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Blood Pressure Lowering, Cardiovascular Inhibitory and Bronchodilatory Actions of *Achillea millefolium*

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Achillea millefolium **Linn. (Asteraceae) is used in folk medicine for the treatment of overactive cardiovascular and respiratory ailments. This study describes its hypotensive, cardio-depressant, vasodilatory and bronchodilatory activities. The crude extract of *Achillea millefolium* (Am.Cr) caused a dose-dependent (1–100 mg/kg) fall in arterial blood pressure of rats under anaesthesia. In spontaneously beating guinea-pig atrial tissues, Am.Cr exhibited negative inotropic and chronotropic effects. In isolated rabbit aortic rings, Am.Cr at 0.3–10 mg/mL relaxed phenylephrine (PE, 1 μM) and high K^+ (80 mM)-induced contractions, as well as suppressed the PE (1 μM) control peaks obtained in Ca^{++}-free medium, like that caused by verapamil. The vasodilator effect of Am.Cr was partially blocked by N_ω-nitro-L-arginine methyl ester in endothelium intact preparations. In guinea-pig tracheal strips, Am.Cr inhibited carbachol (CCh, 1 μM) and K^+-induced contractions. These results indicate that *Achillea millefolium* exhibits hypotensive, cardiovascular inhibitory and bronchodilatory effects, thus explaining its medicinal use in hyperactive cardiovascular and airway disorders, such as hypertension and asthma. Copyright © 2010 John Wiley & Sons, Ltd.**

Keywords: Achillea millefolium; antihypertensive; cardio-suppressant; vasodilator; bronchodilator.

INTRODUCTION

Achillea millefolium Linn. (Asteraceae) is a perennial herb, commonly known as 'yarrow' or 'milfoil'. It occurs mainly in Asia, Europe and USA and blooms from June to September (Nadkarni, 1976; Chandler *et al*., 1982; Radusiene and Gudaityte, 2005). The plant has a folkloric reputation as an analgesic, antihaemorrhagic, antihelmintic, antimicrobial, antipyretic, antiphlogistic, antiseptic, astringent, carminative, diaphoretic, diuretic, emmenagogue and stimulant (Baquar, 1989; Usmanghani *et al*., 1997). It is used traditionally for curing arthritis, cancer, colic, headache, heartburn, hypertension, hyperglycaemia, inflammations, hepatobiliary and congestive respiratory disorders (Duke *et al*., 2002; Hamayun *et al*., 2006). *Achillea millefolium* is known to contain achilleine, achillicin, achillin, achilletine, apigenin, artemitin, austricin, azulene, balchanolide, betonicine, butyric acid, casticin, chamazulene, cineole, caffeic acid, dulcitol, furfural, formic acid, humulene, leucodin, limonene, luteolin, millefin, millefolide, quercetin, rutin, β-sitosterol, sabinene, sesquiterpenes, succinic acid, tannin, terpineol (Duke, 1992), chlorogenic acid, essential oil, mandelic acid, pyrocatechol, salicylic acid, stachydrine, undecylenic acid (Tunon *et al*., 1994), camphor, isoborneol and *p*-cymene (Jaimand *et al*., 2006). The plant is reported to possess antispasmodic, hepatoprotective (Yaeesh *et al*., 2006), antiinflammatory (Benedek *et al*., 2007), antispermatogenic (Montanari *et al*., 1998), antiulcer (Cavalcanti *et al*., 2006), antinociceptive (Pires *et al*., 2009) and mosquito repelling (Tunon *et al*., 1994) properties. This investigation describes the hypotensive, cardio-suppressant, vasodilatory and bronchodilatory activities of the aqueous–methanol extract of *Achillea millefolium* in order to rationalize its medicinal use in hyper-excitable diseases of the cardiovascular and respiratory systems.

MATERIALS AND METHODS

Plant material and preparation of extract. Dried aerial parts of *Achillea millefolium* were obtained in April 2004 from a well-known local herbalist in Karachi. The plant material was identified by Mr Afzal Rizvi, a taxonomist at the Hamdard University; a voucher specimen (AM–PL–03–02–43) has been submitted to the herbarium of the Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi. The plant materials were cleaned, shade dried and coarsely ground. The powdered material (1500 g) was soaked in 70% aqueous–methanol solution in a large container for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper (Williamson *et al*., 1998). This procedure was repeated twice and the combined filtrate was

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evaporated on a rotary evaporator, under reduced pressure, to a thick semi-solid mass of dark brown colour, i.e. the crude extract of *Achillea millefolium* (Am.Cr), yielding approximately 18%. Am.Cr was solubilized both in saline and distilled water.

Chemicals. The following reference chemicals were obtained from the sources specified: acetylcholine chloride (ACh), carbachol chloride (CCh), isoprenaline hydrochloride, N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), norepinephrine hydrochloride (NE), phenylephrine hydrochloride (PE) and verapamil hydrochloride (Sigma Chemical Company, St Louis, MO, USA). Pentothal sodium (thiopental sodium) was obtained from Abbot Laboratories, Karachi, Pakistan. The following chemicals were used to make the physiological salt solution: potassium chloride (Sigma Chemical Company, St Louis, MO, USA), calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride (Merck, Darmstadt, Germany) and ethylenediaminetetra-acetic acid (EDTA) from BDH Laboratory Supplies, Poole, England. The chemicals used in phytochemical analysis include: acetic anhydride, aluminium chloride, ammonium hydroxide, Dragendorff's reagent, ferric chloride (Sigma Chemical Co, St Louis, MO, USA), benzene, chloroform, hydrochloric acid and petroleum ether (BDH Laboratory Supplies, Poole, England). All the chemicals used were of the highest analytical grade available.

Animals. 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 animal house of the Aga Khan University under a controlled environment (23–25°C). Animals were given tap water *ad libitum* and a standard diet. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethical Committee of the Aga Khan University.

Phytochemical analysis. Preliminary screening for the detection of various phytochemical classes, such as alkaloids, saponins, coumarins, sterols, terpenes, flavonoids, tannins and anthraquinones was done according to standard methods (Edeoga *et al*., 2005). Alkaloids were detected by using Dragendorff's reagent. The presence of saponins was detected based on the appearance of froth upon vigorous shaking of diluted samples. The observation of yellow fluorescence under ultraviolet light on the examination of filter paper previously exposed to the vapours from boiling plant material indicated the presence of coumarins. For the detection of sterols and terpenes, the plant material was treated with petroleum ether and subsequently extracted with chloroform. The appearance of green to pink (for sterols) and pink to purple colours (for terpenes) was then noted after treatment of the chloroform layer with acetic anhydride and concentrated hydrochloric acid in succession. Plant material was noted as positive for flavonoids when it gave a yellow colour with aluminium chloride reagent and for tannins, when green or black colour was produced with aqueous ferric chloride. For anthraquinones, the extract was dissolved in 1% hydrochloric acid, then in benzene and the appearance of a pink, violet or red colour with ammonium hydroxide indicated the presence of anthraquinones.

Measurement of blood pressure. These experiments were performed according to the method described previously (Ghayur and Gilani, 2005). Briefly, the rats were anaesthetized with thiopental sodium (Pentothal®, 70–90 mg/kg, i.p.) and the arterial blood pressure (BP) was recorded through carotid artery cannulation via a pressure transducer (P23 XL) coupled with a Grass Model 7 Polygraph. Am.Cr (1, 3, 10, 30, 100 mg/kg), ACh (1 μg/kg) and NE (1 μg/kg) were injected through a sterile cannula inserted into the jugular vein. After a 20 min period of equilibrium, the rats were injected intravenously with 0.1 mL saline (NaCl 0.9%) or with the same volume of test substance. Arterial BP was allowed to return to the resting level between injections. Control responses of standards, such as ACh and NE were obtained before testing the extract. Changes in BP were recognized as the difference between the steady state values before and the lowest readings after injection. Mean arterial pressure (MAP) was calculated as the diastolic BP plus one-third pulse width.

Guinea-pig atria. Right atria isolated from the guinea-pigs, killed by cervical dislocation, were mounted separately in 20 mL tissue baths containing Kreb's solution, at 32°C, and aerated with carbogen (5% CO_2 in O_2). The composition of 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 tissue was allowed to beat spontaneously (due to pacemaker) under the resting tension of 1 g (Khan and Gilani, 2008a). This preparation allows study of the effects on both force and rate of contractions. An equilibrium period of 30 min was given before the application of any drug. The effect on heart rate was measured by increasing the speed of the chart recorder. Control responses of the ACh (1 μM) and isoprenaline (1 μM) were obtained at least in duplicate. Tension changes in the tissues were recorded via force-displacement transducers (FT-03) using a Grass Model 7 Polygraph.

Rabbit aorta. The rabbits were killed by cervical dislocation. The descending thoracic aorta was removed and cut into 2–3 mm wide rings which were individually mounted in 5 mL tissue baths containing Kreb's solution, at 37°C, and aerated with carbogen. The endothelium lining of the tissues was removed mechanically by gentle rubbing. A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 h was allowed before any experimentation (Gilani *et al*., 2005). The tissues were then stabilized with PE (1 μM). The effect of extract was first determined on the resting baseline of the tissue to see if it has any vasoconstrictor effect. The extract was later tested for its ability to relax the contractions induced with PE (1 μM) and high K^+ (80 mM). The ability of the extract to relax K^+ (80 mM)-induced contractions would indicate an L-type voltage-operated calcium channel blocking mode of vasodilation, while inhibition of the PE-induced contractions would signify the blockade of the Ca^{++} influx through receptor-operated calcium channels (Karaki *et al*., 1997). In order to determine if the extract was inhibiting Ca^{++} release from intracellular stores, the effect of increasing concen-

trations of extract was observed on PE (1 μM) peaks obtained in Ca^{++}-free Krebs solution (Ca^{++} omitted and EDTA (0.1 mM) added) to ensure the total elimination of extracellular Ca^{++}. In a Ca^{++}-free Krebs solution, PE acts through stimulation of α_1-adrenergic receptors and then the consequent conversion of phosphatidylinositol to inositol-1,4,5-trisphosphate released Ca^{++} from the intracellular stores, thus bringing about the contraction (Cauvin and Malik, 1984). To study whether or not the vasodilator effect of the test substance is endothelium-dependent, PE (1 μM)-induced contractions in the endothelium intact aortic rings preincubated with L-NAME (0.1 mM) for 60 min were obtained, to explore for the possibility of an endothelium-dependent vasodilator action (Vanhoutte *et al.*, 1986). The endothelial integrity of the aortic ring was indicated by administration of ACh (0.1 μM) upon PE-induced contractions, resulting in vasorelaxation (Furchgott and Zawadski, 1980). Changes in tension were recorded and analysed isometrically, using a force transducer (Fort-10, WPI, UK) coupled to a bridge amplifier (Transbridge TBM4M, WPI) and a PowerLab ML-845 data acquisition system (AD Instruments, Sydney, Australia).

Guinea-pig trachea. Tracheas were dissected out of guinea-pigs and kept in Krebs solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about two cartilages (Khan and Gilani, 2006). Each ring was opened by a longitudinal cut on the ventral side opposite to the smooth muscle layer, forming a tracheal strip with a central part of smooth muscle in between the cartilaginous portions on the edges. The preparation was then mounted in a 20 mL tissue bath containing Kreb's solution, at 37°C, and aerated with carbogen. A tension of 1 g was applied to each of the tracheal strips and kept constant throughout the experiment. The tissue was equilibrated for 1 h after which it was stabilized with CCh (1 μM), allowing the study of the effect of the extract first on the resting baseline of the tracheal strip and later against CCh and K^+-induced contractions. Tension changes in the tissue were recorded via a force-displacement transducer (FT-03) using a Grass Model 7 Polygraph.

Statistical analysis. The data expressed are mean ± standard error of the mean (SEM, n = number of experiment) and the median effective concentrations (EC_{50}) with 95% confidence intervals (CI). Concentration–response curves were analysed by non-linear regression using the GraphPad program (GraphPAD, San Diego, CA, USA). The statistical parameter applied is two-way analysis of variance (ANOVA) followed by Bonferroni test. A value of $p < 0.05$ was noted as significantly different.

RESULTS

Phytochemical screening

The Am.Cr showed the presence of alkaloids, coumarins, flavonoids, saponins, sterols, tannins and terpenes, while testing negative for anthraquinones.

Effect on blood pressure

Intravenous administration of Am.Cr caused a dose-dependent fall in MAP of normotensive rats, under anaesthesia. The basal MAP of rats was 112 ± 0.5 mm Hg (n = 4). The percent fall in MAP at the respective doses of 1, 3, 10, 30 and 100 mg/kg was of the magnitude of 8.3 ± 0.7, 11.8 ± 1.0, 18.7 ± 0.8, 31.5 ± 2.1 and 45.0 ± 2.2 (n = 4). Figure 1A shows a tracing from a typical experiment, whereas combined data from different experiments are presented in Fig. 1B.

Effect on atria

In isolated guinea-pig atria, the Am.Cr exhibited a concentration-dependent inhibitory effect on atrial force and rate of spontaneous contractions (Fig. 2A) with respective EC_{50} values of 1.4 (0.90–2.2, 95% CI, n = 4) and 1.5 mg/mL (0.91–2.5, n = 4). Verapamil was used as a positive control which exhibited negative inotropic and chronotropic effects with EC_{50} values of 0.74 (0.53–1.0, n = 4) and 0.90 μM (0.65–1.2, n = 4), respectively (Fig. 2B).

Effect on aorta

When tested on the resting baseline of the aortic rings, Am.Cr was found to be devoid of any vasoconstrictor

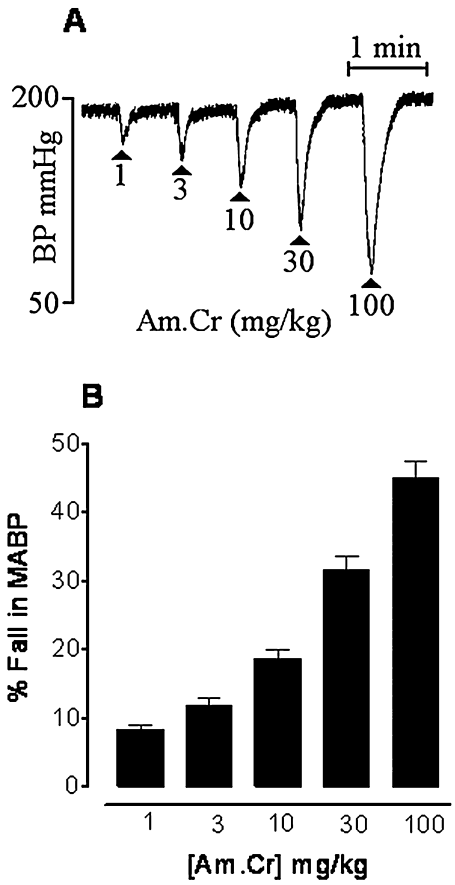

Figure 1. Upper panel [A] shows a typical tracing of *Achillea millefolium* crude extract (Am.Cr) blood pressure (BP) lowering effect, and the lower panel [B] shows a bar chart representing the hypotensive effect of Am.Cr on mean arterial pressure (MAP) in anaesthetized rats. The dose was administered after the response to the preceding one had returned to normal. Values shown represent mean ± SEM, n = 4.

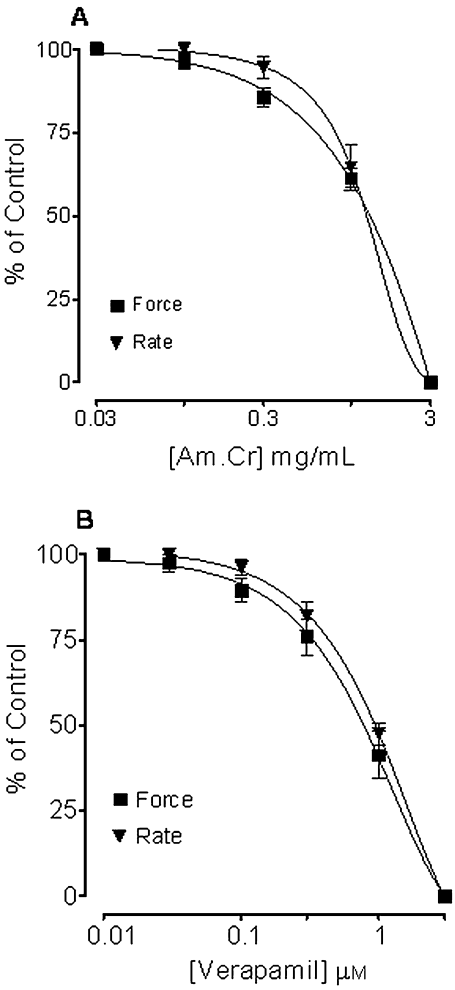

Figure 2. Concentration–response curves showing the inhibitory effect of [A] *Achillea millefolium* crude extract (Am.Cr) and [B] verapamil on force and rate of spontaneous contractions in isolated guinea-pig right atria. Values shown are mean ± SEM, $n = 4$.

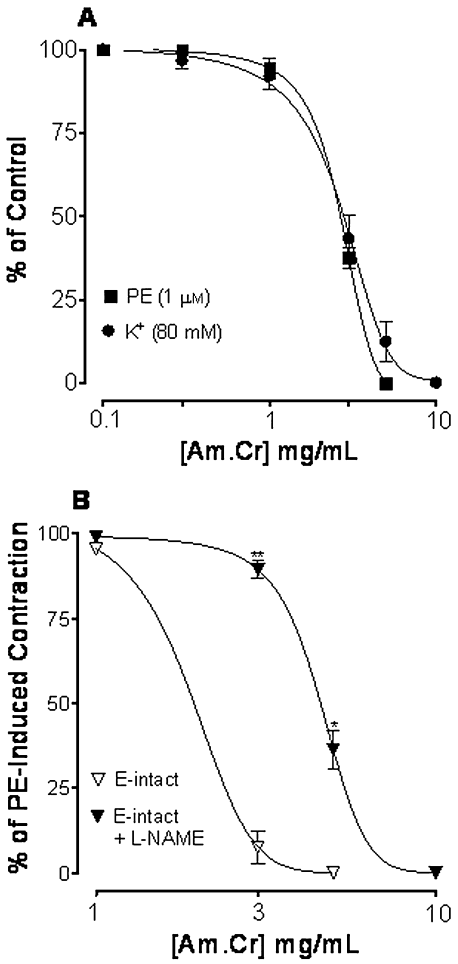

Figure 3. Concentration–response curves showing the effect of *Achillea millefolium* crude extract (Am.Cr) on: [A] phenylephrine (PE) and K^+-induced contractions in endothelium denuded aortic rings and [B] PE (1 μM)-induced contractions in the absence and presence of N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) in endothelium intact aortic preparations from isolated rabbit aorta. Values shown are mean ± SEM, $n = 4$–5. * $p < 0.01$ and ** $p < 0.001$ compared with respective concentrations values in the absence of L-NAME curve, two-way ANOVA followed by Bonferroni test.

effect up to 10 mg/mL. When tested on the PE (1 μM) and K^+ (80 mM)-induced contractions in endothelium denuded aortic tissues, the Am.Cr inhibited these contractions with respective EC_{50} values of 2.8 (1.9–3.4, $n = 4$) and 3.1 mg/mL (2.5–3.6, $n = 5$) as shown in Fig. 3A. In endothelium intact preparations, Am.Cr relaxed the PE (1 μM)-induced contractions with EC_{50} value of 2.1 mg/mL (1.2–3.8, $n = 4$). In the presence of L-NAME, the relaxant effect against PE (1 μM)-induced contraction was partially blocked and the curve was shifted to the right with an EC_{50} value of 4.6 mg/mL (4.4–4.7, $n = 4$) as shown in Fig. 3B. When tested against PE (1 μM) control responses in Ca^{++}-free Krebs solution, Am.Cr and verapamil suppressed the PE (1 μM) peak responses at 1.0–5.0 mg/mL ($n = 4$), and 0.03–0.3 μM ($n = 4$) respectively (Fig. 4A and 4B).

Effect on trachea

The Am.Cr was found to be devoid of any bronchoconstrictor effect up to 10 mg/mL. When tested on agonist-induced contractions, Am.Cr relaxed CCh (1 μM) and high K^+ (80 mM)-induced contractions with respective EC_{50} values of 2.7 (1.5–3.6, $n = 4$) and 2.6 mg/mL (1.7–3.4, $n = 4$) as shown in Fig. 5.

DISCUSSION

The aqueous–methanol extract of *Achillea millefolium* caused a dose-dependent fall in arterial BP of rats, which is in line with its medicinal use in hypertension (Duke *et al.*, 2002). As BP is considered the product of cardiac output and peripheral resistance (Johansen, 1992), the extract was studied in cardiovascular preparations for possible cardiac inhibitory and vasodilatory actions. In guinea-pig atria, *Achillea millefolium* extract depressed the force and rate of spontaneous atrial contractions, similar to verapamil, a standard Ca^{++} antagonist (Fleckenstein, 1977). To see the effect on vascular resistance, Am.Cr was studied in the rabbit thoracic aorta: (a) to evaluate the effect of the extract on K^+ and PE-induced contractions and thus to distinguish between activity at the voltage-operated and

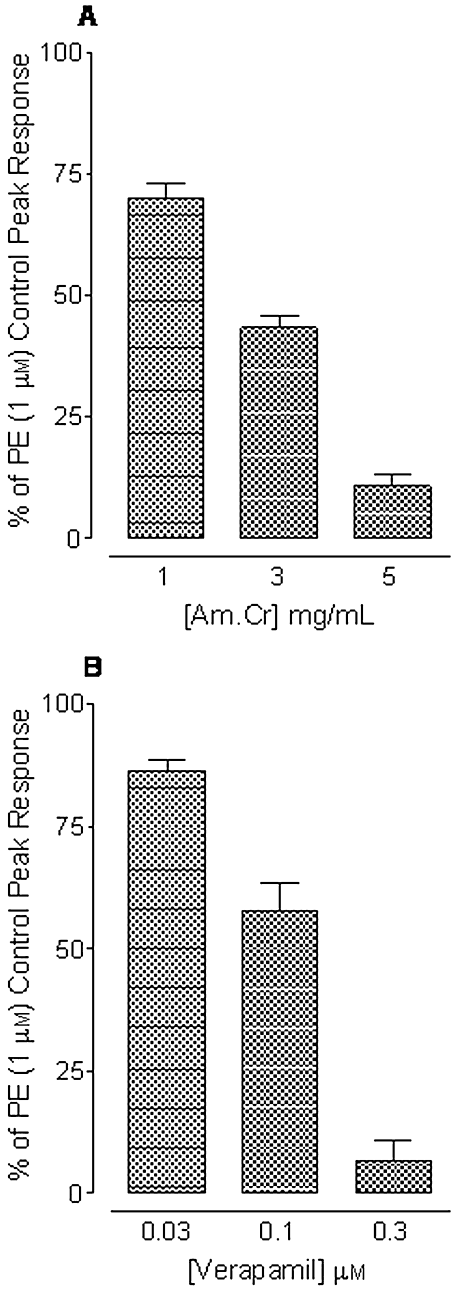

Figure 4. Inhibitory effect of increasing concentrations of [A] *Achillea millefolium* crude extract (Am.Cr) and [B] verapamil on control phenylephrine (PE) peak responses in Ca^{++} free Krebs solution in isolated rabbit aortic ring preparations. Values shown are mean ± SEM, $n = 4$.

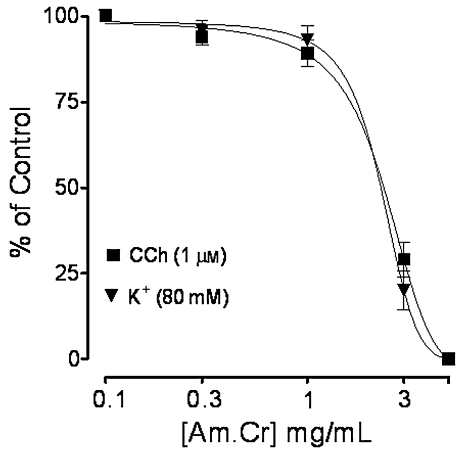

Figure 5. Concentration–response curves showing the inhibitory effect of *Achillea millefolium* crude extract (Am.Cr) on carbachol (CCh) and high K^+-induced contractions in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, $n = 4$.

receptor-operated channels, (b) to determine if the vasodilator effect of Am.Cr is endothelium-dependent or independent and (c) to distinguish between inhibitory effects of the crude extract on membrane bound Ca^{++} channels and those inside the cells. The Am.Cr inhibited PE and high K^+-induced contractions in endothelium denuded rings, at a similar concentration range, indicating that it was acting equipotently through blockade of voltage- and receptor-operated Ca^{++} channels (Musha *et al.*, 2005). The vascular endothelium plays a pivotal role in modulating vascular tone through the release of nitric oxide (NO), which diffuses to the cells in the vicinity to cause relaxation (Chan *et al.*, 2006). This release of NO is mediated by an increase in the cGMP content in the vascular smooth muscle in response to stimulation to guanyl cyclase (Andreopouos and Papapetropoulos, 2000). To see if the vasodilator effect has an endothelium-dependent component, the extract was then tested on endothelium-intact aortic preparations precontracted with PE (1 μM), where it caused a relaxant effect. Pre-incubation of tissue with L-NAME, a standard NO synthase inhibitor (Thorin *et al.*, 1998) resulted in partial blockade of the relaxant effect, shifting the inhibitory concentration–response curve to the right. This showed that the vasodilator effect of Am.Cr is mediated via a combination of endothelium-dependent and -independent pathways. Smooth muscle contraction is brought about by the activation of (1) membrane bound Ca^{++} channels which are: voltage-operated and receptor-operated Ca^{++} channels (Bolton, 1979; Hall *et al.*, 2006), but this is not the only mechanism for contractility. Ca^{++} influx into the cell can also be guided through (2) Ca^{++} release from internal stores, such as the inositol triphosphate (IP_3)-sensitive sarcoplasmic reticulum as well (Burt, 2005). To assess the activity of the extract on Ca^{++} release from intracellular stores, PE control responses were taken in the absence and presence of Am.Cr in a Ca^{++}-free environment. The extract in increasing concentrations suppressed the agonist peaks, inhibiting the Ca^{++} release from the intracellular stores (Khan and Gilani, 2008b).

In tracheal strips, the extract inhibited high K^+ and CCh-induced contractions with a similar potency, suggesting a non-specific bronchodilator effect, mediated possibly through Ca^{++} antagonist mechanism. Calcium channel blockers are known to be beneficial in airway hyperactivity (Barnes, 2006), and the presence of Ca^{++} antagonist content(s) in this plant may explain its medicinal use in the congestive respiratory disorders. Preliminary phytochemical analysis of the extract showed the presence of alkaloids, coumarins, flavonoids, saponins, sterols, tannins and terpenes. Flavonoids are reported to possess hypotensive, vasodilator (through endothelium-dependent and-independent pathways), bronchodilator and Ca^{++} channel blocking actions (Pietta, 1998; Ajay *et al.*, 2003; Ghayur *et al.*, 2007). Alkaloids, coumarins, saponins,

sterols, tannins and terpenes are also known to reduce the BP (Gilani *et al*., 2000; Khan and Gilani, 2008a; Krishnaiah *et al*., 2009). Hence, the observed effects of *Achillea millefolium* might be due to the presence of such phytochemicals.

Thus, this study shows that *Achillea millefolium* exhibits BP-lowering, cardio-suppressant, vasodilator and bronchodilator activities, mediated possibly through Ca^{++} antagonism in addition to an endothelium-dependent relaxant component. This justifies its application in cardiovascular and congestive airway disorders.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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