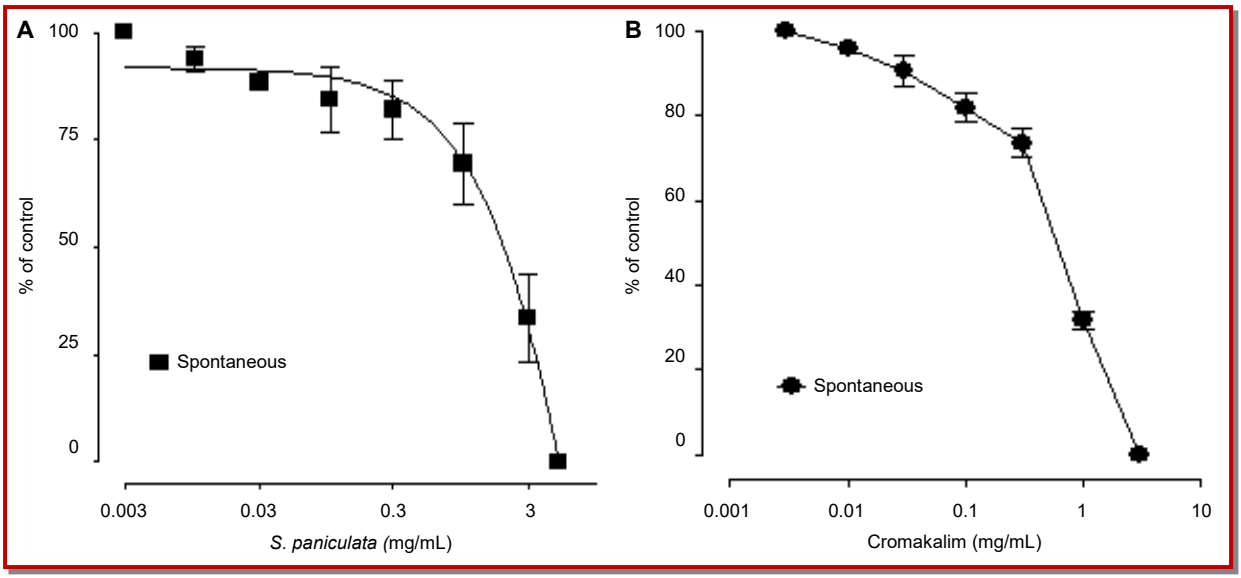

BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 495-500
ISSN: 1991-0088

Antispasmodic activity of *Symplocos paniculata* is mediated through opening of ATP-dependent K^+ channel

Khalid Hussain Janbaz, Saba Akram, Fatima Saqib and Mamoona Khalid

Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.

Article Info

Received: 22 August 2015
Accepted: 19 November 2015
Available Online: 29 April 2016

DOI: 10.3329/bjp.v11i2.24667

Cite this article:
Janbaz KH, Akram S, Saqib F, Khalid M. Antispasmodic activity of *Symplocos paniculata* is mediated through opening of ATP-dependent K^+ channels. Bangladesh J Pharmacol. 2016; 11: 495-500.

Abstract

Symplocos paniculata is a medicinal plant used by native healers to manage gastrointestinal ailments. The crude methanolic extract of *S. paniculata* was screened pharmacologically both *in vitro* and *in vivo* for the validation of its therapeutic potential. It suppressed the spontaneous activity of isolated rabbit jejunum preparations and also caused inhibition of the low K^+ (20 mM)-induced spastic contractions in isolated rabbit jejunum preparations in a manner comparable to cromakalim. The relaxant effect was found to be blocked following glibenclamide exposure of the isolated tissue preparations similar to cromakalim, suggesting that observed response was likely to be mediated through opening of ATP dependent K^+ channels. Following oral administration to mice provided protection against castor oil-induced diarrhea in a manner similar to loperamide. The plant material was found safe in toxicity study up to oral dose of 8 g/kg in mice. Hence, present study provides a scientific basis for the vernacular use of *S. paniculata* in gastrointestinal system.

Introduction

Symplocos paniculata (Syn: *S. chinensis, Pronus paniculata* and *S. crataegoides*; Family: *Symplocaceae*), locally known as Ludh pathani, is a small tree distributed throughout the world but grows luxuriously in moist and moderate climatic conditions prevailed in Northern areas of Pakistan (Dymock et al., 1972). It has dark green deciduous leaves, white hermaphrodite flowers and blue fruits (Huxely, 1992).

The barks has folkloric repute as astringent, tonic, coolant and used traditionally to manage menorrhagia, bowel complaints, eye diseases, bleeding gums and ulcer (Chopra et al., 1956; Facciola, 1990). It is also used to treat diarrhea and lower blood pressure (Nudrat and Usha, 2005). The plant material has been used to extract red or yellow dye (Polunin and Stainton, 1984).

Phytochemical investigations revealed presence of monomethyl pelargonidine glucosides, loturine, collutrine, loturidine, oxalic acid, pytosterols, 3-monoglucofuranoside, betulin, acetyloleanolic acid, oleanolic acid, ellagic acid (Joy et al., 2001), octacos-1-ene, stigmasterol, lupeol and salirepin (Kumar and Jangwan, 2012).

The pharmacological investigations on plant materials explored the presence of antimicrobial, analgesic and anti-inflammatory activities (Semwal et al., 2011). Current study was carried out to provide mechanistic basis for its use in bowel disorders i.e. diarrhea.

Materials and Methods

Chemicals

Acetylcholine, potassium chloride, and loperamide hydrochloride were purchased from Sigma Chemical Co, USA. Cromakalim was purchased from Tocris Ellisville, USA; glibenclamide was obtained from RBI Chemicals Co., USA and castor oil from KCl Pharma

Pakistan. Calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium bicarbonate, sodium chloride, DMSO and Tween 80 were purchased from Merck, Germany. All chemical used were of the reagent grade and soluble in distill water/ saline except cromakalim and glibenclamide, which were dissolved in 10% DMSO. The vehicle used to make the extract soluble was without any effect in the control experiment. Physiological salt solutions like Tyrode's and Krebs provide natural environment to isolated tissues. Stock solutions and subsequent dilutions were made fresh on the day of experiment.

Animals

Animals used in experiments were rabbit (1-2 kg) of local breed of either sex; housed at the Animal House of Faculty of Pharmacy, Bahauddin Zakariya University, Multan. The composition of standard animal diet was as follow: Flour (38%), choker (38%), molasses (1.2%), sodium chloride (0.58%), vegetable oil (3.8%), fish meal (17%), powdered milk (15%) and potassium meta bisulfate (0.12%). The animals were provided with food and water *ad libitum,* maintained at 23-25°C and 12 hours light-dark cycle.

Preparation of the extract

The dried barks of *S. paniculata* was purchased from a local herbal store in Multan and was identified by an a taxonomist (Late Prof. Mumtaz Hussain Bukhari) at The Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, having voucher No. Fl/ P/102-1. The plant material was rendered free of any possible adulterant through manual picking and grinded into coarse powder through electrically driven milling device. The powders were soaked in 80% aqueous-methanol (v/v) for eight days in amber colored glass bottle with shaking on alternate days (Williamson et al., 1998). The soaked materials was passed through muslin cloth to remove vegetative debris. The mark was pressed and the obtained fluid was subsequently filtered through Whatman No. 1 filter paper. The filtrate was evaporated at 37°C on a rotary evaporator (R-210, BUCHI; Switzerland) under reduced pressure (700 mmHg) to a thick, semi-solid mass. The thick paste material was transformed into a dry cake following freeze drying and stored in amber colored air tight bottle. The approximate yield was 2.8%.

Phytochemical analysis

The crude plant extract of *S. paniculata* was subjected to phytochemical analysis to detect possible presence of different classes of secondary metabolites, i.e., alkaloids, saponins, anthraquinones, coumarins, sterols, terpenes, flavonoids, tannins and phenolic compounds (Janbaz and Saqib, 2015; Harborne, 1998).

Isolated rabbit jejunum preparation

Isolated tissues experiments were performed as described elsewhere (Janbaz et al., 2014; Janbaz et al., 2015a). The crude plant extract of *S. paniculata* was screened for possible presence of antispasmodic activities using isolated rabbit jejunum preparation.

The animals were subjected to starvation for 24 hours prior to killing by cervical dislocation. The abdomen of the animal was dissected; jejunum segments of approximately 2 cm in length were excised and placed in a dish containing Tyrode's physiological salt solution. The individual jejunum segments were suspended in 15 mL tissue baths containing Tyrode's solution being at 37°C and continuously bubbled with carbogen (95% O_2 and 5% CO_2).

The preparations were allowed to equilibrate for 30 min at a resting tension of 1.2 g. The tissue responses were recorded on bioscience oscillograph through isotonic transducers. The tissues were repeatedly treated with sub-maximal dose (0.3 µM) of acetylcholine and subsequent washing with fresh Tyrode's solution. The isolated rabbit jejunum preparations exhibit spontaneous rhythmic contractions on which relaxant effect of the test material can be demonstrated without application of an agonist (Janbaz et al., 2013; Saqib et al., 2012).

The test material or standard drug was added to the tissue bath in a cumulative manner and extent of the achieved relaxation was taken as response (Bolton, 1979). In isolated rabbit jejunum preparation, sustained spastic contraction was produced subsequent to exposure of low K^+ (20 mM) or high K^+ (80 mM) tissue bath concentrations, on which test material was applied in cumulative manner to obtain the concentration dependent inhibitory response to elucidate the possible mechanism(s) of action. The extent of relaxation produced in isolated tissue preparation was expressed as percent of the observed contractile response subsequent to exposure to low and high K^+ tissue bath concentration (Janbaz et al., 2015b).

The relaxation of low K^+ (20 mM)-induced contraction in isolated rabbit jejunum preparations following addition of the test material was likely to be mediated through opening of K^+ channel. The low K^+ (20 mM)-induced spastic contractions in isolated rabbit jejunum preparation was relaxed by cromakalim and antagonism of the relaxant effect was observed following addition of glibenclamide (30 µM) the tissue bath. The observed blockade by glibenclamide (30 µM) of the relaxant effect of test material on low K^+ (20 mM)-induced contractions in isolated rabbit jejunum preparation might be a indication test material might have exerted its relaxant effect through opening of the ATP-dependent K^+-channels.

On the other hand, the possible relaxation of high K^+ (80 mM)-induced contractions following test material application to isolated rabbit jejunum preparations was

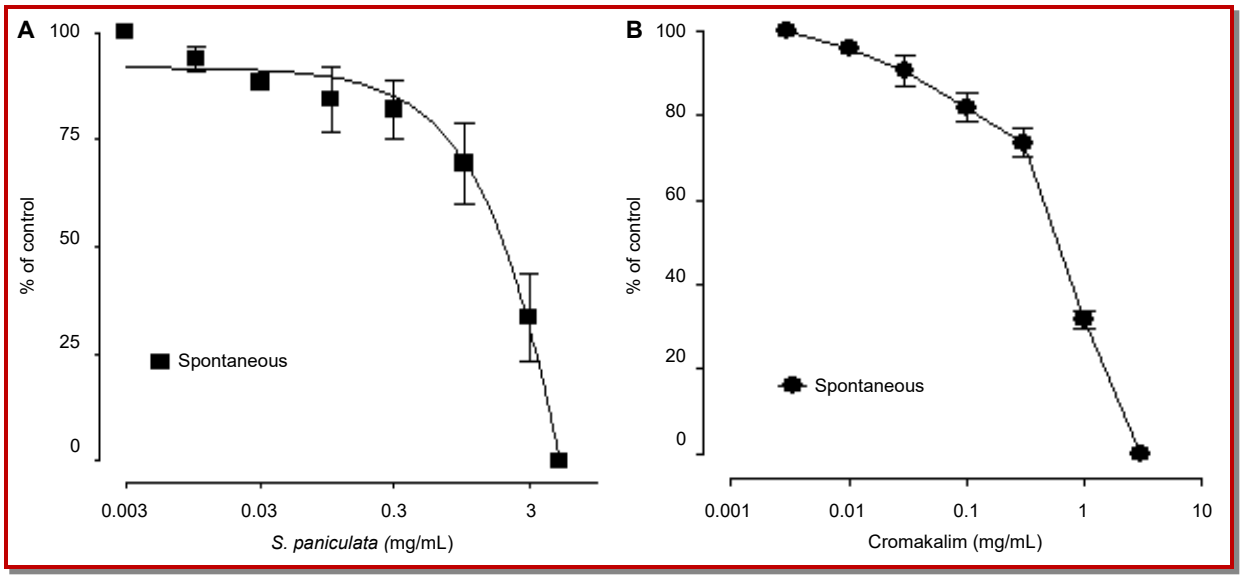

Figure: 1 Effect of *S. paniculata* (A) and cromakalim (B) on spontaneous contractions in isolated rabbit jejunum. Values are expressed as mean ± SEM for n = 5

reported to be mediated through blockade of Ca^{2+} channels (Janbaz et al., 2013)

In vivo experiments

Mice of either sex were divided into 5 groups containing 6 animals each. The animals were housed in individual cages and subjected to fasting for 24 hours prior the experiment. Group I animals were treated with saline (10 mL/kg) as negative control, while Group II animals were treated with loperamide (10 mg/kg) as positive control. Group III, IV and V animals were treated with crude extract of *S. paniculata* by respective oral doses of 100, 300 and 500 mg. One hour after the treatment, each individual animal received castor oil (10 mL/kg) orally. After 4 hours of castor oil treatment, the cages were inspected for typical diarrhea droppings, and the absence of droppings were taken as indicator of protection against castor oil-induced diarrhea.

Acute toxicity study

The experiments were conducted on 20 Swiss albino mice of either sex (16-36 g). The animals were housed into individual cages and maintained at 25°C. Mice were randomly divided into 4 groups, each containing 5 animals. The animals were subjected to fasting for 24 hours prior to experiments. Group I animals served as a control and received an oral dose (10 mL/kg) of normal saline. Group II animals received randomly selected higher initial dose of *S. paniculata* (2 g/kg). Group III animals were treated by an oral dose of *S. paniculata* which was double the dose of Group II (4 g/kg), whereas Group IV animals were treated by an oral dose of *S. paniculata* which was double the dose of Group III (8 g/kg). The animals were monitored for possible behavioral changes, while toxicity was assessed after 24 hours by inspecting the cages and counting the number of dead animals in comparison with the control group.

Statistical analysis

The data was expressed as mean ± standard error of mean and the median inhibitory concentrations (IC_{50}) with 95% confidence intervals. The statistics applied was Student's t-test except in case of castor oil-induced diarrhea where χ^2-test was used and ($p<0.05$) was taken as significant difference. Concentration-response curves were analyzed by non–linear regression using Graph Pad program (USA).

Results

Phytochemical analysis

Preliminary phytochemical studies detected the presence of saponins, flavonoides, anthraqinones, tannins, terpenes and phenolics among the methanol extractable constituents of *S. paniculata*.

Effect on rabbit jejunum

The application of *S. paniculata* to the spontaneous contractions of isolated rabbit jejunum preparations exhibited antispasmodic activity (Figure 1). The low K^+ (20 mM)-induced spastic contraction in isolated rabbit jejunum preparation were completely relaxed on bath in concentration-dependent manner (0.03-5 mg/mL) (Figure 2). The observed relaxant effect was speculated to be mediated through K^+-channel opening activity as cromakalim (a standard K^+ channel opener) caused suppression of the spontaneous contractions and also caused relaxation of low K^+ (20 mM)-induced spastic contractions in isolated rabbit jejunum preparation. The K^+-channel opening activity is known to be mediated through multiple mechanistic pathways but the

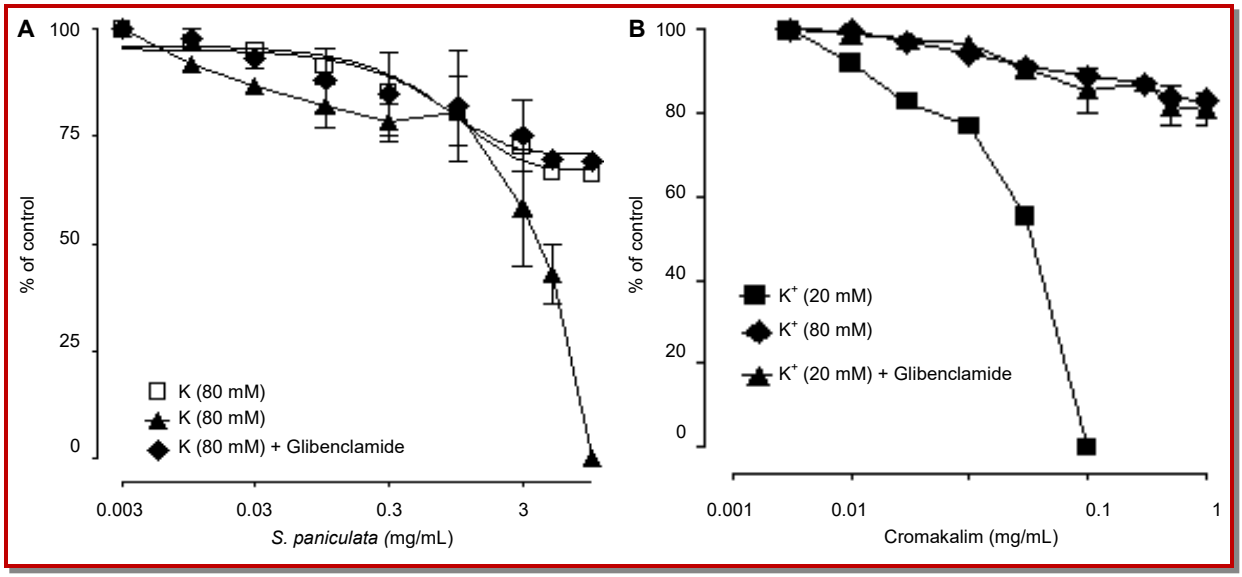

Figure: 2 Effect of *S. paniculata* (A) and cromakalim (B) on low K^+ (20 mM)-, high K^+ (80 mM)- and low K^+ plus glibenclamide-induced contractions in isolated rabbit jejunum preparations. Values are expressed as mean ± SEM for n = 5

observed relaxant profile on low K^+ (20 mM)-induced spastic contraction on the part of *S. paniculata* was noted to be comparable to the relaxant activity exerted by the cromakalim (ATP-dependent K^+-channel opener, Figure 1 and 2). These considerations were supported further by the findings that the relaxant effects of *S. paniculata* and cromakalim on low K^+ (20 mM)-induced contractions were observed to be blocked following treatment with glibenclamide. Furthermore, *S. paniculata* did not exhibit relaxant effect on high K^+ (80 mM)-induced spastic contractions in isolated rabbit jejunum preparations (Figure 2).

Effect on castor oil-induced diarrhea

Diarrhea was observed in all of the Group I mice to whom castor oil (10 mL/kg) was administered subsequent to treatment with normal saline (10 mL/kg). Group II animals were taken as positive control and diarrhea was not observed in any of its member, hence loperamide treatment provided 100% protection against castor-induced diarrhea. Group III, IV and V animals were treated by *S. paniculata* at respective oral doses of 100, 300 and 500 mg/kg prior to castor oil (10 mL/kg) treatment and the observed protection against castor oil was found to be 33, 50 and 67% respectively (Table I).

Table I

Effect of *S. paniculata* on castor oil-induced diarrhea in Swiss albino mice

Treatment	No. of mice	No. of mice with diarrhea
Saline (10 mL/kg) + Castor oil (10 mL/kg)	6	6
Loperamide (10 mg/kg) + Castor oil (10 mL/kg)	6	0
S. paniculata (100 mg/kg) + Castor oil (10 mL/kg)	6	4
S. paniculata (300 mg/kg) + Castor oil (10 mL/kg)	6	3
S. paniculata (500 mg/kg) + Castor oil (10 mL/kg)	6	2

Acute toxicity study

The *S. paniculata* was found to be safe up to the dose of 8 g/kg on administration.

Discussion

The preliminary phytochemical analysis indicated presence of saponins, flavonoides, anthraquinones, tannins, terpenes and phenolics in the hydro-methanol extract of *S. paniculata*. The *S. paniculata* caused suppression of the spontaneous contractions in isolated rabbit jejunum preparations. The smooth muscle contractions are carried out through increase in intracellular Ca^{2+} concentrations, which is responsible for demonstration of contractile responses (Karaki and Wiess, 1983) and the observed antispasmodic effect on the part of *S. paniculata* is likely to be mediated either through blockade of calcium channel(s) (Janbaz et al., 2015b) or through opening of K^+ channel. The *S. paniculata* was unable to relax the high K^+ (80 mM)-induced contractions and exhibited relaxation of the low K^+ (20 mM)-induced contractions. Hence, blockade of Ca^{2+} channels mechanism is ruled out and isolated

tissue preparations are likely to be relaxed through opening of K^+ channel(s) (Hamiltom et al., 1986; Kishii et al., 1992). The K^+ channel openers are drugs of variable chemical entity but with significant therapeutic applications (Quest, 1992; Empfield et al., 1995). The increase in K^+ efflux results in hyperpolarization of the excitable tissues, resulting in the decrease in intracellular Ca^{2+} contents, causing relaxation of the contracted smooth muscles (Quest and Cook, 1989; Weston and Edwards, 1992). Further investigations were carried out to explore the possible target for *S. paniculata* among multiple K^+ channels. The relaxant behavior of cromakalim on low K^+ (20 mM)-induced contractions of isolated rabbit jejunum preparations was found to be comparable with *S. paniculata* as activity of both of these relaxant was found to be mitigated following exposure to glibenclamide. Thus, confirming that suppression of spontaneous contractions and relaxation of low K^+ (20 mM)-induced spastic contraction in isolated rabbit jejunum preparations. The *S. paniculata* also demonstrated inhibitory influence on gut motility through *in vivo* experimentation as oral treatment of mice resulted in dose dependent protection against castor oil-induced diarrhea. The crude methanolic extract of *S. paniculata* was found free of any acute toxic effect up to the dose of 8 mg/kg indicating that it can be used medicinally without any avert risk of toxicity.

Conclusion

S. paniculata possesses antispasmodic activity mediated predominantly through opening of ATP-dependent K^+-channels. Thus, this study provided a rational to the folkloric use of *S. paniculata* in gastrointestinal disorder i.e. diarrhea.

Financial Support

Self-funded

Ethical Issue

The experiments performed were in compliance to the rulings of the Institute of Laboratory Animal Resource, Commission on Life Sciences, National Research Council (1996) and approved by the Ethical Committee of Faculty of Pharmacy, Bahauddin Zakariya University, Multan.

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

The author(s) are very thankful to laboratory staff of Faculty of Pharmacy. Bahauddin Zakariya University, Multan for the provision of research related facilities.

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Author Info
Fatima Saqib (Principal contact)
e-mail: fatima2saqib@yahoo.com