Experimental Studies on the Antidiarrheal Effects of *Anjang-san*

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Objectives: The purpose of the present study was to investigate the antidiarrheal effects of *Anjang-san* in mice and rats.
Methods: We measured the content of condensed tannin in *Anjang-san* extract, and observed the effects of *Anjang-san* on the small intestinal and colonic transport of mice, as well as on mice models of diarrhea induced by castor oil and $MgSO_4$, and on rat models of castor oil-induced enteropooling.
Results: *Anjang-san* showed significant inhibitory effects on abnormally increased small intestinal transit induced by pyridostigmine and neostigmine, and inhibitory effects on large intestinal transit. *Anjang-san* also exhibited antidiarrheal effects on diarrhea induced by $MgSO_4$, and inhibitory effects on castor oil-induced enteropooling. *Anjang-san* also improved castor oil-induced diarrhea based on simple numbers without statistical significance.
Conclusion: These results demonstrate that *Anjang-san* has significant antidiarrheal properties and attests to its possible utility in functional diarrheas, irritable bowel syndrome and other gastrointestinal disorders based upon further studies.

Key Words : *Anjang-san*, antidiarrheal, diarrhea

Introduction

Diarrhea can be diagnosed clinically when the frequency of defecation is more than 4 times per day and the weight of stool per day is higher than 250g. Chronic diarrhea is defined as diarrhea persisting for more than 2-3 weeks in adults, whereas acute diarrhea refers to recently occurred diarrhea which hasn't reached that duration yet[1].

Acute diarrhea is mainly caused by pathogenic microorganisms and drugs, and chronic diarrhea is much more diverse in its causes, including irritable bowel syndrome, chronic inflammatory bowel diseases, post-operation, malabsorption, and pathogenic microorganisms, in order of frequency[1].

In oriental medicine, diarrhea has been mentioned in various terms. Depending on the severity and duration, diarrhea is classified into fulminant diarrhea which belongs in the excessive pattern and chronic diarrhea which belongs in the deficient pattern, and both types share the pathogenic mechanism of excess dampness and spleen deficiency. Fulminant diarrhea is more centered on excess dampness and the therapeutic principle is to resolve the dampness, while chronic diarrhea is centered on spleen deficiency and the principle is to invigorate the spleen[2].

Many herbal prescriptions are being used in the treatment of diarrhea, and there have been experimental studies on the antidiarrheal effect of herbal medicine using animal models. Ryu *et al*. reported the antidiarrheal properties of *Yukgunja-tang* (Liu Jun Zi Tang)[3]; Ryu et al. of *Jisa-tang*[4]; No *et al*. of Dolichoris Semen, Terminaliae Fructus and *Bojanggunbi-tang*[5].

When acute diarrhea progresses into chronic diarrhea, the differential diagnosis becomes complicated and efficient treatment becomes difficult, so it is important to come up with a herbal prescription which can swiftly resolve the diarrhea at an early stage. Thus the Oriental Medicine Research Center in Kyung Hee University Oriental Medicine Hospital developed *Anjang-san* for efficient treatment of diarrhea.

Anjang-san is an experiential prescription created by Ryu for antidiarrheal purposes, and is composed of Geranii Herba, Terminaliae Fructus, Dolichoris Semen, Alpiniae Katsumadaii Semen, Myristicae Semen, Glycyrrhizae Radix, and Zizyphi Semen.

Currently, *Anjang-san* is being used in Kyung Hee University Hospital of Oriental Medicine on acute and chronic diarrhea, functional diarrhea and irritable bowel syndrome, but its effects have not been validated experimentally.

In this study, we investigated the antidiarrheal properties of *Anjang-san* by measuring the content of condensed tannin in *Anjang-san* extract, by observing the effect of *Anjang-san* on the small intestinal and colonic transport of mice, on mice models of diarrhea induced by castor oil and magnesium sulfate ($MgSO_4$), and on rat models of castor oil induced enteropooling.

Materials and Methods

1. Materials and Animals

1) Materials

The ingredients of the herbal prescription used in this study were purchased by Kyung Hee Oriental Medical Center, and were all within standards of the Korean Pharmacopoeia and the Korean Herbal Pharmacopoeia as shown in Table 1.

2) Preparation of Test Solution

The herbal medicine (620g) were put into flasks and mixed with purified water, then were hot reflux extracted in a water bath for 2 hours and filtered while hot. The residue was then mixed with purified water and was hot reflux extracted for 1 hour and filtered while hot, then both filtrates were united. The filtrate was vacuum concentrated in a rotary evaporator until the dry substance was 30%, then was freeze dried and 170.5g (27.5%) of dry powder was obtained. The test solution was diluted as needed in the study.

3) Experimental Animals

The animals used in this study were ICR male mice (Samtako Bio, Korea) weighing 18-24g, and SD rats (Samtako Bio, Korea) weighing approximately 200g. The animals were used after 2 weeks of adaptation period while being fed with solid feed (Samyang Oil & Feed Corporation, Korea) and ample water. The experiments were conducted at 24±2℃ unless specifically mentioned otherwise.

Table 1. Composition of *Anjang-san*

Latin Crude Drug Name	Scientific Name	Oriental Medicinal Name	Content(g)
Geranii Herba	*Geranium thunbergii*	Laoguancao	15
Terminaliae Fructus	*Terminalia chebula*	Hezi	15
Doilichoris Semen	*Dolichos lablab*	Bian dou	12
Alpiniae Katsumadaii Semen	*Alpinia katsumadai*	Caodoukou	6
Myristicae Semen	*Myristica fragrans*	Roudoukou (Nutmeg)	6
Glycyrrhizae Radix	*Glycyrrhiza uralensis*	Gancao (Licorice)	4
Zizyphi Semen	*Zizyphus jujuba Miller var. inermis*	Dazao	4
Total			62

2. Methods

1) Condensed Tannin Content

The quantitative measurement of the condensed tannin in *Anjang-san* water extract was based on the process used by Broadhurst et al[6]. who used a vanillin method. 10mg of each ingredient was dissolved in 10mL of water and used as test solutions. 0.5mL of test solution was mixed with 1 mL of vanillin-H_2SO_4 solution (1% in 7 M H_2SO_4) and was incubated for 15 minutes at 15℃. Then the optical density was measured at 500 nm and the content of condensed tannin was calculated from standard catechin calibration curve.

2) Effects on Intestinal Transport

(1) Effects on Small Intestinal Transport

Each group of 6 mice fasted for 16 hours. The test solution was administered orally and after 30 minutes, 25% barium sulfate ($BaSO_4$) suspension 0.2ml was administered orally. After 20 minutes, each mouse was sacrificed and split according to the above method and the small intestine was extracted. The movement rate of $BaSO_4$ suspension was calculated using the formula below[7,8]. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

$$Movement\ Rate(\%) = \frac{BaSO_4\ Movement\ Distance}{Distance\ Between\ Pylorus\ and\ Cecum} \times 100$$

(2) Effects on Colonic Transport

Based on Ishii's method[7,8], mice were exposed on a filter paper for 1 hour prior to the administration of test solution, and only those which did not defecate were selected to groups of six. The test solution was administered orally and after 30 minutes, 25% $BaSO_4$ suspension 0.1ml/10g was administered orally. Then the time until the $BaSO_4$ was fecally discharged was measured to determine the effect of the test solution.

The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

(3) Effects on Pyridostigmine-induced Accelerated Small Intestinal Transport

Each group of 6 mice fasted for 16 hours. The test solution was administered orally and after 30 minutes, 25% $BaSO_4$ suspension 0.2ml was administered orally. After 15 minutes, pyridostigmine 750μg/kg was injected subcutaneously. After 20 minutes, each mouse was sacrificed and split according to the above method and the small intestine was extracted. The movement rate of $BaSO_4$ suspension was calculated with the formula above[9,10]. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

(4) Effects on Neostigmine-induced Accelerated Small Intestinal Transport

Each group of 6 mice fasted for 16 hours. The test solution was administered orally and after 30 minutes, 25% $BaSO_4$ suspension 0.2ml was administered orally. After 15 minutes, neostigmine 750μg/kg was injected subcutaneously. After 20 minutes, each mouse was sacrificed and split according to the above method and the small intestine was extracted. The movement rate of $BaSO4$ suspension was calculated as the formula above[9,10]. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

3) Experiments on Antidiarrheal Effects

(1) Castor-Oil Induced Diarrhea

Based on Ishii's method[7,8], mice were exposed on a filter paper for 1 hour prior to the administration of test solution, and only those which did not defecate were selected to groups of six. The test solution was administered orally and after 3 hours, 45% castor oil (solvent: olive oil) 0.1ml/10g was administered orally. The hardness of stool was then

observed for 5 hours and was evaluated according to the 5-score criteria of Miyawaki *et al.*[11]; 1 point for normal stool, 2 for bloated stool with stains inside the filter paper, 3 for soft stool with diffuse stains on the filter paper, 4 for muddy stool, and 5 for mucous stool. Then, the total number of feces was counted. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

(2) Magnesium Sulfate (MgSO₄)-induced Diarrhea

Based on Ishii's method[7,8], mice were exposed on a filter paper for 1 hour prior to the administration of test solution, and only those which did not defecate were selected to groups of six. The test solution was administered orally and after 1 hour, MgSO₄ 2.0g/kg was administered orally. The hardness and total number of feces was measured by the method above. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

(3) Castor Oil-induced Enteropooling

Each group of 6 rats fasted for 18 hours. The test solution was administered orally, and after 60 minutes, castor oil 1ml was administered orally. After 2 hours the animals were sacrificed using ether. Then, the pylorus and cecum of the mice were knotted by strings. The weight of the intestines was measured initially and was measured again after removing the intestinal contents into a measuring cylinder. The differential of the two values was

defined as the weight of the intestinal contents[10,12,13]. The test solution was administered orally at 1,000mg/kg and 2,000mg/kg, and atropine 5.0mg/kg was used for comparison.

4) Statistical Analysis

All results are shown using the mean ± standard deviation. Student's *t*-test was used in comparing the groups, and results were determined to be statistically significant when the p value was less than 5%.

Results

1. Condensed Tannin Content

The regression equation of the standard catechin calibration curve was $y=8.1461x+0.0271$ ($r^2=0.9952$) and showed a satisfactory linear tendency. The amount total tannin in *Anjang-san* water extract derived from the regression equation was $1.47 \pm 0.056\%$.

2. Effects on Intestinal transport

1) Effects on Small Intestinal Transport

After extracting the intestines of the BaSO₄-administered mice as described above, the movement distance of BaSO₄ was calculated to determine the rate of intestinal transport. The test solution 1,000mg/kg and 2,000mg/kg groups showed a slight decrease in BaSO₄ movement rate compared to control, but the differences were not statistically significant. The positive control group using atropine showed significant intestinal transport inhibition ($p<0.001$) (Table 2).

Table 2. Effects of *Anjang-san* on Barium Sulfate Transport in the Small Intestine of Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	Transport Rate (%)	Inhibition (%)
Control	-	6	37.7 ± 3.24[a]	-
Sample	1,000	6	32.2 ± 3.10	16.9
Sample	2,000	6	31.2 ± 1.24	20.8
Atropine	5.0	6	26.0 ± 2.36***	44.7

a) Mean ± Standard error
*: Statistically significant compared with control data (***: $p < 0.001$)

2) Effects on Colonic Transport

Suspension was orally administered to the mice and the time until it was fecally discharged was measured to determine large intestinal transport, and the results are as shown in Table 3. Test solution 1,000mg/kg and 2,000mg/kg groups showed significant inhibitory effects on large intestinal transport (p<0.01 and p<0.01), while the atropine 5.0mg/kg positive control group also showed significant inhibitory effects (Table 3).

3) Effects on Pyridostigmine-induced Accelerated Small Intestinal Transport

The movement rate of $BaSO_4$ suspension in the pyridostigmine 750μg/kg-injected control group was 86.4±5.93%, significantly higher than the 56.2±5.41% of the normal group (p<0.01). Test solution 1,000mg/kg and 2,000mg/kg groups both showed significant inhibitory effects on small intestinal transport compared to the pyridostigmine control group (p<0.05 and p< 0.01). The atropine 5.0mg/kg group also showed significant inhibitory effects (p<0.001) (Table 4).

4) Effects on Neostigmine-induced Accelerated Small Intestinal Transport

The movement rate of suspension in the neostigmine 50μg/kg-injected control group was 61.0±4.35%, significantly higher than the 38.3±3.28% of the normal group (p<0.01). The test solution 2,000mg/kg group showed a significant inhibitory effect on small intestinal transport when compared to the neostigmine group (p<0.05), but the test solution 1,000mg/kg group showed only a statistically insignificant decrease. The atropine 5.0mg/kg group showed a significant inhibitory effect (p<0.05) (Table 5).

3. Antidiarrheal Effects

1) Effects on Castor Oil-Induced Diarrhea

The effects of the test solution on castor oil-induced diarrhea were observed as shown in Table 6. the test solution 1,000mg/kg group showed a slight increase in diarrhea score and the test solution 2,000

Table 3. Effects of *Anjang-san* on Barium Sulfate Transport in the Large Intestine of Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	Transport Time (min)	Inhibition (%)
Control	-	6	204.8 ± 23.4[a]	-
Sample	1,000	6	276.3 ± 19.3[*]	34.9
Sample	2,000	6	284.5 ± 13.2[**]	38.9
Atropine	5.0	6	346.2 ± 42.4[**]	42.5

a) Mean ± Standard error
: Statistically significant compared with control data (: p < 0.05 and **: p < 0.01)

Table 4. Effects of *Anjang-san* on Pyridostigmine-induced Accelerated Barium Sulfate Transport in the Small Intestine of Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	Transport Rate (%)	Therapeutic Effect (%)
Normal	-	6	56.2 ± 5.41[a]	-
Control	-	6	86.4 ± 5.93##	-53.7
Sample	1,000	6	66.1 ± 4.10[*]	67.3
Sample	2,000	6	59.1 ± 4.34[**]	90.5
Atropine	5.0	6	29.6 ± 2.69[***]	188.2

a) Mean ± Standard error
#: Statistically significant compared with normal data (##: p < 0.01)
: Statistically significant compared with control data (: p < 0.05, **: p < 0.01 and ***: p < 0.001)
Therapeutic effects are % of protection that is calculated as 100 (values of pyridostigmine control-values of sample)/(values of pyridostigmine control-values of normal)

Table 5. Effects of Anjang−san on Neostigmine−induced Accelerated Barium Sulfate Transport in the Small Intestine of Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	Transport Rate (%)	Therapeutic Effect (%)
Normal	-	6	$38.3 \pm 3.28^{a)}$	-
Control	-	6	$61.0 \pm 4.35^{\#\#}$	-59.3
Sample	1,000	6	54.5 ± 3.24	28.8
Sample	2,000	6	$51.0 \pm 2.19^{*}$	44.2
Atropine	5.0	6	$43.7 \pm 4.46^{*}$	76.4

a) Mean ± Standard error
#: Statistically significant compared with normal data (##: $p < 0.01$)
: Statistically significant compared with control data (: $p < 0.05$)
Therapeutic effects are % of protection that is calculated as 100 (values of neostigmine control-values of sample)/(values of neostigmine control-values of normal)

mg/kg group showed a slight decrease, but both were statistically insignificant. The atropine 5.0mg/kg group also showed a statistically insignificant decrease.

The total count of feces discharged during 5 hours after castor oil administration showed a statistically insignificant decrease in both the test solution 1,000 mg/kg and 2,000 mg/kg groups, whereas the atropine 5.0mg/kg group showed a significant decrease compared to the control ($p<0.05$) (Table 6).

2) Effects on MgSO4-induced Diarrhea

The effects of the test solution on MgSO4-induced diarrhea were observed and are shown in Table 7. The diarrhea score was significantly lower in both the test solution 1,000mg/kg and 2,000mg/kg groups compared to the control ($p<0.05$ and $p<0.05$). The atropine 5.0mg/kg group also showed a significant inhibitory effect ($p<0.001$).

The total count of feces discharged during 5 hours after MgSO$_4$ administration was significantly lower in the test solution 2,000mg/kg group compared to the control ($p<0.05$), but the test solution 1,000mg/kg group only showed a statistically insignificant decrease. The atropine 5.0mg/kg group showed a statistically significant decrease compared to the control ($p < 0.05$) (Table 7).

3) Effects on Castor Oil-induced Enteropooling

Effects of the test solution on intestinal water retention (enteropooling) induced by castor oil were observed as shown in Table 8. The amount of intestinal water retained in the castor oil and normal saline administered control group was 1.53±0.145g/rat, significantly higher than the 0.44±0.048g/rat of the normal group ($p<0.001$). The test solution 2,000mg/kg group showed a significant inhibitory effect compared to the control ($p<0.05$), but only a statistically insignificant decrease showed in the test solution 1,000mg/kg

Table 6. Effects of *Anjang−san* on Diarrhea Induced by Castor Oil in Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	5h After Castor Oil Administration	
			Score	Mean Number of Feces in 5h
Control	-	6	$5.5 \pm 1.76^{a)}$	12.7 ± 1.76
Sample	1,000	6	$6.5 \pm 2.04\ (-18.2)^{b)}$	$12.0 \pm 1.33\ (5.3)$
Sample	2,000	6	$5.2 \pm 1.40\ (6.1)$	$9.0 \pm 0.75\ (28.9)$
Atropine	5.0	6	$5.2 \pm 0.72\ (6.1)$	$8.7 \pm 0.78^{*}\ (31.6)$

a) Mean ± Standard error
b) Parenthesis value represents inhibitory ratio with control data
: Statistically significant compared with control data (: $p < 0.05$)

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Table 7. Effects of *Anjang-san on* Diarrhea Induced by Magnesium Sulfate in Mice

Groups	Dose (mg/kg, p.o.)	No. of Animals	5h After MgSO4 Administration	
			Score	Mean Number of Feces in 5h
Control	-	6	$7.8 \pm 1.37^{a)}$	11.7 ± 1.12
Sample	1,000	6	$4.8 \pm 0.52^{*}$ (38.3)b)	10.8 ± 1.43 (7.1)
Sample	2,000	6	$3.7 \pm 1.00^{*}$ (53.2)	$9.0 \pm 0.57^{*}$ (22.9)
Atropine	5.0	6	$2.8 \pm 0.44^{***}$ (63.6)	$8.7 \pm 0.88^{*}$ (25.7)

a) Mean ± Standard error
b) Parenthesis value represents inhibitory ratio with control data
: Statistically significant compared with control data (: $p < 0.05$ and ***: $p < 0.001$)

group. The atropine 5.0mg/kg group showed a signifi-cant anti-enteropooling effect compared to the control ($p<0.05$) (Table 8).

Discussion

Diarrhea is a common condition frequently seen in clinical practice. Strict definition of diarrhea is difficult due to the individual differences in defecating habits, but it is generally defined as: frequent defecation more than 3 times a day, loose and formless stool, or an abnormal increase in the total amount and water content of stool. Diarrhea can include gastroin-testinal complications such as fever, abdominal pain, or vomiting. It is related to an imbalance in the regu-lation of absorption and secretion in the intestine, and its causes are diverse[1,14,15].

The mechanisms of diarrhea are explained as the following: ① Hypermotility of the intestine leading to rapid transport of intestinal content and keeping water from being properly absorbed. ② Lesions in intestinal mucosa leading to malabsorption of water. ③ Hypersecretion in the intestinal mucosa leading to hypertonic intestinal contents irritating the mucosa[14].

There are many causes of diarrhea: endotoxins from bacterial infection, viral infection, mucosal damage from food, food intolerance such as lactose intolerance, drugs such as antibiotics and hypertension medication, gastrointestinal disorders such as inflammatory bowel disease, irritable bowel syndrome, etc.[1,14,15].

Severe diarrhea can cause loss of water and minerals, and can result in grave complications such as spasms, central nerve system stimulation, or malnutrition, thus the use of antidiarrheals is important. Antidiarrheals refer to drugs which act on the intestines and stop the diarrhea, and can be classified to antimotility agents, antibiotics, absorbents, astringents, and bacterial replacements[14,15].

In oriental medicine, the mechanisms of diarrhea have been explained in various texts. The Huang Di

Table 8. Effects of Anjang-san on Castor Oil-Induced Small Intestine Enteropooling in Rats

Groups	Dose (mg/kg, p.o.)	No. of Animals	Weight of intestinal fluid (g/rat)	Therapeutic effect (%)
Normal	-	6	$0.44 \pm 0.048^{a)}$	-
Control	-	6	$1.53 \pm 0.145^{\#\#\#}$	247.7
Sample	1,000	6	1.11 ± 0.185	38.5
Sample	2,000	6	$0.99 \pm 0.186^{*}$	49.9
Atropine	5.0	6	$0.92 \pm 0.172^{*}$	56.6

a) Mean ± standard error
#: Statistically significant compared with normal data (###: $p < 0.001$)
: Statistically significant compared with control data (: $p < 0.05$)
Therapeutic effects are % of protection that is calculated as 100 (values of castor oil control-values of sample)/(values of castor oil control-values of normal)

Nei Jing (Yellow Emperor's Inner Canon) stresses excess dampness as an external cause of diarrhea, and Zhang Jingyue stated in Jing Yue Quan Shu (The Complete Work of Zhang Jingyue) that dysfunction of the spleen and stomach play crucial roles as an internal cause of diarrhea, as well as the small and large intestines[16,17]. The therapeutic principles of diarrhea are to treat the root by invigorating the spleen and stomach and boosting *qi* and harmonizing the middle, and to treat the tip by eliminating the dampness in the spleen. If the diarrhea persists for a long time the healthy *qi* easily becomes damaged leading to deficiency-oriented complications, so timely administration of a medicine that can check the diarrhea and also secure the healthy *qi* at the same time to promote restoration is important[2,18,19].

There have been reports on antidiarrheal effects of oriental medicine; Ryu *et al*. on *Yukgunja-Tang* (Liu Jun Zi Tang)[3], and Jang *et al*. on *Onbaek-won*[20], Han *et al*. on *Pyungjin-Tang*[21], etc. There have also been studies on antidiarrheal effects of other prescriptions traditionally used on diarrhea such as *Bobi-Tang*[22], *Samryoungbaekchul-san* (*Shen Ling Bai Shu San*)[23], *Gamigwakjung-tang*[24], *Youryung-tang*[25], *Bojanggunbi-tang*[26], *Siljang-san*[27], *Jungri-tang*[28], *Pyung-wee-san* (*Ping Wei San*)[29], *Jisa-tang*, and on herbal medicinal ingredients like Myristicae Semen, Granati Pericarpium, Alpiniae Katsumadaii Semen[30], Dolichoris Semen and Terminaliae Fructus[5].

Those studies have focused on the validation of oriental medicines previously described in traditional texts to have antidiarrheal effects, but there have been few studies on antidiarrheal effects of new oriental medicinal prescriptions used in practice without being mentioned in traditional texts.

Anjang-san is an experiential prescription created by Ryu and developed at the Oriental Medical Research Center in Kyung Hee University Hospital of Oriental Medicine for antidiarrheal purposes, and is composed of Geranii Herba, Terminaliae Fructus, Dolichoris Semen, Myristicae Semen, Alpiniae Katsu-madaii Semen, Glycyrrhizae Radix, Zizyphi Semen. The prescription is manufactured as granule extracts for better patient compliance.

In oriental medical herbology, Geranii Herba (Lao-guancao) is sour and mildly warm, and can remove wind and dampness, quench heat and detoxify, and unblock the meridian[31,32]. It has been reported to show antidiarrheal effects based on animal studies by exhibiting antibacterial, astringent properties and inhibiting gastrointestinal propellant function[33,34,35,36]. Terminaliae Fructus (Hezi)[37,38] is warm, bitter, sour and astringent, and can constrain the lung and prevent collapse, remove distension, and resolve accumulation. It has been reported to have antidiarrheal effects through antibacterial and astringent properties[39,40]. Dolichoris Semen (Bian dou)[37,38] is mildly warm and sweet, and affects the spleen and the stomach, invigorating the spleen and harmonizing the middle, resolving the heat and dampness. It has been shown to have antidiarrheal properties in animal studies[5]. Alpiniae Katsumadaii Semen (Cao dou kou)[37,38] is warm and pungent, and can resolve the dampness and invigorate the spleen, warm the middle and check vomiting. It has displayed antidiarrheal and antibacterial effects in animal studies[30]. Myristicae Semen (Roudoukou)[37,38] is also warm and pungent, and can astringe the intestines and check diarrhea, warm the middle and move *qi*. There have been studies on its antidiarrheal actions, and it has been proposed that it exhibits antidiarrheal effects by stabilizing intestinal electrolyte levels[41,42].

Tannins are plant-derived compounds, and the term is used to describe various polyphenols which react to and bond strongly with proteins, alkaloids, and metal ions and form poorly soluble salts. Tannins such as epicatechin are astringent and pharmacologically known to have antidiarrheal effects[46,47,48,49].

The medicinal ingredients of *Anjang-san* such as Geranii Herba, Terminaliae Fructus, Myristicae Semen are rich in tannins. Some of the typical tannins found are geraniin, dehydrogeraniin and corilagin in Geranii

Herba and chebulinic and gallic acids in Terminaliae Fructus[43,45,49]. We measured the quantity of condensed tannin in *Anjang-san* as it could be an indicator of antidiarrheal effect. The total amount of tannin in *Anjang-san* water extract was 1.47±0.056%. The fact that medicinal ingredients known to have high tannin contents were shown in this study to have less than normal tannin contents can be attributed to the tannins bonding and forming a poorly soluble precipitation with the soy protein found in Dolichoris Semen.

To investigate the effects of *Anjang-san* on gastrointestinal transport, first we observed the effects on intestinal transport of mice.

Small intestinal transport was calculated by observing the movement distance of orally administered $BaSO_4$ in extracted small intestine of mice. Test solution 1,000mg/kg and 2,000mg/kg groups showed slight suppressive effects of 16.9% and 20.8% respectively compared to control, but the results were not statistically significant. On the other hand, the positive control group using atropine 5.0mg/kg showed significant small intestinal transport suppression.

Large intestinal transport was determined by measuring the time until the orally administered $BaSO_4$ was excreted fecally in mice. Test solution 1,000mg/kg and 2,000mg/kg groups showed significant suppressive effects on large intestinal transport of 34.9% and 38.9% respectively compared to control. The positive control group using atropine 5.0mg/kg also showed significant large intestinal transport suppression.

To examine the effects of *Anjang-san* on abnormally stimulated intestinal movement, we induced abnormal intestinal movement by subcutaneously administering parasympathomimetics neostigmine and pyridostigmine to mice and then observing the changes in small intestinal transport using the same method as above. Neostigmine and pyridostigmine are cholinesterase inhibitors which are known to suppress acetylcholine decomposition and thereby stimulate acetylcholine-induced gastrointestinal movement, increasing small intestinal transport[9,10,50,51].

The pyridostigmine control group displayed a significant promoting effect on small intestinal transport of 53.7% compared to the normal group. Test solution 1,000mg/kg and 2,000mg/kg groups showed significant improvement of 67.3% and 90.5% respectively, and the results were dose-dependent. Especially in the high-dose group, small intestinal transport was almost restored to normal levels.

The neostigmine control group showed a significant promoting effect on small intestinal transport of 59.3%. The test solution 2,000mg/kg group showed a significant 44.2% improvement, but the 1,000mg/kg group showed only a statistically insignificant 28.8% improvement. The atropine positive control group also displayed significant improving effects.

Animal models of diarrhea are constructed by using bacterial toxoids, stimulating gastrointestinal movement, applying stress, etc[52,53,54]. In this study we used a pathologic diarrhea model based on abnormally stimulated gastrointestinal movement. Agents known to promote intestinal movement include castor oil, barium chloride, pilocarpine, serotonin, and magnesium sulfate, and they cause diarrhea by different mechanisms[52,53], but ileal and colonic contraction are especially known to play key roles in inducing diarrhea.

We used castor oil and magnesium sulfate ($MgSO_4$) as diarrhea inducers to determine the antidiarrheal effects of *Anjang-san*. Irritant cathartic agents such as castor oil are said to achieve cathartic effects by irritating the intestinal mucosa, acting selectively on intramucosal nerve plexus, affecting intestinal smooth muscle to promote peristalsis, and changing the electrolyte transportability of intestines[51,55]. Castor oil-induced diarrhea is also known to last longer than most other bowel movement stimulating agents[53]. In this study, we examined the diarrhea score and total number of feces during 5 hours after castor oil administration on mice to determine the antidiarrheal effects of *Anjang-san*. Test solution 1,000mg/kg and 2,000mg/kg groups showed a slight tendency to improve the diarrheal markers but it was not statistically

significant.

Magnesium sulfate (MgSO$_4$) causes myogenic contraction of intestinal smooth muscle to achieve cathartic effects. We examined the diarrhea score and total number of feces during 5 hours after MgSO$_4$ administration on mice.

Test solution 1,000mg/kg and 2,000mg/kg groups showed significant 38.3% and 53.2% improvements of diarrhea score compared to control, and the effect was dose dependent. Test solution 2,000 mg/kg group also showed a significant 22.9% improvement of fecal count compared to control, but 1,000mg/kg group only showed a statistically insignificant improvement.

One of the main causes of diarrhea is water retention in the intestines (enteropooling), and agents such as castor oil and prostaglandin E$_2$ (PGE$_2$) are known to increase enteropooling[12,13,56,57]. In this study, castor oil was administered to prepare a pathologic rat model of abnormal enteropooling. The castor oil control group had a significant 247.3% increase in enteropooling compared to the normal group. The test solution 2,000 mg/kg group showed a significant suppressive effect of 46.9%, but the 1,000 mg/kg group showed only a statistically insignificant decreasing tendency. The atropine 5.0mg/kg positive control group showed a significant improvement of 56.6%.

Based on these results, *Anjang-san* showed significant inhibitory effects on abnormally increased small intestinal transit induced by pyridostigmine and neostigmine, and inhibitory effects on large intestinal transit. *Anjang-san* also showed significant antidiarrheal effects on diarrhea induced by MgSO$_4$, and inhibitory effects on castor oil-induced enteropooling. *Anjang-san* also appeared to improve castor oil-induced diarrhea based on simple numbers but it was not statistically significant. Whether this is because *Anjang-san's* antidiarrheal mechanism does not affect diarrhea induced by irritant cathartics such as castor oil, or the concentration of the *Anjang-san* test solutions used in this study was too low to yield significant result is up to speculation and merits further investigation using different kinds of irritant cathartics and more diverse test solution concentration.

Conclusions

To investigate the antidiarrheal effects of *Anjang-san,* we measured the content of condensed tannin in *Anjang-san* extract, observed the effects of *Anjang-san* on the small intestinal and colonic transport of mice, and on mice models of diarrhea induced by castor oil and MgSO$_4$, and on rat models of castor oil induced enteropooling and came to the following conclusions.

1. The condensed tannin content in *Anjang-san* water extract was 1.47±0.056%.

2. *Anjang-san* showed dose dependent inhibitory effects on pyridostigmine-induced stimulated small intestinal transport of mice, and showed significant inhibitory effects on neostigmine-induced stimulated small intestinal transport only in the 2,000mg/kg dose group, but did not show significant inhibitory effects on normal small intestinal transport.

3. *Anjang-san* showed significant inhibitory effects on normal colonic transport, and the effects were dose-dependent.

4. *Anjang-san* showed significant inhibitory effects on MgSO$_4$-induced diarrhea, but did not show significant inhibitory effects on castor oil-induced diarrhea.

5. *Anjang-san* showed significant inhibitory effects on castor oil-induced enteropooling only in 2,000mg/kg dose group.

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