INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Research Article

In-Vitro Evaluation of Antispasmodic Activity Of Rhizomes Of *Bergenia Ligulata*

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ARTICLE INFO

Received: 24 June 2023
Accepted: 05 July 2023
Published: 07 July 2023

Keywords:
Bergenia ligulata, Antispasmodic, antidiarrhoeal, *Bergenia rhizome*, GIT disorders

DOI:
10.5281/zenodo.8121954

ABSTRACT

The study's objective was to evaluate the rhizomes of Bergenia ligulata's antispasmodic properties. This species' roots, leaves, and petals have also been employed for numerous medical conditions. The gastrointestinal tract is in responsible of consuming and digesting food, as well as absorbing nutrients and getting rid of any leftover waste. Numerous illnesses that affect the GI system might negatively affect digestion and overall health. Diarrhoea, IBS (Irritable Bowel Syndrome), constipation, peptic ulcer, and other digestive issues are common. GIT disorders are any disease that affects the gastrointestinal tract. This group includes any symptoms that manifest in the middle or lower gastrointestinal system. The goal of this study was to examine the antispasmodic activity of rhizomes of Bergenia ligulata on the voluntary motility and contractility of the smooth muscle of chicken ileum invitro The study uses chicken ileum to assess the antispasmodic effects of Bergenia ligulata rhizomes on intestinal contraction. Traditional treatments for GIT diseases include the use of botanicals like Zingiber officinale, Glycyrrhiza uralensis, Artemisia vulgaris, etc. As a result of this investigation, it was determined that Bergenia ligulata rhizome methanolic extract has antispasmodic properties in intestinal tissue, suggesting its potential use as an antidiarrheal medication.

INTRODUCTION

The gastrointestinal system is in charge of ingesting and digesting food, absorbing nutrients, and eliminating waste products from food digestion. The mouth, oesophagus, stomach, small and large intestines, and anus make up the GIT[1].

The GIT, liver, pancreas, and gall bladder are all parts of the digestive system. It is a system of blood arteries that transfers nutrients from other body organs and supplies blood to the organs. In addition, nerves and hormones collaborate to control how well the digestive system works. Our

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GI tract's resident bacteria are critical to our digestion, immune system, and general health. Any illness that affects the gastrointestinal tract are referred to as GIT disorders. Any signs that appear in the middle or lower portion of the digestive tract fall under this category[2].

For centuries, ethnic healers and traditional medic al systems have used herbal remedies to treat a va riety of illnesses. Currently, it is believed that 80% of people use herbal traditional remedies to treat a variety of illness. This is in part because of its accessibility, and lack of negative side effects[3].

Gastrointestinal tract diseases are traditionally treated using a variety of plants and medicines. For the treatment, various plant parts like leaves, roots, rhizomes, etc. are utilised. Compared to contemporary treatments, these are less poisonous and more potent.

Some of the plants that are traditionally used in the treatment of GIT disorders are: *Zingiber officinale*[4], *Glycyrrhizauralensis*[5], *Artemisia vulgaris*[6], *etc.*

Many of the plants and herbs that are used traditionally for the curing and preventing GIT disorders have been examined scientifically. It has been found that these plants and herbs contain bioactive compounds such as flavonoids and tannins which has therapeutic effect in human body.

Bergenia ligulata, also known as Pashanbeda, is traditionally used in the treatment of GIT disorders. Communities in the Himalayas use the plant's roots and rhizomes to heal wounds, septic infections, cough and cold, heart conditions, asthma, inflammation, as well as in GIT disorders and many type of urinary issues. This plant also contains tannins and flavonoids[7]in abundant amount which may be responsible for the various activities.

Hence, this study was designed to study the antispasmodic activity of rhizomes of *Bergenia ligulata* on the voluntary motility and contractility of the smooth muscle of chicken ileum invitro. The study evaluates the antispasmodic activity of *Bergenia ligulata* rhizomes on intestinal contraction using chicken ileum.

MATERIALS AND METHODS

Chemicals:

All chemicals used were of analytical grade and purchased from standard companies. Acetylcholine and Atropine were purchased from S D Fine-Chem Ltd, Kolkata. All the ingredients of solution were prepared freshly before the experiment.

Table 1: Chemical requirement for the preparation of physiological salt solution

Compounds	Tyrode (mg)
NaCl (58.45)	40
KCl (74.56)	1
$CaCl_2$(110.99)	1
$MgCl_2$(95.23)	5
$NaHCO_3$	5
NaH_2PO_4(119.97)	0.25
Glucose (180.16)	10

Instruments:

Table 2: List of instruments

Instruments	Manufacturer
Millipore water system	Direct Q3, Merck Millipore,
Organ bath	Instruments and chemicals private ltd, Ambala.
Heating mantle	Zenith glasswares and instruments cooporation, Baghbazaar
Water bath	Bio Techno Lab, Mumbai, Maharashtra
Weighing balance	K. Roy&Co. (EKB 200), Kolkata.

Plant collection:

Bergenia ligulata rhizomes were collected from Dentam (begha) West Sikkim.

Figure 1: *Bergenia ligulata* plant

Preparation of extract:

Extraction of active constituent of *Bergenia ligulata* was performed by maceration method. The rhizomes of plant were chopped and air dried for 4 to 5 days. The air-dried rhizomes were grinded to coarse powder by using grinder. Approximately 40 gm of grinded rhizome were soaked in 70% methanol in water (v/v) at a room temperature for 3 days with occasional stirring with the help of glass rod. After filtering through a single layer of muslin cloth, the final filtrate was collected by passing through a Whatman filter paper (grade 1). This procedure of soaking the rhizome residue and filtration was repeated twice. All the filtrates were combined and evaporated to dryness on a rotator evaporator under reduced pressure to a thick and dark brown material i.e the crude extract of *Bergenia ligulata* rhizome was prepared[8].

Figure 2: Powdered form of *B. Ligulata* rhizomes

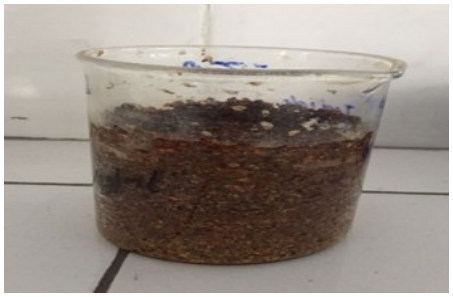

Figure 3: Powdered rhizomes soaked in methanol

Figure 4: Extract of *Bergenia ligulate* rhizome

Calculation of yield:

The yield calculated was = (wt. of the dried extract /wt. of dried powder) x100

= (11.2/40) x 100

= 28%

Phytochemical Screening

Test for alkaloids[9,10]

SL NO.	Test	Observation	Inference
1.	**Mayer's test:** 2ml of extract was taken in a test tube. 0.2ml of diluted hydrochloric acid and 0.1ml of Mayer's reagent was added	Formation of yellowish buff colored precipitate	Presence of alkaloids.
2	**Dragendroff's test:** 0.1ml diluted hydrochloric acid, and 0.1ml Dragendroff's reagent was added in 2ml of extract in a test tube	Formation of orange brown colored precipitate	Presence of alkaloids.

Test for flavonoids

SL NO	Test	Observation	Inference
1	5ml of the extract was hydrolyzed with 10% v/v sulphuric acid and cooled. Then it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted ammonia, 1ml of diluted sodium bicarbonate and 1ml of 0.1N sodium hydroxide were added to the first, second and third test tubes respectively.	Formation of yellow color in each test tube	Presence of flavonoids.

Test for reducing Sugars

Sl No	Test	Observation	Inference
1	**Fehling's test:** 5ml of extract solution was mixed with 5ml of Fehling's solution (equal mixture of Fehling's solution A&B) & boiled.	Formation of brick red precipitate	Presence of reducing sugars.
2	**Benedict's test:** To 5ml of the extract solution, 5ml of Benedict's solution was added in a test tube and boiled for few minutes.	Development of brick red precipitate	Presence of reducing sugar.

Test for tannins

Sl no	Test	Observation	Inference
1	i. 5ml of extract solution was allowed to react with 1ml of 5% ferric chloride solution. ii. 5ml of the extract was treated with 1ml of 10% of aqueous potassium dichromate solution.	Greenish black coloration Formation of yellowish brown precipitate	Presence of tannins. Presence of tannins.

Test for saponins

Sl No	Test	Observation	Inference
1	Foam formation test 1ml solution of the extract was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes	Development of stable foam	Presence of saponins

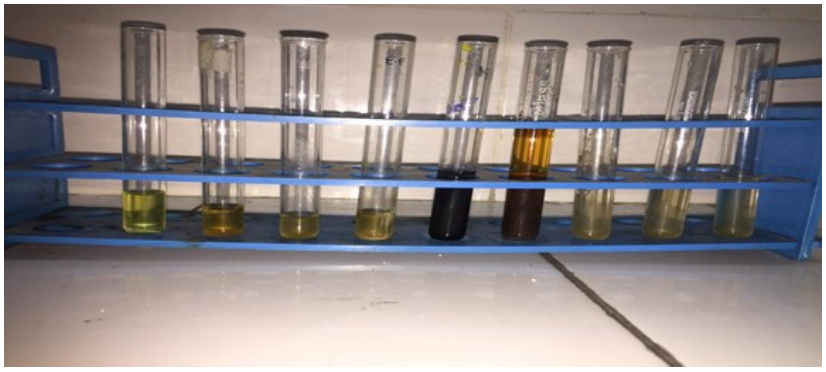

Figure 5: Phytochemical test results

Pharmacological test:

Fresh ileum of healthy chickens was obtained from a nearby slaughterhouse. About 1-1.5cm terminal segments of the chicken ileum was taken and placed in 30ml baths which contained the prepared Tyrode solution (NaCl, 40; KCl, 1; $MgCl_2$, 5; NaH_2PO_4, 0.25; $CaCl_2$, 1; $NaHCO_3$, 5; glucose10). Tyrode solution containing fresh chicken ileum was kept at room temperature and oxygenated continuously. Initial tension was 2 grams. Then, the Tyrode solution containing the chicken ileum was given time to stabilize. Isometric contractions were recorded. Graded doses of acetylcholine ranging from 1, 2, 4µg/ml were added into the organ bath to induce contraction. For each acetylcholine, Control cumulative concentration–response curves were plotted.

Methanolic extract, 2µg/mL of atropine was then added to the bath 10 min before the corresponding concentration–response curve was recorded. Each agonist was tested in the presence of methanolic extracts and standard antagonist (atropine). Extracts and atropine was evaluated against a fixed effective dose of acetylcholine from 1 to 4µg/ml for their antispasmodic activity. Graphs were plotted against the spasmolytic effects on contractile response caused by extracts and standard antagonists.

RESULTS:

Preliminary qualitative phytochemical analysis:

Results of the preliminary phytochemical investigation of methanolic extract of *Bergenia ligulata* exhibited presence of flavonoids and tannins.

Table 3: Preliminary phytochemical screening of *Bergenia ligulata*

Sl. No.	Test	Result
1.	Flavonoids	+ve
2.	Tannins	+ve
3.	Saponins	+ve
4.	Reducing sugars	+ve

Yield: The percentage yield of the extracts was calculated as follows:

Weight of dried extract/ weight of dried rhizome powder x 100 (w/w)

The percentage yield of *Bergenia ligulata*

= (wt. of the dried extract /wt. of dried powder) x100

= (11.2/40) x 100

=28%

Specifications: The environmental conditions were maintained during the experiment were as follows: the bath volume was 30ml, speed of recording was 0.25mm/sec, equilibrium time was 30 minutes, the interval between dose is 1minute, and the dosing method was cumulative.

Table 4: Environmental conditions maintained during the experiment

Experimental conditions	
Bath volume (ml)	30
Speed of recording paper(mm/sec)	0.25
Equilibrium time (min)	30
Interval between dose (min)	1
Dosing method	Cumulative

Response for different doses of acetylcholine alone and in the presence of HDBL, LDBL, Atropine:

Concentration-response curve in Figure 7 showed that both 4 and 2mg/ml of *Bergenia ligulate* as well as 2µg/ml atropine caused concentration dependent decrease in contractile response in comparison to concentration-response curve of Ach alone.

Table 5: Response for different doses of acetylcholine (mm)

Treatment	Response (in mm)
ACh 1µg/ml	14
ACh 2 µg/ml	20
ACh 4 µg/ml	23

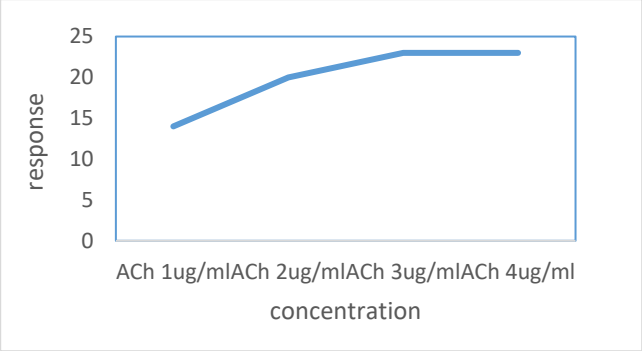

Figure 5: Response for different doses of acetylcholine (mm)

Table 6: Contractile response of acetylcholine alone and in the presence of high and low dose of methanolic extract of *Bergenia ligulata* and atropine on chicken ileum

Treatment	Response (in mm)	Ach+ HDBL (in mm)	ACh+ LDBL (in mm)	ACh+ Atropine (in mm)
Ach 1µg/ml	14	2	3	1
Ach 2µg/ml	20	4	5	2
Ach 4µg/ml	23	5	7	4

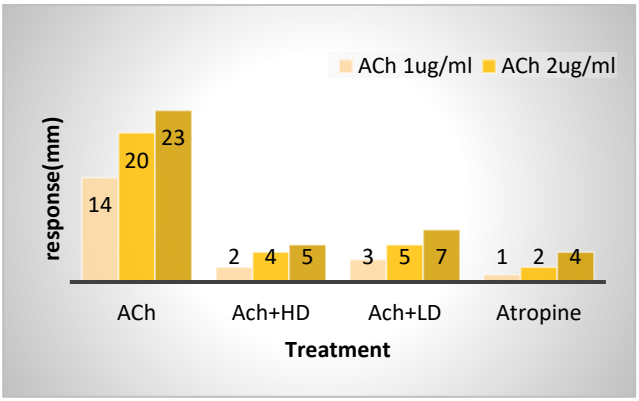

Figure 6: Contractile response of acetylcholine alone and in the presence of high and low dose of methanolic extract of *Bergenia ligulata* and atropine on chicken ileum.

Half Maximal Response (EC50) for acetylcholine alone and in the presence of high and low dose of methanolic extract of *Bergenia ligulate* and atropine

According to the determination of EC50, EC50 of ACh alone was 1.2µg/ml. After addition of 4 and 2mg/mlof *Bergenia ligulata*,EC50 of ACh increased. In the presence of HDBL EC50 was 5.7µg/ml and LDBL EC50 was 4.8µg/mL respectively. EC50 of ACh in the presence of 2µg/ml atropine was 11.7µg/ml.

Table 7: Half Maximal Response (EC50) for acetylcholine alone and in the presence of high and low dose of methanolic extract of *Bergenia ligulata* and atropine on chicken ileum

Treatment	EC50 (µg/ml)
ACh 1-4µg/ml	1.2
ACh+HDBL 4mg/ml	5.7
ACh+LDBL 2mg/ml	4.8
Atropine 2µg/ml	11.7

Force of contraction in gram generated by chicken ileum in response to addition of Ach, high and low dose of methanolic extract of *Bergenia ligulata*and atropine:

The peaks presented in Figure 7 showed the force of contractions in chicken ileum generated by graded doses of Ach from 1, 2 and 4 µg/ml. After addition of 4 and 2mg/ml of *Bergenia ligulata* as well as 2 µg/mL atropine, there were significant reductions in the peaks.

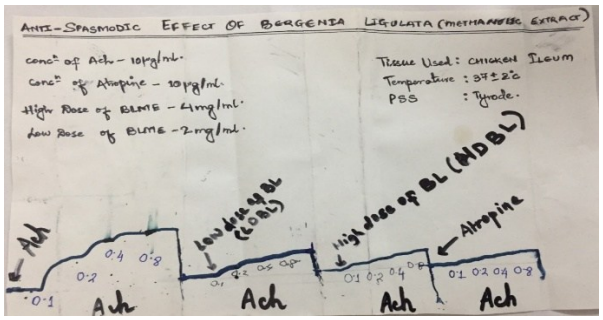

Figure 7: Force of contraction in gram generated by chicken ileum in response to addition of Ach, high and low dose of methanolic extract of *Bergenia ligulata* and atropine

DISCUSSION

The primary objective of this study was to assess the antispasmodic activities of a methanolic extract derived from the rhizome of *Bergenia ligulata*. The experiment was conducted using

chicken ileum and observed that acetylcholine (ACh) caused a concentration-dependent increase in contractions. The maximum contraction was observed at a concentration of 4 µg/ml. Before introducing different concentrations of the extract and an antagonist, the tissue was washed for 3 to 5 minutes to check the recovery of the contractile response.

Concentration-response curves revealed that both 2 and 4 mg/mL of *Bergenia ligulata* extract, as well as 2 µg/mL atropine (an antagonist), caused a concentration-dependent reduction in contractile response of Ach. At higher doses of the extract, the contraction was significantly inhibited compared to the lower dose.

It is known that M2 and M3 receptors play a major role in intestinal contractions induced by acetylcholine. These receptors are found in isolated preparations of intestinal smooth muscles. Hence, it can be inferred that acetylcholine binds to M2 receptors to inhibit the relaxant impact produced by cyclic adenosine monophosphate (cAMP) and it attaches to M3 receptors to promote phosphoinositide hydrolysis and trigger contractions in the intestine[11].

The results suggest that *Bergenia ligulata* extract, at both high and low doses, blocks the M2 and M3 receptors, which inhibits intestinal contractions. Ach reduced the power of contraction more than Ach alone when 4 and 2 mg/mL of Bergenia ligulata extract and 2 µg/mL atropine were present. According to these findings, atropine and *Bergenia ligulata* extract at high and low doses may suppress muscarinic receptors and lessen intestinal spasms. Intestinal smooth muscles are known to relax as an outcome of muscarinic receptor inhibition in the gastrointestinal tract (GIT) is well known for calming the intestine's smooth muscles[12].

The EC50 (effective concentration 50%) of Ach alone was discovered to be 1.2 µg/ml. When 4 and 2 mg/ml of *Bergenia ligulata* extract were added, the EC50 of Ach increased. The *Bergenia ligulate* extract EC50 values were 5.7 µg/mL for high-dose and 4.8 µg/mL for low-dose, respectively. The EC50 of Ach in the presence of 2 µg/mL atropine was 11.7 µg/ml. The EC50 increases as the amount of Ach required to generate 50% of the maximum contractile response increases. In other words, *Bergenia ligulata* extract can stop Ach from causing the GIT to contract. Figure 7 displays the force of contractions in chicken ileum generated by increasing doses of Ach (1, 2, and 4 µg/ml). Significant reductions in the contraction peaks were observed after adding 4 and 2 mg/ml of *Bergenia ligulata* extract, as well as 2 µg/ml atropine. These findings suggested the extract and atropine counteracted the intestinal contractions induced by acetylcholine (Ach).

Plants such as *Baccharis teindalensis* (Asteraceae), *Pentaclethra macrophylla* are known for its antispasmodic activity which aids in the treatment of diarrhoea. Studies have concluded that these plants contain flavonoids and tannins which are known to produce relaxation in the smooth muscles[13].

Phytochemical screening identified the presence of flavonoids in *Bergenia ligulata* rhizome. Moreover, a connection has been established between high levels of flavonoids & tannins &their potential antidiarrheal effects. Therefore, the results of this experiment provide evidence that the rhizomes of *Bergenia ligulata* possesses an antispasmodic effect, possibly due to the presence of phytochemicals such as flavonoids and tannins.

CONCLUSION

Results from this work has signified that both 4mg/ml and 2mg/ml of methanolic extract of *Bergenia ligulata* showed concentration dependent decrease in the contraction of the chicken ileum induced by acetylcholine, hence it has exhibited antispasmodic activity.

Force of contraction induced by ACh in the presence of high and low doses of *Bergenia*

ligulata as well as atropine is less than that induced by ACh alone. These findings proposed that high and low dose of *Bergenia ligulata* extract and atropine may block the muscarinic receptors and decrease intestinal contractions. It is reported that inhibition of muscarinic receptors in GIT can lead to relaxation of intestinal smooth muscles.

The findings support that *Bergenia ligulata* may serve as the potential treatment for gastrointestinal disorders such as diarrhoea as well as IBS. Further studies can be carried out to establish the fact clinically.

ACKNOWLEDGEMENT

We acknowledge Dr. H.P. Chhetri, Director, Himalayan Pharmacy Institute, Sikkim and Dr. N.R. Bhuyan, Principal, Himalayan Pharmacy Institute, Sikkim for providing necessary facilities to carry out the research work.

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HOW TO CITE: Abhijit Mishra, Ankita Adhikari, Benazeer Ali, Pratham Gurung, Rupam Kalita, Ananya Bhattacharjee, In-Vitro Evaluation of Antispasmodic Activity Of Rhizomes Of *Bergenia Ligulata*, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 7, 182-189. https://doi.org/10.5281/zenodo.8121954