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N-2 Polyphenol Targets Vascular Calcium Channels to Exert Antihypertensive Effects: *In Vitro* and *In Vivo* Evaluation

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Abstract

This study investigated the antihypertensive potential of the natural compound N-2 polyphenol using both *in vitro* and *in vivo* approaches. *In vitro* assays focused on its effects on vascular smooth muscle cells, particularly voltage-dependent L-type calcium channels, receptor-operated calcium channels, and endothelium-mediated pathways. N-2 significantly inhibited Ca^{2+} influx through L-type channels, producing 86.2 ± 2.4% relaxation at 50 µM (IC_{50} = 30 µM), and suppressed receptor-operated channel-mediated contractions by 90.5 ± 3.5% at 45 µM. Endothelium-dependent vasorelaxation was partially mediated by nitric oxide (NO), as shown using the NOS inhibitor L-NAME (100 µM). In endothelium-denuded preparations, N-2 still reduced contractility by 38.0 ± 3.1%, suggesting partial NO-cGMP-PKG pathway involvement. *In vivo*, an adrenaline-induced hypertension model in rats showed that intravenous N-2 markedly lowered blood pressure. Three h after administration, systolic and diastolic pressures dropped to 74.7 ± 3.3 and 62.7 ± 4.1 mmHg, respectively. These results demonstrate that N-2 polyphenol exerts strong antihypertensive effects through both calcium channel blockade and endothelium-dependent mechanisms. Its dual mode of action highlights N-2 as a promising candidate for managing hypertension.

Keywords: N-2 polyphenol, Antihypertensive activity, L-type Ca^{2+} channels, Receptor-operated Ca^{2+} channels, Vascular smooth muscle, Nitric oxide (NO), *In vitro*, *In vivo*, Adrenaline-induced hypertension, Calcium transport, Endothelial function, Tail-cuff method

Introduction

Natural bioactive properties by plant-based compounds, mainly polyphenols, have gained large-scale scientific interest in recent times due to their medicinal actions and limited side effects. Polyphenols are considered a valuable and possibly hepatoprotective product to prevent and treat cardiovascular diseases that exhibit antioxidant, anti-inflammatory and improved endothelial functions [1]. Some of these polyphenols may also induce responses that could affect the regulatory mechanisms of blood pressure and regulate pathological mechanisms of injury associated with arterial hypertension [2]. Arterial hypertension is a global health problem, and one of the most important pathophysiological mechanisms of cardiovascular diseases. The identification of the modes of action by naturally derived compounds that lower blood pressure is a major direction of research in pharmacology [3]. Polyphenols, and particularly N-2 polyphenol, are

expected to be beneficial agents to help regulate vascular tone by a pathway involving L-type calcium (Ca^{2+}) channels, nitric oxide (NO) mechanisms, and other secondary messengers. We used experiments *in vivo* and *in vitro* to investigate the effect of N-2 polyphenol on blood pressure. *In vitro*, the effect of N-2 polyphenol on the aortic smooth muscle rings contractile force were determined, and *in vivo* we examined blood pressure changes in the rat model because they are widely used comparative creatures in these testing scenarios [4].

Materials and methods

N-2 polyphenol

The compound designated as N-2 polyphenol is a substituted phenolic ester featuring 3 functional groups on a benzene ring: A methoxy group (-OCH_3), a hydroxyl group (-OH), and an ethyl ester moiety (-$COOC_2H_5$). Its molecular formula is $C_{11}H_{14}O_5$ and the molecular weight is 226 g/mol (**Figure 1**). The spatial arrangement of these substituents confers the compound both antioxidant and potentially hepatoprotective properties, due to its electron-donating phenolic groups and ester-linked functionality. This molecule is structurally related to polyphenolic compounds commonly found in medicinal plants, which are known to scavenge free radicals and modulate redox-sensitive cellular pathways. Its chemical profile suggests potential application as a bioactive agent in oxidative stress-related models and as a lead compound for antiviral or anti-inflammatory drug development.

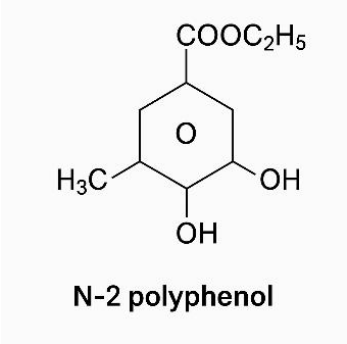

Figure 1 2D chemical structure of N-2 polyphenol ($C_{11}H_{14}O_5$). The compound features a substituted benzene ring bearing an ethyl ester group (-$COOC_2H_5$), a hydroxyl group (-OH), and a methoxy group (-OCH_3). The spatial arrangement of these functional groups contributes to the compound's antioxidant potential and suggests possible bioactivity relevant to oxidative stress and hepatoprotection.

Chemicals

In this study, N-2 polyphenol, isolated from local medicinal plants, was utilized. Additionally, other chemical reagents used in the experiments - phenylephrine (Phe), phentolamine, and verapamil - were purchased from Sigma-Aldrich Chemie, a division of Sigma-Aldrich based in St. Louis, Missouri, USA.

Animal ethics

All preoperative and experimental protocols were read and approved slowly by the Institutional Committee for Animal Use and Care. The animals lived in vivarium rooms under controlled conditions, relative humidity of 55% - 65%, ambient temperature of 22 ± 2 °C, and had free access to water and normal laboratory chow. All animal handling and care procedures rigorously followed the European Directive 2010/63/EU on the protection of animals used for scientific purposes. Ethical approval for this research was provided by the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Animal Ethics Committee (Protocol No. 133/1a/h, 4 August 2016).

Tissue preparation

All the exploration protocols were approved by the Institutional Committee for the care and use of laboratory animals. The experimental protocol was conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes [5]. All surgical procedures were performed under sodium pentobarbital anesthesia to reduce the perception of pain. Euthanasia were conducted by cervical dislocation; then, the thoracic aorta was removed and submerged in a 5 mL organ bath

containing Krebs-Henseleit solution. The Krebs-Henseleit solution contained: NaCl (120.4 mM), KCl (5 mM), $NaHCO_3$ (15.5 mM), NaH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), glucose (11.5 mM) and HEPES adjusted to pH 7.4. In some of the experiments, we also performed a calcium-free Krebs solution with the addition of 1 mM EGTA [6].

The temperature of the solution was held at 37 °C and continuously bubbled with carbogen (95% O_2 and 5% CO_2). The aorta was cleaned of any connective tissue and fat, cut into rings of 3 - 4 mm length and set up for physiological experiments.

Aortic-ring contraction studies

Aortic rings were carefully mounted onto a Radnoti isometric transducer using platinum wire hooks and allowed to equilibrate for 60 min prior to recording measurements. An initial resting tension of 1 g (10 mN) was applied to each tissue ring (**Figure 2**). The contractile responses generated by the rings were detected via a signal amplifier, converted into digital format, and visualized in real time on a computer screen using a Go-Link data acquisition interface. The obtained data were processed and analyzed using OriginPro v8.5 SR1 software (OriginLab Corp., Northampton, MA, USA). The isometric tension values recorded under *in vitro* conditions were normalized and expressed as a percentage of the maximal contraction force (mN) [7].

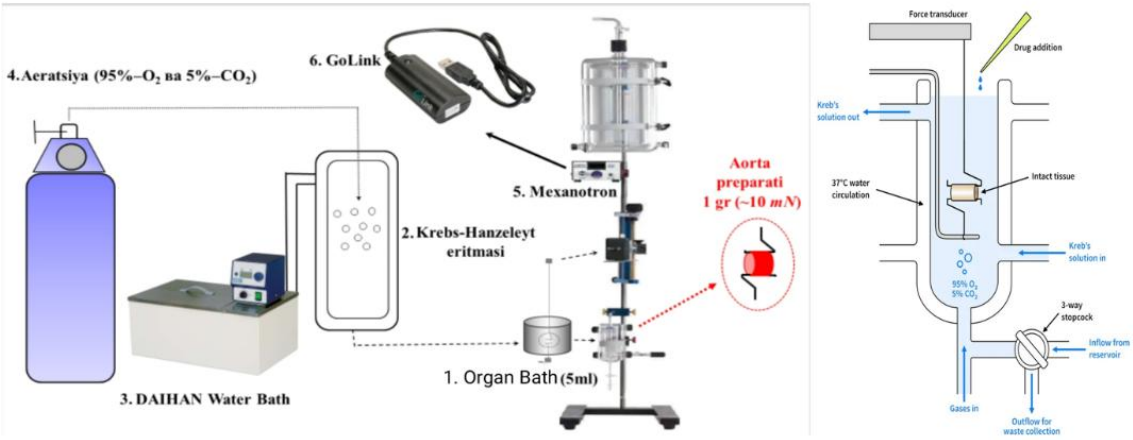

Figure 2 Schematic representation of the apparatus designed for controlling and measuring isometric contraction of isolated rat aortic smooth muscle. 1) The organ bath (5 mL) circulates solution via a specialized reservoir, 2) Krebs-Henseleit solution maintains physiological conditions, 3) A thermostat ensures constant physiological temperature, 4) The system is oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The contractile activity of the aortic preparation is maintained within the experimental chamber, 5) An isometric transducer (Grass Instrument, USA) records the contractions, 6) GoLink devices amplify and support signal acquisition.

Blood pressure measurements

All experiments were conducted using adult male Sprague-Dawley rats, weighing 200 - 250 g at the start of the experiments. Male animals were specifically chosen to reduce hormonal variability and ensure consistency in vascular reactivity and hypertension modeling. Rats' arterial blood pressure was assessed using a routine procedure at the Institute of Bioorganic Chemistry named after O. Sodiqov's "Plant Cytoprotectors Laboratory" and "BFM Pharmacology and Screening Laboratory." The Russian-made "Sistola" (Neurobotics) experimental device was used to perform non-invasive measurements via the tail artery [8].

Non-invasive blood pressure measurement was performed using the tail cuff method. In this technique, a specialized cuff is placed around the tail of the rat, and the pressure within the cuff is gradually increased and then decreased. Blood flow restoration is detected using photoplethysmography, allowing for the determination of systolic blood pressure. During measurements, animals were gently restrained and conditions were optimized to minimize stress and movement. The program "AcqKnowledge 4.2 for MP150" was used to evaluate the data (**Figure 3**).

Rats were given a dose of 0.25 mg/kg of adrenaline hydrochloride to induce experimental hypertension. The

tested polyphenol was administered intravenously via the tail vein at doses of 25 and 50 mg/kg to the experimental group following a 30-minute injection of adrenaline hydrochloride, which sustained stable hypertension for 180 min [9].

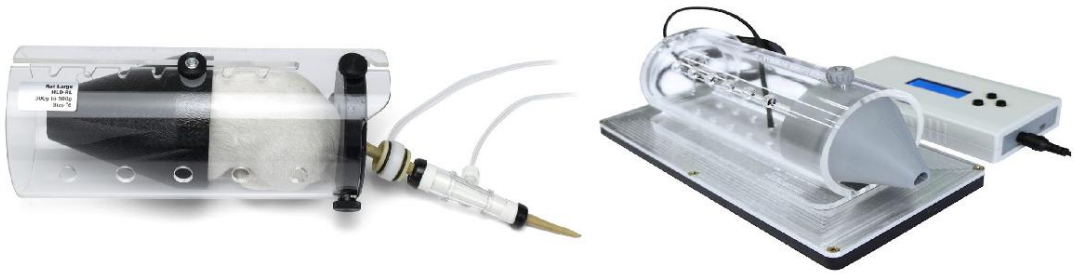

Figure 3 Experimental device "Sistola" (Neurobotics, Russia) used for non-invasive measurement of arterial blood pressure in the tail artery of rats.

Statistical analysis

Statistical analyses and graphing were performed using Origin Pro 9 software (Microsoft, USA). Contraction responses of aortic rings induced by phenylephrine (10 μM) or KCl (50 mM) were expressed as a percentage of the maximal contraction. Results are presented as mean values from 5 - 6 independent experiments (n = 5 - 6). Paired t-tests were used for within-group comparisons, while unpaired t-tests were applied for comparisons between groups. A significance threshold of $p < 0.05$ was adopted.

Results and discussion

***In vitro* experiments on rat aortic smooth muscle**

The contraction of the aortic tissues involved in the KCl (50 mM) response is mediated by opening L-type calcium channels in the smooth muscle cells, which are voltage-dependent [10]. The increase in extracellular K^+ causes depolarization of the cell membrane, which causes L-type calcium channels to open. Aortic rings treated with KCl (50 mM) before N-2 polyphenol treatment demonstrated considerable relaxation [11]. Based on the data obtained, N-2 polyphenol exhibited a reduction in KCl contraction in dose-dependent manner from concentrations of 5 - 50 μM resulting in a decrease in contraction of 5.4 ± 2.5% KCl contraction to 86.2 ± 2.4% KCl contraction. The IC_{50} for N-2 polyphenol in reducing the KCl contraction of aortic rings was estimated to be 30 μM (**Figure 4**).

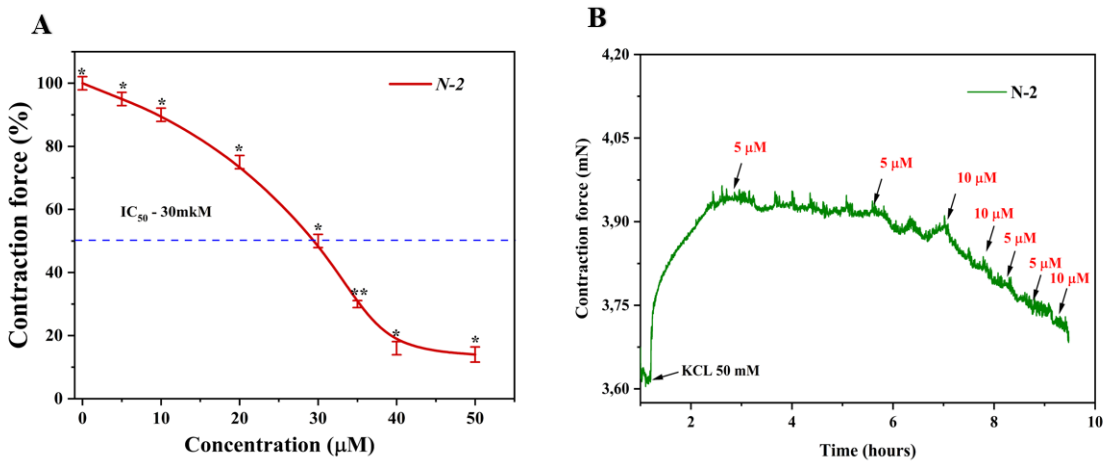

Figure 4 A) Effect of N-2 polyphenol on KCl (50 mM)-induced contraction in isolated rat aortic smooth muscle rings. The contractile response induced by 50 mM KCl was considered 100%, and subsequent relaxation upon cumulative concentrations of N-2 polyphenol (1 - 100 μM) was expressed as a percentage of this maximum contraction. Data are presented as mean ± SEM (n = 3 - 4). All differences compared to KCl-only contraction are statistically significant ($p < 0.05$). B) Representative original tracing showing the relaxation of aortic smooth muscle following N-2 polyphenol administration after contraction by KCl.

In summary, the studies showed that the tested polyphenol inhibited KCl-induced contraction through voltage-dependent Ca^{2+} channel inhibition. To confirm this assumption, assays were performed using Ca^{2+}-free Krebs solution and verapamil (L-type Ca^{2+} channel blocker) [12]. In the absence of extracellular Ca^{2+}, KCl-induced depolarization does not induce contraction; however, with Ca^{2+} (0 - 2.5 mM, the contraction returned) [13]; in our models, we found that N-2 polyphenol (70 and 50 μM) significantly inhibited KCl and Ca^{2+} contraction of aorta rings, when compared with the control group (**Figure 5**).

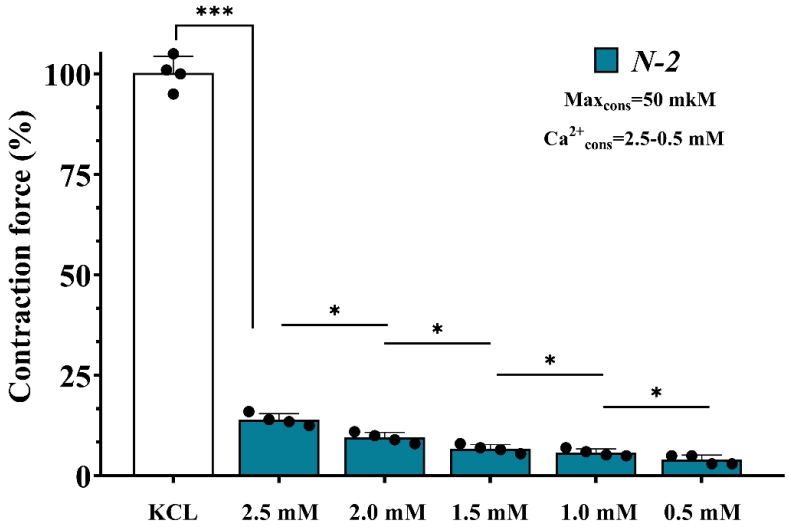

Figure 5 Effect of N-2 polyphenol on the relaxing activity depending on the [Ca^{2+}] concentration in the medium. The vertical axis (ordinate) shows the contraction force of the aortic preparation induced by KCl (50 mM), which is considered as 100%. The horizontal axis (abscissa) represents the Ca^{2+} concentration (0 - 2.5 mM). In all cases, $p < 0.05$; n = 3 - 4.

To evaluate the interaction of the relaxant effect of flavonoids and the specificity of the flavonoid action on voltage-dependent Ca^{2+} channels, we compared their actions with a specific blocker of these channels, verapamil [14]. Verapamil (0.1 µM) is the concentration that causes a half-maximal contraction in aortic preparations that have been contracted by KCl (50 mM). It is probable that the relaxant effect with N-2 polyphenol is through the reduction of influx of Ca^{2+} ions into smooth muscle cells through potential L-type Ca^{2+} channels in the cell membrane. In the interest of evaluating the interaction of this extract with that of verapamil, other experiments were conducted. The concentration of verapamil (0.1 µM) used in these experiments, produces half-maximal contraction and a further evaluation of an additive inhibitory effect of the polyphenol was to be assessed [15]. The effect on KCl (50 mM) induced contraction of aortic preparations in incubation conditions with and without verapamil (0.1 µM) and N-2 polyphenol (30 µM, EC_{50}), with the polyphenol further inhibiting aortic contraction activity by 21.8 ± 3.1%, is shown in **Figure 6**.

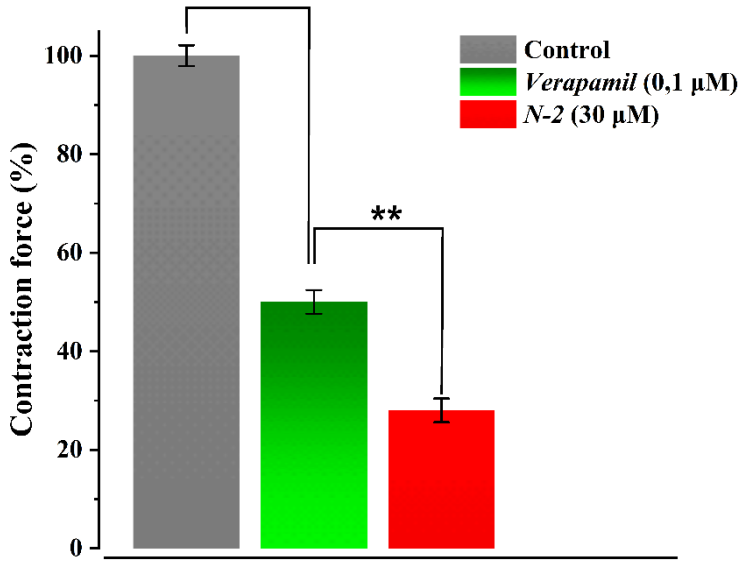

Figure 6 Interaction between N-2 polyphenol and the Ca^{2+} channel blocker verapamil (at EC_{50} concentration) on the contraction of aortic preparations induced by KCl (50 mM). The vertical axis (ordinate) represents the contraction force of the rat aortic smooth muscle induced by KCl (50 mM), normalized to 100%. All values are expressed as mean ± SEM; $p < 0.05$; $n = 3$ - 4.

The polyphenol demonstrated a clear relaxant effect in the experimental results and significantly reduced KCl (50 mM)-induced contractions. These findings suggest that the effect may involve the modulation of voltage-dependent L-type Ca^{2+} channels in the plasma membrane (sarcolemma), potentially limiting the entry of Ca^{2+} ions into the cell and thereby contributing to smooth muscle relaxation [15]. The observed relaxant activity of the polyphenol appears similar to the effect produced by verapamil, a clinically used selective L-type Ca^{2+} channel blocker. While these results imply that the polyphenol may act as a modulator of L-type Ca^{2+} channels, they do not confirm direct binding, and further studies are required to elucidate the precise molecular interaction [16].Significantly, in addition to voltage-dependent L-type Ca^{2+} channels, calcium transport systems such as sarcoplasmic reticulum (SR) pathway also has very important contractile activity in vascular smooth muscle cells.

Thus, in the next experiments, the relaxant effect of N-2 polyphenol on rat aortic smooth muscle preparations was examined by evaluating its effect on the contraction induced by the α-adrenoceptor agonist phenylephrine (1 µM). It is known that the contraction induced by phenylephrine (1 µM) is due to an increase in the intracellular calcium concentration ($[Ca^{2+}]$ in) which results from calcium release from the SR and calcium influx through receptor operated calcium

channels [17]. The experimental results show that N-2 polyphenol at 45 µM inhibited the contraction induced by phenylephrine (1 µM) by 90.5 ± 3.5% compared with control (**Figure 7**). Under these conditions the IC_{50} of N-2 polyphenol was calculated to be approximately 25 µM.

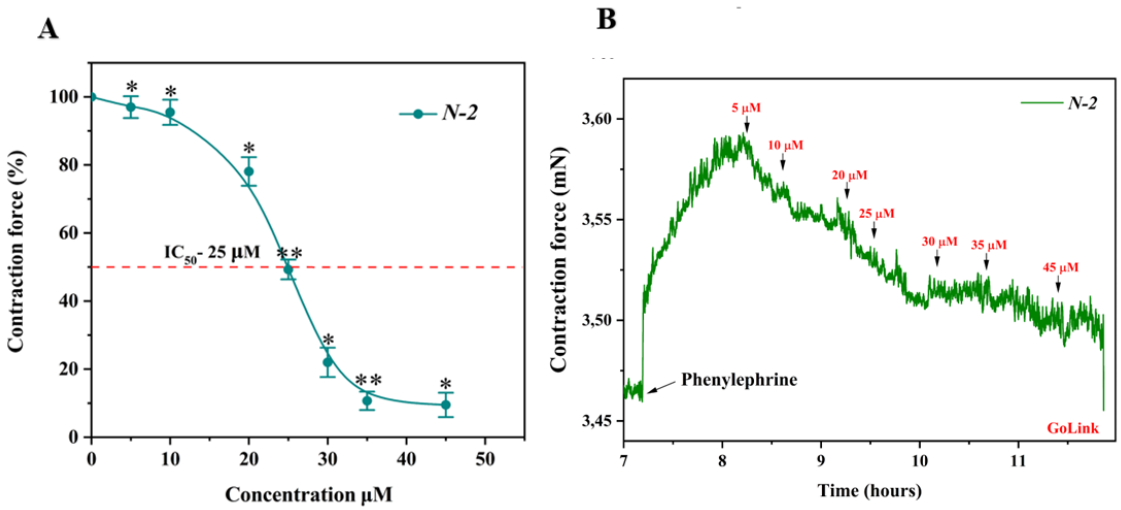

Figure 7 (A) Effect of N-2 polyphenol on contraction of rat aortic smooth muscle induced by phenylephrine (Phe, 1 µM). The vertical axis (ordinate) represents the contraction force induced by Phe (1 µM), normalized to 100%. The horizontal axis (abscissa) indicates the concentration of the extract (µM). All values are statistically significant with $p < 0.05$; n = 3 - 4. (B) Original recording.

The results show that blocking receptor-operated Ca^{2+} channels may explain the relaxant action of the tested polyphenol. The effects of the polyphenol and the α-adrenoceptor blocker phentolamine were evaluated [18] in order to investigate this process more. In the absence of phentolamine, N-2 polyphenol (45 µM) lowered the contraction caused by 1 µM phenylephrine as seen in prior investigations. The contraction generated by 1 µM phenylephrine fell by 83.4 ± 2.7% when 10 µM phentolamine was introduced, compared to the control. Under these circumstances, N-2 polyphenol decreased the contraction by 63.5 ± 3.4% as compared to the control (**Figure 8**).

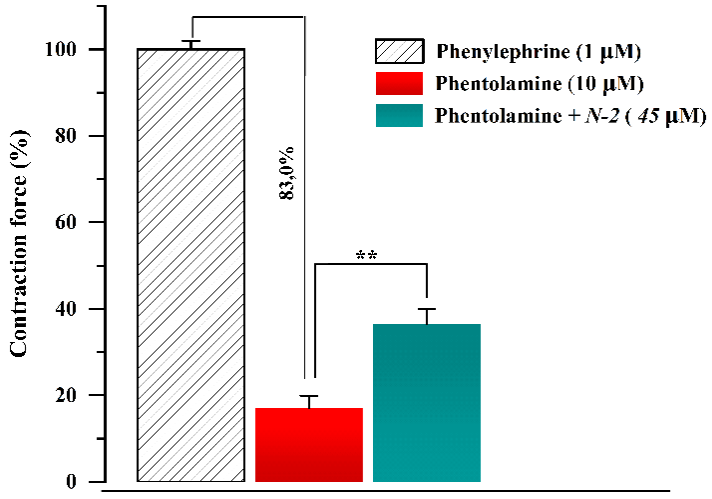

Figure 8 Effect of phentolamine (10 µM) on the relaxant activity of N-2 polyphenol. Aortic contraction was induced by phenylephrine (Phe, 1 µM) and the resulting contractile force was taken as 100%. All values are expressed as mean ± SEM; statistical significance was considered at $p < 0.05$; n = 3 - 4.

In the following experiments, flavonoid impact on Ca^{2+} ion release from the sarcoplasmic reticulum (SR) by IP3 receptors (IP3R) was examined. In the Ca^{2+} ion-free incubation medium, contraction force caused by phenylephrine (1 μM) is a reflection of Ca^{2+} ion release from the SR through IP3R [19]. In our experiment, phenylephrine (1 μM) evoked the contraction force to be 70.3 ± 3.1% of that in normal Krebs solution, the latter being taken as 100%. When the effect of N-2 polyphenol was examined at a dose of 45 μM under these conditions, it was found to reduce the contraction force by 66.7 ± 3.2% from control (**Figure 9**)

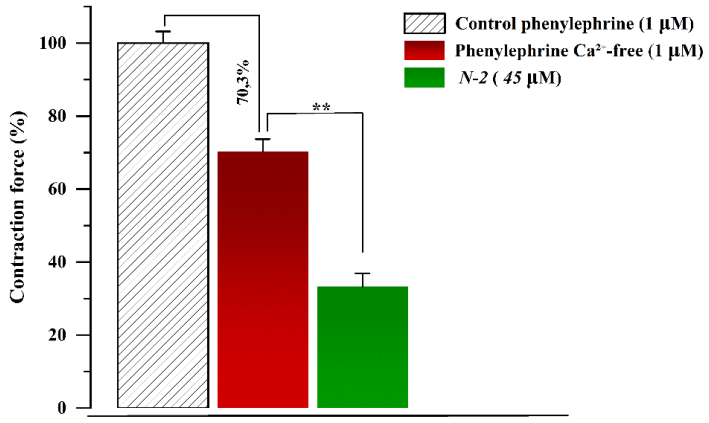

Figure 9 Dose-dependent relaxant effect of PN-2 polyphenol on phenylephrine-induced contraction of rat aorta in Ca^{2+}-free Krebs solution. The contraction of the aorta induced by phenylephrine (1 μM) in Ca^{2+}-free Krebs solution was taken as 100% control (significance levels in all cases: $*p < 0.05$; $p < 0.01$; n = 3 - 4).

These findings suggest that without extracellular Ca^{2+}, N-2 polyphenol significantly reduces the force of contraction caused by phenylephrine (1 μM). This suggests that these kinds of compounds can reduce Ca^{2+} ion release from the SR and consequently influence the intracellular Ca^{2+} concentration ($[Ca^{2+}]$in).

Investigation of the role of the endothelial layer in the vasorelaxant effect of N-2 polyphenol

The endothelium that lines the interior of blood vessels is important in vascular homeostasis and the control of blood flow. Of the numerous signaling molecules that are released by endothelial cells, nitric oxide (NO), a very potent vasodilator, controls vascular pressure and tone.

An imbalance of these signaling molecules is what characterizes endothelial dysfunction (ED), a major causative mechanism in the onset of cardiovascular disease (CVD) such as hypertension and atherosclerosis. With the progression of such events, endothelial cell damage initiates a series of pathologic processes characterized by a uniform decline in vascular function. NO is synthesized by endothelial cells from the amino acid L-arginine by the action of endothelial nitric oxide synthase (eNOS). Calcium and calmodulin characterize the activation mechanism of eNOS. In resting conditions, eNOS is caveolin complexed and membrane microdomain-bound caveolae to a protein referred to as caveolin. When stimulated by stimuli like acetylcholine, bradykinin, or serotonin, eNOS releases caveolin, translocates to the plasma membrane, and becomes activated [20].

Statins, usually prescribed lipid-lowering drugs, also increase production of NO at very low doses (0.1 mM or less). Such an effect is mediated principally by their pleiotropic effect, specifically by inhibiting the binding of eNOS to caveolin-1, allowing eNOS activation. These effects are thought to comprise most of the beneficial effect of statins on endothelial function [21].

Oxidative stress plays a central role in endothelial dysfunction (ED) and atherosclerosis pathogenesis, acting mainly through the breakdown of NO by ROS. Breakdown reduces the vasodilatory effect of NO, with increased vascular constriction as a result. Under normal conditions, NO activates the soluble guanylate cyclase (sGC), which raises intracellular cyclic guanosine monophosphate (cGMP). Increased cGMP,

subsequently, activates the protein kinase G (PKG), with vascular smooth muscle relaxation achieved through decreasing myosin light chain phosphorylation.

Conversely, under conditions of increased oxidative stress, NO bioavailability is decreased due to its spontaneous reaction with ROS. Endothelial cells also regulate vascular tone by producing other signaling molecules, regulation of intracellular Ca^{2+} homeostasis, and effects on vascular smooth muscle cell (VSMC) contractile function. NO spreads into the VSMCs and activates the NO/sGC/cGMP/PKG signaling pathway, which eventually reduces intracellular calcium and leads to relaxation of the smooth muscle. In order to examine the role of the endothelium in the vasorelaxant action of the N-2 polyphenol, experimental research was carried out on isolated rat aortic rings. The endothelial layer was mechanically denuded in certain preparations in order to identify if the action of N-2 is endothelium-dependent or endothelium-independent. To verify the existence of an intact endothelium, 1 μM acetylcholine (ACh) was used [22]. ACh decreased phenylephrine (Phe)-induced contractions by 57.8 ± 4.3% in rings with intact endothelium, whereas in endothelium-denuded preparations (mechanically rubbed with cotton), ACh was virtually ineffective. All contractions were normalized to an amplitude of 10 mN after stimulation with 1 μM Phe. These findings demonstrate that endothelial-derived NO is responsible for a vital role in vascular relaxation. Attenuation of the amplitude of Phe-induced contraction is directly related to the presence of an intact endothelial layer. This attests to the important role of the endothelium for the regulation of vascular tone. These experiments were aimed at investigating the vasorelaxant effects of N-2 polyphenol on rat aortic vessels, with particular focus on the role of the endothelial layer [23].

In functional endothelium preparations, different concentrations of N-2 polyphenol caused relaxation of the vessels, inhibiting phenylephrine-evoked contraction by a mean value of 90.5 ± 3.5% versus control (**Figure 10**). In preparations where the endothelium was mechanically stripped, relaxation activity was greatly inhibited with a mean inhibition of only 38.0 ± 3.1%. All these observations indicate that the vasorelaxant effect of N-2 polyphenol is primarily endothelium-dependent. The surprising decrease in activity of the compound after endothelium removal once again underlines the pivotal position of endothelium in mediating N-2 polyphenol's vasodilatory effects.

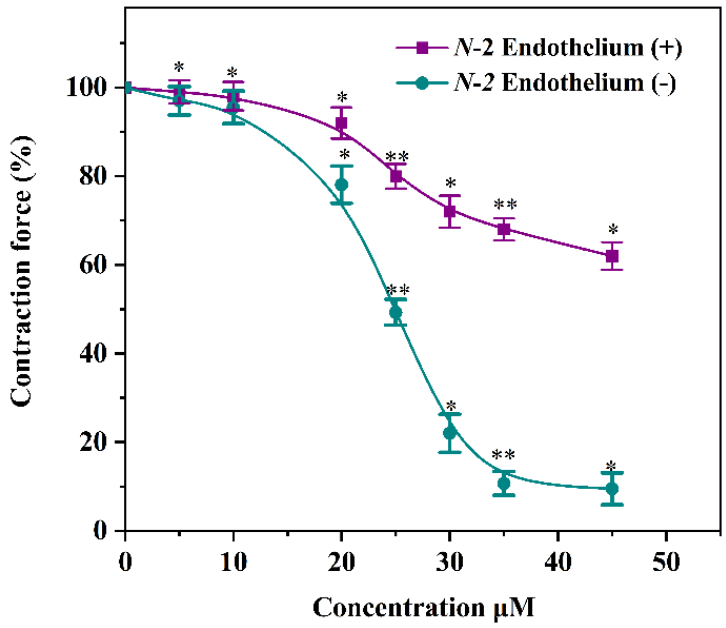

Figure 10 Relaxant effects of N-2 polyphenol on contractions induced by 1 μM phenylephrine (Phe) in the presence (+) and absence (–) of the rat aortic endothelium. The contraction force induced by 1 μM Phe was considered 100% of the control. In all cases, $p < 0.05$; n = 3 - 4.

Our experiments showed that the action of the polyphenol was more or less disparate in aortic preparations without the endothelial layer, implying that such compounds might act directly on the endothelium. To verify this, we performed additional experiments with L-NAME (100 µM), an eNOS inhibitor [24]. The finding indicated that the relaxing action of N-2 polyphenol was abolished from L-NAME-treated aortic preparations. Most significantly, N-2 polyphenol decreased the tension of phenylephrine (Phe)-elicited contraction by 90.5 ± 3.5% in normal endothelial conditions. In the presence of L-NAME, however, this decrease dropped to 36.4 ± 2.4% from the control (**Figure 11**). These findings firmly establish that the relaxation caused by N-2 polyphenol is at least partially through the release of nitric oxide (NO) from the endothelial cells. This is also evidenced by the conspicuous decrease of the relaxation response after inhibiting eNOS with L-NAME and even more so when the endothelium was removed [25].

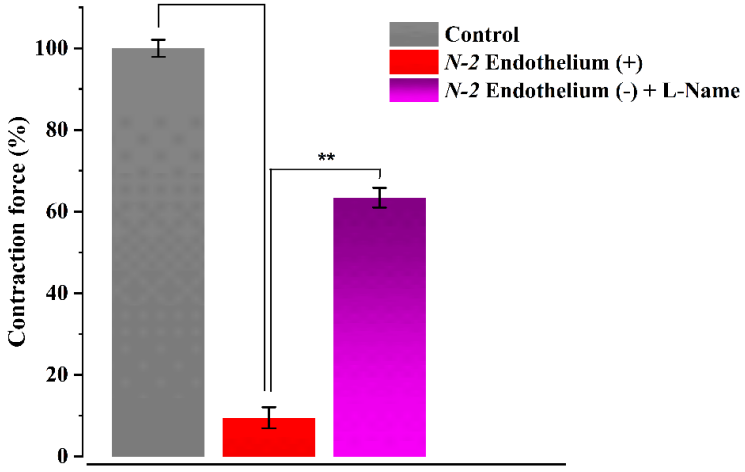

Figure 11 Relaxant effects of N-2 polyphenol and the eNOS inhibitor L-NAME (100 µM) on the contraction of rat aortic preparations under incubation conditions. The contraction force induced by 1 µM Phe was considered 100% of the control. In all cases, $p < 0.05$; n = 3 - 4.

Our experimental results show that the tested polyphenol has an intense relaxant action, whose intensity largely depends on the presence or absence of an endothelial layer. It is reduced considerably if endothelium is eliminated or if NOS is inhibited by L-NAME. This proves the pivotal role played by nitric oxide (NO) in relaxation [26]. The extracts are most likely to cause relaxation by activation of the NOS - cyclic GMP (cGMP) - protein kinase G (PKG) pathway. The pathway functions as follows: Reduces calcium influx through receptor-operated and L-type calcium channels (Ca^{2+} R and Ca^{2+} L) of the plasma membrane [27]. It prevents release of calcium from the sarcoplasmic reticulum (SR). Therefore, intracellular calcium concentration ($(Ca^{2+})_i$) in smooth muscle cells (SMCs) falls, resulting in relaxation of the muscle [28].

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***In vivo* experiments in a rat tail cuff**

In vivo studies were conducted in 2 phases. In the first phase, the hypotensive effect of the N-1 polyphenol and its time-dependent changes in the organism were evaluated. For this purpose, N-2 polyphenol was administered to rats at doses of 25 and 50 mg/kg, and blood pressure was measured hourly over a 4-hour period [29]. Before the experiment, the baseline systolic blood pressure of the rats was 110.3 ± 3.5 mmHg, and the diastolic pressure was 77.8 ± 2.7 mmHg. Following the administration of N-2 polyphenol, a significant decrease in both systolic and diastolic blood pressure was observed (**Table 1**).

Table 1 Effect of N-1 polyphenol at a dose of 25 mg/kg on blood pressure. Statistical processing showed that the greatest blood pressure fall occurred between the 1st - 3rd h interval ($p < 0.05$) after intragastric administration of the 25 mg/kg dose.

Time (h)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
0 (start)	110.3 ± 3.5	77.3 ± 2.7
1st h	94 ± 4.4	59,5 ± 3.4
2nd h	96.3 ± 2.3	69.3 ± 2.2
3rd h	93.5 ± 2.9	71.8 ± 3.0
4th h	109.6 ± 2.8	88.5 ± 2.4

The results show that an active hypotensive action of N-2 polyphenol was present as early as the first h following intragastric administration of the 25 mg/kg dose. The average reduction in systolic blood pressure (SBP) was 16 mmHg, and diastolic blood pressure (DBP) was reduced by 17.8 mmHg. This effect, however, started to wane with time, and blood pressure returned almost to baseline by the 4th h. It implies that N-2 polyphenol has a fast-acting hypotensive effect.

Additionally, from the 4th h onwards, there was a mild rebounding of the blood pressure towards the base level though still below it. This indicates that the compound was being metabolized slowly over time, and its effect was beginning to wear off [30].

At a 50 mg/kg dose, N-2 polyphenol initially displayed hypotensive activity in rats. SBP reduced at the 1st h from 108.0 ± 2.8 to 89.3 ± 8.8 mmHg, while DBP reduced from 78.8 ± 2.6 to 56.3 ± 5.4 mmHg. But from 2nd h onwards, blood pressure improved gradually, and from 4th h, SBP was 115.8 ± 10.5 and DBP was 85.8 ± 8.4 mmHg (**Table 2**).

Table 2 Effect of N-1 polyphenol at a dose of 50 mg/kg on blood pressure.

Time (h)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
0 (start)	104 ± 2.5	78.8 ± 3.7
1st h	89.3 ± 3.4	56.3 ± 2.4
2nd h	111.3 ± 2.3	69.3 ± 3.6
3rd h	101.5 ± 3.9	73.3 ± 2.7
4th h	115.7 ± 2.2	75.5 ± 4.1

The results derived show that a high dose of N-2 polyphenol has a short but effective hypotensive action. But its effect declines from the 2nd h, and at the 4th h, blood pressure again increases above the baseline value. The effect is due to the release of body compensatory mechanisms such as enhanced activity of sympathetic nervous system and renin-angiotensin-aldosterone system. Secondly, the pharmacokinetic profile of N-2 polyphenol (i.e., rapid metabolism or low half-life) may also be a contributing factor to the temporary effect seen (**Figure 12**).

Moreover, there is a dose-effect relationship - at a larger dose, the blood pressure first drops significantly, but then the defensive mechanism of the body either eliminates the effect or even produces a rise in blood pressure [31].

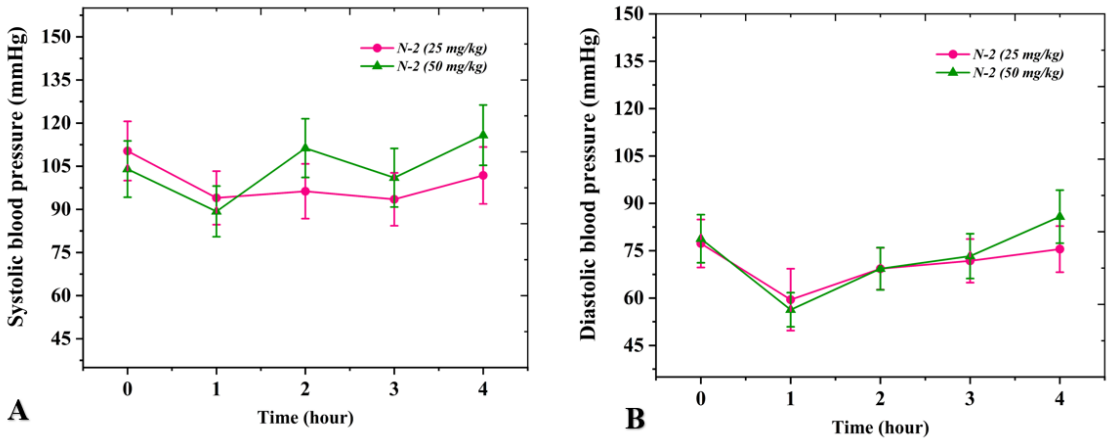

Figure 12 Hypotensive effect of N-2 polyphenol at doses of 25 and 50 mg/kg. A) Systolic blood pressure, B) Diastolic blood pressure (n = 5, $p < 0.05$).

Comparing the 2 doses, the 25 mg/kg dose can be termed as more effective since it produced a controlled and stable hypotensive action, and the blood pressure was kept comparatively low for 4 h without any rebound effect. Although the 50 mg/kg dose caused a more marked short-term reduction, this was not only not sustained but the blood pressure actually increased later. This is to be expected to produce untoward effects with prolonged therapy.

In the subsequent phase of the research, the hypotensive action of N-2 polyphenol was examined in a rat model of adrenaline-induced hypertension. For this, the rats were made into 2 groups, and the blood pressure of all the animals was recorded initially before any treatment (**Figure 13**). Then, adrenaline was administered to both the groups for inducing hypertension. The first group was used as control and received adrenaline alone, whereas the second group was treated with N-2 polyphenol 25 mg/kg after adrenaline. Blood pressure was measured by tail-cuff method at an interval of every hour up to 3 h (**Table 3**).

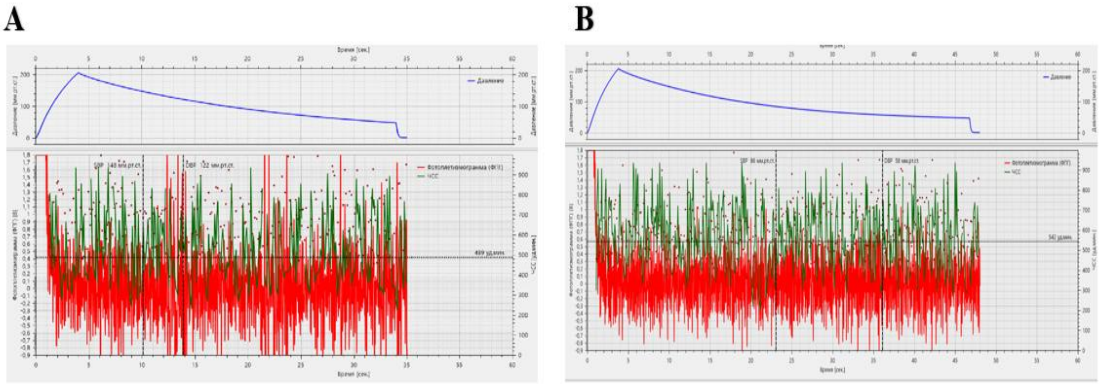

Figure 13 Dynamics of arterial blood pressure in rats (recorded using the "Systole" device). (A) Dynamics of blood pressure and heart rate in rats after adrenaline administration, (B) Arterial blood pressure dynamics in rats after administration of N1 polyphenol.

The research reiterated that N-2 polyphenol is capable of creating a hypotensive effect in adrenaline-induced hypertensive rats. When both groups were given adrenaline, SBP and DBP levels were higher. When the group given N-2 polyphenol received adrenaline, however, blood pressure fell with time, while for the control group, it did not fall (**Figure 14**).

Table 3 Effect of N-2 polyphenol on systolic and diastolic blood pressure (mmHg) in an adrenaline-induced hypertension mode.

Time Point	Control Group (Adrenaline only)		N-2 Group (25 mg/kg after Adrenaline)	
	SBP	DBP	SBP	DBP
Baseline	110.3 ± 3.6	78.5 ± 2.9	92.3 ± 4.2	69.3 ± 2.9
30 min (post-adrenaline)	155.5 ± 3.4	99.5 ± 3.0	155.0 ± 3.4	119.5 ± 2.7
1 h	149.0 ± 2.8	101.0 ± 3.2	143.3 ± 3.6	107 ± 2.2
2 h	130.3 ± 4.6	101.5 ± 4.0	102.5 ± 3.8	72.3 ± 2.8
3 h	126.8 ± 3.6	102.5 ± 3.3	96.3 ± 4.8	66.8 ± 3.3

The administration of N-2 polyphenol after adrenaline significantly reduced both systolic and diastolic blood pressure starting from the second hour, indicating a potential antihypertensive effect of the compound [34].

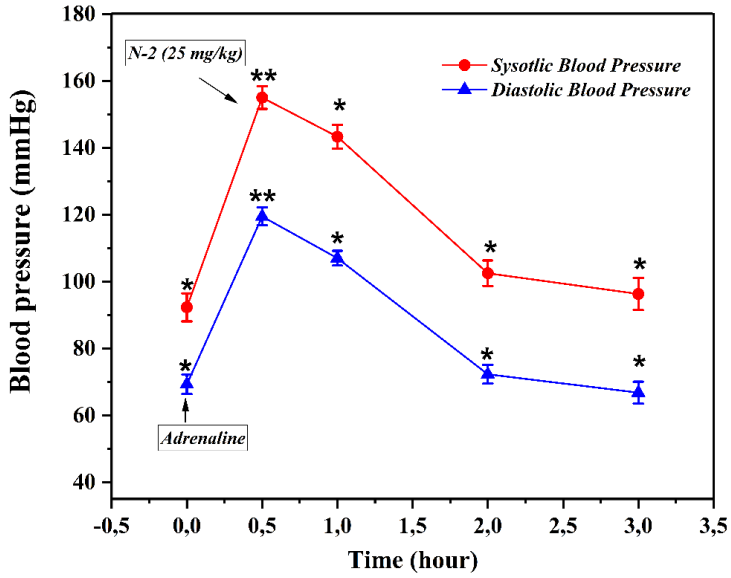

Figure 14 Effect of N-2 polyphenol on systolic blood pressure in an experimental hypertension model (n = 5, * - $p < 0.05$, ** - $p < 0.01$).

Discussion

This study demonstrated that N-2 polyphenol exerts potent vasorelaxant and antihypertensive effects by modulating calcium homeostasis in vascular smooth muscle cells through multiple mechanisms. The compound induced concentration-dependent relaxation in KCl- and phenylephrine-precontracted aortic rings, pointing to its ability to affect both voltage-dependent (VDCC) and receptor-operated calcium channels (ROCC). These results align with previous studies suggesting that natural polyphenols can influence vascular tone by targeting calcium influx pathways [32].

The strong inhibition of KCl-induced contraction implies a primary effect on L-type calcium channels. This was further supported by the additive effect observed when N-2 was combined with verapamil, a standard L-type blocker, suggesting either cooperative binding or a complementary mechanism of action. Likewise, the suppression of phenylephrine-induced contraction - further enhanced by phentolamine - confirms N-2's multi-targeted inhibition of both calcium entry via α_1-adrenergic signaling and internal calcium release.

The inhibition of contraction in calcium-free media indicates that N-2 also interferes with IP_3-

mediated calcium release from the sarcoplasmic reticulum. This ability to reduce intracellular calcium from both extracellular and internal sources highlights its comprehensive calcium-modulatory effect.

Importantly, endothelium-denuded rings showed a marked reduction in relaxation, suggesting that endothelial nitric oxide (NO) contributes significantly to N-2's vasodilatory action. Since endothelial dysfunction is a hallmark of hypertension, N-2's ability to preserve or enhance NO signaling may provide additional vascular protection beyond calcium channel modulation.

The antihypertensive activity observed *in vivo* strengthens these findings. N-2 polyphenol dose-dependently reduced blood pressure, with 25 mg/kg being the most effective within the short observation period. Moreover, its efficacy in reversing adrenaline-induced hypertension - a model reflecting sympathetic overactivity - emphasizes its therapeutic potential for stress-related or neurogenic hypertension [33].

Taken together, the findings suggest that N-2 polyphenol reduces vascular tone and blood pressure by targeting key components of vascular contraction: VDCCs, ROCCs, intracellular calcium release, and endothelial NO production. These multimodal actions underline its promise as a natural antihypertensive agent capable of addressing multiple pathogenic mechanisms in hypertension [34].

Conclusions

Our findings suggest that the N-2 polyphenol possesses promising vasorelaxant and antihypertensive properties, primarily through the inhibition of voltage-gated and receptor-operated calcium channels, and potentially through enhancement of endothelium-derived nitric oxide signaling. These effects were supported by both *in vitro* and *in vivo* data, including dose-dependent blood pressure reduction in normotensive and adrenaline-induced hypertensive rat models. While these results are encouraging, it is important to acknowledge the preliminary nature of the findings. The current study lacks molecular-level confirmation (e.g., eNOS expression and calcium influx assays) and long-term safety evaluation, which limits definitive conclusions about therapeutic potential. The observed effect at 25 mg/kg serves as a basis for future pharmacokinetic and toxicological studies. Additional investigations are necessary to optimize the N-2 structure for improved efficacy and specificity, and to explore potential synergistic effects with existing antihypertensive drugs. With these considerations, N-2 polyphenol may represent a valuable lead compound for future antihypertensive drug development, but further validation is required.

Declaration of generative AI in scientific writing

Only minimal assistance was used from QuillBot for paraphrasing selected sentences. All scientific content, interpretation, and conclusions were developed independently by the authors.

CRediT author statement

Khasanov Alikhon, Izzatullo Abdullaev, Lazizbek Makhmudov, Shokhida Kadirova contributed to the study design, *in vivo* and *in vitro* experimentation, and data collection. **Inomjonov Dolimjon** participated in data analysis, interpretation, and preparation of figures and table. **Muxtorjon Mamajanov and Anvar Zaynabiddinov** provided critical revisions to the manuscript and contributed to the interpretation of results within the physiological context. **Sirojiddin Omonturdiev, Ulugbek Gayibov, Rakhmat Esanov and Alimjan Matchanov** supervised the overall research process, coordinated team efforts, and finalized the manuscript.

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