TRENDS IN SCIENCES 2025; 22(9): 10496
https://doi.org/10.48048/tis.2025.10496 RESEARCH ARTICLE

Channelopathy Activity Of A-41(Propyl Ester of Gallic Acid): Experimental and Computational Study of Antihypertensive Activity

Shodiyakhon Sodiqova[1,2,*], Shokhida Kadirova[3], Anvar Zaynabiddinov[1], Izzatullo Abdullaev[4], Lazizbek Makhmudov[4,5], Ulugbek Gayibov[4], Mukhabbat Yuldasheva[3], Madina Xolmirzayeva[1], Rakhmatilla Rakhimov[6], Azizbek Mutalipov[1] and Hayotbek Karimjonov[1]

[1]*Andijan State University, Andijan, Andijan Region, Uzbekistan*
[2]*Kokand University, Turkiston St 28-A, Kokand, Fergana Region, Uzbekistan*
[3]*National University of Uzbekistan, Gulistonyŭ, Universitet Ko'chasi 4, Toshkent, Uzbekistan*
[4]*A.S. Sadykov Institute of Bioorganic Chemistry of the Science Academy of Uzbekistan "Plant Cytoprotectors Laboratory" and "Pharamacology", Tashkent, Uzbekistan*
[5]*Tashkent Medical Academy, Farabi 2 Tashkent UZ, Uzbekistan*
[6]*Tashkent State Transport University, 25 Sairam Street, Tashkent, Uzbekistan*

([*]Corresponding author's e-mail: shsodiqova@kokanduni.uz)

Received: 28 April 2025, Revised: 12 May 2025, Accepted: 22 May 2025, Published: 10 July 2025

Abstract

This study aimed to evaluate the antihypertensive potential of the natural compound A41, a bioactive molecule, using integrated *in vitro*, *in vivo*, and in silico approaches. The rationale for selecting A41 is based on preliminary screening that suggested its strong calcium-modulating activity, a key factor in vascular tone regulation. *In vitro* experiments were performed on isolated rat aortic rings to investigate A41's effect on vascular smooth muscle ion channels, particularly voltage-operated L-type Ca^{2+} channels and receptor-operated Ca^{2+} channels. The study also explored endothelium-dependent pathways. Selective ion channel blockers were applied to confirm the involvement of specific mechanisms. A41 significantly inhibited Ca^{2+} entry through L-type Ca^{2+} channels under hypertensive conditions, achieving 82.2 ± 2.0 % relaxation at 35 µM, with an IC_{50} of 22.10 µM. Additionally, receptor-operated Ca^{2+}-induced contractions were reduced by 87.0 ± 2.7 % at 50 µM. Molecular docking simulations demonstrated high binding affinity of A41 to calcium-regulating proteins, including the sodium-calcium exchanger (NCX) and plasma membrane Ca^{2+} ATPase, with binding free energies of −6.6 kcal/mol and estimated inhibitory constants (K_i) of 14.558 µM. *In vivo* studies were conducted using male Wistar rats (n = 6) in an adrenaline-induced hypertension model. Intravenous administration of A41 (dose: 50 mg/kg) produced a marked antihypertensive effect, lowering systolic blood pressure to 82.8 ± 8.3 mmHg and diastolic pressure to 61.3 ± 5.9 mmHg within 3 h. Together, these findings demonstrate that A41 exerts antihypertensive effects by modulating calcium transport mechanisms in vascular smooth muscle, involving both ion channels and calcium-handling proteins, supporting its potential as a candidate for hypertension therapy.

Keywords: Endothelium, Ion transport, Isometric contraction, Molecular docking, Smooth muscle, Vasorelaxation, Voltage-gated, Ca^{2+} ion channels

Introduction

Hypertension remains one of the leading global health concerns, contributing significantly to cardiovascular morbidity and mortality worldwide [1,2]. Despite the availability of a wide range of

antihypertensive medications, limitations such as side effects, resistance, and suboptimal efficacy in certain populations underscore the need for novel therapeutic agents with improved safety and multi-target profiles. In this context, natural products have gained increasing attention as alternative or complementary sources of cardiovascular therapy. Their diverse bioactive compounds—especially polyphenols, flavonoids, and tannins-exhibit antioxidant, anti-inflammatory, and vasomodulatory properties, which are beneficial in the management of hypertension. Notably, these compounds have been shown to modulate critical vascular mechanisms, including endothelial function and ion channel activity, particularly voltage-gated Ca^{2+} channels. Since these channels play a pivotal role in vascular smooth muscle contraction and blood pressure regulation, targeting them presents a promising strategy for antihypertensive intervention [3]. However, its direct vascular effects and interaction with key ion transport mechanisms remain insufficiently explored. Therefore, the present study aims to investigate the antihypertensive effects of A41 through a multidisciplinary approach combining *in vitro*, *in vivo*, and in silico methodologies. We examined its influence on calcium handling in vascular smooth muscle, its systemic blood pressure-lowering capacity, and its molecular interactions with calcium transport proteins. This integrated strategy is designed to provide mechanistic insight into A41's mode of action and support its potential development as a novel, nature-derived antihypertensive agent. This study aims to evaluate the antihypertensive potential of A41, a natural bioactive compound, by examining its effects on calcium ion channels through *in vitro*, *in vivo*, and in silico approaches.

Materials and methods

Synthesis of propyl ester of gallic acid - A41.

2 g of gallic acid and 50 mL of propyl alcohol and 1 mL of sulfuric acid for the catalyst were placed in a round-bottom flask with a reverse cooler and heated in a nitrogen atmosphere at 98 - 100 °C for 8 - 10 h [6,7]. Every half hour, the reaction was checked using thin-layer chromatography. After the reaction, the excess alcohol was distilled under pressure and diluted in 10 mL of ethyl acetate. The organic part was washed 1st with 15 mL of NaCl water and then with 15 mL of a 5 % $NaHCO_3$ solution. The separated precipitate was extracted 3 times with ethyl acetate and evaporated under low pressure (**Figure 1**). Propyl ester of gallic acid is a white-yellow solid substance. The reaction yield is 75 %. The product was checked using thin-layer chromatography [8]. (1:1 hexane/EtOAc), Rf = 0.87. Melts at 145 - 147 °C.

Chemicals

Phenylephrine, phentolamine, and verapamil (≥98 % purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animal ethics

All preoperative care procedures and experimental protocols were examined and approved by the Institutional Animal Use Committee. The animals were kept in standard vivarium conditions, including 55 - 65 % humidity, 22 ± 2 °C temperature, and ad libitum access to water and laboratory chow. All animal handling was in accordance with the European Directive 2016/63/EU on the protection of animals used for scientific purposes. Ethical clearance was given by the Animal Ethical Committee of the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan (Protocol No. 133/1a/h, 4 August 2016).

Tissue preparation

Surgery was performed under sodium pentobarbital anesthesia to reduce distress. Experiments were performed on aortic tissue from healthy white male rats (200 - 250 g). Animals were killed by cervical dislocation. Thoracic cavity was opened, and aorta was removed, cleaned of adhering fat and connective tissue, and cut into 3 - 4 mm segments. The aortic rings were put in a 5 mL organ bath filled with Krebs-Henseleit physiological solution with (in mM): NaCl 120.4, KCl 5, $NaHCO_3$ 15.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5 and HEPES-buffered to pH 7.4. For certain experiments, a Krebs solution free of Ca^{2+} and with 1 mM EGTA was used. The bath solution was continuously gassed with carbogen (95 % O_2 and 5 % CO_2) and maintained at 37 °C using a DAIHAN WATER BATH ultrathermostat [9].

Aortic-ring contraction studies

The aortic rings were suspended in a Radnoti isometric transducer system (USA) using platinum wire hooks and equilibrated for 60 min under normal conditions. The preparations were all stretched with an initial load of 1 g (10 mN). Contractile responses were transferred from the transducer to a signal amplifier and were digitally recorded by a Go-link automatic converter interfaced with a computer. Data were analyzed with Origin Pro v.8.5 SR1 (EULA, Northampton, MA, USA). The isometric contraction force (mN) of the rat aortic tissue *in vitro* was calculated as a percentage (%) for statistical analysis. The preparations of vascular smooth muscle were studied on an apparatus. The organ bath (5 mL) was connected via a special reservoir circulating the Krebs-Henseleit physiological solution (**Figure 2**). The physiological temperature was constantly maintained with the help of a thermostat, and the solution was continuously gassed with a mixture of 95 % O_2 and 5 % CO_2. The contractile activity of the aortic vascular segment, mounted in the experimental chamber, was recorded with the help of an ISOMETRIC TRANSDUCER (Grass Instrument, USA) and displayed on the GoLink signal amplifier and support system [10].

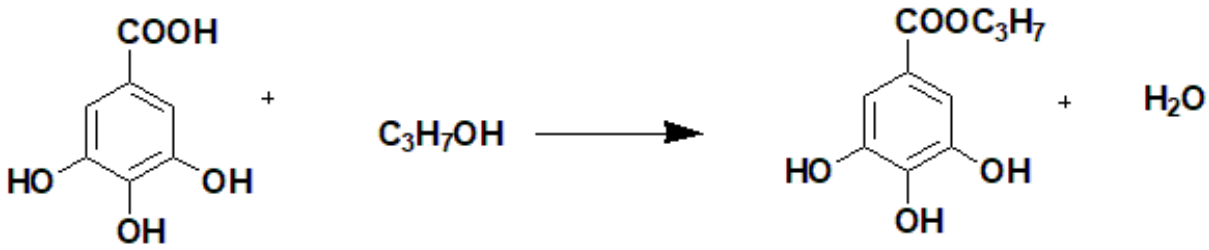


Figure 1 Synthesis scheme of the propyl ester derivative of gallic acid (A41).

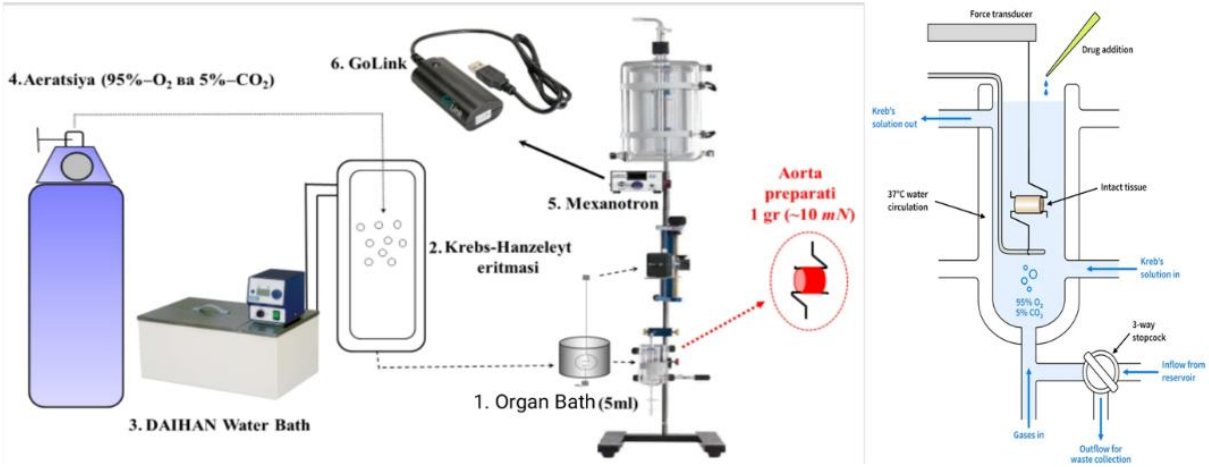


Figure 2 Diagram of the experimental setup used to monitor isometric contractions in isolated rat aortic vascular tissue. (1) The organ bath (5 mL) is connected to a dedicated reservoir for solution circulation. (2) Krebs-Henseleit solution is used to maintain physiological conditions. (3) A thermostat ensures stable temperature regulation. (4) The system is continuously aerated with a gas mixture containing 95 % oxygen and 5 % carbon dioxide. The aortic tissue is mounted within the experimental chamber for contraction assessment. (5) An isometric transducer (Grass Instrument, USA) captures mechanical responses, while (6) GoLink devices handle signal amplification and system integration.

Blood pressure measurements

Tail-cuff plethysmography using the Sistola device (Neurobotics, Russia) was performed after acclimating animals for 3 consecutive days. Blood pressure readings were taken in triplicate per session. The experiments took place at the "BFM Pharmacology and Screening Laboratory" and the "Plant Cytoprotectors" laboratory of the A. Sodikov

Institute of Bioorganic Chemistry. Data were analyzed using the specialized software "AcqKnowledge 4.2 for MP150" (**Figure 3**).

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

Statistics

Statistical evaluation and graphical representations were performed using Origin Pro 8.5 software (USA). Contractile responses were expressed as a percentage of the maximum contraction induced by phenylephrine (10 mM) or KCl (50 mM) and are shown as mean values from 4 to 6 independent trials (n = 4 - 6). Paired t-tests were used for analyzing combined data sets, while unpaired t-tests assessed differences between separate groups. Results were considered statistically significant at the threshold of $p < 0.05$.

Molecular docking "software and databases"

The software utilized in this research is freely accessible for academic purposes [11]. The Protein Data Bank (PDB) serves as a global repository for 3-dimensional structural information of biological macromolecules [12]. Protein structures associated with calcium signaling and regulation were retrieved from the PDB, including the L-type calcium channel Cav1.2 (PDB ID: 6jp5), R-type calcium channel Cav2.3 (PDB ID: 7xlq), sodium-calcium exchanger NCX1 (PDB ID: 8sgi), ryanodine receptor type 2 RyR2 (PDB ID: 5c33), and sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase SERCA (PDB ID: 6rb2). Additionally, the PubChem database-a comprehensive resource integrating pharmacological, pharmaceutical, chemical, and molecular target data (such as sequences, structures, and pathways)-was used to obtain flavonoid compounds and reference molecules for this study [13]. Each DrugCard entry in PubChem contains over 80 data fields, including information on both chemical substances and target proteins (as presented in **Table 1**). Visualization of the retrieved PDB structures was carried out using PyMOL (version 1.2), a molecular graphics tool based on Python [http://www.pymol.org]. PyMOL was also employed to examine and display molecular docking results. For docking analysis, AutoDock 4.2-developed by The Scripps Research Institute (www.scripps.edu)-was used. Preparation of input files and configuration of docking parameters were managed via AutoDock Tools (ADT), a graphical interface supporting user interaction. AutoDock provides a robust platform for simulating the interaction and optimal binding pose of small molecules, such as drugs or natural ligands, with macromolecular targets of known 3D structure.

Calculation of inhibition constant (Ki) from binding energy

In molecular docking, the strength of interaction between a ligand and its target is usually represented by binding energy (ΔG) in kcal/mol [14]. The inhibition constant (Ki) can be derived from this energy using a thermodynamic equation.

$$Ki = e\text{^}\frac{\Delta G \times 1000}{RxT}$$

Where:

- Ki is the inhibition constant (in mol/L)
- ΔG is the binding free energy (in kcal/mol)
- R is the universal gas constant = 1.987 cal/(mol·K)

- T is the temperature in Kelvin (usually 298.15 K)
- The factor 1,000 converts kcal to cal

Results and discussion

The study the role of l-type ca^{2+} and ca^{2+}r-type ion channels in the relaxant effect of a-41

It is firmly established that contraction of aortic smooth muscle tissues by 50 mM potassium chloride (KCl) is mostly mediated through the opening of voltage-dependent L-type calcium (Ca^{2+}) channels. In the presence of higher extracellular concentrations of K^+, the ensuing depolarization of the cell membrane changes the membrane potential, thereby opening the L-type Ca^{2+} channels, permitting the entry of Ca^{2+} ions into the cell and contraction of vascular smooth muscle cells. Here, in the present study, we investigated the possible vasorelaxant effect of the compound A-41 on KCl-induced contraction of isolated rat aortic rings. The results indicated that A-41 caused concentration-dependent relaxation of pre-contracted aortic tissues. In particular, at concentrations between 5 - 35 μM, A-41 effectively suppressed KCl (50 mM)-induced contraction with relaxation percentage ranging from 5.0 ± 2.2 to 82.2 ± 2.0 % when compared with the control (**Figure 4(A)**). These results show that A-41 has a strong ability to interfere with the contractile response to membrane depolarization [15]. The ensuing vasorelaxation suggests that A-41 most probably operates by regulating the influx of calcium via voltage-operated L-type Ca^{2+} channels on the plasma membrane of the smooth muscle cells. Through blockade of intracellular influx of extracellular calcium necessary for contraction, A-41 induces vascular smooth muscle relaxation. To examine more closely if the effect of A-41 is exclusively on L-type Ca^{2+} channels, a pharmacological comparison with verapamil, which is an established L-type calcium channel blocker, was conducted [16]. A submaximal concentration of verapamil (0.1 μM) that caused a partial block of KCl (50 mM)-elicited contractions was added to A-41. Under the experimental conditions used, A-41 was noted to cause an additional relaxing effect, inhibiting the contractile response by 13.0 ± 2.7 %. IC_{50} (half-maximal inhibitory concentration) obtained for A-41 was approximately 22.10 μM (**Figure 4(B)**). The additive action explains the synergistic or complementary action between A-41 and verapamil in inhibiting Ca^{2+} influx via L-type channels. Together, the results of the experiment are strong evidence that the vasorelaxant effect of A-41 is mediated primarily through its action on voltage-dependent L-type calcium channels. The drug is able to inhibit KCl-induced contraction by inhibiting calcium influx and thereby decreasing intracellular calcium concentration and causing relaxation of the smooth muscle. The mechanistic similarity of verapamil and A-41 also supports the finding that A-41 is active via modulation or blockage of L-type Ca^{2+} channels, indicating its promise as a therapeutic agent in the management of vascular disease linked with increased calcium channel activity.

It is established that vascular smooth muscle cell contraction is not exclusively controlled by voltage-dependent L-type Ca^{2+} channels. Intracellular calcium transport mechanisms, i.e., those associated with the sarcoplasmic reticulum (SR), also play a crucial role in calcium regulation and contraction. In this regard, the subsequent experiments were conducted to determine compound A-41's impact on contraction induced by the α-adrenoceptor agonist phenylephrine (1 μM), which stimulates receptor-operated calcium channels [17]. Phenylephrine is known to cause contraction mainly by raising intracellular calcium concentration ($[Ca^{2+}]_i$) due to the mobilization of Ca^{2+} from the SR and also via receptor-operated Ca^{2+} channels on the plasma membrane. In the aforementioned studies, A-41 exhibited potent vasorelaxant activity, markedly inhibiting phenylephrine (1 μM)-induced contractions by 87.0 ± 2.7 % at its highest test concentration (50 μM) in comparison to the untreated control (**Figure 4(C)**). These results implicate that A-41 might be producing the relaxant effect through blockade of receptor-operated Ca^{2+} channels. To explore this possibility further, the activity of A-41 was compared with that of phentolamine, a selective α-adrenoceptor antagonist, and with those flavonoids which are found to antagonize receptor-mediated communication. In the absence of phentolamine, as noted above, A-41 (50 μM) also significantly suppressed the contraction evoked by phenylephrine. Phentolamine (10 μM) suppressed phenylephrine-induced contraction by 75.0 ± 2.4 % compared with the control. Surprisingly, in the presence of both phentolamine and A-41, the contraction force was further decreased to 66.0 ± 2.7 %

(**Figure 4(D)**). These findings are strong evidence that the vasorelaxant action of A-41, as modeled by compound A-41, is mediated at least in part by inhibition of receptor-operated Ca^{2+} channels [18]. The further observation that contraction was also inhibited by the presence of the α-adrenoceptor antagonist phentolamine is further evidence for this mechanism, suggesting that A-41 disrupts α-adrenoceptor-mediated calcium entry mechanisms [18].

The study the role of endothelium in the relaxant effect of a-41

The endothelium plays a critical role in regulating vascular tone by producing local mediators such as nitric oxide (NO), a key endothelial-derived vasodilator. Endothelial dysfunction (ED), which arises from structural and functional impairments of endothelial cells, is a major contributor to the development of cardiovascular diseases like hypertension and atherosclerosis [19,20].

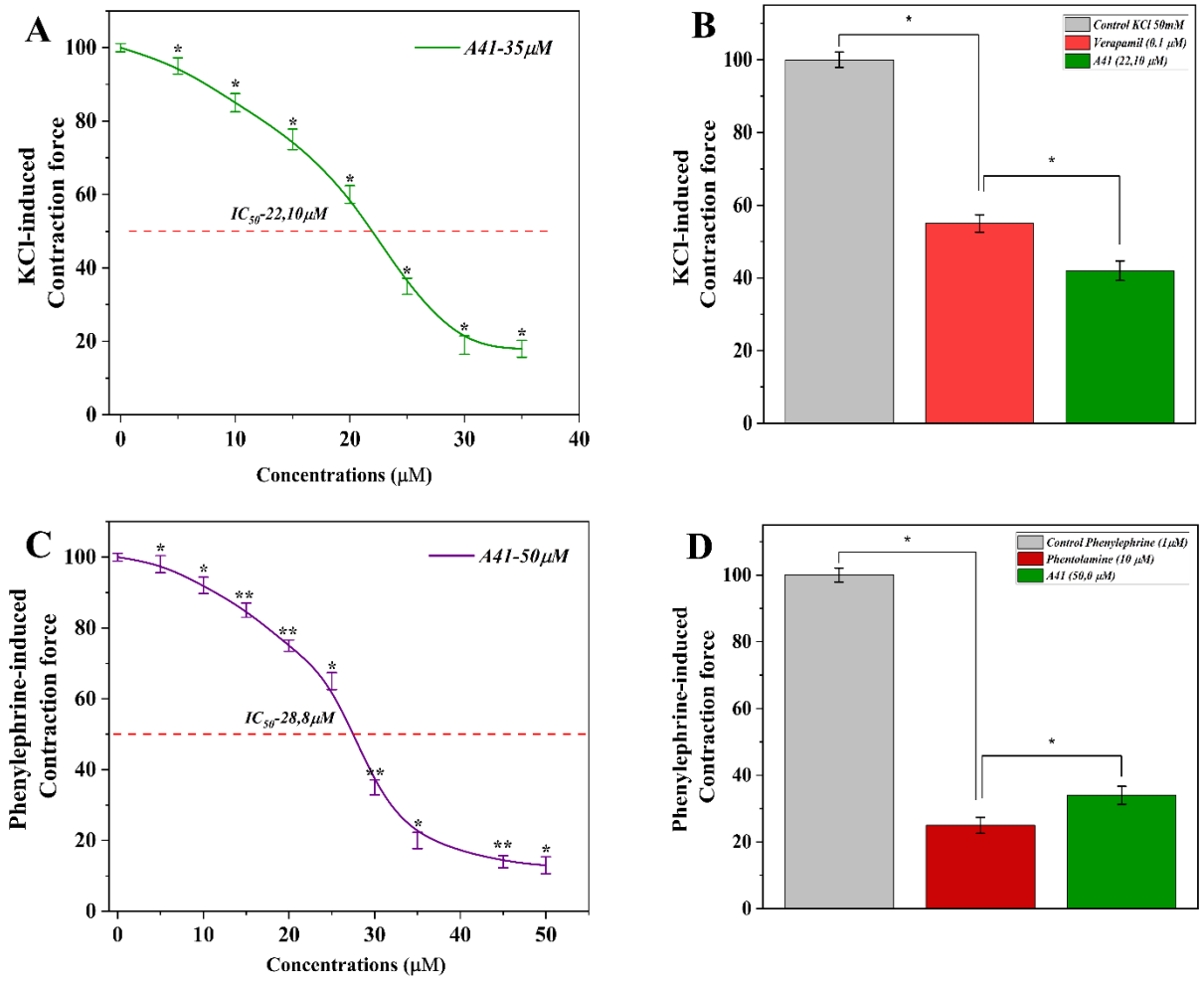


Figure 4 (A) Dose-dependent, linear vasorelaxant effect of compound A-41 on KCl-induced contraction. (B) Comparison of A-41 with the calcium channel blocker verapamil. (C) Effect of A-41 on receptor-operated Ca^{2+} ion channels. (D) Confirmation of A-41's mechanism using the α-adrenergic antagonist phentolamine. *Data represent mean ± SEM, n = 3 - 4, p < 0.05.*

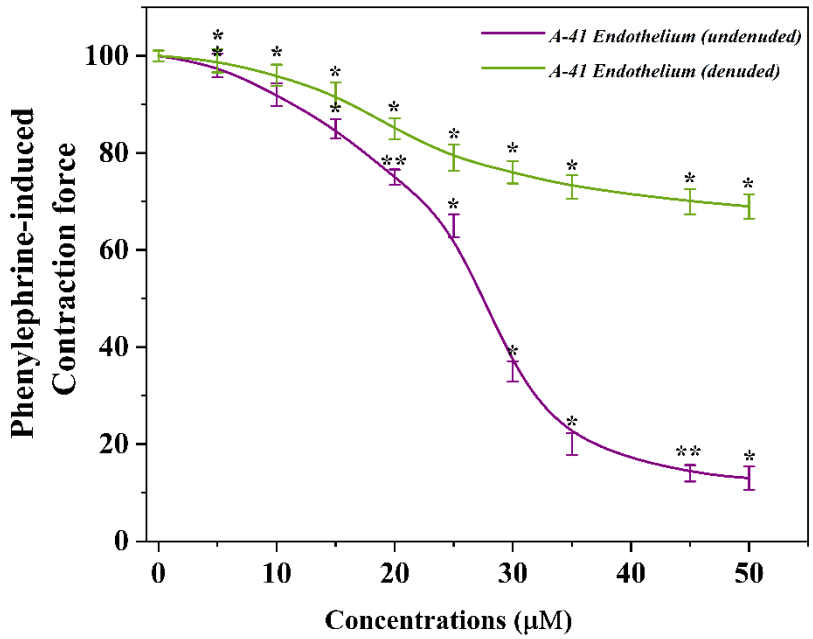


Figure 5 Relaxant effect of A-41 on the contraction induced by Phe 1 μM in the undenuded and denuded of the rat aortic blood vessel endothelial layer. Contraction force elicited by 1 μM Phe was taken as 100 % of control. (in all cases $*p < 0.05$, $**p < 0.01$; n = 5).

ED results from an imbalance between vasodilatory and vasoconstrictive mediators, often triggered by oxidative stress and modifiable risk factors such as smoking, poor diet, and metabolic disturbances. NO is synthesized in endothelial cells from L-arginine via endothelial nitric oxide synthase (eNOS), which is regulated by calcium-calmodulin and inhibited when bound to caveolin. Stimuli such as acetylcholine or bradykinin release eNOS from caveolin, enhancing NO production. Statins at low concentrations can also stimulate NO synthesis by disrupting the eNOS-caveolin complex, contributing to their vascular protective effects. Oxidative stress reduces NO availability by accelerating its degradation through reactive oxygen species, diminishing its vasodilatory effect. NO promotes vascular smooth muscle cell (SMC) relaxation via the NO/cGMP/PKG pathway, lowering intracellular Ca^{2+} levels and enhancing vasodilation. To assess the role of the endothelium in the relaxant effect of A-41, experiments were conducted on endothelium-denuded aortic rings. The removal of the endothelial layer relatively reduced A-41's vasorelaxant effect by 31.0 ± 2.5 %, (**Figure 5**) indicating that the endothelium-and likely NO signaling-plays a key role in mediating its action [21].

Molecular docking study of a-41 with aortic ion channels

To further elucidate the molecular mechanisms underlying our *in vitro* findings, molecular docking studies were conducted. These experiments aimed to assess the interaction of the A-41 compound with key ion channels involved in vascular smooth muscle function. Specifically, we examined the binding affinity and inhibitory constants (Ki) of A-41 with the following aortic ion channels. The docking results provided insight into the binding energies and potential inhibitory interactions of A-41 at the molecular level, supporting its vasorelaxant activity observed *in vitro*. After docking of the A41 ligand with a series of calcium-handling targets - Ca^{2+} L-type, Ca^{2+} R-type, RyR2 receptor, SERCA, Ca^{2+}-ATPase, and NCX channels - the binding energies of the resulting complexes were calculated. The lowest binding energy was –6.6 kcal/mol, with Ca^{2+}-ATPase and NCX, showing the strongest interaction among all the targets investigated. From these binding energies, the inhibitory potencies of the ligand (Ki values) for the proteins were then determined [22]. In order of the rising Ki values (declining affinity), the targets are as follows:

Ca^{2+} R-type > RyR2 receptor > SERCA > L-type Ca^{2+} > Ca^{2+}-ATPase – NCX, a gradient of diminishing interactions among these calcium-regulatory proteins (**Table 1**).

Voltage-gated L-type Ca^{2+} ion channel

Calcium ions (Ca^{2+}) are significant secondary messengers in eukaryotic cells and are crucial in controlling various physiological and biochemical processes. Of special interest is the influx of Ca^{2+} in mediating cellular response in cardiac, smooth muscle, and neural tissues. One of the major routes for the entrance of calcium into cells is through Voltage-Dependent Calcium Channels (VDCCs). Of these, L-type calcium channels (Cav1 family) stand out due to their wide distribution and the key role that they play in cellular communication. L-type channels are particularly very significant in cardiac and vascular smooth muscle cells, mediating calcium influx required in processes like muscle contraction, secretion of hormones, gene expression, and signal transduction. Control and function of L-type channels are of high significance in a wide range of pathophysiological conditions, e.g., cardiovascular diseases, hypertension, neurological disease, and metabolic syndrome. In view of such situations, L-type channels are of high value for physiological and pharmacological/clinical study alike [23,24]. Over the last few years, computational modeling and experimental techniques have been heavily utilized to analyze the structure, functional modulation, and interaction of L-type calcium channels with natural products-particularly flavonoids-with emphasis on their increasing therapeutic significance. In this research, the biological significance of L-type calcium channels, modulators of their activity, and flavonoid-derived ligand inhibitory activity are investigated. When the A41 ligand was docked onto the Ca^{2+} L-type calcium channel, its binding energy was –6.3 kcal/mol, indicating a stable interaction. The inhibitory constant (Ki) was determined from this binding energy as 24.152 μM, which categorizes the ligand as a weak inhibitor for the L-type channel (**Table 1(A)**). To induce functional inhibition, the ligand will have to contact essential amino acid residues necessary for channel function. According to docking results, A41 contacts several such essential residues, which include:

LEU F:269, SER F:265, TYR:396, ASP F:598, PRO A:75, ASP A:586, ARG A:593

Nature of these contacts are: Normal hydrogen bonds, Carbon-hydrogen bonds Unfavorable acceptor–acceptor interactions Alkyl and π-alkyl interactions. These results demonstrate that A41 interacts with structural and potentially functional areas of the L-type calcium channel and reveal its mechanism of action and potential pharmacological relevance.

Receptor mediated Ca^{2+}R-type calcium channels

R-type calcium channels (Cav2.3) are members of the voltage-dependent calcium channel family (VDCCs), or more specifically the Cav2 subfamily. They are "R-type" by designation because they are resistant to most conventional calcium channel blockers and have intermediate electrophysiological properties between T- and L-type channels [25]. Upon docking with R-type calcium channels, the binding energy obtained was calculated to be −5.5 kcal/mol and is a very weak binding. From the binding energy, Ki value or inhibition constant was estimated to be 93.165 μM and A41 was found to be a weak inhibitor of R-type calcium channels (**Table 1(B)**). The docking analysis showed that A41 molecule binds to specific amino acid residues of the R-type calcium channel, i.e., PRO A:1479, GLN A:1174, TYR A:1469, ASN A:1466, and LYS A:1345. These bindings are complemented by a range of non-covalent interactions including normal hydrogen bonds, carbon hydrogen bonds, unfavorable donor–donor interactions, pi-cation, pi–sigma, pi–pi T-shaped, alkyl, and pi-alkyl interactions.

SERCA (sarcoplasmic reticulum Ca^{2+}ion channel)

SERCA is a significant enzyme that transfers calcium ions (Ca^{2+}) from cytoplasm into the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR) in muscle cells utilizing the hydrolysis energy of ATP. The process has a central function in intracellular calcium management, muscle contraction and relaxation, and signaling transduction. SERCA is P-type ATPase as it goes through phosphorylation/

dephosphorylation during the transfer of calcium. It exists as a solitary polypeptide chain with the transmembrane domain that crosses the SR or ER membrane and the cytoplasmic domain that can bind ATP [26]. It uses the energy gained from hydrolysis of ATP to translocate 2 calcium ions per ATP molecule, contrary to the direction of the concentration gradient, to the lumen of the ER or SR. SERCA also plays a critical role in the relaxation of muscles because it actually pumps calcium ions out of the cytoplasm following a contraction, resulting in reduced cytosolic calcium, and thus induces relaxation of muscles. Besides muscle tissue, SERCA is present in many other cell types and performs significant roles in signal transduction, protein folding, and regulation of many calcium-dependent enzymes. Recent studies imply that increasing SERCA activity by pharmacological agents or gene therapy might have therapeutic effects for the treatment of cardiac and skeletal muscle diseases, as well as enhancing calcium homeostasis in neurons. Molecular docking of the A41 ligand into the SERCA (Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase) was −5.9 kcal/mol binding energy, indicating a moderate degree of interaction strength (**Table 1(C)**). From this binding energy, the inhibition constant (Ki value) was estimated to be 40.070 μM, suggesting that A41 is a moderate inhibitor of SERCA. The binding study showed a number of significant interactions between the A41 molecule and specific amino acid residues of SERCA, such as LYS A:158, PRO A:160, ASN A:39, THR A:230, LEU A:41 and GLY A:227. Such interactions were formed by a series of non-covalent interactions, such as classical hydrogen bonds, carbon–hydrogen bonding, unfavorable acceptor acceptor, alkyl relationships, and pi-pi stacking. These findings highlight the molecular mechanism underlying the inhibitory action of ligand to SERCA, and specific amino acid residues are involved in the binding event.

RyR2 (Ryanodine) receptor

Ryanodine receptor 2 (RyR2) is a huge calcium (Ca^{2+}) channel found on the sarcoplasmic reticulum (SR) membrane of cardiac muscle cells. It is very important for the contraction of cardiac muscle. When there is electrical stimulation of the cardiac cell, the L-type calcium channels (Cav1.2) present on the cell membrane open and permit the ingress of some Ca^{2+} into the cell. This calcium influx triggers RyR2 receptors to release a massive amount of Ca^{2+} from the SR calcium reservoir [27]. This process is referred to as calcium-induced calcium release (CICR). The RyR2 receptor is not only required for normal heart function, but it is also required for the maintenance of blood circulation, regulation of blood pressure, and adequate supply of oxygen to the whole body. Its normal functioning stabilizes heart rhythm and is one of the critical determinants in the prevention of heart failure, arrhythmias, and sudden cardiac death. RyR2 function is controlled by inherent mechanisms (phosphorylation and oxidation) and external inputs (drugs and genetic mutations). The receptor's mutation or dysregulation has been linked to catastrophic cardiac diseases like catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure, and sudden cardiac death. Because of its central role in cardiac function, the RyR2 receptor continues to be at the center of numerous scientific studies and is a potential pharmacological target for drug therapy [28,29].

From our research, we noted that A41 ligand binds to RyR2 receptor with the binding energy of –5.9 kcal/mol, which is equivalent to the calculated inhibitory constant (Ki) of 24.152 μM (**Table 2(A)**). The fact that A41 qualifies as a weak inhibitor of the receptor implies that the compound binds to some amino acids that are crucial for the functioning of RyR2 structure. As per our docking results, A41 ligand binds with amino acid residues ALA A:698, PHE A:723, ASP A:721, and ASN A:716 via pi-alkyl, pi-anion, and classical hydrogen bonds. These bindings keep the lipophilic contacts, electrostatic interactions, and binding of the ligand overall in the active site of the receptor stable.

Ca^{2+}-ATPase

Ca^{2+}-ATPase is a membrane-bound enzyme responsible for regulating intracellular concentration of calcium ion (Ca^{2+}). It harnesses the energy derived from the hydrolysis of ATP molecules to transport Ca^{2+} ions from the cytoplasm through to the plasma membrane or into organelles, such as sarcoplasmic and endoplasmic reticulum. The activity aids in calcium homeostasis within the cell. Ca^{2+}-ATPase regulates the transport of calcium ions to prevent excess calcium accumulation in the cytoplasm [30]. By moving Ca^{2+}

away from the cytoplasm or into storage spaces such as the sarcoplasmic or endoplasmic reticulum, it prevents internal imbalance (homeostasis) in the cell. This is particularly necessary in muscle and heart cells, where the enzyme prevents muscle relaxation by pumping calcium out following contraction. Aside from its structural role, calcium takes part in activities like nerve impulse conduction, hormone release, signaling, and gene expression, all of which are Ca^{2+}-ATPase-regulated. When Ca^{2+}-ATPase is impaired, calcium will pile up inside the cell, causing ailments like heart failure, muscle disease, and nervous diseases. Ca^{2+}-ATPase can thus be referred to as an important pharmacological target. Most drugs affecting the heart function by promoting the action of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). In our research, while Ca^{2+}-ATPase was docked in the presence of the ligand A41, binding energy was -6.6 kcal/mol, and it was observed that there is a good interaction between Ca^{2+}-ATPase and A41 molecule. From this binding energy, inhibition constant (Ki value) was observed to be 14.558 µM, which reflects weak inhibitory activity (**Table 2(B)**). The ligand A41 binds to the following amino acids in Ca^{2+}-ATPase: ALA A:516, CYS A:560, LEU A:561, PHE A:487, LYS A:514, ARG A:559, and ARG A:677. These interactions involve traditional hydrogen bonds, pi-sigma bonds, alkyl and pi-alkyl, pi-cation bonds, and donor-donor hydrogen bonds. These non-covalent interactions bind and stabilize the ligand to the enzyme, although the interaction is not strong enough for effective inhibition.

Table 1 Molecular docking results of A-41 with various calcium ion channels. (A) L-type Ca^{2+} channel: 2D and 3D structures, binding energy, and inhibition constant. (B) R-type Ca^{2+} channel: Structural views along with docking parameters. (C) SERCA Ca^{2+} pump: visual representation and corresponding interaction metrics.

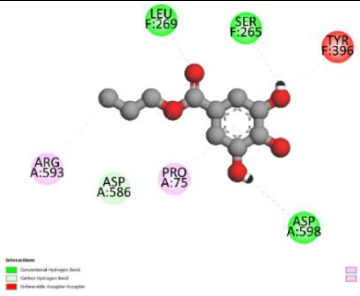
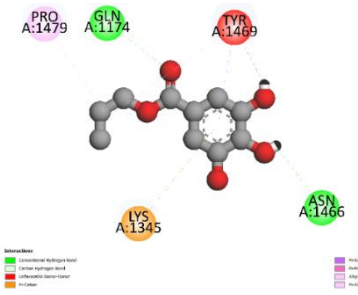
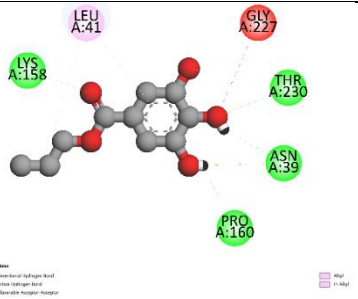
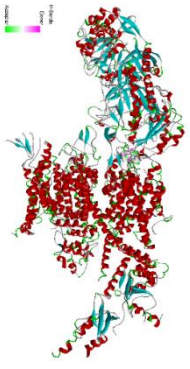
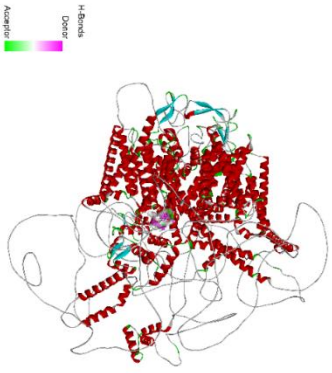
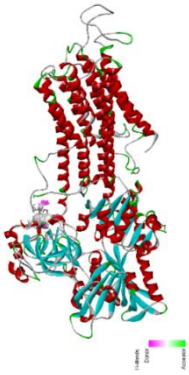
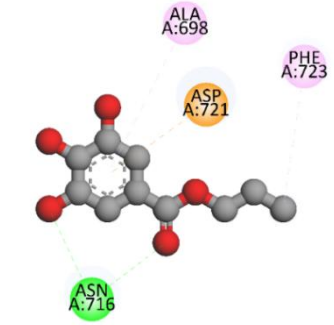
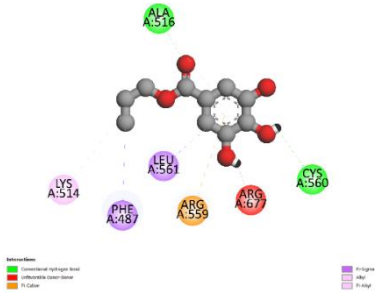
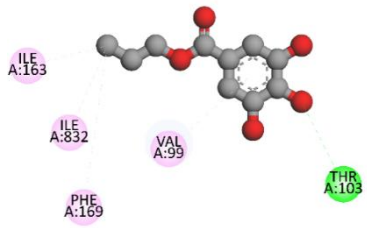
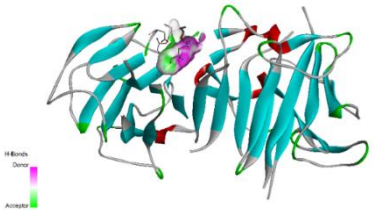
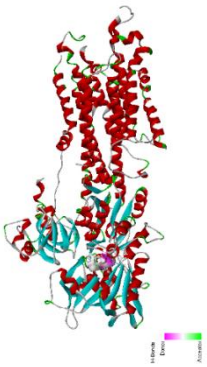
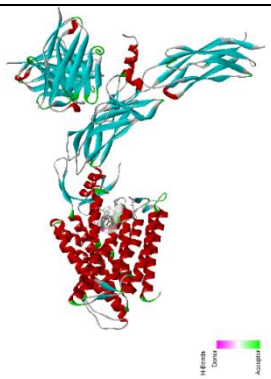
Channels	**L-type Ca^{2+} (A)**	**Ca^{2+} R-type (B)**	**SERCA (C)**
2D			
3D			
Ki value	24.152 µM	93.165 µM	40.070 µM
Affinity (Kcal/mol)	–6.3	–5.5	–6.0

Table 2 Molecular docking analysis of the A-41 compound with selected calcium ion channels. (A) RyR2 Ca^{2+} release channel: 2D/3D structural representations, binding affinity, and inhibitory constant (Ki). (B) Ca^{2+}-ATPase: Docking visualization and key interaction parameters. (C) NCX (Na^{+}/Ca^{2+} exchanger): Structural models and associated binding energies and Ki values.

Channels	**RyR2 receptor (A)**	**Ca^{2+}-ATPase (B)**	***NCX* (C)**
2D			
3D			
Ki value	47.436 µM	14.558 µM	14.558 µM
Affinity (Kcal/mol)	–5.9	–6.6	–6.6

NCX (sodium and calcium exchanger)

Na^{+}/Ca^{2+} exchanger (NCX) is an essential membrane-bound ion transport protein that plays a role in intracellular calcium homeostasis by bidirectional exchange of sodium (Na^{+}) and calcium (Ca^{2+}) ions across the plasma membrane. Having a tendency to work in its forward mode, NCX extrudes one Ca^{2+} ion from the cytoplasm in return for the inward entry of 3 Na^{+} ions powered by the electrochemical gradient of sodium. This electrogenic, ATP-independent antiporter plays a crucial role in excitable cells like cardiac myocytes and neurons to regenerate low cytosolic levels of calcium after the stimulation of a cell. NCX is critical in the egress of intracellular Ca^{2+}, complementing other mechanisms of Ca^{2+} egress to avoid Ca^{2+} overload, which might result in cytotoxicity, mitochondrial impairment, or cell death [31,32]. It is also especially crucial in neuroprotection, synaptic transmission, and cardiac relaxation, through calcium regulation under ischemia, oxidative stress, or excitotoxicity. Changes in NCX function have been shown to be involved in the etiology of a range of diseases, such as cardiac arrhythmias, congestive heart failure, epilepsy, and neurodegenerative diseases, making it a valuable drug target. Current research aims to develop selective NCX modulators to activate or inhibit its function, based on the disease condition. In our molecular docking experiments, the A41 ligand exhibited a binding energy of –6.6 kcal/mol against NCX, a strong interaction and moderate inhibition activity, with a Ki of 14.558 µM. A41 molecule established contacts with the important residues of the NCX protein, including ILE A:163, ILE A:832, PHE A:169, VAL A:99, and THR A:103, by alkyl contacts and normal hydrogen bonds (**Table 2(A)**). Such interactions confirmed the close and stable ligand

binding, which defined its inhibitory function. These findings suggest A41 as a candidate compound for NCX targeting to develop drugs for therapy of calcium-pathology-related diseases in cardiovascular and neurologic disease.

Effect of A41 compound on blood pressure *in vivo* studies

The effect of the A41 compound on blood pressure was evaluated *in vivo* using the tail cuff method. Rats were divided into 4 groups, each receiving different doses of the A41 compound: 10 mg/kg, 20 mg/kg, and 30 mg/kg. Prior to the experiment, the baseline blood pressure (0 h) of all animals was measured. Afterward, the A41 compound was administered intravenously, and its effect on the cardiovascular system was monitored for 3 h, with measurements taken every hour [33].

Effect of A41 Compound at 10 mg/kg dose

In the control group, the baseline systolic blood pressure was 133.0 ± 13.1 mmHg, and the diastolic blood pressure was 98.5 ± 9.7 mmHg. In the group receiving 10 mg/kg of A41, the blood pressure changes were as follows:

1 h: Systolic blood pressure decreased to 132.3 ± 13.1 mmHg, and diastolic blood pressure decreased to 104.5 ± 10.3 mmHg.

2 h: Systolic blood pressure significantly decreased to 93.0 ± 9.2 mmHg, and diastolic blood pressure dropped to 73.0 ± 7.1 mmHg.

3 h: Blood pressure partially recovered, with systolic blood pressure reaching 84.8 ± 8.5 mmHg, and diastolic blood pressure returning to 56.3 ± 5.6 mmHg.

The 10 mg/kg dose showed the most significant decrease in blood pressure, followed by partial recovery. This dose was identified as the optimal dose.

Effect of A41 compound at 20 mg/kg dose

In the control group, systolic blood pressure was 118.0 ± 11.5 mmHg, and diastolic blood pressure was 92.8 ± 9.1 mmHg. In the group receiving 20 mg/kg of A41, the blood pressure changes were as follows:

1 h: Systolic blood pressure decreased to 112.8 ± 11.1 mmHg, and diastolic blood pressure decreased to 79.8 ± 7.8 mmHg.

2 h: Systolic blood pressure decreased significantly to 76.3 ± 7.5 mmHg, and diastolic blood pressure decreased to 55.8 ± 5.4 mmHg.

3 h: Blood pressure partially recovered, with systolic blood pressure reaching 104.5 ± 9.9 mmHg, and diastolic blood pressure returning to 68.3 ± 6.7 mmHg.

The 20 mg/kg dose also resulted in a significant decrease in blood pressure, but compared to the 10 mg/kg dose, the duration of the reduction was shorter. This dose also demonstrated a significant effect.

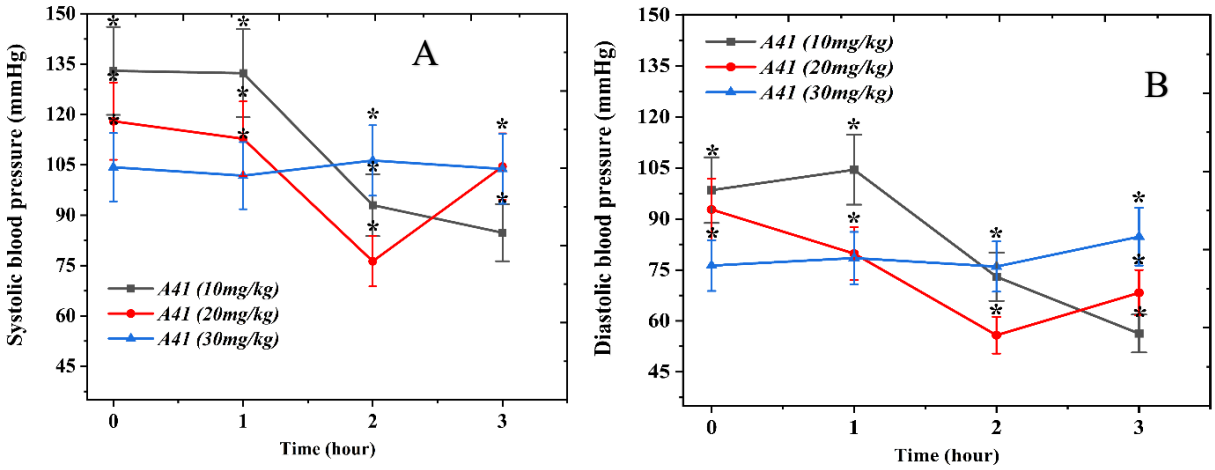


Figure 6 Doses decrease in systolic and diastolic blood pressure was observed following the administration of A41 compound at doses of 10, 20 and 30 mg/kg. These results indicate that the antihypertensive effect of the A41 compound is dose-dependent (n = 4, $p < 0.05$).

Effect of A41 compound at 30 mg/kg dose

The baseline systolic blood pressure was 104.3 ± 10.2 mmHg, and diastolic blood pressure was 76.3 ± 7.5 mmHg. In the group receiving 30 mg/kg of A41, the blood pressure changes were as follows:

1 h: Systolic blood pressure was 101.8 ± 10.0 mmHg, and diastolic blood pressure was 78.5 ± 7.7 mmHg.

2 h: Systolic blood pressure increased to 106.3 ± 10.5 mmHg, and diastolic blood pressure increased to 76.0 ± 7.4 mmHg.

3 h: Systolic blood pressure was 103.8 ± 10.4 mmHg, and diastolic blood pressure was 84.8 ± 8.5 mmHg (**Figures 6(A)** and **6(B)**).

The 30 mg/kg dose did not result in a significant effect on blood pressure, and its efficacy was lower compared to the 20 mg/kg dose. This suggests a lower impact at higher doses. The effect of the A41 compound on blood pressure was evaluated at various doses. At both 10 mg/kg and 20 mg/kg doses, a significant reduction in blood pressure was observed, while at the 30 mg/kg dose, minimal changes occurred. The most effective dose was 10 mg/kg, which showed a significant decrease in blood pressure followed by partial recovery. Further studies are required to explore the mechanism of action of the compound in greater detail (**Figure7**).

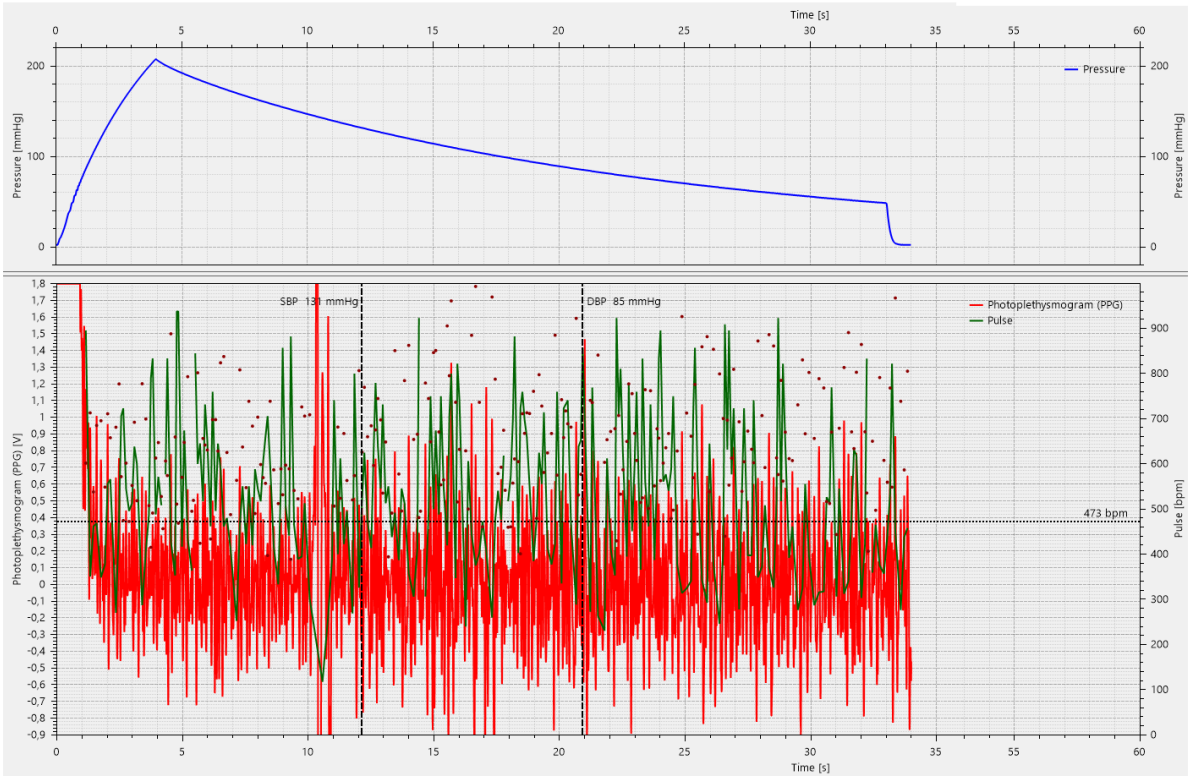


Figure 7 Original systolic recordings obtained using the Neurobotics software. (These results were used as sample data for statistical analysis.) (Control Group).

Evaluation of the effect of A41 compound in the adrenaline-induced hypertension model using the tail-cuff method

Before starting the experiments, the most effective dose of A41 compound was determined, and subsequent studies were conducted based on this dose. During the experiment, the rats were divided into the following groups: healthy control group, adrenaline-induced hypertension control group, and experimental group where A41 compound was administered on an adrenaline background. Each group consisted of healthy male rats (n = 4) weighing 300 - 350 g. Initially, systolic (SBP) and diastolic (DBP) blood pressures of all rats were measured using the Tail-Cuff method. The baseline measurements were as follows: in the control group, SBP was 93.3 ± 8.3 mmHg, DBP was 68.3 ± 5.7 mmHg; in rats receiving 10 mg/kg of A41, SBP was 85.0 ± 6.3 mmHg, DBP was 61.0 ± 5.8 mmHg.

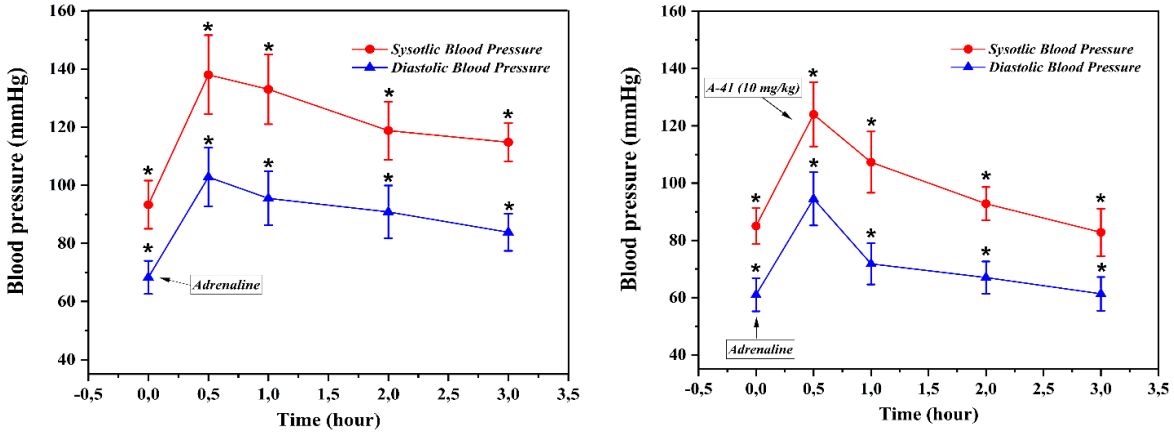


Figure 8 (A) Administration of the A41 compound at doses of 10, 20 and 30 mg/kg resulted in a dose-dependent reduction in systolic and diastolic blood pressure. (B) These findings illustrate the antihypertensive effect of A41 against adrenaline-induced hypertension (n = 4, $p < 0.05$).

Subsequently, adrenaline hydrochloride was intravenously injected into all groups (except the healthy control group), and changes in blood pressure were monitored [34]. After adrenaline administration, blood pressure in the hypertensive model rats increased as follows: SBP 138.3 ± 13.6 mmHg, DBP 102.8 ± 10.1 mmHg. In the experimental group where A41 compound was administered along with adrenaline, the changes in blood pressure were relatively lower: A41 (10 mg/kg) group - SBP 124.0 ± 11.2 mmHg, DBP 94.5 ± 9.3 mmHg. In the next phase, after the intravenous administration of A41 compound, blood pressure was measured hourly in each group. The control group was used solely to show the effect of adrenaline and did not receive any other preparations. In this group, the blood pressure readings were as follows: 1st hour - SBP 133.3 ± 14.9 mmHg, DBP 95.5 ± 9.3 mmHg; 2nd hour - SBP 118.8 ± 10.0 mmHg, DBP 90.8 ± 9.1 mmHg; 3rd hour - SBP 114.8 ± 6.6 mmHg, DBP 83.8 ± 6.4 mmHg (**Figure 8**).

The antihypertensive effects of A41 compound were observed as follows:

The 10 mg/kg dose of A41 compound showed the most effective results:

1 h: SBP 107.3 ± 10.7 mmHg, DBP 71.8 ± 7.2 mmHg

2 h: SBP 92.8 ± 5.8 mmHg, DBP 67.0 ± 5.6 mmHg

3 h: SBP 82.8 ± 8.3 mmHg, DBP 61.3 ± 5.9 mmHg

These results show that the A41 compound was effective in lowering blood pressure in adrenaline-induced hypertension. The 10 mg/kg dose of A41 compound demonstrated a pronounced antihypertensive effect. This finding is significant in determining the potential therapeutic possibilities of A41.

Discussions

The current research explores compound A-41's vasorelaxant effect and probable antihypertensive action and investigates the mechanisms of its action using a battery of *in vitro* and *in vivo* experimental models, supported by molecular docking simulations. Together, the results validate that A-41 exhibits remarkable vasorelaxant effect via several mechanisms, mainly through the regulation of calcium homeostasis in vascular smooth muscle cells. Our results with KCl-induced contractions of isolated rat aortic rings revealed that A-41 elicits concentration-dependent relaxation from moderate (5.0 ± 2.2 %) at 5 µM to significant (82.2 ± 2.0 %) at 35 µM. The calculated IC_{50} value of around 22.10 µM reflects moderate potency. A-41's potent blockade of KCl-induced contraction is highly indicative of interference with voltage-dependent L-type calcium channels, which play a significant role in membrane

depolarization-stimulated contraction of vascular smooth muscle. The mechanism was also pharmacologically verified by comparison with verapamil, an established L-type calcium channel blocker. Of note, A-41 showed additive effects with verapamil, suggesting complementary action at different sites on L-type channels or activation of other mechanisms for calcium regulation.

In addition to voltage-dependent calcium channels, our results show that A-41 also acts on receptor-operated calcium channels. This was demonstrated by its strong inhibition (87.0 ± 2.7 % at 50 μM) of phenylephrine-induced contraction, which is mostly mediated by the stimulation of α-adrenoceptors and the subsequent mobilization of calcium from intracellular stores and also from receptor-operated channels. The additive inhibitory effect of co-administration of A-41 with phentolamine (α-adrenoceptor antagonist) is also a pointer to the multi-target mechanism of action because this would imply that A-41 interferes with α-adrenoceptor-mediated calcium entry processes in addition to intracellular calcium stores.

The endothelium dependency experiments provided a glimpse into A-41's mechanism of action. The marked attenuation (31.0 ± 2.5 %) of vasorelaxant activity following endothelial denudation indicates that endothelium-derived factors, and specifically nitric oxide (NO), are crucial in A-41's vasodilatory effect. This finding is particularly significant considering that endothelial dysfunction is a major contributor to the pathophysiology of cardiovascular diseases like hypertension and atherosclerosis.

The ability of A-41 to potentially cause NO production or enhance its bioavailability presents an additional therapeutic advantage as it can reverse endothelial dysfunction in pathologic states with oxidative stress and reduced availability of NO.

Molecular docking research has offered additional mechanistic insights by elucidating the interactions of A-41 with other calcium-handling proteins. Binding energy analysis revealed various affinities towards targets, with optimal interactions being with NCX and Ca^{2+}-ATPase (both –6.6 kcal/mol), followed by L-type Ca^{2+} channels (–6.3 kcal/mol), RyR2 receptors and SERCA (both –5.9 kcal/mol), and Ca^{2+} R-type channels (–5.5 kcal/mol).

The inhibitory constants (Ki values) calculated were between 14.558 μM against NCX and Ca^{2+}-ATPase and 93.165 μM against R-type calcium channels, and A-41 is thus a weak to moderate inhibitor against these targets. The determination of the exact amino acid residues participating in these interactions offers useful structural information for the further optimization of A-41 derivatives.

This multi-targeting profile of A-41 uncovered by these docking studies is also consistent with our *in vitro* results and predicts a global mechanism for the regulation of calcium homeostasis. By regulating several calcium transport mechanisms simultaneously-voltage-gated channels (L-type and R-type), intracellular calcium release channels (RyR2), calcium pumps (SERCA and Ca^{2+}-ATPase), and exchangers (NCX)-A-41 would theoretically be able to regulate intracellular calcium concentrations effectively through complementary pathways, which could be an advantage over single-target calcium channel blockers.

The blood pressure *in vivo* experiments also confirmed the therapeutic action of A-41 as an antihypertensive compound. The dose-response study identified 10 mg/kg as the most effective dose, which caused profound reduction in blood pressure and recovery, albeit partial, within 3 h. Interestingly, the 30 mg/kg dose failed to exert any significant action, indicating a bell-shaped dose-response curve that should be further investigated. In the model of adrenaline-induced hypertension, A-41 (10 mg/kg) exhibited excellent antihypertensive activity in the form of a gradual decrease in systolic and diastolic blood pressure lasting for 3 h. These results are most comforting as they validate the translatability of A-41's *in vitro* vasorelaxant activity to *in vivo* with therapeutic importance. The demonstrated action of A-41 in the model of adrenaline-induced hypertension is of particular interest considering the pathophysiological importance of this model. Adrenaline-induced hypertension combines α-adrenergic receptor-mediated vasoconstriction with cardiac output augmentation, and both of these actions simulate phenomena of sympathetic hyperactivity commonly encountered in essential hypertension. The implication that A-41 was able to block such actions is conducive to its prospective value in hypertension of high sympathetic tone.

Conclusions

In conclusion, our extensive study demonstrates that compound A-41 displays excellent vasorelaxant and antihypertensive activity through multiple mechanisms of action. A-41 primarily modulates calcium homeostasis through inhibition of voltage-dependent and receptor-operated calcium channels, along with a possible enhancement of endothelium-derived nitric oxide signaling. Molecular docking studies revealed interactions with an array of calcium-handling proteins, confirming A-41's multi-target profile. *In vivo* studies authenticated these findings, demonstrating dose-dependent reduction of blood pressure in normotensive and adrenaline-induced hypertensive models. A-41's multi-modal action is a promising strategy for antihypertensive treatment and has the potential to be more effective and have a better side effect profile than single-target molecules. The 10 mg/kg dose needed for full effect established herein gives a basis for additional pharmacokinetic study and chronic toxicity testing. Additional studies should be aimed at maximizing the structure of A-41 for greater potency and selectivity, chronic safety and efficacy trials, and exploration of possible synergistic interactions with traditional antihypertensive drugs. These findings collectively position A-41 as a lead compound of promise for the development of novel antihypertensive drugs targeting calcium channel modulation and endothelial function with therapeutic application in hypertension and cardiovascular disorders related to it.

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

Shodiyakhon Sodiqova performed the in vitro experiment, **Shokhida Kadirova** was responsible for the extraction of the substances, **Anvar Zaynabiddinov** acted as the PhD supervisor, **Izzatullo Abdullaev** prepared the original draft of the manuscript, **Lazizbek Makhmudov** conducted the in vivo experiments, **Ulugbek Gayibov** supervised the manuscript drafting process, **Mukhabbat Yuldasheva** supervised the substance extraction process, **Madina Xolmirzayeva** served as a scientific supervisor, **Rakhmatilla Rakhimov** contributed to the provision and handling of the substances, **Azizbek Mutalipov** and **Hayotbek Karimjonov** contributed equally to the research and data analysis.

References

[1] MD Cesare, P Perel, S Taylor, C Kabudula, H Bixby, TA Gaziano, DV McGhie, J Mwangi, B Pervan, J Narula, D Pineiro and FJ Pinto. *Global Heart* 2024; **19(1)**, 11.

[2] IZOGL Abdullaev, UG Gayibov, SZ Omonturdiev, SF Azamjonovna, SN Gayibova and TF Aripov. Molecular pathways in cardiovascular disease under hypoxia: Mechanisms, biomarkers, and therapeutic targets. *The Journal of Biomedical Research* 2025; **39(2)**, 1-16.

[3] CJ Hutchings, P Colussi and TG Clark. Ion channels as therapeutic antibody targets. *MAbs* 2019;**11(2)**, 265-296.

[4] AA Abdullaev, DR Inamjanov, DS Abduazimova, SZ Omonturdiyev, UG Gayibov, SN Gayibova and TF Aripov. *Silybum mariánum's* impact on physiological alterations and oxidative stress in diabetic rats. *Biomedical & Pharmacology Journal* 2024; **17(2)**, 1291-1300.

[5] BK Joseph, KM Thakali, CL Moore and SW Rhee. Ion channel remodeling in vascular smooth muscle during hypertension: Implications for novel therapeutic approaches. *Pharmacological Research* 2013; **70(1)**, 126-138.

[6] O Gaibullayeva, A Islomov, D Abdugafurova, B Elmurodov, B Mirsalixov, L Mahmudov, I Adullaev, K Baratov, S Omonturdiev and S Sa'dullayeva. *Inula helenium* L. root extract in sunflower oil: Determination of its content of water-soluble vitamins and immunity-promoting effect. *Biomedical & Pharmacology Journal* 2024; **17(4)**, 2729-2737.

[7] AQQ Azimova, AX Islomov, SA Maulyanov, DG Abdugafurova, LU Mahmudov, IZ o`g`li Abdullaev, AS Ishmuratova, SQQ Siddikova and IR Askarov. Determination of vitamins and pharmacological properties of *Vitis vinifera* L.

plant fruit part (mixed varieties) syrup-honey. *Biomedical and Pharmacology Journal* 2024; **17(4)**, 2779-2786.

[8] AV Mahmudov, OS Abduraimov, SB Erdonov, UG Gayibov and LY Izotova. Bioecological features of *Nigella sativa* L. in different conditions of Uzbekistan. *Plant Science Today* 2022; **9(2)**, 421-426.

[9] OS Zoirovich, AIZ Ugli, ID Raxmatillayevich, ML Umarjonovich, ZM Ravshanovna, G Sabina, Narimanovna, GU Gapparjanovich and AT Fatikhovich. The effect of Ájuga Turkestánica on the rat aortic smooth muscle ion channels. *Biomedical & Pharmacology Journal* 2024; **17(2)**, 1213-1222.

[10] D Inomjonov, I Abdullaev, S Omonturdiev, A Abdullaev, L Maxmudov, M Zaripova, M Abdullayeva, D Abduazimova, S Menglieva, S Gayibova, M Sadbarxon, U Gayibov and T Aripov. *In vitro* and *in vivo* studies of crategus and inula helenium extracts: Their effects on rat blood pressure. *Trends in Sciences* 2025; **22(3)**, 9158.

[11] A Abdullaev, I Abdullaev, A Bogbekov, U Gayibov, S Omonturdiev, S Gayibova, M Turahodjayev, K Ruziboev and T Aripov. Antioxidant potential of rhodiola heterodonta extract: Activation of Nrf2 pathway via integrative *in vivo* and *in silico* studies. *Trends in Sciences* 2025; **22(5)**, 9521.

[12] HM Berman, J Westbrook, Z Feng, G Gilliland, TN Bhat, H Weissig, IN Shindyalov and PE Bourne. The protein data bank. *Nucleic Acids Research* 2000; **28(1)**, 235-242.

[13] H Zohny, J McMillan and M King. Ethics of generative AI. *Journal of Medical Ethics* 2023; **49(2)**, 79-80.

[14] M Iman, A Saadabadi and A Davood. Molecular docking analysis and molecular dynamics simulation study of ameltolide analogous as a sodium channel blocker. *Turkish Journal of Chemistry* 2015; **39(2)**, 10.

[15] J Palacios, D Asunción-Alvarez, D Aravena, M Chiong, MA Catalán, C Parra, F Cifuentes and A Paredes. A new oxime synthesized from Senecio nutans SCh. Bip (chachacoma) reduces calcium influx in the vascular contractile response in rat aorta. *RSC Advances* 2024; **14(14)**, 9933-9942.

[16] J Striessnig, NJ Ortner and A Pinggera. Pharmacology of L-type calcium channels: Novel drugs for old targets? *Current Molecular Pharmacology* 2015; **8(2)**, 110-122.

[17] M Al-Khrasani, DA Karadi, AR Galambos, B Sperlagh and ES Vizi. The pharmacological effects of phenylephrine are indirect, mediated by noradrenaline release from the cytoplasm. *Neurochemical Research* 2022; **47(11)**, 3272-3284.

[18] UG Gayibov, SN Gayibova, HMU Karimjonov, AAU Abdullaev, DS Abduazimova, RN Rakhimov, HS Ruziboev, MA Xolmirzayeva, AE Zaynabiddinov and TF Aripov. Antioxidant and cardioprotective properties of polyphenolic plant extract of *Rhus glabra* L. *Plant Science Today* 2024; **11(3)**, 655-662.

[19] TF Aripov, UG Gayibov, SN Gayibova, AA Abdullaev, DS Abduazimova, NS Berdiev, JF Ziyavitdinov, YI Oshchepkova and SH Salikhov. Antiradical and antioxidant activity of the preparation “Rutan” from *Rhus* coriaria L. *Journal of Theoretical and Clinical Medicine* 2023; **4**, 164-170.

[20] AV Mahmudov, OS Abduraimov, SB Erdonov, AL Allamurotov, OT Mamatqosimov, UG Gayibov and L Izotova. Seed productivity of *Linum usitatissimum* L. in different ecological conditions of Uzbekistan. *Plant Science Today* 2022; **9(4)**, 1090-1101.

[21] AG Vakhobjonovna, KE Jurayevich, Ogli, AIZ Ogli, EN Azamovich, MR Rasuljonovich and AM Islomovich. Tannins as modulators in the prevention of mitochondrial dysfunction. *Trends in Sciences* 2025; **22(8)**, 10436.

[22] PJ Tonge. Drug-target kinetics in drug discovery. *ACS Chemical Neuroscience* 2018; **9(1)**, 29-39.

[23] EM El-Assri, Y El-Assri, RE Brahimi, ME Fadili, A Baghouz, HA Abuelizz, S Er-Rahmani, A Lahkimi and A Bouia. Molecular characterization, chemical profile and biological properties of essential oils from *Chamaemelum nobile* (L.) flowers of Morocco: *In vitro* and *in silico* studies. *Frontiers in Chemistry* 2025; **13**, 1539872.

[24] M Zaripova, I Abdullaev, A Bogbekov, U Gayibov, S Omonturdiev, R Makhmudov, N Ergashev, G Jabbarova, S Gayibova and T Aripov. In Vitro and in Silico Studies of Gnaphalium U. Extract: Inhibition of α-amylase and α-glucosidase as a Potential Strategy for Metabolic Syndrome Regulation. *Trends in Sciences* 2025; **22(8)**, 10098.

[25] UG Gayibov, EJ Komilov, RN Rakhimov, NA Ergashev, NG Abdullajanova, MI Asrorov and TF Aripov. Influence of new polyphenol compound from Euphorbia plant on mitochondrial function. *Journal of Microbiology Biotechnology and Food Sciences* 2019, **8(4)**: 1021-1025.

[26] S Shao and RS Hegde. Membrane protein insertion at the endoplasmic reticulum. *Annual Review of Cell and Developmental Biology* 2011; **27**, 25-56.

[27] EM Capes, R Loaiza and HH Valdivia. Ryanodine receptors. *Skelet Muscle* 2011; **1(1)**, 18.

[28] Y Umidakhon, B Erkin, G Ulugbek, N Bahadir and A Karim. Correction of the mitochondrial NADH oxidase activity, peroxidation and phospholipid metabolism by haplogenin-7-glucoside in hypoxia and ischemia. *Trends in Sciences* 2022; **19(21)**, 6260.

[29] MK Pozilov, UG Gayibov, MI Asrarov, N Abdulladjanova, HS Ruziboev and TF Aripov. Physiological alterations of mitochondria under diabetes condition and its correction by polyphenol gossitan. *Journal of Microbiology Biotechnology and Food Sciences* 2022; **12(2)**, e2224.

[30] L Daverkausen-Fischer and F Pröls. Regulation of calcium homeostasis and flux between the endoplasmic reticulum and the cytosol. *Journal of Biological Chemistry* 2022; **298(7)**, 102061.

[31] Z Shakiryanova, R Khegay, U Gayibov, A Saparbekova, Z Konarbayeva, A Latif1 and O Smirnova. Isolation and study of a bioactive extract enriched with anthocyanin from red grape pomace (Cabernet Sauvignon). *Agronomy Research* 2023; **21(3)**, 1293-1303.

[32] U Gayibov, SN Gayibova, MMK Pozilov, FS Tuxtaeva, UR Yusupova, GMK Djabbarova, ZA Mamatova, NA Ergashev and TF Aripov. Influence of quercetin and dihydroquercetin on some functional parameters of rat liver mitochondria. *Journal of Microbiology, Biotechnology and Food Sciences* 2021; **11(1)**, e2924.

[33] DG Harrison, M Bader, LO Lerman, G Fink, SA Karumanchi, JF Reckelhoff, MLS Sequeira-Lopez and RM Touyz. Tail-cuff versus radiotelemetry to measure blood pressure in mice and rats. *Hypertension* 2024; **81(1)**, 3-5.

[34] M Zaripova, S Gayibova, R Makhmudov, A Mamadrahimov, N Vypova, U Gayibov, S Miralimova and T Aripov. Characterization of *Rhodiola heterodonta* (Crassulaceae): Phytocomposition, antioxidant and antihyperglycemic activities. *Preventive Nutrition and Food Science* 2024; **29(2)**, 135-145.