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Research article

Pharmacological evaluation of *Euphorbia hirta*, *Fagonia indica* and *Capparis decidua* in hypertension through in-vivo and in vitro-assays

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ABSTRACT

Objective: This study determines the efficacy and probable underlying mode of action to the folk usage of *Euphorbia hirta, Fagonia indica* and *Capparis decidua* in hypertension.

Methods: The aqueous-methanol extracts of *E. hirta* (EH.Cr), *F. indica* (FI.Cr) and *C. decidua* (CD.Cr) were tested for antihypertensive effects in rats using *non-invasive* and *in-vasive* blood pressure measuring apparatus. *In-vitro* assays were carried out using isolated rat aortae using PowerLab station.

Results: EH.Cr, FI.Cr and CD.Cr at 500 mg/kg (orally) caused a fall in the mean systolic blood pressure in arsenic-induced hypertensive and normotensive rats, similar to nifedipine. In rat aortae, EH.Cr, CD.Cr and FI.Cr reversed low (20 mM), high (80 mM) K^+ and phenylephrine (P.E)-driven contractions, while *F. indica* partially inhibited high K^+ contractions. In the presence of TEA, *F. indica* remained unable to relax low K^+ contractions. EH.Cr and CD.Cr moved Ca^{++} concentrations response curves to the right, like nifedipine. All fractions of EH.Cr and CD.Cr except aqueous, pet-ether and chloroform fractions of FI.Cr displayed Ca^{++} antagonistic activity. FI.Cr, its ethyl acetate and aqueous fraction exhibited TEA-sensitive potassium channel activation. On baseline tension, test materials also produced phentolamine-sensitive vasospasm.

Conclusion: *E. hirta*, *F. indica* and *C. decidua* possess antihypertensive activity in arsenic-induced hypertensive rats possibly mediated *via* endothelium-dependent vasorelaxation. In normotensive rats, *E. hirta* and *C. decidua* showed antihypertensive activities through endothelium-dependent and Ca^{++} antagonistic pathways, while *F. indica* exhibited potassium channel activation and Ca^{++} antagonistic like effects in its vasorelaxation. Additional weaker vasospastic effects were derived through α-adrenergic like pathways.

1. Introduction

Among the risks factors, which lead to the mortality and disability along the globe, hypertension is one of the strongest candidates in the development of cardiovascular diseases and death. Hypertension has become the main reason of mortality in developing states, as it causes stroke, kidney impairment, atrial fibrillation, congestive heart failure and cognitive loss [1, 2]. Numerous medicines are available in market to cure hypertension, as their extended use and extra-ordinary price produces several adverse effects and results patients' poor compliance and unaffordability [3]. In this situation, herbal medicines are getting more attention as they are safe, affordable, easily accessible and durable because of the existence of dual opposing mechanisms *i.e.* inhibitory and excitatory constituents inbuilt in same herb, which advantages the remedy with less side effects [4, 5]. Many medicinal plants ameliorate the hypertension as a decoction of stem bark of *Rhaptopetalum coriaceum* oliver has been used in folk to treat hypertension [6], Rauwolfia serpentine with active agent reserpine has been widely used to treat the hypertension [7], other herbs include *Zingiber officinale*, *Uncaria rhynchophylla*, *Solanum sisymbriifolium*, *Moringa oleifera*, *Hibiscus sabdariffa* and many other plants [8].

Euphorbia hirta Linn (Euphorbiaceae) has been employed traditionally for treatment of hypertension and edema [9], diarrhea, nausea, vomiting, colic's, dysentery, constipation, intestinal worms, heartburn and

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duodenal ulcers [10]. Numerous constituents like as caffeic acid, benzoic acid, epicatechin 3-gallate acid, quercetin, kaempferol, rutin, myricitrin, β-sitosterol, β-amyrin, euphorbin-A, euphorbin-B, euphorbianin, euphorbin-C, euphorbin-D, gamma-tocopherol, leucocyanidol, camphol, palmitic acid, shikmic acid, pentadecylic acid, gallic acid, niacin, protocatechuic acid, phytol, 24-methylene-cycloartenol, heptacosane, choline, tinyatoxin, squalene, afzelin and quercitrin [11, 12, 13], while dimethoxyageratochromone, n-haxadecanoic acid, caryophyllene oxide, hexahydrofarnesyl acetone and β-caryophyllene are major components of essentials oils (EOs) from *E. hirta*. The sesquiterpenes were found to be the predominant group of ingredients in the EOs of various *Euphorbia* species like *E hirta, E convolvuloides and E heterophylla*, these EOs as natural sources of antioxidants as well as cholinesterase and tyrosinase inhibitors [14].

Numerous activities like antioxidant, anti-inflammatory and anticancer [11], antibacterial [15], antidiabetic [16], anxiolytic [17], angiotensin converting enzyme inhibitory potential [18], antispasmodic, antidiarrheal and laxative [13] has also been reported.

Fagonia indica Burm. f. (Zygophyllaceae) has been used traditionally to cure indigestion and heart problems [19], asthma, fever, anorexia, jaundice, constipation, vomiting, diarrhea, dysentery and body aches [20, 21, 22]. *F. indica* possess ursolic acid, oleanolic acid, kaempferol, quercetin, hederagenin, pinatol, nahagenin, harmine, diosgenin, kryptogenin, Genin A,-B, C, lanosterol, fagogenin, stigmasterol, harman, chinovic acid, fagonin, ascorbic acid, riboflavin, niacin, β-amyrin, β-sitosterol, lupeol and faganilin [20, 21, 22, 23]. The reports indicated that it possesses anti-oxidant, anti-inflammatory and hepato-protective [24], antifungal and antipyretic [25], analgesic and antimicrobial [26] anticancer [27] anti-sickling [21] and laxative activities [22].

Capparis decidua Edgew (Capparidaceae), conventionally engaged to treat hypertension, pile, asthma, ulcer, diarrhea, dysentery, vomiting, inflammation, diabetes, rheumatism- and various stomach problems [28, 29]. A number of bioactive constituents have been found in *C. decidua* like spermidine, n-triacontanol, isocodonocarpine, capparisinine, capparidisine, 15-N-acetyl capparisine, capparisesterpenolide, stachydrine, capparisine, codonocarpine, capparine, cappariline, capparinine, glucocapparin, kaempferol, rutin, isorhamnetin, (methyl, isopropyl, benzyl and sec-butyl isothiocyanates), tetracosanol, hexadecanol, octadecanol, β-amyrin, cycloartenol, gramisterol, citrostadienol, simiarenol, lupeol, taraxerol, N-pentacosane, stigmasterol, sitosterol, campesterol, avenasterol, δ-tocopherol, α-tocopherol, γ-tocopherol, vitamin C, lutein, β-carotene, oleic acid, linoleic acid, amino acids, sugars and different elements like calcium, phosphorous, zinc, potassium, iron and manganese [29, 30, 31]. This multi-purpose plant has its importance as source of functional food and nutrapharmaceuticals like *Capparis spinosa* L with nutraceutical potential in diseases like diabetes and hyperlipidemia [29, 32]. The geological variations also effects the composition of bioactive components as one species of capper (*Capparis spinosa* L.) has shown varying profile of bioactives and associated functionalities in different geographical locations [33, 34]. *C. decidua* showed anti-arthritis, anti--fungal and anti-bacterial [29], antimicrobial, antigiardial, antimalarial, antioxidant, anti-diabetic [35], anti-hypertensive [30], antispasmodic, antidiarrheal and laxative [31] activities. It has been reported that quercetin, kaempferol, rutin, myricitrin and β-sitosterol produce spasmolytic activity may be through calcium channel blocking type action (22, 31). Thus *E. hirta*, *F. indica* and *C. decidua* containing these ingredients, may give blood pressure lowering effects to these plants.

keeping in view the folk effects of *E. hirta*, *F. indica and C. decidua* in cardiovascular disorders like hypertension and to further study the lack in current literature for hypotensive effects of *C. decidua* as mentioned by Shah and Gilani, (2011) [30], that *C. aphylla* exhibited anti-hypertensive effects mediated *via* endothelium-dependent (atropine-sensitive NO pathway) relaxation and CCBs like constituents as vaso-relaxant. The primary aim and objective of the study is to divulge the scientific basis of these effects in cardiovascular system of these aforementioned plants by using standard pharmacological protocols *via in-vivo* and *in-vitro* confirmations/assays. Further, this project has also been extended to elaborate the proportion and efficacy of vessel relaxant constituents in polarity-based fractions of these plants.

2. Methodology

2.1. Preparation of aqueous-methanol extracts and its fractions

Whole herb of *E. hirta* was assembled from the vicinity of Faisalabad Punjab Pakistan in November 2017, stem part of *C. decidua* were assembled from Mochi wala Jhang- Faisalabad road District Jhang Punjab and the entire shrublet of *F. indica* was congregated from the adjoining areas of Shorkot District Jhang Punjab Pakistan. These plants were authenticated by an expert taxonomist Dr. Mansoor Hameed, Associate Professor, Department of Botany, University of Agriculture, Faisalabad. The specimen samples of each plant were put at same University herbarium. These plants were cleaned thoroughly, dried under shade and then dried plants powdered coarsely. Powdered material of each plant was soaked separately with aqueous (30%)-methanol (70%) solvent for three successive days and shaken occasionally. The slurry of each plant was first passed over muslin fabric and then subjected to filter by Whatman (Maidstone, Kent, England) No.1 filter paper. This practice of filtration was executed thrice. The filtrates of each plant were join to evaporate under rotary evaporator (BUCHI, Switzerland). This process produced the crude extracts with the respective percentage yields of *E. hirta* (7.3 % w/w), *C. decidua* (8.4 % w/w), and *F. indica* (9 % w/w).

The further polarity-based fractionation of crude extract of each plant was performed after dissolving twenty five gram of every extract in about one fifty mL of distilled water. Then the solution was passed to separating funnel to fraction the crude extracts of every plant with petroleum ether, chloroform and ethyl acetate, respectively to get organic and aqueous fractions [36].

2.2. Chemicals

Nifedipine, norepinephrine, arsenic, acetylcholine chloride, calcium chloride, phenylephrine, and potassium chloride were attained from sigma chemical company (U.S.A). The chemicals for physiological salt solutions like sodium chloride, potassium dihydrogen phosphate, magnesium sulphate, magnesium chloride, sodium bicarbonate, glucose and sodium dihydrogen phosphate were booked from E. Merck, Darmstadt, Germany, which was prepared freshly every day. Stock solutions of different chemicals and crude extracts were prepared in distilled water/saline and solubilized using DMSO and tween 80. The stock solution was stowed at -20 °C, from which the dilutions were prepared freshly.

2.3. Animals

The animals used in this study were Sprague- Dawley rats, weighing 200–250 g and without restriction of gender. These were provided with temperature (23 ± 5 °C) and humidity (55 ± 5%) in animal house of Government College University Faisalabad, Pakistan. Each animal was placed in special rectangular plastic cages having sawdust bedding. The rats were delivered with standard diet containing g/Kg as nutrivet L 2.5; flour 380; choker 380; NaCl 5.8; vegetable oil 38; molasses 12; potassium meta bisulphate 1.2; powder milk 150 and fish oil 170 and tap water [28]. The study protocol was followed as permitted by the Institutional Review Board (IRB), Government College University Faisalabad, Pakistan and approved on 27.06.2018, vide latter no. GCUF/ERC/1983. We also followed the guidelines of Institute of laboratory Animal Resources Commission on Life Sciences, National Research Council (1996).

2.4. In-vivo investigations

2.4.1. Estimation of antihypertensive activity of plants in arsenic-induced hypertensive rats

The healthy SD rats of either gender were selected by physical randomization and distributed into six groups, six animals per group. First group got normal saline 10 mL/kg *via* oral route per day for successive four weeks and was named as positive control. The rodents of group second to six were injected (intra-peritoneal) with 1.5 mg/kg of arsenic every day for first two weeks. The animals of group third received nifedipine (10 mg/kg) every day during the third and fourth week of experiment, while the animals of group four, five and six were provided with plant extract at dosing of 500 mg/kg *via* oral route [31]. The SBP was counted from the tails of each rat through non-invasive blood pressure (NIBP) measuring technique (ADInstruments, Australia). To monitor the SBP every rat was accurately positioned in rat restrainer, while the tail cuff with pulse sensor was fixed to the appropriate positions on the rat tail. Afterward the tail cuff was inflated *via* air to above SBP, which could be 230- 50 mm of Hg. Then the pressure in the cuff was freed gradually and the pulses were measured *via* PowerLab data system [37].

2.4.2. Estimation of the effects of plants on in-vasive blood pressure in anaesthetized rats

Healthy SD rats without gender restrictions were randomly selected from animal house of same University. Every animal was sedated by using an injection (intra-peritoneal) of thiopental sodium at dosing of 60–70 mg/kg the rat was put over the dissecting tray with supine position, the throat area was incised and open to locate the trachea and then

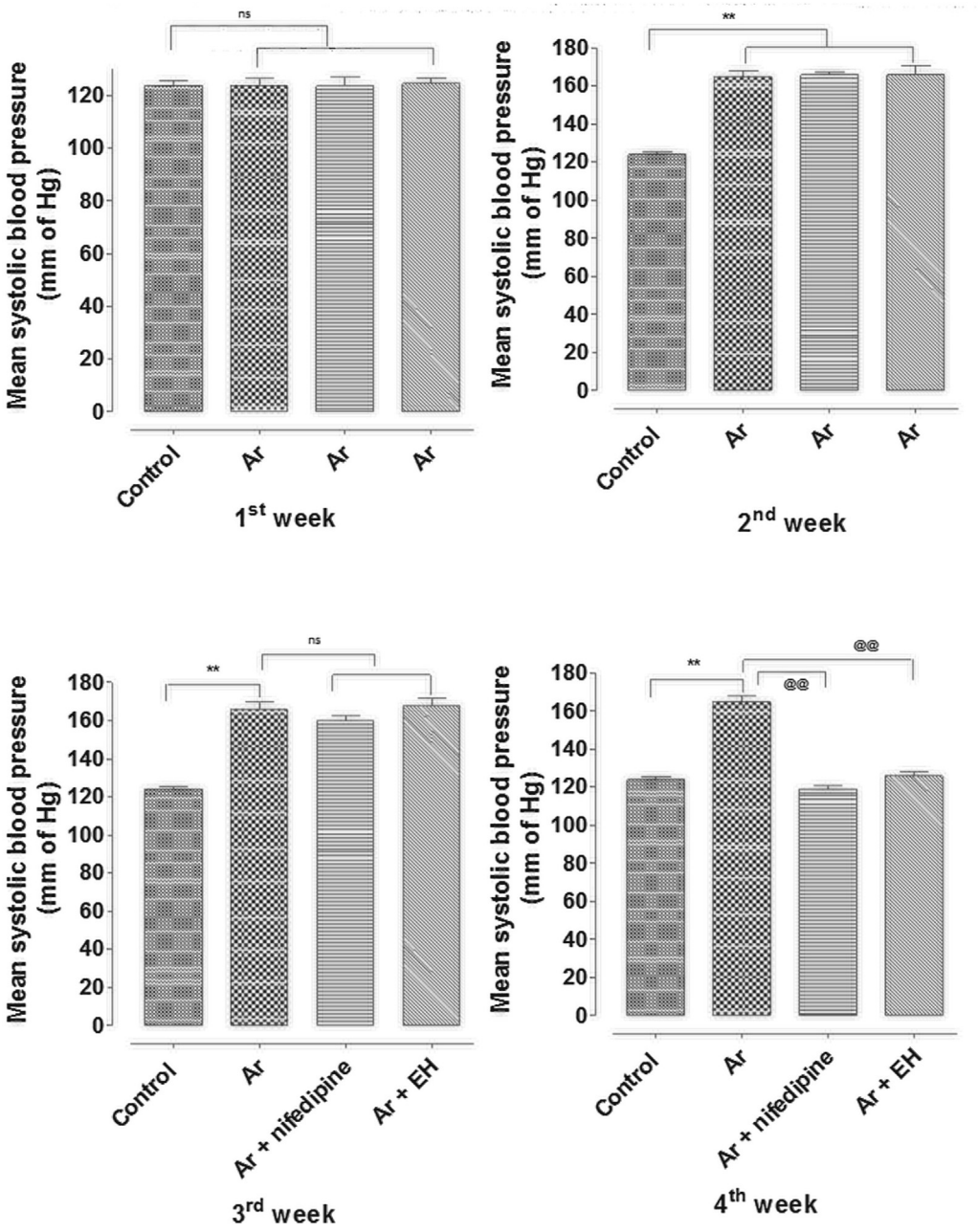

Figure 1. Effects of *E. hirta* extract (500 mg/kg) on SBP in arsenic-induced hypertensive SD rats. 1st week; depicts saline vs arsenic-fed group. 2nd week; ** depicts saline vs arsenic-fed groups. 3rd week; ** depicts saline vs arsenic-fed groups. 4th week; ** saline vs arsenic-fed groups, @@ depicts arsenic + treatment groups vs arsenic-fed groups. ns shows non-significant, Where ** ($p < 0.001$) vs control and @@ ($p < 0.001$) vs control (One-way ANOVA followed by Dunnett's test).

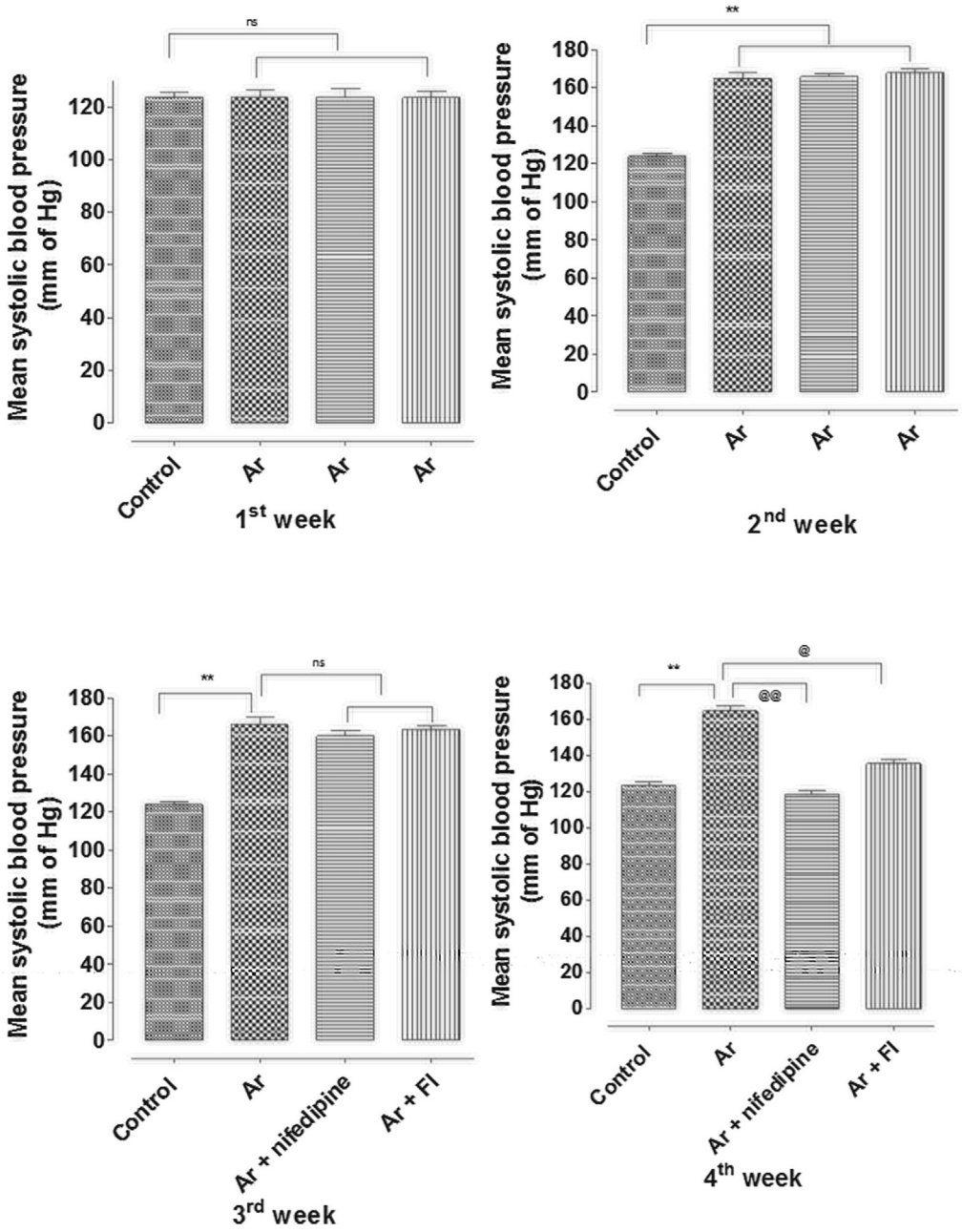

Figure 2. Effects of *F. indica* extract (500 mg/kg) on SBP in arsenic-induced hypertensive SD rats. 1st week; depicts saline vs arsenic-fed group. 2nd week; ** depicts saline vs arsenic-fed groups. 3rd week; ** depicts saline vs arsenic-fed groups. 4th week; ** saline vs arsenic-injected groups, @@ depicts arsenic + treatment groups vs arsenic-fed groups, Where ** ($p < 0.001$) vs control and @ ($p < 0.05$) and @@ ($p < 0.001$) vs control, (One-way ANOVA followed by Dunnett's test), ns represents nonsignificant.

right jugular vein and left carotid artery. This tracheal hole was inserted with transparent plastic tube, which was repeatedly cleaned with cotton swab during whole experiment. The artery was inserted with arterial/intravenous catheters full of heparin solution in normal saline, attached with pressure transducer to PowerLab station (ADInstruments, Australia) to note the arterial blood pressure. On the opposed side the jugular vein was tied with catheters in same manner to administer test material(s) to the animals. Earlier to any test dose, normal saline of same volume of test material was administered to rats and a period of 7–10 min was specified to stabilize the animal. Erstwhile, evaluation of the results of lower to higher doses (1–100 mg/kg) of tested plant extract on BP, the control responses of some standard drugs like acetylcholine (1 μg/kg) and norepinephrine (1 μg/kg) were recorded after administration *via* jugular vein. After every dose of drug and before the administration of next dose, some gap was provided to return the arterial blood pressure would return to its normal/resting level. The difference in BP was presumed by changes in steady-state values before and the peaks after the dose injection. Mean arterial pressure (MAP) was measured as the diastolic pressure plus one third of the pulse pressure {SP-DP, SP-systolic pressure, and DP-diastolic pressure} [38].

2.5. In-vitro investigations

2.5.1. Preparation of endothelial intact rat thoracic aortic rings

The healthy adult Sprague- Dawley rats of 8–12 weeks were isolated randomly and starved for 16 h. Animal as per protocol were sedated by

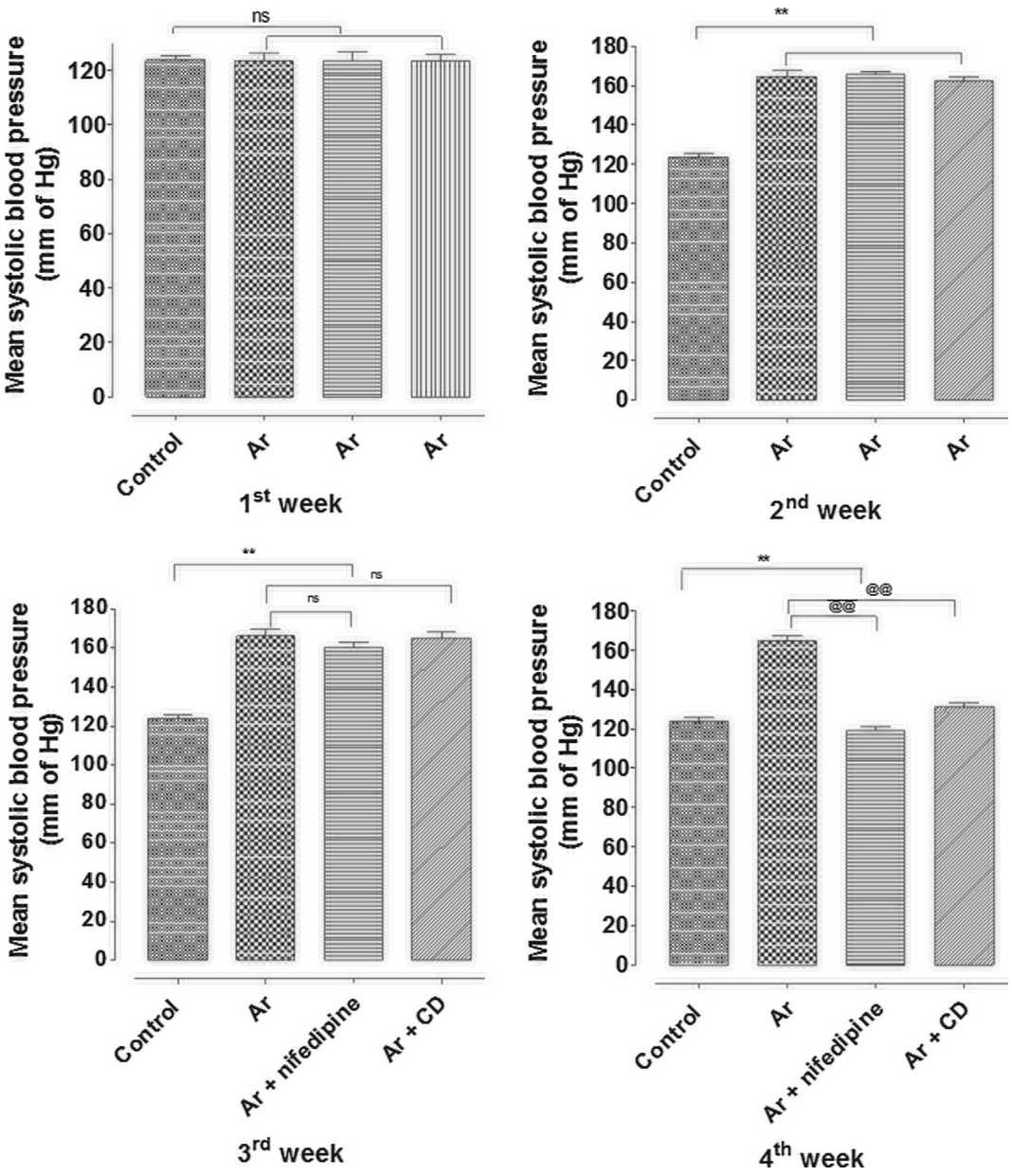

Figure 3. Effects of *C. decidua* extract (500 mg/kg) on SBP in arsenic-fed hypertensive SD rats. 1st week; depicts saline vs arsenic-fed group. 2nd week; ** depicts saline vs arsenic-fed groups. 3rd week; ** depicts saline vs arsenic-fed groups. 4th week; ** saline vs arsenic-fed groups, @@ depicts arsenic + treatment groups vs arsenic-fed groups, Where ** ($p < 0.001$) vs control and @@ ($p < 0.001$) vs control, (One-way ANOVA followed by Dunnett's test), ns represents nonsignificant.

the use of isoflurane (2–5 % v/w) *via* breathing by keeping them in a closed cabin till the deep anesthesia. Once animals were deeply anaesthetized, cervical dislocation was executed with the help of rod stroke placed on the occiput followed by pulling the tail of the animal opposite to the neck, subsequently the rats were incised at thoracic region by using sharp edge blades and thoracic aortae was isolated from thoracic portion of rats. The aortae were sliced into segments of rings of around 2–3 mm width. The individual ring was hung into 5 mL double jacket organ bath holding krebs solution at 37 °C and carbagen was being unceasingly bubbled *via* it. The freshly prepared krebs solution includes the following components in mM as NaCl 118.2; KH_2PO_4 1.3; $NaHCO_3$ 25.0; Glucose 11.7; KCl 4.7; $MgSO_4$ 1.2 and $CaCl_2$ 2.5 with pH 7.4. Every preparation was delivered a pretension of 1 g, as baseline load and permitted to incubate for duration of 45–50 min. The alterations in isometric load were noted and scrutinized using the force transducer attached with PowerLab data system. These aortae were stabilized using phenylephrine (1 μM). The tested drugs were applied to observe vasorelaxant capability against P.E, low K^+ and high K^+-driven contractions in rat aortic strips. The calcium channel blockers (CCBs) type effects were authenticated by building the control Ca^{++} concentration-response curves (CRCs) in calcium-free environment. The Ca^{++} CRCs were reconstructed later to the addition of first lower and then higher concentrations of tested material (s), to detect the nonparallel move in the CRCs of calcium to right direction with repression in maximal response [30, 31].

While a tested material failed to express inhibitory effects against the high K^+-driven contractions, the drug was evaluated against the low K^+-driven contractions, to further elucidate the probable K^+ channel activation type of mode [39]. K^+ channel opener is a new class of drugs which causes an increase in efflux of K^+ and result in hyperpolarization, consequently intra-cellular Ca^{++} contents are decrease and contraction of muscle is reversed. Genarally the following classes of K^+ channels have been studied with their inhibitors like large and small Ca^{++}-activated K^+ (BK_{Ca}) channels blocked by iberiotoxin (100 nM) [40], ATP-sensitive K^+ (K_{ATP}) channels are only slightly voltage-dependent which selectively

blocked by sulphonylurea derivatives, such as glibenclamide, delayed rectifier K^+ (K_{dr}) channels are voltage-dependent and Ca^{++}-independent K^+ channels. K^+ (K_{dr}) channels are blocked by 4-aminopyridine and participate significantly in the initiation of resting membrane potential. The inward rectifier K^+ (K_{ir}) channels are inhibited by Ba^{++} (50 μM for K_{ir} channel), whereas TEA (30 μM) is considered as non-specific blocker [41, 42]. To attest the existence of any vasoconstrictor constituent within the tested material(s), assays were accomplished over the resting baseline tension of aortae. For these experiments, the aortic tissues were stabilized with phenylephrine until a steady state on baseline tension was achieved; vasospastic result of the tested material(s) were noted as percent part of P.E-driven contractions [38, 39].

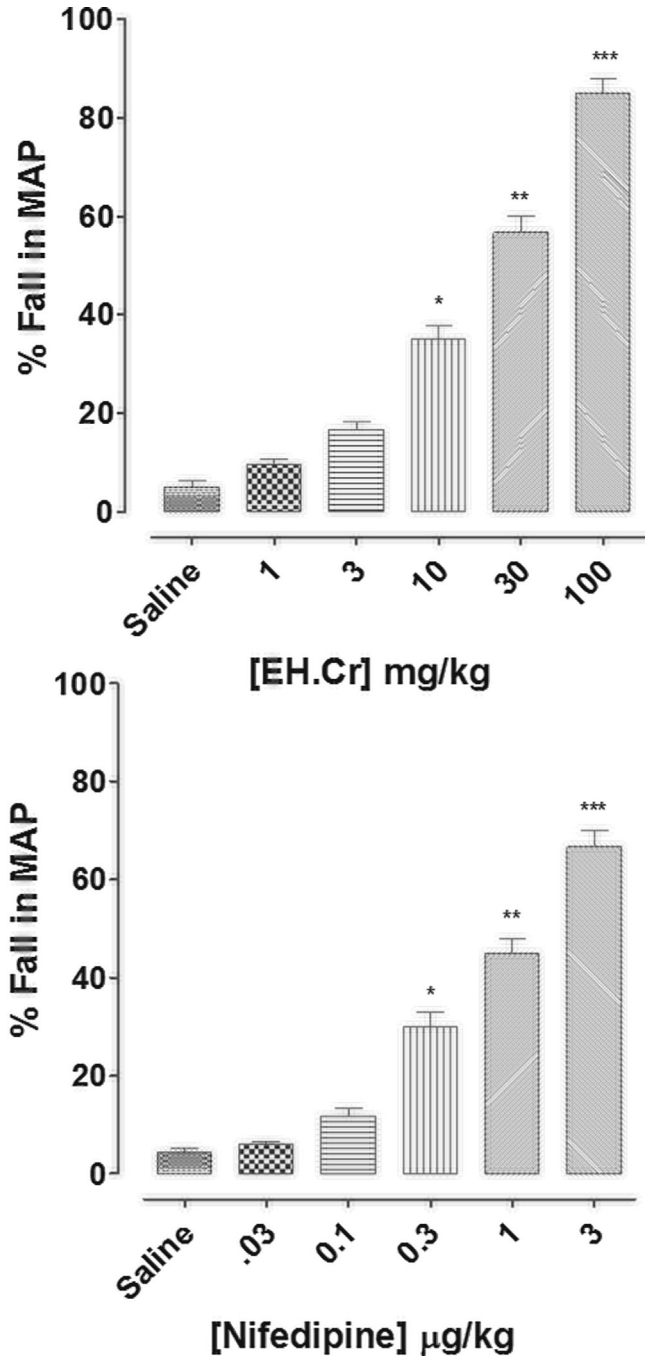

Figure 4. Effects of the crude extracts of *E. hirta* on fall in blood pressure in normotensive sedated rats. The data depicts mean ± SEM of 3–5 experiments. *p < 0.05 and **p < 0.01 vs saline (0.1 mL/kg) as control (One-way ANOVA followed by Dunnett's test).

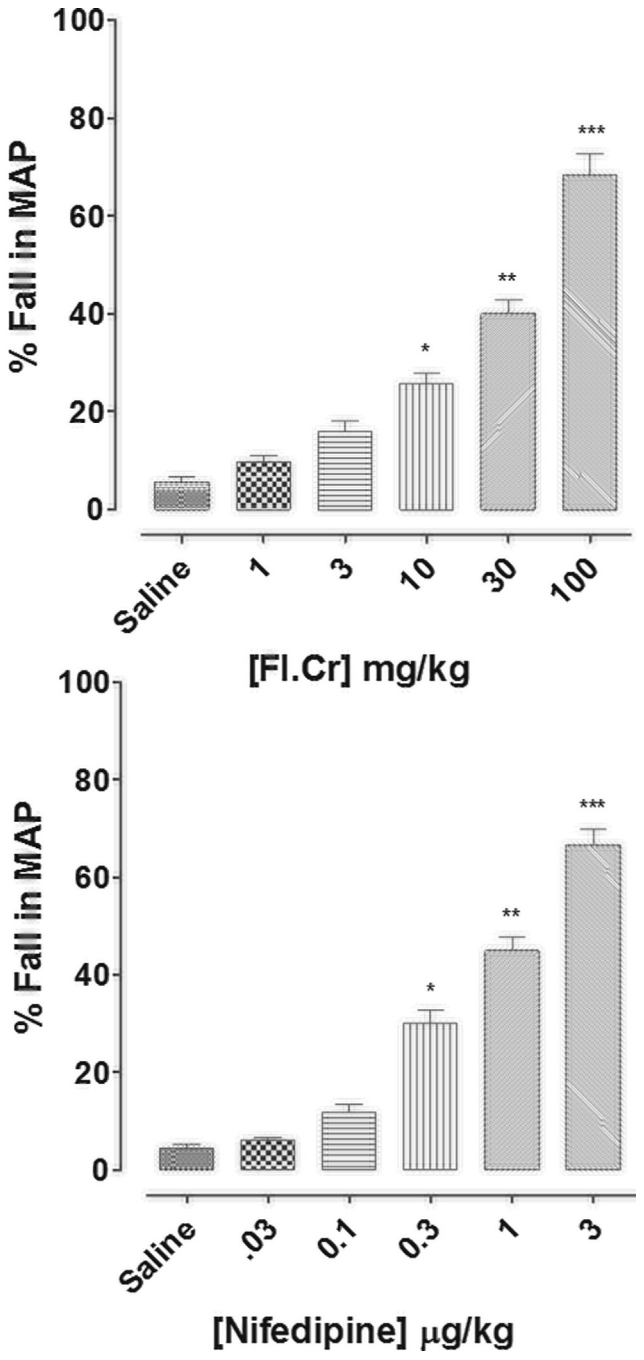

Figure 5. Effects of the crude extracts of *F. indica* on fall in blood pressure in normotensive sedated rats. The data depicts mean ± SEM of 3–5 experiments. *p < 0.05 and **p < 0.01 vs saline (0.1 mL/kg) as control (One-way ANOVA followed by Dunnett's test).

2.5.2. *Preparation of endothelial denuded rat thoracic aortic rings*

In these trials the aortic rings were rubbed between the thumb and fingers to detach the endothelium. These denuded rings were hung into organ bath in same manner as described earlier and now blood vessels were ready to estimate endothelial independent vaso-relaxant activity. The vascular endothelium release some of the chemicals which also induce relaxation and contractions in smooth muscles, NO is one of them which leads to dilatation, while muscarinic receptor M_3 activation also release the NO [43], hence endothelial intact tissues were pre-applied

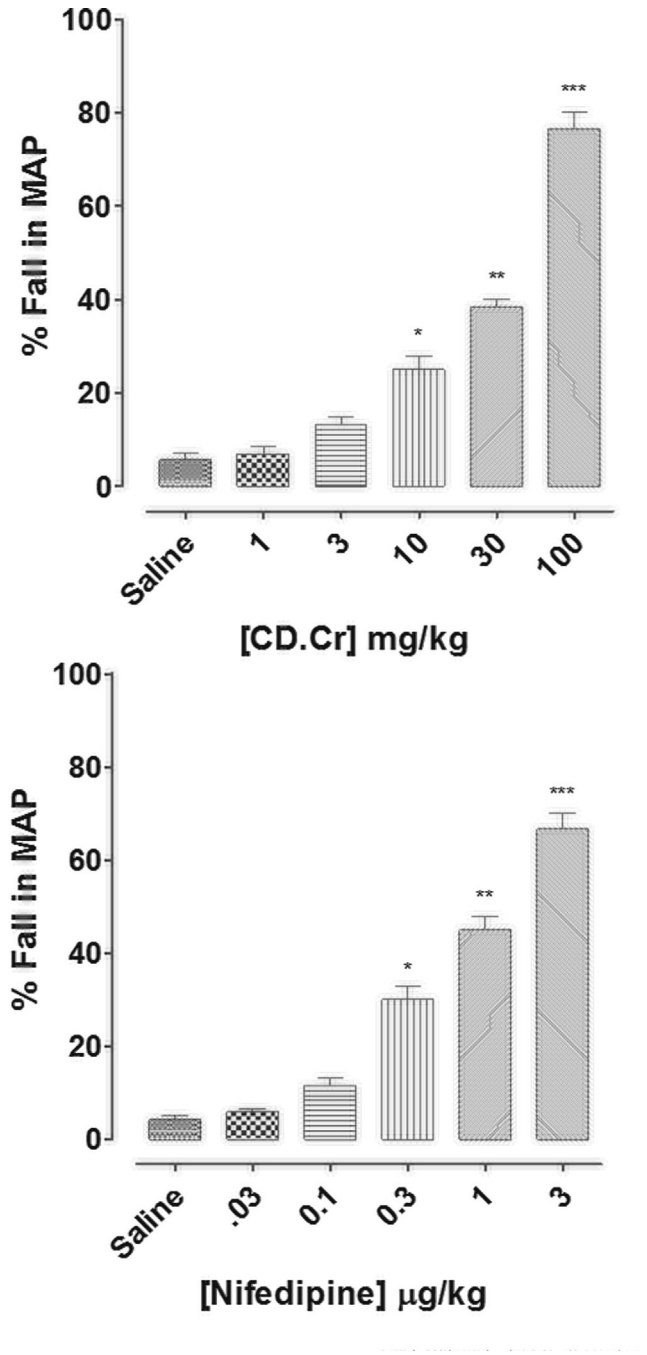

Figure 6. Effects of *C. decidua* extract on fall in blood pressure in normotensive sedated rats. The data presents mean ± SEM of 3–5 experiments. *p < 0.05 and **p < 0.01 vs saline (0.1 mL/kg) as control (One-way ANOVA followed by Dunnett's test).

with L-NAME {NO synthase (NOS) inhibitor} [44] to observe the probable nitregic contributions for vaso-relaxant activities [38].

2.6. Statistical analysis

The results were displayed in mean ± standard error of mean (S.E.M), n = number of tests and EC_{50} (median effective concentration) data shows the geometric means with 95% CI (confidence intervals). CRCs examined by non-linear regression followed by sigmoidal curves. For the purpose of significant differences, one-way ANOVA (analysis of variance) followed by Dunnet's test or two-way ANOVA followed by Bonferroni's post-test correction or unpaired t-test were applied.

Table 1. Percent maximal decline in blood pressure (% E_{max}) by the tested matter in normotensive sedated rats.

Tested matter	Concentration	Percent E_{max}
EH.Cr	100 mg/kg	85.0 ± 2.89 *
FI.Cr	100 mg/kg	75 ± 2.87 *
CD.Cr	100 mg/kg	76.6 ± 3.33 *
Nifedipine	10 mg/kg	66.63 ± 3.34 **

Data indicated mean ± SEM of 3–5 assays. *p < 0.05 and **p < 0.01 vs basal blood pressure (One-way ANOVA followed by Dunnett's test).

Table 2. Proportional relaxant actions of *E. hirta* (EH.Cr) extract against low K^+, high K^+ and P.E-driven contractions, its petroleum ether (Pet.EH), chloroform ($CHCl_3$.EH), ethyl acetate (Et.Ac.EH) and aqueous (Aq.EH) fractions against high K^+ and P.E-driven contractions in isolated rat aortic strips.

Sr. no.	Tested matter	EC_{50} value (95% CI; n = 4–6) mg/mL P.E-induced contractions	EC_{50} value (95% CI; n = 4–6) mg/mL against high K^+-driven contractions
1.	EH.Cr	0.72 (0.50–1.05)	0.73 (0.53–1.13)
2.	Pet.EH	0.09 (0.03–0.11)	0.14 (0.06–0.52)
3.	$CHCl_3$.EH	0.16 (0.13–0.86)	0.18 (0.12–0.86)
4.	Et.Ac.EH	0.8 (0.24–0.9)	0.74 (0.18–0.93)
5.	Aq.EH	N/A	N/A

Data indicated the geometric means with 95% confidence intervals (CI) in parenthesis, "n" indicated number of assays.

Table 3. Comparative relaxant actions of the crude extract of *C. decidua* (CD.Cr), its petroleum ether (Pet.CD), chloroform ($CHCl_3$.CD), ethyl acetate (Et.Ac.CD) and aqueous (Aq.CD) fractions in isolated rat aortae.

Sr. no.	Test matter	EC_{50} value (95% CI; n = 4–6) mg/mL P.E-driven contractions	EC_{50} value (95% CI; n = 4–6) mg/mL against high K^+-driven contractions
1.	CD.Cr	3.01 (1.8–6.3)	2.9 (1.66–4.3)
2.	Pet.CD	0.06 (0.04–0.09)	0.08 (0.05–0.13)
3.	$CHCl_3$.CD	0.20 (0.14–0.28)	0.25 (0.16–0.39)
4.	Et.Ac.CD	0.64 (0.46–0.87)	0.73 (0.54–1.2)
5.	Aq.CD		N/A

Data exhibited in geometric means with 95% confidence intervals (CI) in parenthesis. "n" represents number of assays.

3. Results

3.1. Estimation of antihypertensive activity of plants in arsenic-induced hypertensive rats

The *E. hirta*, *F. indica* and *C. decidua* extract at 500 mg/kg significantly (p < 0.001) dropped elevation of SBP to arsenic-induced hypertensive rats to almost 29.0 ± 1.21, 23.0 ± 2.3 and 26.0 ± 1.40% respectively at week 4 matched to control as presented in Figures 1, 2, and 3.t

3.2. Estimation of the effects of plants on in-vasive blood pressure in anaesthetized rats

Intravenous injection of *E. hirta*, *F. indica* and *C. decidua* extract to the normotensive sedated SD rats exhibited a dose-dependent drop in mean arterial pressure (MAP) at dosing of 1, 3, 10, 30 and 100 mg/kg to almost 85.0 ± 2.89, 70.0 ± 2.02 and 68.0 ± 1.90% at maximum administered dose of 100 mg/kg respectively.

Nifedipine also declined MAP with dose-dependent fashion (0.03–3 µg/kg) as depicted in Figures 4, 5, and 6. The percent maximum effects (E_{max}) of *E. hirta*, *F. indica* and *C. decidua* extract and nifedipine at challenged doses have been tabulated in Table 1.

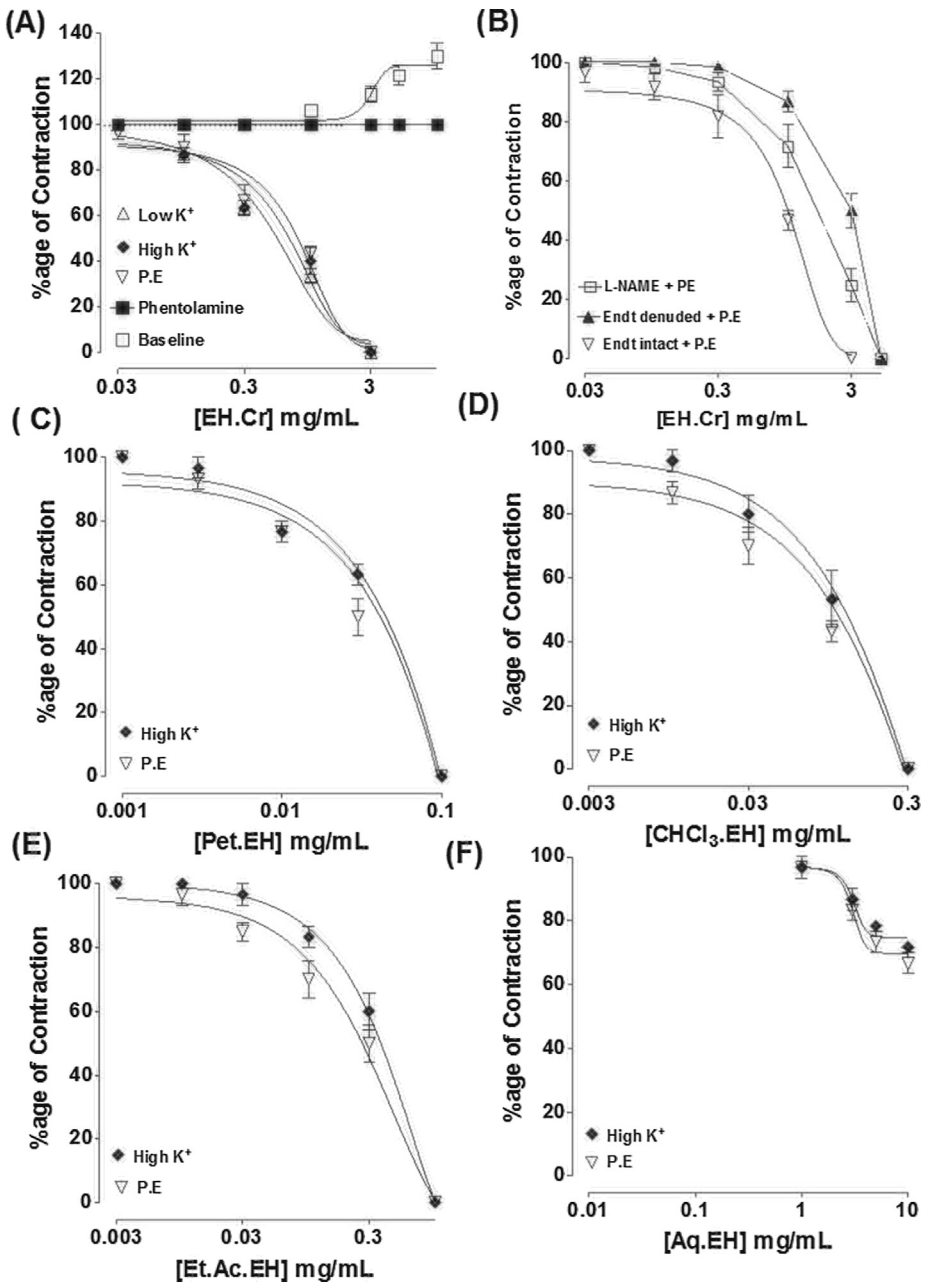

Figure 7. The concentration-specific vaso-modulatory effect of *E. hirta* extract against low K^+ (20 mM), high K^+ (80 mM) and P.E-driven contractions and on baseline with and without phentolamine (A), against P.E in existence of L-NAME, endothelium intact and endothelium denuded (B), petroleum ether (Pet.EH) (C), chloroform ($CHCl_3$.EH) (D), ethyl acetate (Et.Ac.EH) (E) and aqueous (Aq.EH) (F) fractions against high K^+ and P.E-driven contractions on rat aortae. The data indicated mean ± S.E.M, n = 4–6 experiments on aorta of four to five different rats.

3.3. Effects of plants on rat aortic contractions

The *E. hirta* and *C. decidua* extracts were investigated for vaso-relaxant activity against different spasmogens driven contractions *i.e.* P.E (1 μM), low K^+ (20 mM) and high K^+ (80 mM). Both extracts showed concentration-related relaxant effects against P.E, low K^+ and high K^+-driven spasms with respective EC_{50} values as presented in Tables 2 and 3.

All fractions of *E. hirta and C. decidua*, except their aqueous, showed full relaxation against P.E and high K^+-driven contractions in rat aorta, however the aqueous fraction presented partial relaxation as given in Figures 7 and 8. Their respective EC_{50} values are unfolded in Tables 2 and 3.

F. indica extract displayed an incomplete relaxation of high K^+-driven contractions while full inhibition of low K^+-driven contractions with EC_{50} {2.55 mg/mL (1.46–4.48, 95% CI, n = 4–6)}. Although after the addition of TEA, the relaxant actions of *F. indica* extract against low K^+-driven contractions were completely inhibited (Figure 9B).

Further exploration of spasmolytic activity among the fractions of plant ethyl acetate (Et.Ac.FI) and aqueous (Aq.FI) partly blocked the high K^+ and entirely inhibited the low K^+-driven contractions, which were partially inhibited in the existence of 4-aminio pyridine and fully inhibited by TEA. However the pet-ether (Pet.FI) and chloroform ($CHCl_3$.FI) fraction equally relaxed the high K^+ and low K^+-driven contractions (Figure 9). On the baseline or resting tension of rat aorta rings, the *E. hirta, F. indica and C. decidua* extract displayed the phentolamine-sensitive stimulant effects at higher tested doses of plant.

Further *E. hirta and C. decidua* extract were attested against P.E-induced contraction in endothelial intact, denuded and L-NAME pre-applied aortic rings (Figures 7 and 8). These assays depicted that a relatively lower concentration of *E. hirta and C. decidua* compared to endothelial denuded/L-NAME pre-applied aorta was required to relax the endothelium intact aortic rings with respective EC_{50} values shown in Table 4.

The Ca^{++} antagonist effects of *E. hirta* and *C. decidua* were further established when their crude extracts shifted the CRCs of Ca^{++} to the right, similar to nifedipine.

The fractions of *E. hirta* and *C. decidua* except their aqueous and the pet-ether and chloroform fraction of *F. indica* showed Ca^{++} antagonist-like activity and displaces the CRCs of calcium to right as seen in Figures 10, 11, and 12.

4. Discussion

On the account of the established traditional usage of *E. hirta*, *F. indica* and *C. decidua* in cardiovascular disorders like as hypertension [9, 19, 28], the oral administration of *E. hirta, F. indica and C. decidua* extract (500 mg/kg) reduced systolic blood pressure (SBP) significantly in arsenic-induced hypertensive rats with following order of potency; EH.Cr ˃ CD.Cr ˃ FI.Cr. Arsenic induces hypertension in animal *via* endothelial impairment and inactivates the endothelial nitric oxide synthase, consequently attenuating the bio -generation and availability of nitric oxide [45]. This endothelial cells dysfunction is likely to make straight effects on the vascular cells. The chronic arsenic exposure brings inflammatory effects to vascular tissues, increases oxidative stress, exacerbates atherosclerosis and stimulation of vascular redox signaling, which may affect the structure and function of cardiovascular system [46]. Literature studies depict that *E. hirta* and *F. indica* contains flavonoids and ascorbic acid [12, 22], while *C. decidua* contains vitamin E (tocopherols with different isoforms as δ-tocopherol, α-tocopherol and γ-tocopherol), vitamin C (ascorbic acid), and flavonoids [29, 30, 31], which possesses antioxidant effects, while *E. hirta in-vitro* exhibited antioxidant and anti-inflammatory activities [11], *F. indica* and *C. decidua* showed antioxidant actions in different assays [24, 35], which imparts their effects to lessen the deformity induced by arsenic exposure *in-vivo*, *E. hirta* also possess angiotensin converting enzyme inhibitory potential [18] which also give it antihypertensive activities.

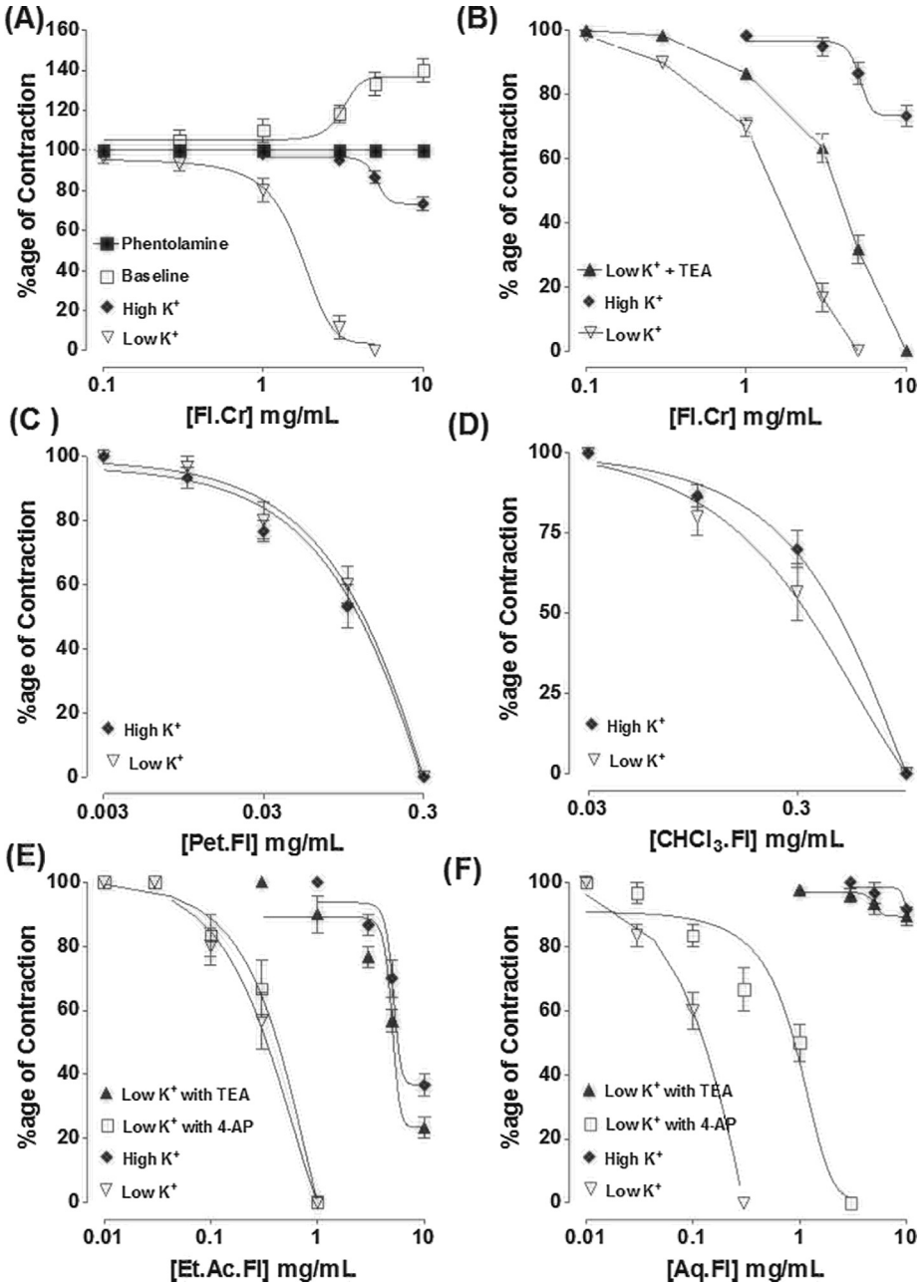

Figure 8. The stimulatory effect of *F. indica* extract on baseline tension in presence and absence of phentolamine and the concentration-specific inhibitory potential of the crude extract of *F. indica* (FI.Cr) (F), its ethyl acetate (Et.Ac.CD) (I) and aqueous (Aq.CD) (J) fractions (in absence and presence of 4-amino pyridine and TEA) against low K^+ (20 mM) and high K^+ (80 mM)-driven contractions, its petroleum ether (Pet.CD) (G) and chloroform ($CHCl_3$.CD) (H) fractions against low K^+ and high K^+-driven contractions on rat aortae. Data is presented in mean ± S.E.M, n = 4–6.

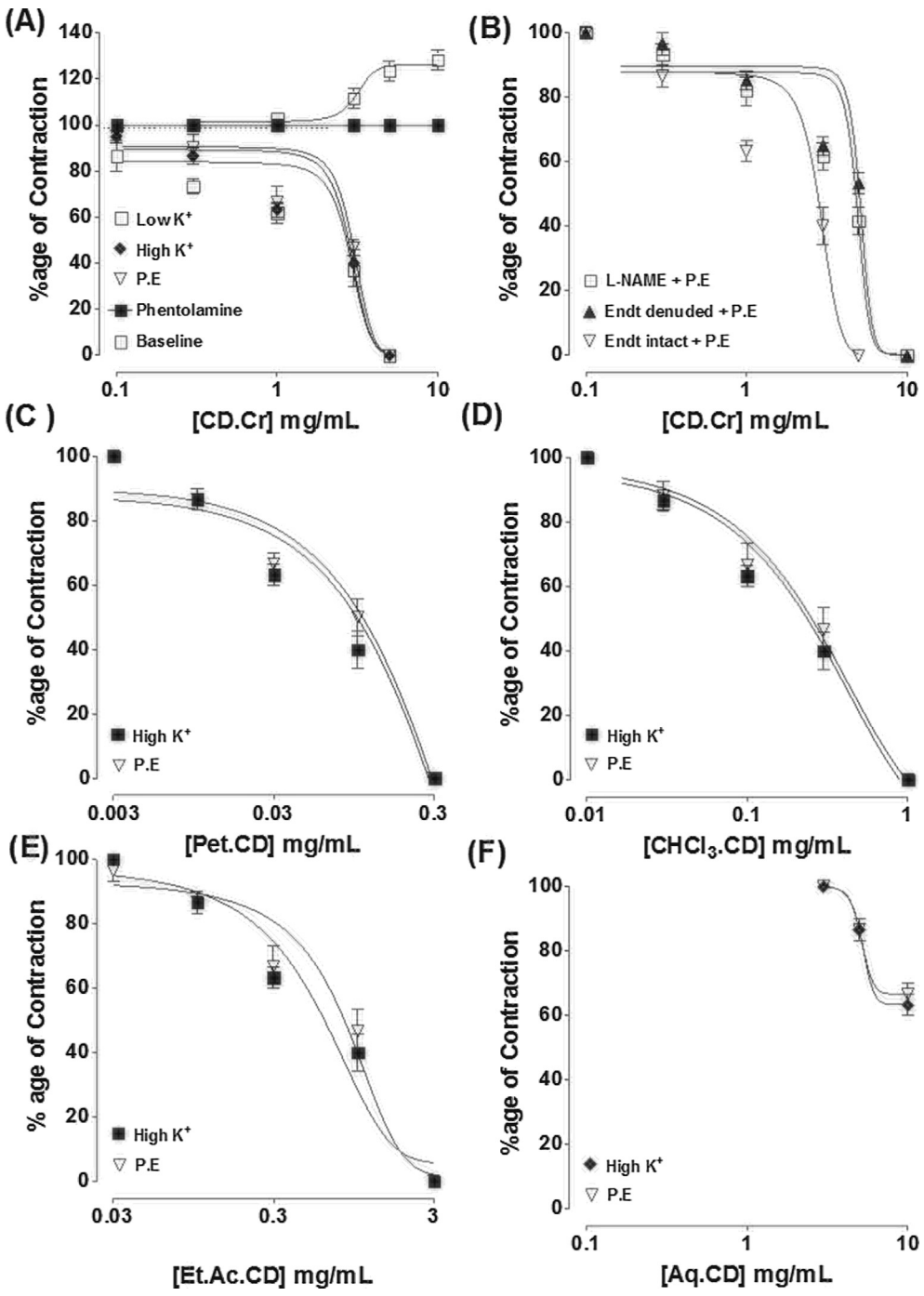

Figure 9. The concentration-specific blocking action of the crude extract of *C. decidua* against low K^+, high K^+, P.E-driven contractions and on -baseline with phentolamine (A), against P.E in existence of L-NAME, endothelium intact and endothelium denuded (B), petroleum ether (Pet.CD) (C), chloroform ($CHCl_3$.CD) (D), ethyl acetate (Et.Ac.CD) (E) and aqueous (Aq.CD) (F) fraction of *C. decidua* against high K^+ and P.E-driven contractions on rat aortae. The data presented as mean ± S.E.M, n = 4–6 individual experiments with four to five different rats.

Table 4. Proportional relaxant action of *E. hirta* (EH.Cr) and *C. decidua* (CD.Cr) extracts on P.E-driven contraction in isolated endothelium intact and denuded/L-NAME treated rat aortae.

Sr. no.	Tested matter	EC_{50} value (95% CI; n = 4–6) mg/mL P.E-induced contractions (intact endt)	EC_{50} value (95% CI; n = 4–6) mg/mL against P.E-induced contractions (denuded/L-NAME treated)	EC_{50} value (95% CI; n = 4–6) mg/mL against P.E-induced contractions (intact endt)
1.	EH.Cr	0.81 (0.54–1.2)	3.4 (1.9–4.6)	3.1 (1.59–5.31)
2.	CD.Cr	0.63 (0.4–0.90)	3.1 (1.93–4.4)	2.98 (1.59–3.31)
3.	Nifedipine	0.11 (0.08–0.40)	0.10 (0.09–0.39)	0.11 (0.10–0.43)

In a new study, in the anesthetized rats, *E. hirta, F. indica and C. decidua* lessened the mean arterial pressure (MAP) in a dose-specific fashion, with percent maximal decline in blood pressure (85.0 ± 2.89%, n = 4–5) of EH.Cr at maximum administered dose (100 mg/kg) as being most potent among these three plants. These verdicts demonstrate a rationale support to the traditional consumption of these plants as cardiotonic or hypotensive. As the alterations in blood pressure are the consequences of variations in cardiac output and peripheral resistance [47]. Hereafter-any escalation with cardiac output or peripheral resistance might develop the hypertension, therefore-the therapeutic agents used to treat the hypertension primarily presents their effects by reducing the both components either alone or in combination [48].

To explore the effects of *E. hirta*, *F. indica* and *C. decidua* extract and their fractions on vessels, the crude extracts of these plants were tested

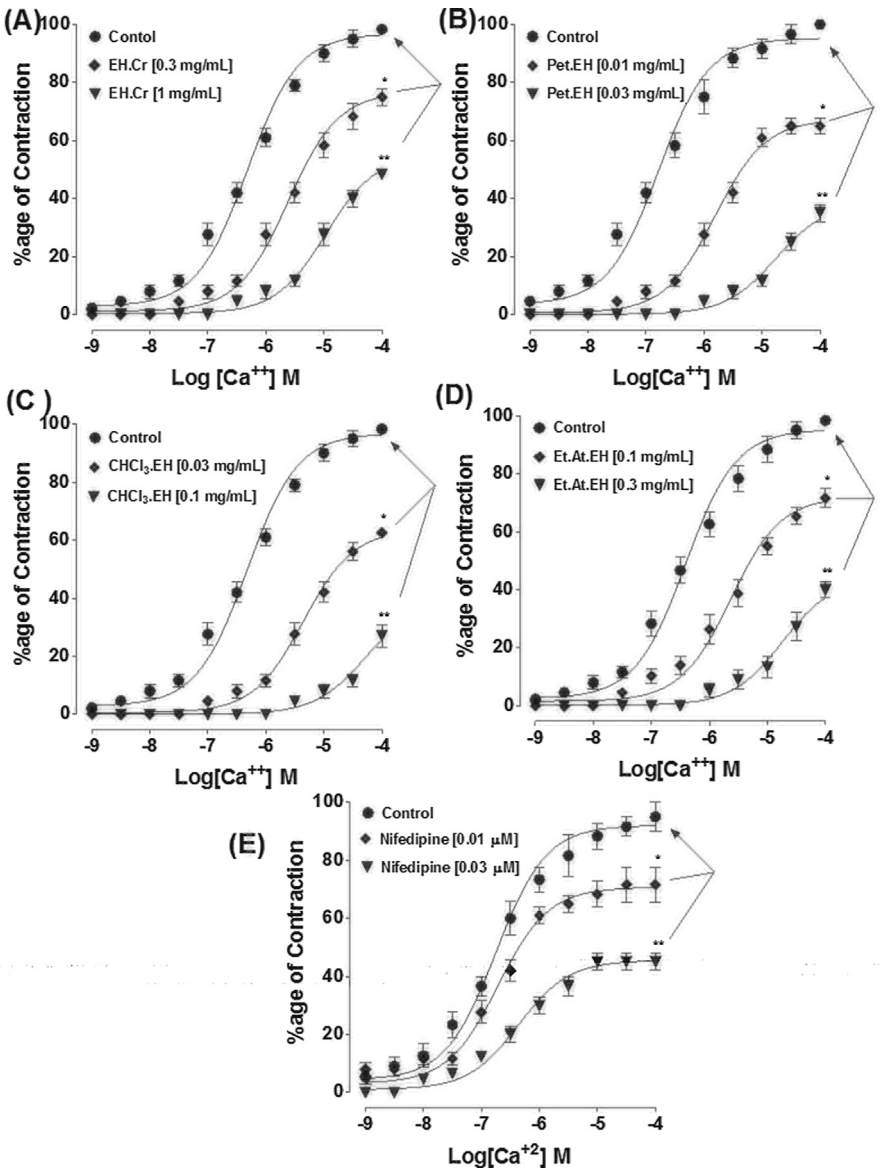

Figure 10. The concentration-response curves of Ca^{++} in lower and then higher concentration of *E. hirta* extract (A), its petroleum ether (Pet.EH) (B), chloroform ($CHCl_3$.EH) (C) and ethyl acetate (Et.Ac.EH) (D) fractions. *$p < 0.05$, **$p < 0.01$ (two-way ANOVA followed by Bonferroni's post-test correction). The data indicated mean ± S.E.M, 'n' showed 4–6 experiments with aorta of four to five different rats.

against induced contractions by different spasmogens in rat aortic strips. *E. hirta* and *C. decidua* presented inhibition of P.E (1 µM), low K^+ (20mM) and high K^+ (80 mM)-driven contractions, and their fractions except aqueous also showed inhibition of high K^+ and P.E-driven contractions, which demonstrated the calcium channel blockers type patterns of activity. While *F. indica* extract partially relax the high K^+-driven contraction and fully relax the low K^+-driven contractions in rat aortae.

Those substances, which particularly block the low potassium driven contractions are named as potassium channel opener, while Ca^{++} entry blockers inhibit both the both spasmogens *i.e.* low and high K^+ equally. Hence these experiments differentiate the K^+ channel activations from calcium channel blockers [31]. The K^+ channel opening effect of *F. indica* was established, when the inhibitory effects of low K^+-driven contraction was fully blocked by TEA, a non-specific K^+ blocker [49]. From these experiments, it has been concluded that the relaxant effects of the *F. indica* are probably driven *via* a combination of K^+ channel openers and Ca^{++}-channel blockers. Potassium channel openers include diverse molecules, which possess a big spectrum of therapeutic usage, as are effective in bronchial and gut spasm, diarrhea, hypertension and enuresis [50]. The potassium channel openers activate the K^+ channels to efflux the potassium and causing membrane hyperpolarization, consequently the intracellular free Ca^{++} decreases and smooth muscle relaxation occurs [31, 40]. To further explore the distribution of Ca^{++} antagonism and K^+ channel openers among the fractions of *F. indica*, it was observed that the low polar fractions as pet-ether and chloroform contain the CCBs-like activity while ethyl acetate and aqueous fractions contain the potassium channel opener constituents.

The vessel diameter and blood pressure are maintained *via* endothelium vasoactive components like cyclooxygenase (COX), nitric oxide (NO), endothelin, endothelium-derived hyperpolarizing factors (EDHFs) and endothelium-derived contracting factors (EDCFs) [51]. To investigate the other mechanism(s) for vasodilatation, *E. hirta* and *C. decidua* were examined on rat aortic rings, which is supposed as a prototype preparation to elucidate the endothelium-dependent or/and independent vasodilatation. On endothelium intact preparations, a comparatively low concentration was used to relax with respect to endothelium-denuded and L-NAME {NO synthase (NOS) inhibitor} [44] pre-applied rat aortic rings against P.E pre-contracted tissues. This aftermath suggest the

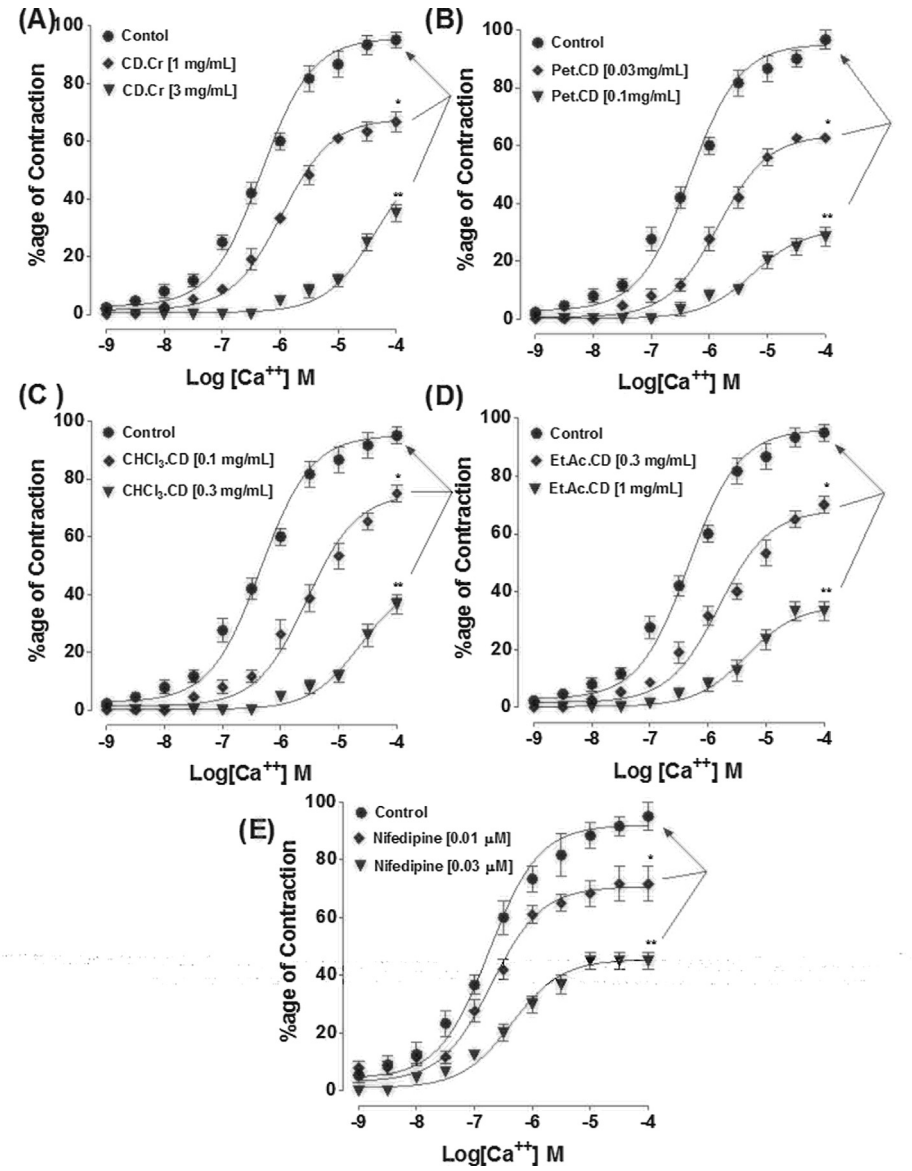

Figure 11. The concentration-response curves of Ca^{++} in lower and then higher concentrations of *C. decidua* extract (A), its petroleum ether (Pet.CD) (B), chloroform ($CHCl_3$.CD) (C), ethyl acetate (Et.Ac.CD) (D) fractions. *p < 0.05, **p < 0.01 (two-way ANOVA followed by Bonferroni's post-test correction). The data presented as mean ± S.E.M, n = 4–6 individual experiments using aorta of 4–5 different rats.

existence of added endothelium-regulated relaxing behavior possibly governed *via* nitric oxide activation like effects of *E. hirta* and *C. decidua*.

To endorse the CCBs contribution in *E. hirta*, *C. decidua* and their fractions except aqueous and the pet-ether and chloroform fraction of *F. indica*, control calcium concentration response curves CRCs were built in rat aorta where these caused rightward move in the CRCs of calcium with repression of maximal response of calcium like the effects of nifedipine pattern, a standard Ca^{++} channel blocker. In further experiments on phenylephrine-driven contractions in rat aortae, to elaborate the interference of herb with intracellular store of calcium, *E. hirta*, *C. decidua* and their fractions except aqueous presented complete relaxation. These relaxant behavior of *E. hirta*, *C. decidua* and their fractions except aqueous on high potassium and P.E-treated pre-contracted rat aortae validated the capability of the herb to hinder the calcium influx to cytosol *via* voltage-dependent calcium channels (VDCCs) and receptor-operated calcium channels (ROCs). Whereas it was established that calcium influx *via* VDCCs and ROCs amplified the calcium contents of cytosol in response to high K^+ and P.E addition to organ bath [52, 53], which resultantly trigger a persistent contractions. It has also been demonstrated that calcium channel blockers possesses antioxidant properties which reduce endothelial dysfunction by restoring NO availability [54, 55]. It has also been demonstrated that nifedipine, a standard CCBs, preserves endothelial integrity in patients with hypertension [56] and hence *E. hirta*, *F. indica* and *C. decidua* showed additional antihypertensive mechanism in arsenic-induced hypertensive rats *via* improving the endothelium damage by additional CCBs type constituents. These studies also presented the existence of vasoconstrictor components in *E. hirta*, *F. indica* and *C. decidua* extract, which was blocked in the existence of phentolamine. Interestingly, the extract ensured that no increase in BP in anesthetized rats, that strengthen the hypothesis that there may be the expression of endogenous mediator which block the vasoconstrictor effects in intact body systems or the existence of strong vaso-relaxant and cardio-depressant constituents within the plant, which does not permit

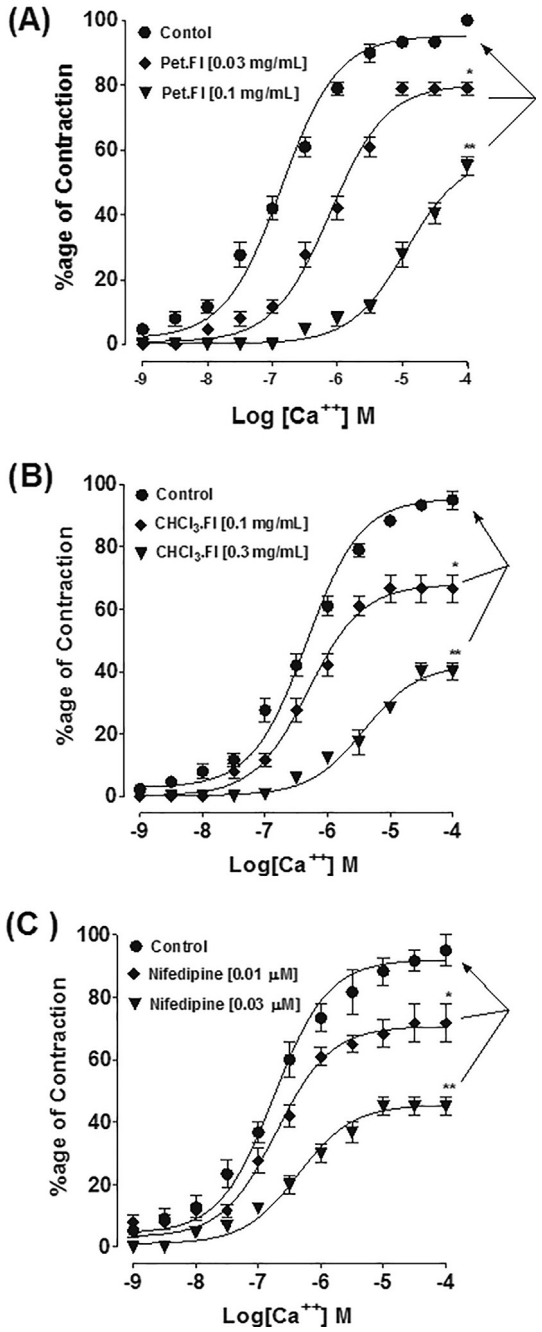

Figure 12. The concentration-response curves of Ca^{++} in the absence and existence of different concentrations of petroleum ether (Pet.FI) (A), chloroform ($CHCl_3$.FI) (B) fractions of *F. indica* and nifedipine (C) in rat aortae. The data represented as mean ± SEM from 4- 6 assays. *$p < 0.05$, **$p < 0.01$ (two-way ANOVA followed by Bonferroni's post-test correction).

the vasoconstrictor components to manifest dominance and originate hypertension in the *in-vivo* study.

To conclude, it shows that nature has installed the dual behavior in herbs, to subside the excessive dilatory effects, preventing the undesired drop in blood pressure, that is a usual phenomenon while treating with standard antihypertensive drugs [42]. The existence of this dual nature of components has been frequently observed in many plants like *Curcuma longa* [57], *Carthamus oxycantha* [53], *Viola odorata* [58], *Piper longum* and *Piper nigrum* [59]. These observed effects also help the statement that herbal medicine retain the synergistic and/or side effects abolishing mixtures [4, 5].

This data displays that *E. hirta*, *F. indica* and *C. decidua* possess blood pressure dropping effects in arsenic-induced hypertensive rats mediated possibly *via* endothelium-dependent vaso-relaxation. In normotensive rats, test materials showed blood pressure lessening activity driven *via* endothelium-dependent and Ca^{++} antagonism-like mechanism(s). Interestingly, *F. indica* showed combination of potassium channel activation and Ca^{++} antagonist-like activity in isolated rat aortae and mild vasospastic effect, which was found driven *via* α-adrenergic receptor activated cascades. This existence of vasospastic components within the same remedy may be intended to defeat the hypotension effects that is associated with longer duration/higher dose use of antihypertensive drugs. Therefore, current study supports to medicinal consumption of *E. hirta*, *F. indica* and *C. decidua* in cardiovascular disorders like hypertension.

Declarations

Author contribution statement

Muhammad Zeeshan Ali: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Malik Hassan Mehmood: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Muhammad Saleem; Muhammad Sajid Hamid Akash; Abdul Malik: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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