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Blood pressure-lowering and cardiovascular effects of plumbagin in rats: An insight into the underlying mechanisms

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ABSTRACT

Background: Plumbagin, a natural phenolic compound is investigated for response against blood pressure and vascular reactivity.

Methodology: Blood pressure lowering effects were observed by *in-vivo* invasive evaluation in normotensive rats, and *in-vitro* experimentation to measure changes of tension in isolated rat aorta and contractility in atria.

Results: The percentage decrease in mean arterial pressure (MAP) observed with plumbagin intravenously at doses of 0.1, 0.5, 1, 5, 10 μg/kg in normotensive rats was 7.16 ± 2.35, 15.5 ± 5.62, 19.5 ± 5.27, 26 ± 6.67, 34.33 ± 8.80, respectively. Plumbagin exerted vasorelaxant effects in rat aorta, unaffected by the removal of vascular endothelium, and $_{L}$-NAME and methylene blue pretreatment. Plumbagin completely inhibited phenylephrine (1 μM) and High K^{+} (80 mM) induced contractions. Similar to a Ca^{+2} channel antagonist, plumbagin caused a rightward shift in the Ca^{+2} concentration-response-curves (CRCs), resembling nifedipine. Pre-incubation with plumbagin, significantly suppressed contractions induced by phenylephrine in Ca^{+2}-free medium via disrupting Ca^{+2} release from intracellular stores. No change in vasorelaxant response was observed with the addition of potassium channel blockers, TEA and $BaCl_2$. In rat atrial strips, plumbagin exerted significant negative inotropic and chronotropic effects. No significant change was observed with atropine and atenolol pretreatment, so the effect appeared independent of muscarinic and beta-adrenergic receptors.

Conclusion: This study suggests the blood pressure lowering effects of plumbagin. That could be contributed by a decrease in vascular resistance via calcium antagonism, interferences in calcium efflux, and depressive effects on the rate and force of cardiac contraction. Further studies would be necessary to probe deeper into the underlying mechanisms.

1. Introduction

Hypertension is a major contributor and modifiable risk factor towards the development of cardiovascular diseases (CVDs) such as stroke, ischemic heart diseases, myocardial infarction, heart failure, and renal disorders (Oparil et al., 2018). Blood pressure is dependent on cardiac output (CO) and total peripheral resistance (TPR) (Whittlesea and Hodson, 2019). Important factors involved in the pathophysiology of hypertension include; an imbalance of cardiac output and total peripheral resistance, dysfunction of the vascular endothelial cells, the subsequent release of vasoactive substances, high levels of reactive oxygen species (ROS) and oxidative stress (Beevers et al., 2001; Korsager Larsen and Matchkov, 2016). Endothelial dysfunction is indicated as characteristic of essential hypertension, and has become potential target for the development of anti-hypertensive drugs (Ghiadoni et al., 2011). Numerous drugs including beta blockers, ACE inhibitors, ARBs, diuretics and calcium channel blockers are being used in the treatment of hypertension (Wecker et al., 2018; Katzung et al., 2019). These are accompanied with various undesirable side effects which limit their use and patient adherence. Other factors such as the availability and affordability of medicines also leads to ineffective control. Herbal medicines prove as suitable alternatives, their availability in areas where other medical facilities are scarce increases their consumption (Ahmad et al., 2018; Kulkarni, 2020; Tashakori-Sabzevar et al., 2016). Owing to their anti-oxidant properties, natural products exert anti-hypertensive effects by decreasing oxidative stress and lowering the availability of free radicals and ROS, which are involved in the development of hypertension and CVDs (Jung et al., 2018).

Phenolic compounds from medicinal plants such as luteolin, quercetin and kaempferol exert vasorelaxant and antihypertensive actions, that protect against CVDs (Maione et al., 2013; Mbaveng et al., 2014; Miranda et al., 2016). Plumbagin (Fig. 1), is a natural phenolic

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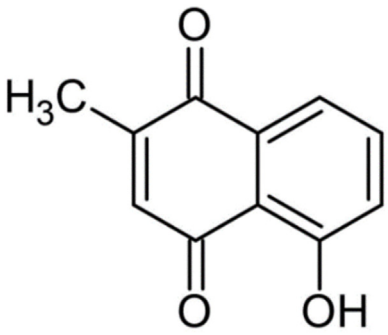

Fig. 1. Structure of plumbagin.

compound (a naphthoquinone), is present in plants of Plumbaginaceae e.g. *Plumbago zeylanica*, *Plumbago indica* and *Plumbago europea* (Tilak et al., 2004; Kapadia et al., 2005). Plumbagin shows many pharmacological actions and possesses anti-cancer (Checker et al., 2018), hypolipidemic, anti-atherosclerotic (Mbaveng et al., 2014; Sharma et al., 1991), anti-oxidant (Tilak et al., 2004), anti-diabetic (Sunil et al., 2012), anti-inflammatory (Rajalakshmi et al., 2018) and cardioprotective properties (Li et al., 2020; Wang et al., 2016). Plumbagin is suggested to be beneficial against experimental pulmonary arterial hypertension (Courboulin et al., 2012). This study investigated the response of plumbagin on blood pressure, vascular tension and atrial contraction.

2. Material and methods

2.1. Chemicals

Plumbagin and Nω-nitro-L-arginine methyl ester ($_L$-NAME) were purchased from Shanghai Aladdin Biochemicals. Nifedipine, Isoproterenol and Norepinephrine were purchased from Shanghai Macklin Other chemicals such as, dimethyl sulfoxide (DMSO), acetylcholine, atropine, atenolol, barium chloride ($BaCl_2$), phenylephrine, NaCl, KCl, $MgSO_4.H_20$, KH_2PO_4, $C_6H_{12}O_6$, $NaHCO_3$, $CaCl_2$ were purchased from Sigma Chemicals Co. Tetraethylammonium (TEA) was purchased from Rhawn Reagent. Ketamine (Ketasol by Indus pharma), diazepam (Valium by Roche) and Normal saline (Medisol) were purchased. Stock solutions of plumbagin and nifedipine were prepared with DMSO (10%) and diluted with distilled water, all solutions were freshly prepared on each day of experimentation.

2.2. Animals

Adult Sprague Dawley rats (250–350 g) were housed at the animal house of the College of Pharmacy, University of Sargodha, Sargodha, Pakistan. Standard laboratory conditions of humidity (40–50%), temperature (23–25 °C), pellet diet and unrestricted access to water was given. Approval was obtained from the Animal Ethical Committee of the University of Sargodha, Sargodha prior to initiation of experimentation (No. IAEC/UOS/2021/46).

2.3. Experimental protocols

2.3.1. Invasive blood pressure measurement in normotensive rats

Rats were anesthetized, the trachea was intubated to facilitate respiration. The right jugular vein and left carotid artery were located, isolated and cannulated. The carotid artery was isolated with extra caution to avoid damaging the vagal nerve, and connected by a catheter to the pressure transducer, subsequently connected with Power Lab and a data acquisition system. Mean arterial blood pressure (MAP) calculated; MAP = DBP + (SBP- DBP)/3. Percentage fall in MAP = Control - Fall/ Control x 100 (Parasuraman and Raveendran, 2012; Bopda et al., 2014; Ahmad et al., 2020).

2.4. Vascular studies

2.4.1. Rat aortic preparations

After cervical dislocation, rat thoracic aorta was carefully excised and transferred to Kreb's solution and continuously aerated with carbogen (95% O_2, 5% CO_2). Krebs solution was as (mM): NaCl 90, KCl 4.7, $MgSO_4.7H_2O$ 1.17, KH_2PO_4 1.17, $C_6H_{12}O_6$ 11.65, $NaHCO_3$ 25.0, $CaCl_2.H_2O$ 2.5 (pH 7.4). 2–3mm wide rings of the aorta were prepared, each ring was individually suspended on hooks in 25 ml tissue baths at a temperature of 37 °C and aerated with carbogen. Resting tension of 1 g was applied to each preparation and an incubation period of 30 min was provided for stabilization. Changes in isometric tension were recorded by a force transducer connected with a bridge amplifier and PowerLab data acquisition system (Shah and Gilani, 2012; Khan et al., 2018).

2.5. Endothelial dependent and independent effects

Intact and denuded aortic endothelium rings were tested. The rings were incubated with 10 μM $_L$-NAME and phenylephrine were used to induce contractions, to observe the possible relaxation effect produced by the drug, added cumulatively (each addition separated by 5–10 min). In some rings, the intimal surface of the aorta was gently rubbed to damage the endothelium, rings were considered denuded when they produced <10% relaxation response to acetylcholine, they were similarly tested (Qayyum et al., 2016; Salma et al., 2018).

2.6. Effect against phenylephrine, K^+ (80 mM)

Phenylephrine (1 μM) and K^+ (80 mM) were used to contract the rings, plumbagin was added and the response was recorded as a percentage of the contractions induced by the agonists (Qayyum et al., 2016).

2.7. Effects on calcium channels and intracellular Ca^{2+} stores

Control concentration response curves (CRCs) of $CaCl_2$ were obtained in Ca^{2+} free Krebs solution, in the absence and presence of plumbagin (30 μM) and reference standard nifedipine (10 μM) to evaluate the effect on calcium channels. The vascular reactivity of plumbagin was evaluated on Ca^{2+} release from internal store(s). Isolated aorta rings were treated with phenylephrine to induce the first contraction, and KCl (60 mM) was added to allow the re-filling of the internal stores, afterwards the solution was replaced with Ca^{2+} free/EDTA solution, followed by an incubation period before the addition of plumbagin and nifedipine respectively. The rings were incubated with increasing concentration of the compound and phenylephrine (1 μM) was added to induce the second contraction (Lee et al., 2013; Qamar et al., 2018).

2.8. Effects on potassium channels

The effect of plumbagin on the vascular endothelium was evaluated in the presence and absence of potassium channel blocker $BaCl_2$ and tetraethylammonium (TEA) (Ahmad et al., 2020).

2.9. Studies on rat atrial contractility

2.9.1. Isolated right atrial preparations

Rat right atria was excised, cleaned and mounted in aerated Krebs solution and maintained at a temperature of 37 °C. The rate and force of contractions were measured via a pressure transducer connected to a bridge amplifier and PowerLab Data Acquisitions system. Resting tension of 1 g and an equilibrium period of 30 min was given. Control responses with isoprenaline (1 μM) and acetylcholine (1 μM) were used to validate the protocol. To determine the involvement of muscarinic and beta adrenergic receptors, atrial strips were pretreated with atropine (1 μM) and atenolol (1 μM) (Salma et al., 2018) respectively.

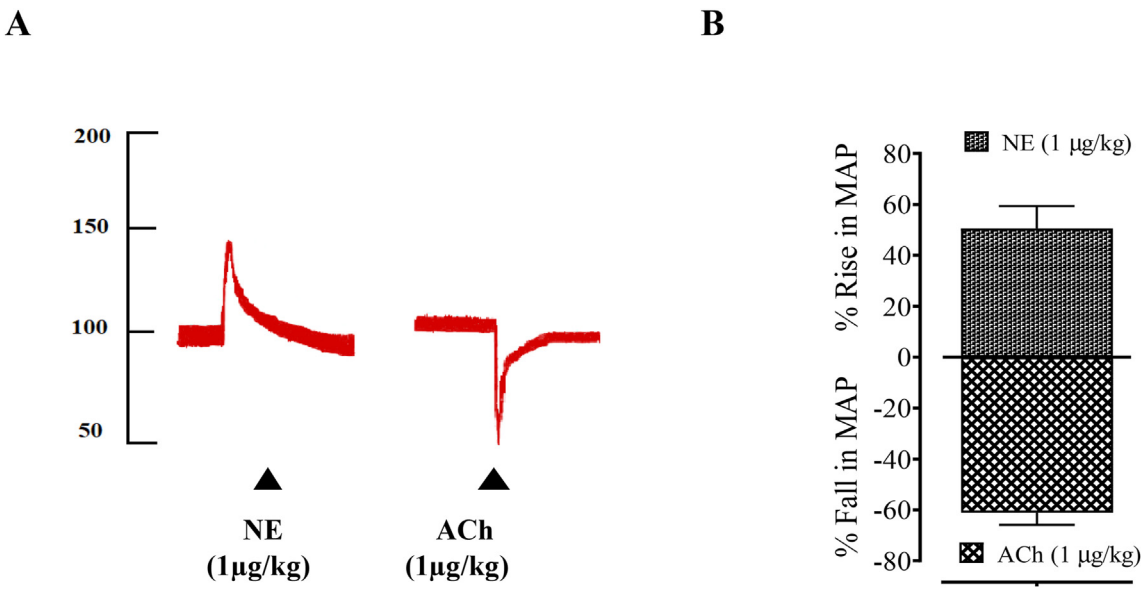

Fig. 2. (**A**) Typical tracing depicting the incline and decline of blood pressure induced by administration of norepinephrine (NE) and acetylcholine (ACh). (**B**) The percentage changes in mean arterial pressure (MAP) after dosing with NE and ACh.

2.10. Statistical analysis

The data was entered and statistical tools were applied using Graph Pad Prism version 8. The data is given as Mean ± standard error of the mean (SEM) for a total of six (6) observations in each experiment conducted on rats (n = 6). The median effective concentrations (EC_{50} values) i.e. the concentration of plumbagin (µM), required to produce a half-maximal reduction in the contraction, are given. They were calculated by interpolation from semi-logarithmic plots, and are expressed as geometric means with 95% confidence intervals.

3. Results

3.1. Effects on blood pressure in normotensive anesthetized rats

To validate the protocol, 1 µg/kg norepinephrine and 1 µg/kg acetylcholine were administered intravenously to confirm the subsequent incline and decline in blood pressure of the live animal, respectively (Fig. 2). Plumbagin induced a dose-dependent fall in MAP after intravenous administration of different doses. The baseline MAP was 100 ± 8.75 mmHg. The percentage fall in MAP was 7.16 ± 2.35, 15.5 ± 5.62, 19.5 ± 5.27, 26 ± 6.67, 34.33 ± 8.80 mmHg at the doses of 0.1, 0.5, 1, 5, 10 µg/kg, respectively (Fig. 3).

3.2. Vascular studies

3.2.1. Endothelium-dependent and independent effects

Endothelium intact rings were contracted with phenylephrine (1 µM) and treated with increasing concentration of plumbagin, a resultant relaxant effect on the vasculature was observed with EC_{50} value of 21.98 µM (17.3–26.5) as compared to Ach EC50 value of 0.08 µM (0.10–0.06). Upon removal of endothelium, there was no significant change in the vasorelaxant response in denuded aortic rings, EC_{50} value was 22.89 µM (16.1–29.7). Pretreatment with L- NAME and methylene blue (MB), were

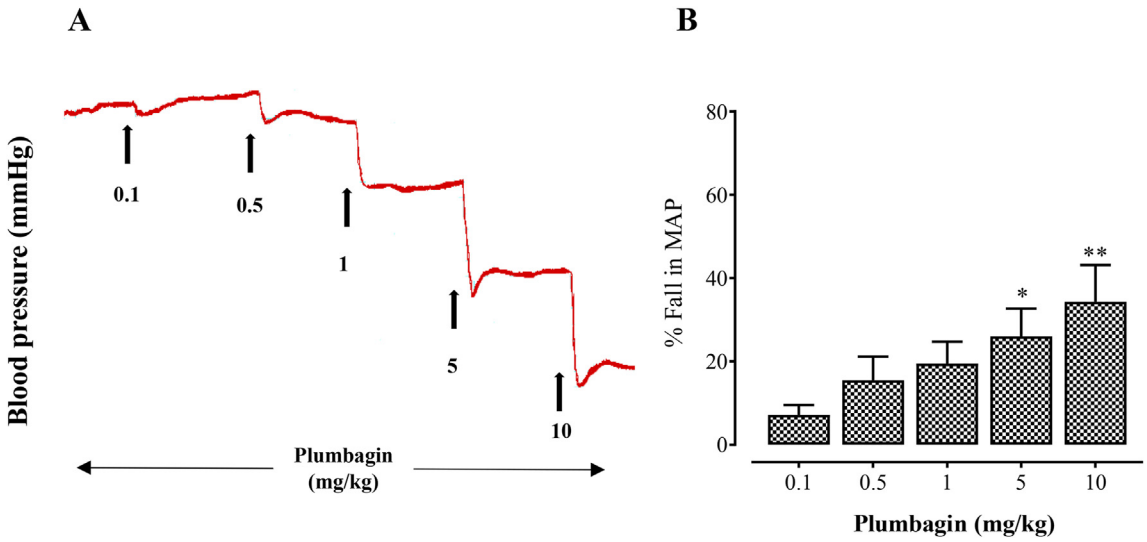

Fig. 3. (**A**) Representative tracing showing the fall in blood pressure induced by intravenous administration of plumbagin in normotensive anesthetized rat (**B**) Blood pressure lowering effect of plumbagin on MAP, bars represent the mean ± SEM for six (6) observations. One-way ANOVA followed by Dunnett's multiple comparison, displayed a statistical difference from the 1st dose, with *$p < 0.05$ and **$p < 0.01$.

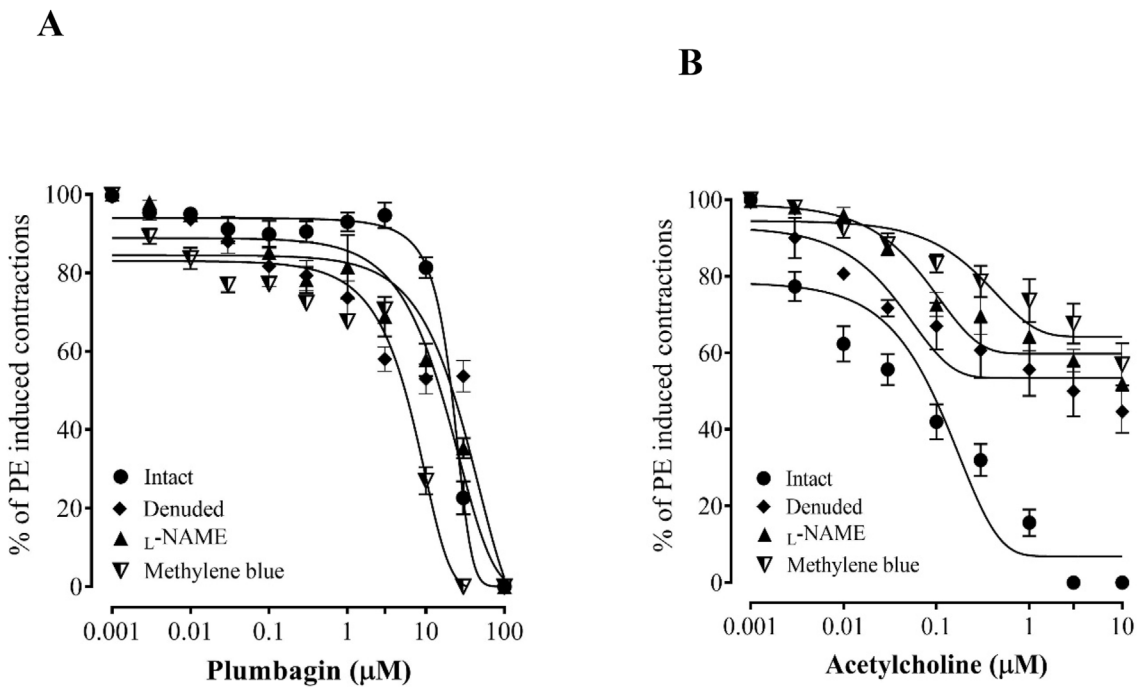

Fig. 4. The response of plumbagin **(A)** and acetylcholine **(B)** on phenylephrine (PE) induced-contractions in aortic rings that were intact, denuded and pretreated with L-NAME and MB.

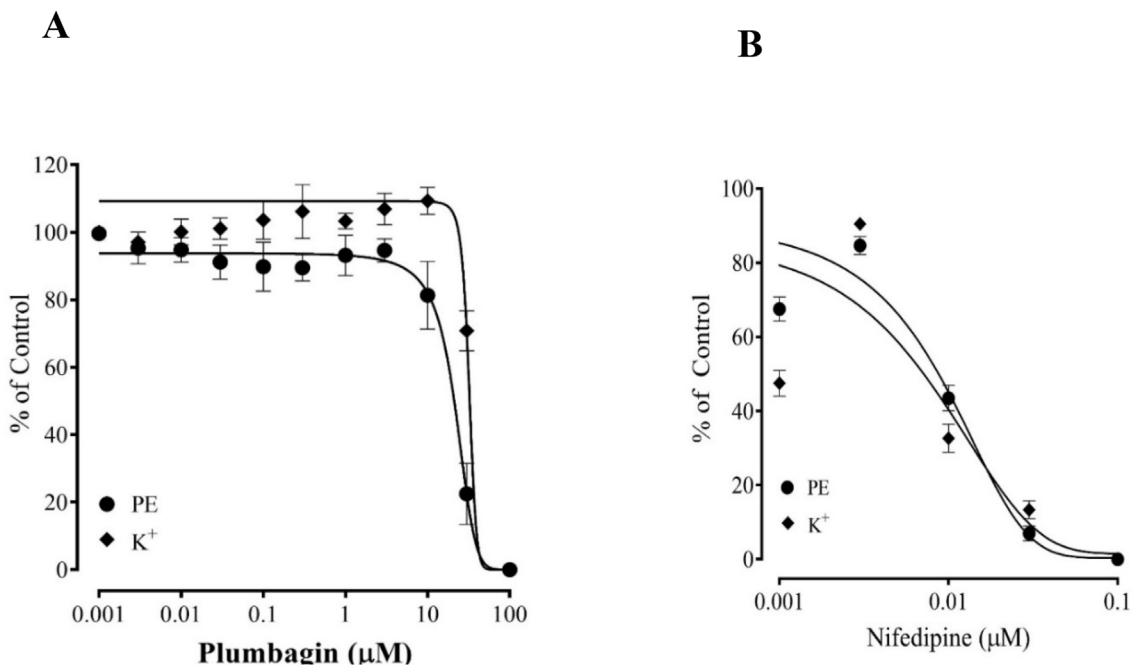

Fig. 5. The graphs show vasorelaxant effect of plumbagin (A) and nifedipine (B) on phenylephrine (PE) and high K^+ (80 mM) pre-contracted rat aortic rings.

performed, which also did not inhibit the vasorelaxant response of plumbagin, EC50 values were recorded as 14.67 μM (11.8–17.5) and 5.3 μM (4.6–6.1), and respectively. The findings and results of plumbagin (Fig. 4A) were compared with the standard drug acetylcholine (ACh) (Fig. 4B).

3.3. Effects against phenylephrine and high K^+ (80 mM) induced contractions

Plumbagin exerted a concentration-dependent vasorelaxation response in isolated rat aortic rings pretreated with phenylephrine (1 μM) and high K+ (80 mM), with EC50 values of 22.2 μM (19.1–25.5) and 33.26 μM (28.9–37.4) (Fig. 5A). The response of plumbagin was compared with calcium channel blocker nifedipine (Fig. 5B).

3.4. Effects on calcium channels

After incubation with plumbagin, aortic rings in Ca^{2+}-free/EDTA medium markedly inhibited calcium concentration response curves, resulting in a rightward shift, similar manner as nifedipine (Fig. 6A and B).

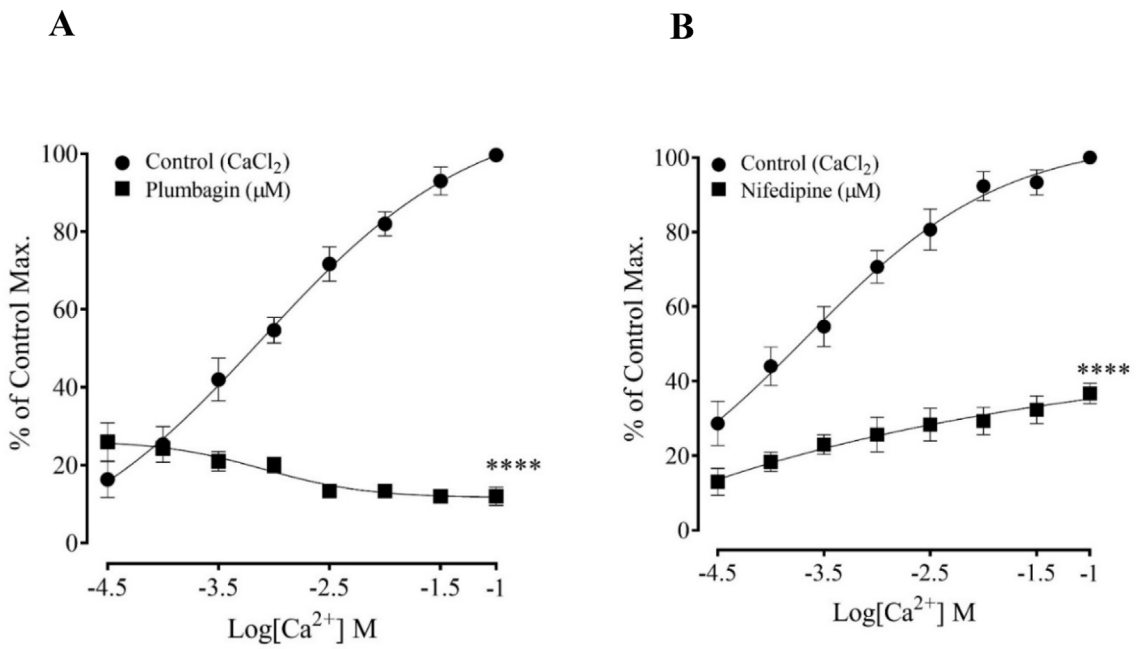

Fig. 6. Graph depicts the inhibitory effect of **(A)** Plumbagin 30 μM and **(B)** and Nifedipine 10 μM in isolated rat preparations against the calcium chloride induced contractions in Ca^{2+}-free/EDTA medium. ****$p < 0.0001$ represents the significant difference between the control and treated groups.

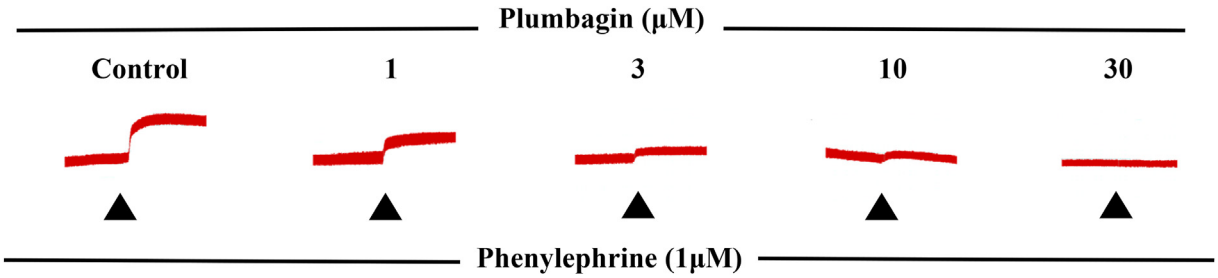

Fig. 7. Representative tracing showing inhibitory effect of increasing concentrations of Plumbagin on the initial peak formation of phenylephrine (PE)-induced contractions in Ca^{2+}-free/EDTA medium.

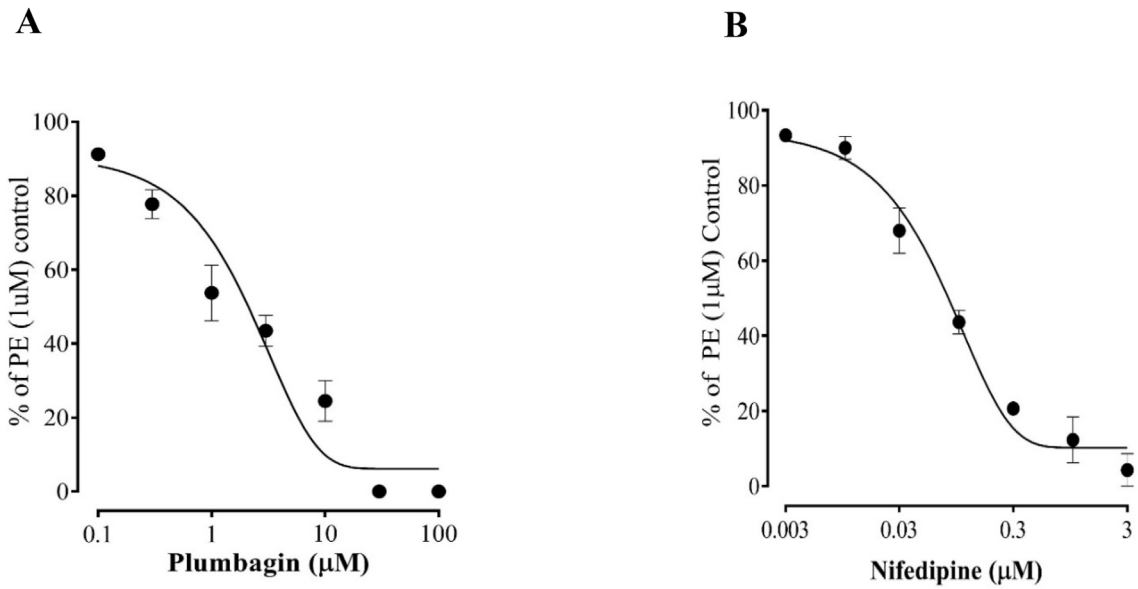

Fig. 8. Graph shows the inhibitory effect of increasing concentrations of (A) plumbagin and (B) nifedipine on the initial peak formation of PE-induced contractions in Ca2+-free/EDTA medium.

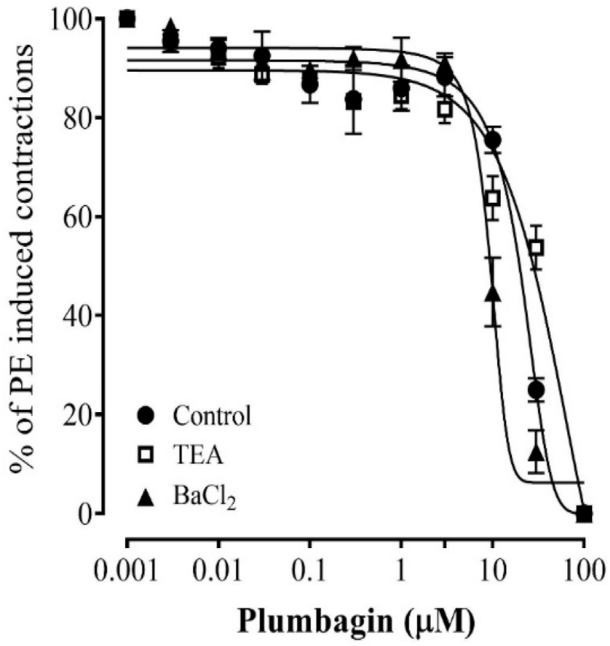

Fig. 9. Graph depicts the vasorelaxant effects of Plumbagin in isolated rat aorta, against 1 μM phenylephrine induced contractions in the absence and presence of potassium channel blockers, tetraethylammonium (TEA) 5 mM and barium chloride ($BaCl_2$) 30 μM.

3.5. Effects on intracellular Ca^+ stores

When investigating the response on cellular calcium stores, pre-treatment of aortic rings with plumbagin (0.1 μM–30 μM) attenuated the individual peaks formed due to phenylephrine (Figs. 7 and 8A), in Ca^{2+}-free/EDTA medium, similar to the standard calcium channel blocker, nifedipine, with EC_{50} values of 2.32 μM (1.96–2.57) and 0.08 μM (0.18–0.09), respectively (Fig. 8B).

3.6. Effects in the absence and presence of K^+ channel blockers

The effects via potassium channels, were tested in pre-contracted aortic tissues in the presence of K^+ channel blockers, tetraethylammonium (TEA) barium chloride ($BaCl_2$) (Fig. 9). The effect of plumbagin remain unchanged in the presence of these potassium blockers, with EC_{50} values for control, TEA and $BaCl_2$ being 20.28 μM (18.35–22.4), 30.3 μM (27.8–33.2) and 8.68 μM (7.59–10.34) respectively.

3.7. Studies on rat atrial contractility

3.7.1. Effects on rate and force of atrial contraction

To validate the protocol 1 μM isoproterenol (ISO) and 1 μM acetylcholine (ACh) were administered intravenously to confirm the subsequent incline and decline in the normal rate and force of atrial contraction, respectively. Plumbagin concentrations were added commutatively to the isolated rat atrial strips, which resulted in complete suppression of the rate and force of atrial contraction (Fig. 10A), with EC_{50} values of 8.7 μM (20.1–11.7) and 30.1 μM (47.9–18.1). The inhibitory response of plumbagin was not significantly altered in the presence of blockers atropine and atenolol (Fig. 10B and C). The EC_{50} values of plumbagin on the rate and force after pretreatment with atropine were as 1.2 μM (1.8–0.6) and 41.2 μM (65.0–23.8) and atenolol were 35.2 μM (57.2–22.0) and 20.1 μM (41.5–21.4).

4. Discussion

Plumbagin, a naturally occurring 1,4-naphthaquinone is widely distributed in many plants of the family Plumbaginaceae. Plumbagin possess a wide variety of pharmacological actions, and has been most extensively investigated against different types of cancers (Checker et al., 2018). Previously, the compound has been identified as an anti-atherosclerotic, hypolipidemic, anti-oxidant and cardioprotective agent (Sharma et al., 1991; Wang et al., 2016; Tan et al., 2011). The hypotensive potential of plumbagin was investigated in this study via *in-vivo* and *in-vitro* experimentation in rats. Hypertension is characterized by an increase in the cardiac output and vascular resistance, that is determined by the contraction or relaxation of the vascular smooth muscle cells (Foex and Sear, 2004). Being a multifactorial disease, HTN occurs due to factors that result in disturbances of the normal compensatory and regulatory mechanisms of blood pressure. For example, an increased circulatory volume which may be due to an imbalance in the sodium retention and excretion process (high salt intake/activation of RAS), would result in high amounts of blood sodium and an increase in blood fluid volume. The excessive salt would cause endothelial dysfunction and vasoconstriction, while high fluid volume would affect the cardiac output. Similarly, autonomic dysfunction with an over-stimulation of sympathetic activity would increase the cardiac output and vascular resistance. Additionally, there may be increased peripheral vascular resistance by the action of vasoconstrictors or alterations in the vasculature (Ohishi, 2018; Soumen, 2018). In this study, the blood pressure lowering-effect of plumbagin was investigated using the invasive blood pressure (IBP) apparatus, which is accepted as the gold standard for the measurement of blood pressure (Parasuraman and Raveendran, 2012). Changes on the vascular tension were monitored by experimenting on the isolated rat thoracic aorta, while cardiac depressant effects were detected by monitoring the force and rate of contraction on the isolated right atrial strips. Plumbagin was injected intra-venously in normal anesthetized rats and lowered blood pressure significantly, by inducing a fall in the mean arterial pressure (MAP) in a dose-dependent manner. The maximum decline in MAP was observed at dose 10 mg/kg of plumbagin having an approximate 34% reduction.

The vascular modulation of blood pressure involves the endothelial cells and the smooth muscles. The endothelium membrane regulates blood pressure by releasing certain vasoactive endothelium derived factors such nitric oxide (NO) and prostacyclin (PGI2), which lead to vasorelaxation (Oparil et al., 2018; Panthiya et al., 2019), and endothelin, which cause vasoconstriction (Beevers et al., 2001). Additionally, ion channels like calcium channels and potassium channels on the smooth muscle cells control blood pressure by influencing ion efflux and influx, these are known as the endothelium-independent pathways (Ch'ng et al., 2017).

The vascular mechanisms which led to the blood pressure lowering activity of plumbagin were explored by *in-vitro* studies on isolated rat aorta. For differentiating whether or not plumbagin acted via endothelium dependent or independent pathways, it was tested against constricted aortic rings with either endothelium intact or removed and damaged. Plumbagin relaxed the phenylephrine (PE) induced peaks both in the intact and denuded rings. This effect was not significantly changed in the rings in which the endothelial lining had been damaged. The involvement of the endothelium was further investigated. Pre-treatment with $_L$-NAME, an inhibitor of nitric oxide synthase (eNOS), was unable to attenuate the vasorelaxant effect of plumbagin. Similarly, pre-treatment with a soluble guanylate cyclase (sGC) inhibitor, methylene blue, did not lead to any suppression in the response by plumbagin. The eNOS catalyzes the formation of nitric oxide (NO) in the endothelium, NO then diffuses across the membrane towards the smooth muscle cells. In the VSMCs, NO stimulates sGC mediated formation of cGMP, which

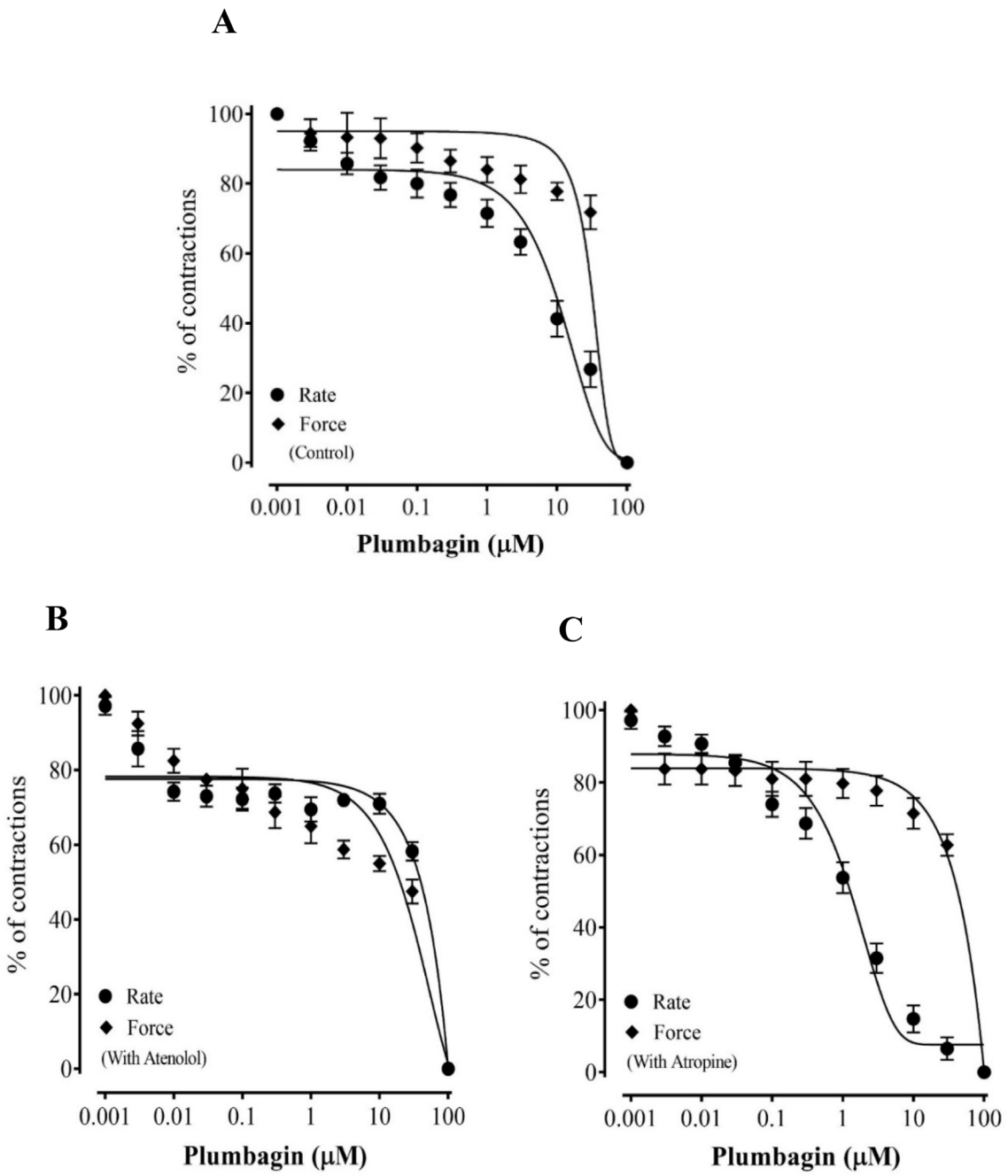

Fig. 10. Graphs showing the responses of plumbagin (A) on the rate and force of atrial contractions, and after pretreatment with (B) atropine (1 μM) and (C) atenolol (1 μM).

promotes vascular relaxation (Panthiya et al., 2019), thus the vaso-relaxant effect with L-NAME and methylene blue pre-treatment indicated that plumbagin did not act on the NO-synthase pathway or via the NO-cSG-cGMP cascade. These important findings ruled out the involvement of the nitric-oxide linked endothelial vasorelaxation and indicated that the blood pressure lowering mechanisms of plumbagin were endothelial independent.

A highly interesting bi-phasic response of plumbagin was observed against vasoconstrictors phenylephrine and High K^+, characterized by a transient increase in vasoconstriction of the aortic tissue, followed by subsequent gradual vasorelaxation. This augmentation of phenylephrine - induced contraction by plumbagin has also been similarly reported in rat renal and mesenteric arteries (Kim et al., 2017). Phenylephrine binds to the α_1-receptors on the smooth muscles and through G-protein coupled mechanisms, induces the activation of (Inositol-1,4,5-triphosphate) IP3 and diacylglycerol (DAG), and the release of calcium from intracellular stores and influx of calcium through the extracellular calcium channels (Karen, 2015). Similarly, high K^+ induces an influx of calcium through the extracellular voltage-dependent L-type Ca^{2+} channels which leads to contraction of the vascular smooth muscles (Qamar et al., 2018). Based on these findings, it could be hypothesized that plumbagin inhibits calcium influx, possibly by acting on voltage dependent calcium channels (VDCC) or by inhibiting the release of calcium from intracellular stores, as it suppressed the contractions induced by high K^+ and PE in tested aortic preparations. To test the activity of the compound against voltage dependent calcium channels (VDCCs), concentration response curves (CRCs) of calcium chloride ($CaCl_2$) were created in a Ca^{2+}-free EDTA-Krebs solution, both in the presence and absence of plumbagin (30 μM).

The dihydropyridine calcium channel blocker, nifedipine (10 μM) was used for comparison. Plumbagin successfully inhibited the contractions induced by calcium chloride and a right-ward shift in the CRCs was observed, resembling nifedipine. This supported the calcium-channel blocking activity of plumbagin. To test if plumbagin possessed any effect on the release of calcium from the intracellular stores, individual phenylephrine peaks were produced both in the presence of plumbagin and the vehicle. This pre-treatment successfully inhibited the phenylephrine-induced contraction, indicating that plumbagin successfully suppressed calcium release from the intracellular stores and further confirmed the calcium-channel blocking activity of plumbagin.

Alongside calcium channels, various potassium channels are present on the vascular smooth muscle cells, which are involved in regulating blood pressure and vascular tone. Plumbagin was tested against two potassium channel blockers i.e. TEA (tetraethylammonium) an inhibitor of calcium-activated K^+ channels (BK_{Ca}), and $BaCl_2$ (barium chloride) a blocker of inward rectifying K^+ channel (K_{ir}) (Ahmad et al., 2020). No significant change in the vasorelaxant response with or without the addition of K^+ channel inhibitors, indicating that these potassium channels were not involvement in the blood pressure lowering effect of plumbagin.

Another variable in blood pressure is cardiac output, a factor that may be influenced by agents having inotropic and chronotropic effects on the heart. Plumbagin exerted negative chronotropic and ionotropic effects, significantly decreasing the rate and force of contraction in spontaneously beating rat atrial strips. To test whether the negative inotropic and chronotropic effects were exerted via blockade of the muscarinic or beta-adrenergic receptors, the effect of plumbagin was observed after pre-treatment with atropine and atenolol, respectively. This pre-treatment did not diminish the lowering and inhibitory effects of plumbagin on the force and rate of contraction, suggesting that the negative ionotropic and chronotropic action of plumbagin could be independent of muscarinic and beta-adrenergic receptors. Possibly plumbagin may produce this response by inhibiting the calcium influx or the intracellular release of calcium in cardiac cells. However, this claim would require further investigation.

Altogether, these new findings on the hypotensive abilities of plumbagin could suggest the possible benefit of the compound against hypertension. Plumbagin is previously reported as an anti-oxidant, anti-inflammatory, anti-atherosclerotic and cardioprotective agent. Such properties in combination with the vasorelaxant and negative chronotropic and inotropic effects may support further studies on the effects of plumbagin against hypertension and cardiovascular diseases.

5. Conclusion

The present study concluded that plumbagin a naturally occurring phenolic compound exerted a significant hypotensive effect in normotensive Sprague Dawley rats. The vascular mechanism for the blood pressure lowering effects were investigated and found to include endothelial-independent pathways, predominantly via suppressing the influx of calcium ions through the calcium channels and release of calcium from the endoplasmic stores. Furthermore, plumbagin also exerted significant negative chronotropic and inotropic effects. These effects may contribute to the blood pressure lowering properties of plumbagin. More investigation would be required to explore the molecular pathways in the blood pressure lowering mechanism(s) of plumbagin.

Credit author statement

Maira Ahmad, Taseer Ahmad: Conceptualization, Methodology, Software, Hafiz Muhammad Irfan,.: Data curation, Writing – original draft: Visualization, Investigation.: Taseer Ahmad, Supervision: Nabeela Noor: Software, Validation: Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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