Effect of Tri-Sa-Maw Recipe on Gastrointestinal Regulation and Motility

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Background: *Tri-sa-maw recipe is comprised of equal proportions of three herbal fruits, including Terminalia chebula Retz., Terminalia sp. and Terminalia bellirica Roxb. The traditional use of this recipe has been reported as a medication for fever, expectorant, relief of tightness in the stomach, laxative and antidiarrheal agent.*
Objective: *To study the effects of Tri-sa-maw recipe extract on gastrointestinal tract in both in vitro and in vivo.*
Material and Method: *Gastrointestinal effect of Tri-sa-maw recipe was studied by using two in vivo models (gastric emptying, gastrointestinal transit) and in vitro isolated guinea pig ileum experiment.*
Results: *Tri-sa-maw recipe showed both stimulatory and inhibitory effects on the stomach function. Not only did the extract at the dose of 1,000 mg/kg inhibit the gastric emptying time, but also stimulate the movement of the digestive tract by increasing the mobility of charcoal. In the isolated guinea pig ileum experiment, the extract at low concentration (0.1 ng/mL) induced the contraction of isolated guinea pig ileum. However, the stimulation effect on contractions of isolated guinea pig ileum was very much decreased at the high concentration (0.2-1 ng/mL) of the extract.*
Conclusion: *The findings of this study support to traditional uses of Tri-sa-maw recipe as a laxative and antidiarrheal agent.*

Keywords: *Tri-sa-maw recipe, Gastric emptying, Intestinal transit*

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Tri-sa-maw recipe is a herbal combination of equal proportions of the three herbal fruits, including *Terminalia chebula* Retz. (Sa-maw-Thai), *Terminalia sp.* (Sa-maw-teh) and *Terminalia bellirica* Roxb. (Sa-maw-phe-phek). These three plants are herbaceous plants in the Combretaceae family[1]. According to Thai traditional medicine, *T. chebula* unripe fruit is used as laxative whereas the ripe fruit as laxative, antidiarrheal, antiemetic agents. The pharmacological activities of *T. chebula* include anti-ulcerogenic[2], spasmogenic[3] and increasing the gastric emptying[4]. *T. bellirica* unripe fruit is often used as carminative, but the mature fruit is for medication of fever and hemorrhoid. Many pharmacological activities of *T. bellirica* has been demonstrated as antispasmodic[5] and anti-ulcerogenic[6]. The fruit of *Terminalia sp.* is also used as a carminative, laxative and antidiarrheal agent.

Tri-sa-maw recipe has long been used in traditional medicine for reducing fever, expectorant, periodic maintenance, and relieving stomach tightness. Nonetheless, the gastrointestinal effects of Tri-sa-maw recipe have never been studied. The aim of the present study is therefore to assess the gastrointestinal effects of Tri-sa-maw recipe using both in vivo and in vitro experiments.

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Material and Method

Preparation of Tri-sa-maw recipe extract

The extracts of *T. chebula*, *T. bellerica* and *Terminalia sp.* were identified by Associate Professor Dr. Noppamas Soonthornchareonnon, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol

University, Bangkok, Thailand. The voucher specimen has been kept at the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The fruits were dried in hot air oven at 50°C, ground into a fine powder and sifted through a sieve (No. 100). Tri-sa-maw recipe was prepared by Thailand Pharmaceutical Company. The quality control of raw materials and the extract was determined according to Thai Herbal Pharmacopoeia including organoleptic examination, % loss on drying, extractive values, total ash and acid insoluble ash[7]. The percent amount of volatile oil, type of chemical constituents in oils (detected by GC/MS), chemical constituents in raw materials and the extract were also analyzed using thin layer chromatography (TLC) following the method of Farnswort[8].

Experimental animals

Male Sprague-Dawley rats (200-250 g), and male Guinea pig (350-400 g) were obtained from the National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. The animals were housed under standard environmental conditions of temperature at 24±1°C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. The experimental protocols were authorized by the Animal Ethics Committee of Faculty of Medicine, Thammasat University, Pathumthani, Thailand (AE 006/2013).

Gastric emptying[9]

Rats were fasted for 16-18 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. The rats were given distilled water, Tri-sa-maw recipe (orally) or Bethanechol (20 mg/kg, ip). Next, all animals were orally fed with 1.5 mL phenol red (1 mg/mL in 1.5% methylcelluose). Thirty minutes later, the rats were sacrificed with ether inhalation and the stomach was removed to collect the gastric content. The absorbance of phenol red in the gastric content was measured by spectrophotometer. The quantity of phenol red was then calculated from standard curve of the concentration and absorbance of phenol red solution. Gastric emptying was calculated as follows:

$$\text{Gastric emptying} = \frac{\text{C standard - C sample}}{\text{C standard}} \times 100$$

Isolated guinea pig ileum experiment[10]

Briefly, Guinea pigs were fasted for 48 h before the experiment. After the animals were sacrificed, the midline incision of the abdomen was made and the ileum was isolated. The ileum was cut into strips of 2 cm long. A piece of ileum was mounted in a bath of 20 mL Tyrode's solution (NaCl = 8.0, KCl = 0.2, $MgCl_2$ = 0.1, $CaCl_2$ = 0.2, NaH_2PO_4 = 0.05, $NaHCO_3$ = 1.0, and glucose = 1 g/L) with a controlled temperature of 37°C and aerated with 95% O_2 and 5% CO_2. Isometric concentrations were recorded under a resting tension of 1 g via a force displacement transducer (FTO3 Grass Instrument Co., Quincy, MA) and displayed on a polygraph (PD7, grass Instrument Co.). After an equilibration period of 30 min, standard contractions produced by acethylcholine (ACh, 20 µg/mL) were recorded. The tissue was then washed out with Tyrode's solution. To test the inhibitory effect, the test substance was added into the organ-bath 3 minutes before the addition of ACh. The tissue was further washed 3-4 times after measuring the contractions at each dose of test substances. Results are expressed as percent of contraction response in isolated Guinea pigs ileum compared with the contraction response of ACh. Moreover, the effect in the isolated-guinea pig ileum by using the atropine pre-treatment was studied.

Small intestinal transit in rats[11]

Rats were fasted 16-18 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. The rats were given distill water, castor oil (4 mL/kg) or Tri-sa-maw recipe (orally). Sixty minutes later, they were orally fed with 0.3 mL of 3% deactivated charcoal (in 0.5% carboxymethylcellulose). Twenty minutes after the deactivated charcoal feeding, the rats were sacrificed with ether inhalation and the gastrointestinal tract was removed. Total length of the small intestine (pylorus to caecum), and the distance of the deactivated charcoal movement were measured. The small intestine transit was calculated and expressed as percentage of the GI transit.

Statistical analysis

Data were reported as mean ± standard error of mean (SEM) and were compared using one-way analysis of variance (ANOVA), followed by Dunnett test. The *p*-values less than 0.05 were considered significant.

Results

In the present study, the % gastric emptying of Tri-sa-maw recipe and bethanechol are shown in Table 1. The % gastric emptying of bethanechol (20 mg/kg) was 70.32, which increased as compared to the control group. The % gastric emptying of Tri-sa-maw

recipe at the doses of 100, 500 and 1,000 mg/kg were 48.67, 38.91 and 32.33, respectively. As shown in Table 2, ACh (20 µg/mL) and Tri-sa-maw recipe (0.1 mg/mL) had the stimulation effect on the contraction of isolated guinea pig ileum. The higher concentration of Tri-sa-maw recipe (0.2-1 mg/mL) caused the inhibitory effect on contractions of isolated guinea pig ileum. Moreover, Tri-sa-maw was further studied in the isolated-guinea pig ileum by using the atropine pre-treatment (12 mg/mL) as shown in Table 3. The inhibition of contractions of isolated guinea pig ileum was more pronounced using atropine with the higher concentration of Tri-sa-maw recipe (0.2-1 mg/mL). In gastrointestinal transit model, Tri-sa-maw recipe at the dose of 1,000 mg/kg increased the % GI transit when compared to that of the control (Table 4).

Discussion

In Thai folk medicine, Tri -sa-maw recipe has been used for the relief of tightness in the stomach.

Table 1. Effects of Tri-sa-maw recipe and bethanechol on gastric emptying in rats

Group	Dose (mg/kg)	Gastric emptying (%)
Control		61.18±7.18
Bethanechol	20	70.32±1.30
Tri-sa-maw recipe	100	48.67±11.40
	500	38.91±14.47
	1,000	32.33±5.61*

Values are expressed as mean ± SEM (n = 6)
* $p<0.05$ compared with control group

Table 2. Effect of Tri-sa-maw recipe on the contraction response in isolated Guinea pigs ileum compared with the contraction response of ACh

Conc. of Tri-sa-maw recipe (mg/mL)	Contraction response in isolated Guinea pigs ileum compared with the contraction response of ACh 20 µg/mL (%)
0.05	24.04±1.31
0.10	78.77±1.80
0.20	55.69±1.49
0.40	41.83±1.96
0.70	35.41±3.91
1.00	27.31±2.03

Values are expressed as mean ± SEM (n = 6)

Table 3. Effect of Tri-sa-maw recipe on the ileum contraction response in atropine-pretreatment compared with the contraction response of ACh

Conc. of Tri-sa-maw recipe (mg/mL)	Contraction response in isolated Guinea pigs ileum compared with the contraction response of ACh 20 µg/mL (%)
0.1	38.27±9.40
0.2	35.58±3.98
0.4	24.29±1.83
0.7	-7.41±0.92

Values are expressed as mean ± SEM (n = 6)
The percent of contraction response of ACh 20 µg/mL was 100%

Table 4. Effects of Tri-sa-maw recipe and castor oil on gastrointestinal transit in rats

Group	Dose	% GI transit
Control		55.22±1.41
Castor oil	4 mL/kg	36.66±3.64*
Tri-sa-maw recipe	100 mg/kg	55.28±0.99
	500 mg/kg	56.48±2.64
	1,000 mg/kg	60.04±1.61*

Values are expressed as mean ± SEM (n = 6)
* $p<0.05$ compared with control group

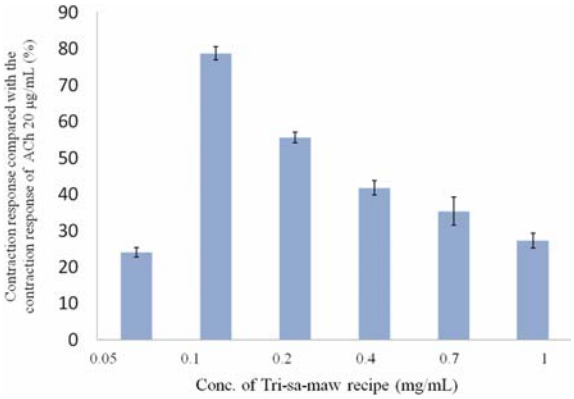

Fig. 1 Effect of Tri-sa-maw recipe on the contraction response in isolated Guinea pigs ileum compared with the contraction response of ACh.

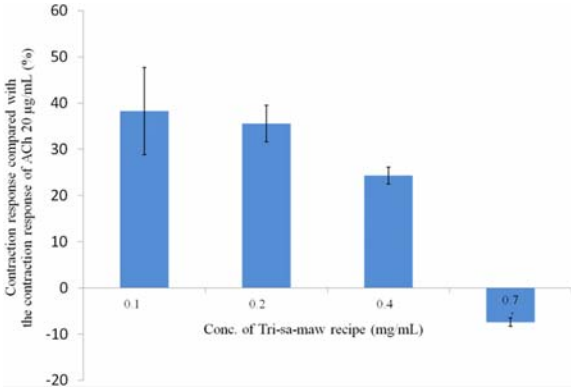

Fig. 2 Effect of Tri-sa-maw recipe on the ileum contraction response in atropine-pretreatment compared with the contraction response of ACh.

The present study is therefore aimed to verifying the gastrointestinal effects of this recipe using three different models; the in vitro (isolate guinea-pig ileum) and the in vivo (small intestinal transit and gastric emptying in rats).

Nerve supply of GI tract has two types, enteric system (myenteric and submucosal plexus) and extrinsic nerves (parasympathetic and sympathetic nerves). The enteric system controls the movement and secretion within the gut. The stimulation of parasympathetic nerves lead to the increase of motility and secretion within the tract and relaxation of the gut sphincters. In contrast, the sympathetic nerves reduced blood flow to the gut, and decreased secretions, motility and contractions[12].

Gastric emptying is a test that measures the time it takes to empty food from the stomach and enter into the small intestine. In this study, the gastric emptying percentages of Tri-sa-maw recipe and bethanechol are shown in Table 1. Bethanechol is a parasympathomimetic choline carbamate that selectively stimulates muscarinic receptor[13]. The % gastric emptying of bethanechol (20 mg/kg) was 70.32, which increased as compared to the control group. This result confirms that the drug is likely to increase the function of the stomach. The % gastric emptying of Tri-sa-maw recipe at the doses of 100, 500 and 1,000 mg/kg were 48.67, 38.91 and 32.33, respectively, suggesting that Tri-sa-maw recipe is likely to decrease the activity of the stomach. Normally, parasympathetic nervous system showed both inhibition and stimulation effects in the stomach. Moreover, reduction of the stomach movement may be the result of hormone gastrin, pentagastrin, secretin, CCK, GIP and VIP.

ACh is a neurotransmitter in the parasympathetic nervous system. It acts on muscarinic and nicotinic cholinergic receptors. This neurotransmitter produces a contractile response in the ileum. As shown in Table 2, ACh (20 µg/mL) and Tri-sa-maw recipe (0.1 mg/mL) had the stimulation effect on the contraction of isolated guinea pig ileum. Interestingly, the higher concentration of Tri-sa-maw recipe (0.2-1 mg/mL) caused the inhibitory effect on contractions of isolated guinea pig ileum. Thus, Tri-sa-maw recipe has a biphasic

effect on either the stimulation or inhibition of contraction of the intestines depending upon doses of the recipe. This effect is likely acting through the muscarinic receptor (cholinergic receptor) as well as ACh and may be the result of the activation of the other receptors. The crude extract of Tri-sa-maw contains various types of substances that are biologically active ingredients, such as flavonoids, hydrolysable tannin, terpenes and saponin. Thus, different substances may act differently on the muscarinic receptor.

Mechanism of action of Tri-sa-maw was further studied in the isolated-guinea pig ileum by using the atropine pre-treatment (12 ng/mL) as shown in Table 3. Atropine is a competitive antagonist for the muscarinic acetylcholine receptor[14]. The effect of atropine on gastrointestinal tract is dramatic on the motility and some of the secretory function of the gut. In the present study, atropine inhibited the stimulation of the contraction of the smooth muscle of ileum by Tri-sa-maw recipe. Moreover, inhibition of contractions of isolated guinea pig ileum was more pronounced using atropine with the higher concentration of Tri-sa-maw recipe (0.2-1 mg/mL). This result indicates that the cholinergic receptor and other receptors may be an important part of Tri-sa-maw's action in regulating bowel function and the small intestine.

The inhibition of ileum contractions, slowing motility and propulsion of intraluminal contents are generally known to take part in anti-transit effect[15]. Gastrointestinal (GI) transit may be coordinated by relaxation of the circular muscle and constriction of the longitudinal muscle[16,17]. A bowel transit time test measures duration for food to travel through the digestive tract. The effects of Tri-sa-maw recipe and castor oil on gastrointestinal transit in rats were shown in Table 4. The castor oil clearly increased the peristalsis activity, decreased the small intestinal transit time and produced a permeability change in the intestinal mucosal membrane to electrolytes and water resulting in a hypersecretory response[18-20]. Moreover, its irritating properties are a cause of the inflammation of intestinal mucosal membrane, leading to the release of prostaglandins, which stimulates the small intestinal movement and water and electrolytes secretion[21,22]. In this experimental model, Tri-sa-maw recipe at the dose of 1,000 mg/kg increased the % GI transit when compared to that of the control. This result suggests that the Tri-sa-maw recipe stimulates the small intestinal movement.

In conclusion, Tri-sa-maw showed the both stimulatory and inhibitory effects on the function of the intestine. These results are correlated to the traditional use for the relief of the stomach tightness, laxative and antidiarrheal. The biphasic action of Tri-sa-maw may be a consequence of a variety of substances present in the crude extract. Moreover, the unknown substances likely affect the muscarinic receptor as well as other receptors.

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Potential conflicts of interest

None.

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ผลของตำรับยาตรีสมอต่อการควบคุมทางเดินอาหารและการเคลื่อนไหว

สุภาพร วรรณศิริ, กาญจนา ใจจ้อย, ณัฏฐกานติ์ จิรัณธนัฐ, นพมาศ สุนทรเจริญนนท์, สีวบูรณ์ สิริรัฐวงศ์

ภูมิหลัง: ตำรับยาตรีสมอประกอบด้วยสัดส่วนที่เท่ากันของผลไม้ที่เป็นสมุนไพร 3 ชนิด ได้แก่ สมอไทย สมอเทศ และสมอพิเภก ตำรับยานี้ใช้เป็นยาลดไข้ ขับเสมหะ ลดอืดแน่นท้องในกระเพาะอาหาร เป็นยาระบาย และแก้ท้องร่วง

วัตถุประสงค์: เพื่อศึกษาผลของสารสกัดตำรับยาตรีสมอในระบบทางเดินอาหารทั้งในหลอดทดลองและในร่างกายสัตว์ทดลอง

วัสดุและวิธีการ: ผลต่อระบบทางเดินอาหารของตำรับยาตรีสมอได้ศึกษาโดยใช้การทดลองในร่างกายของหนูขาว 2 แบบจำลอง (กระเพาะอาหารว่างและการเคลื่อนไหวของลำไส้) และการทดลองในหลอดทดลองโดยใช้ลำไส้เล็ก ส่วนปลายที่แยกออกมาจากหนูตะเภา

ผลการศึกษา: ตำรับยาตรีสมอแสดงให้เห็นว่าผลทั้งกระตุ้นและยับยั้งการทำงานของกระเพาะอาหาร สารสกัดในขนาด 1,000 มิลลิกรัม/กิโลกรัม มีผลลดระยะเวลาในการขนส่งอาหารออกจากกระเพาะอาหาร แต่ยังมีผลเพิ่ม การเคลื่อนที่ของผงถ่าน การทดลองในลำไส้เล็กส่วนปลายของหนูตะเภาที่แยกออกมาพบว่าสารสกัดที่ความเข้มข้นต่ำ (0.1 นาโนกรัม/มิลลิลิตร) ทำให้เกิดการหดตัวของลำไส้เล็กส่วนปลายของหนูตะเภาที่แยกออกมา แต่อย่างไรก็ตามผล ในการกระตุ้นการหดตัวของลำไส้เล็กส่วนปลายของหนูตะเภาจะลดลงอย่างมากที่ความเข้มข้นของสารสกัดที่สูงขึ้น (0.2-1 นาโนกรัม/มิลลิลิตร)

สรุป: ผลการวิจัยสามารถสนับสนุนการใช้ตำรับยาตรีสมอสำหรับใช้เป็นยาระบายและแก้ท้องร่วง