Original Article

Study of the mechanism of *Fomes officinalis* polysaccharides inhibiting the contraction of the isolated duodenum in mice through the myenteric plexus

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Abstract

The objectives of this study were to observe the effect of different concentrations of *Fomes officinalis* polysaccharides (FOPs) on the contractile activity of the isolated duodenal smooth muscle in mice and explore its mechanism. The contractility of the isolated duodenum was recorded by the Medlab biological signal acquisition and processing system before and after administration and the effects of FOPs-treated groups at low, medium and high dose (5, 10 and 20 mg/mL) on the contractile frequency and amplitude of the intestines were observed. Adrenaline hydrochloride (AD) and $CaCl_2$ were selected respectively to be co-incubated with the high dose FOPs to observe the effects on duodenal contraction. The effects of different concentrations of the FOPs on the activities of acetylcholine transferase (ChAT), acetylcholine Esterase (A-CHE), total nitric oxide synthase (TNOS) and the content of Leucine-enkephalin (Leu-enk) in the isolated duodenum of the myenteric plexus in mice were detected by UV-Vis and enzyme-linked immunosorbent assay (ELISA). The results showed that the three FOPs-treated groups had different inhibitory effects on the contractile frequency and amplitude of the isolated duodenum in mice compared with those before administration. The high dose FOPs could synergize (P<0.01) the inhibition effect of AD on the frequency and amplitude of the intestinal contraction and could significantly (P<0.01) inhibit the promotion effect of $CaCl_2$ on it. The effect was equivalent to that of isoprenaline hydrochloride (ISO) or verapamil hydrochloride, respectively. The results of UV-Vis and ELISA showed that compared with the control group, the activity of ChAT and the content of Leu-enk in the three FOPs-treated groups decreased to varying degrees, contrarily, the activities of A-CHE and TNOS significantly increased (P<0.01). All the results suggest that the FOPs can inhibit the contraction of the isolated duodenum in mice, and the mechanism of action is that the FOPs cannot only inhibit the signal transduction pathways of G protein-coupled M receptor-mediated AC-cAMP-PKA and PLC-IP_3- Ca^{2+}, but also the Ca^{2+} signalling systems (such as inhibiting $I_{Ca\text{-}L}$ of the muscle membrane and then inhibiting Ca^{2+}-CaM signalling pathway) through the myenteric plexus. By inhibiting the release of Leu-enk from the motor neurons of the myenteric plexus, the G protein-coupled delta receptor-mediated GTP-cAMP- (PKK or PKC) signalling pathway and Ca^{2+} signalling systems were inhibited. By promoting the release of NOS from the motor neurons of the myenteric plexus, the increased NO was induced, then the enzyme-linked receptor-mediated GC-cGMP-PKG signal transduction pathway was reactivated. The G protein-coupled receptor-mediated AC-cAMP-PKA signal transduction pathway was activated by the myenteric plexus.

Keywords: *Fomes officinalis* polysaccharides, duodenal smooth muscle, G protein-coupled receptor, mechanism, myenteric plexus, Ca^{2+}, neurotransmitters, signal pathways

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Introduction

Gastrointestinal motility disorder often manifests as hyperactivity or hypofunction of the gastrointestinal motility, which causes a variety of gastrointestinal symptoms. The incidence rate of gastrointestinal motility disorder is very high in China, especially when the hyperfunction often leads to an abnormal increase in gastrointestinal gland secretion, which causes suffering in the patient. Gastrointestinal motility diseases are hotspots in gastroenterology and traditional Chinese medicine for gastrointestinal motor function regulation is widely used in clinical practice and the curative effect is definite (Sha *et al*., 2013). The overall concept of "integration of man and nature" and the syndrome differentiation and treatment characteristics of "look, smell, ask and cut" in traditional Chinese medicine have resulted in a prominent advantage in the clinical diagnosis and treatment of gastrointestinal motility disorder (Sha *et al*., 2013).

Fomes officinalis Ames is Phellinuspini, Polyporaceae, Polyporales, Basidiomycetes, which is mainly grown in Western and Northeastern China and is widely distributed in Xinjiang forest areas, especially in Altai. It is not only a common medicine used by Uygur doctors to treat chronic bronchitis, abdominal pain, colds, tuberculosis and various cancers but also a folk prescription by Uyghur, Kazak, and Kirgiz for coughs, asthma and epigastric pain (Sha and Hao, 2019). Additionally, it has been recorded that *Fomes officinalis* has the effect of warming the stomach, resolving phlegm, reducing asthma, eliminating wind and dehumidification, promoting blood circulation, reducing swelling, etc (Sha, 2016). In the early stages, we studied whether or not an aqueous extract of *Fomes officinalis* can affect the contraction of the isolated small intestinal smooth muscle in rabbits and the results showed that an aqueous extract of *Fomes officinalis* could inhibit the contractile activity of the small intestinal smooth muscle (Sha *et al*., 2013). *Fomes officinalis* contains many active components, including polysaccharides, saponins, flavonoids, terpenes, etc. (Feng, 2010), but what it is that plays a role in inhibiting the contraction of the small intestine is not clear. It has been reported that polysaccharides are the main active component of *Fomes officinalis*, which possesses many important pharmacological effects, such as anti-ageing (Abulizi *et al*., 2014), anti-tumor (Guo *et al*., 2010), immune enhancement (Yakum *et al*., 2018), protection from neuron damage (Li *et al*., 2020), etc. Our previous study showed that *Fomes officinalis* polysaccharides (FOPs) also had the effect of anti-fatigue and improving hypoxia tolerance (Sha and Hao, 2019). Therefore, we speculate that the FOPs may be the main component of *Fomes officinalis* that inhibits small intestine contraction. The aim of this research is to investigate the effects of the FOPs on the contractility of isolated duodenal smooth muscle in mice and the mechanism of action and, in addition, the effective components of *Fomes officinalis* in inhibiting gastrointestinal motility and their potential in the treatment of hyperactivity are examined.

Materials and Methods

Animals: Healthy adult Kunming mice (20±2g, n=45 for each sex), were provided by the Animal Experimental Center attached to Xinjiang Medical University (license no. SCXK (Xin) 2011-0004, Urumqi, Xinjiang, China). All mice were housed in conventional cages with free access to standard food and water. The care and use of the mice were approved by the Ethic-Scientific Committee for Experiments on Animals of Chongqing Three Gorges University.

Chemicals and Reagents: *Fomes officinalis* was collected in Koktokay, Fuyun County, Altay District, Xinjiang in August 2012. It was identified by the Institute of Edible Fungi of Tarim University as a medicinal fungus of the Phellinuspini, namely *Fomes officinalis* Ames. Protease inhibitors were purchased from Sigma Chemical Co., USA. Adrenaline hydrochloride (AD), isoprenaline hydrochloride (ISO) and verapamil hydrochloride were obtained from Sinopharm Chemical Reagent Co., Ltd., China. Commercial kits used for the determination of acetylcholine transferase (ChAT), acetylcholine Esterase (A-CHE), total nitric oxide synthase (TNOS) and Coomassie brilliant blue protein were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).The Leucine-enkephalin (Leu-enk) ELISA kit was from Shanghai Jingkang Bioengineering Co., Ltd., China. Locke's solution (KC1 0.42 g, NaC1 9 g, $NaHC0_3$ 0.2 g, $CaC1_2$ 0.24 g, glucose 1.0 g, and distilled water 1000 mL; all reagents used were analytical pure, purchased from Sinopharm Chemical Reagent Co., Ltd., China).

Preparation of the FOPs: The preparation program of FOPs was improved according to our previous research methods (Sha, 2016). The overdried *Fomes officinalis* powders were weighed (passed through a 40 mesh sieve), 6 times the amount of 95% ethanol was added to soak, and they were extracted at reflux at 75°C for 2 hours each time and filtered. The filtered residue was extracted by adding 12 times the amount of distilled water to hot water 3 times, each time for 2 h. The extracts were combined, centrifuged at 4000 rpm for 10 mins with the residue removed, and concentrated. An appropriate amount of 95% ethanol was added to the concentrated solution to make an alcohol concentration of 80% and precipitation was performed. The solution was allowed to stand overnight at 4°C and was filtered. The precipitates were combined for suction filtration and the precipitates were washed with 95% ethanol, anhydrous ethanol, acetone and ether three times each in order and dried to obtain a crude FOPs powder. Dried FOPs coarse powders had the protein removed by Sevag method (the volume ratio of chloroform to n-butanol = 1/5), and were centrifuged at 4000 rpm for 10 mins in a suction filter. The precipitates were washed repeatedly with absolute ethanol, and dried under vacuum at 60°C, obtaining milk brown FOPs. The prepared FOPs (2.00 g) were put into a 100 mL volumetric flask and dissolved in distilled water to obtain the high dose FOPs solution (20mg/mL). Then after two times of dilution, the medium and low doses FOPs solutions of 10 and 5 mg/mL were obtained

respectively. In this study, the concentrations of the FOPs were determined by our previous studies and the pre-experiment. We have previously found that *Fomes officinalis* water extracts of 0.2g/ml can significantly inhibit the contractile activity of the isolated duodenum in rabbits (Sha *et al*., 2013), and the content of polysaccharides in fruit bodies of wild *Fomes officinalis* can reach 6.4%. The FOPs concentrations of 5, 10 and 20 mg/mL have anti-fatigue and anti-hypoxia activity (Sha and Hao, 2019), and through the pre-experiment of the same concentrations, it has also been found that the concentrations of the FOPs have pharmacological activity on the duodenum.

Preparation of isolated mouse duodenal smooth muscle and recording of its contractile activity: After fasting for 24 hours, the mice were sacrificed by cervical dislocation and the upper abdomen was quickly dissected. The duodenal segment close to the pylorus of the stomach was cut off about 2 cm, with the attached adipose tissue being gently peeled away, the contents of intestinal segments were washed with precooled Locke's solution, and then placed in Locke's solution which was bubbled with carbogen at 37℃. After 10 minutes of stabilization, one end of the duodenal segment was connected with a tension transducer and the Medlab biological signal acquisition and processing system (Nanjing Medease Science and Technology Co., Ltd., China), while the other end was attached to an L-shaped hook in the bottom of the test piece of the thermostatic smooth muscle bath (Chengdu Taimeng Software Co., Ltd., China). In the test tube of 18 mL Locke's solution, a piece of cotton thread was adjusted just vertically so that the isolated intestinal section was suspended in the centre of the test tube. The temperature of the thermostatic smooth muscle bath was set to 37℃, and 1~2 bubbles were pumped in every second through the built-in air pump. The contraction curve of intestinal segment was recorded and the experiment was started after 10 minutes of stabilization. The results recorded in the experiment showed that the contraction of the isolated intestinal segments had been completely stabilized at the horizontal baseline during the 10 minute incubation period and the reactivity data (the contraction amplitude and frequency) at the three time points of 3 mins, 6 mins and 9 mins were basically consistent.

In the experiment, one concentration of FOPs was added into the test tube and the effect was observed, then, the test tube with the duodenal segment was rinsed for 2-3 times with Locke's solution at 37℃. After that, another concentration of FOPs was added and the process repeated as above. According to low, medium and high concentrations of FOPs, the effects were successively tested. After each concentration of FOPs had been added into the test tube, it was observed and recorded for 6 mins respectively. The effects of the three FOPs-treated groups on the contractive frequency and amplitude of isolated duodenum in mice (n=9 for each sex) were recorded and the change rates of the contractive frequency and amplitude were calculated. Change rate (%) = (average value after dosing - average value before dosing) /average value before dosing×100%.

In order to explore the mechanism of FOPs' effect on duodenal smooth muscle contraction, the following experiments were designed by the methods described below.

AD induction experiment: AD (0.1μmol/L) was added into the test tube when the duodenal contraction curve of the mice (n=9 for each sex) was stable, and the high dose FOPs was added after 3 minutes of action. During this period, the changes of the isolated duodenal contraction frequency and amplitude were observed and recorded. The ISO (0.1μmol/L) was given as a positive control.

$CaCl_2$ induction experiment: When the duodenal contraction curve of the mice (n=9 for each sex) was stable, $CaCl_2$ (0.1μmol/L) was added into the test tube, and the high dose FOPs was added after 3 minutes of action, and the changes in the contraction frequency and amplitude of the isolated duodenum in the mice were observed and recorded. Verapamil hydrochloride (0.1μmol/L) was used as a positive control.

Determination of ChAT、A-CHE、TNOS activities: A total of 36 mice (n=18 for each sex) were randomly divided into 4 groups, i.e. the control group, FOPs-treated groups of the low, medium, and high dose, 9 in each group. The isolated duodenal smooth muscle samples of mice in each group were prepared and bathed with Locke's solution, low, medium and high doses FOPs respectively. After 6 minutes, the intestinal segments were smashed and placed in 9-fold precooled normal saline, homogenized for 10 mins in an ice water bath to obtain a 10% tissue homogenate, centrifuged at 3000 rpm for 10 mins. The activities of ChAT, A-CHE, TNOS and the content of protein in the supernatants were detected according to the respective detection kits by UV-Vis (Shanghai Lengguang Technology Co., Ltd., China).

Preparation of the duodenal tissues in enzyme-linked immunosorbent assay (ELISA): The isolated duodenum segments in each group were washed with precooled PBS (0.01M, pH = 7.4), then the tissues were cut up after weighing. The cut tissues and PBS of corresponding volume (according to the weight volume ratio of 1:9, it was recommended to add protease inhibitor into PBS) were added into the glass homogenizer, which was grounded on ice. Finally, the homogenate was centrifuged at 5000 rpm for 8 mins and the supernatant was detected.

ELISA: The reaction program of ELISA was carried out according to the Leu-enk ELISA kit instruction. Finally, 50 μl termination solution was added into each hole of the 96 well microtiter plate for 15 min, and the OD value was measured at the wavelength of 450 nm using an iMark Microplate Reader (Bio-Rad, USA). Then, the OD value was substituted into the linear regression equation, and the Leu-enk concentration of the sample was calculated.

Statistical Analysis: SPSS19.0 statistical software (SPSS Inc.) was used for experimental data processing. The results were expressed as the mean plus or minus

standard deviation (SD). Paired t test was used to compare the differences between the two groups before and after administration. One-way ANOVA was used to compare the differences between the experimental and control groups and Dunnett's t-test for further comparison among groups. Differences were regarded as significant at $P<0.05$; extremely significant at $P<0.01$.

Results

Effects of the FOPs on the contractile frequency of the isolated duodenum in mice: Compared with those before administration, the three FOPs-treated groups (5, 10, 20 mg. ml^{-1}) all had an inhibitory effect on the contraction frequency of the isolated duodenum in the mice and the inhibitory rate (8.51%, 13.57% and 24.58% individually) gradually increased with the increase of polysaccharides concentration, which showed a significant dose-dependent effect (Table 1, Figure 1). The difference was significant in the low dose group ($P<0.05$) and very significant in the medium and high dose groups ($P<0.01$).

Effects of the FOPs on the contractile amplitude of the isolated duodenum in mice: As shown in Table 2 and Figure 1, compared with those before administration, the three FOPs-treated groups (5, 10, 20 mg. ml^{-1}) could significantly reduce the contraction amplitude of the isolated duodenum in mice ($P<0.01$) and the inhibition rate (21.27%, 25.00% and 30.49% individually) gradually enhanced with the increase of polysaccharides dose, which showed a significant dose-dependent effect.

Effects of the FOPs on the frequency and amplitude of contraction of the isolated mouse duodenum induced by AD: AD (0.1μmol/L) alone significantly reduced the frequency (11.26±1.05) and amplitude (1.57±0.18) of the isolated duodenal contraction in mice compared with the one before administration ($P<0.01$). After adding the FOPs (20mg/mL), the contractile frequency (9.63±0.24) and amplitude (1.02±0.10) were further reduced, the magnitude of the effect was comparable to ISO (9.35±0.28 and 0.90±0.11, respectively), and the difference was very significant compared with AD ($P<0.01$) (Table 3, Figure 2).

Effects of the FOPs on the frequency and amplitude of contraction of the isolated mouse duodenum induced by $CaCl_2$: Compared with that before administration, $CaCl_2$ (0.1μmol/L) alone significantly increased the frequency (16.60±1.04) and amplitude (5.72±0.65) of the isolated duodenal contraction in mice ($P<0.01$). After the addition of the high dose FOPs (20mg/mL), the contraction frequency (13.78±0.63) and amplitude (2.85±0.26) were very significantly reduced ($P<0.01$) and the effect was equivalent to that of verapamil hydrochloride (Table 4, Figure 3).

Effects of the FOPs on the activities of ChAT, A-CHE, TNOS and the content of Leu-enk in the isolated duodenum of mice: The ChAT activity and the Leu-enk content of the three FOPs-treated groups (5, 10, 20 mg. ml^{-1}) were lower than those of the control group, among which the differences were very significant in the medium (10 mg. ml^{-1}) and high (20 mg. ml^{-1}) dose groups ($P<0.01$), while significant in the low (5 mg. ml^{-1}) dose group ($P<0.05$). The activities of A-CHE and TNOS in the three FOPs-treated groups were significantly higher than those in the control group ($P<0.01$), which were significantly dose-dependent (Table 5).

Table 1 Effects of the FOPs on the contractile frequency of the isolated duodenum in mice ($\bar{x}$ ±SD, n=9)

Group	Concentrations／mg. ml^{-1}	Contraction frequency（times min^{-1}）		Inhibition rate(%)
		Before administration	After administration	
Low	5	13.52±0.65	12.37±0.74*	8.51
Intermediate	10	13.63±0.74	11.78±0.42**	13.57
High	20	13.59±0.63	10.25±0. 68**	24.58

Note: compared with before administration,**$P<0.01$, * $P<0.05$.

Table 2 Effects of the FOPs on the contractile amplitude of the isolated duodenum in mice（ $\bar{x}$ ±SD, n=9）

Group	Concentrations／mg. ml^{-1}	Contractile amplitude（g）		Inhibition rate(%)
		Before administration	After administration	
Low	5	2.21±0.23	1.74±0.19**	21.27
Intermediate	10	2.24±0.34	1.68±0.18**	25.00
High	20	2.23±0.30	1.55±0.21**	30.49

Note: compared with before administration,**$P<0.01$.

Table 3 Effects of the FOPs on the frequency and amplitude of contraction of the isolated mouse duodenum induced by AD ($\bar{x}$±SD, n=9)

	Before administration	AD	High FOPs	ISO positive control
Contraction frequency (times·min^{-1})	13.57±0.61	11.26±1.05**	9.63±0.24$^{\#\#}$	9.35±0.28$^{\#\#}$
Contractile amplitude (g)	2.20±0.29	1.57±0.18**	1.02±0.10$^{\#\#}$	0.90±0.11$^{\#\#}$

AD: Adrenaline hydrochloride; ISO: Isoprenaline hydrochloride.
Note: **indicates comparison before and after administration, P<0.01; $^{\#\#}$ Compared with the intervention of AD, P<0.01.

Table 4 Effects of the FOPs on the frequency and amplitude of contraction of the isolated mouse duodenum induced by $CaCl_2$ ($\bar{x}$±SD, n=9)

	Before administration	$CaCl_2$	High FOPs	Verapamil hydrochloride positive control
Contraction frequency (times·min^{-1})	13.53±0.58	16.60±1.04**	13.78±0.63$^{\#\#}$	13.41±0.67$^{\#\#}$
Contractile amplitude (g)	2.21±0.27	5.72±0.65**	2.85±0.26$^{\#\#}$	2.45±0.19$^{\#\#}$

Note: **indicates comparison before and after administration, P<0.01; $^{\#\#}$ Compared with the intervention of $CaCl_2$, $^{\#\#}P$<0.01.

Table 5 Effects of the FOPs on the activities of ChAT, A-CHE, TNOS and the content of Leu-enk in the isolated duodenum of mice ($\bar{x}$±SD, n=9)

Group	Concentrations /mg. ml^{-1}	ChAT／(U/mL)	A-CHE／(U/mL)	TNOS／(U/mL)	Leu-enk／(pg. ml^{-1})
Control group	—	1.83±0.12	0.09±0.01	0.44±0.06	7.43±0.81
Low FOPs	5	1.62±0.11*	0.16±0. 02**	0.87±0.09**	6.40±0.57*
Intermediate FOPs	10	0.94±0.08**	0.37±0.04**	1.83±0.21**	5.36±0.52**
High FOPs	20	0.52±0.04**	0.62±0.07**	2.35±0.45**	3.79±0.38**

A-CHE: acetylcholinesterase; ChAT: acetylcholine transferase; TNOS: total nitric oxide synthetase; Leu-enk: Leucine-enkephalin.
Note: Compared with the control groups. $^{*}P$<0.05, shows significant difference. $^{**}P$<0.01, shows extremely significant difference.

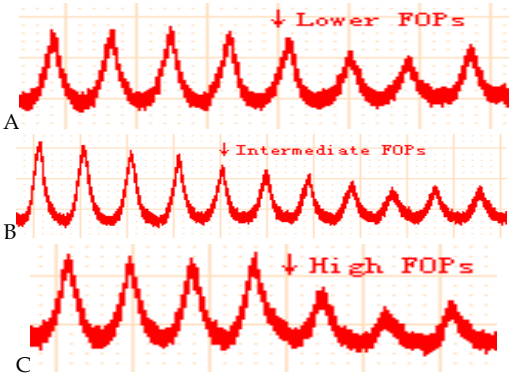

Figure 1 Effects of FOPs on the contraction of the isolated duodenum in mice

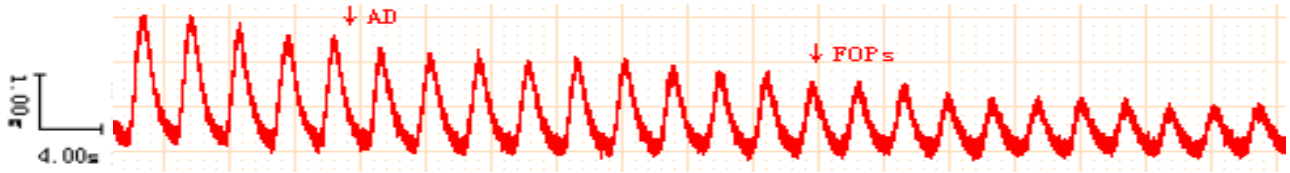

Figure 2 Effects of FOPs on the contraction of the isolated duodenum in mice by AD

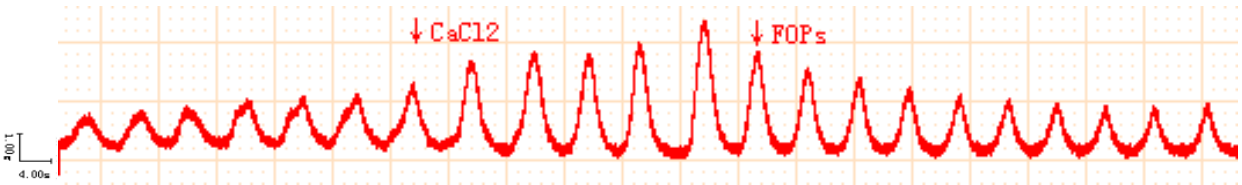

Figure 3 Effects of FOPs on the contraction of the isolated duodenum in mice by $CaCl_2$

Discussion

The reasons for choosing the duodenum in this study include: Firstly, studies have shown that there are differences in the effects of different small intestinal segments on various chemical drugs. Generally, the duodenum has the strongest effect, the jejunum takes second place, and the ileum is the weakest (Sha *et al*., 2013), which is mainly related to the different distribution of nerve density in different segments of the small intestine. The upper part of the small intestine is more than the lower part of the small intestine (Zhang and Lu, 1997). Therefore, the duodenum was selected in this study as being more conducive to study the mechanism of intestinal myenteric plexus inhibiting the contraction of the isolated duodenum in mice. Moreover, the duodenum is a part of the small intestine and the contraction frequency is the fastest, so it is easy to record the contraction curve (Wang, 2018). Secondly, the contents of the duodenum and the pancreatic juices have been washed with precooled Locke's solution, so the influence of the digestive juice can be excluded.

Duodenal activity is influenced by neurological and humoral factors. The nerve factors include the regulation of the external nerves (sympathetic and parasympathetic) and the enteric nervous system (Wang *et al*., 2019). Although the external nerves were lost in the isolated duodenum of the mice, the enteric nervous system still existed (Sanders, 1996). The smooth muscle movement of the duodenum is mainly regulated and controlled by the myenteric plexus (Furness, 2016). The neurons that make up the myenteric plexus regulate the duodenal movement by releasing different neurotransmitters. These neurotransmitters can be divided into two categories. Excitatory neurotransmitters such as ACh, enkephalin (Enk) and substance P can promote the duodenal movement (Wang et al., 2019), and inhibitory neurotransmitters such as adrenaline, nitric oxide (NO) and somatostatin have inhibition (Furness, 2016).

Ach is an excitatory neurotransmitter of the duodenal movement. When binding with the G protein-coupled M receptor on the intestinal muscle membrane, Ach activates adenylate cyclase (AC), which increases the concentration of cAMP in the cell, activates protein kinase A (PKA) and then activates $I_{Ca\text{-}L}$ to increase the extracellular Ca^{2+} influx (Huang et al., 2019). Ach can also activate G protein subtype-coupled receptors when binding to the M receptor, and then activate PLC to generate IP_3 and diacylglycerol (DG) and then activate the IP_3 receptor in the sarcoplasmic reticulum. Ca^{2+} is released into the cytoplasm, resulting in the enhancement of the duodenal smooth muscle contraction (Wallace and Li, 2013). ChAT can catalyse the synthesis of Ach by acetyl-CoA and choline in vivo (Wang et al., 2019), while A-CHE can hydrolyse Ach. The results show that the three FOPs-treated groups could reduce ChAT activity in mice, significantly increase the A-CHE activity (P<0.01), the FOPs can significantly (P<0.01) inhibit the promotion of $CaCl_2$ on the frequency and amplitude of the intestinal contraction, and the inhibition effect was similar to that of verapamil hydrochloride, which has been a commonly used $I_{Ca\text{-}L}$ blocker. Therefore, it showed that the FOPs can inhibit the contraction of the isolated duodenal smooth muscle by inhibiting the synthesis of the cholinergic neurotransmitter ACh and promoting the decomposition of the released Ach, thus not only inhibiting the signal transduction pathways of AC-cAMP-PKA and PLC-IP_3-Ca^{2+} but also the Ca^{2+} signal systems (such as inhibiting $I_{Ca\text{-}L}$ of the muscle membrane and then inhibiting Ca^{2+}-CaM signalling pathway) mediated by G protein-coupled M receptors.

Enk is also an excitatory neurotransmitter of duodenal movement, which belongs to the family of endogenous opioid neuropeptides, mainly including Leu-enk and Met-enk. Our previous study has shown that ENK is an endogenous ligand of the delta opioid receptor (Sha et al., 2013). By binding to delta receptors coupled with the G protein, ENK can activate GTP binding protein (Chakrabarti et al., 1998) on the intestinal muscle membrane, increase intracellular cAMP concentration, activate protein kinase K (PKK) and Ca^{2+} dependent protein kinase C (PKC) (Heagy et al., 1999) and then activate Ca^{2+} signal systems (Martin and Gabrilovac, 1999), resulting in increased contraction of the duodenal smooth muscle. In the ELISA experiment, the Leu-enk content in the isolated duodenal myenteric plexus of the mice in the three FOPs-treated groups was reduced in varying degrees, which indicated that the FOPs could inhibit the release of Leu-enk by inhibiting the motor neurons in the myenteric plexus, thereby inhibiting the GTP-cAMP-(PKK or PKC) signal pathway and Ca^{2+} signal systems mediated by G protein- coupled delta receptor, thus inhibiting the isolated duodenal contraction.

NOS is the synthetase of NO. NO can activate GC by binding with Fe^{2+} in hemoglobin, increase the concentration of cGMP (Ignarro et al., 1982), activate PKG, cause the phosphorylation of related proteins, reduce the Ca^{2+} concentration in cytoplasm and inhibit the contraction of the duodenal smooth muscle (Melamed et al., 1976). The results show that the three FOPs-treated groups could significantly increase the activity of TNOS in mice (P<0.01), which indicated that the FOPs can promote the synthesis of gas neurotransmitter NO by promoting the release of NOS from motoneurons in the myenteric plexus, thus activating the GC-cGMP-PKG signal transduction pathway mediated by the enzyme-linked receptor, leading to a weakening in the contraction of the isolated duodenal smooth muscle.

AD and ISO are both adrenergic beta receptor agonists, which can relax the duodenal smooth muscle by activating the G protein-coupled beta receptor-mediated AC-cAMP-PKA signal transduction pathway (Li et al., 2015). In this study, firstly AD activated the beta-adrenoceptor, and then added the FOPs, in order to observe whether the FOPs can further affect the beta receptor. The known ISO was used as the positive control drug to compare with the FOPs. The results show that the FOPs can synergize (P<0.01) the inhibition of AD on the frequency and amplitude of the intestinal contraction, and the magnitude of the effect was comparable to ISO, indicating that the FOPs may have an exciting effect on beta receptors, which may relax the isolated duodenal smooth muscle by activating G protein-coupled receptor-mediated AC-cAMP-PKA signal transduction pathway. However,

the specific mechanism needs to be confirmed by further research. For example, in order to determine whether the beta receptor is involved or not in the FOPs action, we can block the receptor and verify the effect of the FOPs in the future.

Based on the results of this study, it can be seen that polysaccharides are the effective components of *Fomes officinalis* in inhibiting duodenal movement and the mechanism of action is that the FOPs can not only inhibit the signal transduction pathways of G protein-coupled M receptor-mediated AC-cAMP-PKA and PLC-IP_3- Ca^{2+}, but also the Ca^{2+} signalling systems (such as inhibiting I_{Ca-L} of the muscle membrane and then inhibiting Ca^{2+}-CaM signalling pathway) through the myenteric plexus. By inhibiting the release of Leu-enk from the motor neurons of the myenteric plexus, the G protein-coupled delta receptor-mediated GTP-cAMP- (PKK or PKC) signalling pathway and Ca^{2+} signalling systems were inhibited. By promoting the release of NOS from the motor neurons of the myenteric plexus, the increased NO was induced, then the enzyme-linked receptor-mediated GC-cGMP- PKG signal transduction pathway was reactivated. The G protein-coupled receptor-mediated AC-cAMP-PKA signal transduction pathway was activated by the myenteric plexus. In addition, this may be related to the decrease in the number of transverse bridges involved in the cycle swing, the inhibition of myosin ATPase activity and the chloride or potassium channels on the sarcolemma.

In conclusion, FOPs can inhibit the contraction of the isolated duodenum in mice through the myenteric plexus. It can be concluded that the FOPs can inhibit gastrointestinal spasm, relieve gastrointestinal pain and resist the severe peristalsis of injured intestines and is effective in the treatment of gastrointestinal motility diseases such as diarrhea, abdominal pain and gastrointestinal spasm. This study provided a theoretical basis for the development of the FOPs in the treatment of abdominal pain, diarrhea, increased gas production and other clinical diseases caused by gastrointestinal motility and also increased the medicinal and feeding value of *Fomes officinalis*.

Ethical statement: All applicable international, national and institutional guidelines for the care and use of animals were followed. All of the protocols on living animals used in this paper came from the Animal Experimental Center of Xinjiang Medical University (license no. SCXK (Xin) 2003-0001). Moreover, every effort was made to minimize the suffering of the Mice.

Declaration of interest: The authors have declared that there are no conflicts of interest.

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