EFFECTS OF PHUONG DONG DAI TRANG TABLETS ON INTESTINAL MOTILITY IN EXPERIMENTAL ANIMALS

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Phuong Dong Dai Trang tablets (PDDT) prepared from several medicinal plants, including Hedychium coronarium, Coix lacryma-jobi, Dioscorea persimilis, Cynara Scolymus L., Paeonia lactiflora, Glochidion eriocarpum, are intended to treat gastrointestinal function disorders. This study was carried out to evaluate the effects of PDDT on intestinal motility in vivo and ex vivo. Swiss mice were divided into four groups that were given orally 0.9% sodium chloride, Duspatalin (mebeverine) 80 mg/kg b.w./day, PDDT 1080 mg/kg b.w./day and PDDT 3240 mg/kg/b.w./day, respectively for 5 days. The activated charcoal movements were measured 20 minutes and 40 minutes after all mice were fed with 0.2 ml of 10% activated charcoal. New Zealand White rabbit's isolated intestinal segments were divided into two groups with two concentrations of PDDT 1350 mg/100 ml Tyrode's solution and 2025 mg/100 ml Tyrode's solution, respectively. The intestine's contractile frequency and amplitude were recorded. Our results showed that both doses of PDDT reduced the intestinal motility in mice and reduced the contractile frequency and amplitude in the rabbit's isolated intestinal segments.

Keywords: Phuong Dong Dai Trang tablets, mice, intestinal motility, rabbits, isolated intestinal segments.

I. INTRODUCTION

The gastrointestinal system is a system of many organs, whose function is to break down food and absorb nutrients for human daily activities. Therefore, any organ dysfunction in this system can affect the body's ability to digest food and absorb nutrients.[1] To date, gastrointestinal diseases have become one of the leading causes of burden and death worldwide.[2] Among them, functional gastrointestinal diseases such as functional dyspepsia, and irritable bowel syndrome are common with varied pathological conditions.[3] These diseases impair the patients' health-related quality of life and the ability to work.[4]

The mechanisms of functional gastrointestinal diseases are mostly related to the gastrointestinal motility disorders. There are several chemical drugs used to treat these diseases. However, they can cause a number of adverse effects, that leads to the need for safer and more effective treatment therapies.[5]

Herbal preparations as complementary and alternative medicine have been used to treat these diseases in many countries, including Vietnam. It is found that herbal preparations are composed of multiple herbal ingredients which contain multiple biologically active compounds on multiple pathophysiological targets.[5]

Phuong Dong Dai Trang tablet (PDDT) is a herbal product which is prepared from herbal ingredients including *Hedychium coronarium*, *Coix lacryma-jobi, Dioscorea persimilis*, *Cynara Scolymus* L., *Paeonia lactiflora*, *Glochidion eriocarpum*. These medicinal plants have been used in traditional medicine to treat gastrointestinal diseases, including functional

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disorders.[6] Some of them were demonstrated to have the effects of reducing intestinal spasms, anti-inflammation, and antioxidants.[6-9] Many factors may contribute to the cause of functional gastrointestinal diseases. The combination of various medicinal plants can provide therapeutic benefits through different mechanisms of action. Therefore, the expected indication of this product is to treat functional gastrointestinal diseases such as irritable bowel syndrome. However, there have been no study on the efficacy of this herbal combination on gastrointestinal disorders. Thus, this study was carried out to provide scientific proof to justify the intended medicinal use of PDDT tablets with two main objectives: 1) Investigate the effects of PDDT on mice' intestinal motility; 2) Evaluate the effects of PDDT on rabbits' isolated intestinal segments.

II. MATERIALS AND METHODS

1. Subjects

The investigational product

Phuong Dong Dai Trang tablet (PDDT) is a herbal product manufactured by Phuong Dong Pharmaceutical and Trading Company. Each tablet contains 750mg of the mixed extract of the following herbal plants: *Hedychium coronarium*, *Coix lacryma-jobi, Dioscorea persimilis , Cynara Scolymus* L., *Paeonia lactiflora, Glochidion eriocarpum.*

Intended human dosage: two tablets, three times a day, 30 - 60 minutes before meal.

Experimental animals

Healthy adult *Swiss mice* (weighed 20 ± 2g) and *New Zealand* rabbits (weighed 2,0 ± 0,2kg) were housed in cages and fed the standard certified diet. They were acclimated to housing one week before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

2. Methods

Effects of PDDT on the intestinal motility in mice

In vivo effect of PDDT on intestinal motility was investigated based on the methods of Dobrescu.[10]

Eighty mice were randomly divided into 4 groups (20 mice per group) and were fasted but allowed to drink water for 20 hours before the experiment. Mice of 4 groups were given orally for 5 days:

- Group 1 (control group): 0.9% sodium chloride 20 ml/kg b.w./day.
- Group 2: Duspatalin (mebeverine) 80 mg/kg b.w./day.
- Group 3: PDDT 1080 mg/kg b.w./day (equivalent to the recommended human dose).
- Group 4: PDDT 3240 mg/kg b.w./day (three times as high as the recommended human dose).

One hour after day 5 treatment, each mice was given 0.2ml of 10% activated charcoal (10g of activated charcoal suspended in 100ml of 3% carboxymethyl cellulose).

Either 20 minutes or 40 minutes after activated charcoal feeding, ten mice of each group were dissected and the intestines were retrieved. The distance of charcoal transited from the pylorus to the end of the black streak (color of activated carbon) was measured. The intestinal motility was calculated as the percentage of the intestinal length with activated charcoal per the entire length of the intestine from pylorus to cecum.

Effects of PDDT on isolated intestinal segments in rabbits

Ex vivo effect of PDDT on intestinal motility was investigated based on the methods of Magnus.[10]

Rabbits were anesthetized by thiopental. The 2cm intestinal segments were retrieved.

After an intraluminal flush of Tyrode's solution, intestinal segments were suspended in an organ bath with aeration and controlled temperature (37°C) for 30 minutes to stabilize the tissue. In this experiment, Tyrode's solution was used to maintain the normal peristalsis of isolated intestinal segments after being removed from the rabbits.

The isolated intestinal segments were divided into two groups (6 segments per group) to evaluate the effects of two PDDT concentrations in Tyrode's solution:

Group 1: were added PDDT at the concentration of 1350 mg/100ml Tyrode's solution (approximately 1.8 tablets/100 ml).

Group 2: were added PDDT at the concentration of PDDT 2025 mg/100ml Tyrode's solution (approximately 2.7 tablets /100ml).

The intestinal segment's contractions were recorded on specialized paper before and after PDDT addition. Then the researcher counted the frequency and used a meter to measure the amplitude. Only one researcher evaluated all records to avoid bias.

Statistical analysis

Data were analyzed by the T-test using Microsoft Excel software version 2010. Data were presented as a mean ± standard deviation. A p-value of less than 0.05 is statically significant.

III. RESULTS

1. Effects of PDDT on mice's intestinal motility

Table 1. Effects of PDDT on the movement of activated charcoal

Group	n	Percentage of the intestinal length with activated charcoal per the entire intestinal length	
		20 minutes	40 minutes
Group 1: control group	20	74.17 ± 10.26	80.50 ± 9.33
Group 2: Duspatalin	20	57.56 ± 8.22**	75.06 ± 7.68
Group 3: PDDT 1080 mg/kg b.w.	20	54.43 ±16.68**	75.23 ± 8.14
Group 4: PDDT 3240 mg/kg b.w.	20	52.17 ± 19.65**	69.42 ± 8.43*

**p < 0.05: compared with the control group, **p < 0.01: compared with the control group, ***p < 0.001: compared with the control group*

#p < 0.05: compared with Duspatalin group 2, ##p < 0.01: compared with Duspatalin group, ###p < 0.001: compared with Duspatalin group

As shown in Table 1:

Twenty minutes after taking activated charcoal: Duspatalin, PDDT at both doses decreased the percentage of the intestinal length with activated charcoal per the entire intestinal length compared with the control group. There was no significant difference between two PDDT groups compared with Duspatalin group.

Forty minutes after taking activated charcoal: PDDT 3240 mg/kg/b.w. decreased the percentage of the intestinal length with activated charcoal per the entire intestinal length compared with the control group.

2. Effects of PDDT on rabbits' isolated intestinal segments

Table 2. Effects of PDDT on the contractile frequency of rabbits' isolated intestinal segments

Group	n	Contractile frequency (numbers of contraction per minute)	
		Before PDDT addition	After PDDT addition
Group 1: PDDT (1350 mg/100 ml)	6	13.17 ± 1.17	10.33 ± 1.03**
Group 2: PDDT (2025 mg/100 ml)	6	12.17 ± 0.75	9.50 ± 0.55***

**p < 0.05: compared with the control group*

***p < 0.01: compared with the control group*

****p < 0.001: compared with the control group*

Table 3. Effects of PDDT on the contractile amplitude of rabbits' isolated intestinal segments

Group	n	Contractile amplitude (mm)	
		Before PDDT addition	After PDDT addition
Group 1: PDDT (1350 mg/100ml)	6	34.67 ± 5.92	18.67 ± 6.38**
Group 2: PDDT (2025 mg/100ml)	6	35.83± 4.79	15.17 ± 0.75***

**p < 0.05: compared with the control group*

***p < 0.01: compared with the control group*

****p < 0.001: compared with the control group*

As shown in Table 2 and Table 3, PDDT at both concentrations (1350mg and 2025mg in 100mL Tyrode's solution) decreased the contractile frequency and amplitude significantly.

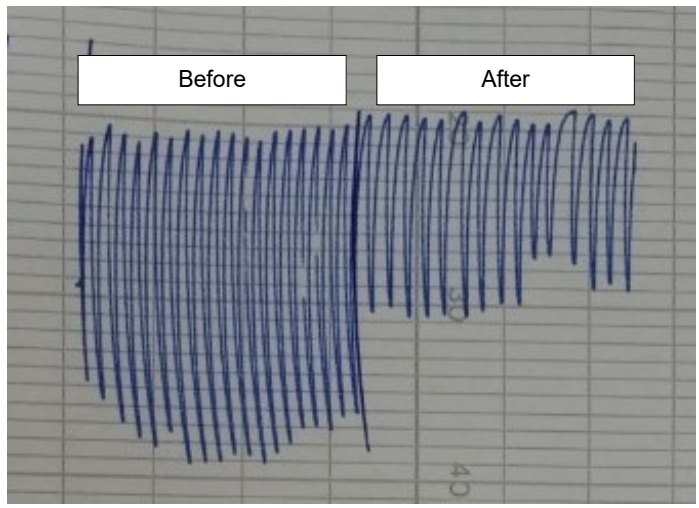

Figure 1. Image of rabbits' intestinal contraction before and after PDDT addition at the concentration of 1350mg in 100ml Tyrode's solution

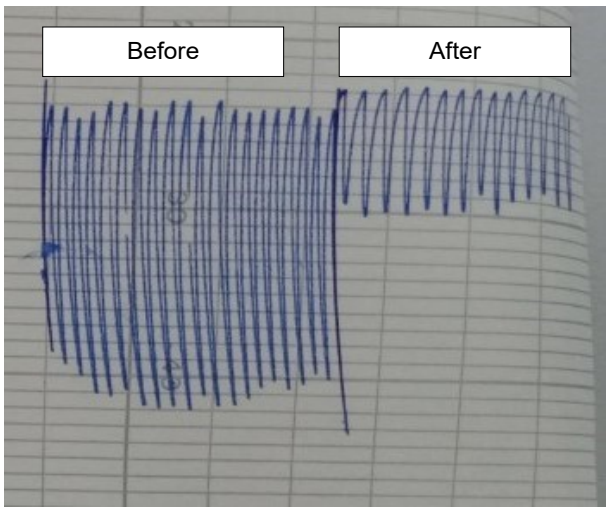

Figure 2. Image of rabbits' intestinal contraction before and after PDDT addition at the concentration of 2025mg in 100ml Tyrode's solution

IV. DISCUSSION

In countries with plentiful resources of herbal plants like Vietnam, medicinal herbs have been used since ancient times to treat diseases, including digestive diseases. PDDT is a combination of medicinal herbs which intended use is to treat gastrointestinal disorders such as irritable bowel syndrome. To initially provide evidence for this use, this study was performed to evaluate the effects of PDDT on intestinal motility *in vivo* and *ex vivo*.

There are three types of movements that take place in the intestines: segmental contractions, peristaltic contractions, and migrating motor complexes. These peristaltic movements stir and mix food with intestinal fluid, increasing absorption efficiency and moving food toward the anus.[1] Assessing each type of intestinal motility separately is complicated, however, it is possible to indirectly assess overall intestinal motility by measuring the movement of food in the digestive tract over a certain period of time. Because it is difficult to accurately determine the distance of food movement, other color indicators, such as activated charcoal, methylene blue, carmine dye, Chinese ink... can be used. Among them, the activated charcoal is the most common indicator becauseits low cost, neutrality, non-absorption and causes no difference on normal or inflamed intestines.[10] For those reasons, the activated charcoal is an appropriate choice for this study to evaluate the intestinal motility. The movement of charcoal depends on many factors such as the concentration of the charcoal, the assessment time after charcoal administration, and digestive motility of the experimental animals.

Mebeverine (brand name Duspatalin) was selected as the positive control drug in our study. It is a synthetic anticholinergic drug that acts directly on gastrointestinal smooth muscle to reduce contraction. The action mechanism of mebeverine is to reduce the ion channel permeability, block noradrenaline reabsorption and alter the water absorption.[11,12]

Our results showed that PDDT decreased the percentage of the intestinal length with activated charcoal per the entire length of the intestine from pylorus to cecum, thus

decreased the activated charcoal movement in the intestines. The effects of PDDT at both doses were not significantly different from Duspatalin 20 minutes after activated charcoal administration. It can be concluded that PDDT 1080 mg and 3240 mg/kg b.w./day might decrease the intestinal motility in mice.

To further evaluate the effect of PDDT, we conducted the research on the rabbit's isolated intestinal segments according to the Magnus method.[10] The contractile frequency and amplitude of the intestines were recorded before and after PDDT addition. In order to prevent the effect of external factors, such as temperature, humidity, moisture, Tyrode's solution was used as the culture for the isolated intestinal segments.

Tyrode's solution is a solution that is isotonic with interstitial fluid and used in physiological experiments and tissue culture. It resembles lactated Ringer's solution, but contains magnesium, glucose as an energy source and uses bicarbonate and phosphate as a buffer instead of lactate. It not only provided the physiologically similar conditions but was also used as the solvent to prepare different concentrations of PDDT in this experiment. Our results showed that PDDT at both concentrations decreased the contractile frequency and amplitude significantly.

Several previous studies showed the antispasmodic effects of some medicinal ingredients in PDDT on smooth muscle of the gastrointestinal tract. The methanolic extract of *Hedychium coccineum* leaves inhibited the intestinal motility and peristalsis in mice.[13] *Dioscorea persimilis* was demonstrated to inhibit the adrenaline-induced contractions on isolated rabbit intestines.[6] *Coix lachryma-jobi* has been used as an antispasmodic agent.[14] To date, there have been few studies evaluating the action mechanisms of herbal ingredients in PDDT on intestines. Thus, further studies are needed to understand the exact mechanism by which PDDT reduces intestinal motility.

It can be concluded from the research that PDDT reduced the intestinal motility both in *vivo* and *ex vivo*. The combination of the medicinal herbs in PDDT has the effect of reducing intestinal motility. Therefore, it has the potential to be developed into a product to treat conditions of increased intestinal motility in patients such as patients with irritable bowel diseases that have diarrhea as the predominant bowel symptom (IBS-D subtype).

The results of this study serve as a premise for further studies to evaluate the effects of PDDT on the experimental models of specific diseases to provide more scientific evidence for its use in patients with gastrointestinal function disorders.

V. CONCLUSION

Our results demonstrated that PDDT at both doses of 1080 mg/kg b.w./day and 3240 mg/kg b.w./day reduced the intestinal motility in *Swiss* mice. Two concentrations of PDDT 1350 mg/100 ml Tyrode's solution and 2025 mg/100 ml Tyrode's solution reduced contractile frequency and amplitude of *New Zealand White* rabbit's isolated intestinal segments.

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