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Original Article

Antispasmodic Activity of Dried Ginger 70% Methanolic Crude Extract in Isolated Smooth Muscle Preparations

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ABSTRACT

Objectives: Ginger (*Zingiber officinale* Roscoe) is a popular edible herb consumed and known globally for its culinary and medicinal properties. Particularly in South Asia, the ginger rhizome is used in several Gastrointestinal (GI) related ailments. In this study, we report preliminary findings on the GI relaxant activity of the dried variety of ginger rhizome. **Materials and Methods:** Ginger rhizome was soaked in 70% aqueous-methanol and dried to give the extract (Gd.Ex). Segments of different isolated smooth muscle preparations were suspended in tissue baths. **Results:** Phytochemistry profiling showed that the extract has terpenoids, phenols, and alkaloids. On baseline of isolated tissues like rat stomach pylorus, rabbit jejunum, guinea-pig ileum and colon, and rat uterus, Gd.Ex was devoid of any stimulant effect up to 10 mg/mL. The extract was able to completely inhibit spasmogenicity induced with K^+ 80 mM in stomach pyloric strips, indicating a Ca^{2+} Channel Blocking (CCB) mechanism. Gd.Ex in bolus (1-10 mg/mL) and cumulative dosing (0.3-3 mg/mL), showed a relaxant effect on spontaneously contracting baseline of rabbit jejunum. The extract was then tested against different standard GI stimulants like acetylcholine (ACh, 0.3 µM), histamine (0.3 µM), and K^+ (50 mM) in guinea-pig ileum. Gd.Ex (3 mg/mL) pretreatment abolished the stimulant responses. A similar inhibitory effect of the extract (0.3-1 mg/mL) was observed in guinea-pig colon and rat uterus against ACh. **Conclusion:** The study shows the spasmolytic potential of dried ginger extract in different smooth muscle preparations. Together with the earlier published results of dried ginger in rat stomach fundus, this explains the benefit of ginger in potentiating gastric emptying and alleviating nausea.

Keywords: *Zingiber officinale*, Rhizome, Antidiarrheal, Rat, Rabbit, Guinea-pig.

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INTRODUCTION

Zingiber officinale Roscoe (family: Zingiberaceae), or ginger as it is commonly called, is a common food additive and condiment used to spice up food all over the world. The pungent smell and sweet/spicy taste give food an added taste when it is added. It is a perennial herb that is indigenous to South Asia, China, Mexico, and Jamaica.[1,2] As popular as it is for its use as a condiment, it is also used as a medicinal plant, particularly in the Traditional Chinese and South Asian systems of medicine. Ginger has been used by Chinese traditional health practitioners for over 2500 years.[1] Today, the total global ginger market revenue stands at $5.3 billion.[3] In 2018, a colossal 564 thousand tonnes of ginger were exported worldwide. China (69% of export) is the biggest exporter of this herb, followed by Thailand, Peru, India, and Brazil. The main importers are the USA, Japan, Netherlands, and UAE.[3] These numbers give an idea of the extent of popularity this herb enjoys.

From the plant, it is the rhizome that is the most prized and valuable part of the herb.[2] Ginger rhizome is used both for its condiment and medicinal properties, in the fresh and dried varieties. Oils are also extracted from the rhizome, while it is also utilized in the preserved form. Dried ginger (locally called 'Sunth' in South Asia) is prepared from fresh ginger. The fresh ginger is sun-dried, cleaned, and then immersed in water. The rhizome external coating is discarded, then the rhizome is again cleaned and sun-dried.[4] Although ginger has many different traditional uses, its most important use is in the disorders of the Gastrointestinal (GI) tract. There are many GI conditions and several ways of using ginger, either alone or in combination with other herbs. The ginger rhizome is used medicinally both as a GI relaxant and stimulant[4,5] in dyspepsia; flatulence; indigestion; nausea/vomiting/retching; colic; bowel and stomach spasms and pain; diarrhea; and to treat low appetite.[2,4,5]

Many different chemicals have been isolated from the ginger rhizome. Broadly speaking, there are both volatile and

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non-volatile components in it.[2] The most common of the non-volatile phenol constituents are the gingerols (most common 6-gingerol) and shogaols (6-shogaol). These are the pungent principles in ginger.[4,6] The volatile component consists of chemicals including zingiberene (responsible for the ginger's flavour), bisbolene, curcumene, farnesene, and many other monoterpenes such as borneol.[2]

Ginger has been investigated and found effective for several medical conditions. Ginger has been found to have antidepressant;[7] weight-reducing;[8,9] anti-inflammatory;[10,11] antifungal;[12,13] antihypertensive and cardioprotective;[14,16] antiulcer;[17] antidiabetic;[18] antiemetic;[19] hepatoprotective;[20] anticancer;[21,22] renoprotective;[23] and antineuropathic[24] properties. Additionally, there are many other studies, of all types, justifying the many traditional uses and other benefits of ginger rhizome.

As discussed earlier, there are different types of ginger, mainly the fresh and the dried variety. The process of drying leads to chemical changes in the rhizome, one such change is the conversion of gingerols to shogaols due to dehydration.[25-27] As most of the pharmacological studies are done on fresh ginger, we wanted to investigate the activity of a dried *Zingiber officinale* 70% methanolic extract on contractility of different isolated GI and uterine smooth muscle tissues. The results from the current study, and an earlier study we reported on dried ginger extract,[28] will be discussed to explain the potential of dried ginger in GI motility, particularly its benefit as an antiemetic agent.

MATERIALS AND METHODS

Chemicals and reagents

The standard reagents purchased from Sigma Chemical Company, USA were: acetylcholine (ACh), estradiol, and histamine dihydrochloride (Hist). Chemicals for constituting the physiologic salt solutions (De Jalon, Krebs, and Tyrode) were acquired from either Sigma Chemical Company or E. Merck, Germany. The composition of salt solutions used is given below:

Tyrode (mM) for rat stomach pylorus, rabbit jejunum, guinea-pig ileum: 2.68 KCl, 136.9 NaCl, 1.8 $CaCl_2$, 5.55 glucose, 11.9 $NaHCO_3$, 1.05 $MgCl_2$, 0.42 NaH_2PO_4.

Krebs (mM) for guinea-pig colon: 4.7 KCl, 118.2 NaCl, 2.5 $CaCl_2$, 11.7 glucose, 25.0 $NaHCO_3$, 1.3 KH_2PO_4, 1.2 $MgSO_4$.

De Jalon (mM) for rat uterus: 5.6 KCl, 154.0 NaCl, 0.5 $CaCl_2$, 2.8 glucose, 6.0 $NaHCO_3$.

Animals

Sprague-Dawley rats (both sexes, weight around 200 g), rabbits (weighing a kg), and guinea-pigs (around 500 g) of either sex were kept in the animal facility of Aga Khan University. The temperature inside the facility was maintained at 23°C and the environment was pathogen-free. The animals were provided drinking water as needed and food (containing Nutri-Vet, flour, salt, fibre, grease, molasses, $K_2S_2O_5$, seafood, and powdered milk), which was withheld a day before running the experiments. All steps were taken to prevent and minimize the suffering of any sort to the animals. Experiments were performed ethically as per the laboratory animal use and care guidelines (EEC Directive 86/609/EEC) of the European Community.

Plant material and extract preparation

The extract to be tested was prepared from 1 kg of dried ginger rhizome (Figure 1) purchased from a local supplier in Karachi, Pakistan. Dried ginger rhizome was cut into small pieces, to enable more contact between the rhizomes and solvent, and then immersed in eight litres of 70% methanol for 72 hr. The solvent was then passed through a piece of porous cloth while the ginger rhizome was soaked in a new batch of solvent for another 72 hr, twice. All the solvent was collected in the end and was passed through Whatman grade-1 filters. This filtrate was evaporated with the use of a rotary evaporator. The extract (labelled as Gd.Ex) weighed ~126 g (yield of 12.6%, weight/weight). The extract was dissolved in saline on the day of the experimentation, and subsequently stock solution and dilutions were prepared. Saline solution served as the negative control, with no effect on the contractility of the tissue preparations (data not shown).

Phytochemical analysis

Phytochemical analysis of Gd.Ex was performed, as described earlier,[29,30] with help of thin layer chromatography. Silica gel G plates were used. Gd.Ex was solubilized and the plates were developed in a solvent followed by spraying the developed plates with an appropriate reagent[31] to detect the presence or absence of classes of chemical compounds (see Figure 2 for details).

Rat stomach pylorus

The experiments were performed using a method described earlier, with modifications.[32] Briefly, rats were sacrificed and the stomach was dissected out of the abdominal cavity. A portion of

Figure 1: Dried ginger rhizome.

the proximal duodenum (around 3 cm) was also left attached to the stomach. The pylorus was sliced in a circle format. This was later cut opened and pyloric strips (5x12 mm) were obtained. These strips were hung in organ baths (preload of 1 g used), immersed in a salt solution with a gas mixture of O_2/CO_2 (95/5%), at 37°C. Metric changes in contractility of the tissue were observed via Harvard oscillographs and force transducers from Harvard Apparatus, Holliston, MA, USA. The tissue was left to stabilize for half an hour. Later, it was given repeated concentrations of the standard agonist ACh 3 µM (Figure 3A). Once consistent ACh responses were observed, the tissue was ready for testing and experimentation.

Rabbit jejunum

The isolated rabbit jejunum preparations were prepared for experiments as described earlier.[33] Briefly, pieces of rabbit jejunum (1-2 cm) were hung with a cotton thread in tissue baths. The tissues were maintained immersed in a salt solution, at physiologic temperature, and bubbled with the gas mixture. A preload of 1 g was used. Isotonic changes were recorded with the use of Harvard equipment. Tissues were left to normalize in the solution for 30 min before any drug was added. Rabbit jejunum, under normal circumstances, beats spontaneously. This behaviour of the tissue helps in testing for muscle relaxants, although stimulants can also be tested. The tissues were stabilized with ACh concentrations of 0.3 µM (Figure 4). Once consistent responses of ACh were noted, the tissue was ready for challenge from the extract (Figures 4, 5). Relaxation from the extract was calculated as a percent change in spontaneous behaviour of the tissue.

Guinea-pig ileum

The tissues were prepared for experiments as described earlier.[34] Briefly, a couple cm long pieces of the tissue (1-2 cm long) were obtained from guinea-pigs. Tissues were maintained in baths with salt solution aerated with the gas mixture. Preload of a gram was used on the tissues. Isotonic changes were noted using Harvard equipment. Tissues were kept for 30 min to normalize and then made stable by using concentrations of a standard agent, ACh 0.3 µM (Figure 6B). A 3 min gap was allowed between the administration of drugs. In contrary to rabbit jejunum, guinea-pig ileum does not beat spontaneously, but rather maintains a quiescent baseline ideal for testing stimulant drugs. Spasmolytic

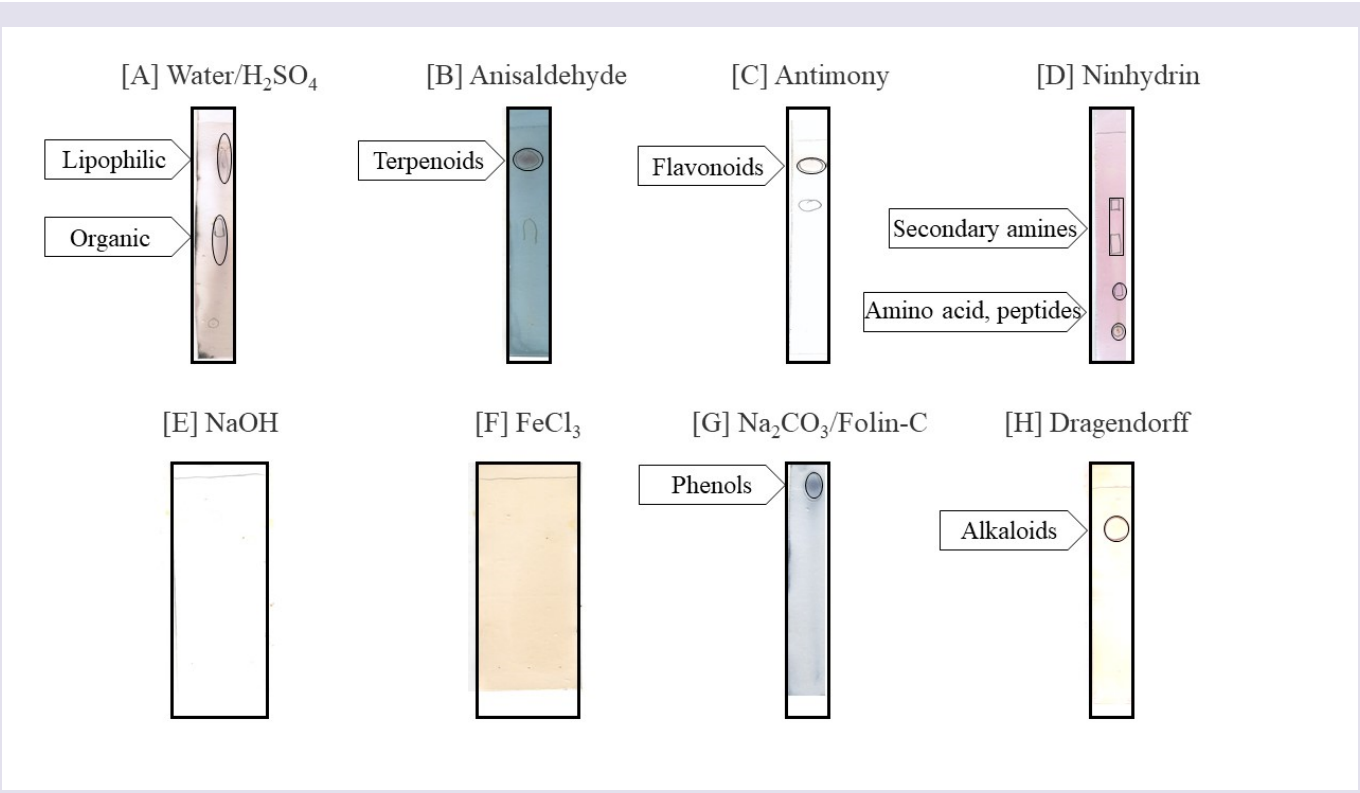

Figure 2: Thin Layer Chromatography (TLC) plates showing the phytochemical analysis for dry ginger methanolic extract (Gd.Ex). Concentration of the extract was 10 mg/mL in $CHCl_3$: MeOH [2:1], while the solvent system was $CHCl_3$: MeOH [3:17]. [A] shows lipophilic (spray: water), organic chemicals (spray: 10% H_2SO_4); [B] presents presence of purple spots for terpenoids (spray: 0.5% anisaldehyde in H_2SO_4, glacial acetic acid + methanol, 5:10:85 v/v); [C] shows flavonoids and terpenoids under UV (spray: 10% antimony trichloride in chloroform trichloride); [D] box shows secondary amines while circles show amines and peptides (spray: 0.5% ninhydrin in acetone); [E] does not show any pink or red spots so absence of anthraquinones (spray: 5% ethanolic NaOH); [F] does not show any blue spots so indicates absence of tannins (spray: aqueous $FeCl_3$); [G] shows blue spots for phenols (spray: 20% aqueous Na_2CO_3 followed by Folin-Ciocalteu reagent); and [H] shows orange spots for presence of alkaloids (spray: Dragendorff). Investigation was performed with naked eye in visible light and below UV (λ = 365 nm).

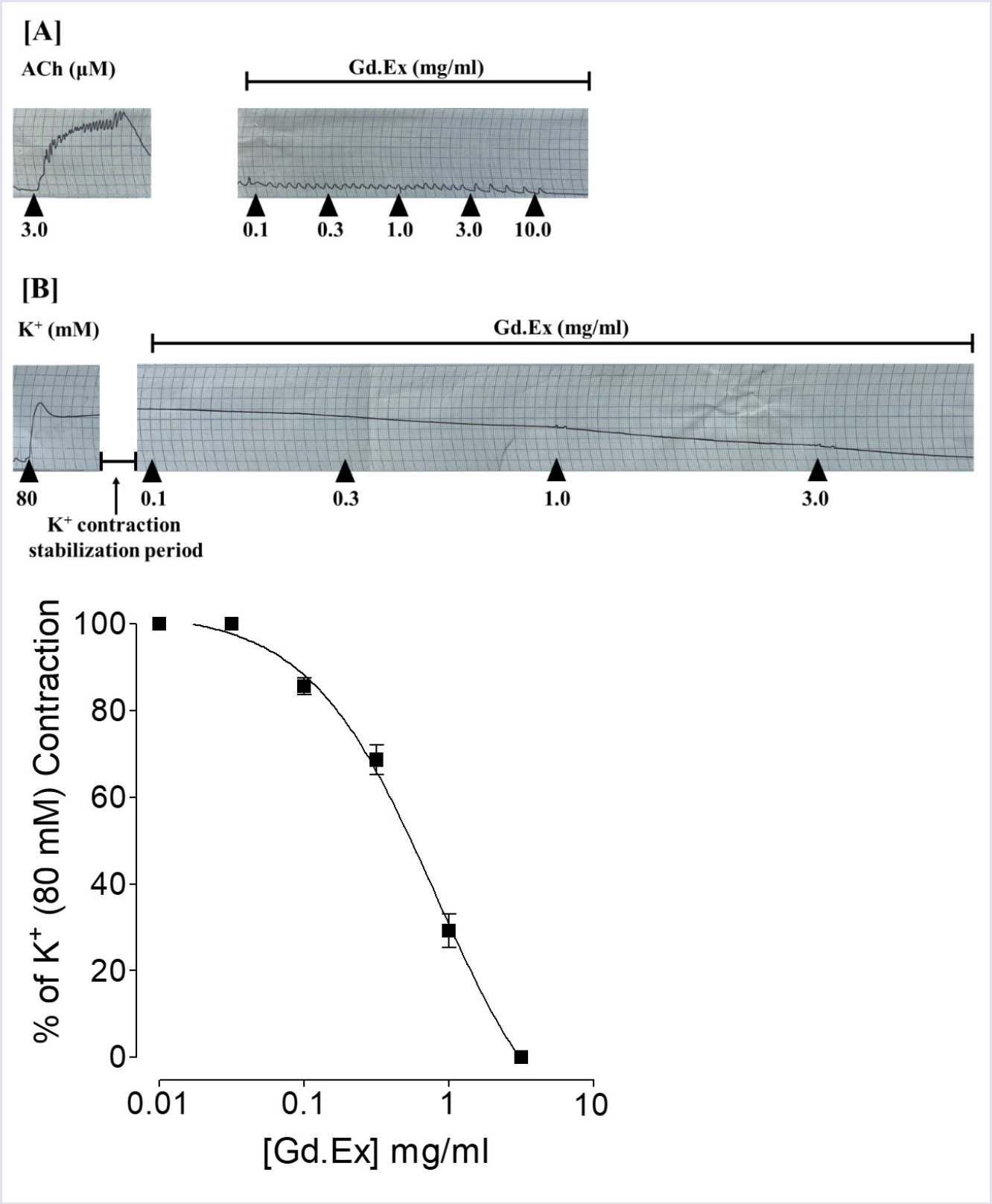

Figure 3: Activity of dried ginger 70% methanolic extract (Gd.Ex) upon rat stomach pyloric strips. Tracing [A] shows stimulant effect of acetylcholine (ACh) 3 μM control followed by influence of escalating concentrations of Gd.Ex on resting baseline of tissue. Tracing [B] and the graph show the relaxant activity of Gd.Ex upon K^+ 80 mM. Values shown are mean ± SEM of 4 observations.

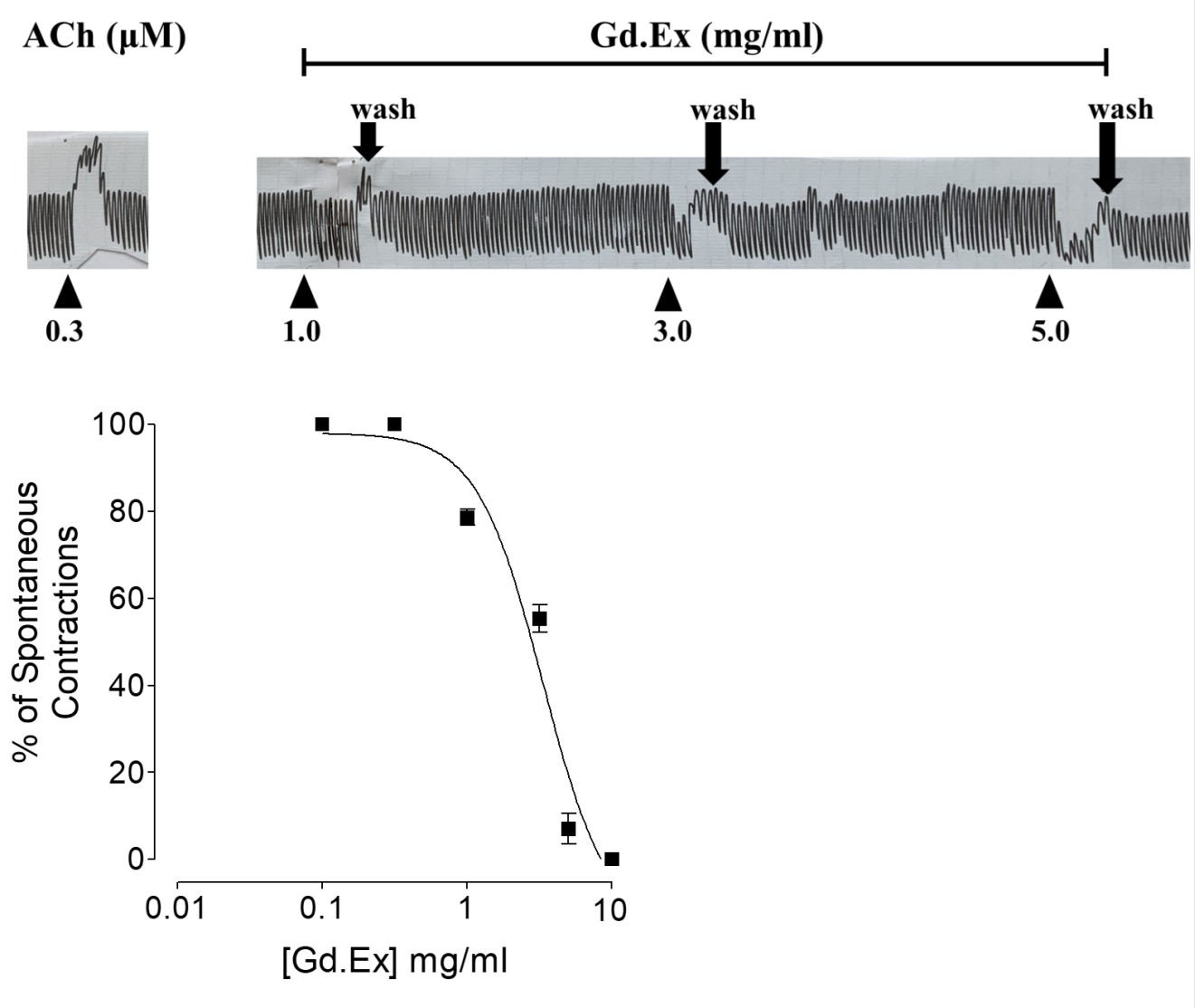

Figure 4: Effect of dried ginger 70% methanolic extract (Gd.Ex) on rabbit jejunum. Tracing shows stimulant effect of acetylcholine 0.3 μM control on spontaneous beating jejunum. Tracing and graph show the relaxant effect of increasing concentrations of Gd.Ex, given in a bolus manner, on baseline of tissue. Values shown are mean ± SEM, 3 observations.

agents can be tested, but with the use of a standard agonist like ACh, histamine, and potassium chloride or K^+ (Figure 6).

Guinea-pig colon

We have described working with the guinea-pig colon in detail previously.[34] Briefly, a decimetre long segment of tissue (around half a decimetre down the starting of colon) was obtained. Pieces around 1-2 cm in length were hung in baths with salt solution at 37°C and bubbled with the gas mixture. Tension of ~2 g was used. Similar Harvard equipment was used for recording. The tissues were left to normalize for half an hour, after which time 0.3 μM of ACh were added to the baths until consistent responses were seen. This indicated that the tissue is ready to be used for testing. Guinea-pig colon is similar to both rabbit jejunum and guinea-pig ileum in terms of spontaneity. It does not completely show a flat baseline, but some disturbance is visible (Figure 7A). This is suitable to examine if a substance has relaxant activity, although stimulants can also easily be noted. Similar to guinea-pig ileum, quantifying a spasmolytic agent requires the use of a standard agonist like ACh (Figure 7).

Rat uterus

The extract was tested on isolated rat uterine horns using the methodology described previously.[35] Briefly, 0.1 mg/kg of β-estradiol was given to female rats subcutaneously 24 hr before the day of testing to get an estrogenized uterus. The two uterine horns were obtained from the abdominal cavity of rats. Uterine tissues were mounted in the organ baths with salt solution,

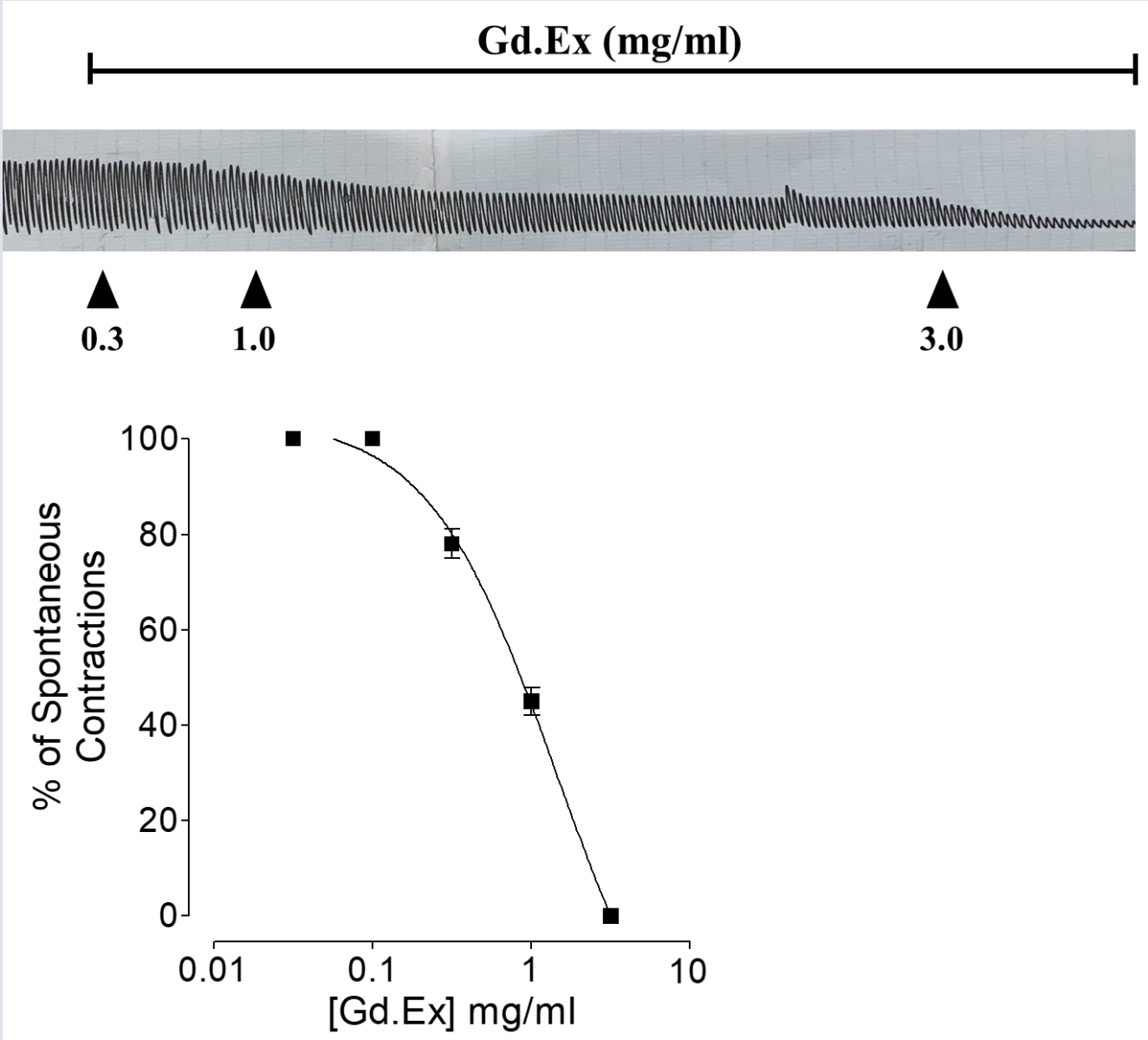

Figure 5: Influence of dried ginger 70% methanolic extract (Gd.Ex) on rabbit jejunum. Tracing and graph show the relaxant effect of increasing concentrations of Gd.Ex, given in a cumulative fashion, on baseline of tissue. Values shown are mean ± SEM of 3 observations.

aerated with the gas mixture at 32°C. Recordings were made using isotonic Harvard transducers and equipment. For this tissue, a preload 0.5 g was used. Similar to the other tissues used, a 30 min period was given for the preparations to normalize. The tissues were challenged with repeated concentrations of the standard uterotonic agent ACh (1 μM) (Figure 8A). When consistent responses were seen, the preparation was considered ready for testing.

Experimental protocol

The extract, in increasing concentrations, was tested for any pharmacological activity on different isolated tissue preparations. The extract was initially investigated using the quiescent tone of all the tissue preparations. This was done to see if the extract has any tone modulatory effects (either relaxant or stimulant). In the case of the rabbit jejunum tissue, it has rhythmic spontaneous contractions, which makes it easy to test for both spasmolytic and spasmogenic agents. For all the other tissue preparations (stomach pylorus, guinea-pig ileum, guinea-pig colon, rat uterus), as they do not have baseline contractility (the stimulant effect can be tested without the use of an agonist but spasmolytic behaviour can only be tested with the use of an agonist), different standard agonists were used to quantify the spasmolytic behaviour of the test extract. Table 1 shows how the different isolated tissue

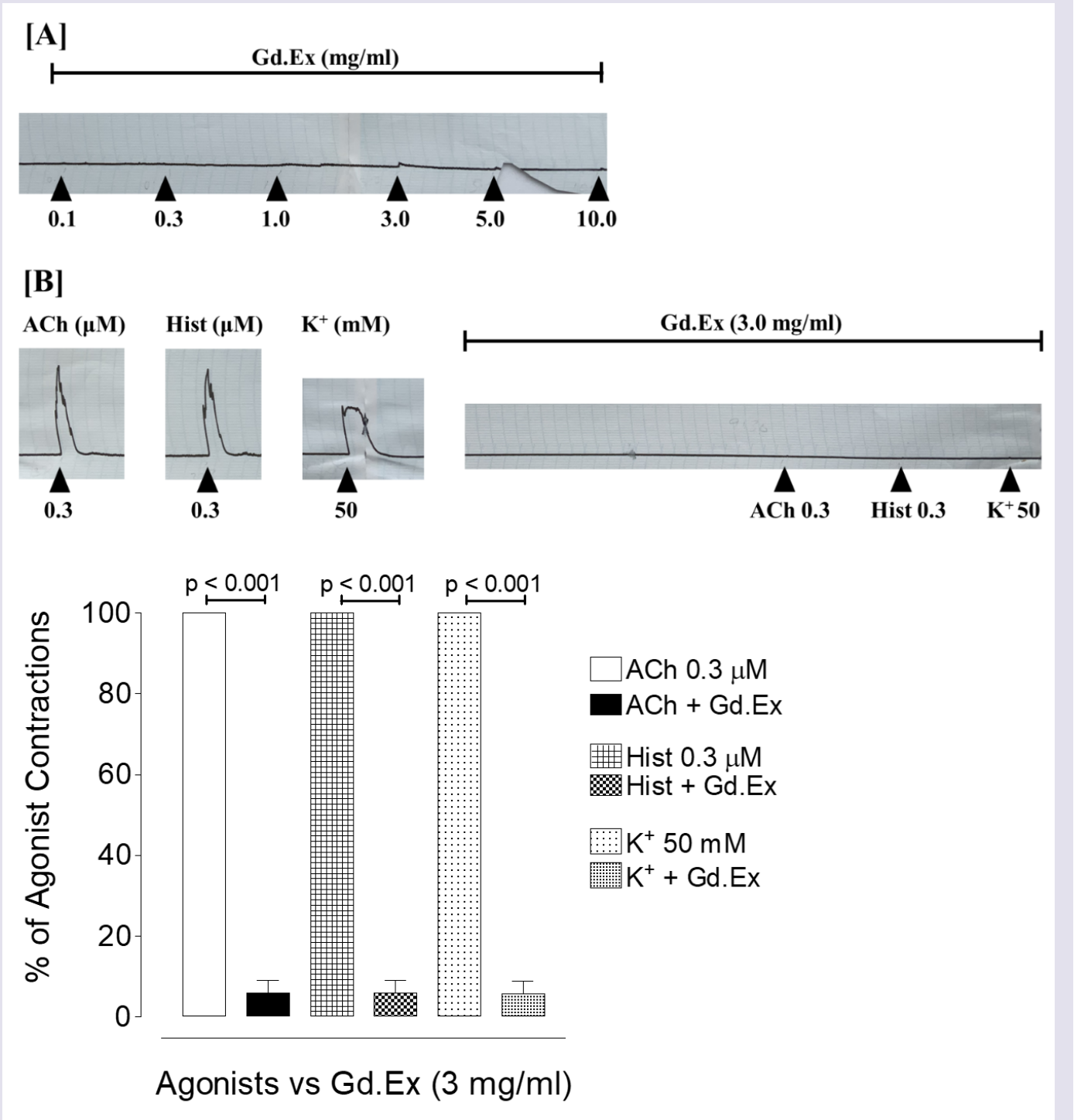

Figure 6: Dried ginger 70% methanolic extract (Gd.Ex) tested on guinea-pig ileum. Tracing [A] shows effect of increasing concentrations of Gd.Ex on resting baseline of tissue. Tracing [B] and the graph show the inhibitory effect of Gd.Ex 3.0 mg/mL (30 min pretreatment) on different standard intestinal stimulants: acetylcholine (ACh) 0.3 µM, histamine (Hist) 0.3 µM and K^+ 50 mM. Values shown are mean ± SEM of 3 observations, one-way ANOVA with Bonferroni's multiple comparisons test.

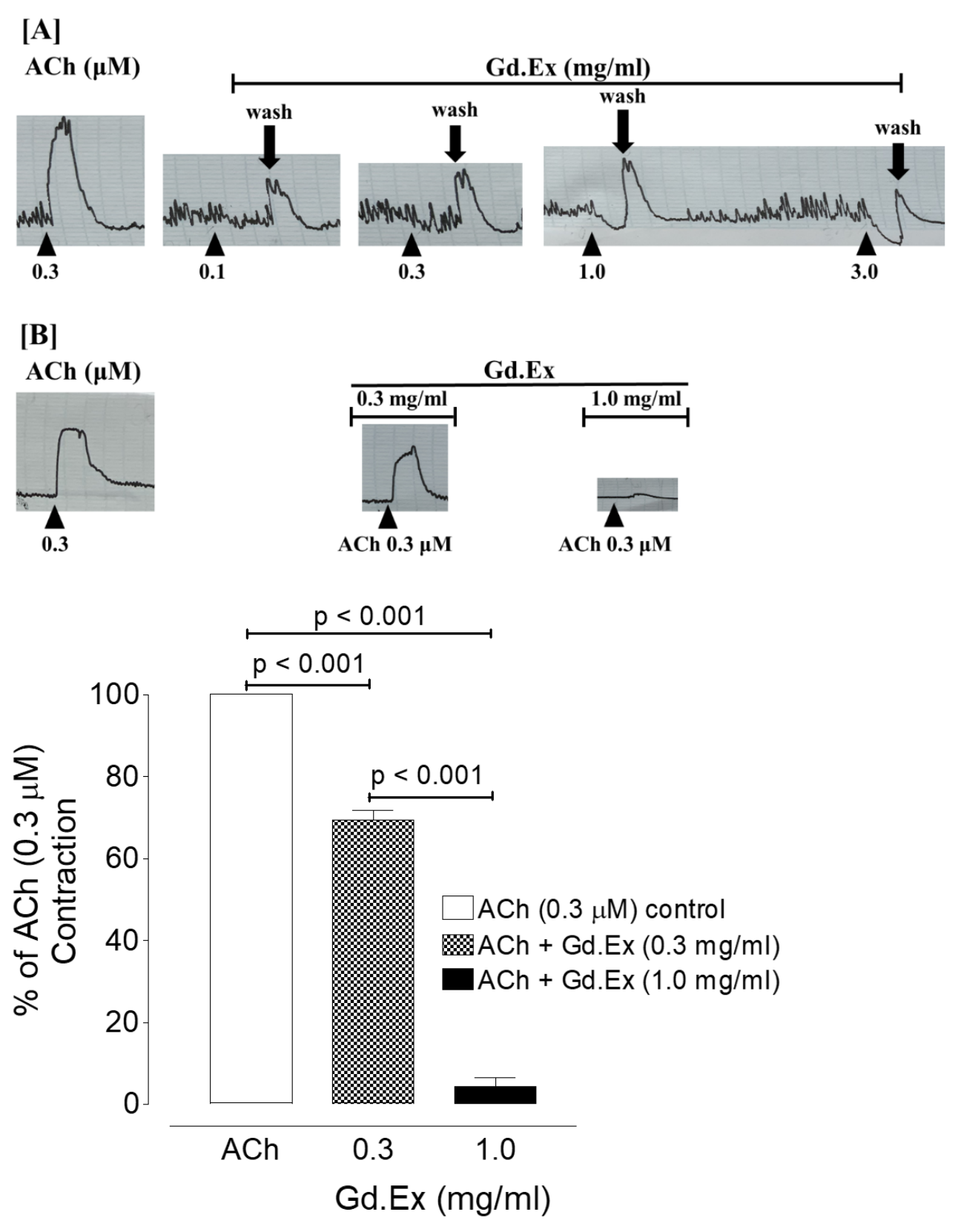

Figure 7: Effect of dried ginger 70% methanolic extract (Gd.Ex) on guinea-pig colon. Tracing [A] shows stimulant effect of acetylcholine (ACh) 0.3 µM control followed by effect of increasing concentrations of Gd.Ex on resting baseline of tissue. Tracing [B] and the graph show the relaxant effect of increasing concentrations of Gd.Ex (0.3-1.0 mg/mL, 30 min pretreatment) on ACh 0.3 µM. Values shown are mean ± SEM of 3 observations, one-way ANOVA with Bonferroni's multiple comparisons test.

preparations were used to elucidate the pharmacology of the test extract.

Statistical analysis

Data mentioned in the paper is mean ± SEM ('n' is the # of observations) and EC_{50} (median effective concentrations) with 95% confidence interval (CI). Stats used: Student's paired t-test and one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. These were applied via GraphPad program from San Diego, CA, USA. A p value of < 0.05 was taken as significantly different.

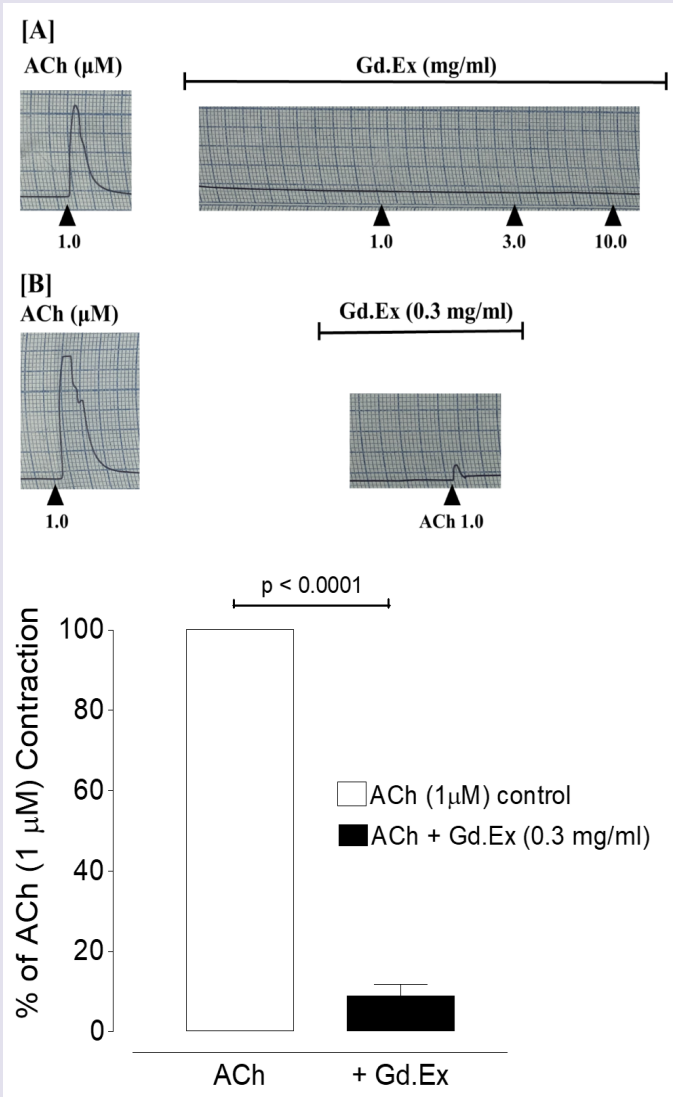

Figure 8: Effect of dried ginger 70% methanolic extract (Gd.Ex) on rat uterine horns. Tracing [A] shows stimulant effect of acetylcholine (ACh) 1.0 µM control followed by effect of increasing concentrations of Gd.Ex on resting baseline of tissue. Tracing [B] and the graph show the relaxant effect of Gd.Ex (0.3 mg/mL, 30 min pretreatment) on ACh 1.0 µM. Values shown are mean ± SEM of 5 observations, paired t-test.

RESULTS

Phytochemical analysis

The phytochemical analysis helped to determine the composition in terms of classes of chemicals in Gd.Ex. It tested positive for most of the chemical classes tested, namely: lipophilic and organic compounds (Figure 2A); terpenoids (Figure 2B); flavonoids (Figure 2C); secondary amines, amino acids, and peptides (Figure 2D); phenols (Figure 2G); and alkaloids (Figure 2H). Gd.Ex did not have detectable anthraquinones (Figure 2E) or tannins (Figure 2F).

Rat stomach pylorus

For the effect of Gd.Ex on the default baseline of stomach pyloric strips, it was found not to have any effect up to the concentration of 10 mg/mL (Figure 3A). There was no stimulant effect, similar to the one shown by the standard agonist ACh 3 µM (Figure 3A). There could be a relaxant effect, but as the baseline of the tissue is almost flat, a relaxant effect can only be observed with the use of an agonist. The sustained contraction was induced in the tissue with high K^+ 80 mM. The extract, concentration-dependently, inhibited the K^+ contraction (Figure 3B). This inhibitory effect was mediated by Gd.Ex from 0.1-3.0 mg/mL (tracing and graph in Figure 3). It was exhibited with an EC_{50} of 0.80 mg/mL (0.61-1.05, 4 observations).

Rabbit jejunum

Rabbit jejunum beats with contractions and relaxations, making it easy to test for GI smooth muscle relaxants. The extract was tested in this tissue via bolus and cumulative dosing. Once tested in a bolus manner (one concentration given and then washed off before the next higher concentration is administered), the extract showed a relaxant effect on spontaneous contractions with increasing concentrations (1-10 mg/mL, Figure 4). There was no stimulant effect, similar to one shown by ACh 0.3 µM (Figure 4). The antispasmodic effect of Gd.Ex (bolus administration) was mediated with EC_{50} of 3.31 mg/mL (2.29-4.77, 3 observations).

Later, Gd.Ex was administered on rabbit jejunum tissues in a cumulative manner (no washing off the extract in between increasing concentrations). Again, the extract showed a spasmolytic effect (Figure 5) with increasing concentrations of 0.3-3 mg/mL. This suppressant activity was mediated with EC_{50} of 1.41 mg/mL (0.68-2.92, 3 observations).

Guinea-pig ileum

As with the previous tissues, the extract was initially examined on the default status of the ileal tissues. Guinea-pig ileum maintains a smooth flat baseline. The extract did not show any stimulant activity up to 10 mg/mL (Figure 6A). A spasmogenic effect was seen with the standard GI stimulants (Figure 6B) ACh (0.3 µM), histamine (0.3 µM), and K^+ (50 mM). To elucidate for a possible spasmolytic action, the extract was tested against the standard

Table 1: Table listing the different isolated tissue preparations used to elucidate the pharmacological actions of the dry ginger 70% aqueous-methanolic crude extract (Gd.Ex). Options in the columns show how the extract was tested on these tissues, the interventions used for quantifying the spasmolytic behaviour of the extract and the significance of those interventions.

Tissue Preparation	Extract Tested on Baseline (in increasing concentrations)	Intervention (to quantify extract spasmolytic effect)	How Extract Used Against the Intervention?	Significance of Intervention
Rat stomach pylorus	Yes	Agonist: K^+ (80 mM) sustained contraction	In increasing concentrations.	Inhibition of K^+ (80 mM)-induced contraction indicates calcium channel blocking (CCB) effect.
Rabbit jejunum	Yes (Bolus and cumulative dosing)	No agonist used	NA	No agonist used as default spontaneous contractions of this tissue help to quantify spasmolytic effect.
Guinea-pig ileum	Yes	Agonists: ACh (0.3 μM), histamine (0.3 μM) and K^+ (50 mM)	Single concentration of 3 mg/ mL	Inhibition of these agonists indicate broad-spectrum relaxant behaviour of extract.
Guinea-pig colon	Yes	Agonist: ACh (0.3 μM)	Two increasing concentrations of extract: 0.3 and 1 mg/ mL.	Inhibitory effect of extract, against agonist, in increasing concentrations.
Rat uterus	Yes	Agonist: ACh (1 μM)	Single concentration of 0.3 mg/ mL.	Relaxant effect of extract.

ACh: acetylcholine; K^+: potassium chloride; NA: not applicable.

GI stimulants. Control responses from ACh (0.3 μM), histamine (0.3 μM) and K^+ (50 mM) were obtained. Once consistent responses were observed, the tissues were administered a 3 mg/ mL concentration of Gd.Ex and left for 30 min. After 30 min, the standard agonists were tested again to see if they exhibit a similar response or not. All the agonist responses were blocked by the extract (n=3, Figure 6). The control (100%) response of ACh (0.3 μM) was reduced to 6.0 ± 3.05% (n=3, Figure 6), that of histamine (0.3 μM) was reduced to 6.1 ± 3.06% (n=3, Figure 6) while that of K^+ (50 mM) was reduced to 5.67 ± 3.18% (n=3, Figure 6). There was a significant difference between the agonist responses with, and without, Gd.Ex 3 mg/mL ($p < 0.001$, Figure 6).

Guinea-pig colon

Gd.Ex was investigated on the default status of the tissues. This preparation, although not explicitly a spontaneously contracting tissue like rabbit jejunum, does have some baseline contractility (Figure 7A). The extract was tested and, as with the previous tissues, it did not show any stimulant behaviour. A stimulant was indeed seen with the standard agonist ACh 0.3 μM (Figure 7A). Rather, Gd.Ex showed a relaxant effect from 0.1-3 mg/mL (Figure 7A). Due to the nature of the tissue (variable spasmogenicity as discussed above), this relaxant effect of Gd.Ex was quantified against a standard stimulant. Control ACh responses were obtained in the absence and then in the presence (tissue treated with extract concentrations of 0.3 and 1.0 mg/mL for 30 min) of Gd.Ex. Increasing concentrations of the extract completely blocked the ACh response (Figure 7). The control (100%) response of ACh was reduced to 69.33 ± 2.33% (n=3, $p < 0.001$ vs ACh alone, Figure 7) by the Gd.Ex concentration of 0.3 mg/ mL. Gd.Ex (1.0 mg/mL) further suppressed the ACh response to 4.33 ± 2.18% (n=3, $p < 0.001$ vs ACh alone, Figure 7). There was also a significant difference between the Gd.Ex 0.3 and 1.0 mg/ mL effects from each other ($p < 0.001$, Figure 7).

Rat uterus

Gd.Ex did not show any effect on the quiescent baseline of the isolated rat uterine tissue (Figure 8A). There was a stimulant effect seen from the standard smooth muscle agonist ACh 1 μM (Figure 8A). The extract was tested for a possible spasmolytic effect against ACh 1 μM control responses (Figure 8B). Once consistent responses were obtained with ACh 1 μM, the tissues were treated with a Gd.Ex concentration of 0.3 mg/mL for 30 min. After this period, the agonist (ACh 1 μM) was again administered to the tissue. The extract concentration of 0.3 mg/mL was able to almost completely block the agonist effect (Figure 8). The ACh control response (100%) was reduced to 8.90 ± 2.88% (n=5, $p < 0.0001$ vs control, Figure 8) by the extract.

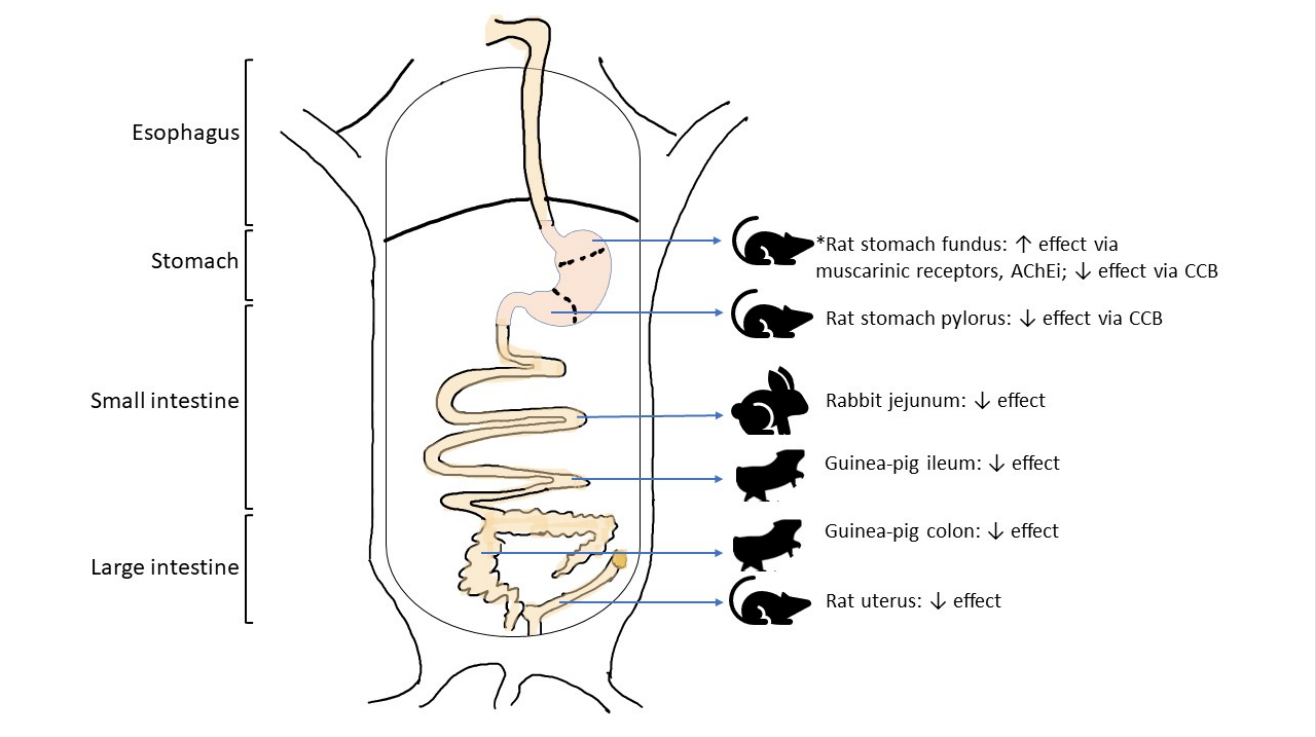

Figure 9: Figure showing effects of dried ginger 70% methanolic extract (Gd.Ex) on different animal organ systems. *From reference [28]; ↑: stimulant; ↓: relaxant; AChEi: acetylcholinesterase inhibition; CCB: Ca^{2+} channel blockade.

DISCUSSION

Ginger is a popular herb known for its culinary and medicinal properties. Although it has several broad-spectrum traditional uses, ginger is especially regarded for its wonders when it comes to disorders of the GI tract. It is known to have both GI spasmogenic and spasmolytic properties,[4,5] which is why it has benefits in nausea/vomiting, diarrhea, and painful/colicky situations of the bowel and stomach.[4,5] This study was undertaken to look into the smooth muscle tone modulatory effects of the extract of dried ginger. For this purpose, different isolated tissues were selected from rats, rabbits, and guinea-pig. Gd.Ex was first passed through some basic phytochemical profiling tests. They revealed that Gd.Ex contains terpenoids, flavonoids; phenols, and alkaloids. All these classes of chemicals have already been reported to be present in ginger.[36] Most notably, the pungent principles in ginger, gingerols and shogaols, are phenolic compounds.[17,37]

In a systematic approach, isolated smooth muscle tissues were selected from the top to the bottom of the GI tract, starting from the murine stomach pyloric strips. How these tissues were used to test the ability of the extract, is tabulated in Table 1. The reason why stomach fundus results are not being reported is that they have already been reported in our earlier communication.[28] The extract did not exhibit stimulant activity when investigated up to 10 mg/mL (Figure 9). This is in total contrast to our earlier reported result for stomach fundus in which the extract showed an increasing spasmogenic action with escalating concentrations (0.03 to 5 mg/mL).[28] This effect was mediated via activation of cholinergic receptors, as it was completely blocked by atropine, a standard non-specific anticholinergic agent.[38,39] To find out if the extract had an antispasmodic effect in the pyloric strips, contractions were induced with K^+ 80 mM. Gd.Ex blocked these K^+-induced contractions when given in a cumulative fashion. High K^+ leads to hyperpolarization of the cell membrane that results in the opening of voltage-gated Ca^{2+} channels. Once the Ca^{2+} channels are open, levels of intracellular Ca^{2+} increase leading to the initiation of muscle contraction.[40] A potential Ca^{2+} Channel Blocker (CCB) would reduce the influx of Ca^{2+} into the cells, thus would relax the K^+ induced contractions. This is how the extract acted, indicating an activity via CCB. This means that the extract has a stimulant effect in the stomach fundus, while a relaxant effect in the stomach pylorus. Ginger is traditionally known as a tonic for the GI tract, a digestive aid, regulates the tone of gastric muscles,[1,4] and an overall gastric protectant.[2] Ginger rhizome has a reputation for speeding gastric emptying. This is why, even when it is used as a condiment in South Asian cooking, it is added to other foods like beans, lentils, and pulses that are known to slow gastric emptying. The process of gastric emptying is a complex combination of coordinated contractions and relaxations of different parts of the GI tract, along with the involvement of several receptors and hormones activated via humoral and neural pathways.[41] The ingestion of a meal follows a long process of the transfer of food from the stomach to the intestines. The time for this transit varies from individual to

individual and is dependent on the type of food. More the acidic and fatty the food, the slower is the gastric emptying rate. In terms of muscle contractility, heightened tonicity of the stomach orad region, strong peristaltic movements in the stomach caudad region while dilation and relaxation of the pylorus, all come together to push the food content of the stomach out into the proximal small intestines.[41] The Gd.Ex seems to be facilitating the similar kind of effects, contraction of proximal stomach muscles, while relaxation of the distal part of the stomach and beyond (Figure 9). Ginger, both dried and fresh varieties, has been shown to increase the intestinal propulsive rate in rats with functional dyspepsia.[26] Our study results demonstrate how it might be happening on an *in vitro* level. The results also explain the already known use of ginger in nausea and vomiting.[19,42] The study further strengthens the possibility of ginger having a local effect (on the level of acting on stomach musculature) in reducing nausea and vomiting compared to an only central effect.[5,43,44]

Along the length of the GI tract, the extract was tested on other GI tissues. Similar to the rat stomach pylorus, Gd.Ex also did not exhibit any stimulant effect on rabbit jejunum. Rabbit jejunum is a different tissue when compared with other tissues in terms of baseline spontaneity. Rabbit jejunum beats/contracts and relaxes spontaneously. This behaviour helps in screening for muscle relaxants.[45] The extract, in increasing concentrations and similar to stomach pylorus, exhibited a relaxant effect via inhibition of rabbit jejunum baseline contractions. This effect of the extract was quantified via both bolus and cumulative dosing formats. When given in a bolus manner, the extract showed the effect from 1-10 mg/mL. Via cumulative dosing, Gd.Ex exhibited the same spasmolytic effect from 0.3-3 mg/mL. This is understandable keeping in mind that in the cumulative format, the individual concentrations of the extract are not washed out and the tissue remains in contact with the extract throughout.

Gd.Ex was next investigated using the guinea-pig ileal tissues. Similar to the results seen with pyloric strips and jejunum, the extract was unable to show any spasmogenic effect in this tissue. Guinea-pig ileum maintains a flat baseline. To quantify a spasmolytic effect from a test substance, it is best to use a standard agonist.[45] Three different standard agonists were used: ACh, histamine, and K^+. all the 3 showed expected stimulant effects in the tissue. Once a response from the agonists was noted, they were then challenged with a dose of the extract. The extract (3 mg/mL) blocked the contractile effect of all the three agonists, again indicating an inhibitory effect of the extract on the GI tissue.

Gd.Ex was also tested on a large intestinal tissue, guinea-pig colon. Guinea-pig colon, rather than showing a completely flat baseline like pylorus or ileum, shows some disturbance or an irregular contractile pattern. Although it is not best to quantify spasmolytic agents without the use of standard agonists, but testing a drug on baseline does give a general idea of whether it has a stimulant or relaxant effect. Gd.Ex did not show a stimulant effect in the colon, similar to pylorus, jejunum, and ileum. But once the higher concentrations of the extract were administered on the baseline, some relaxant activity was observed. This was further investigated using a standard agonist ACh. Consistent responses of ACh were reproduced, and then the tissue was incubated with two escalating concentrations of Gd.Ex. Gd.Ex, from 0.3 to 1 mg/mL, completely inhibited the ACh stimulant effect. This again showed the spasmolytic behaviour of dried ginger extract. Ginger is used traditionally in all kinds of diarrhea.[4] The observed spasmolytic effect of ginger in the colon preparation attests to that folklore antidiarrheal use.

A non-GI tissue, murine uterus, was also selected to look at the effect of the extract outside the GI tract. The extract, similar to all the other tissues, did not show any spasmogenic effect in isolated uterine preparations. As the rat uterus also maintains a flat baseline with no spontaneous contractions, spasmolytic agents can only be evaluated using standard agonists.[45] Contractile responses of ACh were obtained and these were challenged with the extract concentration of 0.3 mg/mL. The extract, after the pretreatment period, completely abolished the control response of ACh. This indicated a similar spasmolytic action of dried ginger extract as seen in the other tissues.

By selecting all the different GI and no-GI tissue preparations, the study was able to show a generalized smooth muscle relaxant effect of dried ginger extract. The extract's spasmolytic effect was observed and quantified on baseline spontaneous contractions of rabbit jejunum via bolus and cumulative dosing without the use of a standard agonist while was also evaluated via the use of standard agonists in rat stomach pyloric strips (against high K^+-induced contractions), guinea-pig ileum (high concentration of extract vs multiple standard agonists), guinea-pig colon (extract increasing concentrations against ACh responses) and in rat uterus (single low concentration of extract against ACh responses).

CONCLUSION

Results from the study show an overall spasmolytic profile of 70% methanolic crude extract of dried *Zingiber officinale*. This spasmolytic effect was investigated in several GI and non-GI isolated smooth muscle preparations. The spasmolytic effect was mediated, possibly via CCB, as it suppressed the 80 mM K^+ contractions in rat stomach pyloric strips. These observations explain how dried ginger might be acting locally in the GI tract to elicit its tone modulatory effect. The combination of its stimulant effect in stomach fundic tissues and the relaxant effect in the pylorus and beyond might explain how ginger is useful in nausea and in speeding up gastric emptying.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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