The Antispasmodic Activity of *Buddleja scordioides* and *Buddleja perfoliata* on Isolated Intestinal Preparations

Alma Rosa CORTÉS,* Alba Jady DELGADILLO, Marcela HURTADO, Adriana Miriam DOMÍNGUEZ-RAMÍREZ, José Raúl MEDINA, and Kazuko AOKI

Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco; Calzada del Hueso 1100, Colonia Villa Quietud, México, D.F., México, C.P. 04960. Received October 5, 2005; accepted March 3, 2006

The antispasmodic activity of extracts from the aerial parts of *Buddleja scordioides* and *Buddleja perfoliata* (family: Scrophulariaceae) was studied on isolated tissue preparations from rabbit and guinea pig intestine. The chloroform extract from the plants exhibited a significant relaxation on the spontaneous contraction of isolated rabbit jejunum at concentrations ranging from 1 to 400 μg/ml, and also caused an inhibitory effect on both K⁺ and Ca²⁺ induced contractions in the same tissue. The extracts at moderate doses (50 μg/ml) reduced 5-hydroxytryptamine (5-HT), acetylcholine and histamine induced contractions on isolated guinea pig ileum. Therefore, *B. scordioides* and *B. perfoliata* possess similar relaxant mechanism of action, in view of the fact that both inhibit K⁺ induce contraction and act through serotoninc, muscarinic and histaminic receptors. So, these data support the idea that the extracts may interfere either with calcium mobilization from intracellular stores, or with calcium interaction with regulatory proteins (e.g., calmodulin), or in other steps in the calcium signaling pathway. This leads us to suggest that the spasmylic effect of both *Buddleja* species on smooth muscular contractility are due to the same or similar compounds occurring in these two species, which might be present in similar quantities.

**Key words**  *Buddleja scordioides*; *Buddleja perfoliata*; spasmylic; calcium antagonist; smooth muscle relaxant

The genus *Buddleja*, included in the family Loganiaceae, and previously classified in a family of its own, the Buddlejaceae, is now classified in the family Scrophulariaceae. Native to Asia, Africa, North and South America, *Buddleja* (also *Buddleia*), is a genus containing approximately 100 species, 50 being distributed in America, of which 16 grow in Mexico.¹

*Buddleja* species are widespread and share some remarkable similarities in their medicinal uses. This may well indicate the presence of the same or similar compounds with a particular pharmacological action. A pattern is emerging about the composition of these compounds; flavonoid and iridoid glycosides being the major secondary metabolites that have been isolated to date, and which are common throughout the genus.²

Due to the interesting phytochemical and ethnopharmacological observations, the genus *Buddleja* has been investigated extensively; a valuable review on chemical composition and biological activity has been published by Houghton.²,³

*Buddleja scordioides* HBK (KUNT), Loganiaceae, is commonly known as escobilla, butterfly-bush, mato, salvia real and salvilla. This plant is widely used for the treatment of diarrheea, stomachache (colic) and gastrointestinal disorders.⁴ In addition, a verbascoside with antibacterial activity,³ triterpenoid saponins and other glycosides,⁵ have been extracted from this species. The presence of some flavonoids such as rutine, quercetin and quercitin has also been reported.⁴

*Buddleja perfoliata* HBK (KUNT), Loganiaceae, known in Spanish as salvia de bolita, hierva de manita, salvia chiquita, salvia de campo, became officially recognised in the 1930 Mexican Pharmacopoeia where it was shown to have antispasmodic activity.²,³ Vague and limited information regarding the types and quantity of chemical compounds found in this plant is available. This plant also contains essential oil, tan-

cic, gallic and oxalic acids.⁴ In folk medicine, it is used in the treatment for tuberculosis as well as for catarrh, ptalamus, and headaches.⁶,⁷

Despite the extensive use of *B. scordioides* for gastrointestinal problems such as intestinal spasms, no pharmacological studies have been carried out to date, for the chemical content relationship, throughout the *Buddleja* genus, where the species *perfoliata* may possibly have similar biological activity.

In the present paper, we evaluated the antispasmodic activity of two *Buddleja* species, *Buddleja scordioides* and *Buddleja perfoliata*, which are widely used in Mexico in traditional medicine.

**MATERIALS AND METHODS**

**Plant Material and Extraction**  *B. scordioides* was collected in May from the North of Mexico (Chihuahua State) and a fresh plant of *B. perfoliata* was purchased from the Mexico city local market. The plants were identified by Dr. Jerónimo Reyes from the Botanical Garden, Institute of Biology, Autonomous National University of Mexico. A voucher of each specimen was retained. The aerial parts of the plants were washed with water, air-dried and milled to a coarse powder. The powders were then extracted twice under reflux for 4 h using chloroform. The extracts were then filtered and dried on a rotary evaporator and stored at −20°C until required.

The crude extracts and polyvinylpyrrolidone 2500 (PVP) (1 : 4 w/w), used as a co-solvent, were weighed and then dissolved separately in a minimum volume of chloroform and methanol, respectively. They were then quantitatively mixed, dried under a vacuum and dissolved in a Tyrode solution pH 7.4. Control experiments demonstrated that the solvents used to dissolve plant extracts did not affect the contractile re-

* To whom correspondence should be addressed. e-mail: cortesar@correo.xoc.uam.mx

© 2006 Pharmaceutical Society of Japan
sponses of the isolated tissues at the final bath concentration.

**Chemicals and Drugs** All chemicals used were of reagent grade, purchased from Merck Mexico. Acetylcholine (Ach), phentolamine, histamine (HIS), 5-hydroxytryptamine (5-HT) and propranolol were obtained from Sigma Chemical Company, U.S.A. Ach and HIS were dissolved in a Tyrode solution, adjusted to pH 4 using ascorbic acid, and all other drugs were prepared in suitable physiological saline solution.

**Animals** Male New Zealand white rabbits (2—2.5 kg) and Dunkin Hartley guinea pigs from both sexes (400—600 g) of local breed were used for this study. These were housed at the Animal House of the Autonomous Metropolitan University-Xochimilco of Mexico, maintained in controlled conditions at 23—25 °C, under a 12 h light/dark cycle, and were given a standard diet and free access to water, however, food was withdrawn 24 h prior to experimentation. The rabbits were sacrificed by a blow to the nape of the neck followed by exsanguination and the guinea pigs euthanized with CO2. Animals were treated according to the guidelines for animal care and use of laboratory animals by official Mexican regulations.

**Isolated Tissue Preparations** The spasmolytic activities of *B. scordioides* and *B. perfoliata* were investigated using isolated tissue preparations from rabbit jejunum and guinea pig ileum. Several segments of jejunum were rapidly removed through a midline abdominal incision. The tissue was cleaned and placed directly in a 10-ml organ chamber and was allowed to equilibrate for 30 min in Tyrode solution, pH 7.4, and was aerated continuously with a gas mixture of 95% O2—5% CO2, and maintained at 37 °C. An initial tension of 0.5 g was applied.

The terminal portion of the guinea pig ileum, the 10 cm portion nearest the caecum being discarded, was cleaned and transferred to the Tyrode solution. Segments of ileum (0.5 cm) were placed directly in a 10-ml organ chamber. The preparations were given an initial load of 1 g and were equilibrated for 1—2 h before the experiments were initiated with a 15 min washout interval.

The composition of the Tyrode solution (mm) was: NaCl (136.9), KCl (2.7), CaCl2 (1.8), MgCl2 (1.0), NaHCO3 (11.9), NaH2PO4 (0.4) and glucose (5.5), pH 7.4.

The experiments were performed on three or four segments of jejunum or ileum taken from each of four or five animals. Segments that did not show spontaneous activity were discarded.

The force of contraction was recorded simultaneously on a 4-channel Narco-Biosystem physiograph, equipped with an isometric F-60 force transducer which had been previously calibrated.

**Relaxing Activity on Isolated Rabbit Jejunum** The activity of the extracts was tested in isolated spontaneously contracting rabbit jejunum, in the absence and presence of several agents including non-selective α and β-adrenergic antagonists: phentolamine (2×10⁻⁶ M) and propranolol (1×10⁻⁶ M). Verapamil (50—280 nm) was used as a positive control. The inhibition of contractions in the test material was assessed as a percentage of the basal spontaneous contractions in the rabbit jejunum.

The spasmolytic effect was studied using potassium depolarized for adding K⁺ (50 mM) to the tissues; the high potassium solution was obtained by an equimolar replacement of NaCl by KCl in the Tyrode solution. A concentration response was obtained by the cumulative addition of the extracts at 7-min intervals after addition of 50 mM KCl. The relaxation of the jejunum was expressed as a percentage of control K⁺-induced contractions. Verapamil (3.2×10⁻⁴—3.2×10⁻⁶ M) was used as a positive control.

To assess whether the spasmolytic activity of *Buddleja* extracts was via a calcium channel blockade, the jejunum was allowed to stabilize in normal Tyrode solution, and was then replaced with a Ca²⁺-free Tyrode solution for 30 min. This solution was replaced with K⁺ rich (50 mM) and Ca²⁺ free Tyrode solution. Following an incubation period of 20 min and after the confirmation of no spontaneous contractions of jejunum, Ca²⁺ was added in a cumulative fashion (1×10⁻⁴—3×10⁻² M), every 3 min, to obtain control concentration—response curves of Ca²⁺. The concentration—response curves of Ca²⁺ were repeated following a 10 min incubation with the extracts.

**Spasmolytic Effect on Guinea Pig Ileum** The influence of the extracts on the contractions induced by 5-HT (1×10⁻⁴—1×10⁻⁵ M), Ach (1×10⁻⁹—1×10⁻⁸ M) and HIS (1×10⁻⁷—3×10⁻⁵ M), was studied in preparations pre-incubated for 10 min with each extract.

**Statistical Analysis** The results obtained from at least four different animals are presented as mean±S.E.M. Two sample comparisons were made by a two-tail unpaired Student’s t-test. Statistical significance was assumed at p levels less than 0.05. The concentrations to produce 50% of the maximal responses (EC₅₀) were calculated by a nonlinear curve fitting procedure using Sigma Plot version 8.0.

**RESULTS**

Chloroformic extracts from the aerial parts of *B. scordioides* and *B. perfoliata* caused both a concentration-dependent antispasmodic effect in the spontaneous contraction of rabbit jejunum, at a concentration of 1—400 μg/ml, which was similar (with respect to inhibition profile) to that caused by verapamil at a concentration range of 50 to 280 nm, however, in isolation, neither the resting tension of the tissue nor the spontaneous contraction frequency was altered (Fig. 1).

The effect of *B. scordioides* and *B. perfoliata* extracts on spontaneous contraction were not significantly different (p>0.05), concentrations that caused 50% inhibition of the spontaneous contractions (EC₅₀) were 62.8±11.8 and 41.35±5.6 μg/ml, respectively (Fig. 2a). The spasmolytic effect was reversible and the spontaneous activity returned to normal after washing the preparation.

Pre-incubation of the tissues with phenolamine and propranolol had no effect on the extract response. However, a high concentration of K⁺ is known to cause smooth muscle contractions through the opening of voltage dependent slow Ca²⁺ channels, thus allowing the influx of extracellular Ca²⁺, causing a contractile effect. When tested in isolated rabbit jejunum, the extract of *B. scordioides* and *B. perfoliata*, within the same concentration range that inhibited the spontaneous contraction, caused a spasmolytic effect on the tissue pretreated with high K⁺ (50 mM), which was similar to that of verapamil. The concentration—response curves show exactly the same pattern for both *Buddleja* extracts, with no signifi-
cant difference in the EC$_{50}$ values of 27.6±3.1 µg/ml and 27.3±4.6 µg/ml, respectively and with the 400 µg/ml bath concentration response to KCl completely abolished by the extracts (Fig. 2b).

Throughout these experiments, there was no significant change in the K$^+$ responses of the preparations treated with an equivalent volume of the vehicle.

A contractile response was obtained by the addition of calcium (1×10$^{-4}$–3×10$^{-2}$ M) to a previously decalcified rabbit jejunum. Under the same conditions, but in presence of the extracts of $B$. scordioides and $B$. perfoliata a decrease in the contractile response was observed, producing a shift in the Ca$^{2+}$ curves (Fig. 3), with maximum inhibition at 50 µg/ml of the extracts reaching 55.8±5.1% and 62.6±4.9%, respectively. Serotonin (5-HT), Ach and HIS caused a rapid contraction of guinea pig ileum, reaching the maxima within 30 s of contact. Similarly, both extracts of $B$. scordioides and $B$. perfoliata at a concentration of 50 µg/ml reduced the tissue response to these drugs without any significant difference between them ($p>0.05$). The spasmylic effect of the extracts in the preparations contracted with 5-HT was more pronounced that those contracted with HIS or Ach (Fig. 4).

The concentration–response curves to 5-HT, in the presence of 50 µg/ml of $B$. scordioides and $B$. perfoliata extracts,
shifted to the right, and the maximal height of contractions decreased to 40.7±1.4% and 35.7±5.1%, respectively (Fig. 4a).

At a same concentration of both extracts (50 μg/ml) the concentration–response curves to Ach and HIS also decreased. The reduction of the maximum response with respect to the acetylcholine control was 57.2±4.4% and 54.3±3.5% (Fig. 4b), and 72.8±6.1% and 64.8±2.6% with respect to the HIS control (Fig. 4c).

When the tissues were pre-incubated with 100 μg/ml of B. scordioides and B. perfoliata (data not shown), the responses of HIS decreased to 52.9±3.4% and 38.7±6.3%, respectively.

**DISCUSSION**

The results from the present study indicate that both crude chloroformic extracts of *B. scordioides* and *B. perfoliata* had a relaxant effect on rabbit jejunum and guinea pig ileum, and reversibly blocked spasms induced by specific and unspecific stimuli. This effect was attained at relatively low concentrations and may be related to the symptomatic relief of acute abdominal pain associated with some gastric disturbances, obtained with medicinal usage of these plants commonly practiced in Mexico. The inhibitory effect was probably due to different components acting separately or together. Apparently, relaxation was not due to postsynaptic stimulation of adrenergic receptors since pre-incubation of the tissues with phentolamine and propranolol had no effect on the extract response.

At identical bath concentrations, the chloroformic extracts of *B. scordioides* and *B. perfoliata* demonstrated an inhibitory effect on the contraction of ileum induced by 5-HT, Ach and HIS (Fig. 4). This suggests that both extracts have no selective inhibitory effect on contractions induced by these spasmodens.

It has been accepted that the contraction of smooth muscle is dependent on an increase in the concentration of cytosolic Ca^{2+}, and the sensitivity of the contractile elements to Ca^{2+} in response to changes in the cell. There is evidence that there is a significant variation in the degree of participation of extra- and intracellular Ca^{2+} in smooth muscle contraction.\(^1\)

The increase in intracellular Ca^{2+} is due to either influx via voltage dependent Ca^{2+} channels (VDCs) or the release from intracellular stores in the sarcoplasmic reticulum. Periodic depolarization and repolarization regulate the spontaneous movements of the intestine and at the height of depolarization, the action potential appears as a rapid influx of Ca^{2+} via VDCs.\(^2\) Organic calcium channel antagonists, such as verapamil inhibit more markedly the entry of calcium through the VDCs.

In order to confirm the interaction of Buddleja extracts with voltage dependent Ca^{2+} channels, the tissue was pretreated with high potassium. Certainly, the contractions induced by KCl are dependent on the entry of Ca^{2+} into the cells through voltage-dependent calcium channels, therefore a substance which can inhibit high K^{+}-induced contraction is, considered to be a Ca^{2+} channel blocker.\(^3\)

However, it might be also possible that *Buddleja* could relax the intestinal smooth muscle by interfering with calcium transport across membranes, or blocking inositol triphosphate (IP3) mediated Ca^{2+} release from internal stores.

In the following, an attempt to summarize the current knowledge about IP3 participation is presented. Ach interacts with muscarinic receptors on ileum smooth muscle cell membranes and thereby increases the activity of membrane bound phospholipase-C enzymes and the generation of inositol triphosphate, which on being released into the cytoplasm, will interact with receptors on intracellular Ca^{2+} store sites and cause the release of calcium.\(^4\)

In addition, histamine-induced contractions generated by H1 receptor activation, locate postsynaptically to produce depolarization and tonic contractions of smooth muscle.\(^5\) The binding of HIS to a H1 receptor in smooth muscle results in the opening of receptor operated channels, thereby allowing sodium influx, which causes a depolarization of the cell membrane. This depolarization opens voltage dependent calcium channels and Ca^{2+} enters the cell, which induces the release of calcium from the sarcoplasmic reticulum. The cytosolic calcium thus binds to calmodulin, which results in contraction,\(^6\) but calcium release from IP3 sensitive stores is negatively regulated by binding of calmodulin to the IP3 receptor.

Finally, the 5-HT contraction is mediated by the release of Ach from the cholinergeric neurone and activation of serotonergic receptors on the smooth muscles of the ileum. 5-HT has been described as participating in the regulation of intestinal peristalsis in different animals, and produces a contractile effect mediated by Ca^{2+} via 5-HT2 receptors (attenuated by simultaneous generation of cAMP via 5-HT4 receptors) which may alter or modulate the intestinal physiology.\(^8\)

Therefore, the effects observed in both *Buddleja* species to these three agonists seemed to be Ca^{2+}-dependent and involves, at least in part, the mobilization of Ca^{2+} from inositol triphosphate (IP3) sensitive intracellular stores.

Consequently, it can be postulated that the therapeutic activity exhibited by Buddlejas must be due to the combined effects of several chemical constituents present in the plants, such as triterpene saponins and triterpenic glycosides isolated from *B. scordioides*,\(^9\) and different flavonoids, such as quercetin which exhibits a spasmytic effect through blockade of calcium channels.\(^10\) In this regard, studies with triterpene saponin and triterpenic glycosides obtained from different medicinal plants, have shown spasmytic activity on isolated tissues such as guinea pig bronchus and ileum. Specifically, Sairogenin A, a triterpenoid saponin, one of the compounds reported for *B. scordioides*,\(^11\) is related to plants from the genus Bupleurum. Besides its observed anti-inflammatory effect,\(^12\) it has also been reported that Sairogenin A can suppress asthmatic bronchoconstriction in sensitized guinea pigs by antagonism of HIS action.\(^13\) Similarly, triterpenic glycosides, could also be participating in the antispasmodic effect.\(^14\)

**CONCLUSIONS**

The results from this study demonstrate that both crude chloroformic extracts of *B. scordioides* and *B. perfoliata* had a relaxant effect on rabbit jejunum and guinea pig ileum, and that this activity may be the basis for some of its uses in tra-
ditional medicine. Therefore, the antispasmodic activity found, supports the rationale use, in both folk and traditional medicine of *B. scordioides* in the treatment of gastrointestinal cramps, spasms and colic. This activity was also observed with *B. perfoliata*, which suggests that it could be used to treat the same afflictions.

Finally, it can be concluded that both *Buddleja* species possess similar smooth muscle relaxant mechanism of action, in view of the fact that both inhibit K⁺ induced contraction and they act through serotoninic, muscarinic and histaminic receptors. Therefore, these data support the idea that this extracts may interfere either with calcium mobilization from intracellular stores, or with the calcium interaction with regulatory proteins (e.g., calmodulin), or at other levels of the calcium signaling pathway as a second messenger, such as, by decreasing the contractile machinery sensitivity to calcium, as discussed previously.

This leads us to suggest that the spasmolytic effect of *B. scordioides* and *B. perfoliata* can be attributed to the same or similar compounds occurring in both species, and which might be present in similar quantities.

REFERENCES AND NOTES