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Rationalization of Traditional Uses of *Berberis lycium* in Gastrointestinal Disorders

Muhammad Shafeeq ur Rahaman[1], Mueen Ahmad Chaudhary[1*], Bashir Ahmad[1] and Alamgeer Alamgeer[2]

[1]Faculty of Pharmacy, University of Lahore, Lahore, Pakistan.
[2]Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Author BA designed the study and supervised the work. Author AA did all literature survey, selection, collection and extraction of plant. Author MSR carried out the experimental work and first draft of manuscript. Author MAC performed statistical analysis and drafted the final manuscript. All authors read and approved the final manuscript for publication.

Research Article

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ABSTRACT

Aims*: Berberis lycium* (Sumbal) is abundantly available in the northern areas of Pakistan and extensively used in local practice for the treatment of several human diseases. The objective of this study was to explore pharmacological basis for its use in gastrointestinal disorders.
Materials and Methods: Crude aqueous (Bl.Aq) and methanolic (Bl.Meth) extracts of *B. lycium* were studied on isolated gut preparations of rabbit (jejunum) and guinea pig (ileum) by using in-vitro techniques. Tissues were mounted in tissue organ baths assembly containing physiological salt (Tyrode's) solution, maintained at 37ºC and aerated with carbogen, to assess the spasmogenic and spasmolytic effect and to find out the possible underlying mechanisms. Responses were measured on BioScience Powerlab data acquisition system by using isotonic transducers.
Results*:* Phytochemical analysis indicates the presence of alkaloids, tannins and saponins in Bl.Aq and Bl.Meth. when tested on spontaneously contracting isolated rabbit jejunum, showed a dose-dependent spasmogenic effect at lower concentration (*0.01-0.1 mg/mL*) and (*0.01-0.03 mg/mL*), which was followed by spasmolytic effect at higher concentration (*0.3-1.0 mg/mL*) and (*0.1-0.3 mg/mL*) respectively. Pretreatment of the

**Corresponding author: Email: mueenahmadch@hotmail.com;*

tissue with atropine (*0.1 µM*) partially suppressed the contractile effect. Bl.Aq and Bl.Meth caused complete inhibition of high K^+ (*80 mM*)–induced contraction at *0.3 mg/mL* and *0.1 mg/mL* respectively and also produced a dose-dependent (*0.01-0.03 mg/mL*) rightward shift in the Ca^{++} concentration-response curve, similar to verapamil. When tested in bolus protocol on isolated guinea pig ileum, Bl.Aq and Bl.Meth caused a dose-dependent spasmogenic effect at *0.01-0.1 mg/mL.* Pretreatment of tissue with atropine (*0.1 µM*) partially suppress the contractile effect.

Conclusions: Results indicate that spasmogenic effect was partially mediated through cholinergic activity and spasmolytic effect was mediated through calcium channel blocking activity (CCB), explain its traditional uses in diarrhea, intestinal cramps and other gastrointestinal intestinal disorders.

Keywords: Berberis lycium; spasmogenic; spasmolytic; cholinergic; Ca^{++} antagonist.

1. INTRODUCTION

Berberis lycium Royle (Sumbal) is commonly known as Berberry (Family: Berberidaceae); a medium sized tree found in the northern areas of Pakistan [1]. Plant is extensively used in local practices for the treatment of several human diseases [2] like intestinal colic, diarrhea, piles, menorrhagia, jaundice, wounds and broken bones. It is used as expectorant, diuretic [3], antitumor and in bacterial dysentery [4], acute conjunctivitis, chronic opthalmia [5] and throat inflammations [1]. The plant is stomachic, aperient, carminative and febrifuge [6]. Fruit and leaves are used for edible purposes [7,8] due to presence of high percentage of various nutritive constituent i.e. protein, fat, fiber, palmetine, calcium, sulphur, berbarine and vitamin C [9].

Different studies have evaluated the presence of antioxidant [10], bone healing [11], antidiabetic [12], antihyperlipidimic [13], antihyperglycemic [14] and antibacterial [15; 16] activities. Berberine a major alkaloid of plant [17], prevented ischemia-induced ventricular tachyarrhythmia, stimulated cardiac contractility, peripheral vascular resistance [18; 19] and left ventricular hypertrophy development [20].

Despite the wide medicinal uses of *B. lycium*, no data is available with respect to its effectiveness in gut motility effect. The present study on the aqueous and methnolic extract of *B. lycium* roots was undertaken to rationalize these traditional uses and to explore mechanistic basis for these medicinal uses.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Crude Extract

Fresh plant root of *B. lycium* was collected from Gilgit (Shikyote) northern area of Pakistan and was then identified by an expert taxonomist of department of biology, University of Sargodha, Pakistan. Plant was shade dried for one week, thoroughly cleaned and coarsely powered by using electric grinder. The aqueous and methanolic extracts were prepared by cold maceration method. Approximately 1Kg coarsely powdered plant material was soaked in distilled water and methanol separately at room temperature for 7days with occasional shaking [21]. Both were filtered through a double layered muslin cloth and subsequently through filter paper. The collected filtrates were concentrated in rotary evaporator separately

at 40ºC under reduced pressure (-760mmHg) to a thick, semi-solid mass, they were transferred to Petri-dishs and placed at room temperature to get rid of remaining solvents. The aqueous and methanolic extracts obtained with approximated yield of 3.85% and4.05% respectively were transfer to glass bottles and stored to refrigerator (-4ºC) until used. The stock solutions and their dilutions were made fresh in distilled water at the day of experiment for purpose of evaluating pharmacological activity.

2.2 Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of Bl.Aq and Bl.Meth was done for the presence of Alkaloids, Anthraquinones, Coumarins, Saponins, and tannins as described by Tona et al., 1998.

2.3 Drugs and Reagents

All the chemicals used in the experiments were of highest purity and research grade and were obtained from the sources specified: acetylcholine chloride, atropine sulfate, verapamil and potassium chloride from sigma chemicals co. St louis, mo, USA. Calcium Chloride, Glucose, Magnesium Chloride, Magnesium Sulphate, Potassium Dihydrogenphosphate, Sodium Bicarbonate, Sodium Dihydrogenphosphate, Methanol from Merck, Darmstadt, Germany. Ammonium Hydroxide, Sodium Chloride, Sodium Hydroxide and BDH Laboratory supplies Poole, England.

2.4 Animals

Rabbits (1.0-1.5kg) and guinea-pigs (500-600g) of local breed and either sex used for experimental work were housed under controlled environmental condition (23-25ºC) at the animal house of University of Sargodha, Sargodha. Animals were given standard diet and tap water. Constituents of diet are shown as following (Table 1)

Table 1. Constituents of standard diet provided to the experimental rabbits and guinea pigs

Constituents	Quantity (g/kg)
Flour	380
Molasses	12
Sodium chloride	5.8
Nutrivet L	2.5
Potassium metabisulphate	1.2
Vegetable oil	38
Fish meal	170
Powdered milk	150

Animals were kept at fasting 24 hr prior to the experiments but had free access to water. Rabbits and guinea-pigs used for *in-vitro* study were sacrificed by blow on the back of head and cervical dislocation respectively. Experiments performed complied with the rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences [23], approved by the Ethical Committee of the University of Sargodha [24].

2.5 Isolated Tissue Experiments

In-vitro experiments were performed according to the protocols as previously followed by Chaudhary et al., 2012; Abdur Rahman et al., 2012.

2.5.1 Rabbit jejunum

Plant extracts were screened for their spasmogenic and spasmolytic activity on isolated tissue of rabbit jejunum. Segments of approximately 2cm length were suspended in 10mL tissue organ bath containing Tyrode's solution, having the following composition in mM: KCl 2.68; NaCl136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8 and glucose 5.55, aerated with Carbogen (95% O_2 and 5% CO_2) at 37°C. Each tissue was given 1g pre-tension and allowed to equilibrate for at least 30 min. and stabilized with the repeated exposure to 0.3µM acetylcholine (3-5 times) and subsequent washing with the Tyrode's solution until the sub-maximal responses were obtained. The dose-response curves of acetylcholine were constructed before the addition of test materials. Maximum response of the tissue was considered to have been achieved when the next higher concentrations of the agonist failed to produce a further increase in response [27]. The contractile effect of the test materials was assessed as the percent of the maximum effect produced by the control drug, acetylcholine (3µM). Intestinal responses were obtained isotonically using BioScience transducers and Powerlab data acquisition system (AD Instruments, Sydney, Australia) attached to a computer installed with labchart software (version 6).

2.5.2 Determination of Ca^{+2} antagonist effect

To assess whether the relaxant effect of the crude extracts was through calcium channel blocking activity, K^+ was used to depolarize the preparations as described by Farre et al., 1991. K^+ (80 mM) was added to induce the sustained contraction. Crude extract was then added to the tissue bath in a cumulative fashion to obtain dose-dependent inhibitory responses [29]. The relaxation of intestinal preparations, pre-contracted with K^+ (80mM) was expressed as percent of the control response mediated by K^+. To confirm the calcium antagonist activity of crud extract, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove calcium from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition in 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. Following an incubation period of 30 min, control concentration–response curves of Ca^{++} were constructed. When the control CRCs of Ca^{++} were found super-imposable (usually after two cycles), the tissue was pretreated with the crude extract for 50 min to test the possible calcium channel blocking effect. The CRCs of Ca^{++} were reconstructed in the presence of different concentrations of the test material, verapamil used as a positive control.

2.5.3 Guinea-pig ileum

Since isolated guinea pig ileum behaves as a quiescent preparation and is considered more suitable for spasmogenic activity [30]. The ileum was dissected out and segments of approximately 2cm length were suspended individually in 10mL tissue organ bath, filled with Tyrode's solution; having composition as described earlier and was aerated with Carbogen at 37ºC. A preload of 1 g was applied to each tissue and kept constant throughout the experiment. Following an equilibration period of 30 min, isotonic contractions to ACh (0.3

μM) were repeated to stabilize the preparation. Stimulant effect of the extract was determined on the resting baseline of the tissue and was assessed as percent of the maximum effect produced by the control drug, ACh (1 μM).

2.6 Statistical Analysis

All the data are expressed as mean ± standard error of the mean (S.E.M., n = number of experiments) and the median effective concentrations (EC_{50} values) are given with 95% confidence intervals (CI). The statistical parameter applied is the Student's *t*-test with $p< 0.05$ considered as significantly. All statistical analysis were done by using the software GraphPad Insatat3

3. RESULTS

3.1 Preliminary Phytochemical Analysis

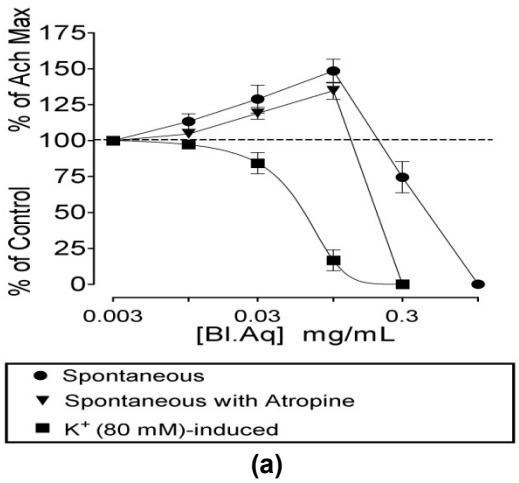
The tests were performed for the presence of different chemical constituents in the crude extracts of *B. lycium* roots and the results are shown as followings (Table 2).

Table 2. Results of preliminary phytochemical screening of crude aqueous (Bl.Aq) and methanolic (Bl.Meth) extracts of *B. lycium* roots

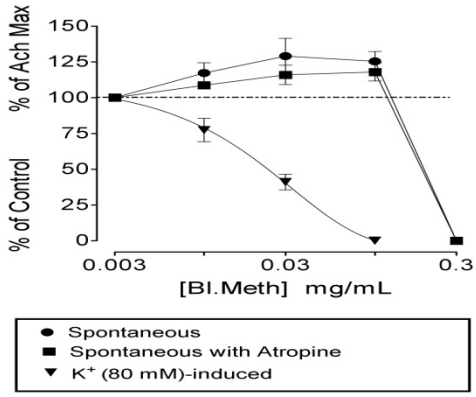
Test	Bl.Aq/ Bl.Meth
Alkaloid Test	+ve
Anthraquinone Test	-ve
Coumarin Test	-ve
Saponin Test	+ve
Tannin test	+ve

3.2 Effect on Rabbit Jejunum

In spontaneously contracting rabbit jejunum, Bl.Aq and Bl.Meth caused a dose-dependent spasmogenic effect at lower concentration (0.01-0.1 mg/mL) and (0.01-0.03 mg/mL) respectively, which was followed by spasmolytic effect at higher concentration (0.3- 1.0 mg/mL) and (0.1-0.3 mg/mL) respectively (Fig. 1a/1b). Spasmogenic effects as % of Ach max. (1.0 μM) was 13.36±2.69, 28.88±4.86 and 48.47±4.13 % (mean ± S.E.M., n= 4) at 0.01, 0.03 and 0.1 mg/mL and 17.26±3.57, and 29.02±6.10 % (mean ± S.E.M., n= 4) at 0.01 and 0.03 mg/mL, respectively. Pretreatment of the tissue with atropine (0.1 μM) partially suppressed the contractile effect of Bl.Aq and Bl.Meth and relaxed the tissue at dose (0.3 mg/mL) with EC_{50} value of 0.21 mg/mL (95% CI, 0.096-0.46, n= 3) and 0.57 mg/mL (95% CI, 0.32-1.01, n= 4), respectively (Fig. 1a/1b). Bl.Aq and Bl.Meth also caused dose-dependent inhibition of high K^+ (80 mM)–induced contraction at dose (0.3 mg/mL) and (0.1 mg/mL) with EC_{50} value of 0.06 mg/mL (0.03-0.10, n = 3) and 0.04 mg/mL (0.03-0.06, n= 3), respectively (Fig. 1a/1b). Similarly, verapamil relaxed both spontaneous and high K^+ (80 mM)-induced contractions with EC_{50} values of 0.28 μM (025-0.31, n=4) and 0.08 μM (0.06-0.09, n= 3), respectively (Fig. 1c). Bl.Aq and Bl.Meth also produced a dose-dependent (0.01-0.03 mg/mL) rightward shift in the Ca^{++} concentration-response curve (CRC) (Fig. 2a/2b), similar to verapamil (Fig. 2c).



(a)



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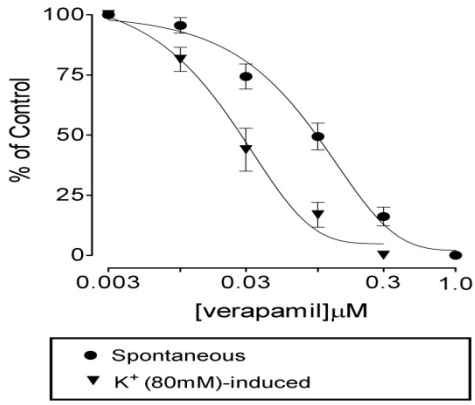

(c)

Fig. 1. Dose-dependent effect of (a) crude aqueous (Bl.Aq) and (b) methanolic extract of *B. lycium* roots (Bl.Meth) in the absence and presence of atropine and (c) the dose-dependent effects of Verapamil on spontaneous and high K^+(80mM)-induced contraction in isolated rabbit jejunum

Values shown are mean ± S.E.M., n= 3-4

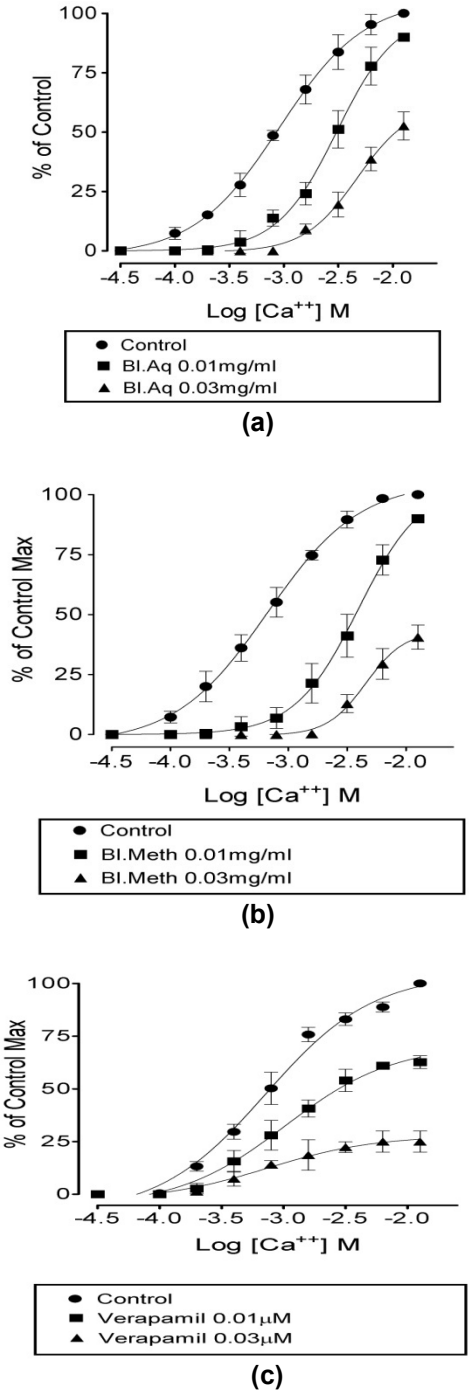

Fig. 2. Concentration Response Curve of Ca^{++} (CRC) in the absence and presence of (a) crude aqueous (Bl.Aq) and (b) methanolic extract of *B. lycium* roots (Bl.Meth) and (c) Verapamil in isolated rabbit jejunum

Values shown are mean ± S.E.M., n= 4.

3.3 Effect on Guinea Pig ileum

Bl.Aq and Bl.Meth caused a dose-dependent spasmogenic effect at the concentration of 0.01 to 0.1 mg/mL. When higher concentrations (3.0-10.0 mg/mL) were added as a bolus protocol on isolated guinea pig ileum, showed no contractile effect. The spasmogenic effect as % of Ach (3 µM) was 21.65±2.91, 66.61±4.24 and 77.68 ± 3.87 % (n= 4) at 0.01, 0.03 and 0.1 mg/mL and 11.33±1.86, 33.33±3.28 and 37.33±2.18 % (n= 3) at 0.01, 0.03 and 0.1 mg/mL, respectively. Pretreatment of tissue with atropine (0.1 µM) partially suppress the contractile effect (Fig. 3a/3b).

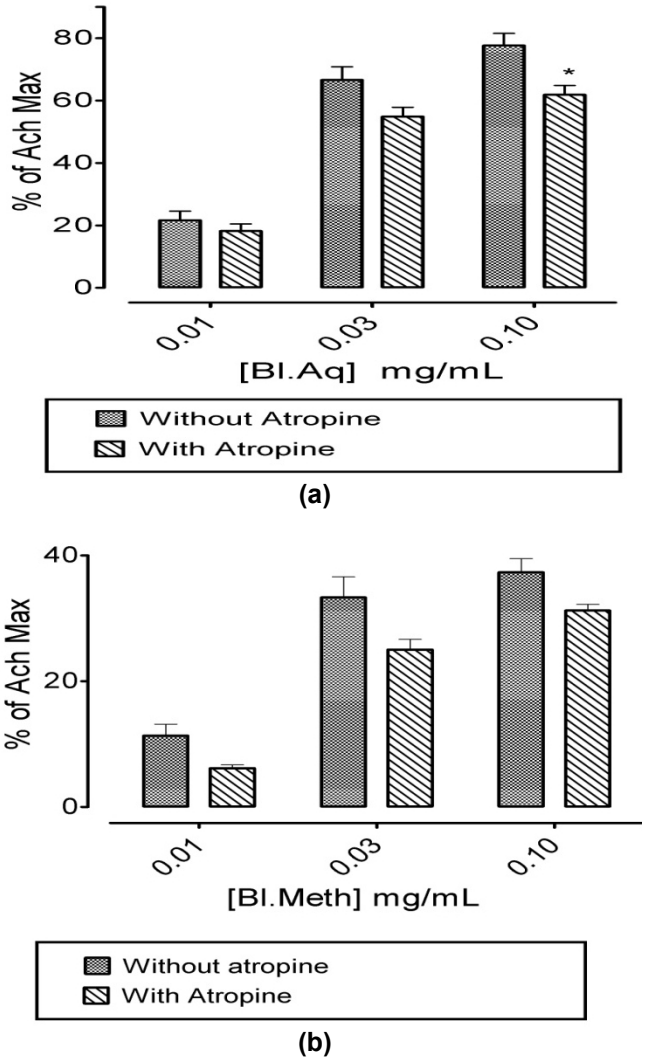

Fig. 3. Bar chart showing the dose-dependent spasmogenic effect of (a) crude aqueous (Bl.Aq) and methanolic extract of *B. lycium* roots (Bl.Meth) in the absence and presence of atropine in isolated guinea pig ileum. Values shown are mean ± S.E.M., n = 4, **P<0.05 compared to effect without atropine (Student's t-test)*

4. DISCUSSION

B. lycium is traditionally used for gastrointestinal disorders [3,6] therefore current study was conducted to validate its uses and underlying mechanisms involved. Bl.Aq exhibit a slight spasmogenic effect at lower concentration, followed by spasmolytic effect at higher concentration which indicates the presence of a combination of gut stimulant and inhibitory constituents in the aqueous extract.

In the presence of atropine; a muscarinic receptor antagonist [31] contractile effect was partially suppressed indicates the presence of cholinergic components in the aqueous extract of *B. lycium.* To see whether the contractile effect was mediated through an Ach-like mechanism, Bl.Aq was further tested on guinea pig ileum; a quiescent preparation [32] and the results were comparable with that of rabbit jejunum; indicates that Bl.Aq causes gut stimulation through multiple components including cholinergic. In GIT smooth muscles, M_3 receptors are present, which increase the intracellular calcium level, leads to increase the gastric motility and secretion [31] and the presence of cholinergic component(s) in the plant extract give the logical reasons for the use of B. lycium as carminative, stomachic and aperient.

K^+ at high concentration (>30mM) is known to cause smooth muscle contraction through opening of voltage-dependent L-type Ca^{2+} Channels, thus allowing an influx of extracellular Ca^{2+} into cytosol causing a contractile effect [33,34] and a substance causing inhibition of high K^+ induced contraction is considered as a blocker of Ca^{2+} influx [35]. Since Bl.Aq inhibited high K^+ induced contractions, which may be due to calcium channel blocking activity. The purposed CCB effect was confirmed further by observing a dose-dependent rightward shift in the Ca^{++} concentration response curve, similar to verapamil, a Ca^{++} channel blocker [36]. Since control of GI smooth muscles is largely dependent on intracellular calcium concentration. While increased intercellular calcium stimulates the secretary processes and motility can leads to diarrhoea and the voltage gated calcium channels trigger this calcium influx [31,37]. So a calcium channel blocker can suppress GI motility, they are useful in hyperactive gut disorders like diarrhea and abdominal cramps [38] and the presence of calcium antagonist activity in plant extract may give the possible explanation of the use of plant in diarrhea and intestinal colic.

To evaluate its usefulness in condition associated with motility of the gut, Bl.Meth was also tested and the effects were almost comparable in all level of experiments performed for Bl.Aq, except with slight difference that spasmogenic effect was slightly depressed and spasmolytic effect was dominant in Bl.Meth as compared to Bl.Aq on rabbit jejunum and guinea pig ileum.

5. CONCLUSION

By taking in consideration the data, obtained from the experimental work it is strongly suggested that aqueous and methanolic extracts of *B. lycium* roots contain non-specific spasmogenic activity, partially mediated through cholinergic substances and further experimental work is required to find out the other components involved, while the spasmolytic effect is likely to be occurring through CCB like activity that supports its traditional uses in diarrhea, intestinal cramps and other gastrointestinal intestinal disorders. On the bases of these findings, it could be recommended that *B. lycium* plant can be used as safer medicine for GIT disorders in Human.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the ethical committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki."

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COMPETING INTERESTS

The authors declare that they have no competing interest.

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