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Effect of jatrorrhizine on delayed gastrointestinal transit in rat postoperative ileus

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Keywords
cholinergic pathway; gastrointestinal transit; 5-HT_4 receptor; jatrorrhizine; postoperative ileus

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Abstract

Objectives Postoperative ileus is major cause of postoperative complication and prolonged hospitalization. Jatrorrhizine, which is a protoberberine alkaloid isolated from the medicinal plants *Berberis aristata* and *Coptis chinensis*, has been found to increase contractility of gastric antral and ileum smooth muscles of rat gastrointestinal tract. We have investigated whether jatrorrhizine could offset gastrointestinal transit in rat with postoperative ileus.

Methods Postoperative ileus was induced by laparotomy with intestinal manipulation under anaesthesia. Gastrointestinal transit was evaluated by measurement of gastric emptying, geometric centre and the migration of Evans blue.

Key findings Postoperative ileus significantly delayed gastric emptying and intestinal transit. Jatrorrhizine dose-dependently (0.1, 0.3 and 1 mg/kg) offset delayed gastric emptying and intestinal transit (geometric centre and the migration of Evans blue) in postoperative ileus. Pretreatment of animals with atropine inhibited the action of jatrorrhizine on gastric emptying and intestinal transit, but pretreatment of animals with SB204070 did not influence the effect of jatrorrhizine on gastric emptying and intestinal transit in postoperative ileus.

Conclusions Jatrorrhizine offset postoperative ileus-induced delayed gastric emptying and intestinal transit in rats, an action mediated via the cholinergic pathway, but not involving activation of 5-HT_4 receptors.

Introduction

Postoperative ileus is a common complication after abdominal surgery and is characterized as inhibition of propulsive intestinal motility, which is accompanied by increased morbidity and prolonged hospitalization, increasing hospital costs.[1–3] It is generally considered that the pathogenesis of postoperative ileus is involved in neurogenic, inflammatory and inflammatory-neuronal interactive mechanisms.[3–5] The pharmacological management of postoperative ileus is important to inhibit morbidity and reduce hospital costs and length of hospital stay.[3] Currently, μ-opioid receptor antagonists and 5-HT_4-receptor agonists have been suggested for development of drugs for the management of postoperative ileus.[6]

Jatrorrhizine is one of the major protoberberine alkaloids isolated from many medicinal plants, such as *Berberis aristata* and *Coptis chinensis*. Traditional Oriental medicine uses the extracts of these plants for the treatment of gastroenteritis and diarrhoea.[7] Pharmacological studies indicate that jatrorrhizine has various bioactivities, including antimicrobial, antiradical, antioxidant, and antifungal activity.[8–10] Jatrorrhizine has been shown also to decrease the blood glucose level in alloxan-diabetic mice, inhibit cytochrome P450 3A4 (CYP3A4), and has shown an acetylcholinesterase inhibitory property.[11–13] Recently, we reported that jatrorrhizine could increase contractility of gastrointestinal tract smooth muscles of isolated gastric antral and ileum strips with a calcium-agonistic effect.[14] However, it is unclear whether jatrorrhizine possesses a gastrointestinal prokinetic action due to its contractility on gastrointestinal tract smooth muscles. We hypothesized that jatrorrhizine may shorten the overall time by stimulating gastric emptying and small intestinal transit. Thus, in this study, we have investigated the effect of jatrorrhizine on delayed gastrointestinal transit in rat postoperative ileus.

Materials and Methods

Animals

To acclimatize to their environment, healthy male Wistar rats (250–300 g; SIPPR/BK Co., Ltd, Shanghai, China) were maintained in a temperature-controlled (22 ± 1°C) room with a 12-h light/dark cycle, with free access to normal food and water for one week. After this time the rats were fasted for 24 h with free access to water until the start of the experiments. All animal handling and procedures were performed according to international guidelines for the use and care of laboratory animals. The experimental protocol was approved by the University Ethics Committee. The number of animals used in the experiment was kept to the minimum necessary for a meaningful interpretation of the data.

Induction of postoperative ileus

Postoperative ileus was induced according to a surgical procedure described by Venkova *et al*.[15] All animals were fasted for a further 48 h with free access to tap water containing 5% glucose. On the day of experiment, the rats were anaesthetized with pentobarbital sodium (35 mg/kg i.p.), the abdomen was shaved and disinfected, and a midline incision was made. The small intestine and the caecum were exteriorized and inspected for 5 min using cotton applicators soaked in sterile saline. After completing the inspection, the intestines were covered with gauze soaked in saline and the abdomen was left open for a total of 10 min. The viscera were then placed back into the abdomen and the incision was closed with silk sutures. The procedure lasted 20–25 min. The animals were allowed to completely recover for 4 h from the anaesthesia before any drug treatment began.

Determination of gastrointestinal transit

Conscious rats received an intragastric gavage of 1 ml Evans blue, a semiliquid non-nutrient dye (50 mg/ml dissolved in 0.5% methylcellulose). After 30 min rats were anaesthetized and killed. The stomach and small intestine were carefully removed. The stomach was numbered as segment 1, and the small intestine was divided into 10 equal segments (numbered as 2–11). Each segment was placed in 25 ml 0.1 M NaOH, minced and put in an ultrasonic bath for 1 h. The resulting suspension was left at room temperature for 1 h. The supernatant (5 ml) was then centrifuged at 1356*g* for 20 min at 4°C. Samples were further diluted (1 : 5 for intestinal specimens and 1 : 50 for the stomach), and absorbance (A) was read at 570 nm.[16] Gastric emptying was calculated by the formula: percentage of gastric emptying = [(A570 reference − A570 sample)/A570 reference] × 100.[17] The stomach obtained from rats killed immediately after orogastric administration of Evans blue served as the reference. The small intestinal transit was assessed by geometric centre calculated as a function of the amount of dye content transported to each segment: geometric center = Σ [% A (segment) × (number of segment)]/100. Transit was also measured from the pylorus to the most distal point of migration and expressed as a percentage of the total length of small intestine.

Experimental protocols

Experiments were performed 4 h after surgery. To study the action of jatrorrhizine on rat postoperative ileus, chemically pure jatrorrhizine hydrochloride (0.1, 0.3 or 1.0 mg/kg; National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China), or a muscarinic receptor agonist carbachol (1.0 mg/kg; Sigma-Aldrich, St Louis, MO, USA) or the 5-HT_4 receptor agonist mosapride (10 mg/kg; Sigma-Aldrich) was orally administrated 30 min before Evans blue administration. Control (intestinal manipulation) and normal (sham) animals were orally administrated the same volume (2 ml/kg) of vehicle or distilled water. To determine the involvement of M_3 receptors, rats were injected subcutaneously with atropine (1.0 mg/kg; Sigma-Aldrich) 15 min before treatment with jatrorrhizine or carbachol. To measure the involvement of 5-HT_4 receptors, rats were injected subcutaneously with SB204070 (10 mg/kg; Tocris, Ellisville, MO, USA) 15 min before treatment with jatrorrhizine or mosapride. Control and normal animals were injected subcutaneously with the same volume (2 mg/kg) of saline.

Statistical analysis

Data are expressed as the mean ± standard error of mean (SEM), and *n* is the number of animals. Significance of difference between groups was analysed with the unpaired Student's *t*-test. For multiple comparisons of groups, one-way analysis of variance test was used. When a probability (*P*) value was less than 0.05, the difference was considered statistically significant.

Results

Effect of jatrorrhizine in postoperative ileus

Postoperative ileus significantly delayed gastric emptying and intestinal transit. Percentage of gastric emptying was significantly decreased in postoperative ileus animals compared with sham animals (28.0 ± 3.2 vs 58.8 ± 3.2, $P < 0.01$, respectively; Figure 1a). The geometric centre of intestinal transit was significantly decreased in postoperative ileus animals compared with sham animals (2.3 ± 0.2 vs 5.3 ± 0.5, $P < 0.01$, respectively; Figure 1b). Percentage of migration length of small intestine was significantly decreased in animals subjected to intestinal manipulation

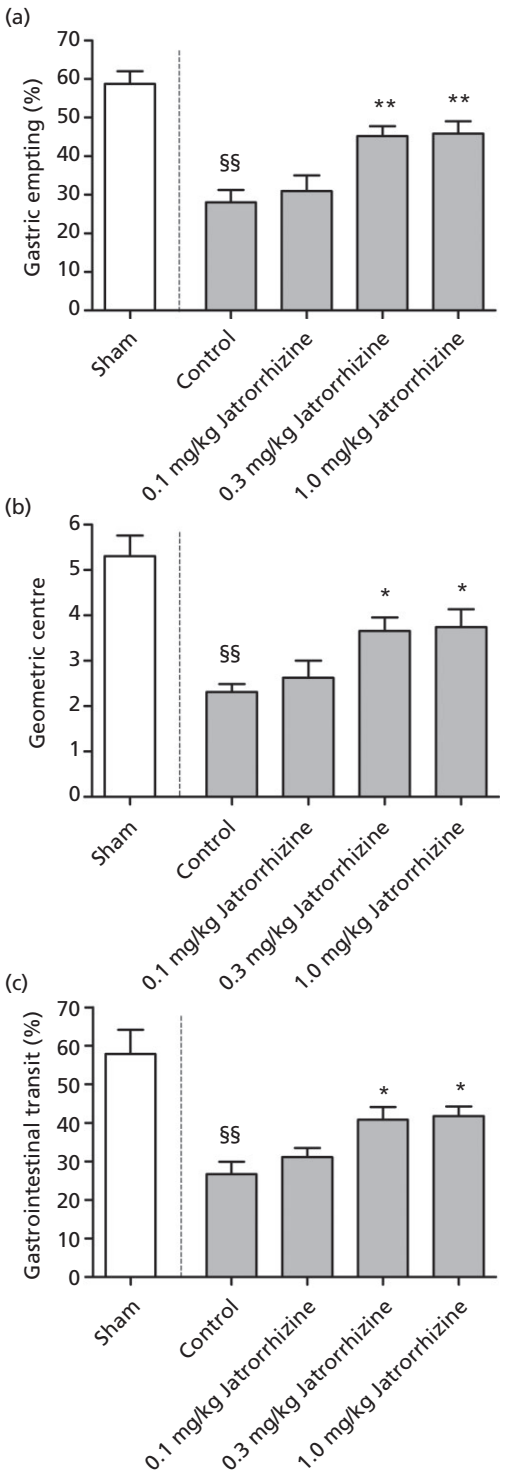

Figure 1 Effect of jatrorrhizine on delayed gastrointestinal transit in postoperative ileus. Effect of jatrorrhizine on (a) gastric emptying, (b) the geometric centres of intestinal transit, and (c) migration length of small intestine in postoperative ileus. Data are expressed as the mean ± SEM of 10 rats. $^{§§}P < 0.01$, compared with sham animals (Student' *t*-test). $*P < 0.05$, $**P < 0.01$, compared with the control animals (one-way analysis of variance).

compared with sham animals (26.8 ± 3.2 vs 57.9 ± 6.3, $P < 0.01$, respectively; Figure 1c).

Jatrorrhizine significantly offset postoperative ileus-induced delayed gastric emptying and intestinal transit. In rats administered 0.1, 0.3 or 1.0 mg/kg jatrorrhizine, percentages of gastric emptying were 30.9 ± 4.1, 45.2 ± 2.6, and 45.9 ± 3.2; the geometric centres of intestinal transit were 2.6 ± 0.4, 3.6 ± 0.3, and 3.7 ± 0.4; and percentages of migration length of small intestine were 31.1 ± 2.3, 40.8 ± 3.3, and 41.8 ± 2.4, respectively. As seen from Figure 1, the 0.3 and 1.0 mg/kg doses of jatrorrhizine had almost identical effects in increasing gastric emptying and accelerating intestinal transit.

Influence of atropine on effect of jatrorrhizine in postoperative ileus

Atropine was used to determine if the cholinergic pathway was involved in the effect of jatrorrhizine in postoperative ileus. After the subcutaneous injection of atropine (1 mg/kg) 15 min before the administration of jatrorrhizine 0.1, 0.3 or 1.0 mg/kg, percentages of gastric emptying were 18.6 ± 3.2, 22.5 ± 2.6, and 38.0 ± 2.3; the geometric centres of intestinal transit were 2.3 ± 0.1, 2.5 ± 0.2, and 3.0 ± 0.2; and percentages of migration length of small intestine were 27.0 ± 1.5, 32.8 ± 2.6, and 46.8 ± 3.1, respectively (Figure 2). As compared with percentages of gastric emptying, the geometric centre of intestinal transit and percentages of migration length of small intestine in the absence of atropine (Figure 1), the offset actions of jatrorrhizine of 0.1 and 0.3 mg/kg in the presence of atropine were strongly inhibited (Figure 2). The actions of carbachol were also compared with the actions of jatrorrhizine in postoperative ileus. Carbachol at 1 mg/kg significantly normalized postoperative ileus-induced delayed gastric emptying and intestinal transit, which were almost completely inhibited by atropine (1 mg/kg). The results suggested that the offset of jatrorrhizine on postoperative ileus was mediated via the cholinergic pathway.

Influence of SB204070 on effect of jatrorrhizine in postoperative ileus

To determine if 5-HT_4 receptors were involved in effect of jatrorrhizine in postoperative ileus, a 5-HT_4 receptor antagonist SB204070 was used. After the subcutaneous injection of SB204070 (10 mg/kg) 15 min before the administration of jatrorrhizine 0.1, 0.3 or 1.0 mg/kg, percentages of gastric emptying were 27.0 ± 2.6, 36.1 ± 2.7, and 46.5 ± 3.2; the geometric centres of intestinal transit were 2.4 ± 0.2, 3.1 ± 0.2, and 3.6 ± 0.2; and percentages of migration length of small intestine were 20.7 ± 2.2, 35.4 ± 1.4, and 40.5 ± 2.6, respectively (Figure 3). As compared with percentages of gastric emptying, the geometric centres of intestinal transit and percentages of migration length of small

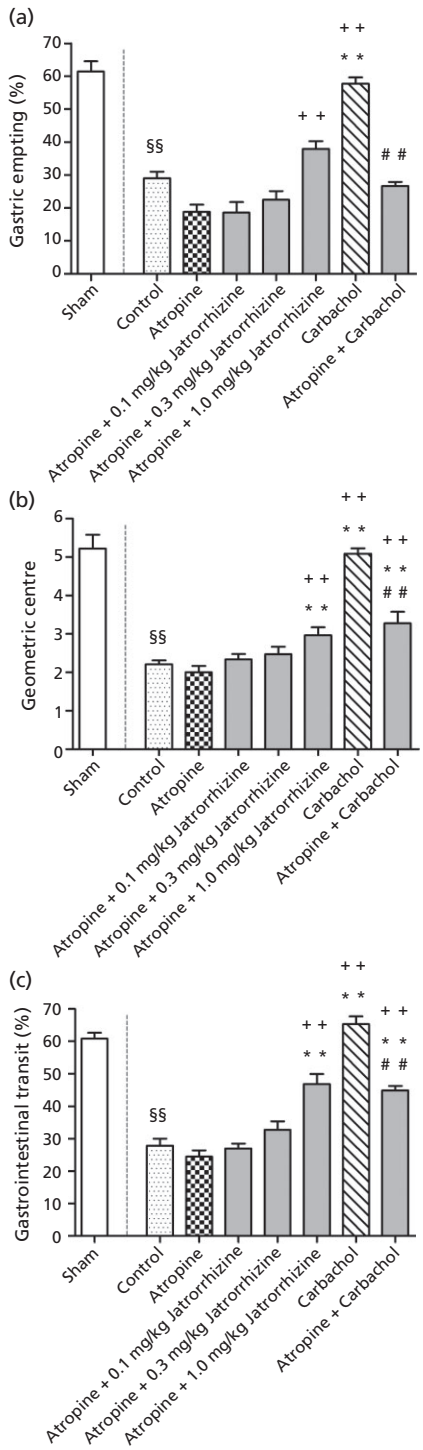

Figure 2 Influence of atropine on the action of jatrorrhizine on delayed gastrointestinal transit in postoperative ileus. Influence of atropine on the action of jatrorrhizine on (a) gastric emptying, (b) the geometric centres of delayed intestinal transit and (c) migration length of small intestine in post-operative ileus. Data are expressed as the mean ± SEM of 10 rats. $^{\S\S}P <$ 0.01, compared with sham animals. $^{**}P < 0.01$, compared with control animals (one-way analysis of variance). $^{++}P < 0.01$, compared with the atropine-treated animals. $^{\#\#}P < 0.01$, compared with carbachol-treated animals. Multiple group comparisons used one-way analysis of variance.

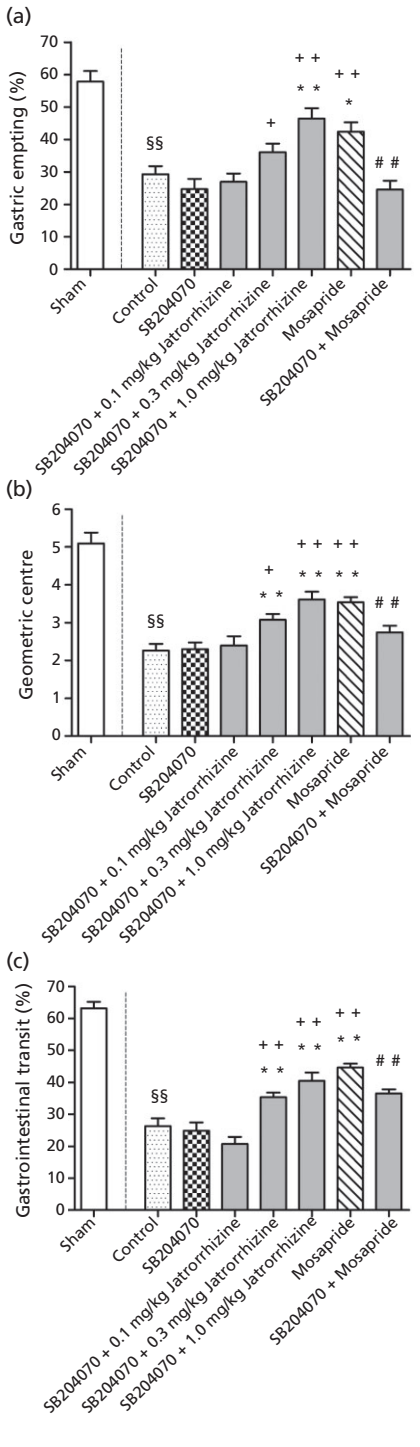

Figure 3 Influence of SB204070 on the action of jatrorrhizine on delayed gastrointestinal transit in postoperative ileus. Influence of SB204070 on the effect of jatrorrhizine on (a) gastric emptying, (b) the geometric centres of delayed intestinal transit, and (c) on migration length of small intestine in postoperative ileus. Data are expressed as the mean ± SEM of 10 rats. $^{\S\S}P < 0.01$, compared with sham animals. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control animals (one-way analysis of variance). $^{+}P < 0.05$, $^{++}P < 0.01$, compared with SB204070-treated animals. $^{\#\#}P < 0.01$, compared with mosapride-treated animals. Multiple group comparisons used one-way analysis of variance.

intestine in the absence of SB204070 (Figure 1), the offset actions of jatrorrhizine of 0.3 and 1.0 mg/kg in the presence of SB204070 were not different from those in the absence of SB204070 (Figure 3). Actions of mosapride were also compared with the actions of jatrorrhizine in postoperative ileus. Mosapride at 10 mg/kg significantly enhanced the postoperative ileus-induced delayed gastric emptying and intestinal transit, which were inhibited by SB204070 (10 mg/kg). The results suggested that the offset of jatrorrhizine on postoperative ileus was not involved in activation of 5-HT_4 receptors.

Discussion

This study has demonstrated that; postoperative ileus significantly delayed gastric emptying and intestinal transit; that jatrorrhizine, at effective doses, was able to offset postoperative ileus-induced delayed gastric emptying and intestinal transit; pretreatment of animals with atropine inhibited the effect of jatrorrhizine on gastric emptying and intestinal transit in postoperative ileus; and pretreatment of animals with SB204070 did not influence the action of jatrorrhizine on gastric emptying and intestinal transit in postoperative ileus.

Until now, the precise mechanism of postoperative ileus has not been completely understood, it was known that impairment of gastrointestinal motility induced by surgery was the result of multiple causes, including stimulation of afferent neurons and consequent activation of noradrenergic, nonadrenergic–noncholinergic (NANC) and tachykinergic neuronal pathways as well as the induction of an intestinal inflammatory response.[15] The activation of α_2-adrenoceptors inhibited the release of acetylcholine from cholinergic nerve terminals and impaired smooth muscle contraction of intestine and stomach.[18] Delayed gastrointestinal transit was significantly offset by the pretreatment of guanethidine or yohimbine, an α_2-adrenoceptor antagonist in postoperative ileus, suggesting that inhibition of α_2-adrenoceptor led to activation of cholinergic receptors releasing acetylcholine in postoperative ileus.[19] Activation of 5-HT_4 receptors had been demonstrated to increase gastrointestinal transit and to inhibit the inflammatory responses in postoperative ileus.[20,21] A recent study confirmed that stimulating the 5-HT_4 receptor accelerated acetylcholine release from cholinergic myenteric neurons, which subsequently activated acetylcholine receptors on activated monocytes/macrophages to inhibit their inflammatory reactions in the muscle layer and to exert a gastroprokinetic action.[3] Selective activation of ghrelin receptors overcame the motility dysfunction in postoperative ileus, which may have been involved in the activation of the cholinergic receptor pathway, because burn-induced delayed gastrointestinal transit was normalized by application of ghrelin that could be antagonized by atropine.[2,22]

Cholinergic nerves and 5-HT_4 receptors have been found to play an important role in postoperative ileus.[21,23] Cholinergic signalling is mediated by muscarinic acetylcholine receptors expressed in the muscle layers of gastrointestinal tract, which is involved in the regulation of excitatory action of gastrointestinal smooth muscles.[24,25] Among muscarinic acetylcholine receptor subtypes in the gastrointestinal tracts, both M_2 and M_3 mediated the intestinal smooth muscle contraction, which is considered as the basis of functional gut movements, including peristalsis.[26–29] Previously, we reported that jatrorrhizine-induced contractions were greatly attenuated by pretreatment with atropine, 4-diphenylacetoxy-N-methylpiperidine methiodide or darifenacin (M_3 receptor antagonists), but not with AF-DX116 (M_2 receptor antagonist) in the rat ileum longitudinal muscles. Those results suggested that jatrorrhizine-induced ileum longitudinal muscle contractions could be mediated by activation of muscarinic acetylcholine receptors, probably the M_3 receptor.[14] Therefore, we examined the role of the cholinergic pathway in the mechanism of action of jatrorrhizine. The results showed that pretreatment of animals with the acetylcholine nicotinic/muscarinic receptor antagonist atropine strongly inhibited the effect of jatrorrhizine on delayed gastrointestinal transit, suggesting that the offset of jatrorrhizine on postoperative ileus was mediated via the cholinergic pathway.

With regard to 5-HT receptor subtypes (5-HT_1–5-HT_7), 5-HT_3 and 5-HT_4 subtypes are distributed in enteric neurons and gastrointestinal smooth muscles, and have important roles in regulating gastrointestinal tract physiological functions. Activation of 5-HT_3 receptor leads to a rapid depolarization of enteric neurons by increasing acetylcholine release. 5-HT_4 receptor expressed in the enteric plexus, the interstitial cells of Cajal and smooth muscle cells of gastrointestinal tracts stimulates the intestinal peristaltic reflex by enhancing the neurotransmitter release including acetylcholine, substance P and vasoactive intestinal peptides, etc.[30,31] Our recent study showed that pretreatment with tropisetron (5-HT_3 receptor antagonist) or SB204070 (5-HT_4 receptor antagonist) did not influence jatrorrhizine-induced ileum longitudinal muscle contractions in the rat.[14] In this study, we also found that pretreatment of animals with SB204070 did not influence the effect of jatrorrhizine on delayed gastrointestinal transit, suggesting that the offset of jatrorrhizine on postoperative ileus could not be mediated by the 5-HT_4 receptor pathway.

Conclusion

Jatrorrhizine dose-dependently offset postoperative ileus-induced delayed gastric emptying and intestinal transit in rats, an action mediated via the cholinergic pathway, but was not involved in activation of the 5-HT_4 receptor. Jatrorrhizine

may have a therapeutic potential for gastric and intestinal dysmotility in patients with postoperative ileus. Further studies are needed to investigate other mechanisms of action, through which jatrorrhizine may exert its effect in postoperative ileus.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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