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# *Bacopa monnieri* and its constituents is hypotensive in anaesthetized rats and vasodilator in various artery types

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ABSTRACT

*Ethnopharmacological relevance: Bacopa monnieri* (Brahmi) provides traditional cognitive treatments possibly reflecting improved cerebral hemodynamics. Little is known about the cardiovascular actions of Brahmi. We sought to assess its effects on blood pressure and on isolated arteries, thus providing insights to clinical applications.

*Materials and methods:* Intravenous Brahmi (20–60 mg/kg) was tested on arterial blood pressure and heart rate of anaesthetized rats. *In vitro* vasorelaxation was assessed in arteries, with and without blockers of nitric oxide synthase (L-NAME), cyclooxygenase (indomethacin), and mechanical de-endothelialisation. The effects of Brahmi on $Ca^{2+}$ influx and release from stores were investigated.

*Results:* Intravenous Brahmi extract (20–60 mg/kg) decreased systolic and diastolic pressures without affecting heart rate. Brahmi evoked relaxation in isolated arteries in order of potency: basilar (IC50 = 102 ± 16 μg/ml) > mesenteric (171 ± 31) > aortae (213 ± 68) > renal (IC50 = 375 ± 51) > tail artery (494 ± 93) > femoral arteries (>1000 μg/ml). Two saponins, bacoside A3 and bacopaside II, had similar vasodilator actions (IC50 = 8.3 ± 1.7 and 19.5 ± 6.3 μM). In aortae, without endothelium or in L-NAME (10–4 M), Brahmi was less potent (IC50 = 213 ± 68 to 2170 ± 664 and 1192 ± 167 μg/ml, respectively); indomethacin (10–5 M) was ineffective. In tail artery, Brahmi inhibited $K^+$-depolarization induced $Ca^{2+}$ influx and $Ca^{2+}$ release from the sarcoplasmic reticulum by phenylephrine (10–5 M) or caffeine (20 mM).

*Conclusions:* Brahmi reduces blood pressure partly via releasing nitric oxide from the endothelium, with additional actions on vascular smooth muscle $Ca^{2+}$ homeostasis. Some Brahmi ingredients could be efficacious antihypertensives and the vasodilation could account for some medicinal actions.



## 1. Introduction

*Bacopa monnieri* (L.) Wettst. (Brahmi) is an Ayurvedic medicinal plant traditionally used in India and Pakistan to promote mental health, enhancing memory, and has also been used as a cardiotonic drug (Thorne Research, 2004; Russo and Borrelli, 2005; Prasad et al., 2008; Gohil and Patel, 2010). The Brahmi extract has potent cognitive enhancing properties and neuroprotective effects (Das et al., 2002; Dhanasekaran et al., 2007; Limpeanchob et al., 2008; Uabundit et al., 2010; Vollala et al., 2010). Very few studies have sought to explain its apparent cardiotonic effects. Recent research showed that a hydro-alcoholic extract of Brahmi provided cardioprotection against isoproterenol-induced myocardial necrosis in rats (Nandave et al., 2007). Previous work reported that an ethanolic extract of Brahmi relaxed isolated guinea-pig and rabbit pulmonary arteries, and rabbit aorta (Dar and Channa, 1997, 1999). Calcium-induced contractions in rabbit pulmonary artery and aorta were attenuated by the plant extract, implying an inhibitory effect on $Ca^{2+}$ influx into vascular smooth muscle cells (Dar and Channa, 1999; Channa et al., 2003). Nevertheless, it is unclear how Brahmi produces vasodilation and whether these actions are manifest as a reduction in systemic blood pressure. Therefore, in the present study, we examined the effect of Brahmi *in vivo* on cardiovascular function, and its *in vitro* effect on endothelial/vascular smooth muscle signaling and role in $Ca^{2+}$ signaling in vascular smooth muscle. The actions of two important single entity constituents of Brahmi extract were also investigated.

*Abbreviations:* PE, phenylephrine; E, endothelium; L-NAME, *N*G-nitro-L-arginine methyl ester; ACh, acetylcholine; SR, sarcoplasmic reticulum.

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## 2. Materials and methods

### 2.1. Preparation of Brahmi extract

Brahmi was collected from Phetchaburi province, Thailand. It was identified by Associate Professor Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, Thailand. The voucher specimen (Phrompittayarat 001) was kept at the Pharmaceutical Botany Mahidol Herbarium, Mahidol University, Thailand. The aerial part of Brahmi was collected, cut into small pieces, dried in a hot-air oven (12 h at 50 °C) and then crushed. The dried powder was soaked in water (24 h, 1:10, w/v), then compressed to squeeze out the water and sonicated with 95% ethanol (1 g in 6 ml). This extraction was repeated another 2 times. The combined alcoholic extract was dried in a rotary evaporator under reduced pressure. The total saponin content was determined using high pressure liquid chromatography as previously reported (Phrompittayarat et al., 2007a,b). The total recovered mass was 10% of the starting dried material and of this extract, 6.25% (w/w) was total saponins comprising bacoside $A_3$ (0.87%), bacopaside I (1.03%), bacopaside II (1.82%) bacopaside X (0.80%), and bacopasaponin C (1.73%). The extract was stored at 5 °C in a dark bottle until used.

### 2.2. Animals

Male Wistar rats (body weight (BW): 200–250 g) were used for all experiments and were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhorn Pathom, Thailand or Monash University Central Animal Services, Victoria, Australia. Experiments were approved by the Animal Ethics Committee (Naresuan University, Phitsanulok, Thailand) and the Monash University animal ethics committee, and complied with the National Health and Medical Research Council of Australia code of practice for the care and use of animals for scientific purposes.

### 2.3. Blood pressure measurement

The rats were anaesthetized with pentobarbital (50 mg/kg BW) by intraperitoneal injection and supplemented as needed. Forelimbs and hind limbs were fixed on the operating pad with adhesive tape. The femoral triangle, consisting of the femoral nerve, femoral artery and femoral vein, was exposed by skin incision and retraction. The femoral vein and artery were cannulated with polyethylene tube (PE50, 0.58 mm i.d. × 0.96 mm o.d.) filled with heparinized (50 units/ml) saline for the injection of experimental drugs and measurement of blood pressure. The arterial catheter was connected to a pre-calibrated pressure transducer and pressure outputs were recorded by a bridge amplifier connected to a personal computer equipped with an analog to digital converter board, Maclab A/D converter and running Chart v5 software (A.D. Instruments, Castle Hill, Australia). After cardiovascular parameters had stabilized, blood pressure was continuously recorded before and during intravenous infusion of Brahmi extract (20, 40, 60 mg/kg BW total doses dissolved in a constant 0.3 ml) at 1 ml/min (i.e., over 18 s). Successive infusions were separated by enough time (>30 min) to allow full recovery of cardiovascular parameters. The intravenous route was used to minimize the effect of metabolism.

### 2.4. Vascular function

#### 2.4.1. Conduit arteries

Rats were killed by cervical dislocation. The thoracic aorta or caudal (tail) artery were excised, cleaned of surrounding loose connective tissue and cut into rings 2–5 mm in length. In some experiments, endothelial cells were mechanically removed by gently rubbing the lumen with a stainless steel wire (Chootip et al., 2002). The rings were mounted on a pair of intraluminal wires in organ chambers containing Krebs' solution (mM): NaCl, 122; KCl, 5; [N-(2-hydroxyethyl) piperazine N′-(2-ethanesulfonic acid)] HEPES, 10; $KH_2PO_4$, 0.5; $NaH_2PO_4$, 0.5; $MgCl_2$, 1; glucose, 11; and $CaCl_2$, 1.8 (pH 7.3), at 37 °C and bubbled with air. The vessel segments were allowed to equilibrate for 1 h at a resting tension of 1 g during which time the solution was replaced every 15 min. Changes in isometric tension were measured using a force transducer (CB Sciences Inc., Milford, USA) connected to a MacLab A/D converter (Chart V5), stored and displayed on a personal computer. Following stabilization, the arterial ring was tested for viability by the application of $10^{-6}$ M PE. Upon development of a steady contraction, the endothelial status was tested using $10^{-5}$ M acetylcholine (ACh). Arteries that produced relaxations greater than 80% were considered to have an intact endothelium intact. Removal of the endothelium was confirmed by loss of the relaxant response to ACh.

#### 2.4.2. Small arteries

Rats were briefly anaesthetized in a chamber containing chloroform and then decapitated. The small intestine, kidney, femoral muscle and brain were removed and placed in cold physiological salt solution (mM): NaCl, 120; KCl, 5; $NaHCO_3$, 25; $KH_2PO_4$, 1; $MgSO_4$, 1.2; $CaCl_2$, 2.5 and glucose 11 and bubbled with 95% $O_2$–5% $CO_2$. The mesenteric, renal lobar, femoral and basilar arteries were excised, cleaned of surrounding loose connective tissue and cut into ring segments, 1–2 mm in length. Arterial rings were mounted on a wire myograph (610 M, Danish Myo Technology, Denmark) for measurement of isometric tension (Mazzuca et al., 2010). Rings were stretched in increments to a tension equivalent to a transmural pressure of 60–70 mmHg. Bathing solution was replaced every 15 min and the temperature was maintained at 37 °C. The integrity of the smooth muscle was tested by applying high $K^+$ solution (isotonic replacement of NaCl with 100 mM KCl) before and at the end of each experiment. The presence of a functional endothelium was assessed by determining the degree of relaxation to $10^{-5}$ M ACh in vessels submaximally constricted with PE ($10^{-6}$ M) or against the spontaneous myogenic tone in the basilar arteries, as described previously (Mazzuca et al., 2010). The vessels were allowed to equilibrate for 30 min and the effects of Brahmi extract (50–1000 μg/ml) and its active compounds (0.05–50 μM) on vascular function were studied.

#### 2.4.3. Experimental protocols

*2.4.3.1. Vasodilator effects of Brahmi extract and active compounds.* Following stabilization, endothelium intact rings of aorta, mesenteric, renal lobar and femoral arteries were submaximally pre-contracted with $10^{-6}$ M PE. The basilar artery was submaximally pre-contracted with $10^{-6}$ M 5-hydroxytrptamine (5-HT) only when spontaneous myogenic tone did not develop. After the contraction had stabilized, Brahmi extract was added cumulatively to the vessels. In some experiments, endothelium-denuded aorta or tail arteries were used. Successful endothelial denudation was confirmed by the absence of relaxation when the endothelium was stimulated with ACh ($10^{-5}$M). In some experiments, endothelium-intact aortic preparations were pretreated with blockers, *N*G-nitro-L-arginine methyl ester (L-NAME, $10^{-4}$ M), an inhibitor of nitric oxide synthase, or indomethacin ($10^{-5}$ M), an inhibitor of cycloxygenases, for 30 min prior to PE exposure.

There are two types of saponin in the Brahmi extract: jujubogenins and pseudojujubogenins (Fig. 3). We tested from each group a compound found in the extract in substantial amounts (bacoside $A_3$ and bacopaside II, respectively) for vasodilator action using endothelium-intact mesenteric arteries. The Brahmi extract