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ORIGINAL ARTICLE

Pharmacological Basis for the Medicinal Use of Psyllium Husk (Ispaghula) in Constipation and Diarrhea

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Abstract
Background The objective of this study was to determine the pharmacological basis of the medicinal use of psyllium husk (Ispaghula) in gastrointestinal motility disorders.
Methods In-vivo studies were conducted on mice, and isolated rabbit jejunum and guinea-pig ileum were used in in-vitro experiments.
Results The crude extract of Ispaghula (Po.Cr) had a laxative effect in mice at 100 and 300 mg/kg, which was partially sensitive to atropine or SB203186 (5-HT_4 antagonist). At higher doses (500 and 1,000 mg/kg), Po.Cr had antisecretory and antidiarrheal activity. In guinea-pig ileum, Po.Cr (1–10 mg/ml) had a stimulatory effect, which was partially sensitive to atropine or SB203186. In rabbit jejunum, Po.Cr had a partially atropine-sensitive stimulatory effect followed by relaxation at 10 mg/ml. The relaxation was inhibited by the presence of L-NAME, a nitric oxide (NO) synthase inhibitor, or methylene blue, a guanylyl cyclase inhibitor. Similarly, the relaxant effect of Po.Cr on K^+ (80 mM)-induced contractions, was attenuated in the presence of L-NAME or methylene blue. Activity-directed fractionation of Po.Cr revealed that the gut stimulatory and inhibitory constituents were widely distributed in the aqueous and organic fractions.
Conclusion This study demonstrates that Ispaghula has a gut-stimulatory effect, mediated partially by muscarinic and 5-HT_4 receptor activation, which may complement the laxative effect of its fiber content, and a gut-inhibitory activity possibly mediated by blockade of Ca^{2+} channels and activation of NO-cyclic guanosine monophosphate pathways. This may explain its medicinal use in diarrhea. It is, perhaps, also intended by nature to offset an excessive stimulant effect.

Keywords *Plantago ovata* · Psyllium husk · Ispaghula · Spasmodic · Laxative · Antispasmodic · Antidiarrheal

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Introduction

Gastrointestinal diseases are major public health issues throughout the world and are estimated to affect 70% of the general population [1]. Constipation and diarrhea, in particular, are commonly prevailing disorders. Constipation is known to affect approximately 27% of the population, being more prevalent in women than in men [2]. Dietary and lifestyle measures are first-line therapy for chronic constipation worldwide [3]. The second choice of treatment is the use of various prescription and consumer laxatives; these, however, are not considered safe for long-term use [4].

Diarrhea is one of the leading causes of mortality in developing countries [5]. Globally, it is estimated that there are 3.2 episodes of diarrhea per child per year [6]. In developing countries, the death rate due to diarrhea is high (1.5–2 million/year) among children under five years of age [7, 8]. Medicinal plants are usually preferred to treat gastrointestinal disorders, for example constipation and diarrhea, because they contain multiple constituents with effect-enhancing and/or side-effect-neutralizing potential [9], and, hence, are considered relatively safe in prolonged use.

The seed husk of *Plantago ovata* (psyllium husk), known locally as Ispaghula, is well known for its effectiveness in chronic constipation and is an essential component of many commercially available laxative products, for example Metamucil [10, 11]. In fact, Ispaghula is unique in three different ways:

1 it is equally popular among traditional healers and modern physicians, as a first-line treatment for chronic constipation;
2 it is effective in both constipation and diarrhea [12], two opposite states of the gut; and
3 it is one of the most common forms of self-medication throughout the world, and is used to relieve constipation as an empirical therapy.

Psyllium contains active principals, for example 4-*O*-methylglucuronic acid, aucubin, campesterol, linoleic acid, oleic acid, palmitic acid, L-cystine, L-asparagine, mucilage, rhamnose, sterol, β-sitosterol, tannins [13], polysaccharides [14], and arabinoxylans with high gel-forming property [15]. Refined psyllium is also used as a prebiotic [16]. Ispaghula has also been reported to be effective in amoebic dysentery [17], irritable bowel syndrome [18], and intestinal inflammation [19], along with its other health benefits.

During the past quarter of a century, several studies [3, 20–24] have demonstrated the clinical usefulness of Ispaghula in the treatment of constipation. However, to the best of our knowledge, no details are known about the possible underlying mechanism(s), responsible for its medicinal use in constipation, except a preliminary report [25], and a general understanding that its effectiveness in constipation is attributed to its high fiber and mucilaginous content. Similarly, there are some reports validating the medicinal use of psyllium husk in the treatment of diarrhea [26–29], but no evidence is available explaining the mode of action of its antidiarrheal effect.

This study provides the first evidence that the gut-stimulatory effect of Ispaghula is mediated through multiple pathways, including the activation of muscarinic and 5-HT_4 receptors. We also report here the first study explaining the possible mechanism(s) underlying the antispasmodic effect of Ispaghula, mediated through blockade of Ca^{2+} channels and activation of nitric oxide/cyclic guanosine monophosphate (NO-cGMP) pathways, thus providing a rationale for its medicinal use in the treatment of diarrhea. The plant extract was also studied in vivo for its laxative, antidiarrheal, and antisecretory activity.

Materials and Methods

Preparation of the Crude Extract and Its Fractions

The seed husk of *Plantago ovata* was purchased from a local market (Jouria Bazaar) of Karachi, Pakistan. It had been brought from the fields of Jamshoro (Sindh, Pakistan). A specimen, voucher # Po-SH-09-06-81, was preserved in the herbarium of the Natural Product Research Division, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. By following a previously described method [30] with slight modification, psyllium husk was soaked in 70% methanol for three days and filtered through muslin cloth and Whatman (Maidstone, UK) no. 1 filter paper, simultaneously. This procedure was repeated three times, and all the filtrates were combined and evaporated on a rotary evaporator (model RE-111; Buchi, Flawil, Switzerland) under reduced pressure (−760 mm Hg) to obtain, finally, a crude extract of Ispaghula (Po.Cr). The yield of thick, dark brown, pasty-like mass was 1.75% (*w*/*w*).

Activity-directed fractionation of the crude extract was carried out by following standard phytochemical procedures using different organic solvents [31]. Approximately 20 g Ispaghula extract was dissolved in 150 ml distilled water. An equal volume of petroleum spirit was added and the mixture was shaken vigorously in a separating funnel. After shaking the mixture separated into two layers and the petroleum spirit layer (upper) was removed. Extraction with petroleum spirit was repeated two more times. The petroleum spirit extracts were combined and evaporated to furnish the petroleum spirit fraction (Po.Pet). The lower separated layer was placed in a separating funnel; chloroform was added, and extraction and separation were performed by following the previous procedure. The lower layer, in this case chloroform, was separated, and the procedure was repeated two times. The chloroform extracts were combined and evaporated to obtain the chloroform fraction (Po.$CHCl_3$). Ethyl acetate was added to the remaining, upper, layer and extraction and separation, followed by evaporation, were performed, resulting in the ethyl acetate fraction (Po.Et.Ac). The remaining lower layer was collected and concentrated to obtain the aqueous fraction (Po.Aq).

Phytochemical Screening

Phytochemical analysis of the crude extract of *Plantago ovata* was performed, qualitatively, for the presence of

alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols, tannins, and terpenes according to standard methods [32].

Standard Drugs

Acetylcholine perchlorate (ACh), atropine sulfate, carbamylcholine (CCh), histamine hydrochloride, 5-hydroxytryptamine (5-HT), pyrilamine maleate, N^G-nitro-L-arginine methyl ester (L-NAME), loperamide hydrochloride, and components of physiological salt solution (calcium chloride ($CaCl_2$), glucose ($C_6H_{12}O_6$), magnesium chloride ($MgCl_2$), sodium chloride (NaCl), ethylenediamine-tetraacetic acid (EDTA), aluminum chloride, ammonium hydroxide, Dragendorff's reagent, and ferric chloride) were purchased from Sigma–Aldrich Chemicals, St Louis, MO, USA. SB203186 (1-piperidinylethyl-1*H*-indole-3-carboxylate) was purchased from Tocris, Ballwin, MO, USA. Sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), and solvents (benzene, chloroform, hydrochloric acid, and petroleum ether) were obtained from E. Merck (Darmstadt, Germany). Castor oil was purchased from Karachi Chemical Industries F/25 S. I. T. E., Karachi (Pakistan). All the chemicals used were of analytical grade and dissolved in distilled water/saline except the organic fractions which were dissolved in 10% DMSO. The vehicle used for solubility was found to be inert in the in-vivo and in-vitro experiments. Stock solutions of all chemicals were prepared fresh on the day of experiment.

Animals

BALB/c mice (weighing 20–25 g), and locally bred rabbits (weighing 1–1.5 kg) and guinea-pigs (weighing 400–600 g) of both sexes, were housed at the animal house of Aga Khan University under controlled environmental conditions (23–25°C). The animals were kept in plastic cages (47 × 34 × 18) with sawdust (changed at every 48 h) and fasted for 24 h before the experiment, whereas they were given tap water and standard diet routinely. The experiments were performed in accordance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [33] and approved by the Ethical Committee of Aga Khan University.

In-Vivo Experiments

Laxative Activity A previously described method [34] was followed with slight modification. Mice (weighing 20–25 g, $n = 54$) of both sexes were given no food for 24 h with free access to water before starting the experiment. The animals were placed individually in cages lined with clean filter paper and were divided into nine groups ($n = 6$, each). The first group received saline (10 ml/kg, p.o.) serving as negative control. The second and third groups were positive controls and given carbachol (1 mg/kg, i.p.) and serotonin (1 mg/kg, i.p.), respectively. The fourth and fifth groups received the plant extract (100–500 mg/kg) orally. Of the remaining four groups, two were pretreated with atropine (10 mg/kg) and the other two were administered SB203186 (1 mg/kg) 30 min before redetermining the positive effect of Po.Cr on wet feces. After 6 h, feces production (total number of feces and total wet feces/group) was measured and the percentage increase in wet feces to that of total fecal output was regarded as the laxative effect.

Intestinal Fluid Accumulation By following an earlier method [35], overnight fasted mice (weighing 20–25 g, $n = 30$) of both sexes were housed in cages in five equal groups ($n = 6$). The first two groups received saline in a solubilizing vehicle (10 ml/kg, p.o.) and acted as negative control. The animals in group three were treated with loperamide (10 mg/kg) intraperitoneally, using a detachable U-100 insulin syringe with a 25G × 1″ (0.50 × 25 mm) needle, as positive control. The fourth and fifth groups were treated with increasing doses of the plant extract (500 and 1,000 mg/kg, i.p). One hour after the treatment, each animal received castor oil (10 ml/kg) orally, except the animals in the first group. All the animals were sacrificed 30 min later by cervical dislocation and the intestine was dissected out carefully, not allowing any intestinal fluid to leak out, and weighed. The results were expressed as $(Pi/Pm) \times 1{,}000$, where *Pi* is the weight (g) of the intestine and *Pm* is the weight (g) of the animal.

Castor Oil-Induced Diarrhea To assess antidiarrheal activity, a previously described method [36] was followed. Mice (weighing 20–25 g, $n = 30$) of both sexes were fasted for 24 h before the experiment. The animals were housed in individual cages and divided into six groups, each group containing five animals. The first group received saline in vehicle (10 ml/kg, p.o.) as a negative control. Subsequent groups were administered different doses of the crude extract (500 and 1,000 mg/kg), Further groups received verapamil (30 and 100 mg/kg) or loperamide (10 mg/kg), serving as positive control. One hour after the treatment, each animal received castor oil (10 ml/kg, p.o.) through a feeding needle. After 6 h, the cages were inspected for the presence of typical diarrheal droppings; the absence was regarded as a positive result, indicating protection from diarrhea.

Isolated Tissue Experiments

Rabbit Jejunum Locally bred rabbits (weighing 1–1.5 kg, $n = 19$) of both sexes were fasted for 24 h then sacrificed by a blow on the back of head. The jejunum was dissected out, immersed in Tyrode's solution and cleaned of mesenteries. Segments of rabbit jejunum approximately 2 cm long were mounted in a 10-ml tissue organ bath containing Tyrode's solution, maintained at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (carbogen). A preload of 1 g was applied to each tissue segment and spontaneous contractions were recorded by use of an isotonic transducer 50-6360 (Harvard Apparatus, Holliston, MA, USA) coupled with either a student oscillograph (Harvard Apparatus) or PowerLab (ML-845) data-acquisition system (AD Instruments; Sydney, Australia) and a computer using chart software (version 5.3). The tissues were left to equilibrate for 30 min before addition of any drug, and then stabilized with a sub-maximum concentration of acetylcholine (ACh, 0.3 μM). Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, enabling testing of the relaxant (spasmolytic) activity directly, without the use of any agonist.

For elucidation of the mechanism of spasmolytic activity, high K^+ (80 mM), low K^+ (25 mM), and carbachol (CCh, 1 μM) were used as spasmodic agents, producing sustained contractions which enable one to determine different inhibitory mechanisms, for example Ca^{2+} channel blockade, K^+ channel activation, and anticholinergic pathways [37–39]. The test material and standard drugs were added in a cumulative fashion to obtain the concentration-dependent inhibitory responses. The relaxation of isolated tissue preparations was expressed as a percentage of the control response mediated by the spasmogen added to the tissue bath.

To confirm the Ca^{2+} channel-blocking (CCB) activity of the test material, the tissue was left to stabilize in normal Tyrode's solution, which was then replaced with Ca^{2+}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{2+} from the tissue. This solution was further replaced with K^+-rich and Ca^{2+}-free Tyrode's solution of composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. After an incubation period of 30 min, the control concentration–response curves (CRCs) for Ca^{2+} were obtained. When the CRCs of Ca^{2+} were found to be superimposable (usually after two cycles), tissue was then preincubated with the test material for 60 min to test the possible CCB-like effect. The CRCs of Ca^{2+} were reconstructed in the presence of different concentrations of the test material.

In order to explore whether nitric oxide/cyclic guanosine monophosphate (NO-cGMP) pathways were involved in the antispasmodic effect of the test material, the tissues were pretreated with L-NAME (0.1 mM) or methylene blue (10 μM) 30 min before redetermining the effect of the test substance [40].

Guinea-Pig Ileum Locally bred guinea-pigs (weighing 400–550 g; $n = 10$) of both sexes were fasted for 24 h then caringly sacrificed by cervical dislocation and the ileum was isolated. The tissues were prepared, equilibrated and stabilized by following the procedure described above (Rabbit Jejunum). The contractile responses of the tissues were recorded using similar isotonic transducers as used for rabbit jejunum. To characterize the spasmodic effect of the test material, tissues were pretreated with different antagonists (atropine, SB203186, hexamethonium, or pyrilamine) simultaneously, 30 min previously, to redetermine the stimulatory effect of test substance [41].

Data Analysis and Statistics Results are expressed are mean ± standard error of the mean (SEM; n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals. The χ^2 test was used to differentiate results in the antidiarrheal activity assay. $P < 0.05$ was considered significantly different using one-way ANOVA and/or an unpaired t-test to differentiate results from measurement of antisecretory or laxative activity. Concentration–response curves (CRCs) were analyzed by non-linear regression. Two-way ANOVA followed by Bonferroni's post-test correction was used for multiple comparisons of CRCs with the respective control. All graphing, calculations, and statistical analysis were performed by use of GraphPAD software (GraphPAD, San Diego, California, USA).

Results

Phytochemical Analysis

Preliminary phytochemical analysis of the crude extract revealed the presence of alkaloids, flavonoids, weak saponins, terpenes, and coumarins.

In-Vivo Studies

Laxative Activity Administration to mice of Po.Cr at 100 and 300 mg/kg resulted in production of 12.8 and 21.9% wet feces, respectively. At the next higher dose of 500 mg/kg there was a decline in the production of wet and total feces (data not shown). The positive controls carbachol (1 mg/kg) and serotonin (1 mg/kg) resulted in 40.3

and 29.1% wet feces, respectively, and the saline-treated group produced only 9.5% wet feces. When Po.Cr (100 and 300 mg/kg) was studied for its positive effect on wet feces in mice pretreated with atropine, the effect declined to 10.6 and 15.8%. Similarly, wet feces production in response to Po.Cr was attenuated to 11.6 and 17.8% in animals pretreated with SB203186 (Table 1).

Intestinal Fluid Accumulation In the enteropooling assay, castor oil caused a considerable increase of fluid accumulation in mice, with a (*Pi*/*Pm*) × 1,000 value of 150 ± 8.0, whereas for saline as negative control the value was 105 ± 4.5 ($P < 0.001$). Po.Cr at 500 and 1,000 mg/kg resulted in significant ($P < 0.001$) reduction in castor oil-induced fluid accumulation with (*Pi*/*Pm*) × 1,000 values of 100 ± 3.2 and 90 ± 2.4, respectively, similar to loperamide at a dose of 10 mg/kg (84 ± 3.2). All (*Pi*/*Pm*) × 1,000 values are expressed as mean ± SEM, $n = 6$ (Fig. 1).

Antidiarrheal Activity The crude extract had an antidiarrheal effect in terms of percentage protection against castor oil-induced wet feces. In the castor oil-treated group wet feces were observed for all animals whereas for the groups of animals pretreated with Po.Cr at 500 and 1,000 mg/kg protection from diarrhea was 60 and 80%, similar to the effect of verapamil, for which protection was by 40 and 100% at 30 and 100 mg/kg, respectively. Loperamide at the a of 10 mg/kg resulted in complete protection.

In-Vitro Studies

Guinea-Pig Ileum Po.Cr (3–10 mg/ml) had a concentration-dependent spasmodic effect reaching a maximum of 71.2 ± 9.9% (mean ± SEM; $n = 5$) of acetylcholine (ACh, 1 μM)-induced contraction. To characterize the

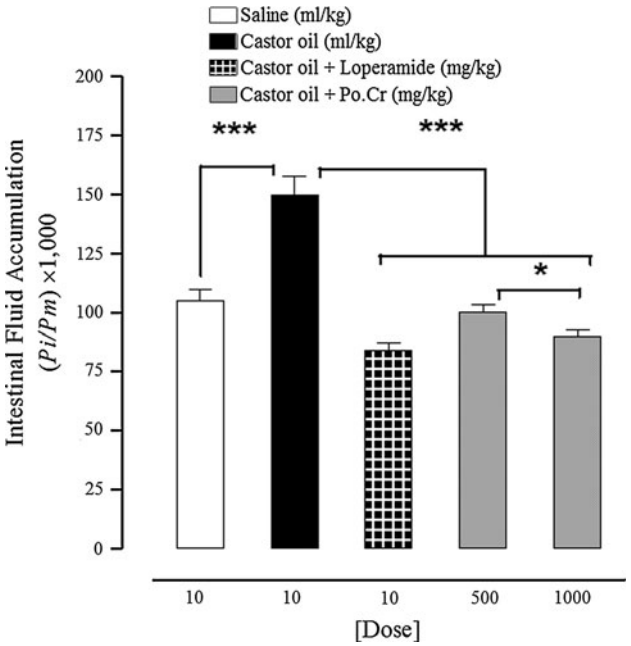

Fig. 1 The dose-dependent inhibitory effect of the crude extract of the seed husk of *Plantago ovata* (Po.Cr) on castor oil-induced fluid accumulation in the small intestine of mice. Intestinal fluid accumulation is expressed as *Pi*/*Pm* × 1,000, where *Pi* is the weight of the small intestine and *Pm* is the weight (in g) of the mouse. The values are mean ± SEM, $n = 6$. *$P < 0.05$ and ***$P < 0.001$ (one-way ANOVA followed by Dunnett's test or unpaired *t* test)

stimulatory effect of the psyllium extract, tissues were pretreated with atropine (0.1 μM), SB203186 (1 μM), hexamethonium (0.3 mM), or pyrilamine (1 μM) before redetermining the effect of Po.Cr. The spasmodic effect of Po.Cr (10 mg/ml) was diminished ($P < 0.001$) to 20.5 ± 4.6% vs. 71.2 ± 9.9% in the presence of atropine, and the presence of SB203186 reduced ($P < 0.01$) the effect to 55 ± 3.9% vs. 71.2 ± 9.9%, but no change was observed with pyrilamine or hexamethonium (data not shown). In the presence of the combined treatment of the tissue with

Table 1 The laxative effect of the crude extract of the seed husk of *Plantago ovata* (Po.Cr) in mice, without and with atropine or SB203186

Group no.	Treatment	Dose (mg/kg)	Mean defecation/group	Mean amount of wet feces/group	% Wet feces
1	Saline (mL/kg)	10	1.9 ± 0.24	0.18 ± 0.11	9.5
2	Carbachol (i.p.)	1	10.5 ± 1.34**	4.23 ± 0.43**	40.3
3	Serotonin (i.p.)	1	8.6 ± 0.54**	2.5 ± 0.36**	29.1
4	Po.Cr (p.o.)	100	3.5 ± 0.55*	0.45 ± 0.19*	12.8
5	Po.Cr	300	5.6 ± 0.39**	1.23 ± 0.16**	21.9
6	Po.Cr + atropine (i.p.)	100 + 10	3 ± 0.44	0.32 ± 0.39	10.6
7	Po.Cr + atropine	300 + 10	3.6 ± 0.24**	0.57 ± 0.04**	15.8
8	Po.Cr + SB203186 (i.p.)	100 + 1	3.1 ± 0.29*	0.36 ± 0.23*	11.6
9	Po.Cr + SB203186	300 + 1	4.2 ± 0.18*	0.75 ± 0.09*	17.8

Values are mean ± SEM, $n = 6$. *$P < 0.05$ and **$P < 0.01$ show comparison of groups 2–5 vs. group 1, groups 6 and 8 vs. group 4, and groups 7 and 9 vs. group 5 (one-way ANOVA followed by Dunnett's test or unpaired *t* test)

atropine and SB203186, the stimulatory effect of Po.Cr was significantly ($P < 0.001$) reduced to 10.85 ± 2.56% vs. 71.2 ± 9.9%, as shown in Fig. 2a.

Among the fractions obtained, the aqueous fraction (Po.Aq) at 0.3–10 mg/ml had a concentration-dependent stimulatory effect, reaching a maximum of 85.7 ± 5.0% of ACh response at the highest tested concentration (10 mg/ml). Pretreatment of the tissue with atropine (0.1 μM) significantly ($P < 0.001$) inhibited the spasmodic effect of Po.Aq, with the maximum effect declining to 37.5 ± 3.2% vs. 85.7 ± 5.0%. Similarly, the presence of SB203186 (1 μM) also inhibited ($P < 0.001$) the spasmodic effect of Po.Aq (57.5 ± 2.4% vs. 85.7 ± 5.0%). Similar to the crude extract, the contractile effect of Po.Aq was also partially ($P < 0.001$) blocked by combined treatment with atropine and SB203186, with the stimulatory effect reduced to 18.5 ± 3.4 vs. 85.7 ± 5.0%, as shown in Fig. 2b.

The petroleum fraction (Po.Pet) was more potent; its stimulatory effect was apparent at distinctly lower concentrations (0.003–0.03 mg/ml), but less efficacious, as its maximum effect was only 40 ± 4% ($P < 0.01$ vs. baseline status of the tissue) of ACh (Fig. 2c), followed by relaxation (data not shown). The stimulatory effect of Po.Pet was abolished in the presence of atropine (0.1 μM), as shown in Fig. 2c, and it remained unaltered in the presence of SB203186. Pretreatment of the tissue with pyrilamine or hexamethonium did not change the spasmodic effects of the aqueous or petroleum fractions (data not shown).

The ethyl acetate and chloroform fractions were devoid of any stimulatory effect in guinea-pig ileum.

Rabbit Jejunum Po.Cr had a concentration-dependent (1–10 mg/ml) stimulatory effect, reaching a maximum of 55.2 ± 5.3% of the ACh (1 μM) response followed by slight relaxation at 10 mg/ml. In atropinized (0.1 μM) tissue, the spasmogenic effect was markedly ($P < 0.05$) attenuated, with a maximum contractile effect of 33 ± 7.4% vs. 55.2 ± 5.3% (Figs. 3a, 4). Pretreatment of tissue with SB203186 or pyrilamine did not alter the spasmodic effect of Po.Cr (data not shown). When the mild relaxant effect of Po.Cr, observed at 10 mg/ml, was redetermined in tissue pretreated with L-NAME or methylene blue (inhibitors of the NO-cGMP pathway), it was blocked, and the stimulatory effect was potentiated with maximal of 82 ± 7.4 and 74 ± 3.9%, respectively, vs. 55.2 ± 5.3%. However, the effect of Po.Cr remained unaltered in the presence of phentolamine, as seen in Fig. 4.

When tested on high-K^+ (80 mM)-induced contractions, Po.Cr caused relaxation with an EC_{50} value of 0.29 mg/ml (95% confidence interval, 0.21–0.32; $n = 6$). The relaxant effect was attenuated in the presence of L-NAME or methylene blue with respective EC_{50} values of 0.95 mg/ml

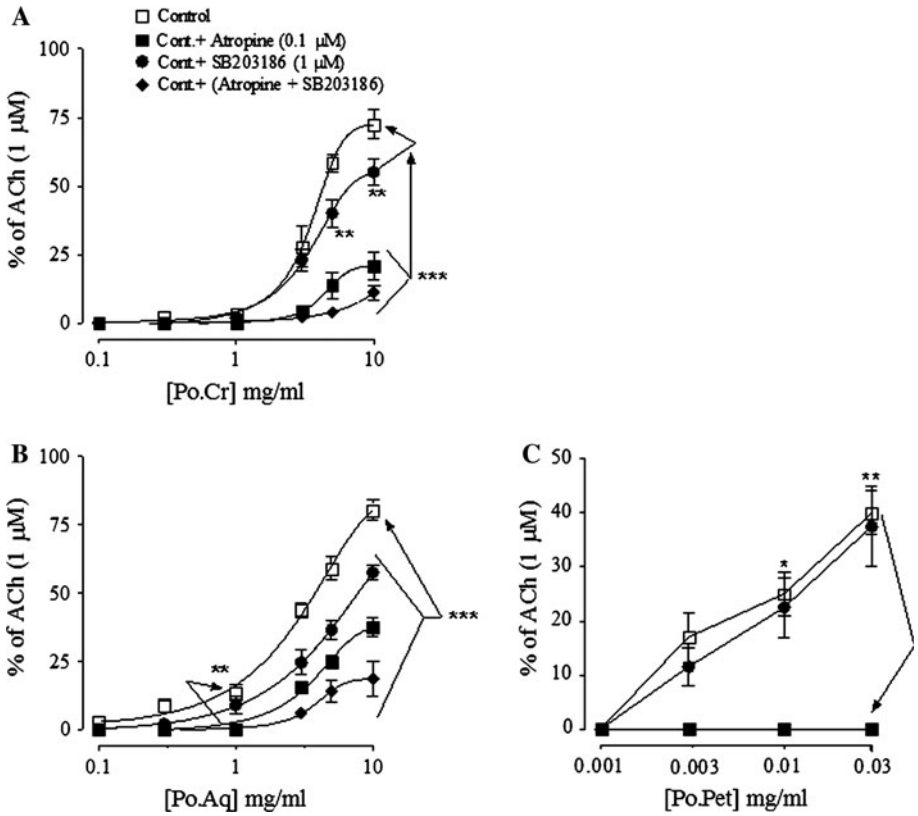

Fig. 2 The concentration-dependent spasmodic effects on isolated guinea-pig ileum preparations of **a** the crude extract of the seed husk of *Plantago ovata* (Po.Cr), and **b** its aqueous (Po.Aq) and **c** its petroleum (Po.Pet) fractions, without and with atropine (0.1 μM), SB203186 (1 μM), and atropine + SB203186. The values are mean ± SEM from 4–7 measurements. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (two-way ANOVA followed by Bonferroni's post-test correction was applied for **a** and **b**, to compare the curves with the control CRC, and one-way ANOVA followed by Dunnett's test was applied for **c**, to compare the control effects with the effects achieved in the presence of atropine)

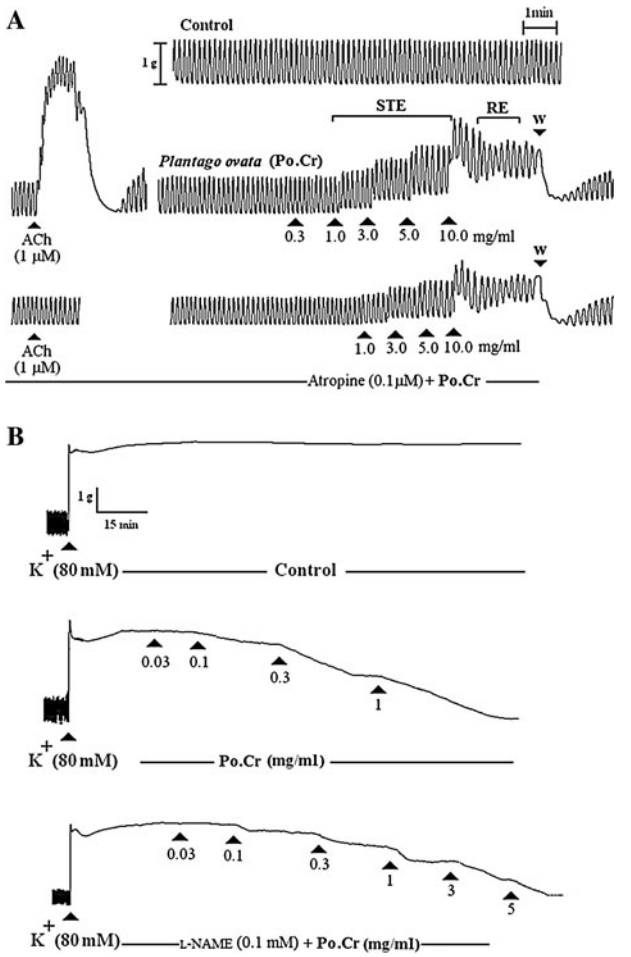

Fig. 3 Typical tracings showing the effect of the crude extract of the seed husk of *Plantago ovata* (Po.Cr,) in the absence and presence of atropine (0.1 μM) on **a** spontaneous, and **b** in the absence and presence of L-NAME (0.1 mM) on K^+ (80 mM)-induced contractions, in isolated rabbit jejunum preparations. "*W*" denotes washing of the tissue with fresh bathing solution, and "*STE*" denotes stimulatory effect whereas "*RE*" denotes relaxant effect

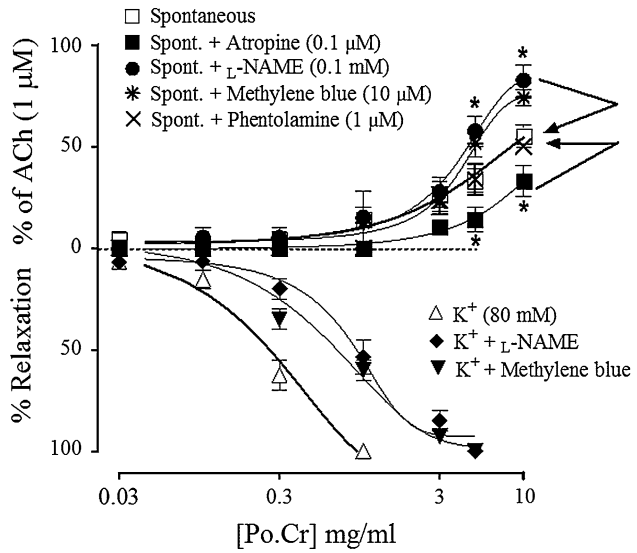

Fig. 4 The concentration-dependent stimulatory and relaxant effects of the crude extract of the seed husk of *Plantago ovata* (Po.Cr) without and with atropine (0.1 μM), L-NAME (0.1 mM), or methylene blue (10 μM) on spontaneous and K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. The values are mean ± SEM from 4–7 determinations. $^*P < 0.05$ (two-way ANOVA followed by Bonferroni's post-test correction)

(0.76–1.20, $n = 4$) and 0.60 mg/ml (0.49–0.75, $n = 3$), as shown Figs. 3b and 4, whereas it remained unchanged in the presence of phentolamine (data not shown).

When tested in Ca^{2+} CRCs for the CCB activity, Po.Cr caused a rightward non-parallel shift in the CRCs of Ca^{2+} with suppression of the maximum response at 0.03 ($P < 0.05$) and 0.1 mg/mL ($P < 0.01$), respectively, as seen in Fig. 5a. Similarly, verapamil produced a non-parallel shift in the CRCs of Ca^{2+} to the right with suppression ($P < 0.01$) of the maximum response at 0.03 and 0.1 μM, respectively, as shown in Fig. 5b.

Among the fractions, the aqueous fraction had a concentration-dependent (1–10 mg/ml) spasmodic effect reaching a maximum of 80 ± 5.7% of ACh response. Pretreatment of tissue with atropine significantly ($P < 0.001$) reduced the maximum contractile effect of Po.Aq to 54.5 ± 5.8% vs. 80 ± 5.7% at 10 mg/ml, whereas the presence of L-NAME did not cause significant ($P > 0.05$) change in its contractile effect. Po.Aq caused relaxation of high-K^+-induced contractions, with an EC_{50} value of 3.13 mg/ml (2.49–3.93, $n = 6$) as shown in Fig. 6a, whereas the effect remained unaltered in the presence of L-NAME.

The ethyl acetate fraction (Po.EtAc) caused an atropine-sensitive contractile effect at lower concentrations (0.01–0.1 mg/ml) followed by relaxation at 0.3 and 1 mg/ml. Pretreatment of tissue with L-NAME did not alter ($P > 0.05$) the effect of Po.EtAc. In high K^+-induced contractions, Po.EtAc caused relaxation with an EC_{50} value of 0.21 mg/ml (0.13–0.28, $n = 4$), as seen in Fig. 6b. However, the relaxant effect of Po.EtAc was unaffected by the presence of L-NAME (data not shown).

The chloroform fraction (Po.$CHCl_3$) was devoid of any spasmodic effect, up to the maximum tested concentration (1 mg/ml), but it caused relaxation of spontaneous and high-K^+-induced contractions with respective EC_{50} values of 0.28 mg/ml (0.23–0.35, $n = 4$) and 0.11 mg/ml (0.09–0.13, $n = 5$). Pretreatment of tissue with L-NAME inhibited the relaxant effect of Po.$CHCl_3$ with EC_{50} values of 3.35 mg/ml (2.32–4.85, $n = 3$) for spontaneous contractions and 0.81 mg/ml (0.59–1.11, $n = 4$) for K^+-induced contractions. Similarly, the relaxant effect of Po.$CHCl_3$ in spontaneous and K^+-induced contractions was attenuated in the presence of methylene blue, with respective EC_{50} values of 1.56 mg/ml (1.06–2.29, $n = 4$) and 0.49 mg/ml (0.29–0.82, $n = 5$), as seen in Fig. 6c.

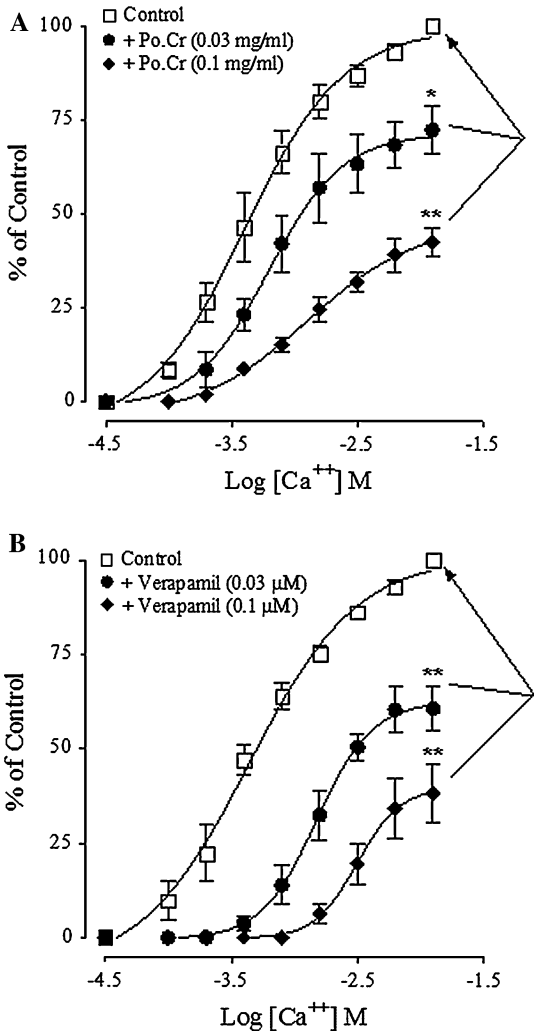

Fig. 5 The concentration–response curves of Ca^{2+} in the absence and presence of increasing concentrations of **a** the crude extract of the seed husk of *Plantago ovata* and **b** verapamil. The values are mean ± SEM from 4–6 determinations. $*P < 0.05$ and $**P < 0.01$ (two-way ANOVA followed by Bonferroni's post-test correction)

The petroleum spirit fraction (Po.Pet) had a partially ($P > 0.01$) atropine-sensitive stimulatory effect at lower concentrations (0.003–0.1 mg/ml) followed by relaxation at 0.3 and 1 mg/ml. The effect remained unaltered in the presence of L-NAME. Po.Pet inhibited K^+-induced contractions with an EC_{50} value of 0.07 mg/ml (0.04–0.08, $n = 4$), as shown in Fig. 6d. The inhibitory effect of Po.Pet was not altered when studied in the presence of L-NAME (data not shown).

Discussion

Ispaghula is well known for its superior effect on chronic constipation, and the general belief is that the laxative effect is because of its high fiber content, which may be true but the question arises why its effect is so prompt compared with other bulk-forming laxatives. We hypothesized that additional mechanisms might be involved. This was confirmed when an aqueous–methanolic extract of Ispaghula at 100 and 300 mg/kg increased the production of wet feces in mice, an effect similar to that caused by carbachol, a muscarinic agonist [42] or 5-HT, a serotonergic receptor agonist [43]. Pretreatment of animals with atropine, a muscarinic receptor antagonist [44], partially blocked the production of wet feces caused by Ispaghula, indicating the presence of ACh-like components. These results are in agreement with observations showing that medicinal plants have a gut-stimulant effect predominantly by activation of muscarinic receptors [41, 45, 46]. Interestingly, the positive effect of Po.Cr on wet feces was also partially blocked in the presence of SB203186, a 5-HT_4 receptor antagonist [47], suggesting the involvement of a serotonergic receptor-mediated stimulatory effect.

5-HT_4 agonists are documented to augment peristaltic movements in the gastrointestinal tract and, hence, are considered suitable for treatment of chronic constipation [48–50]. However, diarrhea is a common side-effect associated with the administration of 5-HT_4 agonists [11], with some indication of the possibility of cardiac-stimulatory risk factors [51]. Interestingly, the presence of the combination of the gut-relaxant constituents (CCB-like) in addition to the stimulatory components in Ispaghula may have a neutralizing effect on such side-effects attributed to serotonergic agents, because the CCBs are known for their antidiarrheal [52, 53] and cardiac-inhibitory effects [54].

At a higher dose (500 mg/kg), Po.Cr was found to have a blunting influence on its laxative effect in mice. In view of the fact that the herb is traditionally known for its antidiarrheal effect [12], and that there was an indication of inhibitory component(s), the crude extract was tested for its possible antisecretory and antidiarrheal effects in mice. Interestingly, Po.Cr (500 and 1,000 mg/kg) inhibited the intestinal fluid contents and diarrhea induced by castor oil, which induces intestinal fluid accumulation and diarrhea by causing giant contractions of the transverse and distal colon, because of its indirect effect via recinolic acid formation. In this way, castor oil causes changes in the transport of electrolytes and water, resulting in a laxative effect [55]. Thus, inhibition of fluid accumulation, and protection from castor oil-induced diarrhea indicate the antidiarrheal effect of Ispaghula.

In humans, 2.5–4 g/day of psyllium husk may be consumed, and the dose can even be increased to a level of 30 g/day until a beneficial effect on gut motility dysfunction [11, 56] has been achieved. The effective doses (100–1,000 mg/kg) of the extract which have gut-modulating effects in mice may seem higher, but it should also be noticed that the metabolic rate to body weight ratio for

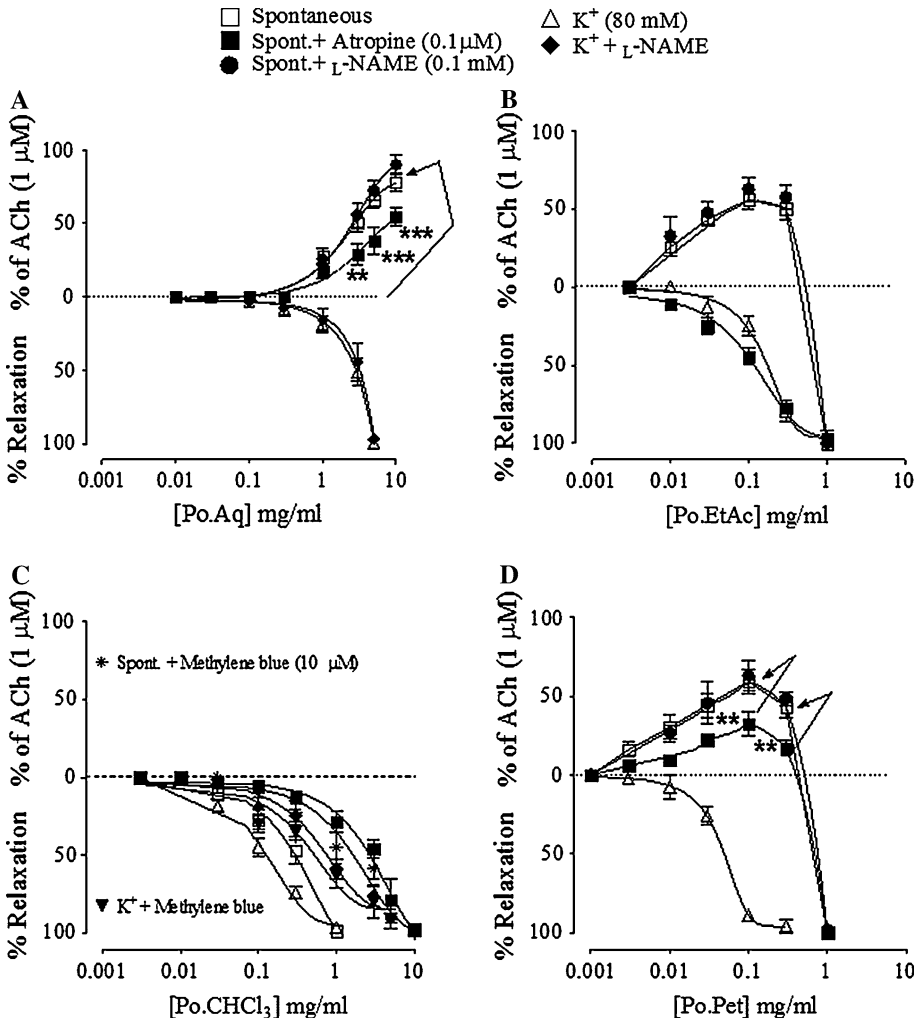

Fig. 6 Concentration-dependent stimulatory and inhibitory effects on spontaneous and K^+ (80 mM)-induced contractions, in isolated rabbit jejunum preparations, of **a** the aqueous (Po.Aq), **b** ethyl acetate (Po.EtAc), **c** chloroform (Po.$CHCl_3$), and **d** petroleum (Po.Pet) fractions of the crude extract of *Plantago ovata*, in the absence and presence of atropine (0.1 μM), L-NAME (0.1 mM), or methylene blue (10 μM). The values are mean ± SEM from 3–5 determinations. $^{**}P < 0.01$ and $^{***}P < 0.001$ (two-way ANOVA followed by Bonferroni's post-test correction)

small animals is much greater (approximately 10 times) than in humans [57]. Thus, for an individual of average weight (70 kg) a postulated dose (after observing the results of this investigation) of the plant extract may be close to the dose usually consumed by people in traditional medicine practice (2.5–30 g).

Isolated preparation of guinea-pig ileum was used to study the possible mechanism(s) underlying its laxative effect. Guinea-pig ileum (a quiescent preparation) is considered suitable for assessment of the spasmogenic effect of the test material [41, 46]. Po.Cr had a stimulatory effect, mediated partially through muscarinic and 5-HT_4 receptor activation, which was evident from its partial blockade in the presence of atropine or SB203186. Co-administration of atropine and SB203186 failed to completely block the stimulatory effect of the plant extract and its aqueous fraction, suggestive of some additional mechanism(s) in the spasmodic effect of Ispaghula. To further characterize the stimulatory effect of Po.Cr, the tissue was pretreated with pyrilamine, an H_1 receptor blocker [58], and hexamethonium, a ganglion blocker [59], but the fact that neither of these affected the stimulatory effect of Po.Cr indicates a third component of the stimulatory effect of Ispaghula involving neither histaminic nor nicotinic receptor activation; this remains to be elucidated.

When tested in spontaneously contracting rabbit jejunum, considered a suitable preparation to test the dual (spasmodic and antispasmodic) effect of the test material [46], the plant extract had a concentration-dependent stimulatory effect followed by mild relaxation, indicating the presence of stimulatory and inhibitory constituents. As in guinea-pig ileum, the spasmodic effect of Po.Cr was attenuated in the presence of atropine but was resistant to SB203186, suggesting that the spasmodic effect is independent of 5-HT receptor activation. This observation demonstrates the tissue or species-specific gut-stimulatory effects of Ispaghula, mediated through 5-HT receptors. Such a tissue-specific 5-HT-mediated response could be explained by multiple studies [47, 60, 61] in which guinea-pig ileum was used to characterize the 5-HT_4 receptor mediated responses instead of using rabbit jejunum, which may indicate that the former preparation has a higher

density of serotonergic receptors. Moreover, the mild relaxant effect of Po.Cr observed at 10 mg/ml was blocked with a resultant increase in its stimulatory effect in the presence of L-NAME, an NOS-inhibitor [62], showing a NO-mediated relaxant effect.

The animal or human gut is innervated by enteric non-adrenergic and noncholinergic (NANC) inhibitory neurons. NO is synthesized from the amino acid L-arginine and produces relaxation in the gut muscles through NO-cGMP mechanisms [63]. To study the possible involvement of the cGMP pathway, the plant extract was studied for its spasmolytic effect in the presence of methylene blue, a guanyl cyclase inhibitor [64], which altered the effect of Po.Cr in a manner similar to that of L-NAME. However, it seems that the enhanced spasmodic effect of Po.Cr in the presence of L-NAME or methylene blue is possibly due to interference with the NO-cGMP mediated relaxant effect, which indirectly promotes the stimulatory effect.

When tested on high K^+ (80 mM)-induced contractions, the plant extract caused concentration-dependent relaxation. K^+ at high concentrations (>30 mM) is known to cause smooth-muscle contractions by opening of voltage-dependent Ca^{2+} channels (VDCs), allowing influx of extracellular Ca^{2+} to exert the contractile effect [65]. Thus, a substance causing inhibition of high K^+-induced contraction is regarded as a Ca^{2+} antagonist. The presence of Ca^{2+} antagonist-like constituent(s) was confirmed when the extract of Ispaghula caused a concentration-dependent rightward shift in the CRCs of Ca^{2+}, similar to that caused by verapamil, a standard Ca^{2+} channel blocker [66]. Ca^{2+} antagonists are also considered useful in diarrhea [42], hence, the presence of CCB-like constituents in Ispaghula might be contributing to its antidiarrheal effect, though additional mechanism(s) cannot be ruled out. When the inhibitory effect of Po.Cr was redetermined in K^+-induced contractions, in tissue pretreated with L-NAME or methylene blue, the relaxant effect was attenuated, suggesting the involvement of NO-cGMP pathways in the antispasmodic effect of Ispaghula, in addition to the CCB-like spasmolytic activity.

In addition to the medicinal value of Ispaghula in diarrhea, the presence of spasmolytic component(s) is, perhaps, also intended by nature to offset the excessive stimulant effect, which could otherwise have been harmful, and which is usually observed when chemical drugs are used to treat constipation [11]. Acetylcholine is one of the most important physiological mediators with physiological importance in the peristaltic movement of the gut [42]; however, cholinergic drugs are not used in the treatment of constipation because of their side-effects, for example diarrhea and abdominal cramps [11].

Activity-guided fractionation of the crude extract revealed that its petroleum fraction was the most potent in imparting a spasmodic effect in guinea-pig ileum, because the effect was mediated at concentrations 100–1,000 times lower than required for the parent extract or the aqueous fraction. However, this fraction was the least efficacious in mediating its maximum stimulatory effect (40 ± 4% of ACh). The aqueous fraction was approximately ten times more potent and more efficacious ($P < 0.05$) in producing a stimulant effect (85.7 ± 5.0%) when compared with that of the parent extract (71.2 ± 9.9%).

In rabbit jejunum, use of the fractions of Po.Cr revealed that the antispasmodic effect (CCB and NO-cGMP-mediated) had been removed from its chloroform fraction, whereas the other fractions had dual (spasmogenic and spasmolytic) activity. Amongst these, the aqueous and petroleum fractions had a partially atropine-sensitive stimulant effect, and the stimulatory effect of the ethyl acetate fraction was fully atropine-sensitive. However, the efficacy of the stimulant effects of the petroleum and ethyl acetate fractions was found to be similar ($P > 0.05$). The aqueous fraction was more efficacious ($P < 0.01$) for its stimulatory effect (80 ± 5.7%) than the parent extract (55.2 ± 5.3) or the other fractions. The relaxant constituent(s) in Ispaghula or its fractions were found to have the following order of potency: Po.Pet > Po.$CHCl_3$ ≥ Po.EtAc ≥ Po.Cr > Po.Aq.

Taken together, the results obtained from the experiments on the guinea-pig ileum and rabbit jejunum were indicative of a tissue or species-selective gut-modulating effect of Ispaghula extract, which was evident in the following ways:

1 the stimulatory effect of the crude extract was greater ($P < 0.01$) in the ileum than in the jejunum;
2 the 5-HT receptor-mediated stimulatory effect of the crude extract and its aqueous fraction was seen only in the ileum;
3 the ethyl acetate fraction had a stimulatory effect only in the jejunum; and
4 the indication of a fully atropine-sensitive stimulatory effect of the petroleum fraction in the ileum whereas a partially atropine-sensitive spasmodic effect was seen in jejunum.

Such types of tissue or species-selective effects have also been found in previous studies [46, 67, 68].

Results from preliminary phytochemical analysis, which show the presence of different phytochemicals (saponins with known spasmogenic effect [69] and tannins and flavonoids with antisecretory, antidiarrheal, and antispasmodic activity [70–72]), also support the gut-modulating (stimulatory and inhibitory) activity of Ispaghula. However, in normal gut status, the laxative effect of Ispaghula is dominates its antidiarrheal activity, probably because of its high fiber and mucilaginous content.

Conclusion

These results suggest that psyllium husk has gut-stimulatory components mediated through muscarinic or 5-HT_4 receptor activation, along with uncharacterized component(s), and that the gut-inhibitory components possibly involve the CCB and NO-cGMP pathways, which may explain its dual efficacy in constipation and diarrhea.

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Conflict of interest No competing financial interests exist.

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