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Pharmacodynamic Evaluation of *Terminalia bellerica* for Its Antihypertensive Effect

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ABSTRACT

Terminalia bellerica has been used as a folk medicine in a variety of ailments including hypertension. Our aim was to investigate the possible mechanism of its blood pressure (BP)-lowering effect. The crude extract of *Terminalia bellerica* fruit (Tb.Cr) which tested positive for flavonoids, sterols and tannins induced a dose-dependent (10-100 mg/kg) fall in the arterial BP of rats under anaesthesia. In isolated guinea-pig atria, Tb.Cr inhibited the force and rate of atrial contractions. In rabbit thoracic aorta, Tb.Cr relaxed the phenylephrine (PE, 1 μM) and K^+ (80 mM)-induced contractions as well as suppressed the PE (1 μM) control peaks in the Ca^{++}-free medium, similar to that caused by verapamil. The vasodilator effect of Tb.Cr was endothelium-independent as it was not opposed by N_ω-nitro-L-arginine methyl ester in endothelium-intact rat aortic preparations and it occurred at the similar concentration in the endothelium-denuded tissues. These results indicate that *Terminalia bellerica* lowers BP through Ca^{++} antagonist mechanism and thus provides a sound mechanistic background for its medicinal use in hypertension.

Key words: *Terminalia bellerica*, antihypertensive, Ca++ antagonist

INTRODUCTION

Terminalia bellerica Roxb. (family: Combretaceae), commonly known as belleric myrobalan and locally known as bahera, is an edible plant found throughout Central Asia[1]. Its fruit has been used in traditional medial system for anemia, asthma, cancer, colic, constipation, diarrhoea, dysuria, headache, hypertension, inflammations, and rheumatism[2,3]. It contains termilignan, thannilignan, 7-hydroxy-3′,4′-(methylenedioxy) flavone, anolignan B[4], gallic acid, ellagic acid, ß-sitosterol[5], arjungenin, belleric acid, bellericoside[6] and cannogenol 3-*O*-ß-D-galactopyranosyl-(1→4)-*O*-α-L-rhamnopyranoside[7].

Terminalia bellerica is known to lower the lipid levels in hypercholesterolemic animals[8]. The ethanolic extract of *Terminalia bellerica* was found effective against several pathogens including *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*[9]. *Terminalia bellerica* exhibited inhibitory effect on human immunodeficiency virus-1 reverse transcriptase[10]. The leaves and fruits of *Terminalia bellerica* showed antioxidant activity[11]. On the other hand, methanolic extract (75%) of *Terminalia bellerica* reduced the serum glucose level both in normal and alloxan-induced diabetic rats[12], showing preventive effect against the myocardial necrosis in rats[13]. A water soluble fraction obtained from the defatted fruits of *Terminalia bellerica* caused hepatoprotection against CCl_4-induced hepatotoxicity[14].

Srivastava *et al.*[15] and Dwivedi *et al.*[16] reported that *Terminalia bellerica* lowers blood pressure (BP) but the precise mode of action remains to be elucidated. In this study, we explored the mechanism underlying its hypotensive effect. The present report further supports the previous findings on the use of *Terminalia bellerica* as an antihypertensive agent.

MATERIALS AND METHODS

I. *Plant Material and Extraction*

Fruits of *Terminalia bellerica* were bought at a local market in Dhaka (Bangladesh) and the sample voucher (TB-FR-10-95-30) was submitted to the herbarium of the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. After cleaning of adulterant material, 432 g of the fruits were crushed and soaked in

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the 70% aqueous-methanol for three days with vigorous shaking. The mixture was filtered through a muslin cloth and then through a Whatman qualitative grade 1 filter paper[17]. This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator under reduced pressure to a semi-solid mass of brown colour, i.e. the crude extract (Tb.Cr), yielding approximately 9.25%. Tb.Cr was completely solubilized both in distilled water and saline for experimental use *in vitro* and *in vivo*.

II. *Drugs and Animals*

The following reference chemicals were obtained from the sources specified: acetylcholine chloride (ACh), N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), phenylephrine hydrochloride (PE) and verapamil hydrochloride (Sigma Chemical Company, St. Louis, MO, USA). Thiopental sodium and heparin injections were purchased from Abbot Laboratories, Karachi, Pakistan and Rotex Medica, Trittau, Germany, respectively. The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company, St. Louis, MO, USA), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and ethylenediaminetetra acetic acid (BDH Laboratory Supplies, Poole, England). All chemicals were of analytical grade. Stock solutions of the drugs were made in distilled H_2O/saline and subsequent dilutions were prepared fresh on the day of experiment.

Animals used in this study such as Sprague-Dawley rats (180-200 g), guinea-pigs (450-500 g) and rabbits (1-1.5 kg) of either sex and local breed were housed at the Aga Khan University animal house under a standard diet and tap water ad labitum in a controlled environment (23-25°C). Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council(18) and approved by the Ethical Committee of the Aga Khan University.

III. *Phytochemical Analysis*

Preliminary screening of the plant extract for various phytochemical classes was carried out based on a modified version of the reported methods[19-21]. Alkaloids were tested using Dragendorff's reagent. Yellow colour with $AlCl_3$ reagent and green or black colour with aqueous $FeCl_3$ detected flavonoids and tannins respectively. Plant material treated with petroleum ether and extracted with $CHCl_3$ was noted for green to pink or pink to purple colour after reaction with acetic anhydride and HCl to detect sterols and terpenes respectively. Saponins were detected on the basis of froth upon vigorous shaking. The observation of yellow florescence under UV light on filter paper impregnated with the vapours from boiling extract indicated the presence of coumarins. Benzene extract prepared from acidified plant material was treated with NH_4OH for anthraquinones based on the appearance of pink, violet or red colour. The total phenolic and flavonoid contents were determined according to Singleton *et al*.[22] and Huang *et al*.[23] respectively.

IV. *Measurement of BP in Anaesthetized Rats*

Measurements were taken according to the method previously described[24]. Rats were anaesthetized with thiopental sodium (Pentothal®, 70-90 mg/kg, i.p.) and the arterial BP was recorded through carotid artery cannulation via pressure transducer (P23 XL) coupled with a Grass model 7 Polygraph (Grass instrument company, Quincy, MA, USA). Drugs were administered through a cannula inserted into the jugular vein. After 20 min of equilibrium, the rats were injected with 0.1 mL of saline (NaCl 0.9%) or with the same volume of test substance. Arterial BP was allowed to return to the initial level between injections. Changes in BP were recognized as differences between the steady state before injection and the lowest readings after injection. Mean arterial blood pressure (MABP) was calculated as the diastolic BP plus one-third pulse width.

V. *Guinea-pig Atria*

Right atria isolated from the guinea-pigs killed by cervical dislocation, were mounted individually in 20 mL of tissue bath containing Kreb's solution[25] at 32°C and aerated with carbogen (5% CO_2 in O_2). Composition of the Kreb's solution was (mM): NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). The tissues were allowed to beat spontaneously (due to pacemaker) under the resting tension of 1 g. An equilibrium period of 30 min was given before the application of any drug. Tension changes in the tissue were recorded via force-displacement transducer (model FT-03) using Grass Model 7 Polygraph.

VI. *Rabbit Aorta*

Rabbit descending thoracic aorta was removed and cut into 2-3 mm wide rings which were mounted individually in 20 mL of tissue bath containing Kreb's solution at 37°C and supplied with carbogen[26]. A basal tension of 2 g was applied to each tissue. After 1 hr of equilibrium period, the tissues stabilized with a fixed concentration of PE (1 μM). Changes in isometric tensions of the rings were measured via FT-03 transducer using Grass Model 7 Polygraph. The extract was tested for its ability to relax the contractions induced with PE (1 μM) or high K^+ (80 mM). The inhibition of PE and high K+-induced sustained contractions indicated a blockade of Ca^{++} influx through membrane bound receptor-operated and

voltage-sensitive calcium channels, respectively[27,28]. The plant material was than tested against PE (1 μM)-evoked peaks in the Ca^{++}-free Kreb's solution to observe its effect on the intracellular stores. As in the Ca^{++}-free medium, PE acts through stimulation of α_1-adrenergic receptors and then the consequent conversion of phosphatidylinositol to inositol-1,4,5-triphosphate which releases Ca^{++} from the sarcoplasmic reticulum resulting in a tonic contraction[29,30].

VII. *Rat Aorta*

The procedure of Furchgott and Zawadski[31] was followed with some modifications. Thoracic aorta was isolated from the rat and cut into the rings, which were mounted individually in 5 mL of tissue bath, maintained at 37°C and aerated with carbogen. A preload of 1 g was applied to each preparation and incubated for 30 min. 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 (AD Instruments, Sydney, Australia). The tissues were than stabilized with PE (1 μM). After stabilization, an induced contraction was obtained with PE (1 μM). Once plateau was achieved, ACh (0.3 μM) was than tested on PE-induced contraction to observe the endothelium integrity. The endothelium lining of the tissues was removed by gentle rubbing, which resulted in the disappearance of the relaxation caused by ACh in the endothelium-intact preparations.

VIII. *Statistical Analysis*

Data were expressed as mean ± standard error of mean (SEM, n = number of experiment). The statistical parameter applied was one-way ANOVA followed by Tukey test using GraphPad program (GraphPAD, San Diego, CA, USA). $P < 0.05$ was considered statistically significant. Concentration-response curves were analyzed by non-linear regression (GraphPAD program).

RESULTS

I. *Phytochemical Screening*

Tb.Cr was tested positive for flavonoids, sterols and tannins while negative for the rest of classes. Total phenolic and flavonoid contents were 258.4 ± 5.8 and 11.0 ± 0.4 mg of quercetin equivalent/g of Tb.Cr.

II. *Effect on BP in Rats*

Tb.Cr at the doses of 10, 30 and 100 mg/kg caused a respective fall of 15.6 ± 2.0, 25.1 ± 2.3 and 44.7 ± 3.1% in MABP of rats under anaesthesia. Figure 1A shows tracing from a typical experiment, whereas the combined data from different experiments is plotted in Figure 1B. Pre-treatment of animals with atropine (1 mg/kg) did not alter the effect of Tb.Cr (data not shown).

III. *Effect on Guinea-pig Atria*

In isolated guinea-pig atria, Tb.Cr exhibited a concentration-dependent inhibitory effect on the atrial force and rate of contractions (Figure 2A) with respective EC_{50} values of 4.5 ± 1.2 and 5.9 ± 1.3 mg/mL (Figure 2B). Similarly, verapamil caused concentration-dependent inhibitory effect with EC_{50} values of 0.7 ± 1.2 and 1.0 ± 1.2 μM respectively (Figure 2 A and C).

IV. *Effect on Rabbit Aorta*

When tested on the resting base line of rabbit aorta, the extract was devoid of vasoconstrictor effect up to 10

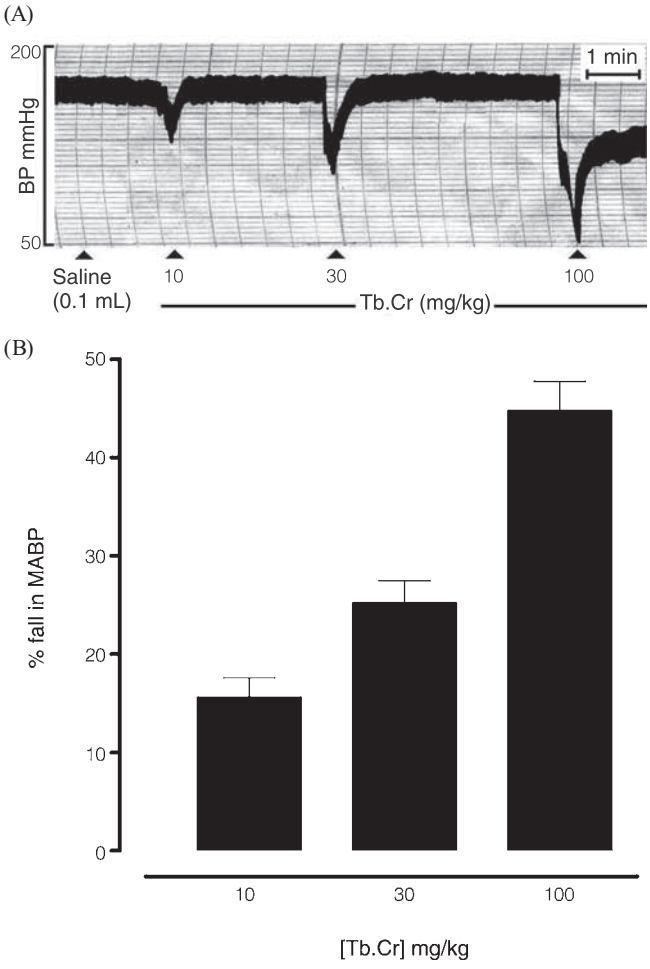

Figure 1. Upper panel (A) shows the typical tracing of *Terminalia bellerica* crude extract (Tb.Cr) for its blood pressure lowering effect and lower panel (B) shows the effect of Tb.Cr on mean arterial blood pressure (MABP) in anesthetized rats. The dose was administered after response of the preceding one returned to the normal. Symbols represent mean ± SEM, n = 4.

Journal of Food and Drug Analysis, Vol. 16, No. 3, 2008

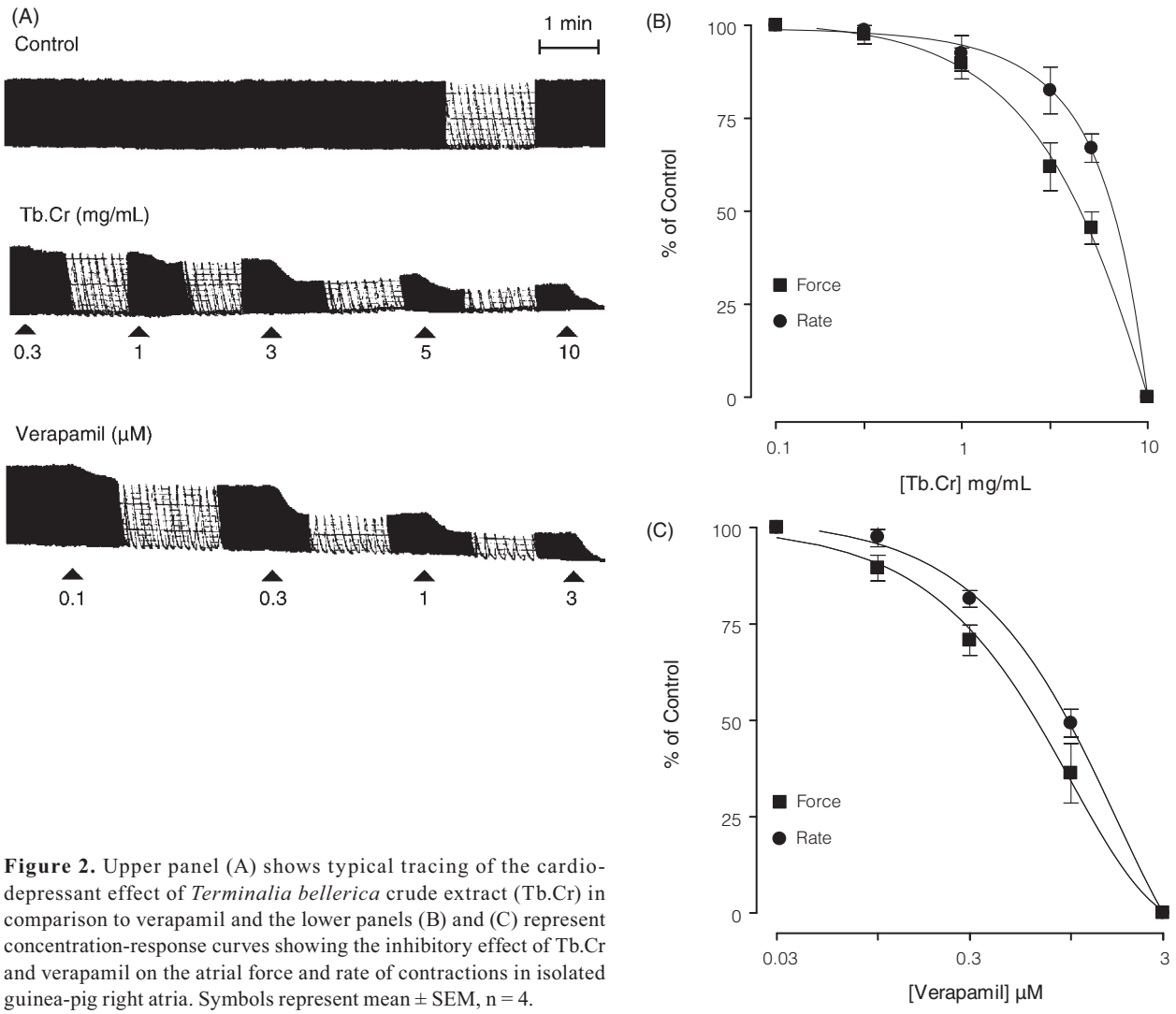

Figure 2. Upper panel (A) shows typical tracing of the cardiodepressant effect of *Terminalia bellerica* crude extract (Tb.Cr) in comparison to verapamil and the lower panels (B) and (C) represent concentration-response curves showing the inhibitory effect of Tb.Cr and verapamil on the atrial force and rate of contractions in isolated guinea-pig right atria. Symbols represent mean ± SEM, n = 4.

mg/mL. Tb.Cr relaxed the PE (1 μM) and K^+ (80 mM)-induced contractions with respective EC_{50} values of 6.4 ± 1.3 and 7.5 ± 1.3 mg/mL. Verapamil was also free of any vasoconstrictor effect and inhibited the PE (1 μM) and K^+ (80 mM)-induced contractions with EC_{50} values of 1.2 ± 1.2 and 1.0 ± 1.2 μM respectively. Figure 3A shows typical tracings, whereas the combined data from different experiments is presented in Figure 3B and C. In the Ca^{++}-free medium, Tb.Cr and verapamil suppressed the PE (1 μM) peak responses at the concentration ranges of 3-10 mg/mL and 0.03-0.3 μM respectively (Figure 4).

V. *Effect on Rat Aorta*

In endothelium-intact rat aortic rings, Tb.Cr concentration-dependently relaxed the PE (1 μM)-induced contractions in absence of any intervention and in presence of L-NAME (0.1 mM) with respective EC_{50} values of 3.5 ± 1.1 and 3.8 ± 1.2 mg/mL. In endothelium-denuded preparations, Tb.Cr relaxed the PE (1 μM)-induced contractions with EC_{50} value of 3.01 ± 1.2 mg/mL. Verapamil inhibited these contractions with respective EC_{50} values of 7.1 ± 1.2, 5.6 ± 1.2 and 6.6 ± 1.3 μM (Figure 5).

DISCUSSION

The aqueous-methanolic extract of *Terminalia bellerica* induced a dose-dependant fall in the BP of normotensive rats under anaesthesia as expected. It is customary to use isolated tissue preparations to evaluate the underlying mechanism of action, as response interference from intact reflex is obliterated[32]. BP is considered the product of cardiac output and peripheral resistance[33], hence the extract was further studied in isolated heart and vascular preparations. In guinea-pig atria, Tb.Cr exhibited a negative inotropic and chronotropic effect, similar to that caused by verapamil, a standard Ca^{++} channel blocker[34]. Calcium antagonists are known to cause cardiac depression through inhibiting the slow

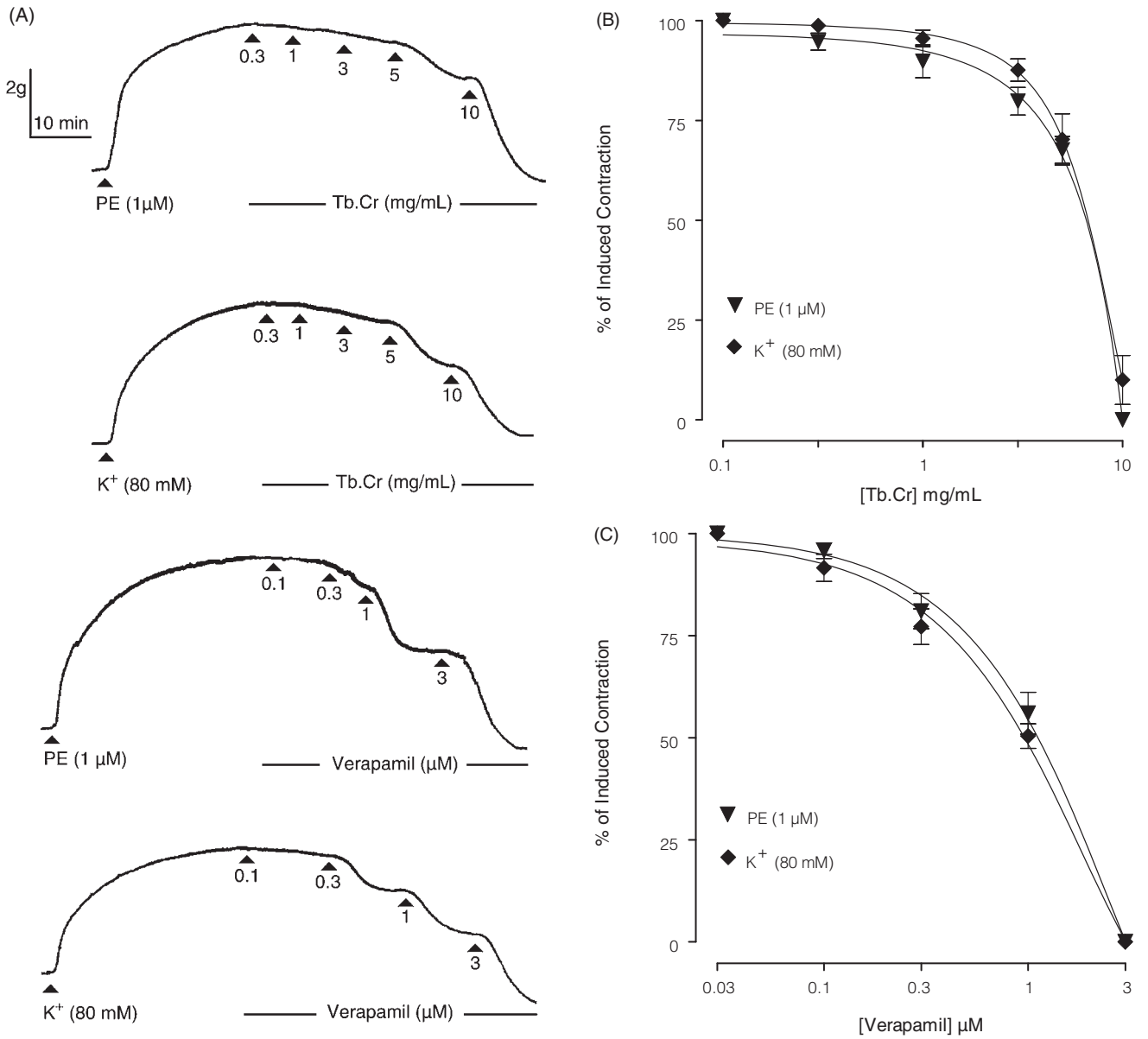

Figure 3. Typical tracings showing the relaxant effect of *Terminalia bellerica* crude extract (Tb.Cr) on phenylephrine (PE) and K^+-induced contractions in comparison to verapamil (A). (B) and (C) concentration-response curves representing the inhibitory effect of Tb.Cr and verapamil on PE and K^+ -induced contractions in isolated rabbit aortic ring preparations. Symbols represent mean ± SEM, n = 4-5.

inward current during the action potential plateau[35]. The cardiac inhibitory action of the extract may be due to the Ca^{++} antagonist effect leading to decrease in cardiac out put and thus falling BP.

The plant extract was tested in two different types of vascular tissues. Rabbit aorta is routinely used for screening of Ca^{++} antagonists[36]. Tb.Cr inhibited the high K^+ and PE-induced contractions of rabbit aorta at similar concentration, indicating that it was equipotently blocking the Ca^{++} influx through voltage- and receptor-operated calcium channels[37]. In addition to the Ca^{++} influx through membrane bound calcium channels, smooth muscle contraction also occurs via Ca^{++} release from the intracellular sarcoplasmic reticulum[38,39]. When control PE responses were obtained in the Ca^{++} free medium, extract in increasing concentrations suppressed the agonist peaks, thus inhibiting the Ca^{++} release from the internal stores[40]. The results were similar to those obtained with verapamil.

The second type of vascular preparation used was rat aorta, which is a prototype tissue for evaluating the endothelium-dependent vasodilation[41]. The vasodilator effect of Tb.Cr was endothelium-independent, evident from the fact that its inhibitory effect on the endothelium-intact tissues was resistant to L-NAME, a nitric oxide synthase inhibitor[42] and that the effect occurred

Journal of Food and Drug Analysis, Vol. 16, No. 3, 2008

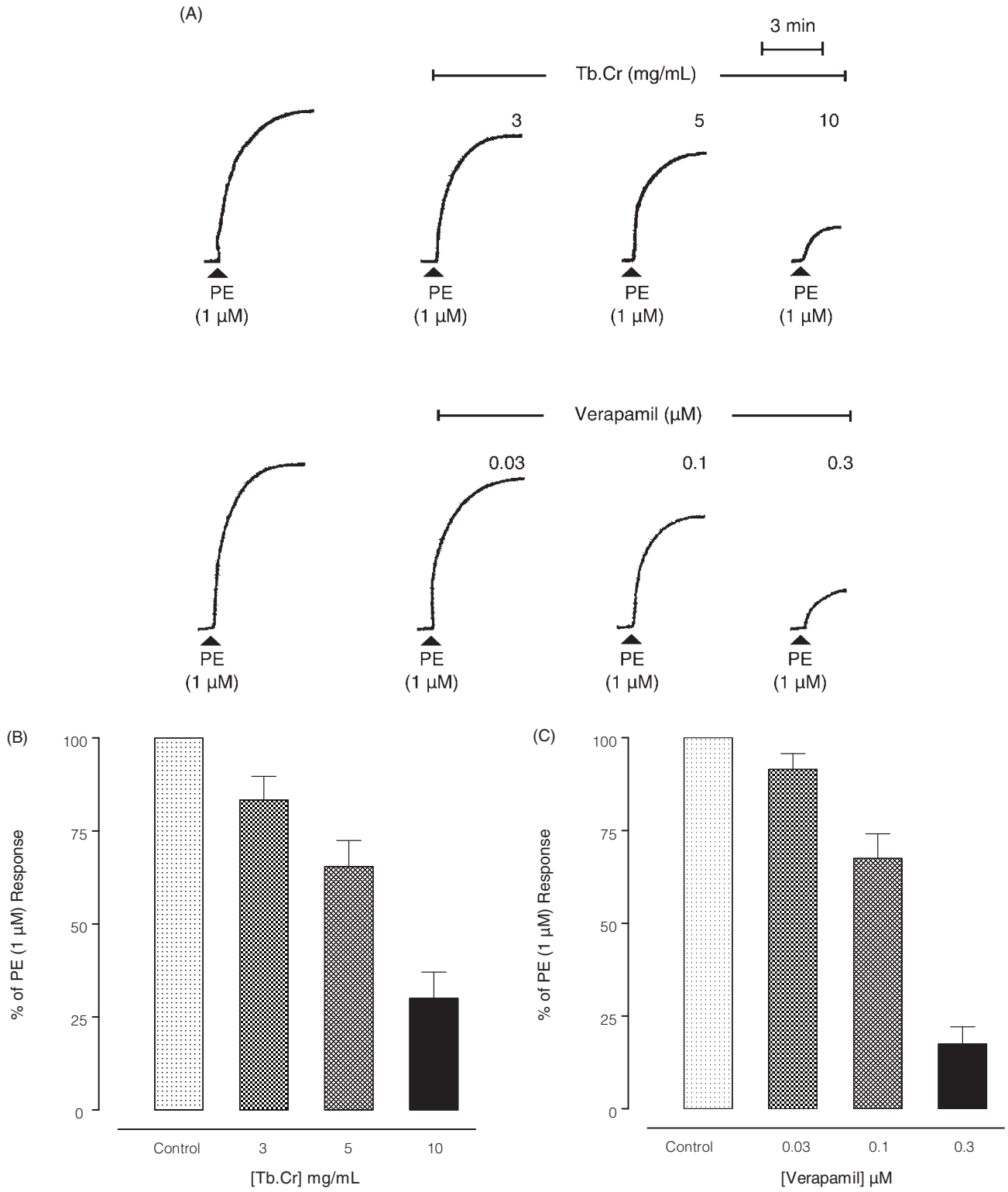

Figure 4. Upper panel (A) shows typical tracings of the concentration-dependant suppressant effect of *Terminalia bellerica* crude extract (Tb. Cr) and verapamil on control phenylephrine (PE) peaks in the Ca^{++} free Kreb's solution while the lower panels (B) and (C) represent bar chart for the inhibitory effect of Tb.Cr and verapamil on PE responses in isolated rabbit aortic ring preparations. Symbols represent mean ± SEM, n = 4.

in endothelium-denuded preparations at the same concentration, similar to the fashion of a standard Ca^{++} channel blocker.

Terminalia bellerica extract was found to contain flavonoids, sterols and tannins. Flavonoids and tannins are reported to possess Ca^{++} antagonist effect[43,44] and

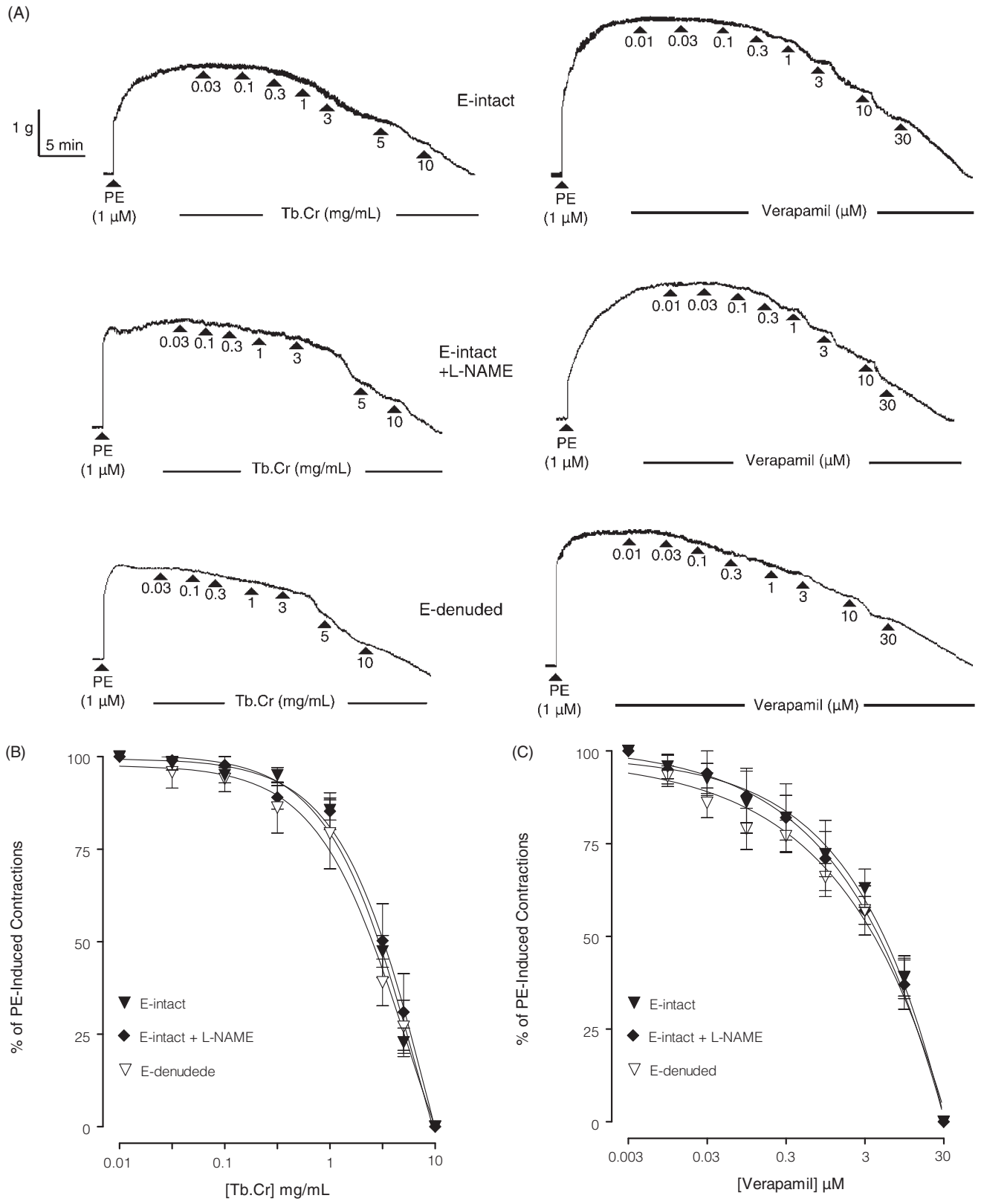

Figure 5. Upper panel (A) shows traces for the relaxant effect of *Terminalia bellerica* crude extract (Tb.Cr) and verapamil on phenylephrine (PE)-induced contractions of endothelium (E)-intact aortic rings in the absence and presence of N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) and E-denuded preparations. (B) and (C) shows concentration-response curves representing the inhibitory effect of Tb.Cr and verapamil on PE-induced contractions in the absence (▼) and presence of L-NAME (◆) in E-intact and E-denuded (▽) isolated rat aorta preparations. Symbols represent mean ± SEM, n = 4-5. The curves were not significantly different from each other ($P > 0.05$); one way ANOVA.

the presence of such compounds in *Terminalia bellerica* might be contributing in its cardiovascular effects.

CONCLUSIONS

This study showed that *Terminalia bellerica* exhibits BP-lowering effect possibly mediated through inhibition of Ca^{++} influx via membranous calcium channels and its release from the intracellular stores and thus explains its medicinal use in hypertension.

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