

EFFECTS OF DRACOCEPHALUM DIVERSIFOLIUM EXTRACT AND N-1 POLYPHENOL ON CALCIUM ION INFLUX IN AORTIC SMOOTH MUSCLE

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Article Received: 01 October 2025 | Article Revised: 22 October 2025 | Article Accepted: 14 November 2025

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DOI: <https://doi.org/10.5281/zenodo.17637989>

How to cite this Article: Alikhon Khasanov, Shoxista Melieva, Shahlo Ismailova, Kamila Eshbakova, Azizbek Abdullayev, Izzatullo Abdullaev, Kuvonchoy Tojiboeva, Sirojiddin Omonturdiyev, Muxtorjon Mamajanov, Ulugbek Gayibov (2025). EFFECTS OF DRACOCEPHALUM DIVERSIFOLIUM EXTRACT AND N-1 POLYPHENOL ON CALCIUM ION INFLUX IN AORTIC SMOOTH MUSCLE. World Journal of Pharmaceutical Science and Research, 4(5), 1122-1130. <https://doi.org/10.5281/zenodo.17637989>



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ABSTRACT

This study investigated the effects of the ethyl acetate extract of *Dracocephalum diversifolium* and its main component, N-1 polyphenol, on rat aortic smooth muscle. The aim was to elucidate the vasorelaxant properties of these compounds and their interaction mechanisms with voltage-dependent Ca^{2+} channels (VDCCs). Experiments were conducted in vitro on aortic rings isolated from male Wistar rats. Aortic contractions were recorded using an isometric transducer, and depolarization was induced by 50 mM KCl solution. *Dracocephalum diversifolium* extract (10–130 $\mu\text{g/ml}$) and N-1 polyphenol (5–30 μM) significantly reduced aortic contraction in a concentration-dependent manner. The IC_{50} values of the extract and N-1 polyphenol were 54.8 $\mu\text{g/ml}$ and 20 μM , respectively. In Ca^{2+} -free Krebs solution, KCl failed to induce contraction, whereas the addition of Ca^{2+} ions (0–2.5 mM) restored contractile responses. Under these conditions, the presence of the extract or N-1 polyphenol significantly attenuated Ca^{2+} -dependent contractions compared to control. These results indicate that both compounds induce vasorelaxation by inhibiting VDCC activity and limiting Ca^{2+} influx. The findings suggest that *Dracocephalum diversifolium* extract and N-1 polyphenol may serve as natural Ca^{2+} channel blockers and potential antihypertensive agents.

KEYWORDS: *Dracocephalum diversifolium*, N-1 polyphenol, aortic smooth muscle, Ca^{2+} channels, vasorelaxation.

INTRODUCTION

The genus *Dracocephalum* L. (family Lamiaceae) comprises over 70 species worldwide, of which 15 are found in the flora of Uzbekistan. These species are rich in biologically active compounds such as essential oils, flavonoids, phenolic acids, and alkaloids, and have traditionally been used in folk medicine for their sedative, antihypertensive, antiseptic, and anti-inflammatory properties.^[1]

Dracocephalum diversifolium Rupr. is a plant native to Central Asia, with its aerial parts containing flavonoids and essential oils. These compounds have been reported to exert beneficial effects on the cardiovascular system; however, the precise mechanisms underlying the plant's action on vascular smooth muscle remain insufficiently studied.^[2]

One of the primary mechanisms regulating vascular tone is the activity of L-type voltage-dependent Ca^{2+} channels (VDCCs) located in the membrane of aortic smooth muscle cells. Activation of these channels increases Ca^{2+} influx into the cells, leading to muscle contraction and elevated blood pressure. Conversely, blockade of L-type Ca^{2+} channels induces smooth muscle relaxation. Therefore, these channels represent key targets for antihypertensive drugs such as verapamil and nifedipine.^[3]

In recent years, numerous polyphenolic compounds have been shown to exert vasorelaxant and antihypertensive effects through mechanisms including inhibition of L-type Ca^{2+} channels, enhancement of endothelial nitric oxide (NO) production, and modulation of intracellular signaling pathways in smooth muscle. Given the high flavonoid content of *Dracocephalum diversifolium*, its extract is hypothesized to relax vascular smooth muscle by reducing Ca^{2+} influx via L-type Ca^{2+} channels.^[4]

Therefore, the present study aimed to investigate the vasorelaxant effects of *Dracocephalum diversifolium* extract on rat aortic rings and to compare these effects with those of its polyphenolic component. The study specifically focused on elucidating the interaction mechanisms with L-type Ca^{2+} channels.^[5]

MATERIALS AND METHODS

Plant Sample and Extract Preparation

The aerial parts (leaves and stems) of *Dracocephalum* L. (family Lamiaceae) used in this study were shade-dried and ground into a fine powder. Ethyl acetate was used as the extraction solvent. A total of 100 g of dried plant material was macerated in 500 ml of ethyl acetate for 48 hours, with the mixture being shaken every 12 hours. After filtration, the solution was concentrated under reduced temperature using a rotary evaporator. The resulting ethyl acetate extract (EA-extract) was dried under vacuum and stored at $-20\text{ }^{\circ}\text{C}$ until further use in experiments.^[6]

N-1 Polyphenol

N-1 polyphenol ($\text{C}_{12}\text{H}_{16}\text{O}_5$, $\text{Mw} = 240$) is a phenolic ester containing an aromatic ring with three methoxy ($-\text{OCH}_3$) groups and one ethyl carboxylate ($-\text{COOC}_2\text{H}_5$) group. Chemically, it belongs to the polymethoxybenzoate class and is considered a bioactive polyphenolic compound. The aromatic core and functional groups confer antioxidant, vasorelaxant, and Ca^{2+} -modulating properties to the molecule.

N-1 polyphenol is well soluble in organic solvents such as DMSO, ethanol, and ethyl acetate. In experiments, it was dissolved in DMSO and diluted to the required concentrations with Krebs–Henseleit solution, ensuring that the final

DMSO concentration did not exceed 0.1%. This polyphenol was used to evaluate its effects on the activity of L-type Ca^{2+} channels in vascular smooth muscle.^[7]

Chemicals

In addition to the ethyl acetate extract of *Dracocephalum* L. and N-1 polyphenol isolated from local medicinal plants, other chemicals used in the experiments, including phenylephrine, phentolamine, and verapamil, were purchased from Sigma-Aldrich Chemie (Sigma-Aldrich, St. Louis, Missouri, USA).

Animal Ethics

All pre-surgical and experimental protocols were reviewed and approved by the Institutional Committee on the Use and Care of Animals. Experimental animals were housed under controlled conditions in a vivarium, with a relative humidity of 55–65% and a temperature of 22 ± 2 °C. They had free access to drinking water and standard laboratory chow.^[8] Animal handling and care procedures fully complied with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. Ethical approval for this study was granted by the Committee on Animal Ethics of the Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan (Protocol № 133/1a/h, August 4, 2016).

Tissue Preparation

All experimental protocols used in this study were approved by the Institutional Committee on the Care and Use of Laboratory Animals, and procedures were conducted in full compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.^[9] All surgical procedures were performed under sodium pentobarbital anesthesia to minimize pain. Euthanasia was carried out by cervical dislocation, after which the aorta was excised from the thoracic cavity and placed in a 5 mL organ bath containing Krebs–Henseleit solution.^[10]

The composition of the Krebs–Henseleit solution was as follows: 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 certain experiments, 1 mM EGTA was added to prepare Ca^{2+} -free Krebs solution.^[11] The solution temperature was maintained at 37 °C and continuously aerated with carbogen (95% O_2 and 5% CO_2).^[12]

The aorta was carefully cleared of connective tissue and fat and then cut into rings 3–4 mm in length, ready for physiological experiments.

Measurement of Aortic Ring Contraction

Aortic rings were mounted on platinum wire hooks connected to a Radnoti isometric transducer and equilibrated for 60 minutes before the start of measurements. An initial resting tension of 1 g (10 mN) was applied to each ring (Figure 1). Contraction responses were recorded through a signal amplifier, digitized, and displayed in real time on a computer screen using the Go-Link data acquisition interface.

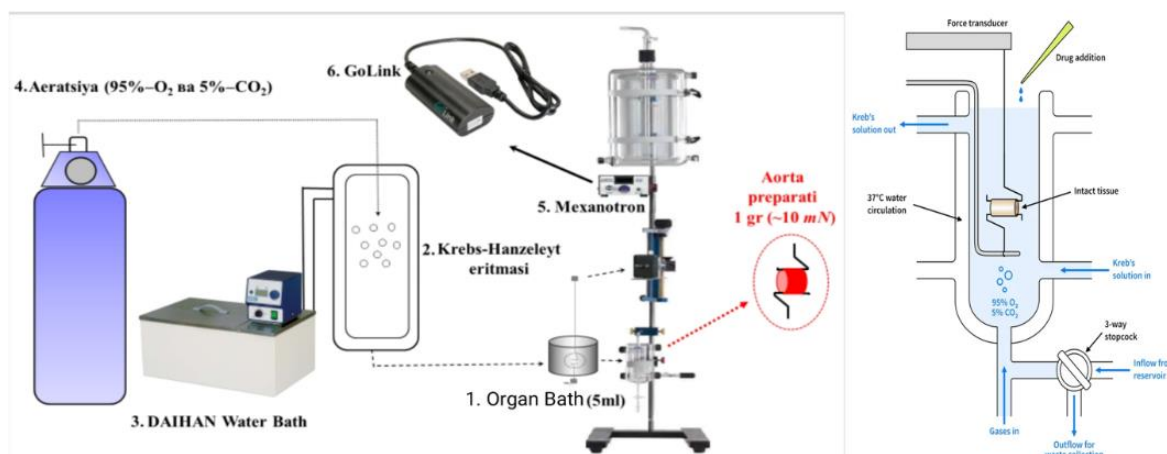


Figure 1: Schematic representation of the apparatus designed for controlling and measuring isometric contraction of isolated rat aortic smooth muscle. 1) The organ bath (5 mL) circulates solution via a specialized reservoir, 2) Krebs-Henseleit solution maintains physiological conditions, 3) A thermostat ensures constant physiological temperature, 4) The system is oxygenated with a gas mixture of 95% O₂ and 5% CO₂. 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.

Data Analysis

Collected data were processed and analyzed using OriginPro v9.0 SR1 software (OriginLab Corp., Northampton, MA, USA). The isometric tension values recorded under in vitro conditions were normalized and expressed as a percentage relative to the maximum contractile force (mN).^[13]

RESULTS AND ANALYSIS

The contractile force of smooth muscle cells (SMCs) is directly dependent on the intracellular Ca²⁺ concentration. The amount of Ca²⁺ within the cells is regulated through several mechanisms, including voltage-dependent Ca²⁺ channels in the membrane, Ca²⁺ release from the sarcoplasmic reticulum, and ion exchange systems.^[14]

According to the literature, contraction of aortic smooth muscle in response to 50 mM KCl is primarily associated with the activation of voltage-dependent L-type Ca²⁺ channels.^[1] Under these conditions, an increase in extracellular K⁺ ions induces membrane depolarization, which opens Ca²⁺ channels and enhances Ca²⁺ influx into the cell. Consequently, cytoplasmic Ca²⁺ concentration rises, leading to an increase in contractile force of the muscle tissue.^[15]

In this study, the vasorelaxant effects of the ethyl acetate extract of *Dracocephalum* L. and N-1 polyphenol were investigated. The extract reduced contraction in a concentration-dependent manner over the range of 10–130 µg/ml. Specifically, at 10 µg/ml, contraction decreased by 9.1 ± 3.7%, while at 100 µg/ml, contraction was reduced by 86.7 ± 3.1%. The half-maximal relaxant concentration (IC₅₀) of the extract was 54.8 µg/ml.

Similarly, N-1 polyphenol exhibited a strong concentration-dependent relaxant effect. At 5 µM, contraction decreased by 2.2 ± 2.4%, whereas at 30 µM, relaxation reached 91.2 ± 3.7% (Figure 2). The IC₅₀ value of N-1 polyphenol was determined to be 20 µM.

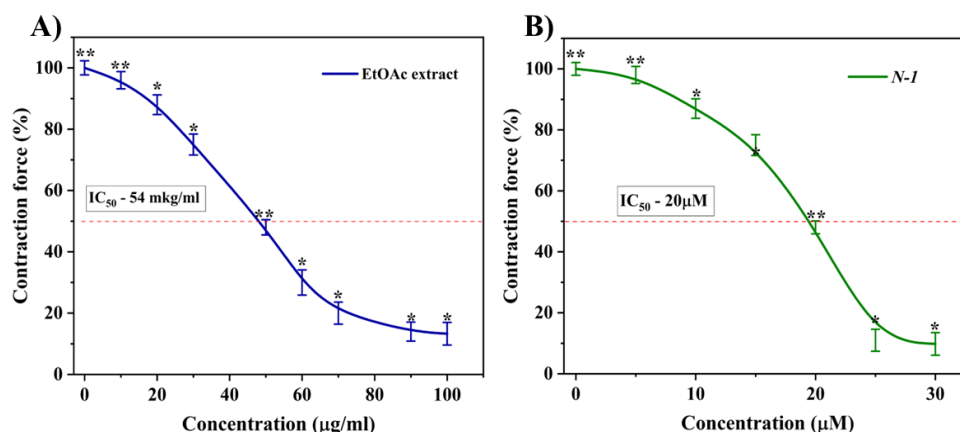


Figure 2: Effects of the ethyl acetate extract of *Dracocephalum diversifolium* (A) and N-1 polyphenol (B) on the contractile force of rat aortic smooth muscle preparations induced by 50 mM KCl. The ordinate represents aortic contraction induced by 50 mM KCl (taken as 100%), and the abscissa represents the concentration of the extract (µg/ml) or N-1 polyphenol (µM). Data are presented as mean ± SEM; **p* < 0.05, ***p* < 0.01; *n* = 5–6.

The obtained results indicate that both the *Dracocephalum* L. extract and N-1 polyphenol effectively inhibit depolarization-induced Ca²⁺ influx. This suggests that these compounds may act as voltage-dependent L-type Ca²⁺ channel blockers. Consequently, they could exert vasorelaxant and hypotensive effects by reducing Ca²⁺-dependent contraction of vascular smooth muscle.^[15]

To further clarify this hypothesis, subsequent experiments were conducted using Ca²⁺-free Krebs solution and the L-type Ca²⁺ channel blocker verapamil. It is known that in Ca²⁺-free Krebs solution, an increase in KCl concentration does not induce contraction in aortic smooth muscle. However, under these conditions, the addition of Ca²⁺ ions (0–2.5 mM) to the Krebs solution restores contractile responses in aortic preparations.^[16]

In our experiments, aortic preparations in Krebs solution containing the *Dracocephalum diversifolium* ethyl acetate extract (100 µg/ml) or N-1 polyphenol (30 µM) exhibited contractile responses upon Ca²⁺ addition in the presence of 50 mM KCl. Notably, the presence of either the extract or N-1 polyphenol significantly attenuated the Ca²⁺-induced contraction compared to control (Figure 3).

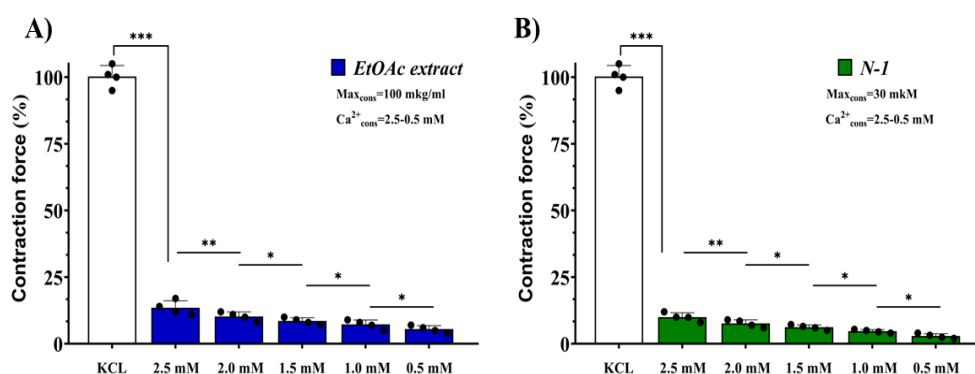


Figure 3: Effects of changes in extracellular [Ca²⁺] on the relaxant activity of the ethyl acetate extract of *Dracocephalum diversifolium* (A) and N-1 polyphenol (B). The ordinate represents aortic contraction induced by 50 mM KCl (taken as 100%), and the abscissa represents Ca²⁺ concentration (0–2.5 mM). Data are presented as mean ± SEM; **p* < 0.05, ***p* < 0.01; *n* = 5–6.

These results indicate that the *Dracocephalum diversifolium* extract and N-1 polyphenol may inhibit the activity of voltage-dependent Ca^{2+} channels.^[17]

To further verify this hypothesis, subsequent experiments were conducted using Ca^{2+} -free Krebs solution and the L-type Ca^{2+} channel blocker verapamil. It is known that in Ca^{2+} -free Krebs solution, an increase in KCl concentration does not induce contraction in aortic smooth muscle. However, under these conditions, the addition of Ca^{2+} ions (0–2.5 mM) to the Krebs solution restores contractile responses in aortic preparations.^[18]

In our experiments, aortic preparations in Krebs solution containing either the *Dracocephalum diversifolium* ethyl acetate extract (100 $\mu\text{g}/\text{ml}$) or N-1 polyphenol (100 μM) exhibited contractile responses upon Ca^{2+} addition in the presence of 50 mM KCl. Moreover, the presence of the extract or N-1 polyphenol significantly attenuated Ca^{2+} -induced contraction compared to control (Figure 4).

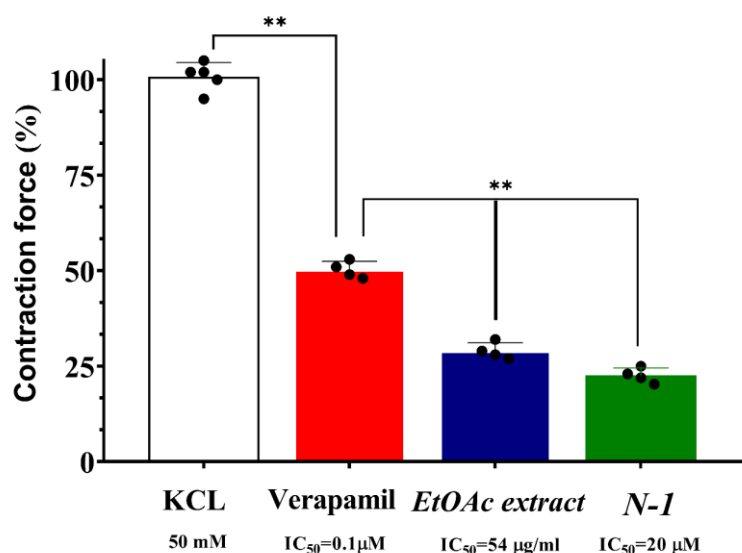


Figure 4: Interaction effects of the ethyl acetate extract of *Dracocephalum diversifolium* (A), N-1 polyphenol (B), and the Ca^{2+} channel blocker verapamil (IC_{50}) on contraction of aortic preparations induced by 50 mM KCl. The ordinate represents the contraction of aortic smooth muscle preparations induced by 50 mM KCl (control taken as 100%). Data are presented as mean $\pm$ SEM; * $p < 0.05$, ** $p < 0.01$; $n = 4-5$.

These results indicate that the *Dracocephalum diversifolium* extract and N-1 polyphenol may inhibit the activity of voltage-dependent Ca^{2+} channels.

The findings of this study demonstrate that the ethyl acetate extract of *Dracocephalum diversifolium* and its constituent N-1 polyphenol significantly attenuate Ca^{2+} -dependent contraction in rat aortic smooth muscle. Although no contraction was observed in Ca^{2+} -free Krebs solution under the influence of 50 mM KCl, the addition of Ca^{2+} ions (0–2.5 mM) restored contractile activity in aortic preparations. However, in the presence of the extract or N-1 polyphenol, the increase in Ca^{2+} concentration led to a significant reduction in contraction compared to control, indicating a potential inhibitory effect on voltage-dependent Ca^{2+} channels (VDCC).^[19]

The *Dracocephalum diversifolium* extract is a complex mixture containing flavonoids, phenolic acids, and other bioactive compounds, which may act synergistically to enhance VDCC blockade. Therefore, the extract exhibits overall vasorelaxant effects by reducing Ca^{2+} influx in vascular smooth muscle.^[20]

In contrast, the effect of N-1 polyphenol appeared more selective and specific. Its significant reduction of contraction upon Ca^{2+} addition suggests a direct interaction with VDCC, inhibiting channel activity.^[21] Additionally, the antioxidant properties of N-1 polyphenol may indirectly limit Ca^{2+} entry by reducing membrane depolarization and enhancing endothelial NO production.^[22]

Overall, these findings suggest that the *Dracocephalum diversifolium* extract and N-1 polyphenol may exert antihypertensive effects by attenuating voltage-dependent Ca^{2+} channel activity in vascular smooth muscle. This observation supports their potential as naturally derived Ca^{2+} channel blockers with clinical relevance.^[23]

CONCLUSION

The present study demonstrated that the ethyl acetate extract of *Dracocephalum diversifolium* and its N-1 polyphenol component significantly reduce Ca^{2+} -dependent contraction of aortic smooth muscle. This effect is associated with the inhibition of voltage-dependent Ca^{2+} channels and highlights the vasorelaxant and antihypertensive potential of these compounds. The findings suggest that *Dracocephalum diversifolium* extract and N-1 polyphenol may serve as promising naturally derived bioactive Ca^{2+} channel blockers.

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