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Blood Pressure Lowering Effect of *Morus alba* is Mediated Through Ca^{++} Antagonist Pathway

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Abstract: *Morus alba* has been used in traditional medicine system for the treatment of hypertension. Our objective was to provide scientific basis for the medicinal use of *M. alba* in hypertension. The crude extract of *Morus alba* (Ma.Cr) induced a dose-dependent (10-100 mg kg^{-1}) fall in the arterial BP in anaesthetized rats. In isolated guinea-pig atria, Ma.Cr caused inhibition of atrial force and rate of spontaneous contractions, similar to that exhibited by verapamil. When tested in rat aortic ring preparations, Ma.Cr at concentration range of 0.1-10 mg mL^{-1} relaxed high K^+ (80 mM) and phenylephrine (PE, 1 μM)-induced contractions and shifted the Ca^{++} dose-response curves to right, like caused by verapamil. These data indicate that the blood pressure lowering action of *Morus alba* occurred via., Ca^{++} channel blockade pathway, which provides evidence for the pharmacological basis to justify its effectiveness in hypertension.

Key words: *Morus alba*, Ca^{++} antagonist, hypertension, cardiovascular inhibitor

INTRODUCTION

Morus alba Linn. (family Moraceae), known as "Mulberry" is found in all over Pakistan and other parts of the world. *M. alba* is considered useful in folk medicine as antihypertensive, antiasthmatic, antidiarrheal, antibacterial, aphrodisiac, antirheumatic, diuretic, expectorant, antiulcer, hepatoprotective and sedative agent (Nadkarni, 1976; Baquar, 1989; Usmanghani *et al*., 1997; Wiart, 2002).

The plant is known to exhibit antidiabetic (Chen *et al*., 1995), anti-inflammatory (Hong *et al*., 2002), hepatoprotective (Consolini *et al*., 1999), anticancer (Kim *et al*., 2000), antihyperlipidimic (Doi *et al*., 2001), antispasmodic and bronchodilatory (Khan *et al*., 2012) activities.

Morus alba has been widely used by traditional healers medicinally as antihypertensive agent, but it has not been studied scientifically for its effectiveness in hypertension. The present study was carried out to see, if *Morus alba* effective in lowering blood pressure and what is the possible mechanism of action?

MATERIALS AND METHODS

Plant material and extraction: The fruit of *M. alba* was collected from trees located in Univrsity of Malakand Dir Lower, Khyber Pakhtun Khwa in April 2008, with voucher specimen Ma/FF/04/07/106, deposited at the herbarium of the Department of Biological and Biomedical Sciences, The Aga Khan University. A total of 1.72 kg of the plant material was soaked in the aqueous-methanol (30:70) for 24 h. This procedure of soaking and filtration was repeated twice more. All the filtrates were combined and evaporated to dryness on a rotary evaporator to the crude extract of *M. alba* (Ma.Cr), yielding approximately 14.61%.

Animals: Animals used in this study include adult Sprague-Dawley rats (180-200 g) and guinea-pigs (450-500 g) of local breed and either sex, housed in the Animal House of the Aga Khan University under controlled environment (23-25°C). Animals were given tap water *ad libitum* and a standard diet. The experiments were performed with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996).

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Chemicals: Acetylcholine chloride (ACh), isoprenaline hydrochloride, norepinephrine hydrochloride (NE), phenylephrine hydrochloride (PE) and verapamil hydrochloride were purchased from (Sigma Chemical Company, St. Louis, MO, USA). Pentothal sodium (thiopental) was obtained from Abbot Laboratories, Karachi, Pakistan.

Measurement of blood pressure in anaesthetized rats: These experiments were performed according to method described previously (Gilani *et al*., 1991, 2006). Rats were anaesthetized with thiopental sodium (Pentothal®, 70-90 mg kg^{-1}, i.p). Trachea was cannulated with a polyethylene tubing Pe-20 to maintain the spontaneous respiration. The right jugular vein was cannulated with polyethylene tubing Pe-50 to facilitate the intravenous administration of drugs. The left carotid artery was cannulated with similar tubing filled with heparinized saline 60 IU mL^{-1} and connected to a pressure transducer (MLT 0380/D Reusable BP-Transducer) coupled to ML 224 Quad Bridge Amplifier and Power-Lab ML 4/25 data acquisition system (AD Instruments, Sydney, Australia) for BP recording. The exposed surface for cannulation was covered with a piece of gauge moistened in warm saline. Rats were injected with heparinized 0.1 mL saline (0.9% NaCl) to prevent blood clotting. Following 20 min period of equilibrium, rats were injected intravenously with the test substance. Arterial BP was allowed to return to resting level between injections. Changes in BP were recognized as difference between the steady state values before and the peak readings after injection. Mean Arterial Pressure (MAP) was calculated as the diastolic BP plus one-third of the pulse width (systolic BP-diastolic BP).

Isolated guinea-pig atria: Right atria isolated from the guinea-pigs were mounted in 20 mL tissue baths containing Kreb's solution at 32°C and aerated with carbogen. Each tissue was allowed to beat spontaneously (due to pacemaker) under the resting tension of 1 g (Taqvi *et al*., 2006). An equilibrium period of 45 min was allowed before the application of any drug. The drug-induced changes in force of atrial contractions were measured as the percent change in base-line values. Tension changes in the tissue were recorded via., Force-displacement Transducer (FT-03) using Grass Model 7 Polygraph.

Rat aorta preparations: Rats were sacrificed by cervical dislocation. Thoracic aorta was dissected out, cleaned of fat and adipose tissues and cut into 3-5 mm long rings and individually mounted in 5 mL tissue bath containing Kreb's solution as described previously (Tep-Areenan and Sawasdee, 2011). The tissues were then stabilized with repeated exposure to high KCL solution. The ability of extract to relax K^+ (80 mM)-induced contractions would indicate L-type voltage-operated Calcium Channel Blocking (CCB) mode of vasodilation, while inhibition of the PE-induced contractions, would signify blockade of the Ca^{++} influx through receptor-operated calcium channels (Godfraind *et al*., 1986). To confirm Calcium Channel Blockade (CCB) activity, Concentration-Response Curves (CRC_S) of Ca^{++} were constructed (Jabeen *et al*., 2007). For this purpose tissue was stabilized in normal Kreb's solution and then placed in Ca^{++}-free Kreb's solution, containing EDTA (0.1 mM) for 30 min to remove calcium from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Kreb's solution. Following an incubation period of 1 h, control CRC_S of Ca^{++} were obtained. The Ca^{++}-CRC_S were reconstructed in presence of different concentrations of the test material. Changes in tension were recorded and analyzed isometrically through a force transducer (Fort-10, WPI, UK) coupled to a bridge amplifier (Transbridge TBM 4M, WPI) and PowerLab ML 845 data acquisition system.

RESULTS

Effect on blood pressure: Ma.Cr at the doses of 10, 30 and 100 mg kg^{-1} caused a respective fall of 18±2.0, 28.5±6.5 and 45±3% (n = 3) in the MABP of anaesthetized rats. Figure 1a shows tracing from a typical experiment, whereas the combined data from different experiments are presented in Fig. 1b.

Effect on guinea-pig atria: In isolated guinea-pig paired atria, Ma.Cr exhibited a concentration-dependent inhibitory effect on the spontaneous rate and force of contractions (Fig. 2a) with respective EC_{50} values of 5.2 mg mL^{-1} (5.1-5.5, n = 3) and 4.7 (4.4-5.0, 95% CI, n = 3). Similarly, verapamil caused concentration-dependent inhibitory effect on force and rate of atrial contractions with respective EC_{50} values of 0.7 (0.6-0.9, n = 3) and 1.1 μM (0.9-1.3, n = 3) as shown in Fig. 2b.

Effect on rat aorta: When tested on the base-line of rat aortic preparations, the extract was found devoid of any vasoconstrictor effect up to 10 mg mL^{-1}. When tested on K^+ (80 mM) and PE (1 μM)-induced contractions, Ma.Cr produced non-specific vasodilator effect with respective EC_{50} values of 2.7 (2.2-3.3, n = 3) and 2.3 mg mL^{-1} (1.5-3.2, n = 3) as shown in Fig. 3a. Verapamil also inhibited the K^+ (80 mM) and PE (1 μM)-induced vascular contractions with EC_{50} values of 0.3 (0.2-0.3, n = 3) and 0.8 μM (0.6-1.1, n = 3), respectively (Fig. 3b). Ma.Cr (0.3-1.0 mg mL^{-1}) shifted the Ca^{++}-CRCs to the right

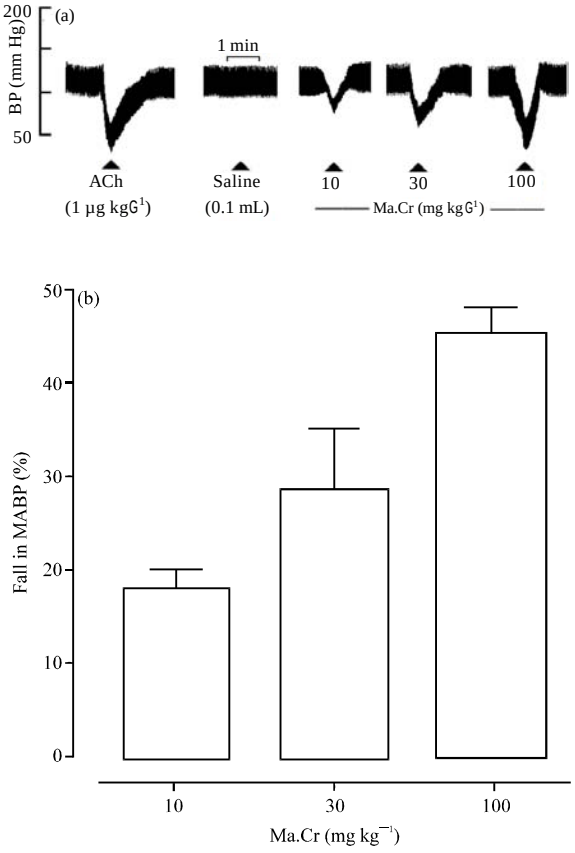

Fig. 1(a-b): Upper panel (a) shows a typical tracing of *Morus alba* crude extract (Ma.Cr) blood pressure (BP)-lowering effect and the lower panel (b) shows a bar chart representing hypotensive effect of Ma.Cr on Mean Arterial Blood Pressure (MABP) in anesthetized rats. The dose was administered after the response to the preceding one had returned to normal. Values are Mean±SEM, n = 3

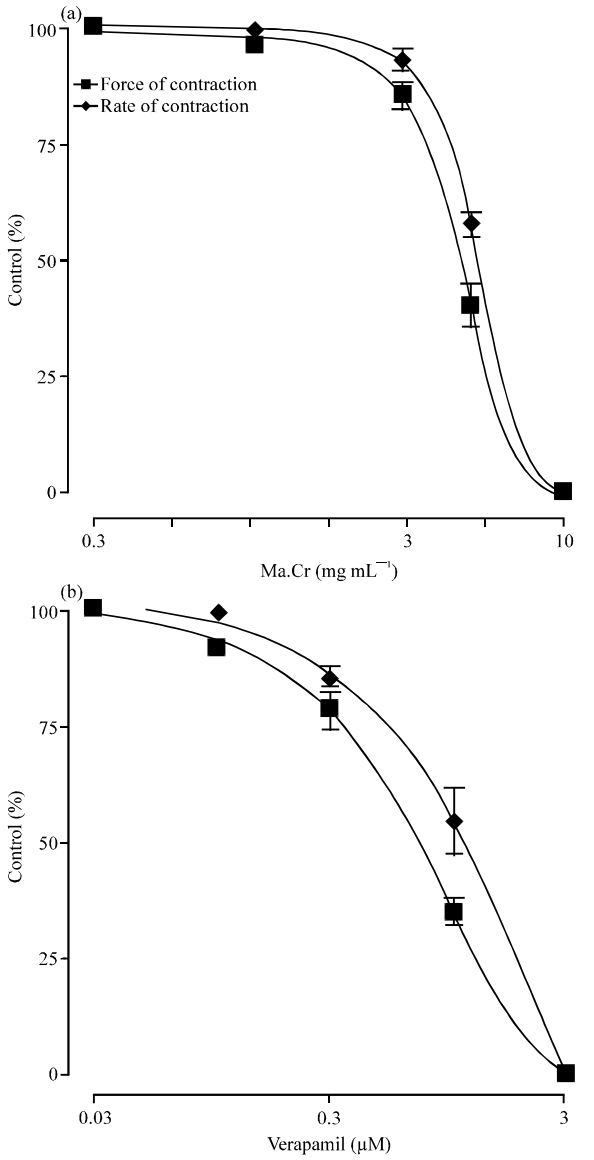

Fig. 2(a-b): Concentration-response curves showing the inhibitory effect of (a) *Morus alba* crude extract (Ma.Cr) and (b) Verapamil on force and rate of spontaneous contractions in isolated guinea pig right atria. Values are Mean±SEM, n = 3

with suppression of the maximum response (Fig. 4a). This rightward shift in Ca^{++}-curves was similar to that obtained with pretreatment of verapamil (0.01-0.03 µM, n = 3) as shown in Fig. 4b.

DISCUSSION

In view of medicinal use of *Morus alba* in hypertension, its extract was evaluated for the possible blood pressure lowering action in rats under anesthesia and the underlying pharmacological mechanism was explored using isolated cardiovascular tissues preparations. The intravenous administration of the crude extract of *Morus alba* caused fall in the arterial BP of rats, which is in line with its folk use in hypertension. It is customary to use isolated tissue preparations to evaluate the underlying mechanism of action, as response interference from intact reflex is obliterated (Ajay *et al*., 2007). Knowing that the BP is the product of cardiac output and peripheral resistance, the extract was further studied in isolated heart and vascular preparations.

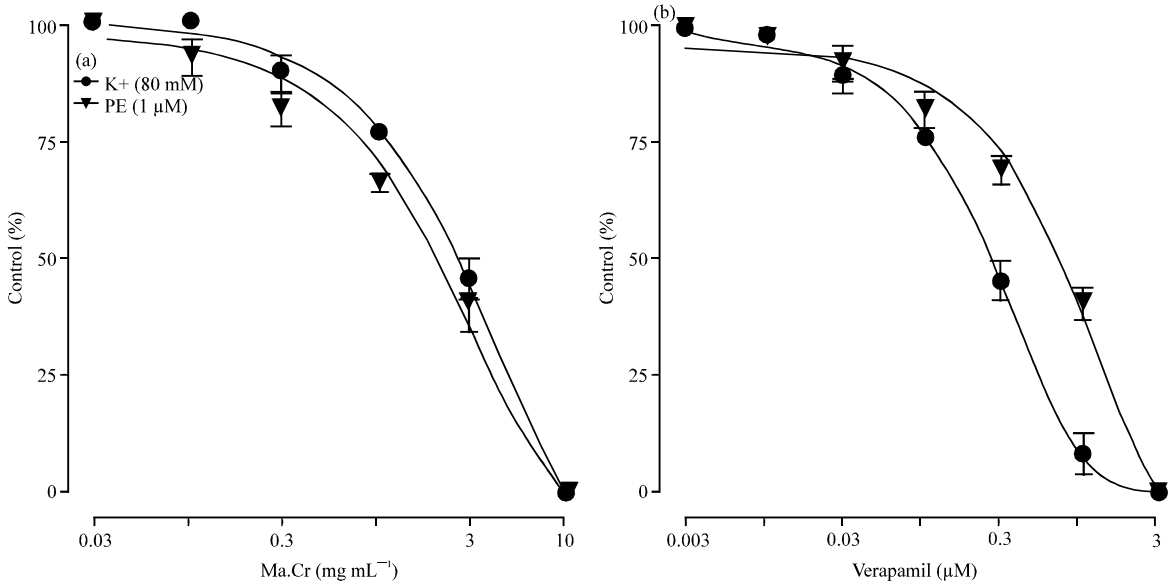

Fig. 3(a-b): Concentration-dependent relaxant effects of (a) *Morus alba* crude extract (Ma.Cr) and (b) Verapamil on high K^+ and phenylephrine (PE)-induced contractions in isolated rat aortic ring preparations. Values are Mean±SEM, n = 3

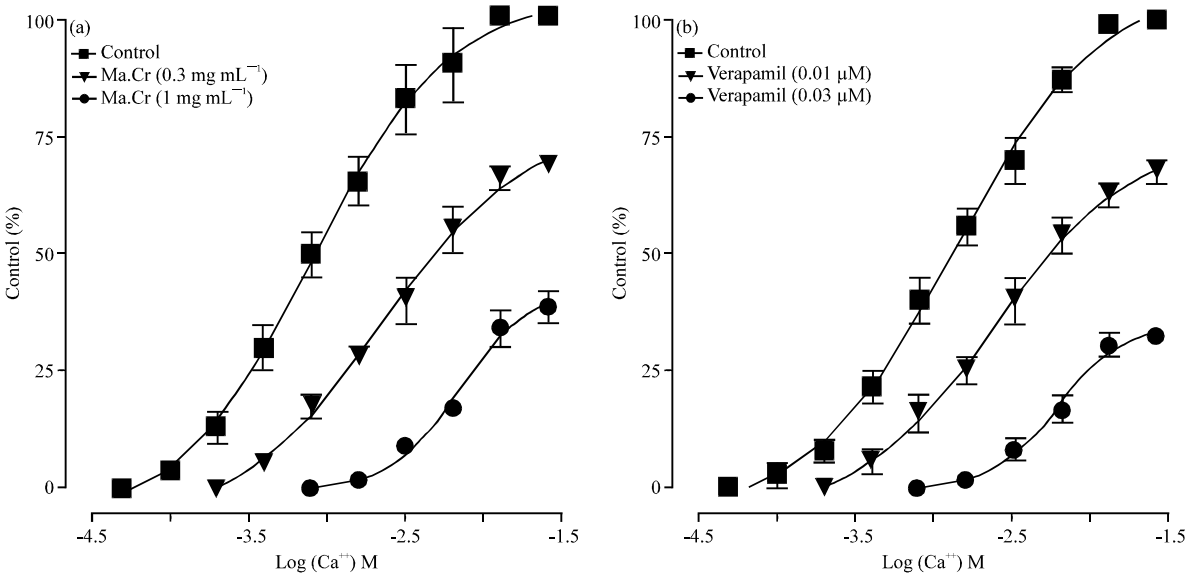

Fig. 4(a-b): Concentration-response curves of Ca^{++} in the absence and presence of different concentrations of (a) *Morus alba* crude extract (Ma.Cr) and (b) Verapamil in isolated rat aortic ring preparations. Values are Mean±SEM, n = 3

The effect of Ma.Cr on vascular resistance was studied in rat thoracic aorta, which is a prototype tissue routinely used for evaluating underlying pharmacodynamic of BP-lowering effect. Rat aorta was selected to evaluate effect of the extract on K^+ and PE-induced contractions and thus to distinguish between activity at voltage-operated and receptor-operated calcium channels. Ma.Cr inhibited the PE and high K^+-induced contractions in aortic rings at similar concentration range, indicating that it was acting

equi-potently through blockade of voltage and receptor-operated Ca^{++} channels (Interaminense *et al*., 2007; Gilani *et al*., 2010) like those obtained with verapamil. The CCB activity of the extract was confirmed when it shifted the Ca^{++}-CRCs, constructed in Ca^{++}-free medium to the right. Verapamil also caused similar rightward of Ca^{++}-curves in concentration-dependent manner.

In guinea-pig atria, Ma.Cr exhibited a negative inotropic and chronotropic effect, similar to that caused by verapamil, a standard Ca^{++} channel blocker (Fleckenstein, 1977). Ca^{++} antagonists are known to cause cardiac depression through inhibiting the slow inward current during the action potential plateau (Johansen, 1992). The cardiac inhibitory action of the extract may be due to the Ca^{++} antagonist effect leading to decrease in cardiac output and thus falling BP.

In conclusion, results of this study show that *Morus alba* exhibits BP lowering, cardio-depressant and vasodilator effects, mediated possibly through inhibition of Ca^{++} ingress via., membranous Ca^{++} channels, thus pharmacologically explains its folkloric repute as antihypertensive agent.

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