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Vascular Responsiveness to *Macrosolen cochinchinensis* Extracts in Isolated Rat Thoracic Aorta

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Abstract: The aim of the current investigation was to examine the vascular responsiveness to different extracts obtained from *M. cochinchinensis* using isolated Sprague Dawley (SD) rat aortic rings preparations. The fresh aerial parts of the plant were dried, pulverized into powder and sequentially extracted with petroleum ether, chloroform, methanol and water using hot extraction method. The effects of three concentrations (0.5, 1 and 2 mg mL^{-1}) of each extract on rat thoracic aorta were tested using cumulative concentrations of noradrenaline (NA). The data showed that all the extracts had the ability to relax vascular smooth muscle; however, high concentrations of the methanol and water extracts caused the most significant ($p < 0.05$) reduction in NA-induced vasoconstriction as compared to petroleum ether and chloroform extracts. Polyphenolic content, HPLC profiling and IR spectra were indicative of the presence of diterpenoid constituents. The results collectively suggested the presence of some biologically active ingredients of possible diterpenoid nature that have the ability to modulate the action of naturally occurring vasoactive agents such as NA on vascular smooth muscle responses *in vitro*.

Key words: Aortic rings, Loranthaceae, noradrenaline, polyphenolic content, vasoconstriction

INTRODUCTION

Macrosolen cochinchinensis (syn.: *Loranthus cochinchinensis, Elytranthe globosa* or *Elytranthe cochinchinensis*) from the Loranthaceae family is a bushy plant distributed all the way from India to Indio-China and throughout Malaysia. It is typically parasitic on many species of dicotyledonous trees and attaches itself to the host tree by modified roots (Ameer *et al.*, 2009; Barlow, 1991; Burkhill, 1966; Huaxing and Gilbert, 2003). The members of the Loranthaceae (about 74 genera) are generally known as mistletoes. In Malaysia, this plant is locally known as Benalu (Barlow, 1991; Lohézic-Le-Dévéhat *et al.*, 2002). Preparations from *M. cochinchinensis* are widely used in folk medicine for several therapeutic purposes. As a reputable home remedy and beside its use for treatment of hypertension, the tea obtained from the leaves of *M. cochinchinensis* are used for poulticing for headache while the juice of the medicinal stems is commonly employed to expel the after-birth. Moreover, fruits from *M. cochinchinensis* are considered useful for symptomatic treatment of cough (Burkhill, 1966; Lohézic-Le-Dévéhat *et al.*, 2002;

Perry, 1980). However, no earlier studies have been conducted to experimentally characterize the effect of this plant on vascular smooth muscle reactivity.

It has been scientifically proven that *M. cochinchinensis* possesses some antiviral and cytotoxic activities (Lohézic-Le-Dévéhat *et al.*, 2002). Previous general phytochemical studies on this plant demonstrated the presence of various natural components such as flavonoids, steroids and high concentrations of condensed tannins (Lohézic-Le-Dévéhat *et al.*, 2002).

Against this backdrop, we report in this investigation the vascular effects of different extracts obtained from *M. cochinchinensis* using an *in vitro* experimental approach. Present technique encompassed the utilization of isolated rat thoracic aorta in an attempt to investigate the folklore use of this plant in the treatment of hypertension.

MATERIALS AND METHODS

Plant materials: Fresh aerial parts (leaves, flowers, twigs, stems and berries) of *M. cochinchinensis* were used in

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this study. The plant (4.5 kg) was collected from a rain forest in Penang Island, Malaysia in March 2007 and authenticated by Mr. Adnan Bin Jaafar, a taxonomist in the herbarium institute, School of Biological Sciences, Universiti Sains Malaysia (USM).

Preparation of crude extracts: The collected plant material was cleaned and dried in the oven at 42°C for 4 days. The dried plant material was ground into a fine powder using a milling machine. To obtain the plant extracts, two methods of hot extraction were used:

Soxhlet extraction: In this method, the powdered plant material (400 g) was packed into a cellulose thimble which was subsequently fitted into the extracting chamber of a Soxhlet apparatus (Schott, Duran, Germany). Using solvents of graded polarity, a sequential extraction process was carried out starting with the non-polar petroleum ether (60-80°C) and chloroform (60-65°C) and ending with the relatively polar methanol (60-65°C).

Reflux extraction: The marc plant material obtained at the end of Soxhlet extraction was then packed into a round bottom flask of a reflux apparatus (Schott, Duran, Germany). Subsequently, the plant material was extracted with distilled water at 100°C.

In the end, all extracts were concentrated on a rotary evaporator (Büchi, Switzerland) under reduced pressure (-760 mmHg) and subsequently freeze-dried.

Drugs and solutions: For extraction process, petroleum ether, chloroform and methanol were purchased from Fisher Scientific, UK. NA, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich, Germany. Polyethylene glycol 400 (PEG400) was purchased from Merck-Schuchardt, Germany, while NaCl, KCl, $CaCl_2.2H_2O$, $MgSO_4$, KH_2PO_4, glucose, $NaHCO_3$ and EDTA were purchased from R and M Chem., UK. Potassium bromide (KBr) for IR spectroscopy was purchased from Merck, Germany. Just before use, methanol and water extracts and NA were immediately dissolved in Kreb's physiological solution (KPS) of the following composition (in mM): NaCl 118.2, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7, $NaHCO_3$ 25.0 and EDTA 0.026, while petroleum ether and chloroform extracts were dissolved using 0.5% DMSO and 0.5% PEG400 in Kreb's solution, respectively.

Animals: Animals used in this study consisted of adult male Sprague Dawley (SD) rats (250-300 g) which were bred and obtained from the USM Animal House Facility, Penang, Malaysia. The animals were housed in standard cages with 12:12 h light-dark cycle and fed with normal commercial rat chow (Gold Coin Feed mills Sdn Bhd, Malaysia) and water *ad libitum*. Before being used for

any experiment, the rats were allowed to acclimatize in the animal transit room for a minimum period of one week. All procedures on animals and their handling were carried out in accordance with the guidelines of the Animal Ethics Committee, USM, Penang, Malaysia. The number of determinations for each *in vitro* isolated rat aortic ring experiment was 8.

Preparation of rat thoracic aortic rings and experimental protocol: The rats were anesthetized with pentobarbitone sodium (60 mg kg^{-1} of body weight, i.p.) and subsequently bled and exsanguinated. The thoracic aorta was exposed and carefully isolated, cleaned of the adherent fat and connective adipose tissues and cut into 3-5 mm long rings. Then rings were immediately suspended horizontally in tissue chambers containing 10 mL of KPS. The tissue-bath solution was aerated continuously with 95% O_2 and 5% CO_2 (Carbogen) at 37°C. Aortic rings were allowed to stabilize under an optimal tension of 1 g for a 30 min period. Throughout this period, KPS was replaced every 15 min and if required, the tension was readjusted to 1 g. Before starting the experimental protocol, contractile responsiveness of the aortic rings was assessed by repeated exposure to NA solution to obtain final bath concentrations of 1.0E-10, 3.0E-10, 1.0E-9, 3.0E-9, 1.0E-8, 3.0E-8, 1.0E-7, 3.0E-7, 1.0E-6, 3.0E-6, 1.0E-5 and 3.0E-5M. Responses were recorded isometrically by a force–displacement transducer (P23 ID Gould, Statham Instrument, UK) connected to Grass polygraph model 79D (Quincy, Mass., USA) (Ajay *et al.*, 2003; Ameer *et al.*, 2009).

Validation of the experimental protocol: A stability study (time control study) was performed by testing the contractile response of the aortic rings following repeated exposure (4 times) to cumulative NA concentrations (1.0E-10-3.0E-5 M) over the entire length of the experiment.

Experimental protocol: Successive doses of NA were added to the tissue bath when the tissues had acquired steady state equilibrium. Upon achieving the maximum response, NA was washed out of the bath and complete relaxation of the tissue was allowed. Three concentrations (0.5, 1 and 2 mg mL^{-1}) of each extract were then added successively to the tissue bath and allowed to equilibrate with the tissue for 20 min. The tissue was then exposed to the same concentrations of NA and concentration-response curves were subsequently obtained for all the concentrations of the extracts.

Effect of solubiliting agent on the contractile response of isolated rat aortic rings: Since, DMSO and PEG400 were used to enhance the solubility profiles of petroleum ether and chloroform extracts, these experiments were

conducted to eliminate the possibility of any toxic effects from the action of these chemicals on aortic rings. The experiments were performed using the same experimental protocol mentioned in the previous section; however, the aortic rings were incubated with solutions of either DMSO (0.05, 0.1 and 0.2%) or PEG-400 (0.25, 0.5 and 1%). Ultimately, concentration-response curves of NA were obtained in the presence of these chemicals.

Chemistry: To define the possible chemical composition of the plant extracts, the following analytical procedures were performed:

Determination of total phenolic content: The total soluble phenolic content in *M. cochinchinensis* extracts were determined by using Folin-Ciocalteu reagent and gallic acid (also known as 3, 4, 5-trihydroxybenzoic acid) as a standard according to the method of Slinkard and Singleton (1977). A solution of 4 mg mL^{-1} of each plant extract in methanol and solutions of 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 mg mL^{-1} of gallic acid in methanol were prepared. Twenty microliters of extract and each concentration of gallic acid solution were pipetted in separate test tubes followed by the addition of 1.58 mL of distilled water and 100 µL of 2N Folin-Ciocalteu reagent. Subsequently, the test tubes were mixed thoroughly. After 8 min, 300 µL of 20% sodium carbonate solution were added. The mixture was then allowed to stand for 2 h with intermittent shaking. The absorbance of solutions was measured at 765 nm with a Hitachi U-2000 spectrophotometer (Hitachi, Japan).

The concentration of total phenolic compounds in the extracts was determined as milligrams of gallic acid equivalent by using an equation which was obtained from a standard gallic acid graph.

HPLC profiling: The high performance liquid chromatography (HPLC) system consisted of a HP-1100 Agilent Technologies equipped with a quaternary pump, online degasser, auto sampler, automatic injector, column heater and ultraviolet (UV) detector. Chromatographic separations of the extractives were performed on a Nucleosil C-18 column (250×4.6 mm, 5 µm). Isocratic solvent was used with a mobile phase combining solvent A (acetonitrile) and solvent B (water). The column temperature was maintained at 25°C, the injection volume was 10 µL and the flow rate was 1 mL min^{-1}. The effluents were monitored at 210 nm with the UV detection.

Lyophilized extracts were dissolved in methanol, sonicated for 15 min and then made up to a concentration of 10 mg mL^{-1} with methanol. The samples were filtered through 0.45 µm filter (Whatman). The separation of the peaks in petroleum ether, chloroform, methanol and water extracts were achieved with a mobile phase containing acetonitrile and deionised water (80:20) on a 5 µm

Nucleosil C-18 column (250×4.6 mm) at a flow rate of 1 mL min^{-1} and detection at 210 nm with 20 min separation time. Samples were also checked in absorbance 254 nm.

IR spectroscopy: About 1 to 2 mg of each extract were triturated with 100 mg Kbr and pressed into pellets for which IR spectra were obtained using Thermo-Nicolet AVATAR 360 fourier transform infrared spectrophotometer (USA).

Data presentation and statistical analysis: All data were expressed in terms of Mean±SEM. Contractile responses to NA are expressed as the relative percentage increase in the respective contraction. R_{max} (response at highest concentration of respective agonist tested) and EC_{50} (median effective concentration) values were calculated using computer software (Prism version 5.0, Graphpad software, USA). The statistical analysis of the overall maximum responses to NA was done using two-way ANOVA while R_{max} values were analyzed using one-way ANOVA followed by the Bonferroni/Dunnett (all mean) post hoc test (SuperANOVA, Abacus Concepts, Inc., Berkeley, CA, USA). The differences between the means were considered significant at the 5% level.

RESULTS

Stability study: It was observed that the addition of repetitive cumulative concentrations of NA did not significantly (p>0.05) alter the contractile response in rat aortic rings. The overall mean of the percentage of maximum contraction repeated for 4 times were 48.9±4.0, 45.9±4.6, 45.8±4.8 and 45.4±4.40% (Fig. 1).
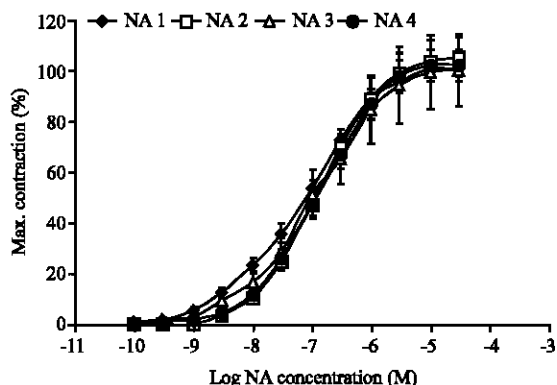

Fig. 1: The effect of repetitive cumulative additions of noradrenaline (NA) on the maximum contractile response in vascular smooth muscle of isolated rat aortic rings preparations. All data presented as Mean±SEM (n = 8). Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test
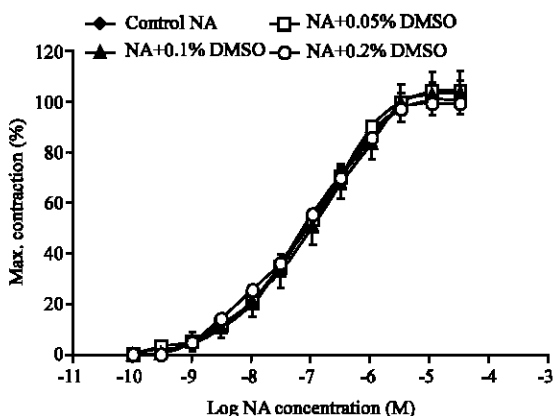
Fig. 2: The effect of dimethyl sulfoxide (DMSO) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test
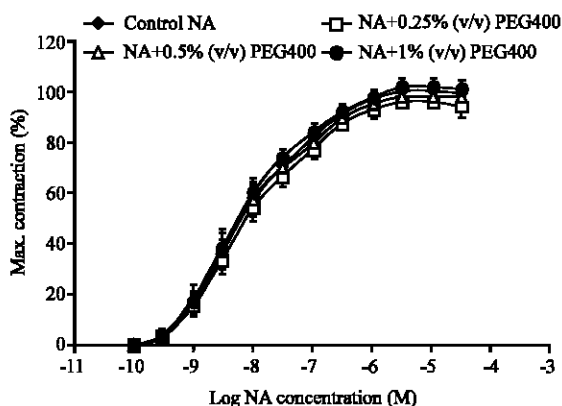

Fig. 3: The effect of polyethylene glycol 400 (PEG400) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test

DMSO and PEG400: The effect of all the tissue bath concentrations of 0.05, 0.1 and 0.2% DMSO used to solubilized petroleum ether extract on NA-induced aortic rings concentration were not significantly different from the control (48.6±4.2, 47.8±4.1 and 48.6±4.2%, respectively vs. 48.0±4.1%; p>0.05) (Fig. 2). Similarly, PEG400 bath concentrations of 0.25, 0.5 and 1% did not contribute to any significant change in the pattern of NA action on rat aortic rings contraction as compared to the basal control values (59.7±3.8, 62.0±3.9 and 62.9±3.9%, respectively vs. 62.8±3.9%; p>0.05) (Fig. 3).
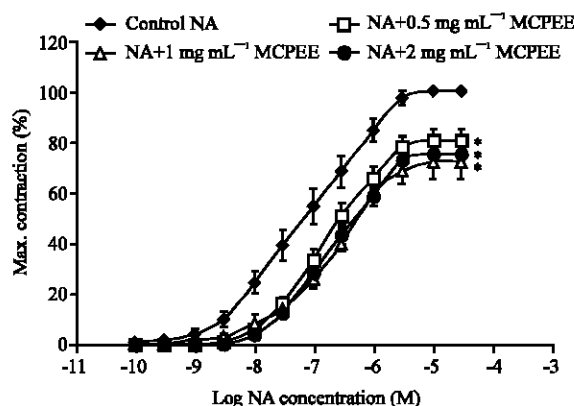

Fig. 4: The effect of *M. cochinchinensis* petroleum ether extract (MCPEE) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). *p<0.05: Significant difference from NA alone at corresponding concentrations. Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test

Petroleum ether extract: Independent of the tissue bath concentration (0.5, 1 and 2 mg mL^{-1}), all the concentrations of *M. cochinchinensis* petroleum ether extract (MCPEE) showed a significant reduction in NA-induced contraction of isolated rat aortic rings as compared to the control (34.4±3.5, 30.3±3.1 and 30.8±3.3%, respectively vs. 48.7±4.1%; p<0.05); yet, such pattern of inhibition was found to be concentration-independent (Fig. 4).

Chloroform extract: Similar to the action of petroleum ether extracts, all the concentrations (0.5, 1 and 2 mg mL^{-1}) of *M. cochinchinensis* chloroform extract (MCCE) showed a significant concentration-independent reduction in NA-induced aortic rings contraction when compared to the control (25.5±2.6, 27.0±3.0 and 42.3±4.0%, respectively vs. 50.3±3.9%; p<0.05) (Fig. 5).

Methanol extract: Although, a tissue bath concentration of 1 mg mL^{-1} methanol extract showed insignificant drop in NA vasoconstricting effect as compared to the control (52.7±5.1 vs. 54.8±3.8%; p>0.05), concentrations of 0.5 and 2 mg mL^{-1} of *M. cochinchinensis* methanol extract (MCME) demonstrated significant attenuation in this effect (42.3±5.0 and 22.0±2.9%, respectively vs. 54.8±3.8%, p<0.05) (Fig. 6).

Water extract: Only a concentration of 2 mg mL^{-1} of *M. cochinchinensis* Water Extract (MCWE) showed a
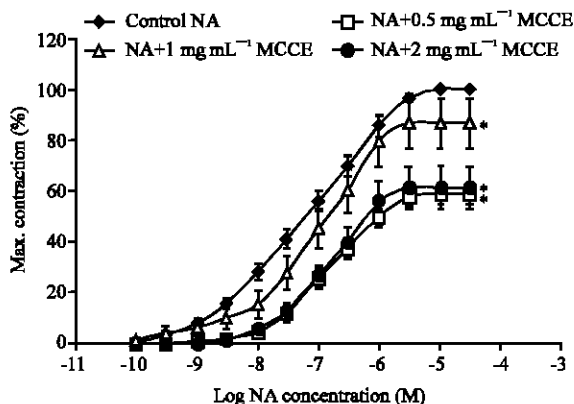
Fig. 5: The effect of *M. cochinchinensis* Chloroform Extract (MCCE) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). *p<0.05: significant difference from NA alone at corresponding concentrations. Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test

Fig. 6: The effect of *M. cochinchinensis* Methanol Extract (MCME) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). *p<0.05: significant difference from NA alone at corresponding concentrations. Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test

highly significant reduction in NA-induced contraction of rat aortic rings as compared to the control (7.9±1.7 vs. 43.2±4.1%; p<0.05). A concentration of 0.5 mg mL^{-1}, on the other hand, significantly augmented aortic rings
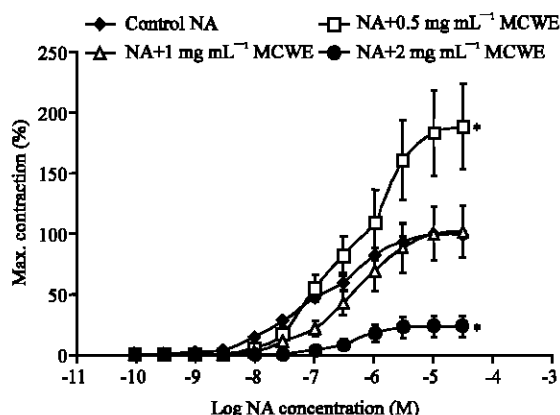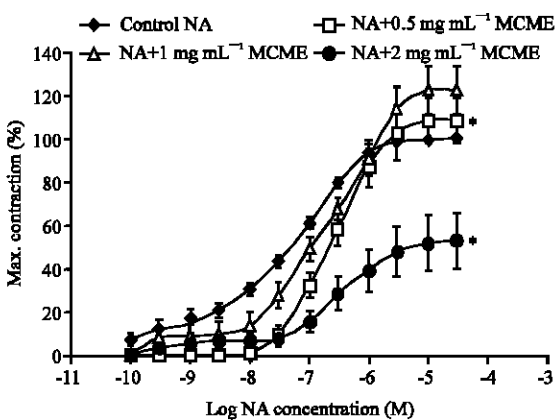

Fig. 7: The effect of *M. cochinchinensis* Water Extract (MCWE) on the maximum contractile response in NA-induced vascular smooth muscle contraction of isolated rat aortic rings preparations. Data presented as Mean±SEM (n = 8). *Indicates p<0.05: significant difference from NA alone at corresponding concentrations. Data were analyzed by two-way ANOVA followed by Bonferonni/Dunnett post-hoc test

contraction in response to NA (66.0±9.1% vs. 43.2±4.1%; p<0.05). No significant change in the action of NA on aortic rings was observed with a concentration of 1 mg mL^{-1} MCWE when compared to the basal control values (36.0±5.2 vs. 43.2±4.1%; p>0.05) (Fig. 7).

R_{max} and EC_{50} values: The results clearly showed a significant (p<0.05) concentration-independent drop in the maximal contraction at all tissue bath concentrations of MCPEE. MCCE displayed a significant (p<0.05) reduction in the R_{max} value at tissue bath concentrations of 0.5 and 1 mg mL^{-1}. In contrast, only a tissue bath concentration of 2 mg mL^{-1} of either MCME or MCWE showed a significant (p<0.05) decrease in the R_{max} value (Table 1).

Phenolic content of the extracts: The total phenolic contents of *M. cochinchinensis* petroleum ether, chloroform, methanol and water extracts were 1.1, 2.9, 23.8 and 21.7%, respectively.

HPLC profiles of the extracts: HPLC chromatograms for *M. cochinchinensis* extracts are shown in (Fig. 8). With all the extracts, absorbance reached maximum at a detection wave length of 210 nm.

IR spectra of the extracts: IR spectra of the petroleum ether, chloroform extract and methanol extracts showed
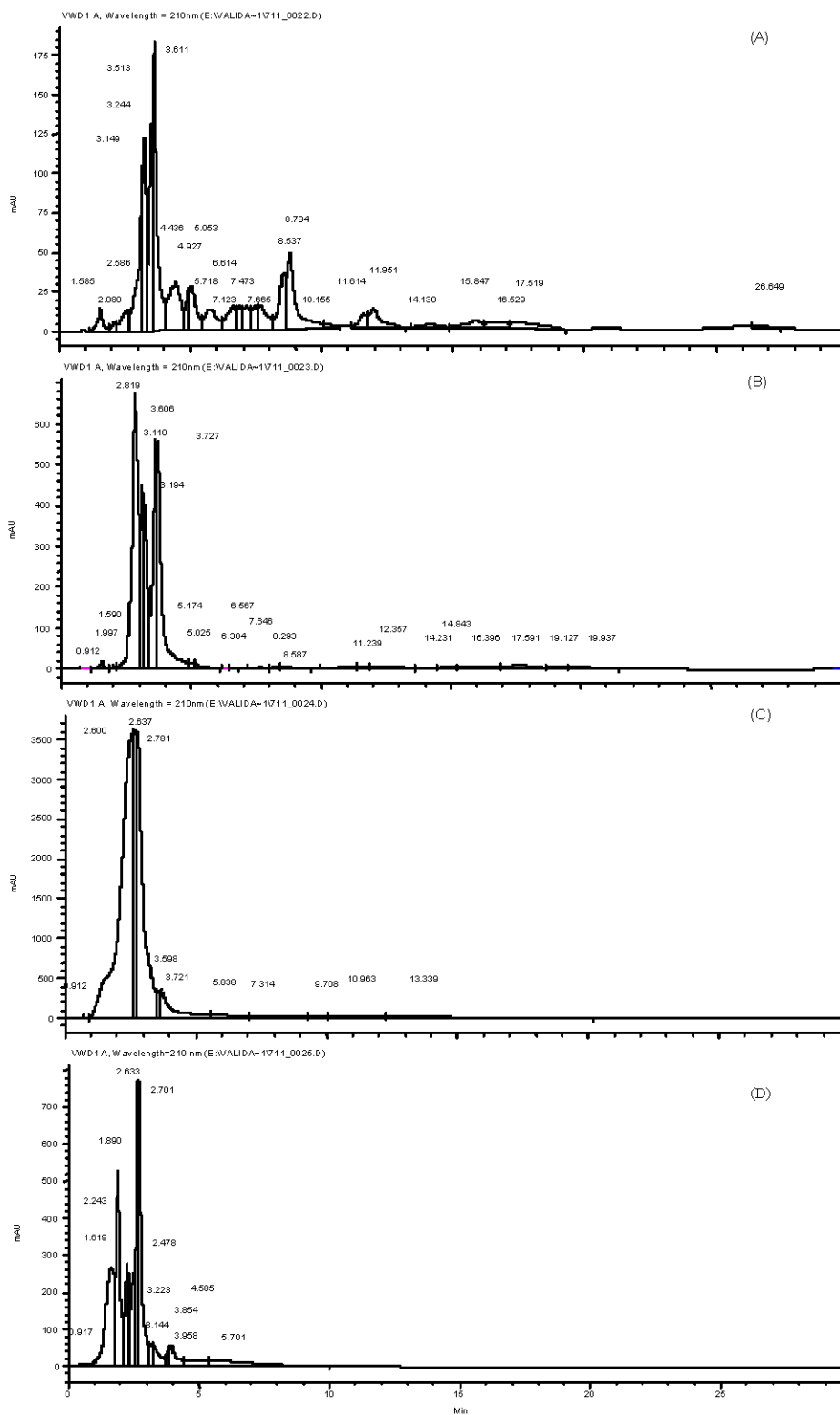
Fig. 8: HPLC chromatograms of *M. cochinchinensis* (A) petroleum ether extract, (B) chloroform extract, (C) methanol extract and (D) water extract

Table 1: Maximal contractile responses (R_{max}) and median effective concentrations (EC_{50}) for noradrenaline (NA) in isolated Sprague Dawley (SD) aortic rings in the presence of different tissue bath concentrations of *M. cochinchinensis* extracts

MC extract	Bath concentration (mg mL^{-1})	EC_{50} (mg mL^{-1})	R_{max} (%)
MCPEE	Control	7.40E-08	100.0±0.0
	0.5	1.56E-07	80.7±4.2*
	1	2.28E-07	71.9±6.6*
	2	2.00E-07	75.3±4.5*
MCCE	Control	6.47E-08	100.0±0.0
	0.5	1.43E-07	58.5±3.9*
	1	1.41E-07	61.0±8.5*
	2	9.35E-08	86.4±9.7
MCME	Control	6.10E-08	100.0±0.0
	0.5	2.52E-07	108.2±10.3
	1	2.07E-07	122.1±11.1
	2	2.56E-07	52.7±12.9*
MCWE	Control	1.85E-07	100.0±0.0
	0.5	5.20E-07	186.5±35.0*
	1	4.47E-07	100.2±4.5
	2	4.60E-07	22.4±8.7*

MC: *Macrosolen cochinchinensis*, MCPEE: *Macrosolen cochinchinensis* petroleum ether extract, MCCE: *Macrosolen cochinchinensis* chloroform extract, MCME: *Macrosolen cochinchinensis* methanol extract, MCWE: *Macrosolen cochinchinensis* water extract. EC_{50} was obtained from the concentration-response curve of *M. cochinchinensis* extracts and was taken as the concentration required to elicit 50% drop in the maximum contraction of the respective agonist. R_{max} values represent Mean±SEM of 8 determinations. *$p<0.05$ vs. control (NA alone)

the presence of hydroxyl group at 3400 cm^{-1}, ester carbonyl at 1720 cm^{-1} and phenyl ring at 1460 cm^{-1}. However, these groups were missing in the water extract spectrum.

DISCUSSION

Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap, available and their use depends on ancestral experience (Amos, 2003; Marin-Bettulo, 1980). Medicinal plants represent a great deal of untapped reservoir of drugs and the structural diversity of their component molecules makes a valuable source of novel lead compounds (Amos, 2003; Farnsworth, 1989; Harvey, 1999). There is a growing interest in the utilization of phytoceuticals because many compounds of plant origin are known to possess important phytoceuticals or nutraceutical properties. Natural product scientists are now intensifying efforts towards scientific evaluation of medicinal plants used in traditional remedies.

Accordingly, this study aimed to investigate the vascular responsiveness of isolated rat aortic rings to various extracts obtained from *M. cochinchinensis* in an attempt to meet the challenge to explain its traditional use in hypertension.

In present experimental protocol, the concentration-response curves were constructed based on the measurement of percent change in the aortic tissue

contraction in response to the action of NA. NA is suggested as a mixed α-adrenergic agonist and is widely used to cause vasoconstriction in different vasculatures. The exogenously administered NA causes vasoconstriction that elegantly resembles the effect of endogenous one in the vasculature. It is further suggested that NA-mediated contractile responses are predominantly mediated by α$_1$-adrenoceptors (Jarajapu *et al.*, 2001; Zacharia *et al.*, 2004). NA at a concentration range of 10E-10-10E-5M has been shown to increase aortic rings contraction in a concentration-dependent manner and with a maximum response at around 10E-5M (Suvitayavat *et al.*, 2005). This action was practically utilized in our study to investigate the ability of the crude extracts to alter the response pattern to NA action on aortic rings following the incubation of the latter with the plant extract.

Initially and before commencing this experimental protocol, we endeavored to validate the effectiveness and the reproducibility of the investigational technique employed despite the abundance of information on using this method (Ameer *et al.*, 2009; Berrougui *et al.*, 2006; Fang *et al.*, 2006; Ghayur *et al.*, 2005; Gonzales *et al.*, 2000). In relation to that, a time control study was performed in which the aortic rings were subjected to repetitive exposure of cumulative concentrations of NA alone for the entire length of the actual study. Results from these experiments showed the absence of any significant alterations in the response pattern of aortic rings to NA throughout the experiments, which had successfully proven the reliability of this technique in recording the changes in response of aortic rings to various plant extracts based on the prerequisites of our experimental design.

It was also crucial to eliminate the possibility of any adverse reactions on aortic rings contraction in response to DMSO and PEG400 used to enhance the solubility profiles of both petroleum ether and chloroform extracts, respectively.

PEG is generally considered to be inert and possess a low order of toxicity in animals and humans. Administration of 0.5 g high-molecular-mass PEG kg^{-1} in the form of an aqueous solution has been found to cause no visible signs of intoxication and no mortality cases. Furthermore, a dose of 2.5 g kg^{-1} of high-molecular-weight PEG has not been shown to be lethal to rats or mice (Cherkasova *et al.*, 1977; Sheftel, 2000).

The cosolvent DMSO due to its solvent properties which are characterized by partial solubility in both aqueous and organic media (Stammati *et al.*, 1996) is also used to increase the solubility of poorly water-soluble compounds but has some irremediable toxic effects

through its interaction with the metabolism and membrane of cells resulting in severe cell damage (Brayton, 1986; Penninckx *et al.*, 1983; Violante *et al.*, 2002). Despite its toxic effect, it has been demonstrated that 5% DMSO is appropriate in chamber experiments and does not affect the membrane integrity of isolated rat tissue (Watanabe *et al.*, 2000). A publication by Violante *et al.* (2002) has further shown that DMSO can be used at higher concentrations of up to 10%.

In spite of the fact that the concentrations of PEG-400 and DMSO used were much less than the maximum safe concentrations reported in the literature, it was crucial to further ensure the lack of any adverse reactions from these chemicals on aortic rings integrity before commencing the determined experimental protocols. Based on our findings, tissue bath concentrations of as high as 1% PEG-400 and 0.2% DMSO produced no marked alterations in NA-induced aortic rings contraction which proved the absence of any toxic consequences form these chemical in the concentration range employed.

The present study revealed that *M. cochinchinensis* contains possible vasoactive constituents that might have exerted their actions through vasodilatation mechanism as almost all the extracts to a degree displayed the ability to inhibit NA-induced aortic rings contraction. Based on the polarity range of the extracting solvents (highly non-polar petroleum ether-highly polar water), these aortic-ring-relaxing components appeared to be of both lipophilic and hydrophilic nature. As compared to the petroleum ether and chloroform extracts, the highest concentrations of the water and methanol extracts of *M. cochinchinensis* showed the most potent inhibition in the aortic rings contraction in response to NA. These effects can provide evidence that the tea traditionally obtained from this plant possesses the ability to possibly lower blood pressure via vasodilatation mechanism since the hydrophilic components of the water and methanol extracts that experimentally displayed a profound drop in the aortic rings contraction pattern can most likely be obtained by the hot infusion commonly prepared by people. Despite the fact that the lowest concentration of the water extract of *M. cochinchinensis* produced an enhancement in the action of NA on aortic rings with an eventual augmented vasoconstriction, the inhibitory action on NA-induced vasoconstriction seen in all the extracts appeared to significantly predominate.

The phenolic contents of the methanol and water extracts were suggestive of the presence of polyphenolic compounds like diterpenoids. HPLC profiling of extractives suggested that polar components cover the area in the range 1.8-2.9 min. Peaks in the case of water

extract excluded the presence of flavonoids. This further suggested that peaks between 3.1-3.7 min could have been responsible for the noted activity. Thus, in the light of IR spectra and HPLC profiling at detection 210 nm one can assume that some terpenoids-related compound(s) were likely to have played a role in the good activity of petroleum ether, chloroform, methanol extracts and water extracts. The presence of high concentration of these constituents was possibly responsible for the highly significant pattern of inhibition in aortic rings contraction observed with the highest concentrations of *M. cochinchinensis* methanol and water extracts. These findings are in agreement with several studies which have shown that the terpeniod compounds bear the ability to induce vascular smooth muscle relaxation (Chattipakorn *et al.*, 2007).

In summary, polar and non-polar extracts obtained from *M. cochinchinensis* contains sufficient concentrations of active constituents that can inhibit isolated rat aortic rings contraction. The methanol and water extracts of *M. cochinchinensis* possess the most potent vasorelaxing properties at high concentrations. Vasodilatation is one of the possible mechanisms by which these extracts perform their actions. The observed actions of the extracts may possibly be due to their substantial terpenoid contents. These findings provide a preliminary rationalization for the traditional use of this plant for management of hypertensive conditions, however; further *in vivo* studies need to be carried out. Active constituents from *M. cochinchinensis* are likely to have a promising therapeutic potential. These constituents, once isolated and purified, can possibly result in a dose-dependent therapeutic action to treat elevated blood pressure in human.

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