Antispasmodic Effects of Junsibaekchul-San In Vivo and In vitro

Jin Il Hur, Joon Seok Byun, Dae Jun Kim*

Department of Internal Medicine, College of Oriental Medicine, Daegu Haany University

In Vivo and In vitro antispasmodic effects of Jun-Si-Baek-Chul-San, a Traditional Korean Polyherbal Medicineconsisted of 7 types of herbs were observed in the present study. To clarify the effects of Jun-Si-Baek-Chul-San, on accelerating small intestinal movement induced by the stimulation of cholinergic neurotransmission, we evaluated the effects of Jun-Si-Baek-Chul-San on In vivo carbachol (an acetylcholinergic agent)-accelerated mice small intestinal transit and on In vitro contractions induced by low-frequency electrostimulation, KCl, histamine or acetylcholine using isolated guinea pig ileum. To induce the acceleration of mice small intestinal transit, Carbachol 1 mg/kg was once subcutaneously dosed 15min before last administration of the test drugs. In the present study, Jun-Si-Baek-Chul-San 500, 250 and 125 mg/kg or domperidone 20 mg/kg were orally pretreated on the carbachol-accelerated mice small intestinal transit once a day for 7 days and the small intestinal transit rateof activated charcoal powder were monitored. In vitro assays, Jun-Si-Baek-Chul-San1, 0.1, 0.01 and 0.001 mg/ml or domperidone 2×10^{-5}M were treated 10min before ileal contraction was induced by filed stimulation, acetylcholine, KCl and histamine, and the % changes of contractions were observed compared to the treatment of inducer alone. In spontaneous contraction, the % changes of contractions were observed compared to treatment of vehicle alone at 10min after Jun-Si-Baek-Chul-San or domperidone treatment. The efficacy of Jun-Si-Baek-Chul-San was compared to those of domperidone. High concentration, 1 mg/ml of Jun-Si-Baek-Chul-San was found to decrease the spontaneous contraction of the isolated guinea-pig ileum. In addition, Jun-Si-Baek-Chul-San decrease contractions induced by electrostimulation, acetylcholine, histamine and KCl in the isolated guinea-pig ileum. In addition, Jun-Si-Baek-Chul-San effectively inhibited the accelerated small intestinal movement induced by carbachol stimulation of cholinergic neurotransmission in In vivo. Based on the results, although the exact molecular or action mechanism and which herbs or compound in Jun-Si-Baek-Chul-San are responsible for actions, it was concluded that Jun-Si-Baek-Chul-San normalization in the accelerated intestinal motility might be interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specificly. Therefore, it is expected that Jun-Si-Baek-Chul-San will be promising as a prescription of clinical treatment of digestive tract disorders such as accelerated the motility of intestine, diarrhea or intestinal painful contractions.

Key words : Junsibaekchul-San, domperidone, gastric motility, polyherbal medicine

Introduction

Gastric motility may be regulated by multiple neural systems. Studies on the mechanisms involving the neuronal systems suggest the involvement of cholinergic[1] and noradrenergic systems[2]. Additionally, various studies have suggested the existence of dopamine neurons and receptors in these tracts[3-7]. Domperidone, a dopamine D_2-receptor antagonist[8], is also clinically effective in treating functional gastrointestinal disorders such as gastroesophageal reflex, gastritis, gastric atony, gastroptosis, dyspepsia, anorexia, nausea and vomiting[9], and has been used as a reference drug for detecting the antispasmodic effects[10].

Junsibaekchul-San is a traditional Korean polyherbal medicine and is a mixture of 7 herbs Puerariae Radix, Ginseng Radix, Atractylodis Macrocephalae Rhizoma, Poria, Aucklandiae Radix, Glycyrrhizae Radix and Pogostemonis Herba, and this formula is known for its immunomodulatory effect on the humoral and cell-mediated immune responses in mice and have been used as anti-diarrhea agent in Korean Medicine. However, the antispasmodic effects of

* To whom correspondence should be addressed at : Dae-Jun Kim, Department of Internal Medicine, College of Oriental Medicine, Daegu Hanny University, 165, Sang-dong, Suseong-gu, Daegu, Korea

· E-mail : emdkdj@naver.com, · Tel : 053-627-9380

· Received : 2009/12/18 · Revised : 2010/01/11 · Accepted : 2010/01/25

Junsibaekchul-San have not been established yet with the In vivo effects on the abnormal intestinal motilities[11-12].

In the present study, to clarify the effects of Junsibaekchul-San, on accelerating small intestinal movement, we evaluated the effects of Junsibaekchul-San on In vivo carbachol(an acetylcholinergic agent)-accelerated mice small intestinal transit and on In vitro contractions induced by low-frequency electrostimulation, acetylcholine, KCl and histamine using isolated guinea pig ileum. The effects were compared to those of domperidone. In the present study, Junsibaekchul-San effectively inhibited the accelerated small intestinal movement in In vivo and these results are considered as well corresponded to the results of In vitro studies. We obtained statistically significant results, so we report them.

Materials and Methods

1. Preparations of Junsibaekchul-San

Junsibaekchul-San: Appropriate proportion of pulverized Puerariae Radix, Ginseng Radix, Atractylodis Macrocephalae Rhizoma, Poria, Aucklandiae Radix, Glycyrrhizae Radix and Pogostemonis Herba, which were purchased from Hermax Co.(Seoul, Korea) after confirm the morphology under microscopy as listed in Table 1, were mixed and used in the present study. Prepared Junsibaekchul-San were stored in a desiccator protected from light and moisture until used.

Table 1. Composition of Junsibaekchul-San

Scientific Name	Botanical Name	Amounts (g)
Puerariae Radix	Pueraria thunbergiana (SIEB.et ZUCC) BENTH	8
Ginseng Radix	Panax schinseng NEES	4
Atractylodis Macrocephalae Rhizoma	Atractylodes macrocephala KOIDZ.	4
Poria	Poria cocos (SCHW.) WOLF	4
Aucklandiae Radix	Saussurea lappa CLARKE	4
Pogostemonis Herba	Agastache rugosa (FISCH. et MEYER) O. KUNIZE	4
Glycyrrhizae Radix	Glycyrrhiza uralensis FISCH	4
Total	7 types	32

2. In vitro assays

1) Reagents and Animals

The sources of the drugs were domperidone(Kyowa Hakkou Co., Ltd., Tokyo, Japan), acetylcholine chloride(Ovisot, Daiichi Pharm. Inc., Osaka, Japan); histamine dihydrochloride (Wako Pure Chemical Industries Inc., Osaka, Japan); Potassium chloride(KCl, Wako Pure Chemical Inc., Osaka, Japan). All other drugs were commercially obtained in Sigma(MO, USA). The composition of the Kreb's solution used in this study was as follows(mmol/l): NaCl 118, KCl 4.8, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11. 10 heads of Male Hartley guinea-pigs(SLC, Japan) weighing between 350～450 g were used. All guinea-pigs housed individually in hanging wire cages under a constant light cycle (12hr : 12hr) at 20～25℃ and food and water were available free to access.

2) Guinea-pig myenteric plexus-longitudinal muscle preparation

The animals were stunned and decapitated and each ileum(about 3 cm) was quickly isolated about 10 cm away from the ileocaecal junction. The myenteric plexus-longitudinal muscle(MPLM) was prepared by a method described previously[10]. Briefly, a glass rod was inserted into the lumen of an intestinal segment and the MPLM was removed by rubbing the segment with a cotton swab soaked in Krebs' solution. The preparations(2～2.5 cm in length) were suspended at a resting tension of 300～500 ㎎ in a 5 ㎖ organ bath between platinum ring electrodes(3.5 cm apart), placed at the top and the bottom of the bath. The bath contained Krebs' solution, at 37℃, and was bubbled with 95% O_2/5% CO_2. The experiments started after a 60 min equilibration.

3) Effect on the spontaneous contraction

After 60 min of equilibration, Junsibaekchul-San 1, 0.1, 0.01 and 0.001 ㎎/㎖ or domperidone 2×10^{-5}M in Kreb's solution were added to prepared isolated guinea-pig ileum, and then the responses were recorded isometrically on an SP-H5P recorder(Riken Denshi Co., Tokyo, Japan) using an SD-1T force displacement transducer(Nihon Kohden Co., Tokyo, Japan). The % changes of contractions were observed and compared to treatment of vehicle alone at 10 min after Junsibaekchul-San or domperidone treatment. All data are represented as the mean±S.D. of ten independent experiments.

4) Effect on the electrical field stimulation-induced contractions

Ten min after treatment of Junsibaekchul-San 1, 0.1, 0.01 and 0.001 ㎎/㎖ or domperidone 2×10^{-5}M on the prepared isolated guinea-pig ileum, rectangular electrical pulse field stimulations were applied at 100 mA, 0.1 Hz, 0.5 ms pulse width and maximum intensity using a DPS-160B stimulator(NEC-Sanei Co., Tokyo, Japan) with a DPS-122 isolator(NEC-Sanei Co., Tokyo, Japan), and then the responses were recorded isometrically as previous[10]. The % changes of contractions were observed and compared to treatment of electrical field stimulation alone. All data are represented as the mean±S.D. of ten independent experiments.

5) Effect on the acetylcholine-induced contractions

Ten min after treatment of Junsibaekchul-San 1, 0.1, 0.01 and 0.001 ㎎/㎖ or domperidone 2×10^{-5}M on the prepared

isolated guinea-pig ileum, acetylcholine 10^{-7}M were added, and then the responses were recorded isometrically as previous[10,13]. The % changes of contractions were observed and compared to treatment of acetylcholine alone. All data are represented as the mean±S.D. of ten independent experiments.

6) Effect on the KCl-induced contractions

Ten min after treatment of Junsibaekchul-San 1, 0.1, 0.01 and 0.001 ㎎/㎖ or domperidone 2×10^{-5}M on the prepared isolated guinea-pig ileum, KCl 2×10^{-3}M were added, and then the responses were recorded isometrically as previous[10,13]. The % changes of contractions were observed and compared to treatment of KCl alone. All data are represented as the mean±S.D. of ten independent experiments.

7) Effect on the histamine-induced contractions

Ten min after treatment of Junsibaekchul-San 1, 0.1, 0.01 and 0.001 ㎎/㎖ or domperidone 2×10^{-5}M on the prepared isolated guinea-pig ileum, histamine 5×10^{-7}M were added, and then the responses were recorded isometrically as previous[10]. The % changes of contractions were observed and compared to treatment of acetylcholine alone. All data are represented as the mean±S.D. of ten independent experiments.

3. Carbachol-accelerated mice small intestinal transit

1) Animals and husbandry

Sixty male ICR mice(6 wk old upon receipt, SLC, Japan) were used after acclimatization for 10 days. Animals were allocated 5 per polycarbonate cage in a temperature(20～25℃) and humidity(40～45%) controlled room. Light : dark cycle was 12hr : 12hr and feed(Samyang, Korea) and water were supplied free to access. All animals were treated according to the Guide for the Care and Use of Laboratory Animals by Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, USA on 1996, Washington D.C.

2) Grouping and administration of test drugs

Animals were divided 10 animals per 6 groups as follows :

Intact control : vehicle pretreated non-carbachol-accelerated small intestinal transit group

Vehicle control : vehicle pretreated carbachol-accelerated small intestinal transit group

Junsibaekchul-San 500 : Junsibaekchul-San 500 ㎎/㎏ pretreated carbachol-accelerated small intestinal transit group

Junsibaekchul-San 250 : Junsibaekchul-San 250 ㎎/㎏ pretreated carbachol-accelerated small intestinal transit group

Junsibaekchul-San 125 : Junsibaekchul-San 125 ㎎/㎏ pretreated carbachol-accelerated small intestinal transit group

Domperidone 20 : domperidone 20 ㎎/㎏ pretreated carbachol-accelerated small intestinal transit group

In the present study, Junsibaekchul-San 500, 250 and 125 ㎎ or domperidone 20 ㎎ were dissolved or suspended in distilled water and orally pretreated on the carbachol-accelerated small intestinal transit mice once a day for 7 days at a volume of 10 ㎖/㎏(of body weight) using a sonde attached to 1 ㎖ syringes containing test drugs.

3) Carbachol treatment and small intestinal transit rate measurement

To induce the acceleration of mice small intestinal transit, Carbachol 1 ㎎/㎏ was once subcutaneously dosed 15 min before last administration of the test drugs. The animals were then killed 20 min later, and the small intestine completely removed. The small intestinal transit rate were obtained after dividing the migrating length of activated charcoal powder by the total length of the small intestine as previous[13].

4) Body weight changes

Changes of body weight and its gains were calculated at 1 day before test drug administration, at dosing and 1, 2, 5 and 6 days after dosing with at sacrifice. At dosing and sacrifice day, experimental animals were overnight fasted(water was not; about 18hr) to reduce the erratum of feeding. In addition, Body weight gains during dosing period were calculated as following equation.

Equation 1.

Weight gains(g) =(Body weight at sacrifice - Body weight at dosing)

4. Statistical analyses

All data was calculated as mean±S.D. Statistical analyses was conducted using Mann-Whitney U-Wilcoxon Rank Sum W test(MW test) with SPSS for Windows(Release 6.1.3., SPSS Inc., USA). The percentage changes compared to that of vehicle control was calculated to help the understanding of the efficacy of test materials on differences between vehicle control and test groups, and the differences between intact and vehicle control were also calculated in case of carbachol-accelerated mice as follows.

Equation 2.

Percentage Changes vs intact control(%) =((Data of vehicle control - Data of intact control)/Data of intact control) × 100

Equation 3.

Percentage Changes vs vehicle control(%)=((Data of test groups - Data of vehicle control)/Data of vehicle control) × 100

Results

1. Results of In vitro assays

1) Effects on the spontaneous contractions

Significant($p<0.05$) decrease of % spontaneous contractions was detected in Junsibaekchul-San 1 ㎎/㎖ treated groups compared to that of vehicle control similar to that of domperinone 2×10^{-5}M treated group, in which a significantly($p<0.05$) decrease of % spontaneous contractions was also detected and compared to that of vehicle control. In Junsibaekchul-San 0.1, 0.01 and 0.001 ㎎/㎖ treated groups, no meaningful changes on the spontaneous contractions were detected in the present study, respectively. The % spontaneous contractions were changes compared to that of vehicle control as -248.28, -0.99, -0.49, -3.45 and -357.98% in Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated groups, respectively. The % spontaneous contractions of vehicle control, Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated were detected as 0.20±0.37, -0.30±0.47, 0.20±0.47, 0.20±0.31, 0.20±0.32 and -0.52±0.72% compared to contractions of treatment of vehicle alone, respectively(Fig. 1).

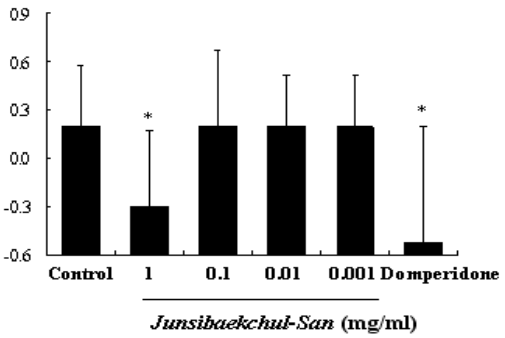

Fig. 1. Effects on the spontaneous contractions. * p<0.05 compared to that of vehicle alone by MW test.

2) Effects on the electrostimulation induced contractions

Significant($p<0.01$ or $p<0.05$) decreases of % electrostimulation induced contractions were detected in Junsibaekchul-San 1 and 0.1 ㎎/㎖ treated groups compared to that of electrostimulation vehicle control. Quite similar to the results of Junsibaekchul-San treated group, a significantly($p<0.01$) decrease of % contractions was detected in domperidone treated group compared to that of electrostimulation vehicle control. In Junsibaekchul-San 0.01 and 0.001 ㎎/㎖ treated groups, no meaningful changes on the electrostimulation induced contractions were detected in the present study, respectively. The % electrostimulation induced contractions were changes compared to that of electrostimulation vehicle control as -40.87, -10.47, -1.83, -1.39 and -23.66% in Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated groups, respectively. The % electrostimulation induced contractions of electrostimulation alone, Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated were detected as 82.80±9.05, 48.96±13.66, 74.13±8.13, 81.28±7.35, 81.65±6.91 and 63.21±5.35% compared to contractions of treatment of vehicle alone, respectively(Fig. 2).

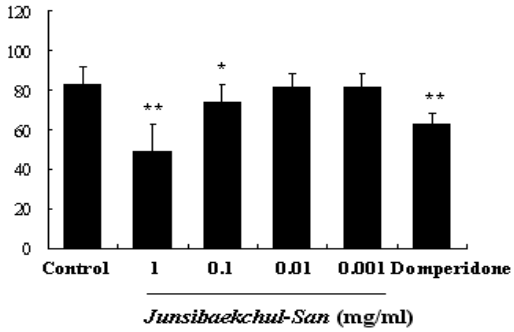

Fig. 2. Effects on the electrostimulation induced contractions. * p<0.05 and ** p<0.01 compared to that of vehicle alone by MW test.

3) Effects on the acetylcholine induced contractions

Significant($p<0.01$) decreases of % acetylcholine induced contractions were detected in Junsibaekchul-San 1 ㎎/㎖ treated groups compared to that of acetylcholine vehicle control. Quite similar to the results of Junsibaekchul-San treated group, a significantly($p<0.01$) decrease of % acetylcholine induced contractions was also detected in domperidone treated group compared to that of acetylcholine vehicle control. In Junsibaekchul-San 0.1, 0.01 and 0.001 ㎎/㎖ treated groups, no meaningful changes on the acetylcholine induced contractions were detected in the present study, respectively. The % acetylcholine induced contractions were changes compared to that of electrostimulation vehicle control as -23.11, -5.52, 6.41, 4.33 and -28.22% in Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated groups, respectively. The % acetylcholine induced contractions of acetylcholine alone, Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated were detected as 73.41±7.22, 59.45±9.93, 69.36±7.85, 78.12±6.29, 76.59±7.48 and 52.70±9.29% compared to contractions of treatment of vehicle alone, respectively(Fig. 3).

4) Effects on the KCl induced contractions

Significant($p<0.01$) decreases of % KCl induced contractions were detected in Junsibaekchul-San 1 ㎎/㎖ treated groups compared to that of KCl vehicle control. Quite similar to the results of Junsibaekchul-San treated group, a significantly($p<0.01$) decrease of % KCl induced contractions was also detected in domperidone treated group compared to that of KCl vehicle control. In Junsibaekchul-San 0.1, 0.01 and 0.001 ㎎/㎖ treated groups, no meaningful changes on the KCl

induced contractions were detected in the present study, respectively. The % KCl induced contractions were changes compared to that of electrostimulation vehicle control as -31.63, 1.19, 5.19, 1.48 and -29.56% in Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated groups, respectively. The % KCl induced contractions of KCl alone, Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated were detected as 62.03±6.96, 42.41±4.40, 62.77±10.99, 65.25±5.90, 62.94±5.73 and 43.69±6.60% compared to contractions of treatment of vehicle alone, respectively(Fig. 4).

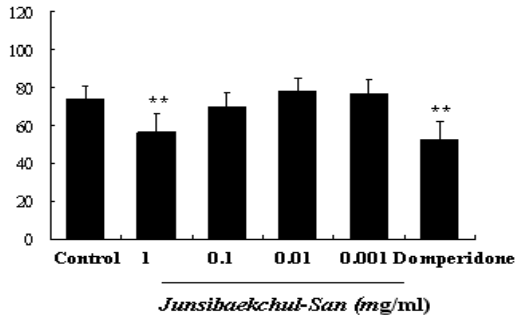

Fig. 3. Effects on the acetylcholine induced contractions ** p<0.01 compared to that of vehicle alone by MW test.

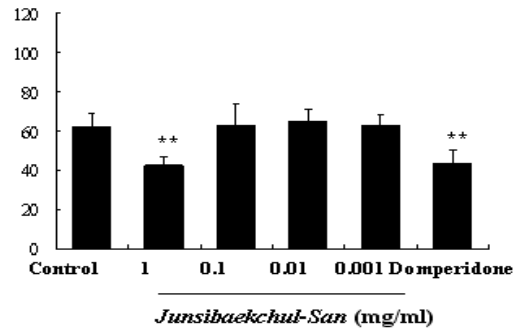

Fig. 4. Effects on the KCl induced contractions. ** p<0.01 compared to that of vehicle alone by MW test.

5) Effects on the histamine induced contractions

Significant(p<0.01) decrease of % histamine induced contractions was detected in Junsibaekchul-San 1 ㎎/㎖ treated groups compared to that of histamine vehicle control. Quite similar to the results of Junsibaekchul-San treated group, a significantly(p<0.01) decrease of % histamine induced contractions was also detected in domperidone treated group compared to that of histamine vehicle control. In addition, non-significantly decrease on the % histamine induced contractions were also detected in Junsibaekchul-San 0.1 ㎎/㎖ treated groups compared to that of histamine vehicle control. However, Junsibaekchul-San 0.01 and 0.001 ㎎/㎖ treated groups, no meaningful changes on the histamine induced contractions were detected in the present study, respectively. The % histamine induced contractions were changes compared to that of electrostimulation vehicle control as -52.04, -19.03, 1.80, 2.40 and -53.11% in Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated groups, respectively. The % histamine induced contractions of histamine alone, Junsibaekchul-San 1, 0.1, 0.01, 0.001 ㎎/㎏ and domperidone 2×10^{-5}M treated were detected as 66.88±6.47, 32.08±7.64, 54.15±14.17, 68.08±5.84, 68.48±9.87 and 31.36±4.77% compared to contractions of treatment of vehicle alone, respectively(Fig. 5).

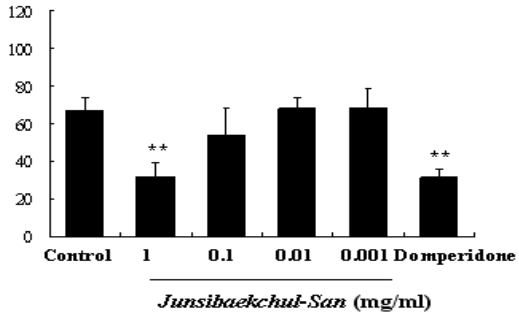

Fig. 5. Effects on the histamine induced contractions ** p<0.01 compared to that of vehicle alone by MW test.

2. Results of In vivo assays

1) Changes of body weights

No meaningful changes on the body weight and gains were detected in all test drug dosing groups compared to those of intact and carbachol controls, respectively. In addition no meaningful changes on the body weight and gains were detected between intact and carbachol controls(Table 2, 3). In intact control, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.47±1.72, 30.81±1.68, 34.68±1.66, 34.84±1.34, 35.99±0.92, 36.41±1.07 and 32.93±1.56 g/head, respectively. In carbachol control, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.38±2.50, 30.60±1.96, 34.86±1.93, 35.11±1.74, 35.90±1.84, 36.67±2.21 and 32.88±2.51 g/head, respectively. In Junsibaekchul-San 500 ㎎/㎏ pretreated group, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.69±2.25, 31.20±2.78, 35.02±1.92, 35.55±2.09, 36.09±1.78, 36.98±2.01 and 33.11±2.48 g/head, respectively. In Junsibaekchul-San 250 ㎎/㎏ pretreated group, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.63±3.04, 30.97±3.03, 35.26±2.72, 35.88±2.48, 36.50±2.44, 37.21±2.26 and 33.20±2.31 g/head, respectively. In Junsibaekchul-San 125 ㎎/㎏ pretreated group, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.36±3.24, 30.50±3.22,

34.81±2.87, 35.37±2.89, 36.17±2.83, 36.93±2.96 and 32.95±2.87 g/head, respectively. In domperidone 20 ㎎/㎏ pretreated group, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were detected as 34.51±2.17, 30.89±2.06, 34.84±1.88, 35.35±1.55, 36.02±1.49, 36.60±1.43 and 32.82±1.32 g/head, respectively(Table 2). The body weight gains during dosing periods(at dosing ~ sacrifice) of intact and carbachol controls, Junsibaekchul-San 500, 250, 125 ㎎/㎏- and domperidone 20 ㎎/㎏- pretreated groups were detected as 2.12±1.01, 2.28±1.46, 1.91±1.14, 2.23±1.47, 2.45±1.26 and 1.93±1.60 g/head, respectively(Table 3).

Table 2. Changes on the body weight

Body weight	1 day before dosing	At Dosing	Days after dosing				At Sacrifice
			1 day	2 days	5 days	6 days	
Controls							
Intact	34.47 ±1.72	30.81 ±1.68	34.68 ±1.66	34.84 ±1.34	35.99 ±0.92	36.41 ±1.07	32.93 ±1.56
Vehicle	34.38 ±2.50	30.60 ±1.96	34.86 ±1.93	35.11 ±1.74	35.90 ±1.84	36.67 ±2.21	32.88 ±2.51
Junsibaekchul-San							
500 mg/kg	34.69 ±2.25	31.20 ±2.78	35.02 ±1.92	35.55 ±2.09	36.09 ±1.78	36.98 ±2.01	33.11 ±2.48
250 mg/kg	34.63 ±3.04	30.97 ±3.03	35.26 ±2.72	35.88 ±2.48	36.50 ±2.44	37.21 ±2.26	33.20 ±2.31
125 mg/kg	34.36 ±3.24	30.50 ±3.22	34.81 ±2.87	35.37 ±2.89	36.17 ±2.83	36.93 ±2.96	32.95 ±2.87
Domperidone							
20 mg/kg	34.51 ±2.17	30.89 ±2.06	34.84 ±1.88	35.35 ±1.55	36.02 ±1.49	36.60 ±1.43	32.82 ±1.32

Table 3. Changes on the body weight gains

Gains	Body weight gains during dosing(at dosing~sacrifice)
Controls	
Intact	2.12 ± 1.01
Vehicle	2.28 ± 1.46
Junsibaekchul-San(mg/kg)	
500 mg/kg	1.91 ± 1.14
250 mg/kg	2.23 ± 1.47
125 mg/kg	2.45 ± 1.26
Domperidone(mg/kg)	
20 mg/kg	1.93 ± 1.60

2) Changes of small intestinal activated charcoal transit

Significantly($p<0.01$) increase of % regions of activated charcoal transit in small intestine was detected in carbachol control compared to that of intact control. However, significant ($p<0.01$ or $p<0.05$) decreases of % regions of activated charcoal transit were dose-dependently observed in 500 and 250 ㎎/㎏ of Junsibaekchul-San or domperidone-pretreated groups. No meaningful change on the % regions of activated charcoal transit was detected in 125 ㎎/㎏ of Junsibaekchul-San pretreated group compared to that of carbachol control. In carbachol control, % regions of activated charcoal transitin small intestine were showed 65.41% changes vs intact control, and they showed % changes vs cabarchol control as -21.55, -11.66, -1.81 and -24.34% in Junsibaekchul-San 500, 250, 125 ㎎/㎏- and domperidone 20 ㎎/㎏-pretreated groups, respectively. The % regions of activated charcoal transit in small intestine of intact and carbachol controls, Junsibaekchul-San 500, 250, 125 ㎎/㎏- and domperidone 20 ㎎/㎏- pretreated groups were detected as 52.13±7.37, 86.22±7.29, 67.64±6.21, 76.17±8.48, 84.66±6.44 and 65.24±7.94%, respectively(Table 4).

Table 4. Changes on % regions of activated charcoal transit

% regions	% regions of activated charcoal transit
Controls	
Intact	52.13 ± 7.37
Vehicle	86.22 ± 7.29**
Junsibaekchul-San	
500 mg/kg	67.64 ± 6.21**,##
250 mg/kg	76.17 ± 8.48**,#
125 mg/kg	84.66 ± 6.44**
Domperidone	
20 mg/kg	65.24 ± 7.94**,##

** $p<0.01$ compared to that of Sham by MW test; # $p<0.05$ and ## $p<0.01$ compared to that of vehicle control by MW test.

Discussion

Plants have been a constant source of drugs and recently much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs[14]. Until now, numerous herb extracts have been shown the beneficial pharmacological effects on the intestinal motilities, among them Aegle marmelos unripe fruit extract[15], Thymus piperella[16], aqueous leaf extract of Irvingia gabonensis[17], Acalypha phleoides extracts[18], aqueous extract of African mistletoe, Tapinanthus sessilifolius leaf[19], methanolic extract of Cassia tora leaf[20], Viguiera hypargyrea root extracts[21], methanolic extract of Ficus platyphylla[22], Aurantii fructus immaturus extracts[23], methanol extract from Monadenium lugardiae[24] and Teucrium polium boiled leaf extract[25] were have been revealed. In addition, poly herbal formula, Dai-Kenchu-To[13,26-28] and Liu-Jun-Zi-Tang[10] also showed favorable effects on the gastrointestinal motilities, and they used to treat various gastrointestinal disorders.

Junsibaekchul-San has been considered as one of the most useful Korean medicine when people has lost lots of body fluids during consistent diarrhea[11]. In the present study, the effects of Junsibaekchul-San, a traditional Korean Polyherbal formula consisted of 7 types of herbs, on the In vivo carbachol-accelerated mice small intestinal transit and In vitro contractions induced by low-frequency electrostimulation, KCl, histamine or acetylcholine using isolated guinea pig ileum.

We used the isolated guinea-pig ileum MPLM system due to the usefulness for analysis of the contraction reaction of

intestinal smooth muscles[10,13]. In the present study, high concentration, 1 ㎎/㎖ of Junsibaekchul-San was found to decrease the spontaneous contraction of the isolated guinea-pig ileum quite similar to that of domperidone and Liu-Jun-Zi-Tang[10]. It has been established that the spontaneous contractions of the intestinal smooth muscle are regulated by cycles of depolarization and repolarization. Action potentials are generated at the peak of depolarization and constitute a fast influx of calcium ions through the voltage-activated calcium channels[29,30]. Therefore, it is possible that Junsibaekchul-San contains some compounds which, decrease with the calcium channels activity.

And the Junsibaekchul-San decreases contractions induced by electrostimulation, acetylcholine, histamine and KCl in the isolated guinea-pig ileum. This effect was concentration-dependent similar to those of domperidone in which, spontaneous contraction, electrostimulation, acetylcholine, histamine and KCl-induced contractions of guinea-pig ileum were significantly($p<0.01$ or $p<0.05$) decreased. As shown in Fig. 2, the 0.1 Hz-induced contraction response was also inhibited by 1 and 0.1 ㎎/㎖, but not by 0.01 and 0.001 ㎎/㎖, of Junsibaekchul-San. As it is well known that the contraction response is due to acetylcholine output from coaxially stimulated myenteric neurons[31], this finding suggests the possibility that Junsibaekchul-San inhibits the release of acetylcholine. Acetylcholine and histamine cause depolarization and tonic contractions of intestinal smooth muscles. It is generally accepted that an increase in concentration of cytoplasmic-free calcium ions is indispensable for smooth muscle contraction. The activation of muscarinic receptors of longitudinal smooth muscle of guinea-pig small intestine produces an increased frequency of action potential discharge and depolarisation which results in a contraction[32].

The acetylcholine-evoked contraction is generally regarded as mediated via M_3 subtype of muscarinic receptor although the muscle has a preponderance of M_2 subtype muscarinic binding sites. Whereas, histamine-induced contraction happens via H1 receptor activation[33], and contraction induced by KCl is due to an increase in K+ and depolarisation of smooth muscle fibers, leading to increased influx of calcium through L-type voltage-operated channels[34]. In short, calcium ions gain access to the cytoplasm through voltage-activated or receptor-operated calcium channels[35].

According to our observations, when the isolated guinea-pig ileum preparations were contracted with histamine, the relaxation induced by the Junsibaekchul-San was very higher than that in the presence of acetylcholine quite similar to those of domperidone. However, the spasmolytic activity of the Junsibaekchul-San could not be attributed solely to any pure antagonistic effect, since the tissue contracted by KCl was also relaxed after exposure to the Junsibaekchul-San. Therefore, Junsibaekchul-San might be interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specificly similar to domperidone.

It was reported that trimebutine enhanced intestinal motility in the suppressed condition and inhibited intestinal motility in the accelerated condition via enteric opoid receptors on cholinergic and adrenergic nerves[36] and Dai-Kenchu-to also intestinal motility in the suppressed condition and inhibited intestinal motility in the accelerated condition but the exact mechanisms are unclear[13,27,28]. In Dai-Kenchu-to, ginseng one of main components of Dai-Kenchu-to and Junsibaekchul-San used in the present study, inhibit the acetylcholine releasing without suppressing its action[13] but Zanthoxylum fruit extracts improves intestinal motilitywhen it is inhibited. Therefore, the dual action on the intestinal motility of Dai-Kenchu-to is due to its different component[37,38]. The possibility, Junsibaekchul-San also showed these dual actions, was could not exclusive because Puerariae Radix a major components contain the acetylcholine. However, we do not observed the effects of Junsibaekchul-San on the intestinal motility when it is inhibited in the present study. Further investigation is also need to identify whether Junsibaekchul-San has dual actions or not.

In addition, further investigation is also needed to identify which herbs and chemical components of Junsibaekchul-San are responsible for these actions. Junsibaekchul-San is a mixture of 7 herbs Puerariae Radix, Ginseng Radix, Atractylodis Macrocephalae Rhizoma, Poria, Aucklandiae Radix, Glycyrrhizae Radix and Pogostemonis Herba. And Puerariae Radix contains daidzin, daidzein, 4, 7-diglucoside, puerarin, puerarin-7-xyloside, 4,6-di-o-acetylpuerarin, genistein, formonetin, soya-sapogenol A, kudzusapogenol B, kudzusaponin B, allantoin, acetylcholine and starch. Ginseng Radix contains more than 30 kinds of ginsenosides. Atractylodis Macrocephalae Rhizoma contains atracylone, furfural, 3beta-acetoxyatractylone, 3beta-hydroxyatractylone, atractyle-nolode I, II, III and 2-furaldehyde. Poria contains ergosterol, histidine, caprylic aicd, dodecenoic acid, lauric acid, palmitic acid, undecanoic acid, lecithin, pachyman, choline, adenien, eburicoic acid, dehydroeburicoic acid, tumulosic acid, pachymic aicd, 3β-hydroxyl-lanosta-7,9(11),24-trien-21-oic acid and carboxy-protease. Aucklandiae Radixcontains costuslactone, costic acid, costol, costene and inulin. Glycyrrhizae Radix

contains glycyrrhizin, isoflavonoids glycyrrhizic/glycyrrhizic acid, saponins, coumarine, starch, essential oils, phytostrol, tannin, beturic acid, oleanolic acid, liquiritigenin, isoliquiritigenin, liquiritin, licuraside andrhamnoliquiritin. Pogostemonis Herba contains patchouli alchol, eugenol and cinnamic aldehyde[39].

In the present study, Junsibaekchul-San also effectively inhibited the accelerated small intestinal movement induced by stimulation of cholinergic neurotransmission(carbachol) in In vivo and these results are considered as well corresponded to the results of In vitro studies. Clinical conditions such as ileus and Irritable Bowel Syndrome(IBS) exhibit various symptoms depending on critical factors. For example, in relation to adhesive ileus, it is known that the intestinal motility is different on the proximal side compared with the distal side at the obstruction[40]. Intestinal dysmotility due to dysfunction of the autonomic nervous system is considered one etiology of postoperative ileus and IBS. The present results seem to indicate that Junsibaekchul-San has an effect that normalization in the accelerated condition. Hence, the prescription of Junsibaekchul-San is useful from the viewpoint of clinical treatment of digestive tract disorders such as accelerated the motility of intestine, diarrhea or intestinal painful contractions.

Conclusion

Based on the results, although it is unknown that the exact molecular action mechanism and which herbs compound in Junsibaekchul-San are responsible for actions, it was concluded that Junsibaekchul-San normalization in the accelerated intestinal motility might be interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specificly. Therefore, it is expected that Junsibaekchul-San will be promising as a prescription of clinical treatment of digestive tract disorders such as the accelerated motility of intestine, diarrhea or intestinal painful contractions.

References

1. Costall, B., Naylor, R.J., Tan, C.C. Field stimulation-induced responses of circular smooth muscle from guinea-pig stomach. Characterisation of the receptor mechanisms involved. Naunyn Schmiedebergs Arch Pharmacol. 323: 155-161, 1983.
2. Sahyoun, H.A., Costall, B., Naylor, R.J. Catecholamines act as alpha 2-adrenoceptors to cause contraction of circular smooth muscle of guinea-pig stomach. J. Pharm Pharmacol. 34: 381-385, 1982.
3. Hay, A.M., Man, W.K. Effect of metoclopramide on guinea pig stomach: critical dependence on intrinsic stores of acetylcholine. Gastroenterology 76: 492-496, 1979.
4. Van Nueten, J.M., Schuurkes, J.A. Studies on the role of dopamine and dopamine blockers in gastroduodenal motility. Scand J. Gastroenterol Suppl. 96: 89-99, 1984.
5. Fernandez, A.G., Massingham, R. Peripheral receptor populations involved in the regulation of gastrointestinal motility and the pharmacological actions of metoclopramide-like drugs. Life Sci. 36: 1-14, 1985.
6. Hernandez, D.E., Adcock, J.W., Orlando, R.C., Patrick, K.S., Nemeroff, C.B., Prange, A.J.Jr. Prevention of stress-induced gastric ulcers by dopamine agonists in the rat. Life Sci. 35: 2453-2458, 1984.
7. Kusunoki, M., Taniyama, K., Tanaka, C. Dopamine regulation of [3H]acetylcholine release from guinea-pig stomach. J. Pharmacol Exp Ther. 234: 713-719, 1985.
8. Kohli, J.D., Glock, D., Goldberg, L.I. Selective DA2 versus DA1 antagonist activity of domperidone in the periphery. Eur J. Pharmacol. 89: 137-141, 1983.
9. Reyntjens, A.J., Niemegeers, C.J., Van Nueten, J.M., Laduron, P., Heykants, J., Schellekens, K.H., Marsboom, R., Jageneau, A., Broekaert, A., Janssen, P.A. Domperidone, a novel and safe gastrokinetic anti-nauseant for the treatment of dyspepsia and vomiting. Arzneimittelforschung. 28: 1194-1196, 1978.
10. Ozaki, M., Nagatomo, T., Maeda, T., Kishioka, S., Yamamoto, H. Pharmacological differences between Liu-Jun-Zi-Tang, a traditional Chinese herbal medicine, and domperidone on isolated guinea-pig ileum. Biol Pharm Bull. 29: 1349-1954, 2006.
11. Heo Jun. Dong-Eui-Bo-Gam. Han-mi-eui-hak, Seoul, p 1035, 2001.
12. Shim, M.K., Park, E.J. Effect of Junsibaekchulsan on the humoral and cell-mediated immune responses in mice. J. Korean Oriental Pediatrics 8: 39-58, 1994.
13. Satoh, K., Kase, Y., Hayakawa, T., Murata, P., Ishige, A., Sasaki, H. Dai-kenchu-to enhances accelerated small intestinal movement. Biol Pharm Bull. c24: 1122-1126, 2001.
14. Fatehi, M., Farifteh, F., Fatehi-Hassanabad, Z. Antispasmodic and hypotensive effects of Ferula asafoetida gum extract. J Ethnopharmacol. 91: 321-324, 2004.
15. Dhuley, J.N. Investigation on the gastroprotective and antidiarrhoeal properties of Aegle marmelosunripe fruit extract. Hindustan Antibiot Bull. pp 45-46, 2003, pp 41-46, 2004.

16. Marti, D., Villagrasa, V., Martinez-Solis, I., Blanquer, A., Castillo, E., Royo, L.M. Hystological and pharmacological study of Thymus piperella(L.). Phytother Res. 19: 298-302, 2005.
17. Abdulrahman, F., Inyang, I.S., Abbah, J., Binda, L., Amos, S., Gamaniel, K. Effect of aqueous leaf extract of Irvingia gabonensis on gastrointestinal tract in rodents. Indian J. Exp Biol. 42: 787-791, 2004.
18. Astudillo, A., Hong, E., Bye, R., Navarrete, A. Antispasmodic activity of extracts and compounds of Acalypha phleoides Cav. Phytother Res. 18: 102-106, 2004.
19. Tarfa, F.D., Amos, S., Temple, V.J., Binda, L., Emeje, M., Obodozie, O., Wambebe, C., Gamaniel, K. Effect of the aqueous extract of African mistletoe, Tapinanthus sessilifolius(P. Beauv) van Tiegh leaf on gastrointestinal muscle activity. Indian J. Exp Biol. 40: 571-574, 2002.
20. Chidume, F.C., Kwanashie, H.O., Adekeye, J.O., Wambebe, C., Gamaniel, K.S. Antinociceptive and smooth muscle contracting activities of the methanolic extract of Cassia tora leaf. J. Ethnopharmacol. 81: 205-209, 2002.
21. Zamilpa, A., Tortoriello, J., Navarro, V., Delgado, G., Alvarez, L. Antispasmodic and antimicrobial diterpenic acids from Viguiera hypargyrea roots. Planta Med. 68: 281-283, 2002.
22. Amos, S., Binda, L., Chindo, B., Akah, P., Abdurahman, M., Danmallam, H.U., Wambebe, C., Gamaniel, K. Evaluation of methanolic extract of Ficus platyphylla on gastrointestinal activity. Indian J. Exp Biol. 39: 63-67, 2001.
23. Takase, H., Yamamoto, K., Hirano, H., Saito, Y., Yamashita, A. Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine, Aurantii fructus immaturus. Jpn J. Pharmacol. 66: 139-147, 1994.
24. Gundidza, M. Effect of methanol extract from Monadenium Lugardiae on contractile activity of guinea-pig ileum. Cent Afr J. Med. 37: 141-144, 1991.
25. Suleiman, M.S., Abdul-Ghani, A.S., Al-Khalil, S., Amin, R. Effect of Teucrium polium boiled leaf extract on intestinal motility and blood pressure. J. Ethnopharmacol. 22: 111-116, 1988.
26. Nakamura, T., Sakai, A., Isogami, I., Noda, K., Ueno, K., Yano, S. Abatement of morphine-induced slowing in gastrointestinal transit by Dai-kenchu-to, a traditional Japanese herbal medicine. Jpn J. Pharmacol. 88: 217-221, 2002.
27. Satoh, K., Hashimoto, K., Hayakawa, T., Ishige, A., Kaneko, M., Ogihara, S., Kurosawa, S., Yakabi, K., Nakamura, T. Mechanism of atropine-resistant contraction induced by Dai-kenchu-to in guinea pig ileum. Jpn J. Pharmacol. a86: 32-37, 2001.
28. Satoh, K., Hayakawa, T., Kase, Y., Ishige, A., Sasaki, H., Nishikawa, S., Kurosawa, S., Yakabi, K., Nakamura, T. Mechanisms for contractile effect of Dai-kenchu-to in isolated guinea pig ileum. Dig Dis Sci. b46: 250-256, 2001.
29. Walsh, J.V.Jr., Singer, J.J. Calcium action potentials in single freshly isolated smooth muscle cells. Am J. Physiol. 239: C162-174, 1980.
30. Brading, A.R. How do drugs initiate contraction in smooth muscle Trends Pharmacol Sci. 2: 262-265, 1981.
31. Paton, W.D. The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. Br J. Pharmacol Chemother. 12: 119-127, 1957.
32. Reddy, H., Watson, N., Ford, A.P., Eglen, R.M. Characterization of the interaction between muscarinic M2 receptors and beta-adrenoceptor subtypes in guinea-pig isolated ileum. Br J. Pharmacol. 114: 49-56, 1995.
33. Zavecz, J.H., Yellin, T.O. Histamine receptors in the myenteric plexus-longitudinal muscle of the guinea-pig ileum: H1- and H2-receptor-mediated potentiation of the contractile response to electrical stimulation. J. Pharmacol Exp Ther. 223: 177-182, 1982.
34. Gilani, A.H., Janbaz, K.H., Zaman, M., Lateff, A., Tariq, S.R., Ahmad, H.R. Hypotensive and spasmolitic activites of crude extract of Cyperus scariosus. Arch Pharmacol Res. 17: 145-149, 1994.
35. Triggle, D.J. Calcium ions and respiratory smooth muscle function. Br J. Clin Pharmacol. 20 Suppl 2: 213S-219S, 1985.
36. Taniyama, K., Sano, I., Nakayama, S., Matsuyama, S., Takeda, K., Yoshihara, C., Tanaka, C. Dual effect of trimebutine on contractility of the guinea pig ileum via the opioid receptors. Gastroenterology 101: 1579-1587, 1991.
37. Hayakawa, T., Kase, Y., Saito, K., Hashimoto, K., Ishige, A., Komatsu, Y., Sasaki, H. Pharmacological studies of the effect of Dai-kenchu-to on spontaneous contraction of isolated rabbit jejunum. J.Smooth Muscle Res. 35: 55-62, 1999.
38. Shibata, C., Sasaki, I., Naito, H., Ueno, T., Matsuno, S. The herbal medicine Dai-Kenchu-Tou stimulates upper gut motility through cholinergic and 5-hydroxytryptamine 3 receptors in conscious dogs. Surgery. 126: 918-924, 1999.
39. Bae, K.W. The medicinal plants of Korea. Kyo-Hak-Sa: Seoul, pp 1-589, 2000.
40. Cullen, P.T., Storey, B.E., Cuschieri, A., Campbell, F.C. Detection of clustered gastrointestinal contractions in partial intestinal obstruction by surface vibration analysis. Ann Surg. 210: 234-238, 1989.