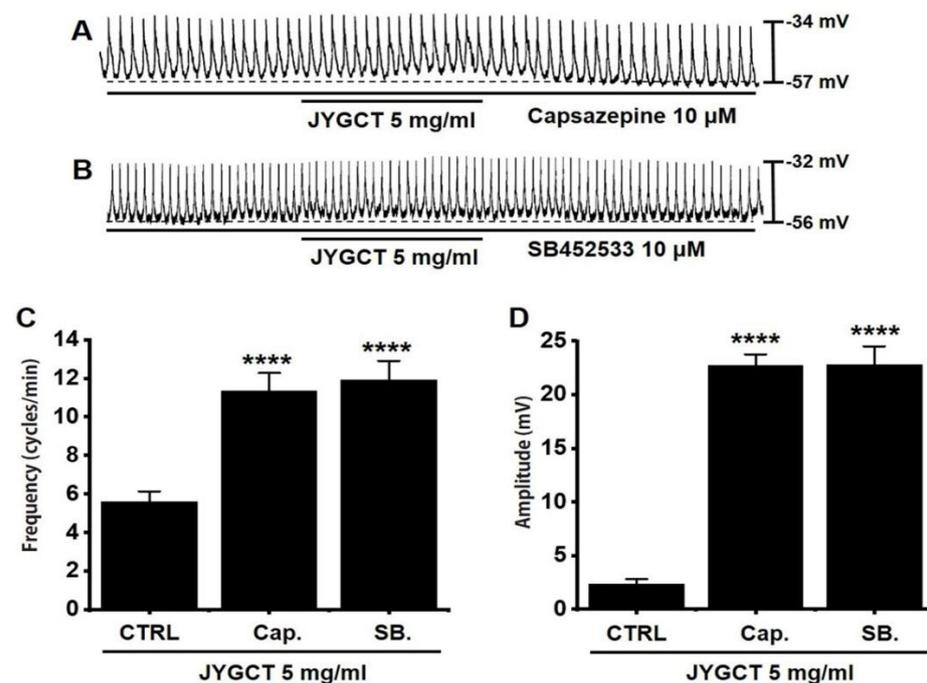


Figure 3. Effect of capsazepine or SB452533 on the pacemaker potential inhibition by JYGCT in the ICC. (A) Pretreatment with capsazepine blocked the pacemaker potential inhibition by JYGCT; (B) SB452533 blocked the pacemaker potential inhibition by JYGCT; (C,D) Summary of the inhibitory frequency and amplitude effects of pretreatment with capsazepine or SB452533. Bars represents the mean \pm SE. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; Cap: capsazepine; SB: SB452533; CTRL: control.

3.4. Cyclic Adenosine Monophosphate (cAMP) Is Involved in the JYGCT-Induced Pacemaker Potential Inhibition in the ICC

The cAMP and guanosine monophosphate (cGMP)-dependent pathway was checked by SQ-22536, an inhibitor of adenylate cyclase, and ODQ, an inhibitor of guanylate cyclase. In the presence of SQ-22536 (10 μ M), JYGCT did not inhibit the pacemaker potential of the ICC (Figure 4A). However, upon treatment with ODQ (10 μ M), JYGCT inhibited the pacemaker potential (Figure 4B). Additionally, in the presence of KT-5823 (a protein kinase G (PKG) inhibitor; 1 μ M), JYGCT inhibited the pacemaker potential (Figure 4C). The frequencies of the pacemaker potential with JYGCT and SQ-22536, ODQ, or KT-5823 were 11.3 ± 1.0 cycles/min ($p < 0.0001$), 6.4 ± 0.5 cycles/min, or 5.9 ± 0.9 cycles/min, respectively (Figure 4D). The amplitudes of the pacemaker potential with JYGCT and SQ-22536, ODQ, or KT-5823 co-treatments were 24.8 ± 1.3 mV ($p < 0.0001$), 2.5 ± 0.7 mV, or 2.7 ± 0.6 mV (Figure 4E). Thus, these findings indicate that cAMP mediates the JYGCT-induced responses.

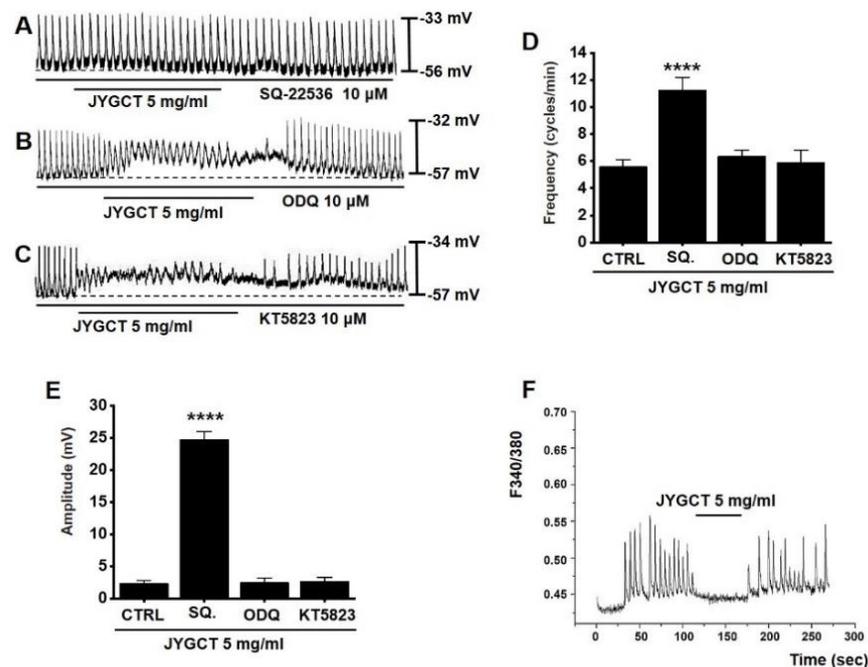


Figure 4. Effect of SQ-22536, ODQ, KT-5823 and intracellular Ca^{2+} flux on the pacemaker potential inhibition by JYGCT in the ICC. (A) Pretreatment with SQ-22536 blocked the pacemaker potential inhibition by JYGCT; (B,C) Pretreatment with ODQ or KT-5823 did not block the pacemaker potential inhibition by JYGCT; (D,E) Summary of the inhibitory frequency and amplitude effects of pretreatment with SQ-22536, ODQ, or KT-5823; (F) Under normal conditions, $[Ca^{2+}]_i$ oscillations were induced and JYGCT blocked these $[Ca^{2+}]_i$ oscillations. Bars represent the mean \pm SE. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; CTRL: control; SQ: SQ-22536; ODQ: 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one.

3.5. Effect of JYGCT on Intracellular Ca^{2+} ($[Ca^{2+}]_i$) Oscillations

Spontaneous $[Ca^{2+}]_i$ oscillations were observed in the ICC; JYGCT administration (5 mg/mL) suppressed these spontaneous responses (Figure 4F).

3.6. Association of the TMEM16A Proteins, and MTL and SP Hormones in JYGCT-Induced ITR Increase

The normal ITR was $51.3 \pm 2.6\%$ and JYGCT increased the ITR ($53.7 \pm 3.2\%$ at 0.01 g/kg, $60.6 \pm 2.9\%$ ($p < 0.001$) at 0.1 g/kg, and $64.8 \pm 2.0\%$ ($p < 0.0001$) at 1 g/kg) (Figure 5A). Next, we generated the GMD mouse model. AA decreased the ITR ($25.9 \pm 1.8\%$ ($p < 0.0001$), Figure 5B). However, JYGCT at 0.01, 0.1, and 1 g/kg restored this response to $51.3 \pm 2.6\%$ ($p < 0.0001$), $51.7 \pm 3.9\%$ ($p < 0.0001$), and $58.3 \pm 1.7\%$ ($p < 0.0001$), respectively (Figure 5B). The reactions in ICC are mainly produced through non-selective cation channels, i.e., TRPM7, TMEM16A or c-Kit in the murine small intestine [15,22–24]. The effects of JYGCT on the TMEM16A, c-Kit or TRPM7 proteins were examined in the GI tract; it was observed that the expression of TMEM16A increased significantly after JYGCT treatment (Figure 5C(a,b)). However, TRPM7 and c-Kit were unchanged (Figure 5C(c,d)). In addition, the levels of MTL and SP were significantly elevated (Figure 5D(a,b)) but the levels of SS (Figure 5D(c)), and VIP (Figure 5D(d)) showed no changes after JYGCT administration. Thus, these results indicate that the JYGCT-induced GI motility increase depends on the activation of the TMEM16A and MTL and SP hormones.

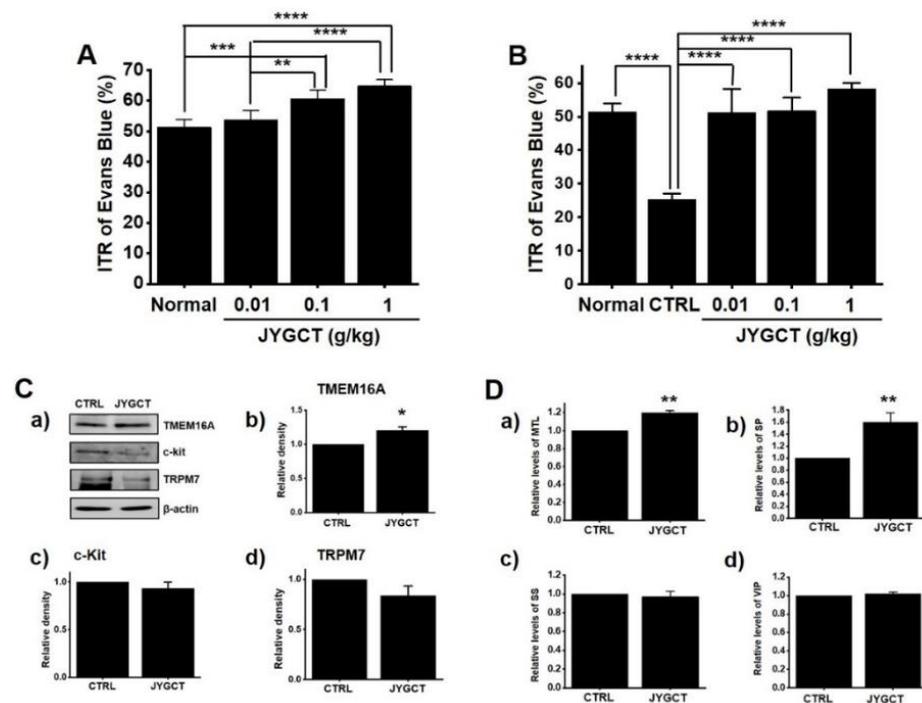


Figure 5. Effects of JYGCT on protein expressions and gut hormones in ITR. (A,B) JYGCT increased ITR; (C) The expression of TMEM16A increased but that of c-Kit or TRPM7 was unchanged; (D) Level changes of GI hormones, (a) MTL, (b) SP, (c) SS, and (d) VIP. Bars represents the mean \pm SE. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; CTRL: control.

3.7. Effects of JYGCT on Macroscopic Score of Zymosan-Induced Colon Changes

We checked the colon length, weight, and stool status by JYGCT in the large intestine administered with zymosan. Administration of zymosan reduced the length of the colon compared to normal mice, and thus it can be seen that colitis was induced. However, when the mice administered with JYGCT were compared with mice administered with zymosan, the length of the colon was increased (Naïve: 9.45 ± 0.8 cm, CTRL: 7.55 ± 0.6 cm ($p < 0.05$), JYGCT: 9.36 ± 0.7 cm ($p < 0.05$), Figure 6A). In addition, although the colon weight increased compared to normal, JYGCT decreased the colon weight (Naïve: 0.25 ± 0.01 g, CTRL: 0.30 ± 0.02 g ($p < 0.05$), JYGCT: 0.23 ± 0.01 g ($p < 0.05$), Figure 6B). The feces of normal mice are dark brown and have the form of hard lumps, but in mice administered with zymosan, it can be seen that diarrhea is induced in light brown or yellow color with water-like mucus. The stool score was increased by comparing the zymosan-administered mice with the normal mice, but decreased by JYGCT (Naïve: 0.09 ± 0.03 , CTRL: 2.46 ± 0.11 g ($p < 0.0001$), JYGCT: 0.54 ± 0.11 ($p < 0.0001$), Figure 6C). Thus, these results indicate that JYGCT might inhibit zymosan-induced colitis and diarrhea.

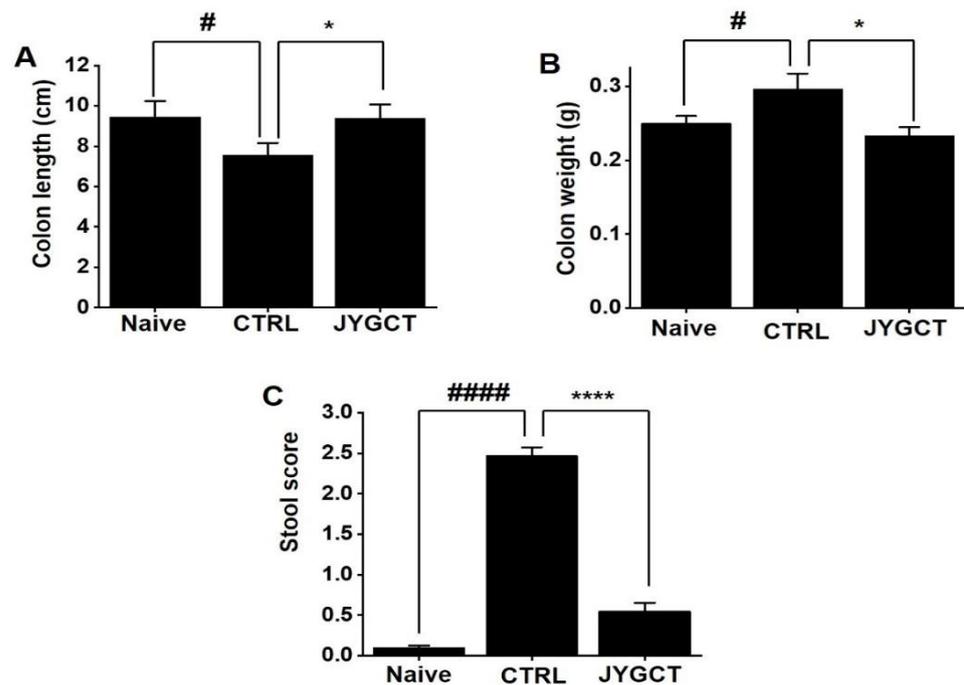


Figure 6. Effects of JYGCT on zymosan-induced colonic changes. (A) Colon length; (B) Colon weight; (C) Stool scores were analyzed. Bars represents the mean \pm SE. * $p < 0.05$. # $p < 0.05$. #### $p < 0.0001$. **** $p < 0.0001$. JYGCT: Jakyakgamcho-tang; CTRL: control.

4. Discussion

In this study, we investigated the effect of JYGCT on the pacemaker potential in the ICC obtained from the murine small intestine. JYGCT inhibited the pacemaker potential via KATP, the TRPV1, the cAMP pathway, and intracellular Ca^{2+} regulation, indicating that JYGCT can affect the ICC and may be a novel prokinetic agent in the GI tract.

JYGCT is a traditional medicine for various purposes [10]. It has been applied to treat various disease conditions; it has been used in the prevention of muscle cramps and the inhibition of skeletal muscle contractions [7], uric acid reduction, autonomic function regulation [9], and intestinal smooth muscle relaxation [25]. JYGCT has the function of decreasing the neuropathy [3] and shows an anti-inflammatory effect [1]. It also has antispasmodic effect on skeletal muscle [8], reduces the serum uric acid levels, and increases the sympathetic activities [9]. However, although JYGCT has been used as an herbal remedy to regulate the GI tract [11,12], its regulation of GI motility has not yet been reported. In this study, we demonstrated that JYGCT modulated the pacemaker potential in the ICC. Therefore, it can be suggested that JYGCT may regulate GI motility via its effects on the ICC pacemaker potential. In a forthcoming study, we plan to investigate the effects of JYGCT's components, Jakyak and Gamcho, on the pacemaker potential in the ICC.

Ion channels are important in the physiological control of GI motility [26]. Among the various ion channels, KATP channels are enriched at various locations in the GI tissues [27,28]. KATP channels in the ICC are composed of SUR2B and Kir 6.2 in the small intestine and SUR2B and Kir 6.1 in the colon [29]. Previous studies have shown that KATP channels exist in cultured murine small intestinal ICC and reduce GI motility by causing relaxation of the smooth muscles; this makes them potential candidates in the treatment of GI motor diseases [30]. In addition, the TRPV1 channels are expressed throughout the alimentary canal where they play important roles in GI motility through the ICC [18]. Various studies show that the TRPV1 channels are involved in the treatment of gastric esophageal reflux disease and GI pain hypersensitivity in the GI tract [18,31]. In this study, JYGCT inhibited the pacemaker potential in the ICC via KATP (Figure 2) and the TRPV1 (Figure 3). Therefore, we believe that KATP and the TRPV1 channels may get involved in the JYGCT-induced inhibition of pacemaker potential. Next, we intend to study

the specific processes and molecular control strategies of KATP and the TRPV1 channels in the ICC. The intracellular regulation of Ca²⁺ flux has a role in the pacemaker activity in the ICC [24,32]. Ca²⁺ flux regulation is important for generating the pacemaker activity in the ICC [33]. In the present study, spontaneous [Ca²⁺]_i responses were inhibited by JYGCT (Figure 5). Therefore, we concluded that JYGCT inhibited the pacemaker potential and probably regulates the pacemaker activity through intracellular Ca²⁺ modulations. In addition, three cell surface proteins, TRPM7, c-Kit, and TMEM16A, are used for identifying the ICCs activation [15,22–24]. In this study, JYGCT increased the ITR and the expression of TMEM16A was only increased by JYGCT, but the expression of c-Kit and TRPM7 was not (Figure 5A,B). In addition, we looked at changes in GI hormones. GI hormonal changes can alter GI motility [34,35]. The roles of GI hormones are diverse and extensive, and although not fully understood yet, they are known to have a significant impact on the physiological functions of the GI tract [36]. In particular, it is known that hormones are responsible for regulating the process of food being digested and sent down through the GI tract [37]. These hormones are believed to regulate the functions of the GI tract as well as the bile and pancreas [38]. GI hormones are thought to have many unexplained functions and are attracting attention as a new research field in the future. Currently, studies on how these GI hormones act when certain GI diseases occur are being actively conducted, and, in particular, the use of hormones as therapeutic agents for GI motility diseases in food digestion is being attempted. In the present study, changes in four representative hormones MTL, SP, SS, and VIP were investigated. By JYGCT, MTL and SP hormone levels increased, but SS and VIP hormone levels did not (Figure 5C). Therefore, it is thought that JYGCT-induced ITR increase is induced by the increase of TMEM16A protein and MTL and SP hormones. In this experiment, ICR mice that are good for observing the GI tract were used for the study of ICC because they are large in size, which are generally used for GI-related experiments. However, in the IBS-related study, C57/BL6 mice, which are mainly used for observing biochemical changes given relatively gentle and specific conditions, were used as black mice. We do not think that there is any particular difference, but the experiment was conducted using commonly used animal species.

Abnormalities and reductions in ICC are often found in GI diseases [17]. A recent study reports that JYGCT protects the ICC in the sphincter of Oddi (SO) and regulates the movement of SO [39]. In addition, the most important IBS mechanisms are GI dysmotility, and, therefore, ICC may be involved in the pathogenesis of IBS [40,41]. In this study, JYGCT restored colon length, weight, and stool status to normal in zymosan-induced IBS-like symptoms (Figure 6). However, research on the association between GI motor disease and the ICC is still underway. Therefore, traditional medicine, which has fewer side effects due to its naturalistic approach, is a very attractive alternative [42,43]. Currently, there is a keen interest in treating GI tract diseases using natural medicine; accordingly, natural substances have the potential to reveal the mechanisms of action of pacemaker potential generation in the ICC and assist in the development of new drugs.

5. Conclusions

The present study shows that JYGCT inhibits the pacemaker potential of the ICC via KATP, the TRPV1 channels, the cAMP pathway, and intracellular Ca²⁺ regulation, increases ITR and alleviates IBS, and indicates that herbal medicine can be used for GI motor control. This study is on the GI tract efficacy of JYGCT in mice, showing the potential for use in GI diseases in mice. However, studies on the efficacy of JYGCT in the human GI tract have not yet been conducted. It is thought that further studies on the efficacy of JYGCT in humans are needed in the future.

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Informed Consent Statement: The study did not involve humans.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lee, Y.M.; Kim, D.S. The Extraction Solvent Influences the Anti-Inflammatory Effects of Jakyakgamcho-Tang in Lipopolysaccharide-Stimulated Macrophages and Mice with Gouty Arthritis. *Int. J. Mol. Sci.* **2020**, *21*, 9748. [CrossRef] [PubMed]
2. Xu, C.H.; Wang, P.; Wang, Y.; Yang, Y.; Li, D.H.; Li, H.F.; Sun, S.Q.; Wu, X.Z. Pharmacokinetic comparisons of two different combinations of Shaoyao-Gancao Decoction in rats: Competing mechanisms between paeoniflorin and glycyrrhetic acid. *J. Ethnopharmacol.* **2013**, *149*, 443–452. [CrossRef] [PubMed]
3. Shen, L.; Hu, R.; Lin, X.; Cong, W.; Hong, Y.; Feng, Y.; Xu, D.; Ruan, K. Pharmacokinetics of characteristic effective ingredients from individual and combination Shaoyao and Gancao treatment in rats using HPLC fingerprinting. *Eur. J. Drug Metab. Pharmacokinet.* **2012**, *37*, 133–140. [CrossRef] [PubMed]
4. Yin, Q.; Wang, P.; Zhang, A.; Sun, H.; Wu, X.; Wang, X. Ultra-performance LC-ESI/quadrupole-TOF MS for rapid analysis of chemical constituents of Shaoyao-Gancao decoction. *J. Sep. Sci.* **2013**, *36*, 1238–1246. [CrossRef]
5. Jeong, S.J.; Lim, H.S.; Seo, C.S.; Kim, J.H.; Jin, S.E.; Yoo, S.R.; Shin, H.K. Traditional herbal formula Jakyakgamcho-tang (*Paeonia lactiflora* and *Glycyrrhiza uralensis*) impairs inflammatory chemokine production by inhibiting activation of STAT1 and NF- κ B in HaCaT cells. *Phytomedicine* **2015**, *22*, 326–332. [CrossRef]
6. Hidaka, T.; Shima, T.; Nagira, K.; Ieki, M.; Nakamura, T.; Aono, Y.; Kuraishi, Y.; Arai, T.; Saito, S. Herbal medicine Shakuyaku-kanzo-to reduces paclitaxel-induced painful peripheral neuropathy in mice. *Eur. J. Pain* **2009**, *13*, 22–27. [CrossRef]
7. Hinoshita, F.; Ogura, Y.; Suzuki, Y.; Hara, S.; Yamada, A.; Tanaka, N.; Yamashita, A.; Marumo, F. Effect of orally administered shao-yao-gan-cao-tang (Shakuyaku-kanzo-to) on muscle cramps in maintenance hemodialysis patients: A preliminary study. *Am. J. Chin. Med.* **2003**, *31*, 445–453. [CrossRef]
8. Tsuji, S.; Yasuda, K.; Sumi, G.; Cho, H.; Tsuzuki, T.; Okada, H.; Kanzaki, H. Shakuyaku-kanzo-to inhibits smooth muscle contractions of human pregnant uterine tissue in vitro. *J. Obstet. Gynaecol. Res.* **2012**, *38*, 1004–1010. [CrossRef]
9. Wu, T.H.; Chen, L.C.; Yang, L.L. Hypouricemic effect and regulatory effects on autonomic function of Shao-Yao Gan-Cao Tang, a Chinese herbal prescription, in a symptomatic hyperuricemic vegetarians. *Rheumatol. Int.* **2007**, *28*, 27–31. [CrossRef]
10. Kim, J.H.; Shin, H.K.; Seo, C.S. Chemical interaction between *Paeonia lactiflora* and *Glycyrrhiza uralensis*, the components of Jakyakgamcho-tang, using a validated high-performance liquid chromatography method: Herbal combination and chemical interaction in a decoction. *J. Sep. Sci.* **2014**, *37*, 2704–2715. [CrossRef]
11. He, J.X.; Goto, E.; Akao, T.; Tani, T. Interaction between Shaoyao-Gancao-Tang and a laxative with respect to alteration of paeoniflorin metabolism by intestinal bacteria in rats. *Phytomedicine* **2007**, *14*, 452–459. [CrossRef] [PubMed]
12. Shao, Y.Y.; Guo, Y.T.; Gao, J.P.; Liu, J.J.; Chang, Z.P.; Feng, X.J.; Xu, D.; Deng, G.F.; Hou, R.G. Shaoyao-Gancao Decoction Relieves Visceral Hyperalgesia in TNBS-Induced Postinflammatory Irritable Bowel Syndrome via Inactivating Transient Receptor Potential Vanilloid Type 1 and Reducing Serotonin Synthesis. *Evid. Based Complement. Alternat. Med.* **2020**, *2020*, 7830280. [CrossRef] [PubMed]
13. Li, L.; Zou, C.; Zhou, Z.; Wang, X.; Yu, X. Phenotypic changes of interstitial cells of Cajal after intestinal obstruction in rat model. *Braz. J. Med. Biol. Res.* **2019**, *52*, e8343. [CrossRef] [PubMed]
14. Huizinga, J.D.; Thuneberg, L.; Kluppel, M.; Malysz, J.; Mikkelsen, H.B.; Bernstein, A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* **1995**, *373*, 347–349. [CrossRef]
15. Sanders, K.M. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* **1996**, *111*, 492–515. [CrossRef]
16. Kim, B.J.; Lim, H.H.; Yang, D.K.; Jun, J.Y.; Chang, I.Y.; Park, C.S.; So, I.; Stanfield, P.R.; Kim, K.W. Melastatin-Type Transient Receptor Potential Channel 7 Is Required for Intestinal Pacemaking Activity. *Gastroenterology* **2005**, *129*, 1504–1517. [CrossRef]
17. Al-Sajee, D.; Huizinga, J.D. Interstitial Cells of Cajal: Pathology, injury and repair. *Sultan Qaboos Univ. Med. J.* **2012**, *12*, 411–421. [CrossRef]
18. Han, D.; Kim, J.N.; Kwon, M.J.; Han, T.; Kim, Y.T.; Kim, B.J. *Salvia miltiorrhiza* induces depolarization of pacemaker potentials in murine small intestinal interstitial cells of Cajal via extracellular Ca²⁺ and Na⁺ influx. *Mol. Med. Rep.* **2021**, *23*, 348. [CrossRef]
19. Holzer, P. TRP channels in the digestive system. *Curr. Pharm. Biotechnol.* **2011**, *12*, 24–34. [CrossRef]
20. Storozhuk, M.V.; Moroz, O.F.; Zholos, A.V. Multifunctional TRPV1 Ion Channels in Physiology and Pathology with Focus on the Brain, Vasculature, and Some Visceral Systems. *BioMed Res. Int.* **2019**, *2019*, 5806321. [CrossRef]

21. Du, Q.; Liao, Q.; Chen, C.; Yang, X.; Xie, R.; Xu, J. The Role of Transient Receptor Potential Vanilloid 1 in Common Diseases of the Digestive Tract and the Cardiovascular and Respiratory System. *Front. Physiol.* **2019**, *10*, 1064. [[CrossRef](#)] [[PubMed](#)]
22. Sanders, K.M.; Koh, S.D.; Ro, S.; Ward, S.M. Regulation of gastrointestinal motility—Insights from smooth muscle biology. *Nat. Rev. Gastroenterol. Hepatol.* **2012**, *9*, 633–645. [[CrossRef](#)] [[PubMed](#)]
23. Huang, F.; Rock, J.R.; Harfe, B.D.; Cheng, T.; Huang, X.; Jan, Y.N.; Jan, L.Y. Studies on expression and function of the TMEM16A calcium-activated chloride channel. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21413–21418. [[CrossRef](#)] [[PubMed](#)]
24. Zhu, M.H.; Kim, T.W.; Ro, S.; Yan, W.; Ward, S.M.; Koh, S.D.; Sanders, K.M. A Ca(2+)-activated Cl(-)conductance in interstitial cells of cajal linked to slow wave currents and pacemaker activity. *J. Physiol.* **2009**, *587*, 4905–4918. [[CrossRef](#)]
25. Satoh, H.; Tsuru, K. Pharmacological Modulation by Shakuyakukanzoto (Shao-Yao-Gan-Cao-Tang) and the Ingredients in Rat Intestinal Smooth Muscle. *Chin. Med.* **2011**, *2*, 62–70. [[CrossRef](#)]
26. Radulovic, M.; Anand, P.; Korsten, M.A.; Gong, B. Targeting Ion Channels: An Important Therapeutic Implication in Gastrointestinal Dysmotility in Patients with Spinal Cord Injury. *J. Neurogastroenterol. Motil.* **2015**, *21*, 494–502. [[CrossRef](#)]
27. Standen, N.B.; Quayle, J.M.; Davies, N.W.; Brayden, J.E.; Huang, Y.; Nelson, M.T. Hyperpolarizing vasodilators activate ATP-sensitive K⁺ channels in arterial smooth muscle. *Science* **1989**, *245*, 177–180. [[CrossRef](#)]
28. Mourre, C.; Widmann, C.; Lazdunski, M. Sulfonylurea binding sites associated with ATP-regulated K⁺ channels in the central nervous system: Autoradiographic analysis of their distribution and ontogenesis, and of their localization in mutant mice cerebellum. *Brain Res.* **1990**, *519*, 29–43. [[CrossRef](#)]
29. Na, J.S.; Hong, C.; Kim, M.W.; Park, C.G.; Kang, H.G.; Wu, M.J.; Jiao, H.Y.; Choi, S.; Jun, J.Y. ATP-sensitive K⁺ channels maintain resting membrane potential in interstitial cells of Cajal from the mouse colon. *Eur. J. Pharmacol.* **2017**, *809*, 98–104. [[CrossRef](#)]
30. Choi, S.; Yeum, C.H.; Chang, I.Y.; You, H.J.; Park, J.S.; Jeong, H.S.; So, I.; Kim, K.W.; Jun, J.Y. Activating of ATP-dependent K⁺ channels comprised of K(ir) 6.2 and SUR 2B by PGE₂ through EP2 receptor in cultured interstitial cells of Cajal from murine small intestine. *Cell. Physiol. Biochem.* **2006**, *18*, 187–198. [[CrossRef](#)]
31. Yu, X.; Yu, M.; Liu, Y.; Yu, S. TRP channel functions in the gastrointestinal tract. *Semin. Immunopathol.* **2016**, *38*, 385–396. [[CrossRef](#)] [[PubMed](#)]
32. Zhu, M.H.; Sung, I.K.; Zheng, H.; Sung, T.S.; Britton, F.C.; O’Driscoll, K.; Koh, S.D.; Sanders, K.M. Muscarinic activation of Ca²⁺-activated Cl⁻ current in interstitial cells of Cajal. *J. Physiol.* **2011**, *589*, 4565–4582. [[CrossRef](#)] [[PubMed](#)]
33. Zhu, M.H.; Sung, T.S.; O’Driscoll, K.; Koh, S.D.; Sanders, K.M. Intracellular Ca(2+) release from endoplasmic reticulum regulates slow wave currents and pacemaker activity of interstitial cells of Cajal. *Am. J. Physiol. Cell. Physiol.* **2015**, *308*, C608–C620. [[CrossRef](#)] [[PubMed](#)]
34. Camilleri, M. Gastrointestinal Hormones and Regulation of Gastric Emptying. *Curr. Opin. Endocrinol. Diabetes Obes.* **2019**, *26*, 3–10. [[CrossRef](#)]
35. Kitazawa, T.; Kaiya, H. Regulation of Gastrointestinal Motility by Motilin and Ghrelin in Vertebrates. *Front. Endocrinol.* **2019**, *10*, 278. [[CrossRef](#)]
36. Rehfeld, J.F. The new biology of gastrointestinal hormones. *Physiol. Rev.* **1998**, *78*, 1087–1108. [[CrossRef](#)]
37. Baynes, K.C.R.; Dhillon, W.S.; Bloom, S.R. Regulation of food intake by gastrointestinal hormones. *Curr. Opin. Gastroenterol.* **2006**, *22*, 626–631. [[CrossRef](#)]
38. Jensen, C.Z.; Bojsen-Møller, K.N.; Svane, M.S.; Holst, L.M.; Hermansen, K.; Hartmann, B.; Albrechtsen, N.J.W.; Kuhre, R.E.; Kristiansen, V.B.; Rehfeld, J.F.; et al. Responses of gut and pancreatic hormones, bile acids, and fibroblast growth factor-21 differ to glucose, protein, and fat ingestion after gastric bypass surgery. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2020**, *318*, G661–G672. [[CrossRef](#)]
39. Zhu, G.Y.; Jia, D.D.; Yang, Y.; Miao, Y.; Wang, C.; Wang, C.M. The Effect of Shaoyao Gancao Decoction on Sphincter of Oddi Dysfunction in Hypercholesterolemic Rabbits via Protecting the Enteric Nervous System-Interstitial Cells of Cajal-Smooth Muscle Cells Network. *J. Inflamm. Res.* **2021**, *14*, 4615–4628. [[CrossRef](#)]
40. Eshraghian, A.; Eshraghian, H. Interstitial cells of Cajal: A novel hypothesis for the pathophysiology of irritable bowel syndrome. *Can. J. Gastroenterol.* **2011**, *25*, 277–279. [[CrossRef](#)]
41. Yang, B.; Zhou, X.C.; Lan, C. Impact of the alterations in the interstitial cells of Cajal on intestinal motility in post-infection irritable bowel syndrome. *Mol. Med. Rep.* **2015**, *11*, 2735–2740. [[CrossRef](#)] [[PubMed](#)]
42. Zhang, J.; Onakpoya, I.J.; Posadzki, P.; Eddouks, M. The safety of herbal medicine: From prejudice to evidence. *Evid. Based Complement. Alternat. Med.* **2015**, *2015*, 316706. [[CrossRef](#)] [[PubMed](#)]
43. Son, J.; Kim, C.; Park, M.; Choi, D.; Yun, S. Traditional Medicine Analysis and Sustainable Use of Korean Pond Wetland Plants in the Agricultural Landscape. *Sustainability* **2020**, *12*, 5963. [[CrossRef](#)]