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# Aqueous-methanolic extract of sweet flag (*Acorus calamus*) possesses cardiac depressant and endothelial-derived hyperpolarizing factor-mediated coronary vasodilator effects

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**Abstract** This investigation was aimed to probe the pharmacological base of medicinal use of *Acorus calamus* in ischemic heart diseases. Effect on heart parameters was studied in isolated rabbit heart while coronary vasodilator effect was studied in isolated bovine coronary arterial rings, suspended in tissue baths filled with Krebs solution, maintained at 37°C, aerated with carbogen and responses were measured on PowerLab data acquisition system. In Langendorff's perfused rabbit heart, the crude extract of *Acorus calamus* (Ac.Cr) at 0.01–10 mg/mL partially suppressed force of ventricular contractions (FVC), heart rate (HR) and coronary flow (CF). The ethylacetate fraction completely suppressed FVC, partially suppressed HR and CF, while the *n*Hexane fraction exhibited similar effect on FVC and HR but increased CF, similar to methacholine and arachidonic acid. In bovine coronary arterial preparations, Ac.Cr caused inhibition of U46619 (20 nM)-precontractions, which was blocked in presence of increasing concentration of K<sup>+</sup> (4.8–20 mM), tetraethylammonium (1 μM) and SKF525A (10 μM), similar to arachidonic acid and methacholine, indicating K<sup>+</sup> channels activation and possible involvement of endothelial-derived hyperpolarizing factor (EDHF). Activity-directed fractionation revealed that EDHF-mediated activity is concentrated in the *n*Hexane fraction. When tested against high K<sup>+</sup>, the ethylacetate fraction was found more potent than parent crude extract

and *n*Hexane fraction. These data indicate that Ac.Cr mediates coronary vasodilator effect primarily through EDHF, responsible for the increase in CF, while the cardiac depressant effects may be due to the presence of additional cardiac depressant constituent(s), thus provides possible mechanistic basis to its medicinal use in ischemic heart diseases.

**Keywords** *Acorus calamus* · EDHF · K<sup>+</sup> channels · Cardio-suppressant · Coronary dilator

## Introduction

*Acorus calamus* L. (Araceae), commonly known as “Sweet flag”, has been known since 2000 years, Ibn Sina called it waj and Dioskurides akopov. A description of the plant exists in the Theatrum Botanicum of J. Parkinson (1640) and it has been listed since then in a number of Pharmacopoeia and Codices, like British Pharmaceutical Codex, 1934 [1]. The plant was established in England as a wild plant, commonly found in moist habitats throughout North America, Europe and Central Asia. It has been taken over the centuries as a remedy to treat various ailments [2]. The roots and rhizomes of this plant have been used in the Ayurvedic medicine, as well in this part of the world for cardiovascular disorders, such as, ischemic heart diseases [3].

Phytochemistry of the plant revealed the presence of glucoside, alkaloid and essential oil containing calamen, calamenol, calameon, asarone and sesquiterpenes [4]. It is also known to contain a better glycoside named acorine along with eugenol, pinene and camphene [5].

The plant is reported in the past for many biological activities, including, antimicrobial [6], antidiarrhoeal [7],

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dyslipidemic [8], neuroprotective [9], antioxidant [10], anticholinesterase [11], wound healing [12] and we reported as spasmolytic [13] hypotensive and vascular modulator [14]. As an extension of our previous work [14], this investigation was undertaken to study the mechanism(s) responsible for the medicinal use of *Acorus calamus* in ischemic heart diseases by using isolated rabbit heart and bovine coronary artery. We have determined the functional nature of the coronary vascular relaxation being of endothelial origin and mediated through the endothelial-derived hyperpolarizing factor (EDHF).

## Materials and methods

### Plant materials

Rhizomes of *Acorus calamus* (3.12 kg) were collected in Swat District, KhyberPukhtonkhwa (formally, N.W.F.P.), Pakistan in November 2004 and authenticated by Assistant Professor Mr. Mehboob-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 crude extract and fractionation

The powdered material about 1.70 kg was soaked in 70% aqueous-methanol for 3 days with occasional shaking. It was then filtered through a muslin cloth and then through a Whatman qualitative grade 1 filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator at 35–40°C under reduced pressure (–760 mmHg) to a thick, semi-solid mass of dark brown color; i.e. the crude extract (Ac.Cr), yielding approximately 10%.

Activity-directed fractionation of the crude extract was carried out by standard phytochemical procedures, using different organic solvents [15–17]. A known quantity of the extract (49.44 g) was dissolved in 100 mL of distilled water. This was transferred to a separating funnel. *n*Hexane (50–70 mL) was then added. The mixture was shaken vigorously with regular allowing the air to escape out. It was kept for about 30 min to let the two layers separate. The upper layer of *n*Hexane was acquired and the same procedure was repeated twice more and all the *n*Hexane layers were collected and concentrated in a rotary evaporator to obtain the *n*Hexane fraction (23.6%). Similarly, ethylacetate fraction (30%) was also obtained.

Ac.Cr and its fractions were solubilized in distilled water and 10% dimethyl sulfoxide (DMSO), respectively, for use in the in vitro experiments.

### Drugs and standards

The following reference chemicals were obtained from the sources specified: arachidonic acid, methacholine chloride, 9-11-dideoxy-9 $\alpha$ , 11 $\alpha$  methanoepoxyprostaglandin F<sub>2 $\alpha$</sub>  solution (U46619), tetraethylammonium (TEA) bromide, and SKF525A hydrochloride (Wako Chemicals, Japan). Heparin from Leo Pharmaceutical Products, Denmark. Verapamil hydrochloride, glibenclamide, indomethacin, *N* $\omega$ -nitro-L-arginine methyl ester (L-NAME) and DMSO from Sigma Chemical Company, USA. All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water/suitable solvent and the dilutions were made fresh on the day of experiment.

### Animals

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 Aga Khan University Karachi. Local male rabbits (1–1.5 kg) used in the study were bred and housed at the Animal house of Aga Khan University under controlled environment (23–25°C). Animals were given tap water ad libitum and a standard diet.

The bovine hearts were collected from freshly slaughtered animal in normal Krebs solution and coronary vessel was excised out and trimmed from the extravascular tissues in the laboratory.

### Isolated tissue preparations

#### *Langendorff's perfused isolated rabbit heart*

As described previously [19, 20] male healthy rabbits (1–1.5 kg) were injected (i.p) heparin (5000 IU) 1 h prior of cervical dislocation and isolation of the heart. The thorax was opened by an incision and the heart was removed immediately. The pericardium and other adjacent tissues were removed quickly and aorta was cut at the point of bifurcation, so that about 1 cm of aorta remained intact. The isolated heart was immediately placed in a petri dish containing the Krebs–Henseleit buffer at 37°C and aerated with 5%CO<sub>2</sub> in O<sub>2</sub> (carbogen). The composition of Krebs–Henseleit buffer was (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.25 CaCl<sub>2</sub>, 24 NaHCO<sub>3</sub>, 1.1 KH<sub>2</sub>PO<sub>4</sub> and 10 glucose with pH 7.4.

After gentle squeezing, the heart was attached to the cannula of Langendorff's apparatus through aorta. The aerated and thermostatically maintained perfusion fluid is allowed to flow into the heart through aorta. A thread is attached through a clip to the apex of the heart and passed

over a freely moving pulley; another end of the thread was attached to the lever of the isotonic BioScience transducer, which was connected through the amplifier to the oscillograph.

### Protocol

Following cannulation, the retrograde perfusion through the aorta was allowed. Once the heart was mounted on the experimental station, perfusion pressure was adjusted to achieve the satisfactory spontaneous heart beat. Usually 10–15 min was sufficient to attain the steady state. Initially, the normal rhythmic and synchronous cardiac activity and the coronary flow (CF) were observed and recorded, which served as control. All the parameters were observed for another 20 min before employing the test sample.

Not more than one full dose–response curve was determined on one preparation. Any heart which shown abnormal pattern of the activity was discarded. In some experiments L-NAME (0.01 mM) and indomethacin (0.01 mM) were added to the buffer to avoid the influence of prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) and nitric oxide (NO) on the effect of test substances. All these antagonists were used to avoid complications in data interpretation [21].

### Dose cycle

After a control period, different doses of the crude extract of *Acorus calamus* and its fractions (mg/mL) and standard drugs were injected gently into the rubber tubing attached to the cannula at the interval of 5–10 min. Measurements were performed both in the controls and in the test. The maximal effect is taken into account during 10 min time following the administration of each dose of test sample(s). Rate of the ventricular contractions, as heart rate (HR) was measured from number of beats per min. Force of ventricular contractions (FVC) was calculated in centimeter from the baseline. CF was calculated from the total number of drops per min, as described previously [19, 20].

### Bovine coronary artery

The protocol of Campbell et al. [22] was followed with some modifications. Bovine hearts were purchased from a local slaughterhouse, and sections of the left anterior descending coronary artery were dissected and cleaned of connective tissue. The artery was cut into 2-mm-diameter rings. Arterial rings were equilibrated in normal Krebs buffer consisting of (in mM) 119 NaCl, 4.8 KCl, 2.4 NaHCO<sub>3</sub>, 3.8 CaCl<sub>2</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, 11 glucose and 0.02 EDTA at 37°C, aerated with carbogen and

equilibrated for 1.5 h. KCl (40 mM) was added to the chamber until reproducible maximal contractions were maintained. U46619 (20 nM) was used to induce sustained contraction.

### Experimental protocol

The integrity of endothelium was confirmed by relaxation to bradykinin (1 μM). In some vessels, the endothelium was deliberately removed by gently rubbing the lumen with forceps and the success of procedure was confirmed by the inability of bradykinin to induce vasodilatation [22]. The rings were suspended on a pair of stainless steel hooks in a 5 mL organ chamber. One hook was anchored to a steel rod, and the other was attached to a force transducer (Fort-100, WPI, UK). Vessels were equilibrated for 1.5 h under 2 g of resting tension. KCl (40 mM) was added until reproducible contractions were obtained. The thromboxane mimetic, U46619 (20 nM) was then administered to induce sustained contraction. Cumulative additions of the test materials along with standards, such as methacholine and arachidonic acid, were made. To establish the mechanisms of relaxation, some vessels were treated with TEA (1 μM), SKF525A (10 μM), glibenclamide (1 μM), indomethacin (1 μM) or vehicle, and the concentration–response curve was determined. Similar experiments were performed with [K<sup>+</sup>] in the Krebs solution reduced to 0 mM or increased to 4.8–20 mM. Equivalent changes in [Na<sup>+</sup>] were made to maintain isotonicity. Results were expressed as percent relaxation, with 100% relaxation representing the basal pre-U46619 tension. Changes in isometric tension were recorded and analyzed through a force transducer (Fort-100, WPI, UK), coupled with bridge amplifier (Transbridge TBM4) and PowerLab (ML845) data acquisition system (ADInstruments, Sydney, Australia).

### Effect on high K<sup>+</sup> induced contractions in bovine coronary arterial preparations

To assess the inhibitory effect of *Acorus calamus* extract on Ca<sup>2+</sup> movement through voltage dependent Ca<sup>2+</sup> channels, K<sup>+</sup>, as KCl, was used which produced sustained contractions by depolarization of the preparations [23]. The *Acorus calamus* extract and its fractions were used in a cumulative fashion, to obtain concentration-dependent inhibitory responses. The relaxation of K<sup>+</sup> (40 mM) pre-contraction was expressed as percent of the control response. Changes in isometric tension were recorded and analyzed through a force transducer (Fort-100, WPI, UK), coupled with bridge amplifier (Transbridge TBM4) and PowerLab (ML845) data acquisition system (ADInstruments, Sydney, Australia).

## Statistics

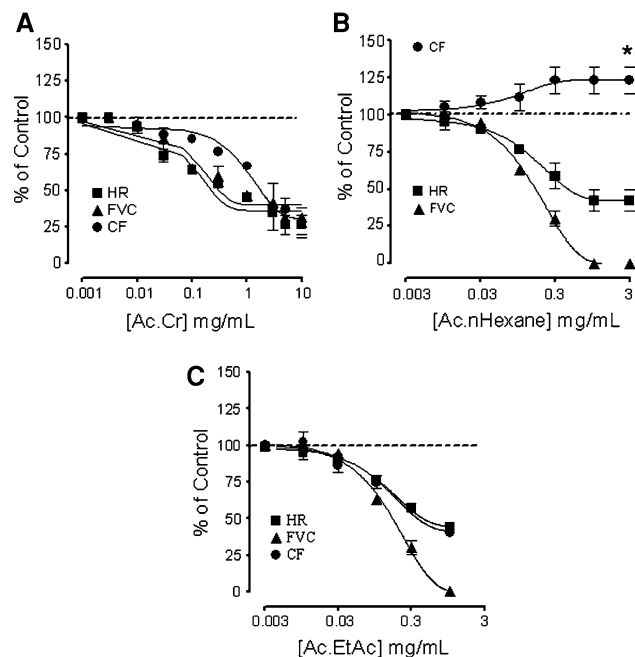
All the data expressed are mean  $\pm$  standard error of the mean (SEM), and the median effective concentrations ( $EC_{50}$  values) are given with 95% confidence intervals (CI). The statistical parameter applied is the student *t* test with  $P < 0.05$  noted as significantly different (GraphPad program, GraphPad, San Diego, CA, USA), concentration response curves were analyzed by non-linear regression (GraphPad program).

## Results

### Langendorff's experiments

In isolated perfused rabbit heart the effect of Ac.Cr and its fractions were observed on three different parameters, such as, force of ventricular contraction (FVC), HR and CF. When tested, Ac.Cr partially (55–60%) suppressed HR, FVC and CF, with respective  $EC_{50}$  values of 0.13 (0.07–0.24), 0.11 (0.06–0.19) and 1.10 mg/mL (0.52–1.80), as shown in Fig. 1a. The suppressant effect of Ac.Cr on CF was weak as compared to its effect on HR and FVC.

The Ac.*n*Hexane completely suppressed FVC, partially suppressed HR, with respective  $EC_{50}$  values of 0.17



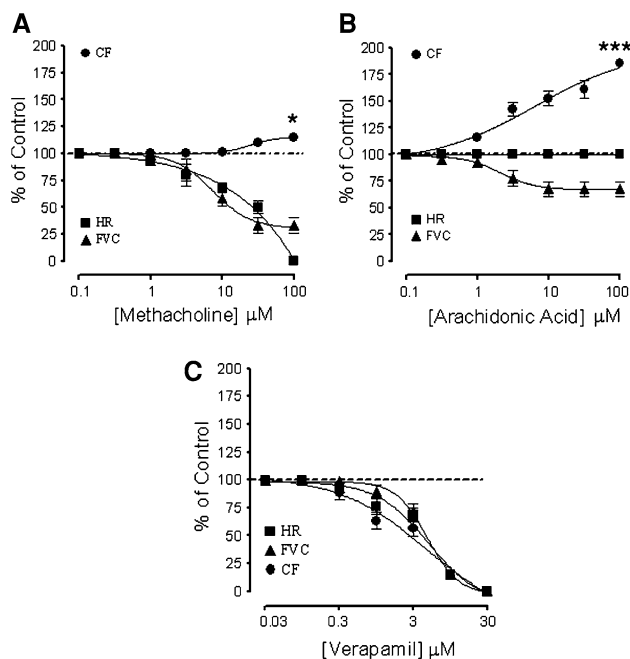
**Fig. 1** Effect of **a** the crude extract of *Acorus calamus* (Ac.Cr) and its fractions; **b** *n*Hexane (Ac.*n*Hexane) and **c** ethylacetate (Ac.EtAc) on the heart rate (HR), force of ventricular contraction (FVC) and coronary flow (CF) in isolated rabbit heart preparations in Langendorff's experiment. \* $P > 0.05$  versus base line. Values shown are mean  $\pm$  SEM ( $n = 3-4$ )

(0.13–0.22) and 0.82 mg/mL (0.43–1.55) while caused modest increase in CF (Fig. 1b). Ac.EtAc completely suppressed FVC and partially inhibited both HR and CF, with respective  $EC_{50}$  values of 0.17 (0.13–0.23), 0.70 (0.49–1.0) and 0.54 mg/mL (0.33–0.87) as shown in Fig. 1c.

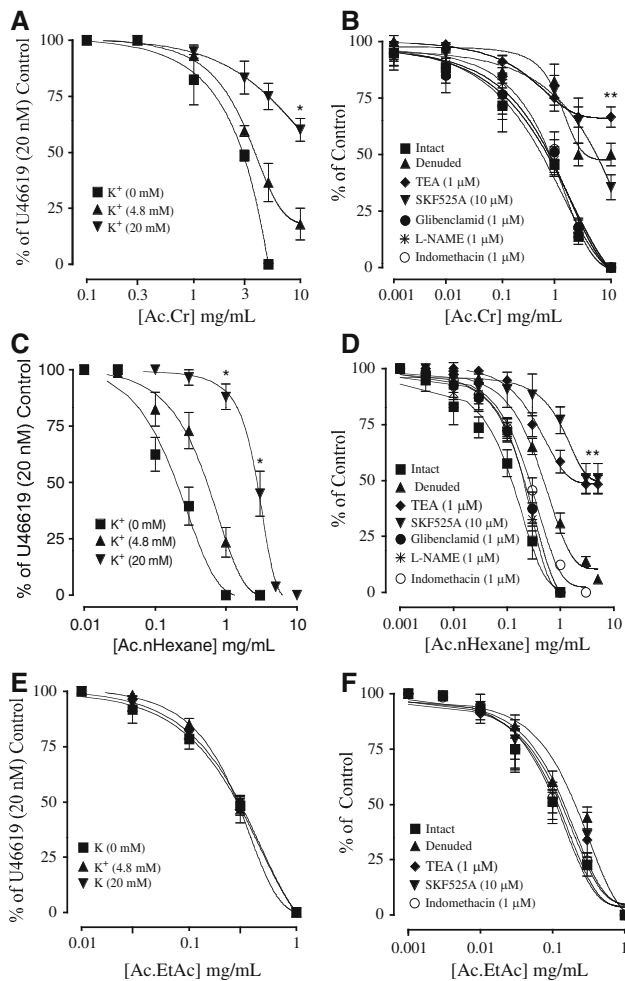
Methacholine partially inhibited FVC, completely suppressed HR while caused a modest increase in CF (Fig. 2a). Arachidonic acid exhibited weak inhibitory effect on FVC, no effect on HR, while caused a dominant increase in CF, which was about 80% of the control (Fig. 2b). Verapamil caused complete suppression of FVC, HR and CF (Fig. 2c).

### Effect on bovine coronary artery

The bovine coronary vessels that were pre-contracted with U46619 (20 nM) produced endothelium-dependent relaxation to Ac.Cr, with  $EC_{50}$  value of 2.5 mg/mL (1.8–3.11). The relaxation was inhibited in vascular ring preparations without functional endothelium (denuded) and in the presence of increasing concentrations (4.8 and 20 mM) of  $K^+$  in the buffer (Fig. 3a); similar effects were observed with methacholine (Fig. 4a) and arachidonic acid (Fig. 4c). Pretreatment of the arterial rings with TEA (1  $\mu$ M) and SKF525A (10  $\mu$ M), did not alter basal tension or contractions to U46619, however, inhibited the relaxations to



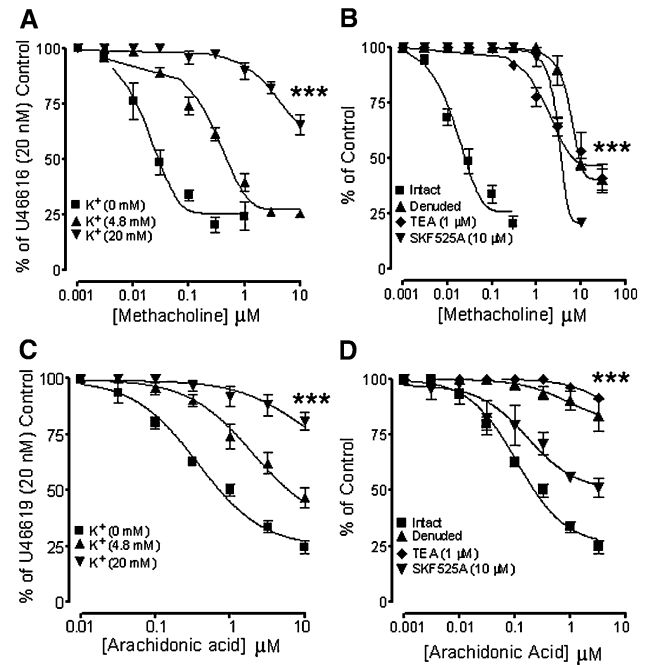
**Fig. 2** Effect of **a** methacholine, **b** arachidonic acid and **c** verapamil on the heart rate (HR), force of ventricular contraction (FVC) and coronary flow (CF) in isolated rabbit heart preparations in Langendorff's experiment. \* $P > 0.05$  versus base line; \*\*\* $P > 0.0001$  versus base line. Value shown are the mean  $\pm$  SEM ( $n = 4$ )



**Fig. 3** Isolated bovine left descending coronary arterial rings with intact and denuded endothelium were constricted with U46619 (20 nM) and cumulative effect of the crude extract of *Acorus calamus* (Ac.Cr) and its fractions was determined. **a**, **c**, and **e** depict, respectively, the effect of Ac.Cr and its *n*Hexane (Ac.*n*Hexane) and ethylacetate (Ac.EtAc) fractions in presence of varying concentrations (4.8 and 20 mM) of K<sup>+</sup>, in the Krebs’ solution. **b**, **d**, and **f** show, respectively, the effect of Ac.Cr, Ac.*n*Hexane and Ac.EtAc in the intact arterial rings in the absence and presence of TEA (1 μM), SKF525A (10 μM), glibenclamide (1 μM) and L-NAME (1 μM) and in the denuded rings preparations. \**P* > 0.05 versus base line; \*\**P* > 0.001 versus base line. Value shown are mean ± SEM (*n* = 5–10)

Ac.Cr (Fig. 3b), similar to methacholine (Fig. 4b) and arachidonic acid (Fig. 4d). The relaxant effect of Ac.Cr remained unchanged in arterial rings pretreated with glibenclamide (1 μM), L-NAME (1 μM) and indomethacin (1 μM).

Ac.*n*Hexane also caused relaxation of U46619-induced vasoconstrictions in a concentration-related manner, with EC<sub>50</sub> value of 0.21 mg/mL (0.17–0.29). The relaxation to Ac.*n*Hexane was inhibited in the presence of increasing concentrations (4.8 and 20 mM) of K<sup>+</sup> in the buffer (Fig. 3c) and rings pretreated with TEA (1 μM) and



**Fig. 4** Isolated bovine left descending coronary arteries were constricted with U46619 (20 nM) and cumulative effect of methacholine and arachidonic acid was determined in intact and denuded endothelium rings. **a** and **c** depict the effect of methacholine and arachidonic acid, respectively, in the arterial rings in presence of varying concentrations (4.8 and 20 mM) of K<sup>+</sup> in the Krebs’ solution. **b** and **d** show, respectively, the effect of methacholine and arachidonic acid in denuded and in intact arterial rings preparations in the absence and presence of TEA (1 μM) and SKF525A (10 μM). \*\*\**P* > 0.0001 versus base line. Values shown are mean ± SEM (*n* = 8–14)

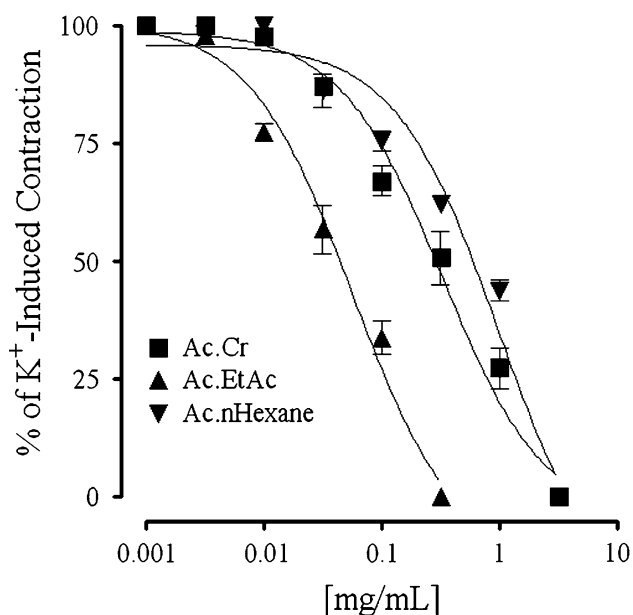
SKF525A (10 μM) (Fig. 3d) and remained unchanged in rings pretreated with glibenclamide (1 μM), L-NAME (1 μM) and indomethacin (1 μM).

Ac.EtAc also produced relaxation to U46619-induced vasoconstrictions in a concentration-related manner but the effect remained unchanged in presence of increasing concentrations of K<sup>+</sup> (4.8 and 20 mM) in the buffer (Fig. 3e) and in rings pretreated with TEA(1 μM), SKF525A(10 μM) and indomethacin (1 μM) (Fig. 3f).

#### Effect on high K<sup>+</sup> precontractions in bovine coronary artery

In isolated bovine coronary arterial rings, cumulative addition of crude extract of *Acorus calamus* caused inhibition of the high K<sup>+</sup> (20 mM) precontractions with EC<sub>50</sub> value of 0.34 mg/mL (0.28–0.49) (Fig. 5). Among the tested fractions, the ethylacetate fraction was more potent than the parent crude extract and *n*Hexane fraction, with respective EC<sub>50</sub> values of 0.06 (0.04–0.08) and 0.98 mg/mL (0.64–1.48) (Fig. 5).





**Fig. 5** Effect of the crude extract of *Acorus calamus* (Ac.Cr) and its ethylacetate (Ac.EtAc) and *n*Hexane (Ac.*n*Hexane) fractions on high  $K^+$  (20 mM)-induced contractions in isolated bovine left descending coronary arterial preparations. Values shown are mean  $\pm$  SEM ( $n = 10$ –16)

## Discussion

As an extension of a previous study [14], the crude extract and fractions of *Acorus calamus* were further studied based on its medicinal use in ischemic heart diseases [3]. Langendorff's perfused rabbit heart and isolated bovine coronary arterial preparations were used. The aim here was to study the functional nature of the pharmacological effect of Ac.Cr and its fractions on CF, as mediated through EDHF, in addition to their effect on FVC and HR. The effect of other mediators of endothelial origin, such as, NO and  $PGI_2$  were ruled out by the addition of L-NAME, a NO synthase inhibitor [24] and indomethacin, a  $PGI_2$  inhibitor [25] to the Krebs–Henseleit solution. Under these experimental conditions, Ac.Cr caused partial ( $\geq 60\%$ ) inhibition of FVC, HR and, CF. Verapamil, a standard calcium channel blocker [26], caused complete suppression. Ac.EtAc completely suppressed FVC and partially inhibited HR and CF. The *n*Hexane fraction unlike the crude extract suppressed FVC, partially inhibited HR while caused modest increase in CF, similar to methacholine and or arachidonic acid. The complete or partial suppression of the cardiac parameters by the crude extract and its fractions may involve inhibitory effect on calcium channels.

When tested in isolated bovine coronary arterial ring preparations pre-contracted with high  $K^+$  (20 mM), the ethylacetate fraction was found to be more potent than the parent crude extract and the *n*Hexane fraction thus

suggesting that the possible presence of  $Ca^{2+}$  channel blocking component observed in terms of inhibitory effect against high  $K^+$  [27], as reported in the previous study [14] is concentrated in the ethylacetate fraction. Calcium channel blockers, such as verapamil are known to possess negative inotropic and chronotropic effects [28, 29] and depress CF in isolated heart preparations, where CF is largely dependent on myocardial contractility [30], so the direct effect of such drugs on CF is not evident [31]. The partial inhibitory effect of the crude extract on cardiac parameters might be of potential clinical advantage, as complete inhibitory effect, like that of verapamil, can lead to myocardial suppression, as a side effect.

The functional nature of the effect on CF was further studied in isolated bovine coronary arterial preparation. The endothelium has emerged as an important regulator of vascular tone. Several soluble mediators released by the endothelium are involved in these vascular effects, such as prostacyclin, NO and EDHF. The EDHF release hyperpolarizes the underlying smooth muscle cells by activation of  $K^+$  channels, the most frequent being  $K_{Ca}$  channels [32]. The activity of EDHF may be distinguished from NO; EDHF activity is blocked by TEA, inhibitors of  $K_{Ca}$  [21], or by high  $K^+$  but not by L-NAME or glibenclamide, inhibitor of  $K_{ATP}$  channel [33].

When tested on the U46619, thromboxane prostanoid receptors activator [34], precontractions in bovine coronary arterial preparations with intact endothelium, Ac.Cr exhibited downstream effect, which was inhibited in presence of increasing concentrations of  $K^+$  in the buffer, similar to methacholine and arachidonic acid. Potassium causes vasodilatation at low concentrations ( $<14$  mM) through hyperpolarization and activation of  $Na^+$ – $K^+$  ATPase [34]. At higher ( $>14$  mM) concentrations  $K^+$  cause depolarization and contraction of the smooth muscle because potassium at high concentration inactivates all  $K^+$  channels [35] and allows voltage-gated calcium channels to open [27]. Thus  $K^+$  functions as an EDHF within the concentration window of 4.8–14 mM [34, 36]. The inhibition of the relaxant effect of Ac.Cr in the presence of increasing concentration of  $K^+$ , suggests that Ac.Cr mediates the downstream effect through activation of  $K_{Ca}$  and may act as EDHF. This hypothesis was further confirmed when the induced relaxation of crude extract was blocked in presence of TEA and remained unchanged in the presence of glibenclamide and L-NAME, excluding the involvement of  $K_{ATP}$  channels or NO.

Methacholine, a known EDHF releasing agent [37], is also known to stimulate the release of several arachidonic acid metabolites which further causes relaxation and hyperpolarization, the effect is sensitive to inhibitors of cytochrome  $P_{450}$ , such as, SKF525A [21]. Interestingly, the downstream effect of Ac.Cr was also attenuated when

repeated in the presence of SKF525A. This indicates that Ac.Cr caused the EDHF-mediated downstream effect possibly via the release of arachidonic acid metabolites, similar to methacholine. Arachidonic acid, in coronary endothelial cells, is known to metabolize by cytochrome P<sub>450</sub> activity to epoxyeicosatrienoic acids (EETs) [38]. Studies from a number of laboratories indicate that EETs function as an EDHF [39]. The EDHF mediated hyperpolarization and subsequent vascular relaxation is uninfluenced in the presence of L-NAME, NO synthase inhibitor or indomethacin, a cyclooxygenase inhibitor. However, the relaxation induced by arachidonic acid is blocked by inhibitors of cytochrome P<sub>450</sub> [38], as well as TEA and high K<sup>+</sup>. When tested in another set of experiment, the downstream effect of Ac.Cr remained unchanged in the presence of L-NAME and indomethacin, suggesting the release of EDHF from the endothelial cells, similar to that observed with methacholine and arachidonic acid.

Ac.nHexane caused an endothelium-dependent downstream effect against U46619-induced contractions in coronary arteries, which was attenuated in the presence of high K<sup>+</sup>, TEA and SKF525A, similar to that observed with methacholine and arachidonic acid. The Ac.nHexane was about ten times more potent in inhibiting U46619-induced contractions than the parent crude extract, which indicate that the EDHF-mediated relaxant activity is concentrated in this particular fraction.

Interestingly, the ethylacetate fraction caused inhibition of the U46619-induced contractions but the effect remained unchanged in the presence of high K<sup>+</sup>, TEA, SKF525A or indomethacin. This suggests that this effect may be due to the presence of calcium channel blocking (CCB) constituent in the extract of *Acorus calamus*, because the ethylacetate fraction was more potent in inhibiting high K<sup>+</sup> precontraction in isolated bovine coronary arterial rings than the crude extract and nHexane fraction.

Endothelium-dependent relaxation and hyperpolarization mechanism is impaired in ischemic heart diseases [40] and different K<sup>+</sup> channels have been identified in myocardial cells [41] where their activation may function as a downstream mechanism in ischemic preconditioning and reduction in myocardial ischemic injury [32]. Endothelium-dependent hyperpolarization and subsequent vascular relaxation are inhibited by blockers of K<sub>Ca</sub> channels in coronary arteries [42], suggesting that the activation of K<sub>Ca</sub> channels plays an important role in coronary vasodilatation [43]. Thus, the vascular relaxant effect of the crude extract and its nHexane fraction in coronary artery, which is supposed to be mediated via the release of EDHF, strongly support and provides pharmacological rationale to the medicinal use of *Acorus calamus* in ischemic heart diseases, though further chemical isolation and molecular

characterization, would provide more insight into the effect.

In conclusion, these data indicate that the crude extract of *Acorus calamus* possesses coronary vasodilator effect, mediated possibly through EDHF. The partial cardiac depressant effect may be of added value as complete suppression may lead to cardiac arrest. This study provides pharmacological rationale, with respect to the medicinal use of *Acorus calamus* in ischemic heart diseases.

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