Effects of Rhizoma Parisdis total saponins and its main compounds on gastric emptying via regulating muscarinic receptors in vitro and in vivo

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The aim of this study was to explore the inhibitory effect of Rhizoma Parisdis total Saponins (RPS) and the main monomer compounds (polyphyllin I, II, VI and H) on gastric emptying and gastrointestinal motility in vitro and in vivo. The in vivo experiments demonstrated that mosapride (2.25 mg kg\(^{-1}\)) and neostigmine (0.1 mg kg\(^{-1}\)) could promote gastric emptying, while RPS (250 and 500 mg kg\(^{-1}\)), atropine (0.3 mg kg\(^{-1}\)), atropine (2 mg kg\(^{-1}\)) and dopamine (1 mg kg\(^{-1}\)) inhibited gastric emptying. Neostigmine markedly enhanced the delayed gastric motility induced by RPS in mice. The delaying effect of RPS was abolished by atropine and dopamine treatments but not atropine. RPS reduced gastric emptying by several pathways, which involved regulating muscarinic receptors. From the in vitro experiments, we found that RPS and the main monomer compounds (polyphyllin I, II, VI and H) (20–160 \(\mu\)g ml\(^{-1}\)) concentration-dependently inhibited the contractions in the antral circular strip compared to untreated controls. Besides, RPS and polyphyllin I, II, VI and H partly inhibited the stimulatory effect of acetylcholine (10 \(\mu\)M) but RPS-induced relaxation was significantly reduced by pretreatment with atropine (10 \(\mu\)M) on gastric antral smooth muscle contractility (GASMC). In addition, we also found that polyphyllin I and II had a stronger inhibitory effect on GASMC than that of polyphyllin VI and H. The experiment indicated that RPS could inhibit the gastric emptying, with polyphyllin I, II, VI and H being the major active ingredients. Meanwhile, the inhibition of gastric emptying and contractions of the antral circular strip by RPS predominantly involves muscarinic receptors.

Introduction

Among the traditional systems of medicine, traditional Chinese medicine (TCM) is the most extraordinary one with unique theory and thousands of years of practice in promoting people’s health and alleviating diseases. As treatments for diseases, herbal medicines have advantages in reducing side effects, promoting the immune system, and prolonging survival time of people with cancer. In recent years, steroidal saponins isolated from herbs have attracted scientific attention because of their structural diversity and significant biological activities. Rhizoma Paridis total Saponins (RPS) as the active parts of P. polyphylla Smith var. yunnanensis has been used as antitumor and hemostatic agents in China for a long time. In previous work, RPS was identified as the main effective components of Rhizoma Paridis. We investigated the in vitro antitumor effect and the safety evaluation of RPS, and found that it showed good cytotoxicity and adverse effects on the gastric area. However, the effects of RPS on gastrointestinal function have not been reported in detail.

According to the research on the pharmacological activities of P. polyphylla Smith var. yunnanensis, saponins in P. polyphylla Smith var. yunnanensis are both active constituents and toxic constituents. In fact, there are reports showing that ginseng saponins, which are isolated from the ginseng root, have effects on reducing acetylcholine. Acetylcholine (ACH), an important neurotransmitter on regulating the gastrointestinal motility, can be combined with the acetylcholine receptors on gastrointestinal smooth muscle cells, then promote gut mobility via the stimulation of fast excitatory synaptic transmission. In most parts of the gastrointestinal tract, acetylcholine (ACH) is the important neurotransmitter which initiate...
excitatory events in response to mechanical or chemical stimuli.\textsuperscript{5,16} Cholinergic antagonism is a conventional approach to produce antispasmodic effects for inhibiting gastric emptying.

Our previous work has demonstrated that RPS inhibited gastric emptying in vivo\textsuperscript{17} while it is unknown which constituents or pure compounds serve as stimulators or as inhibitors in the RPS. The aim of this study is to examine the activity of RPS and the main monomer compounds (polyphyllin I, II, VI and H) on gastric emptying and gastrointestinal motility in vitro and in vivo. Gastric emptying is closely associated with smooth muscle contraction, and antral smooth muscle layer in the stomach is the thickest which may perform a key role in gastric emptying. Thus we investigate the effect of RPS on isolated antral smooth muscle motility. Furthermore, some antagonists and agonists, such as acetylcholine, adrenalin hydrochloride, atropine, dopamine, mosapride citrate and neostigmine, are used to identify the possible pathways of RPS-induced inhibitory effect on gastric emptying. Study on these issues not only offers better guidance for the clinical application of RPS but also provides foundation for new drug discovery.

Materials and methods

Drugs and solution

RPS was prepared by the previously reported method.\textsuperscript{3} Other drugs used in this study were acetylcholine, adrenalin hydrochloride (Kunning Pharmaceutical Group Co., Ltd., China), atropine, dopamine, mosapride citrate (Lunanbeite Pharmaceutical co., Ltd., China) and neostigmine (Qilu Pharmaceutical co., Ltd., China). Polyphyllin I (PubChem CID: 44429646), polyphyllin II (PubChem CID: 72960700), polyphyllin VI (PubChem CID: 71307571) and polyphyllin H (PubChem CID: 101615586) were purchased from Chinese food and Drug Inspection Institute. For oral administration, RPS was suspended in distilled water. For intraperitoneal (ip) injection, acetylcholine, adrenalin hydrochloride and so on, were dissolved in physiological saline. The Krebs solution used in this study contained (in millimolar): NaCl, 119; KCl, 4.7; CaCl\textsubscript{2}, 2.5; MgSO\textsubscript{4}.7H\textsubscript{2}O, 1.2; NaHCO\textsubscript{3}, 25; NaH\textsubscript{2}PO\textsubscript{4}, 1.2; glucose, 11, pH = 7.4. The Krebs solution was bubbled continuously with a mixture of 95\% O\textsubscript{2}/5\% CO\textsubscript{2} (vol/vol).

Animals

Kunning mice (weighing 18 to 22 g for females) and wistar rats (weighing 180 to 220 g for female) were purchased from the Institute of Tianjin Laboratory Animal Center, Tianjin. Mice were kept at 25 ± 1 °C under a 12 hours light/dark cycle condition, while freely access to food (standard pellet diet) and water ad libitum. All experimental protocols were approved by the Animal Ethics Committees of the Faculty of Medicine, Tianjin University, Tianjin, China, and carried out in accordance with “Principles of Laboratory Animal Care and Use in Research” (State Council of China, 1988).

Analysis of the RPS by HPLC-ELSD

The method used in this experiment was same to the previous reported.\textsuperscript{16} The chemical composition of RPS were analyzed by HPLC (Agilent 1100, USA) equipped with a Kromasil RP-C18 column (4.6 × 250 mm, 5 μm, Kromasil C18). The analytical column temperature was kept at 35 °C. Acetonitrile (A) and water (B) under gradient conditions (0–5 min, 33–36\% A; 5–12 min, 36–45\% A; 12–18 min, 45–50\% A; 18–42 min, 50–43\% A; 42–45 min, 43–55\% A; 45–70 min, 55–100\% A) was the mobile phase at a flow rate of 1 ml min\textsuperscript{-1}. The injection volume was 20 μL. The drift tube temperature for ELSD was set at 110 °C, and the nebulizing gas flow rate was 2.9 L min\textsuperscript{-1}.

Measurement of gastric emptying and small intestinal transit

After 16 h of food deprivation, RPS (250 or 500 mg kg\textsuperscript{-1}), mosapride citrate (2.25 mg kg\textsuperscript{-1}), or distilled water was administered orally to Kunming mice. Adrenalin hydrochloride (0.3 mg kg\textsuperscript{-1}, subcutaneous injection, sc), atropine (2 mg kg\textsuperscript{-1}, intraperitoneal injection, ip), dopamine (1 mg kg\textsuperscript{-1}, intraperitoneal injection, ip), or neostigmine (0.1 mg kg\textsuperscript{-1}, intraperitoneal injection, ip) was injected 40 min later, and phenol red meal (1.5\%, 0.3 ml) was administered orally 5 min after the dopamine (ip) injection, 15 min after the adrenalin, atropine, neostigmine, or 20 min after mosapride citrate. All the animals were euthanized by cervical dislocation immediately 20 min after phenol red. Total stomach was removed after cardiac and pylorus ligated. The stomach was cut into pieces and homogenized with its contents in 25 ml of 0.1 N NaOH. The homogenate was allowed to settle for 1 h at room temperature, and 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic acid to precipitate proteins. A 8 ml of the supernatant was added to 1 ml of 33\% of trichloroacetic ac...
along the midline and the stomach was removed in a pre-
oxygenated Krebs solution. We cut the long axis of the
stomach parallel to the circular muscle fibers and strips of the
circular muscle layer (2–10 mm) were prepared. Then the
stomach muscle strips were suspended in longitudinal direc-
tion in a chamber containing 10 ml Krebs solution (37 °C) and
bubbled with 95% O2 and 5% CO2. A computerized integration
system (BIOPAC MP150; BIOPAC system, Inc., USA) was used to
measure the spontaneously contractile activity of gastric antral
circular strip.

**Effect of RPS on gastric antral smooth muscle contractility (GASMC)**

The contractile responses of the strips to RPS were measured
according to the previously described method. Briefly, an
initial tension of 0.5 g for circular muscles was applied before
treating with drugs. All tissues were incubated for 1 hour until
a stable baseline was attained and then the experiment started.
Four different concentrations of RPS (20, 40, 80 and 160
µg ml⁻¹) were administrated consecutively into the bath to
note the responses of strips. The mean tension was measured
for each concentration in the same way. For the further study,
an appropriate agent (acetylcholine) at the final concentration
of 10 µM was pre-incubated with strips for 5 min and the responses
of strips were recorded, followed by consecutive administration of concentrations of polyphyllin I, II, VI and H respectively as stated in the previous part. The method was similar to that of experiment we described above.

**Effect of acetylcholine on RPS-induced relaxation of isolated gastric antral smooth muscle**

The gastric antral smooth muscle was processed as described
above. After an equilibration period of 1 hour, isolated gastric
antral smooth muscle was incubated with RPS or the main
compounds (160 µg ml⁻¹) for 5 min. Then, cumulative-
concentration response curves for acetylcholine (a muscarinic
receptor agonist, 10⁻⁹–10⁻⁴ M) were obtained. The non-
incubation with muscarinic receptor agonist was considered
as control group.

**Effect of atropine on RPS-induced relaxation of isolated gastric antral smooth muscle**

To assess whether RPS and the main compounds produced
relaxation of isolated gastric antral smooth muscle through the
inhibition of muscarinic receptors, isolated gastric antral
smooth muscle was incubated with atropine (a muscarinic
receptor antagonist, 10 µM) for 5 min, followed by consecutive
administration of concentrations of RPS or the main
compounds as stated in the previous part. The non-incubation
with muscarinic receptor antagonist was considered as control
group.

**Statistical analysis**

Data were expressed as means ± standard error (S.E.M.) or
percentage and analyzed for statistical significance using
one-way analysis of variance (ANOVA) followed by Student’s t-
test. Tests were performed using SPSS 17.0 system. P-Value
less than or equal to 0.05 was considered to be statistically
significant.
Results

Chemical composition analysis

The chemical composition of RPS was qualitatively identified utilizing HPLC-ELSD by retention time with standard mixture solution. Fig. 1 shows the HPLC chromatograms of RPS. The contents of polyphyllin I ($t_R$ 41.823 min, Fig. 1-2), polyphyllin II ($t_R$ 31.110 min, Fig. 1-2), polyphyllin VI ($t_R$ 19.674 min, Fig. 1-2), polyphyllin H ($t_R$ 18.964 min, Fig. 1-2) were 24.18 ± 1.40 mg g⁻¹, 6.46 ± 0.16 mg g⁻¹, 2.70 ± 0.12 mg g⁻¹ and 24.91 ± 0.67 mg g⁻¹ respectively.

Gastric emptying and small intestinal motility of RPS

In normal mice, gastric emptying was remarkably inhibited by RPS (250 or 500 mg kg⁻¹, Fig. 3–5). Mosapride and neostigmine significantly promoted the gastric emptying, while RPS, adrenalin, atropine and dopamine evoked a significant decrease of gastric emptying (Fig. 3 and 4). The gastric emptying was stimulated by mosapride and neostigmine, while RPS, adrenalin, atropine and dopamine significantly inhibited the gastric emptying. Fig. 2

Fig. 2 Chemical structure of polyphyllin I (A), polyphyllin II (B), polyphyllin VI (C), polyphyllin H (D), diosgenins (E) and pennogenins (F).
emptying improved by neostigmine and mosapride was significantly abolished by administration with RPS (500 mg kg\(^{-1}\), \(P = 0.000\)). The present results shows that adrenalin inhibited the gastric emptying and the inhibitory effect was significantly enhanced after pre-treatment with RPS (Fig. 4, \(P = 0.016\), \(P = 0.000\)), but the inhibitory effect is similar to RPS alone. Administration of RPS with pretreatment by atropine or dopamine had significantly effect on gastric emptying with dose dependent, but the inhibition rates were weaker than RPS alone (Fig. 4A).

Under basal conditions, geometric center of control group was 3.36 \pm 0.10. RPS showed no effect on the geometric center compared with control group (\(P > 0.05\)). Mosapride and neostigmine gave little effect on the intestinal transit (Fig. 5A). The intestinal transit under adrenalin, atropine and dopamine treatment in the absence or the presence of RPS were shown in Fig. 5B. The intestinal transit was reduced by adrenalin alone, while increased with RPS. Atropine without or with RPS significantly attenuated intestinal transit and dopamine showed similar trend to adrenalin (Fig. 5B).

**Effects of RPS on GASMC**

Since the antral smooth muscle layer plays an important role in gastric emptying, we have investigated the effect of RPS on isolated antral smooth muscle motility. In order to investigate the effect of RPS on the spontaneous contractility of rat antral smooth muscle, RPS was administered at increasing concentrations (20, 40, 80 and 160 \(\mu\)g ml\(^{-1}\)) respectively. The basal levels (before using the drugs) served as control and the mean amplitude was tested for each concentration in the same way.

Here we found that RPS (20–160 \(\mu\)g ml\(^{-1}\)) concentration-dependently reduced the mean tension of contractions in the antral circular strip compared to untreated controls (\(n = 6\)) (Fig. 6). The percentage of changing from control values were 

\[-14.00 \pm 1.61\% (20 \mu g \text{ ml}^{-1}), -18.70 \pm 0.71\% (40 \mu g \text{ ml}^{-1}), -26.05 \pm 0.86\% (80 \mu g \text{ ml}^{-1}) \text{ and } -39.14 \pm 0.25\% (160 \mu g \text{ ml}^{-1})\]

respectively. The results indicated that the RPS had the effect of inhibiting smooth contractions. Significant differences between different concentrations of RPS with control were observed (Fig. 6).

Fig. 7 and Table 1 showed that pretreatment with acetylcholine (ACH) on the response of antral smooth muscle layer in RPS (20–160 \(\mu\)g ml\(^{-1}\)). The maximum contraction elicited by ACH was 5.07 \pm 0.2g, which was attenuated to 4.42 \pm 0.05 g (RPS 20 \mu g \text{ ml}^{-1}, P < 0.001), 4.00 \pm 0.02 g (RPS 40 \mu g \text{ ml}^{-1}, P < 0.001), 2.70 \pm 0.03 g (RPS 80 \mu g \text{ ml}^{-1}, P < 0.001) and 2.06 \pm 0.02 g (59.34% reduction, \(P < 0.001\)) at cumulative concentrations without washing between the administrations. As the Fig. 7B showed, the percentages of control values were 279.11 \pm 1.74\% (ACH 10 \mu M), 230.66 \pm 3.62\% (ACH 10 \mu M + RPS 20 \mu g \text{ ml}^{-1}), 199.77 \pm 1.67\% (ACH 10 \mu M + RPS 40 \mu g \text{ ml}^{-1}), 102.30 \pm 2.00\% (ACH 10 \mu M + RPS 80 \mu g \text{ ml}^{-1}) and 54.14 \pm 1.55\% (ACH 10 \mu M + 160 \mu g \text{ ml}^{-1}). It was indicated that RPS (20–160 \mu g ml\(^{-1}\)) had effect on smooth muscle contraction after treatment with acetylcholine (10 \mu M).

**Effects of polyphyllin I, II, VI and H on GASMC**

In order to investigate the major active component of RPS on GASMC, polyphyllin I (Fig. 2A) and II (Fig. 2B) with polyphyllin VI (Fig. 2C) and H (Fig. 2D) which are the representative of diosgenins (Fig. 2E) and pennogenins (Fig. 2F) in RPS respectively, were performed in GASMC. Based on the above results, we compared the effects of the 4 individual components (polyphyllin I, II, VI and H) with RPS in the *in vitro* model. Polyphyllin I, II, VI and H (20, 40, 80 and 160 \mu g ml\(^{-1}\)) were administered into the bath to note the responses of strips, respectively. As shown in Fig. 8, polyphyllin I and II (20–160 \mu g ml\(^{-1}\)) concentration-dependently reduced the mean tension of contractions in the antral circular strip compared to untreated controls (\(n = 6\)). The percentage of change from control values were from \(-22.00 \pm 1.57\% \text{ to } -58.21 \pm 0.22\% \) (polyphyllin I) and from \(-18.51 \pm 0.97\% \text{ to } -41.63 \pm 0.48\% \) (polyphyllin II), respectively. However, polyphyllin VI (20–160 \mu g ml\(^{-1}\)) or polyphyllin H (20–160 \mu g ml\(^{-1}\)) used alone only had slighter effect on smooth muscle contraction. When compared to the control, the percentage of polyphyllin VI was \(-28.32 \pm 0.87\% \) (160 \mu g ml\(^{-1}\)) and of polyphyllin H was \(-27.46 \pm 0.38\% \) (160 \mu g ml\(^{-1}\)). Apparently, the effect of polyphyllin I and II (20–160 \mu g ml\(^{-1}\)) on GASMC is stronger than that of polyphyllin VI and H (20–160 \mu g ml\(^{-1}\)) when used alone. The inhibitory effect of RPS on GASMC is also more significant than polyphyllin VI or H but weaker than polyphyllin I or II. Therefore, we may suspect that polyphyllin I and II which are saponins with diosgenin have a stronger inhibitory effect on GASMC than saponins with pennogenins.

On the basis of above results, acetylcholine (10 \mu M), was used as a positive control to track down the possible sites of diosgenins and pennogenins' activity on gastric antral smooth muscle contractility. As shown in Fig. 9, pretreatment of gastric antral smooth muscle strips with acetylcholine (10 \mu M) for
5 min was partly abolished by polyphyllin I, II, VI and H (20–160 μg ml⁻¹) induced relaxation in smooth muscle strip contraction. The percentage of changing from control values after administrated by RPS were from 269.11 ± 1.74% to 54.14 ± 1.55% (214.97% reduction, P < 0.001, Fig. 9A). As shown in Fig. 9B, compared to the control, the maximum sustained percentage elicited by acetylcholine (10 μM) was 264.38 ± 8.13% which was attenuated to 42.70 ± 7.68% (221.68% reduction, P < 0.001) in the presence of 160 μg ml⁻¹ polyphyllin I. The maximum contraction induced by acetylcholine (10 μM) was 268.54 ± 9.39% which was attenuated to 49.56 ± 3.11% (218.98% reduction, P < 0.001, Fig. 9C) in the concentration of 160 μg ml⁻¹ polyphyllin II. The maximum contraction induced by acetylcholine (10 μM) was 268.76 ± 8.77%, which was attenuated to 94.63 ± 4.60% (174.13% reduction, P < 0.001, Fig. 9D) in the presence of polyphyllin VI. The maximum contraction induced by acetylcholine (10 μM) was 268.22 ± 5.61%, which was attenuated to 83.02 ± 3.48% (185.20% reduction, P < 0.001, Fig. 9E) in the presence of polyphyllin H.
The order of reduced percentage of changing from acetylcholine (10 μM) in GASMC was the following: polyphyllin I > polyphyllin II > RPS > polyphyllin VI > polyphyllin H. The inhibitory effect of polyphyllin I and II on antral smooth muscle constriction was stronger than that of polyphyllin VI and H. The present study has indicated that diosgenins showed significantly inhibitory activity compared to pennogenins with pretreatment of antral smooth muscle strips with acetylcholine (10 μM).

**Effect of acetylcholine on RPS-induced relaxation of isolated gastric antral smooth muscle**

After incubated with RPS, polyphyllin I, polyphyllin II, polyphyllin VI or polyphyllin H (160 μg ml⁻¹) for 5 min, cumulative-concentration response curves for acetylcholine (10⁻⁹–10⁻⁴ M) were obtained. As shown in Fig. 10, the cumulative concentration–response curves for acetylcholine applied to the isolated gastric antral smooth muscle were
As illustrated in Fig. 11A, RPS-induced relaxation was significantly reduced by pretreatment with atropine (10 μM) in a concentration-dependent manner with the maximum value of 20.83 ± 2.39% (n = 6) at a concentration of 160 μg ml⁻¹ (P < 0.01). The relaxation induced by polyphyllin I, polyphyllin II, polyphyllin VI and polyphyllin H (20–160 μg ml⁻¹) on isolated gastric antral smooth muscle were also significantly attenuated (Fig. 11B–E).

**Discussion**

In recent years, steroidal saponins isolated from herbs have attracted scientific attention because of their structural diversity and significant biological activities. The rhizome of *Paris polyphylla* Sm. var. yunnanensis, found in southwestern China, is widely used in traditional Chinese medicine for the treatment of cancer and abnormal uterine bleeding.² Our group previously provided the first report that Rhizoma Paridis total Saponins (RPS) have an effect on inhibition of gastric emptying *in vivo*.³

The present results demonstrated that RPS and its main compound paris saponin (polyphyllin I, II, VI and H) exhibited inhibitory activities on gastric emptying *in vivo* and isolated antral smooth muscle motility *in vitro*. Meanwhile, from the corresponding effective concentrations, it appeared that the effectiveness of RPS in relaxing ACH-induced contraction which was firstly reported.

The organization of gastric emptying is complex and involves the coordination of motor activity in the proximal stomach, the antrum, the pylorus and duodenum, as well as passive forces generated by intragastric volume.²¹ Neurological and gastrointestinal hormone regulation is involved in the process. From the *in vivo* experiments we knew that gastric emptying was delayed under the influence of RPS alone, this delay became more prominent when the concentration of RPS was increased to 500 mg kg⁻¹. Both of mosapride and neostigmine enhanced the gastric emptying. Neostigmine, an acetylcholinesterase (AChE) inhibitor and mosapride, a serotonin 5-HT₄ receptor agonist, have been reported to improve gastrointestinal motility in dogs or patients.²² As shown in Fig. 3, the gastric emptying improved by neostigmine and mosapride was significantly abolished by administration with RPS (500 mg kg⁻¹, P = 0.000). Neostigmine and mosapride promoted the gastric emptying *via* increasing the content of acetylcholine. Thereby, we speculated that the inhibition effect of RPS on gastric emptying was related to acetylcholine.

Treatment of the animals with adrenalin, atropine and dopamine also reduced the rate of gastric emptying. Restraint stress significantly inhibited gastric emptying. Adrenalin as the α- and β-adrenoceptor agonist could suppress the gastric emptying. In the further study, α₁, α₂, β₁, and β₂-adrenoceptor antagonists did not affect this restraint stress-induced inhibition of gastric emptying, β₂-adrenoceptor antagonist significantly cancelled the restraint stress-induced inhibition of gastric emptying.²³ The present results showed that adrenalin inhibited the gastric emptying and the inhibitory effect was significantly enhanced after pre-treatment with RPS (Fig. 4, *P* =

**Effect of atropine on RPS-induced relaxation of isolated gastric antral smooth muscle**

Atropine, an antagonist of muscarinic receptors, was used to check the involvement of muscarinic receptors in inhibitory effect on isolated gastric antral smooth muscle of RPS and its main compounds.

After pretreatment of the smooth muscle strips with atropine for 15 min, RPS, polyphyllin I, polyphyllin II, polyphyllin VI or polyphyllin H (20–160 μg ml⁻¹) were administrated for 5 min. competitively antagonized by RPS and the main compounds (160 μg ml⁻¹, *P* < 0.05). Moreover, the antagonistic effects on acetylcholine of polyphyllin I and polyphyllin II were stronger than that of polyphyllin VI and H. The data suggested that the contraction of isolated gastric antral smooth muscle elicited by acetylcholine was mainly mediated by RPS and the main compounds.

**Fig. 6** Effects of RPS (20–160 μg ml⁻¹) on the isolated gastric antral smooth muscle. ****P < 0.001 compared to control using unpaired Student's t-test or one-way ANOVA. Representative recording from gastric antral smooth muscle strips induced by RPS is shown in (A). Group summary data of RPS-induced changes in the amplitude is shown in (B).
0.016, \( P = 0.000 \)), which implied that the inhibitory effect of RPS on gastric emptying had no connection with adrenoceptor.

Dopamine as a dopamine and adrenoceptor agonist regulated food intake by modulating food reward and motivation without crossing the blood–brain barrier. The inhibitory effect of dopamine on gastric motility is thought to be mediated via

![Diagram](image)

**Fig. 7** Effects of RPS on ACH-induced contraction of isolated gastric antral smooth muscle. After pretreatment of the smooth muscle strips with ACH (10 \( \mu \text{M} \)) for 10 min, cumulative additions of RPS (20–160 \( \text{mg ml}^{-1} \)) was treated for 5 min, respectively. **\( P < 0.01 \), ***\( P < 0.001 \) compared to ACH (10 \( \mu \text{M} \)).

![Diagram](image)

**Table 1** Inhibitory effects of RPS on isolated gastric antral circular smooth muscle

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<thead>
<tr>
<th>Group</th>
<th>Tension (g)</th>
<th>Reduction (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.34 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>ACH 10 ( \mu \text{M} )</td>
<td>5.07 ± 0.02</td>
<td></td>
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<tr>
<td>ACH 10 ( \mu \text{M} ) + RPS 20 ( \mu \text{g ml}^{-1} )</td>
<td>4.42 ± 0.05**</td>
<td>12.78</td>
</tr>
<tr>
<td>ACH 10 ( \mu \text{M} ) + RPS 40 ( \mu \text{g ml}^{-1} )</td>
<td>4.00 ± 0.02***</td>
<td>21.07</td>
</tr>
<tr>
<td>ACH 10 ( \mu \text{M} ) + RPS 80 ( \mu \text{g ml}^{-1} )</td>
<td>2.70 ± 0.03***</td>
<td>46.64</td>
</tr>
<tr>
<td>ACH 10 ( \mu \text{M} ) + RPS 160 ( \mu \text{g ml}^{-1} )</td>
<td>2.06 ± 0.02***</td>
<td>59.34</td>
</tr>
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\( ^* \) Results as means ± S.E.M. (\( n = 6 \)). **\( P < 0.01 \), ***\( P < 0.001 \) significantly different when compared with the ACH (10 \( \mu \text{M} \)) group.

![Diagram](image)

**Fig. 8** Effects of RPS, polyphyllin I (Pol I), polyphyllin II (Pol II), polyphyllin VI (Pol VI) and polyphyllin H (Pol H) (20–160 \( \mu \text{g ml}^{-1} \)) on gastric antral circular smooth muscle motility. The average amplitude were measured by isometric force transducers before (5 min) and after (15 min) treatment of RPS. The values are represented as a percentage of the measurement before administration of RPS (% of control, mean ± S.E.M.). Paired or unpaired Student’s t-test was used (\( n = 6 \)).
a decrease in acetylcholine release resulting from stimulation of enteric neuronal dopamine receptors. In the research, dopamine showed inhibitory effect on gastric emptying and the inhibitory effect was significantly enhanced after pre-treatment with RPS (Fig. 4A, $P = 0.037$), but the effect was weaker than RPS alone. The results indicated that dopamine may weaken the inhibitory effect of RPS on gastric emptying. Numerous physiological and pharmacological studies have shown that dopamine has an inhibitory effect on gastric motility by a decrease in acetylcholine release resulting from the stimulation of peripheral dopamine receptors. However, previous studies have shown that the delay in gastric emptying that is induced by dopamine receptor agonists is mainly inhibited by centrally acting dopamine receptor antagonists. It can speculate that RPS may have the effect to competitive antagonism the dopamine receptor when administrated with dopamine. The mechanism of gastric emptying administrated with RPS and dopamine need further studies.

![Graphs showing effects of RPS (A), polyphyllin I (Pol I, B), polyphyllin II (Pol II, C), polyphyllin VI (Pol VI, D) and polyphyllin H (Pol H, E) on acetylcholine (10 μM) induced contraction of isolated antral smooth muscle strips.](image)

Fig. 9 Effects of RPS (A), polyphyllin I (Pol I, B), polyphyllin II (Pol II, C), polyphyllin VI (Pol VI, D) and polyphyllin H (Pol H, E) on acetylcholine (10 μM) induced contraction of isolated antral smooth muscle strips. The isolated tissues were preincubated with DMSO (vehicle control), acetylcholine (10 μM) for 5 min before priming with polyphyllin I, II, VI or H (20–160 μg ml⁻¹). Values are expressed as mean ± S.E.M. ($n = 6$). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to ACH (10 μM) using two-way ANOVA with Student’s t-test.
in vitro pretreatment by atropine had significant effects on gastric antral smooth muscle contraction, the effect of RPS was connected with acetylcholine receptor. In this study, RPS and the main compounds (160 μg ml⁻¹, Fig. 10), which suggested that the acetylcholine receptors might be implicated in the relaxation effect of RPS, polyphyllin I, polyphyllin II, polyphyllin VI and polyphyllin H on isolated gastric antral smooth muscle. Moreover, incubation of isolated gastric antral smooth muscle with atropine, a selective muscarinic receptor antagonist, may stimulate the gastric emptying by stimulating the muscarinic acetylcholine receptor. Atropine, a selective muscarinic receptor antagonist, may inhibit the gastric emptying by antagonism the muscarinic acetylcholine receptor. In this study, RPS and the main compounds caused a concentration-dependent relaxation in isolated gastric antral smooth muscle (Fig. 8). We also found that the contraction effect induced by acetylcholine (10 μM) was attenuated by RPS, polyphyllin I, polyphyllin II, polyphyllin VI and polyphyllin H (Fig. 7 and 9). The present study showed that ACH-induced contraction was significantly reduced by pretreatment with RPS and the main compounds (160 μg ml⁻¹, Fig. 10), which suggested that the acetylcholine receptors might be implicated in the relaxation effect of RPS, polyphyllin I, polyphyllin II, polyphyllin VI or polyphyllin H on isolated gastric antral smooth muscle. Moreover, incubation of isolated gastric antral smooth muscle with atropine, a selective muscarinic receptor antagonist, significantly blunted RPS and the main compounds induced relaxation (Fig. 11). Thereby we suspect that RPS, polyphyllin I, polyphyllin II, polyphyllin VI and polyphyllin H inhibiting as antagonist of muscarinic receptors on gastric antral smooth muscle and the muscarinic receptor inhibition is one of the mechanisms responsible for the gastric emptying properties of RPS and the main compounds.

Saponins are well known to exhibit cytotoxic, hemolytic and gastroprotective activities, which are strongly interrelated with the nature of both the aglycone and sugar side chains. Saponins with diosgenin, penangogen and their congeners as the aglycones constitute the most abundant types of steroid saponins in PRS. Several papers reported the correlation of the activities for penangogenins and diosgenins from the structure-activity relationship studies. Meanwhile, diosgenin and penangogen, two major constituents in PRS, were reported to have gastroprotective effect on ethanol-induced gastric lesions in rats, which was associated with the structure of saponins. To our best knowledge, this is the first report of RPS with the significantly inhibitory effect on gastric antral smooth muscle contractility which may be related to the muscarinic receptors. Furthermore, the main pure compounds in RPS serve as inhibitors which were relevant to structure had never reported before.
In summary, our present results suggest Rhizoma Parisdis total Saponins (RPS) and its two kinds of saponins have inhibitory effect on gastric emptying and gastric antral smooth muscle contractility and the muscarinic receptors may be the major site of RPS on inhibiting gastric emptying and gastric antral smooth muscle contractility. Moreover, the 17-hydroxyl group of spirostan may influence the inhibitory effect of both gastric emptying and gastric antral smooth muscle contractility. Further studies are needed to elucidate the exact mechanisms and the structure-activity relationship of Paris polyphylla saponin that are responsible for these pharmacological observations.

**Conflict of interest**

We have no conflict of interest in this research.

**Abbreviation**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACH</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>5-HT₄</td>
<td>5-Hydroxytryptamine 4</td>
</tr>
<tr>
<td>AD</td>
<td>Adrenalin hydrochloride</td>
</tr>
<tr>
<td>AT</td>
<td>Atropine</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
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<tr>
<td>GASMC</td>
<td>Gastric antral smooth muscle contractility</td>
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References