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Presence of Blood-Pressure Lowering and Spasmolytic Constituents in *Buddleja crispa*

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This aim of this study was to investigate the crude extract of *Buddleja crispa* (Bc.Cr) and its active constituent(s) for their antihypertensive and antispasmodic activities. The Bc.Cr caused a dose-dependent (3–10 mg/kg) fall in mean arterial pressure in rats under anesthesia. In rabbit aorta preparations, Bc.Cr (0.03–1 mg/mL) caused inhibition of high K+ (80 mM) precontractions. The Bc.Cr (0.03–1 mg/mL) also inhibited spontaneous and high K+ precontractions in rabbit jejuneum preparations, suggestive of calcium channel blocking (CCB) activity. CCB activity was further confirmed when pretreatment of the tissues with Bc.Cr (0.03–0.10 mg/mL) caused a rightward shift in Ca++ concentration response curves, similar to verapamil. Among the pure compounds, BdI-H3 was more potent against the high K+ than spontaneous contractions and was around eight times more potent than Bc.Cr against the spontaneous contractions while the other two compounds, BdI-2 and BH-3 were inactive. Activity-directed fractionation revealed that the hexane fraction was more potent against K+ precontractions. These data indicate that Bc.Cr possesses a blood-pressure lowering effect, mediated possibly through CCB, though additional mechanism(s) cannot be ruled out. Among the pure compounds, BdI-H3 is likely to be the active compound involved in the spasmolytic and possibly BP lowering effect of the parent crude extract.

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Keywords: *Buddleja crispa*; fractionation; pure compounds; hypotensive; spasmolytic; calcium antagonist.

INTRODUCTION

*Buddleja crispa* Benth. (Buddlejaceae), is a densely tomentose shrub endemic in Pakistan (North-west Himalaya and Baluchistan), Afghanistan and North India (Abdullah, 1974). The genus Buddleja comprises about 100 species endemic throughout the warmer temperate parts of Southern Asia, South and East Africa and America. The genus has widespread medicinal use and several of its species have been used in a variety of health conditions in different parts of the world; however, pharmacological studies related to *B. crispa* are still lacking. *B. crispa* has been reported to contain some new compounds, such as buddlejoside (iridoid galactoside), buddlejone (sesquiterpene), aryl esters along with known compounds, β-sitosterol and ursolic acid (Abdullah, 1974; Ahmad et al., 2004). In view of the medicinal use of the genus Buddleja in cardiovascular and gastrointestinal disorders, the present study was undertaken to evaluate the blood-pressure lowering and antispasmodic effects of *B. crispa* along with its pure compounds, BdI-H3, BdI-2 and BH-3.

MATERIALS AND METHODS

Plant material

The whole plant material was collected in March 2003 from Baluchistan and identified as *Buddleja crispa* Benth., by Taxonomist at the Department of Botany, University of Baluchistan, Pakistan. A voucher specimen (BBU-101) was deposited at the herbarium of the same Department.

Extraction and fractionation

The shade-dried whole plant (30 kg) was chopped and extracted with methanol at room temperature (23–25 °C) for three days thrice with occasional shaking. The methanolic extract was filtered through muslin cloth and finally through filter paper (Wattman qualitative grade 1). The combined extract was then evaporated in rotary evaporator at 35–40 °C under reduced pressure (~760 mmHg) to a dark-greenish residue, the crude extract of *B. crispa* (Bc.Cr), with the approximate yield of 0.11%.

For activity-directed fractionation, about 500 g of the crude extract was dissolved in water and successively partitioned to give n-hexane (Be.Hex) and ethylacetate
(Bc.EtAc) fractions with approximate yields of 14 and 15.60% respectively. The crude extract and fractions were solubilized in normal saline, distilled water and 10% dimethyl sulfoxide (DMSO) respectively for use in the in vivo and in vitro experiments.

**Pure compounds**

Pure compounds, Bdl-2, Bdl-H3 and BH-3 were isolated from B. crispa, as described previously (Ahmad et al., 2005) (Fig. 1).

**Drugs and standards**

The following reference chemicals were obtained from the sources specified: acetylcholine chloride, norepinephrine bitartrate, potassium chloride and verapamil (Sigma Chemical Company, St Louis, MO, USA). All chemicals used were of the highest purity grade. Stock solutions of all chemicals were made in fresh normal saline (NaCl 0.9%) and distilled water for the in vivo and in vitro experiments respectively, on the day of the experiment.

**Animals**

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) and approved by the Ethical Committee of Aga Khan University Karachi. Sprague-Dawley rats (200–250 g) and local rabbits (1.5–2 kg) of either sex used in the study were bred and housed in the animal house of Aga Khan University under a controlled environment (23–25 °C). Animals were given tap water ad libitum and a standard diet consisting of (g/kg): flour 380, chokar 380, molasses 12, salt 5.8, nutrivet L 2.5, potassium meta bisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

**Blood pressure measurement in normotensive anesthetized rats**

Arterial blood pressure was studied in normotensive anesthetized rats as described previously (Gilani et al., 2007). The animals were anesthetized with an intraperitoneal injection of sodium thiopental (Pentothal, 70–90 mg/kg body weight) and were fixed in a supine

![Figure 1. Structure of the pure compounds isolated from B. crispa; compound 1 (Bdl-2), Compound 2 (Bdl-H3) and 3 (BH-3).](image-url)
position on a dissecting table, under an overhead lamp to maintain body temperature. Arterial blood pressure was recorded through carotid cannulation using a pressure transducer (P23 XL) coupled with a Grass model 7 Polygraph. The left jugular vein was cannulated with similar tubing to facilitate the intravenous (iv) injection of the drugs and test materials. After a 20-min period of equilibrium, the rats were injected intravenously with 0.1 mL saline or with the same volume of test substance. Standard drugs and the plant extract (prepared in saline) were then administered intravenously and followed by a flush of 0.1 mL saline. Control responses of standards, such as acetylcholine (ACh; 1 μg/kg) and norepinephrine (NE; 1 μg/kg) were obtained before testing the extract.

A change in mean arterial pressure (MAP) was recognized as the difference between the steady-state value before and the lowest reading after injection of the test material. MAP was determined from the sum of diastolic blood pressure plus 1/3 pulse amplitude. Changes in MAP were expressed as percentages of control values obtained immediately before the administration of test substance. The blood pressure was allowed to return to normal before the next dose was administered and the doses mentioned in the text represent the actual dose administered each time.

Isolated tissues preparations

Rabbit aorta. The experiments on rabbit aorta preparation were performed as described previously (Gilani et al., 2005). Rabbits were sacrificed by cervical dislocation and the descending thoracic aorta was removed, freed from extra vascular tissues and cut into 2–3 mm wide rings, which were individually mounted in 10 mL tissue baths containing normal Krebs solution maintained at 37 °C and aerated with carbogen. The composition of Krebs solution was (mM): NaCl 118.2, KCl 2.7, MgCl2 1.1, NaHCO3 11.9, NaH2PO4 0.4, Glucose 5.55 and CaCl2 1.8 (pH 7.4). A preload of 1 g was applied and the tissues kept undisturbed for an equilibrium period of 30 min after which control responses to a sub-maximal dose of ACh (0.3 μM) were obtained and the tissue presumed stable only after the reproducibility of the said responses.

Determination of calcium channel blocking activity

To assess whether the spasmyolytic activity of the test substances was through calcium channel blockade (CCB), high K+ (80 mM), as KCl, was used to depolarize the preparations (Farre et al., 1991). K+ was added to the tissue bath, which produced a sustained contraction. Test substances were then added in a cumulative fashion to obtain inhibitory response curves, which are expressed as a percent of the control response produced by high K+. Substances which produce relaxation against high K+-induced contractions are considered to be calcium channel blockers (Bolton, 1979).

To confirm the CCB activity of the test substances, the tissues were allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca2+-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 Ca2+-free Tyrode’s solution, having the following composition: NaCl 91.04, KCl 50, MgCl2 1.05, NaHCO3 11.90, NaH2PO4 0.42, glucose 5.6 and EDTA 0.1 mM. Following an incubation period of 30 min, control concentration-response curves (CRCs) of Ca2+ were obtained. When the control CRCs of Ca2+ were found super-imposable (usually after two cycles), the tissue was pretreated with the test substance for 1 h to test the possible CCB effect. The CRCs of Ca2+ were reconstructed in the presence of different concentrations of the test material.

Statistics

All the data expressed are mean ± standard error of the mean (SEM), and the median effective concentrations (EC0n values) are given with 95% confidence intervals (CI). The statistical parameter applied is the student t-test with p < 0.05 noted as significantly different.

RESULTS AND DISCUSSION

Intravenous administration of the crude extract of Buddleja crispa caused a fall in MAP of normotensive rats under anesthesia at the dose range of 3–10 mg/kg (Fig. 2A). The percent fall in MAP at the respective doses of 3, 5 and 10 mg/kg was 17.0 ± 3.14, 23.33 ± 3.12 and 30.0 ± 5.40 mmHg (Fig. 2B). The underlying mechanism of blood-pressure lowering effect of Bc.Cr was studied in isolated vascular preparations because interference due to the intact reflexes is not the problem.

When tested in isolated rabbit aorta preparations, cumulative addition of the Bc.Cr caused inhibitory effect against the high K+-induced contractions, with EC50 value of 0.20 mg/mL (0.11–0.31, 95% CI) (Fig. 2C). K+ at high concentrations (>30 mM) is known to cause smooth muscle contractions through opening of voltage-dependent Ca2+ channels, allowing influx of extracellular Ca2+ causing a contractile effect and a substance which can inhibit the K+-induced contractions is considered to be a calcium channel blocker (Godfraind et al., 1986). Thus the inhibitory effect of the crude extract against the K+-induced vascular contractions can be visualized.
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![Figure 2. A tracing (A) showing the blood pressure lowering effect of the crude extract of *Buddleja crispa* (Bc.Cr) in anesthetized rats. Fig. 2B depicts the blood pressure lowering effect of Bc.Cr in normotensive anesthetized rats. Fig. 2C shows the effect of the Bc.Cr on the high K⁺-induced contractions in isolated rabbit aorta preparations. Values shown are mean ± SEM of 4 determinations each.](image)

as the CCB effect, though additional mechanism(s) cannot be ruled out.

Based on the medicinal use of the Buddleja genus in different disorders (Evans, 2005), the crude extract was further studied in gut preparations. When tested in isolated rabbit jejunum, cumulative addition of the crude extract of *B. crispa*, its fractions and a pure compound, Bdl-H3, caused inhibition of the spontaneous and high K⁺-induced contractions to a varying degree, similar to that of verapamil (Figs 3 and 4), a standard calcium channel blocker (Fleckenstein, 1977). The CCB effect was confirmed when pretreatment of the tissues with crude extract caused a rightward shift of the Ca²⁺ concentration response curves (Fig. 5A), similar to that caused by verapamil (Fig. 5B). The Bc.Cr and its fractions along with pure compound (Bdl-H3) all possessed similar potency against the K⁺. The hexane fraction was less potent for its spasmylytic effect on spontaneous contractions while the pure compound (Bdl-H3) was found distinctly more potent when compared with inhibitory effect against K⁺, which indicates that some additional mechanism(s) are involved in its spasmylytic effect. Unfortunately, limited supply of the pure compound did not allow further study in detail for the precise mode of action. The other two pure compounds (Bdl-2 and BH-3) isolated from this plant, were found without effect on the spontaneous and K⁺-induced contractions (data not shown).

In conclusion, these data indicate the presence of blood-pressure lowering and spasmylytic constituents in the crude extract of *Buddleja crispa*, mediated possibly through CCB effect, though additional mechanism(s) cannot be ruled out.

![Figure 3. A typical tracing showing the effect of the crude extract of *Buddleja crispa* (Bc.Cr), its pure compound (Bdl-H3) and verapamil on the spontaneous contractions in isolated rabbit jejunum preparations.](image)

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Figure 4. Concentration-response curves showing the effect of (A) Buddleja crispa crude extract (Bc.Cr); its (B) hexane (Bc.Hex) and (C) ethylacetate (Bc.EtAc) fractions and (D) pure compound (BdI-H3) and (E) verapamil on spontaneous and high K+ induced contractions in isolated rabbit jejunum preparations. The values shown are mean ± SEM of 4–5 determinations each.

Figure 5. Concentration-response curves showing the effect of (A) the crude extract of Buddleja crispa (Bc.Cr) and (B) verapamil on Ca²⁺ concentration-response curves, constructed in Ca²⁺ free medium in isolated rabbit jejunum preparations. The values shown are mean ± SEM of 4–6 determinations each.
REFERENCES


