บรรณานุกรม

กระทรวงสาธารณสุข. ข้อมูลเผยแพร่โดยศาสตราจารย์เกียรติคุณ นพ.ไกรสิทธิ์ ตันติศิรินทร์ ในการประชุมวิชาการโภชนาการแห่งชาติ ครั้งที่ 2 ณ ศูนย์ไบเทค บางนา เมื่อวันที่ 5 ตุลาคม 2550.

กรองกาญจน์ ชูทิพย์. รายงานการวิจัยฉบับสมบูรณ์ เรื่อง การศึกษาฤทธิ์ทางเภสัชวิทยาของสมุนไพรพรมมิที่มีต่อหลอดเลือด. 2550

Anbarasi K, Kathirvel G, Vani G, Jayaraman G, and Shyamala Devi CS. Cigarette smoking induces heat shock protein 70 kDa expression and apoptosis in rat brain: Modulation by bacoside A. *Neuroscience*. 138(4):1127-35. 2006a.

Anbarasi K, Vani G, Balakrishna K, and Devi CS. Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. *Life Sci.* 16;78(12):1378-84. 2006b.

Anbarasi K, Vani G, Balakrishna K, Devi CS. Creatine kinase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of Bacoside A. *Vascul Pharmacol.* 42(2):57-61. 2005c.

Anbarasi K, Vani G, Balakrishna K, Devi CS. Effect of bacoside A on membrane-bound ATPases in the brain of rats exposed to cigarette smoke. *J Biochem Mol Toxicol.* 9(1):59-65. 2005b.

Anbarasi K, Vani G, Devi CS. Protective effect of bacoside A on cigarette smoking-induced brain mitochondrial dysfunction in rats. *J Environ Pathol Toxicol Oncol.* 24(3):225-34. 2005a.

Channa, S., Dar, A., Yaqoop, M., Anjum, S., Sultani, Z., Rahman, A. Broncho-vasodilatory activity of fractions and pure constituents isolated from *Bacopa monniera. J. Ethno. Pharmacol.* 86: 27-35. 2003.

Chattipakorn, S., Pongpanparadorn, A., Pratchayasakul, W., Pongchaidacha, A., Ingkaninan, K. and Chattipakorn, N. J Ethnopharmacol. 2007; 110(1): 61-68.

Dar, A. and Channa, S. Calcium antagonistic activity of *Bacopa monniera*on on vascular and intestinal smooth muscles of rabbit and guinea-pig. *J. Ethno. Pharmacol.* 66: 167-174. 1999.

Dar, A. and Channa, S. Relaxant effect of ethanol extract of *Bacopa monniera* on trachea, pulmonary artery and aorta from rabbit and guinea-pig. *Phytother. Res.* 11: 323-325. 1997.

Das, A. et al A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Gingko biloba* anticholinesterase and cognitive enhancing activities. *Pharmacology, Biochemistry and Behavior.* 73: 893-900, 2002.

Garai, S., Mahoto, S.B., Ohtani, K., Yamasaki, K.: Bacosaponin D-a pseudojujubogenin glycoside from *Bacopa monniera. Phytochemistry.* 42: 815-920, 1996a.

Garai, S., Mahoto, S.B., Ohtani, K., Yamasaki, K.: Damnarane-type triterpenoid saponins from *Bacopa monniera*. *Phytochemistry*. 42: 815-920, 1996b.

Goel RK, Sairam K, Babu MD, Tavares IA, Raman A. In vitro evaluation of Bacopa monniera on anti-Helicobacter pylori activity and accumulation of prostaglandins. *Phytomedicine.* 10(6-7):523-7. 2003.

Ingkaninan, K., Changwijit, K. and Suwanborirux, K. J Pharm Pharmacol. 2006; 58(6): 847-852.

Kishore K, Singh M. Effect of bacosides, alcoholic extract of Bacopa monniera Linn. (brahmi), on experimental amnesia in mice. *Indian J Exp Biol.* 43(7):640-5. 2005

Phrompittayarat, W., Wittaya-areekul, S., Jetiyanon, K., Putalun, W., Tanaka, H., and Ingkaninan, K. Determination of saponin glycosides in Bacopa monnieri by reversed phase high performance liquid chromatography. *Srinakharinwirot Journal of Pharmaceutical Sciences* 2: 26-32., 2007.

Sairam, K. Dorababu, M., Goel, R.K., Bhattacharya, S.K. Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. *Phytomedicine*. 9: 207-211, 2002.

Stough et al. The chronic effect of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. *Psychopharmacology*. 1-6, 200.

Zhou Y, Shen YH, Zhang C, Su J, Liu RH, Zhang WD. Triterpene saponins from Bacopa monnieri and their antidepressant effects in two mice models. *J Nat Prod.* 70(4):652-5. 2007.

Output ที่ได้จากโครงการ

1. สร้างองค์ความรู้ใหม่เกี่ยวกับสรรพคุณของสมุนไพรพรมมิที่มีต่อระบบหัวใจร่วมหลอดเลือด
2. นำเสนอผลงานในที่ประชุมวิชาการระดับชาติ Poster presentation ในที่ประชุมสรีรสมาคมแห่งประเทศไทย 2-4 พฤษภาคม 2555
3. นำเสนอผลงานในที่ประชุมวิชาการนานาชาติ The Eighth Asian Congress for Microcirculation Bangkok, October 26-28, 2011
4. ตีพิมพ์ผลงานระดับชาติและนานาชาติ

ภาคผนวก

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Oral *Bacopa monnieri* Is Antihypertensive In Rats Chronically Treated With L-NAME

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Key words: hypertension, *Bacopa monnieri*, L-NAME, vasodilatation, captopril

Introduction. We have previously reported that intravenous injection of *Bacopa monnieri* (Brahmi) reduced blood pressure in normotensive anaesthetised rats, suggesting that Brahmi could be an effective antihypertensive. Therefore, the present study sought to show that orally administered Brahmi could lower blood pressure in rats made chronically hypertensive by N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) which blocks endothelial nitric oxide synthase.

Methods. Male Wistar rats (200-250g) were divided into 4 groups: 1) Normotensive control, 2) L-NAME (50mg/kg), 3) L-NAME plus Brahmi (60mg/kg/day), 4) L-NAME plus captopril (20mg/kg/day). L-NAME was administered via the drinking water for 8 weeks. After 4 weeks, animal groups 3 and 4 also received Brahmi ethanolic extract or captopril in the drinking water for the remaining 4 weeks. Systolic blood pressure (SBP) and heart rate were measured weekly, whilst conscious, using an inflatable tail cuff. To elucidate the mechanism of action of Brahmi, we studied the vasodilator effects of Brahmi on phenylephrine (10μM) pre-contracted isolated mesenteric artery by organ bath technique using a separate group of normotensive rats.

Results. L-NAME produced a sustained elevation of SBP from 94.7±7.5mmHg (week0, n=7) to 166.6±3.5mmHg (week8, n=6, $p<0.001$). A further 4 weeks of Brahmi reduced blood pressure from 162.8±4.9mmHg (week4) to 129.9±6.8mmHg (week8, $p<0.01$, n=6-8) and captopril from 166.4±7.2mmHg (week4) to 140.4±5.8mmHg (week8, $p<0.01$, n=6-7) but had no effect on normotensives. There was no difference in heart rate among the 4 groups studied. In isolated mesenteric artery, Brahmi extract elicited endothelial independent vasorelaxation, suggesting that it acts directly on the vascular smooth muscle cells.

Conclusion. These data show that Brahmi is an effective antihypertensive animals, but unlikely to be nitric oxide-mediated. Thus Brahmi or its active ingredients may make a clinically efficacious antihypertensive treatment.

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Introduction

Hypertension is an important determinant of cardiovascular disease. Many plant products are vasoactive and thus have potential antihypertensive applications. *Bacopa monnieri* (Brahmi) is an Ayurvedic medicine traditionally used to improve mental health (1), learning and memory (2, 3) and is cardiotonic (4). Several studies have focused on its procognitive and neuroprotective properties (1, 2, 3), but its cardiovascular actions are yet to be fully explored.

Brahmi has been shown to cause relaxation in blood vessels from a wide range of tissues by an action on both endothelial cells and a direct effect on vascular smooth muscle (5, 6, 7, 8). It is also cardioprotectant against isoproterenol-induced myocardial necrosis in rats (9). Recently, we have showed that acute intravenous injection of Brahmi reduced blood pressure of anaesthetised rats (8). However, there is no information on the effects of Brahmi in chronic hypertension. Therefore, the present study aimed to explore the antihypertensive action of an ethanolic extract of Brahmi using rats made hypertensive by chronic oral administration of N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME). The vasodilator action of Brahmi was also investigated in order to elucidate its mechanism of action.

Methods

Preparation of Brahmi extract

Brahmi was collected from Phetchaburi province, Thailand and its ethanolic extract was prepared as described previously (8). In brief, Brahmi was cut, dried and crushed. The dried powder was soaked in water, compressed, sonicated with 95% ethanol and filtered. The ethanolic extract was dried, evaporated under reduced pressure and contained 6.25% (w/w) saponins, including 0.87% bacoside A_3, 1.03% bacopaside I, 1.82% bacopaside II, 0.80% bacopaside X, 1.73% bacopasaponin C. The extract was stored at 5°C in a dark bottle until used.

Animals

Male Wistar rats (200-250g) were used for all experiments and were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhorn Pathom, Thailand. Rats were housed under standard conditions (25±2°C, 12 hours light-dark cycle) and received tap water and commercial rat diet *ad libitum*. Experimental protocols were approved by the Animal Ethics Committee (Naresuan University, Phitsanulok, Thailand).

Hypertensive model and blood pressure measurement

Rats were rendered hypertensive by blocking endothelial nitric oxide synthase (eNOS) with L-NAME administered via the drinking water (50mg/kg/day) for 8 weeks. When all the water had been consumed, extra plain water was provided. Over 4 weeks, this produced a sustained hypertension with systolic blood pressure (SBP) $\geq$ 140 mmHg (10), while the animals given plain water maintained a constant 'normal' blood pressure. After the initial 4 weeks, some animals received additionally either Brahmi ethanolic extract (60mg/kg/day) or captopril (angiotensin converting enzyme inhibitor (ACE), 20mg/kg/day) also in the drinking water. SBP and heart rate was measured weekly, whilst conscious, using an inflatable tail cuff (NIBP controller, ADInstrument, Australia). This method cannot measure diastolic blood pressure. The trial used 4 matched groups of rats: 1) normotensive controls, 2) hypertensives (L-NAME), 3) hypertensives (L-NAME) + Brahmi extract and 4) hypertensives (L-NAME) + captopril.

Vasodilator effects of Brahmi extract

To evaluate vasodilatory action of Brahmi, a separate group of normotensive rats (200-250g) were killed by cervical dislocation. The mesenteric artery was excised, cleaned of

surrounding loose connective tissue and cut into rings 2-5 mm in length for tension measurement. In some experiments, endothelial cells were mechanically removed by gently rubbing the lumen with a stainless steel wire (11). The rings were mounted on a pair of wires in organ baths 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 hr 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 rings were tested for viability by the application of 10μM phenylephrine and the endothelial status was tested using 10 μM acetylcholine. Arteries that produced relaxations to >80% to baseline were considered to have an intact endothelium. Removal of endothelium was confirmed by loss of the relaxant response to acetylcholine. The vessels were allowed to equilibrate for 30 min and the effects of Brahmi extract (10-1,000 μg/ml) on vascular relaxation were studied. Relaxation was expressed as the percentage of the pre-contraction evoked by 10μM phenylephrine.

Drugs and solution

Phenylephrine, acetylcholine, L-NAME and captopril were obtained from Sigma (St. Louis, MO, USA) and dissolved in distilled water. Brahmi extract was dissolved in bathing solution for vascular function study and were freshly prepared immediately before the experiment.

Statistical analysis

All data are expressed as mean ± standard error of mean (SEM) of *n* animals. Statistical significance was assessed using paired or un-paired Student's *t*-test or analysis of variance (ANOVA) followed by Tukey's test, as appropriate. In all comparisons, *p* values less than 0.05 were accepted as significant.

Results

Brahmi was Antihypertensive

L-NAME (50mg/kg) produced progressively increased SBP, which appeared to be sustained at weeks 3-4 (Figure 1). Throughout the 8 weeks, L-NAME increased SBP from 94.7±7.5mmHg (week0, n=7) to 166.6±3.5mmHg (week8, n=6, $p<0.001$) while the control animals maintained a constant 'normal' blood pressure (Figure 1). Treatment with either Brahmi (60mg/kg) or captopril (20mg/kg) clearly reduced SBP within one week of treatment. SBP dropped from 162.8±4.9 to 133.2±3.4mmHg (Brahmi, $n=8$, $p<0.001$) and from 166.4±7.2 to 137.7±10.3mmHg (captopril, $n=7$, $p<0.05$). The lower blood pressures then appeared to be sustained: treatment with Brahmi or captopril after week5 produced no further reduction of SBP (Figure 1). There was no difference in heart rate among 4 groups of animals studied (Table 1). The heart rate of all animals was slower comparing between week0 and week8 (390±6 to 349±12 BPM, control; 389±5 to 337±18 BPM, L-NAME; 402±5 to 362±12 BPM, L-NAME+Brahmi and 403±7 to 369±26 BPM, L-NAME+captopril) commensurate with age as previously reported (12).

Vasodilator effect of Brahmi

Brahmi extract (10-1,000 μg/ml) produced a concentration-dependent relaxation in isolated mesenteric artery pre-contracted with 10 μM phenylephrine (Figure 2). Removal of endothelium had no detectable effect on the vasodilatory action of Brahmi as judged by the EC_{50} and maximum relaxation to Brahmi in endothelium intact (EC_{50} =165.5±86.8 μg/ml, max response =94.4±9.2%) and endothelium denuded (EC_{50} =165.5±86.8 μg/ml, max response =98.9±1.8%) arterial rings (Figure 2).

Discussion

The present study showed that Brahmi maintained an antihypertensive action in L-NAME induced hypertensive rats. Heart rate was unaffected, thus the hypotensive action of Brahmi was more likely via a systemic vasodilatation.

It is well documented that chronic treatment with L-NAME produced sustained elevation of systemic blood pressure by inhibiting eNOS which prevents NO production leading to vasoconstriction and increased total peripheral resistance. Therefore, several studies used L-NAME treated rat as a hypertensive model to study herbal medicines (13, 14, 15). In the present study, L-NAME produced progressively increased blood pressure during the first week of treatment and thereafter sustained for 3 weeks (and 8 weeks in the hypertensive controls). Treatment with either Brahmi or captopril, an ACE inhibitor, almost normalised blood pressure of hypertensive rats within one week. In this respect, Brahmi appeared to be as effective as captopril in reduction of blood pressure.

These data are in agreement with our previous study showing that intravenous injection of Brahmi extract (20, 40, 60mg/kg) produced an acute reduction of blood pressure in normotensive anaesthetised rats, without affecting heart rate (8). Moreover, we demonstrated that chronic oral treatment of Brahmi for 8 weeks had no effect on heart rate or blood pressure of normotensive rats (12) but was active against hypertension in the present study. Thus, the failure of Brahmi to have any effect on heart rate suggests that the major *in vivo* effect was due to peripheral vasodilatation which is supported by Brahmi causing a concentration dependent vasorelaxation in isolated mesenteric artery. Removal of endothelium had no effect on vasorelaxant response to Brahmi indicating that NOS, prostacyclin and other endothelial pathways did not make a major contribution to the vasorelaxation. Direct action of Brahmi on vascular smooth muscle cells via inhibition of L-type Ca^{2+} channel or release of Ca^{2+} from sarcoplasmic reticulum have been reported by several studies which supports our findings (5, 6, 7, 8). Nevertheless, further investigation on intracellular mechanisms of Brahmi on vascular smooth muscle dilation, as well as pharmacological studies, is needed.

Conclusion

Brahmi showed a clear, prompt and constant antihypertensive action and was at least as effective as the clinically used captopril. The mechanism of action is via vasodilatation, but not substantially via the endothelium. Thus this extract or an active ingredient may lead to an efficacious and novel treatment for human primary hypertension.

References

1. Russo A, Borrelli F. *Bacopa monniera*, a reputed nootropic plant: an overview. Phytomedicine 2005; 12: 305-317.
2. Uabundit N, Wattanathorn J, Mucimapura S, Ingkaninan K. Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease model. J Ethnopharmacol 2010; 127: 26-31.
3. Vollala VR, Upadhya S, Nayak S. Effect of *Bacopa monniera Linn.* (brahmi) extract on learning and memory in rats: A behavioral study. J Vet Behav 2010; 5: 69-74.
4. Thorne Research. *Bacopa monniera.* Altern Med Rev 2004; 9: 79-85.
5. Dar A, Channa S. Relaxant effect of ethanol extract of *Bacopa monniera* on trachea, pulmonary artery and aorta from rabbit and guinea-pig. Phytother Res 1997; 11: 323-325.
6. Dar A, Channa S. Calcium antagonistic activity of *Bacopa monniera* on vascular and intestinal smooth muscles of rabbit and guinea-pig. J Ethnopharmacol 1999; 66: 167-174.
7. Channa S, Dar A, Yaqoob M, Anjum S, Sultani Z, Rahman A. Broncho-vasodilatory activity of fractions and pure constituents isolated from *Bacopa monniera.* J Ethnopharmacol 2003; 86: 27-35.

8. Kamkaew N, Scholfield CN, Ingkaninan K, Maneesai P, Parkington HC, Tare M, Chootip K. Bacopa monnieri and its constituents is hypotensive in anaesthetised rats and vasodilator in various artery types. J Ethnopharmacol 2011; 137: 790-795.
9. Nandave M, Ojha SK, Joshi S, Kumari S, Arya DS. 2007. Cardioprotective effect of *Bacopa monneira* against isoproterenol-induced myocardial necrosis in rats. Int J Pharmacol 2007; 3: 385-392.
10. Colonna VDG, Rigamonti A, Fioretti S, Bonomo S, Manfredi B, Ferrario P, Bianchi M, Berti F, Muller EE, Rossoni G. Angiotensin-converting enzyme inhibition and angiotensin AT_1-receptor antagonism equally improve endothelial vasodilator function in L-NAME-induced hypertensive rats. Eur J Pharmacol 2005; 516: 253-259.
11. Chootip K, Ness KF, Wang Y, Gurney AM, Kennedy C. Regional variation in P2 receptor expression in the rat pulmonary arterial circulation. Brit J Pharmacol 2002; 137: 637-646.
12. Kamkaew N, Scholfield CN, Ingkaninan K, Taepavarapruk N, Chootip K. *Bacopa monnieri* increases cerebral blood flow in rat independent of blood pressure. Phytother Res DOI: 10.1002/ptr.4685.
13. Nguelefack-Mbuyo PE, Nguelefack TB, Dongmo AB, Afkir S, Azebaze AGB, Dimo T, Legssyer A, Kamanyi A, Ziyyat A. Anti-hypertensive effects of the methanol/methylene chloride stem bark extract of Mammea africana in l-NAME-induced hypertensive rats. J Ethnopharmacol 2008; 117: 446-450.
14. Kang DG, Sohn EJ, Lee YM, Lee AS, Han JH, Kim TY, Lee HS. Effects of bulbus Fritillaria water extract on blood pressure and renal functions in the L-NAME-induced hypertensive rats. J Ethnopharmacol 2004; 91: 51-56.
15. Bahgat A, Abdel-Aziz H, Raafat M, Mahdy A, El-Khatib AS, Ismail A, Khayyal MT. Solanum indicum ssp. distichum extract is effective against L-NAME-induced hypertension in rats. Fundam Clin Pharmacol 2008; 22: 693-699.

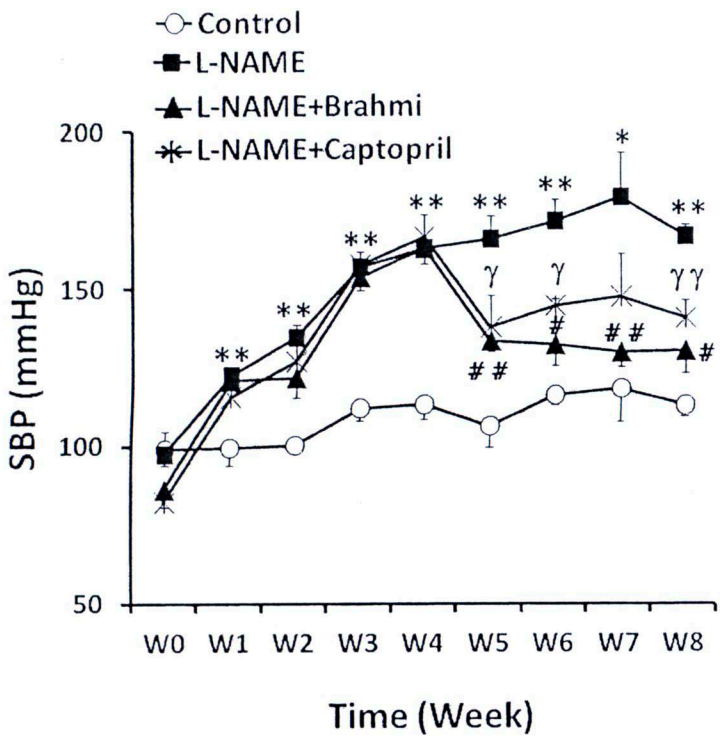

Figure 1

Effect of chronic oral administration of L-NAME (50mg/kg), L-NAME+Brahmi (60mg/kg) and L-NAME+captopril (20mg/kg) on systolic blood pressure (SBP). Rats were treated with L-NAME for 4 weeks and then some animals received additionally either Brahmi ethanolic extract (60mg/kg/day) or captopril (20mg/kg/day) for the remaining 4 weeks (n=5-8). (W, Week) All data points are mean±SEM. $^{*}p<0.01$, $^{**}p<0.001$ comparing with W0 (L-NAME), $^{\#}p<0.01$, $^{\#\#}p<0.001$ comparing with W4 (L-NAME+Brahmi), $^{\gamma}p<0.05$, $^{\gamma\gamma}p<0.01$ comparing with W4 (L-NAME+captopril)

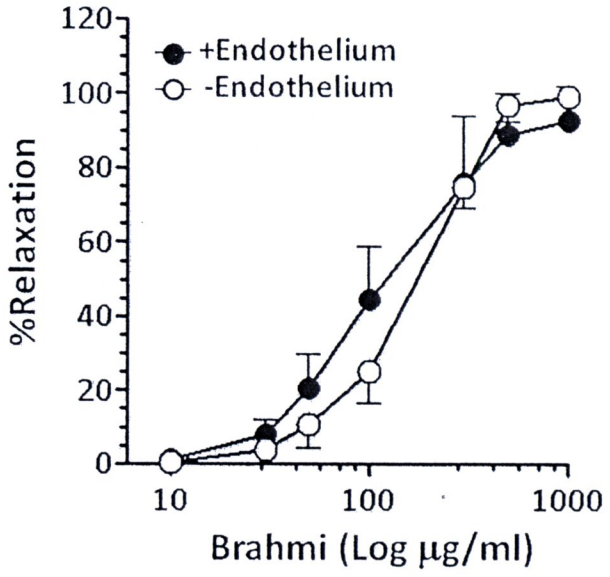

Figure 2

Concentration-relaxation curves for Brahmi extract (0-1000 µg/ml) in endothelium-intact (+Endothelium) and endothelium-denuded (+Endothelium) mesenteric arteries of normotensive rats. Arterial rings were pre-contracted with 10 µM phenylephrine. All data points are mean±SEM (*n*=6-7).

Treatment	Heart Rate (beat/min)		
	W0	W4	W8
Control	390±6	362±9	349±12
L-NAME	389±5	367±6	337±18
L-NAME+Brahmi	402±5	357±10	362±12
L-NAME+captopril	403±7	372±13	369±26

Table 1

Effect of chronic oral administration of L-NAME (50mg/kg), L-NAME+Brahmi (60mg/kg) and L-NAME+captopril (20mg/kg) on heart rate. Rats were treated with L-NAME for 4 weeks and then some animals received additionally either Brahmi ethanolic extract (60mg/kg/day) or captopril (20mg/kg/day) for the remaining 4 weeks (n=5-8). (W, Week) All data points are mean±SEM.