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Blood Pressure-lowering and Vascular Modulator Effects of Acorus calamus Extract Are Mediated Through Multiple Pathways

Abdul Jabbar Shah, PhD*† and Anwarul Hassan Gilani, PhD†

Abstract: This investigation was aimed to provide a pharmacologic basis to the medicinal use of Acorus calamus in cardiovascular disorders. In normotensive anesthetized rats, crude extract of Acorus calamus and its ethylacetate and nHexane fractions caused a fall in mean arterial pressure. In rabbit aorta rings, crude extract was more potent against high K⁺ (80 mM), ethylate against phenylephrine (1 μM), whereas nHexane fraction was equipotent against both precontractions. Crude extract exhibited a vasoconstrictor effect on baseline. Pretreatment of aortic rings with crude extract and its fractions shifted Ca²⁺ concentration-response curves to the right, similar to verapamil. Crude extract and ethylacetate fraction suppressed phenylephrine peak formation in Ca²⁺-free medium. In rat aorta preparations, crude extract exhibited endothelium-independent relaxation with a vasodilatory effect against high K⁺. nHexane fraction caused an endothelium-dependent NO-nitro-L-arginine methyl ester-sensitive vasoconstrictor effect on baseline tension and partially inhibited nHexane fraction-shifted Ca²⁺ responses. Crude extract, antihypertensive, vasomodulator, Ca²⁺ antagonist, NO-mediated

(J Cardiovasc Pharmacol 2009;54:38–46)

INTRODUCTION

Acorus calamus L. (Araceae) has been known for more than 2000 years; Ibn Sina called it “waj” and Dioskurides “akopov.” A description of the plant exists in the Theatrurn Botanicum of J. Parkinson (1640) and has been listed since then in a number of Pharmacopoeias and Codices, like British Pharmaceutical Codex, 1934.¹ The plant is established in the United Kingdom as a wild plant and commonly found in moist habitats throughout North America, Europe, and Asia, particularly in the northern areas of Pakistan. It has been used medicinally for centuries as a remedy for moderate blood pressure² and used as circulatory stimulant in addition to various other medicinal uses.³

Acorus calamus has been reported for the presence of glucoside, alkaloid, and essential oil containing calamen, clamenol, calameon, asarone, and sesquiterpenes.⁴ It is also known to contain a bitter glycoside named acorine along with eugenol, pinene, and camphene.⁵

The plant has been reported to possess tranquilizing,⁶ antimicrobial,⁷ antidiarrheal,⁸ antidysslipidemic,⁹ neuroprotective,¹⁰ antioxidant,¹¹ anticholinesterase,¹² and spasmyloytic¹³ activities. In a previous study of preliminary nature,¹⁴ extract of Acorus calamus has exhibited a moderately antihypertensive effect in rats without any effort to explore the possible mechanism(s) involved. The plant is also believed to be a circulatory stimulant, but no scientific studies have been carried out in this direction. Therefore, this investigation was aimed at providing pharmacologic evidence to explore possible mechanism(s) involved in the blood pressure-lowering and vascular effects of Acorus calamus; the results showed that the plant contains blood pressure-lowering and vasomodulating effects, mediated through multiple pathways.

METHODS

Plant Materials

The rhizomes of Acorus calamus were collected in District Swat, Pakistan, in November 2004 authenticated by Assistant Professor Melhoob-ur-Rehman at the Department of Botany, Govt. PG Jehanzeb College Saidu Sharif Swat, Pakistan. A voucher specimen (A 200) was deposited at the herbarium of the same department.

Preparation of the Crude Extract and Fractionation

The plant material was shade-dried and approximately 3 kg was cleaned off adulterant and coarsely ground into powder. Approximately 2 kg of the powdered material was macerated in aqueous methanol (70%) for an initial 3 days
with occasional shaking. The material was filtered first through a muslin cloth and then through Whatman qualitative grade 1 filter. This process was repeated twice more and all the filtrate was combined and then finally evaporated on rotary evaporator at 35 to 40°C under reduced pressure of ~760 mmHg, which provided a thick, semisolid mass of dark brown color, called the crude extract (Ac.Cr), yielding approximately 10%.

Further activity-directed fractionation of the crude extract was carried out by using standard phytochemical procedures using different organic solvents. A measured amount of the crude extract (50 g) was dissolved in distilled water, which was then transferred to a separating funnel and an equal volume of nHexane was then added; the mixture was shaken vigorously regularly allowing the air to escape. The mixture was then allowed to settle, the nHexane layer was separated above, collected, and a similar procedure was repeated twice. Then all the nHexane layers were concentrated in a rotary evaporator to obtain the nHexane (Ac.nHexane) fraction. Similarly, ethylacetate (50 mL) was then added to the remaining layer and the same process was repeated as with nHexane. Finally, we obtained the ethylacetate (Ac.EtAc) fraction. The yield of both fractions was 23.6% and 30%, respectively, whereas the remaining layer was discarded.

Ac.Cr and its fractions were solubilized in distilled water and 10% DMSO, respectively, and dilutions were made fresh in normal saline for use in the in vivo and in vitro studies on the day of the experiments.

**Drugs and Standards**

The following reference chemicals were obtained from the sources specified: acetylcholine chloride, atropine phosphate, caffeine, ryanodine, potassium chloride, indomethacin, phenylephrine hydrochloride, norepinephrine bitartrate, N-nitro-l-arginine methyl ester (l-NAME) hydrochloride, verapamil hydrochloride, and ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (Sigma Chemical Company, St. Louis, MO). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made fresh in distilled water/suitable solvent and the dilutions were made in normal saline for use in the in vivo and in vitro studies on the day of the experiments.

**Animals**

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council and approved by the Ethical Committee of Aga Khan University Karachi. Local rabbits (1–1.5 kg; n = 20) and male Sprague-Dawley rats (200–250 g; n = 50) of either sex used in the study were bred and housed in the animal house of Aga Khan University under a controlled environment (23–25°C). Animals were given tap water ad libitum and a standard diet.

**In Vivo Blood Pressure Measurement in Normotensive Anesthetized Rats**

These experiments were performed on male Sprague-Dawley rats (200–250 g; n = 50) as described. Animals were anesthetized with an intraperitoneal injection of sodium thiopental (pentothal, 80–100 mg/kg) fixed in a supine position on a dissecting table; a small midtracheal incision (approximately 1 cm) was made to expose trachea, right jugular vein, and left carotid artery. The trachea was cannulated with a polyethylene tubing PE-20 and cleaned from time to time to maintain the spontaneous respiration. The right jugular vein was cannulated with a polyethylene tubing PE-50 to facilitate the intravenous injections of the standard drugs and test materials. The left carotid artery was cannulated with similar tubing filled with heparinized saline (60 IU/mL) and connected to a pressure transducer P-23 XL coupled with a Grass Model 7 Polygraph, (Instruments Co Quincy, MA). This connection was used for blood pressure recording. The exposed surface for the cannulation was covered with a piece of tissue paper moistened in warm saline. Rats were injected with heparinized saline (0.1 mL) to prevent blood clotting. Body temperature of the animal was maintained by using an overhead lamp.

**Experimental Protocol**

After 20 to 30 minutes of the equilibrium period, acetylcholine and norepinephrine were used to check the stability of the animals toward hypotensive and hypertensive responses, respectively. Acetylcholine (1 μg/kg) in a volume of 0.1 mL was slowly administered followed by a flush of 0.1 mL saline, which caused a fall in blood pressure. After approximately 5 to 10 minutes, when the normal pattern of blood pressure was resumed, norepinephrine (1 μg/kg) was slowly administered followed by a flush of 0.1 mL saline, which caused an increase in blood pressure. After resuming the normal pattern of blood pressure, rats were then injected intravenously with 0.1 mL saline or with the same volume of test substances. The mean arterial pressure (MAP) was allowed to return to the resting level between injections. Drugs, extract, and their fractions were then administered intravenously and followed by a flushed with 0.1 mL saline. Changes in MAP were recognized as the difference between the steady-state values before and the lowest readings after injection. The MAP was calculated as the diastolic blood pressure (BP) plus one third pulse width (systolic BP–diastolic BP).

**Isolated Tissue Preparations**

**Rabbit Thoracic Aorta**

As described previously, rabbits were killed by a blow on the back of head; the thoracic aorta was removed and cut into rings of approximately 2 to 3 mm width. Aortic rings were suspended between a pair of stainless steel hooks in 5-mL organ baths, one hook was anchored to a steel rod at the bottom and the other was attached to a force transducer (Fort-100, WPI, UK). The tissues were suspended in normal Kreb’s solution, maintained at 37°C, and continuously bubbled with 5% CO₂ in O₂ (carbogen). The composition of Kreb’s solution was as follows (mM): NaCl 118.2, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 1.3, MgSO₄ 1.2, and glucose 11.7 (pH 7.4). A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 hour was allowed before studying the effect of test materials. Phenylephrine (PE; 1 μM) was used to stabilize the preparations. Changes in isometric tension were recorded and analyzed through a force transducer (Fort-100) coupled with a bridge amplifier (Transbridge TBM4) and PowerLab (ML845) data acquisition system (ADInstruments, Sydney, Australia).
Effect on Contraction Induced by Phenylephrine and High K⁺

The protocol of Chan et al.²⁰ was followed with some modifications. Phenylephrine (1 μM) or high K⁺ (80 mM) was used to induce steady-state contractions. The plant extract and its fractions were added cumulatively to obtain concentration response relationship and the relaxation was expressed as percent of agonist-induced contractions. Vascular reactivity of the extract was evaluated on Ca²⁺ influx either through voltage-dependent (VDCs) or receptor-operated Ca²⁺ channels (ROCs) and Ca²⁺ release from internal store(s).

Determination of Calcium Channel Blocking Activity

In the first set of experiments, an attempt was made to see if the relaxation induced by the extract involved Ca²⁺ influx through VDCs. Aortic rings were washed four to five times with Ca²⁺-free solution before the construction of control CRCs of Ca²⁺ (as CaCl₂). When the control CRCs of Ca²⁺ were found superimposable (usually after two cycles), then tissue was pretreated with the plant extract for 30 to 45 minutes to test the possible calcium channel blocking effect. A parallel control was also run under similar experimental conditions.

A second set of experiments was used to elucidate whether Ac.Cr and its fractions induce relaxation through ROCs. Aortic rings were washed four to five times with normal Kreb’s solution and the effect of Ac.Cr and its fractions was determined on PE (1 μM)-induced sustained contractions.

Effect on Intracellular Ca²⁺ Stores and Baseline Tension

In a set of experiments, the aim was to clarify whether the relaxation induced by Ac.Cr and its fractions is related to inhibition of intracellular Ca²⁺. The rings were exposed to Ca²⁺-free solution for 15 minutes before the application of PE (1 μM) to induce the first transient contraction. The composition of Ca²⁺-free/EGTA Kreb’s solution was (mM): NaCl 118.2, NaHCO₃ 25.0, KCl 4.7, KH₂PO₄ 1.3, MgSO₄ 1.2, EGTA (0.05 mM), and glucose 11.7 (pH 7.4). The rings were then washed three times with normal Kreb’s solution and incubated for at least 40 minutes for refilling of the intracellular stores. Subsequently, the medium was rapidly replaced with Ca²⁺-free solution and the rings were incubated for another 15 minutes. The second contraction was then induced by PE (1 μM) in the presence of Ac.Cr and its fractions (mg/mL), which were added cumulatively to obtain concentration response relationship and the relaxation was expressed as percent of PE (1 μM)-induced sustained contractions. Finally, both contractions were compared.

For the study of vasoconstrictor effect of the test materials, PE (1 μM) was used as the positive control and the underlying mechanism was studied in the presence of phentolamine.

Rat Aorta Preparation

The procedure of Furchgott and Zawadski²¹ was followed with some modifications. Thoracic aorta was isolated from Sprague-Dawley rats. Care was taken to avoid any damage to the endothelium. The aorta was then transferred into the Kreb’s solution aerated with carbogen. It was then carefully cleaned off fats and other connective tissues and then made into rings 2 to 3 mm wide. In some rings, the endothelium was deliberately removed by gentle rubbing of the intimal surface with forceps. The rings with intact endothelium that produced less than 20% relaxation in response to acetylcholine (1 μM) were discarded. Individual rings were suspended in 5 mL tissues baths at 37°C bubbled with carbogen. A preload of 1 g was applied to each preparation and incubated for 30 minutes. Changes in isometric tension were recorded and analyzed through a force transducer (Fort-100) coupled with a bridge amplifier (Trans-bridge TBM4) and PowerLab (ML845) data acquisition system (ADInstruments).

Endothelium-Dependent and Independent Effects

A series of experiments was conducted to assess endothelium-dependent or independent effects of Ac.Cr and its fractions on isolated aortic rings. When the tension was at resting state or reached a plateau induced by PE (1 μM), Ac.Cr and its fractions (mg/mL) were cumulatively added into the organ bath. The rings with intact or denuded endothelium were always tested in parallel. To determine the underlying mechanisms, endothelium-intact rings were incubated with L-NAME (10 μM), atropine (1 μM), and indomethacin (1 μM) for 30 minutes before the addition of PE. The test material was then added cumulatively and the CRCs were constructed for the inhibitory responses.

High K⁺ (80 mM) was also used to depolarize the tissue, which produced sustained contractions, which allowed studying the effect on the VDCs. The test material was then added cumulatively and relaxation was expressed as the percent of the contractions induced by K⁺.

Effect on Baseline Tension

Preparations both with the endothelium intact and denuded were suspended in the organ bath. Tissues were stabilized with PE (1 μM) in both Ca²⁺-free and normal Kreb’s solutions. At a steady-state baseline, the effect of Ac.Cr and its fractions were evaluated and expressed as percent of PE (1 μM)-induced contractions. To determine the underlying mechanisms responsible for the contractile effect on the baseline, preparations were serially washed and equilibrated for 60 minutes in normal and Ca²⁺-free Kreb’s solutions and then the effect of Ac.Cr and its fractions was tested. A time control (same protocol as stated previously except for the omission of Ac.Cr and its fractions to the second application of PE or caffeine) was always run in parallel. After the initial control response in Ca²⁺-free medium and before the second test contraction (to caffeine), a high K⁺-induced contraction in normal Kreb’s solution was obtained to refill the Ca²⁺ stores. Control CRCs of Ac.Cr and its fractions were constructed in the absence and presence of ryanodine and were compared with caffeine (5–15 mM).

Statistics

All the data are expressed are mean ± standard error of the mean (SEM), and the median effective concentrations (EC₅₀ values) are given with 95% confidence intervals. The
statistical parameter applied is the Student t test with $P < 0.05$
noted as significantly different.

### RESULTS

#### Effect on Blood Pressure in Normotensive Anesthetized Rats

Before the administration of the crude extract and fractions of *Acorus calamus* and standard drugs such as acetylcholine and norepinephrine were used, it caused a fall and rise in MAP, respectively (Fig. 1A–B). In normotensive rats under anesthesia, intravenous administration of Ac.Cr caused a fall in MAP (Fig. 1A). The percent fall in MAP at the respective doses of 10, 30, and 50 mg/kg was $18.86 \pm 0.48$, $27.50 \pm 0.97$, and $42.25 \pm 1.0$ (Fig. 1C) and was statistically different ($P < 0.05$) at doses of 30 and 50 mg/kg in comparison to 10 mg/kg.

The Ac.EtAc, at similar doses, caused a fall in MAP with percent fall of $12.46 \pm 0.43$, $23.20 \pm 0.64$, and $34.45 \pm 0.70$ at the respective doses of 10, 30, and 50 mg/kg (Fig. 1D). Similarly, Ac.nHexane also caused a fall in MAP at similar doses with percent fall of $14.33 \pm 0.36$, $18.57 \pm 0.47$, and $23.05 \pm 0.56$, respectively (Fig. 1E). The percent fall in each case was statistically different ($P < 0.05$) at doses of 30 and 50 mg/kg in comparison to 10 mg/kg.

#### Effect on Isolated Rabbit Aorta

Phenylephrine (1 $\mu$M) and high K$^+$ (80 mM) produced steady-state contractions in isolated rabbit aortic rings, in which a cumulative addition of Ac.Cr induced a vasodilator effect against both spasmogens with respective EC$_{50}$ values of $0.24 (0.12–0.48)$ and $0.04$ mg/mL ($0.02–0.10$) (Fig. 2A). Pretreatment of the tissues with Ac.Cr ($0.03–0.1$ mg/mL) shifted the Ca$^{2+}$ CRCs to the right (Fig. 2E) constructed in Ca$^{2+}$-free medium, similar to that caused by verapamil (Fig. 2H), which was also shown to be more potent in inhibiting high K$^+$ than PE precontractions (Fig. 2D).

Unlike the parent crude extract, Ac.EtAc was comparatively more potent in inhibiting PE than high K$^+$ precontractions with EC$_{50}$ values of $0.05 (0.03–0.07)$ and $0.54$ mg/mL ($0.36–0.79$), respectively (Fig. 2B) as well as shifted the Ca$^{2+}$ CRCs to the right (Fig. 2F) constructed in Ca$^{2+}$-free medium.

The Ac.nHexane, unlike Ac.Cr and Ac.EtAc, was equipotent against both PE and high K$^+$ precontractions with
EC$_{50}$ values of 0.26 (0.18–0.40) and 0.27 mg/mL (0.19–0.41), respectively (Fig. 2C). Pretreatment of the tissues with Ac.nHexane caused rightward displacement of the Ca$^{2+}$ CRCs (Fig. 2G).

When tested on the baseline tension, only the crude extract caused a mild contractile effect (Fig. 2A), which remained unchanged in the presence of phentolamine (data not shown).

In a series of experiments designed to show the effect of Ac.Cr on the transient contractile response induced by PE (1 μM), pretreatment of the tissues with Ac.Cr (0.1–1 mg/mL) suppressed the PE peak formation (Fig. 3A–B) in Ca$^{2+}$-free medium, similar to that caused by verapamil (Fig. 3C). The Ac.EtAc (0.3–1 mg/mL) pretreatment also suppressed the transient contractile response of PE (Fig. 3B), whereas Ac.nHexane was without effect (data not shown).

**Effect on Isolated Rat Aorta Preparations**

**Endothelium-Dependent and Independent Effects**

When tested on the PE (1 μM)-induced contractions in the isolated aortic rings with intact and denuded endothelium, cumulative addition of Ac.Cr (0.1–3 mg/mL) induced an endothelium-independent vasodilator effect with EC$_{50}$ values of 1.41 (0.75–2.52) and 2.80 mg/mL (2.05–5.33), respectively (Fig. 4A), similar to verapamil (Fig. 4D).

When tested against PE-induced contractions in isolated aorta rings with intact endothelium, Ac.EtAc caused a dual response, a strong vasoconstrictor effect at lower concentrations (0.03–1 mg/mL) followed by a relaxant effect at next higher concentrations (3–10 mg/mL). In the denuded aortic rings, the contractile effect of Ac.EtAc was abolished and the tissue got relaxed at lower concentration and shifted the CRC to the left (Fig. 4B).

In aorta rings with intact endothelium precontracted with PE (1 μM), cumulative addition of Ac.nHexane induced slowly developing relaxation to a maximum at the concentration range of 0.003 to 0.1 mg/mL. Pretreatment of intact aortic rings with l-NAME (10 μM) inhibited the effect of Ac.nHexane without modifying the maximum effect (Fig. 4C). The respective EC$_{50}$ values of Ac.nHexane in the absence and presence of l-NAME were 0.02 (0.01–0.03) and 0.48 mg/mL (0.39–0.51). The relaxant effect of Ac.nHexane was not affected by indomethacin (1 μM) or atropine (1 μM) pretreatment (data not shown). In aorta rings with denuded endothelium precontracted with PE (1 μM), the induced relaxation of Ac.nHexane was blocked at lower concentrations (0.01–0.10 mg/mL) without modifying the maximum effect at higher concentrations and shifted the CRCs to the right (Fig. 4C).

When tested against high K$^{+}$ precontractions in rat aorta preparations, unlike in rabbit aorta, Ac.Cr was least potent than its ethylacetate and nHexane fractions with respective EC$_{50}$ values of 7.46 (4.20–13.28), 3.17 (1.72–5.82), and 1.25 mg/mL (1.20–3.12) as shown in Figure 4F. The induced relaxation of nHexane fraction was more potent but incomplete (Fig. 4F).

**Effect on Baseline Tension**

When tested on the resting baseline of the isolated rat aorta preparations, cumulative addition of Ac.Cr and Ac.EtAc,
in normal Ca$^{2+}$ and Ca$^{2+}$-free media, was found devoid of contractile effect at concentrations as high as 10 mg/mL. However, in the experiment with Ca$^{2+}$-free medium, Ac.nHexane (3–10 mg/mL) elicited a vasoconstrictor effect. Pretreatment of the tissues with ryanodine (10 μM) blocked the induced vasoconstrictor effect of Ac.nHexane (Fig. 4C), similar to that observed with caffeine (5–15 mM), as shown in Figure 4E.

**DISCUSSION**

The crude extract of the *Acorus calamus* and its fractions (Ac.EtAc and Ac.nHexane) were studied in normotensive anesthetized rats for their blood pressure-lowering effect and were found active, in which the parent extract was slightly more effective than the fractions. Isolated vascular preparations were used for studying the possible mechanism of a blood pressure-lowering effect, because the interference resulting from intact reflexes is not the problem.

When added cumulatively to the aortic rings precontracted with PE or high K$^+$, Ac.Cr was more potent against K$^+$ precontractions than PE, a typical characteristic of calcium channel blockers (CCB) as shared here by verapamil, a standard calcium channel blocker. The CCB effect was further confirmed when pretreatment of the tissues with Ac.Cr caused a rightward shift in the Ca$^{2+}$ CRCs constructed in the Ca$^{2+}$-free medium, similar to verapamil. These data indicate that Ac.Cr mediates its vasodilator effect through inhibition of Ca$^{2+}$ influx through VDCs. Influx of extracellular Ca$^{2+}$ through VDCs and ROCs and release of Ca$^{2+}$ from sarcoplasmic reticulum is caused by high K$^+$ and PE, respectively, resulting in increased intracellular Ca$^{2+}$, which causes contraction. The relaxation of PE precontractions by Ac.Cr also reflects inhibitory effect on Ca$^{2+}$ movements through ROCs and/or release from the internal store(s). Interestingly, pretreatment of the tissues with Ac.Cr caused concentration-dependent inhibition of the PE peak formation in Ca$^{2+}$-free medium, similar to verapamil indicating its inhibitory effect on the release of Ca$^{2+}$ from the internal store(s) as well.

Unlike Ac.Cr, Ac.EtAc was relatively more potent against PE than K$^+$ precontractions, suggesting preferential inhibitory effect on the Ca$^{2+}$ movements through ROCs and release from the internal store(s). When tested on the initial peaks formation of PE in Ca$^{2+}$-free medium, pretreatment of the tissues with Ac.EtAc caused a concentration-dependent inhibitory effect, indicating its inhibitory effect on the release of Ca$^{2+}$ from internal store(s). The inhibitory effect of Ac.EtAc against high K$^+$-induced contractions also indicates CCB activity, which was further confirmed when pretreatment of the

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**FIGURE 3.** Typical tracing showing inhibitory effect of (A) increasing concentrations of the crude extract of *Acorus calamus* (Ac.Cr) on the initial peak formation of phenylephrine-induced contractions in Ca$^{2+}$-free medium. (B) The combined effect of Ac.Cr and its ethylacetate (Ac.EtAc) fraction. (C) The effect of verapamil in isolated rabbit aorta preparations. Values shown are mean ± SEM. Five to six determinations.
tissues with Ac.EtAc caused a rightward shift in the Ca$^{2+}$ CRCs. However, Ac.nHexane was found equipotent against PE and high K$^+$ precontractions as well as pretreatment of the tissues with Ac.nHexane caused a rightward shift in the Ca$^{2+}$ CRCs but was without an inhibitory effect on the initial PE peak formation in Ca$^{2+}$-free media. These data indicate that Ac.nHexane possesses a CCB effect on Ca$^{2+}$ influx through VDCs but lacks an inhibitory effect on intracellular Ca$^{2+}$ stores.

The Ac.Cr and its fractions were further studied for their possible effect on baseline tension. When added cumulatively on steady-state baseline, Ac.Cr caused a vasoconstrictor effect, which remained unchanged in the presence of phentolamine, an α-adrenoceptor antagonist, ruling out the involvement of α-adrenoceptor stimulation. Under similar experimental conditions, the fractions were without a contractile effect on the baseline tension, which indicates that apparently both the fractions are devoid of such effect, although an endothelial-dependent effect, if any, cannot be ruled out.

In isolated rat aorta preparations, cumulative addition of Ac.Cr caused endothelium-independent inhibition of the PE precontractions while inhibiting high K$^+$ at distinctly higher concentrations. The Ac.Cr was found approximately 186 times less potent in rat aorta than in rabbit aorta in inhibiting K$^+$ precontractions indicating species selectivity for its vasodilator effect, which may be the result of the heterogeneous nature of VDCs in different species. Similarly, when tested on baseline tension in rat aorta preparations, Ac.Cr, unlike in the rabbit aorta, was without a vasoconstrictor effect, showing species selectivity also for its vasoconstrictor effect.

In isolated rat aortic rings with intact endothelium, Ac.EtAc exhibited a strong vasoconstrictor effect on PE precontractions at lower concentrations followed by an inhibitory effect at next higher concentrations. In denuded
aortic rings, the contractile effect of Ac.EtAc was abolished and the tissues became relaxed at lower concentrations, indicating an endothelium-dependent vasoconstrictor effect. When tested against high K+ precontractions, Ac.EtAc caused an inhibitory effect at higher concentrations, which can possibly explain its endothelium-independent effect mediated possibly through inhibition of VDCs. This less potency for vasodilator effect may be the result of the presence of a contractile effect seen at lower concentrations possibly interfering with the relaxant effect at similar concentrations.

With the discovery of nitric oxide (NO), the obligatory role of endothelial cells in the relaxation of arteries has been well established.\textsuperscript{27,28} NO is a potent vasodilator synthesized in the endothelium\textsuperscript{29} by NO synthase and causes vascular relaxation.\textsuperscript{30} The results from the present study show that Ac.nHexane, concentration-dependently relaxed PE precontractions in rings with endothelium intact or denuded but with greater potency in the endothelium-intact aorta, indicating some role of endothelium-derived relaxing factors such as NO. Pretreatment of intact aortic rings with L-NAME, an inhibitor of NO synthase,\textsuperscript{31} reduced Ac.nHexane-induced relaxation to such an extent that it was similar to the relaxation in the denuded rings. Endothelium-dependent vascular relaxation can also be mediated by prostaglandin I\textsubscript{2} (PGI\textsubscript{2}) of endothelium origin\textsuperscript{32} or acetylcholine.\textsuperscript{21} In the presence of indomethacin (1 \textmu M), an inhibitor of PGI\textsubscript{2} (Moncada et al, 1978) or atropine (1 \textmu M), a muscarinic receptor blocker,\textsuperscript{33,34} the relaxant effect of Ac.nHexane remained unaltered, which indicates that the endothelium-dependent relaxant effect of Ac.nHexane is not mediated through prostaglandin or cholinergic pathways and the endothelial-derived NO, possibly explains partly its relaxant effect.

When tested against high K+-induced contractions, Ac.nHexane caused incomplete inhibition of the induced contractions even at highest concentrations. This may possibly be the result of the simultaneous releasing effect of Ac.nHexane on the Ca\textsuperscript{2+} stores that interfere with the relaxant effect because high K+ in the medium induces Ca\textsuperscript{2+} influx through VDCs, which further activates Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release through the ryanodine receptor.\textsuperscript{35–37} To test this speculation, Ac.nHexane was studied for its possible vasoconstrictor effect in Ca\textsuperscript{2+}-free medium. When tested on the baseline tension, Ac.nHexane exhibited a modest contractile effect. Interestingly, the contractile effect of Ac.nHexane was blocked when repeated in the presence of ryanodine similar to caffeine, an activator of the ryanodine receptor.\textsuperscript{38} These data show that Ac.nHexane exhibited a ryanodine-sensitive vasoconstrictor effect with a weak inhibitory effect on Ca\textsuperscript{2+} influx through VDCs.

The endothelium-dependent vasoconstrictor effect exhibited by the ethylacetate fraction and ryanodine-sensitive vasoconstrictive effect by the nHexane fraction of \emph{Acorus calamus} possibly provide a pharmacologic basis to its medicinal use as “circulatory stimulant.” However, the presence of a combination of vasoconstrictor and vasodilator constituents in the crude extract and its fractions appropriately explains the vascular modulating effect of the extract because the excessive vasodilatation is likely to be offset by the associated vasoconstrictor constituent(s). The presence of vasoconstrictor constituents might be the contributing factor for limiting the excess drop in BP beyond a certain limit as was the case with earlier evidence with this plant, which was shown to cause a moderate fall in BP.\textsuperscript{39} This gets further strength when the resultant two fractions were shown to be slightly less effective in lowering BP, which are shown to contain a relatively stronger vasoconstrictor effect, although with a different mode of action.

Plant-derived alkaloids have been reported to mediate vascular relaxation through multiple pathways such as inhibition of Ca\textsuperscript{2+} release from Ca\textsuperscript{2+} stores\textsuperscript{40} and the NO pathway\textsuperscript{41} in addition to many other mechanisms. Additionally, alkaloids have also been found to exhibit a vasoconstrictive\textsuperscript{42} effect mediated through the release of Ca\textsuperscript{2+} and through endothelium-dependent pathway.\textsuperscript{44} Glucosides and sesquiterpene\textsuperscript{45} are also known to possess an inhibitory effect on cardiovascular system. The current findings on the cardiovascular activities of \emph{Acorus calamus} can be possibly correlated to its chemical constituents, particularly alkaloids in addition to glucosides and sesquiterpenes, which might be responsible for the antihypertensive and vasomodulatory effects because the other chemical constituents such as acorine and asarone are not known to possess effects related to the cardiovascular system.

In summary, these data indicate that the crude extract of \emph{Acorus calamus} possesses a unique combination of constituents, relaxant effects mediated through Ca\textsuperscript{2+} antagonism and NO pathways, which possibly explain the fall in BP, whereas the presence of vasoconstrictor effects is probably meant to counteract the excessive vasodilatation. These findings possibly provide a pharmacologic base to the medicinal use of \emph{Acorus calamus} as an antihypertensive, circulatory stimulant, and vascular modulator.

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