https://doi.org/10.48048/tis.2025.9158

In Vitro and *In Vivo* Studies of *Crategus* and *Inula helenium* extracts: Their Effects on Rat Blood Pressure

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Received: 16 October 2024, Revised: 25 October 2024, Accepted: 1 November 2024, Published: 10 January 2025

Abstract

The contraction of aortic tissues induced by 50 mM KCl is primarily mediated through the activation of voltage-dependent L-type Ca^{2+} channels in smooth muscle cells. In this study, the effects of *Crategus Rosaceae* and *Inula helenium* extracts on KCl-induced contraction were investigated. Extracts of *Crataegus* attenuated the KCl-induced contraction by 15.0 ± 2.6 and 70.0 ± 2.8 % at concentrations ranging from 20 - 70 μg/mL, while *Inula helenium* extract reduced the contraction by 5.0 ± 3.1 and 80.0 ± 2.8 % at concentrations of 10 - 50 μg/mL. These findings suggest that the extracts inhibit KCl-induced contraction by modulating L-type Ca^{2+} channels. Further experiments were conducted in Ca^{2+}-free Krebs solution and in the presence of the L-type Ca^{2+} channel blocker, verapamil. *Crategus Rosaceae* and *Inula helenium* extracts significantly reduced aortic contractions in the presence of KCl and Ca^{2+}, with reductions of 45.0 ± 2.2 and 38.0 ± 2.4 %, respectively, suggesting that the relaxant effects are mediated by reduced Ca^{2+} influx through L-type Ca^{2+} channels. Additionally, *Crataegus* and *Inula helenium* extracts inhibited phenylephrine (1 μM)-induced contraction, with reductions of 75.0 ± 2.4 and 88.0 ± 2.2 %, respectively, further suggesting an effect on both receptor-controlled and L-type Ca^{2+} channels. When combined with the α-adrenoceptor blocker phentolamine, the extracts reduced phenylephrine-induced contraction by 20.0 ± 2.1 and 10.0 ± 2.3 %, respectively. Endothelium-dependent relaxant effects of the extracts were confirmed in experiments with L-NAME (100 μM), where the relaxant effects of *Crataegus* and *Inula helenium* were significantly reduced to 15.0 ± 2.9 and 24.3 ± 2.4 %, respectively, in the absence of the endothelial layer. These findings suggest that the relaxant effects are mediated through the nitric oxide (NO)-cGMP-PKG signaling pathway. *In vivo* experiments using the tail cuff method demonstrated a dose-dependent decrease in systolic blood pressure (SBP) in hypertensive rats treated with *Crataegus* extract at doses of 50, 100 and 200 μg/mL, further supporting the extract's potential antihypertensive effects.

Keywords: Vasorelaxation, Ca^{2+} L-type channels, Ca^{2+} R-type channels, Phenylephrine, Verapamil, Phentolamine, Tail cuff, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Adrenaline-hydrochloride

Introduction

Biologically active compounds, often derived from natural sources, have been increasingly studied for their potential therapeutic effects. Plant extracts such as those from *Crataegus* and *Inula helenium* contain a rich variety of bioactive substances, including flavonoids, phenolic acids and terpenoids, which can influence various physiological processes, including blood pressure regulation [1]. These extracts are particularly

promising for cardiovascular applications due to their antioxidant, anti-inflammatory and vasodilatory properties [2].

Hypertension, a major risk factor for cardiovascular complications, remains a prevalent global health issue. Identifying natural compounds that can modulate blood pressure is crucial for therapeutic advancements [3]. Both *Crataegus* and *Inula helenium* extracts have been shown to affect vascular tone by targeting pathways such as L-type Ca^{2+} channels and nitric oxide synthesis, offering potential as natural antihypertensive agents.

In this study, we focus on investigating the effects of specific bioactive compounds on rat blood pressure, utilizing both *in vitro* and *in vivo* experimental models. The *in vitro* studies allow for a controlled analysis of how these compounds interact with vascular tissues, while *in vivo* studies provide insights into their systemic effects in a living organism. Rats, being a well-established model for studying cardiovascular physiology, offer a relevant platform for understanding how these compounds could potentially translate to human health benefits [4].

This work aims to bridge the gap between laboratory findings and their practical applications by examining the mechanisms through which these bioactive compounds exert their effects. We also compare the outcomes from *in vitro* experiments with those observed *in vivo*, highlighting any variations or consistencies in the physiological responses.

Materials and methods

Chemicals

Crategus Rosaceae and *Inula helenium*, widely utilized medicinal plants in Uzbekistan, have been employed in our research experiments. The extracts of *Crategus Rosaceae* and *Inula helenium* used in these experiments were supplied by "Bioton" LTD, located in Tashkent, Uzbekistan. Additional chemical reagents, including phenylephrine, phentolamine, and verapamil, were sourced from Sigma-Aldrich Chemie, a division of Sigma-Aldrich based in St. Louis, MO, USA.

Tissue preparation

All experimental protocols were approved by the institutional animal care and use committee. The animals were maintained under standard vivarium conditions, with humidity levels between 55 and 65 %, and a controlled temperature of 22 ± 2 °C. They were provided unrestricted access to water and standard laboratory chow. All experimental procedures adhered to European Directive 2010/63/EU, which governs the use of animals in scientific research [5]. The experimental protocols were specifically approved by the Animal Ethics Committee of the Institute of Bioorganic Chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 4, 2014). Surgical interventions were performed under sodium pentobarbital anesthesia, with precautions to minimize animal discomfort. Male white rats weighing between 200 and 250 g were used in the study [6]. Euthanasia was conducted via cervical dislocation, followed by the excision of the aorta, which was then placed in a 5 mL organ bath containing Krebs-Henseleit solution (**Figure 3**). The solution consisted of 120.4 mM NaCl, 5 mM KCl, 15.5 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 11.5 mM glucose and HEPES, with the pH adjusted to 7.4. In some experiments, a Ca^{2+}-free Krebs solution supplemented with 1 mM EGTA was used [7]. The bath temperature was maintained at 37 °C using a DAIHAN WATER BATH ultrathermostat, and the solution was continuously oxygenated with a carbogen mixture (95 % O_2 and 5 % CO_2). Connective tissue and fat were carefully removed from the aorta, which was subsequently cut into 3 - 4 mm rings for experimentation.

Aortic-ring contraction studies

The aortic rings were attached to a Radnoti Isometric Transducer using platinum wire hooks and allowed to equilibrate for 1 h before measurements. Each ring was initially set to a resting tension of 1 g (10 mN) (**Figure 1**). The contraction forces generated by the aortic rings were transmitted to a signal amplifier and displayed digitally on a PC using a Go-Link automated converter. Data analysis was performed using "OriginLab OriginPro v.8.5 SR1" (EULA, Northampton, MA, USA). The isometric contraction forces (mN) of the aortic rings were recorded under *in vitro* conditions and presented as percentages (%) [8].

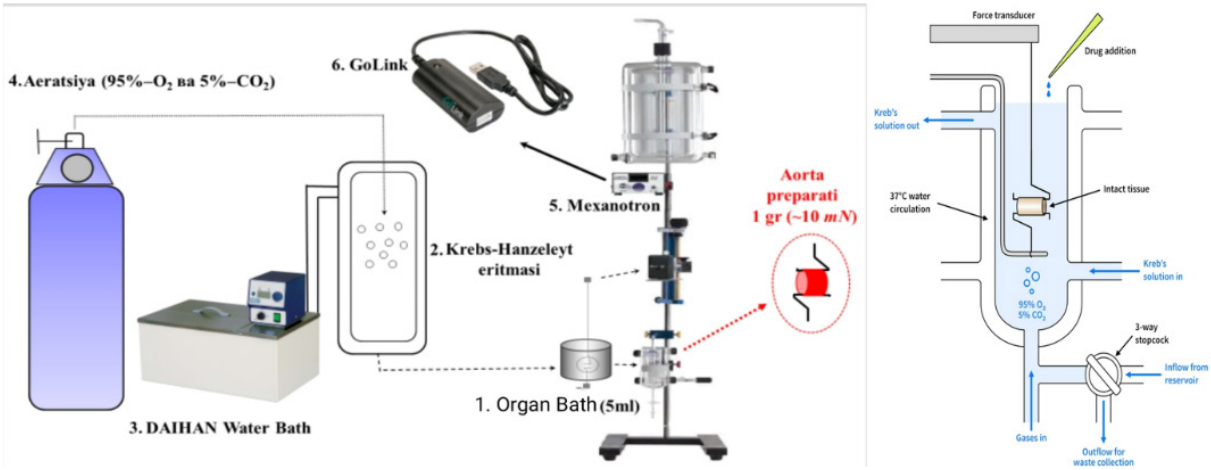

Figure 1 A schematic illustration of the apparatus used to regulate and measure the isometric contraction of isolated rat aortic vascular muscle. 1) The organ bath (5 mL) circulates through a specialized reservoir. 2) The Krebs-Henseleit solution ensures physiological conditions. 3) A thermostat maintains a constant physiological temperature. 4) The system is aerated with a gas mixture of 5 % CO_2 and 95 % O_2. The contractile activity of the aortic preparation is secured in the experimental chamber. 5) An isometric transducer (Grass Instrument, USA) monitors the contraction, and 6) GoLink devices provide signal amplification and support.

Blood pressure measurements

Recording of arterial blood pressure in rats was carried out in the "BFM Pharmacology and Screening Laboratory" and the "Plant Cytoprotectors" laboratory of the Institute of Bioorganic Chemistry named after O. Sodikov in a standard method, using the experimental device "Sistola" (Neurobotics, RF) in a non-invasive way in the blood vessel of the tail artery [10], and the results were analyzed using the special program "AcqKnowledge 4.2 for MP150" (**Figure 2**).

Figure 2 Experimental device "Sistola" (Neurobotics, RF) for noninvasive recording of arterial blood pressure in the blood vessel of the tail artery in rats.

Experimental hypertension was induced in rats by injection of adrenaline hydrochloride (0.25 mg/kg). In the experimental group, 30 min after injection of adrenaline hydrochloride (0.25 mg/kg) (an experimental hypertensive effect was observed to be stable for 180 min), medicinal plant extracts (50, 100 and 200 mg/kg) were injected intraperitoneally [9].

Statistics

The statistical analysis and graphical illustrations were carried out using Origin Pro 8.5 software (Microsoft, USA). The contractile responses were quantified as percentages of the maximal contraction elicited by phenylephrine (10 mM) or KCl (50 mM) and are presented as mean values from 5 to 8 independent experiments (n = 5 - 8). A paired t-test was applied to

analyze combined data, while an unpaired t-test was used for comparisons between individual groups. Statistical significance was determined at 2 levels: $p < 0.05$.

Results and discussion

In vitro experiments in rats aortic smooth muscle

The KCl (50 mM)-induced contraction in aortic tissues is primarily mediated through the activation of voltage-dependent L-type Ca^{2+} channels in smooth muscle cells. The elevated extracellular K^+ concentration depolarizes the membrane, leading to the opening of these channels. In our experiments, extracts from *Crataegus* and *Inula helenium* were observed to significantly relax aortic rings precontracted with KCl (50 mM) [11]. Specifically, *Crataegus* extract attenuated KCl-induced contraction by 15.0 ± 2.6 and 70.0 ± 2.8 % in a dose-dependent manner (20 - 70 µg/mL), while *Inula helenium* extract reduced contraction by 5.0 ± 3.1 and 80.0 ± 2.8 % at concentrations ranging from 10 - 50 µg/mL (**Figure 3**).

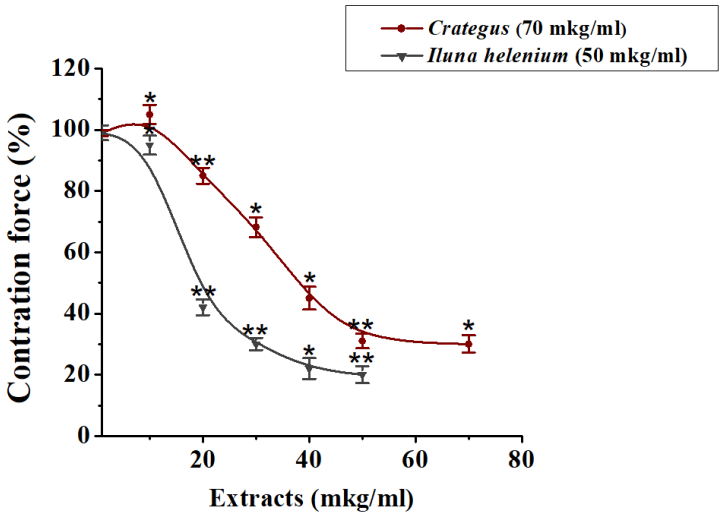

Figure 3 The effects of *Crategus Rosaceae* and *Inula helenium* extracts on the contraction of rat aortic smooth muscle induced by KCl (50 mM). The ordinate axis represents the contraction force of the aortic preparation induced by KCl (50 mM), considered to be 100 %, while the abscissa axis indicates the concentration of the extracts (µM). In all cases $p < 0.05$; n = 5 - 6.

These findings suggest that the extracts effectively inhibit KCl-induced contraction by modulating voltage-dependent Ca^{2+} channels. To confirm this hypothesis, experiments were conducted using Ca^{2+}-free Krebs solution and the L-type Ca^{2+} channel blocker verapamil [12]. It is known that KCl-induced depolarization in the absence of extracellular Ca^{2+} does not induce contraction, but the reintroduction of Ca^{2+} (0 - 2.5 mM) restores contractility [13]. In our experiments, *Crataegus* and *Inula helenium* extracts (70 and 50 µg/mL) significantly reduced the contraction of aortic rings in the presence of KCl and Ca^{2+}, compared to controls (**Figure 4**).

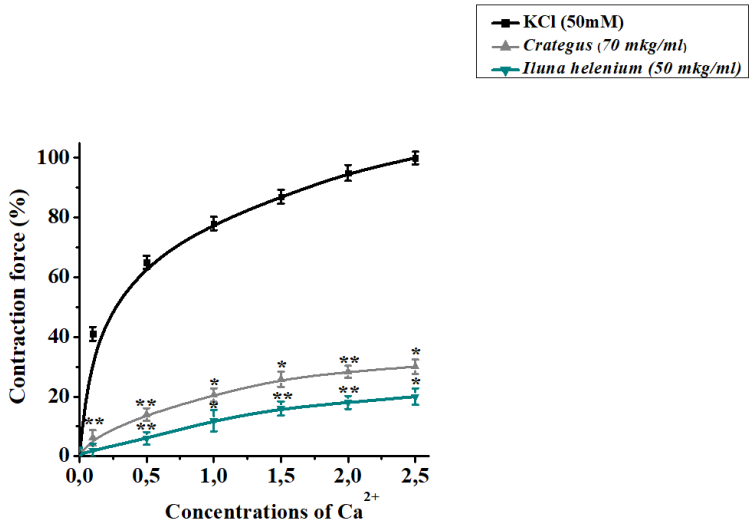

Figure 4 Effects of [Ca^{2+}] concentration in the medium on the relaxant activity of *Crategus Rosaceae* and *Inula helenium* extracts. The ordinate axis shows the contraction force induced by KCl (50 mM) in the aortic preparation, calculated as 100 %, and the abscissa axis represents the concentration of Ca^{2+} (0 - 2.5 mM). In all cases $p < 0.05$; n = 5.

The relaxant effects of *Crataegus* and *Inula helenium* are likely mediated by a reduction in Ca^{2+} influx through L-type Ca^{2+} channels in the smooth muscle cell membrane. Further experiments were conducted to investigate the interaction of these extracts with verapamil. Verapamil at a concentration of 0.1 µM, which induces half-maximal contraction, was used to evaluate the additional inhibitory effects of the extracts [14]. It was observed that *Crataegus* and *Inula helenium* extracts further attenuated aortic contractions by 45 ± 2.2 and 38 ± 2.4 %, respectively, with IC_{50} values of 38.0 and 19.5 µg/mL (**Figure 5**).

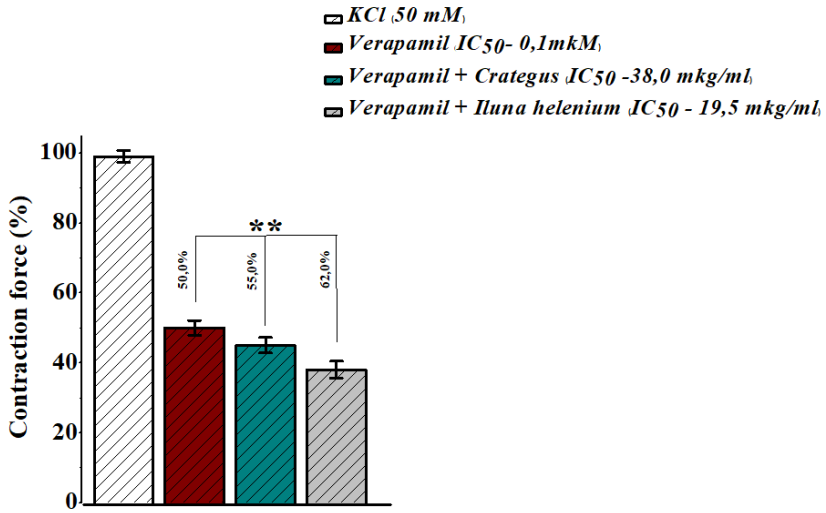

Figure 5 Interaction of *Crategus Rosaceae* and *Inula helenium* extracts with the Ca^{2+}-channel blocker verapamil (EC50) on the contraction of aortic preparations induced by KCl (50 mM) under present conditions. The ordinate axis represents the contraction force of the aortic smooth muscle induced by KCl (50 mM), considered to be 100 %. In all cases $p < 0.05$; n = 5.

Based on the experimental findings, the extract demonstrated a potent relaxant effect, significantly reducing the contraction induced by KCl (50 mM). This effect is attributed to the inhibition of potential-dependent L-type Ca^{2+} channels in the plasmalemma, which subsequently limits the influx of Ca^{2+} ions,

leading to muscle relaxation [15]. The experiment confirmed that the relaxing action of this extract is indeed associated with the blockade of L-type Ca^{2+} channels, as evidenced by comparable outcomes in experiments utilizing verapamil, a specific L-type Ca^{2+} channel blocker.

The inhibitory effects of these extracts on L-type Ca^{2+} channels, as demonstrated by their interaction with verapamil, indicate their potential for modulating vascular smooth muscle contractility. Additionally, it is recognized that calcium transport mechanisms within the sarcoplasmic reticulum (SR) play a crucial role in vascular smooth muscle contraction [16]. Therefore, the effect of *Crataegus* and *Inula helenium* extracts on phenylephrine (1 μM)-induced contraction, which is dependent on both extracellular and SR-derived Ca^{2+}, was also studied. At a concentration of 50 μg/mL, both extracts reduced phenylephrine-induced contraction by 75.0 ± 2.4 and 88.0 ± 2.2 %, respectively (**Figure 6**).

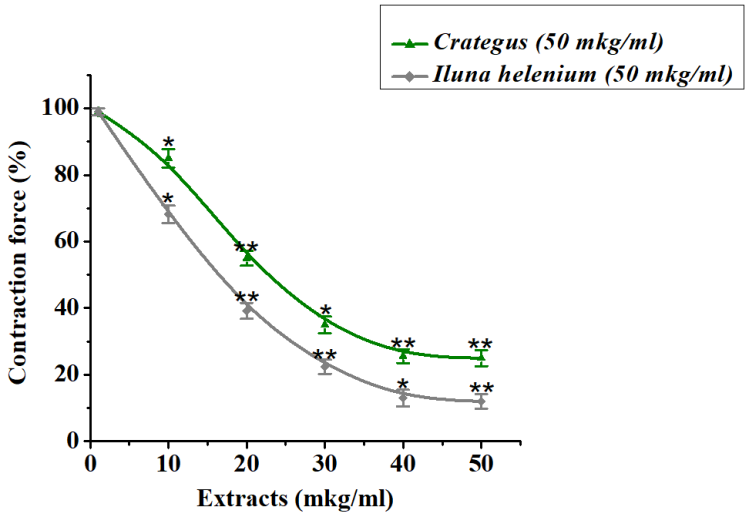

Figure 6 Effects of the extracts on the contraction of rat aortic smooth muscle induced by Phe (1 mM). The ordinate axis shows the contraction force induced by Phe (1 mM), considered to be 100 %, and the abscissa axis represents the concentration of the extracts (μM). In all cases $p < 0.05$; n = 5 - 6.

The obtained results suggest that the relaxant effect of the studied extract may be due to the blockade of receptor-controlled Ca^{2+} channels. To further investigate this, the effects of the α-adrenoceptor blocker phentolamine (PE) and the extracts were compared [17]. It was observed that in the absence of phentolamine, 1 μM phenylephrine induced a reduction in aortic contraction force at the concentration of *Crategus Rosaceae* and *Inula helenium* (50 μg/mL), as seen in previous experiments. When 10 μM phentolamine was added, the contraction force induced by 1 μM phenylephrine was reduced by 80.7 ± 3.1 % compared to control. Under these conditions, the extracts of *Crategus Rosaceae* and *Inula helenium* further reduced the contracture to 20.0 ± 2.1 and 10.0 ± 2.3 %, respectively (**Figure 7**) [18].

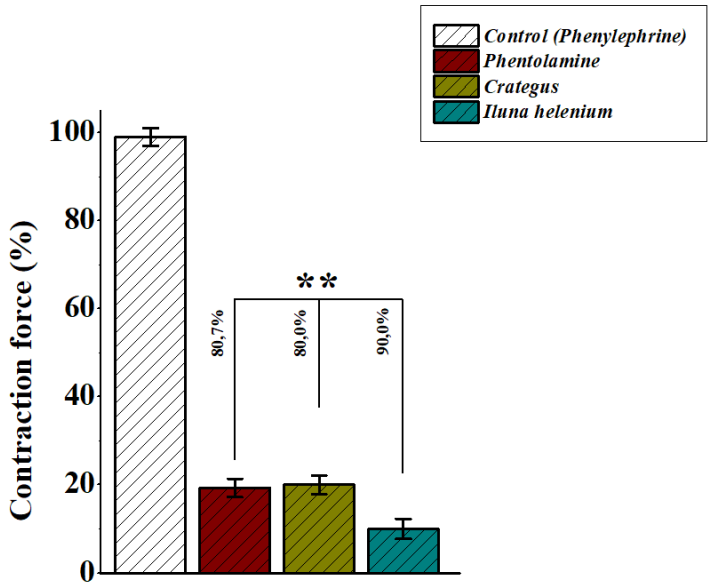

Figure 7 The effect of phentolamine (10 μM) on the relaxant effects of *Crategus Rosaceae* and *Inula helenium* extracts. The aortic contraction was induced by 1 μM Phe, with the contraction strength considered as 100 %. In all cases ($p < 0.05$; n = 5).

Study the role of endothelium in the relaxant effect of *Crategus Rosaceae* and *Inula helenium* extracts

The endothelial layer lining blood vessels plays a critical role in maintaining vascular function and regulating blood flow. Endothelial cells produce various signaling molecules, including nitric oxide (NO), a powerful vasodilator, which helps control blood pressure and vessel tone. Endothelial dysfunction (ED), a disturbance in the balance of these signaling molecules, is implicated in the development and progression of cardiovascular diseases like hypertension and atherosclerosis [19]. Early damage to endothelial cells can initiate a cascade of pathological changes leading to widespread vascular dysfunction. Nitric oxide (NO), a crucial vasodilator, is produced in endothelial cells by the enzyme nitric oxide synthase (eNOS) using the amino acid L-arginine as a substrate. The activation of eNOS by calcium and calmodulin triggers the conversion of L-arginine into NO in tiny amounts (picomoles). Normally, eNOS is bound to a protein called caveolin, which keeps it inactive and associated with caveolae (small membrane pockets). However, when certain stimuli, such as acetylcholine, bradykinin, or serotonin, activate receptors on the cell surface, they release eNOS from its complex with caveolin, allowing it to move to the plasma membrane and become active [20]. Statins, drugs commonly used to lower cholesterol levels, can also promote NO production at very low concentrations (0.1 mM). They achieve this by interfering with the interaction between eNOS and caveolin-1, a major inhibitor of eNOS activity. This effect, known as a pleiotropic effect, contributes to the beneficial effects of statins in treating endothelial dysfunction (ED). The development of atherosclerosis and endothelial dysfunction (ED) is significantly influenced by oxidative stress. Specifically, reactive oxygen species, often referred to as free radicals, play a major role in the breakdown of nitric oxide (NO), a crucial vasodilator. This breakdown of NO diminishes its ability to relax blood vessels, contributing to the development of ED. NO is produced by nitric oxide synthase (NOS) within endothelial cells, the lining of blood vessels. It acts as a key mediator in vascular smooth muscle relaxation, playing a crucial role in regulating blood flow [21]. NO activates guanylate cyclase (GC), leading to increased levels of cyclic GMP (cGMP). cGMP then activates protein kinase G (PKG), ultimately causing smooth muscle relaxation through phosphorylation of a myosin light chain. Interestingly, an increase in local oxygen concentration further reduces the amount of biologically active NO. This is because NO and reactive oxygen species react with each other, neutralizing each other's activity. This further

exacerbates the negative effects of oxidative stress on vascular health [22]. Endothelial cells play a vital role in maintaining vascular tone by producing various vasoactive factors, with NO being a key player. They also control calcium (Ca^{2+}) homeostasis and the contractile activity of smooth muscle cells (SMCs). When NO diffuses into SMCs, it activates the NO/cGC/cGMP/PKG signaling pathway, leading to reduced intracellular calcium levels and relaxation. To investigate the role of the endothelium in mediating the relaxant effects of certain plant extracts, such as *Crategus Rosaceae* and *Inula helenium*, researchers conducted experiments on aorta preparations. These experiments involved removing the endothelial layer to isolate the effects of these extracts on vascular relaxation, independent of endothelial contributions. In conclusion, oxidative stress and the resulting breakdown of NO contribute significantly to the development of endothelial dysfunction and atherosclerosis. Understanding the complex interplay between these factors and the role of the endothelium in vascular health is crucial for developing effective strategies to prevent and manage these conditions. The researchers used a standard method to determine if plant extracts could influence the functionality of the vascular endothelial layer. They employed aortic smooth muscle preparations and induced contractions using 1 μM phenylephrine (Phe). These contractions were standardized to an amplitude of 10 mN. To assess the presence or absence of the endothelial layer, 1 μM acetylcholine (Ach) was applied [23]. In preparations with an intact endothelial layer, 1 μM Ach significantly reduced the contraction amplitude induced by Phe by 57.8 ± 4.3 %. Conversely, in preparations where the endothelial layer was mechanically removed using a cotton swab, 1 μM Ach had a negligible effect on the contraction amplitude induced by Phe. These findings indicate that the endothelium plays a crucial role in mediating vascular relaxation. The presence of a functional endothelium, as demonstrated by the response to Ach, was essential for reducing the contraction amplitude induced by Phe. These results underscore the importance of the endothelial layer in regulating vascular tone.

The experiments investigated the effect of *Crategus Rosaceae* and *Inula helenium* extracts on the relaxation of rat aorta blood vessels, specifically examining the role of the endothelial layer. When the endothelial layer was present, various concentrations of *Crategus Rosaceae* extract reduced the relaxation effect by an average of 65.2 ± 3.5 % compared to the control group. However, when the endothelial layer was removed, the reduction in relaxation effect was significantly less, averaging 15.0 ± 2.9 % compared to the control. This suggests that the endothelial layer plays a substantial role in mediating the relaxant effect of *Crategus Rosaceae*. Similar to *Crategus Rosaceae* and *Inula helenium* extract showed a reduced relaxant effect when the endothelial layer was absent. Different concentrations of the extract reduced the relaxation effect by an average of 55.8 ± 3.0 % with the endothelium present, but only by 24.3 ± 2.4 % when it was removed. This further supports the importance of the endothelial layer in mediating the extract's effects (**Figure 8**). These findings indicate that both *Crategus Rosaceae* and *Inula helenium* extracts exert their relaxant effects primarily through mechanisms that involve the endothelial layer. The reduction in their effectiveness when the endothelium is removed highlights the crucial role of the endothelial layer in mediating their vasodilatory actions.

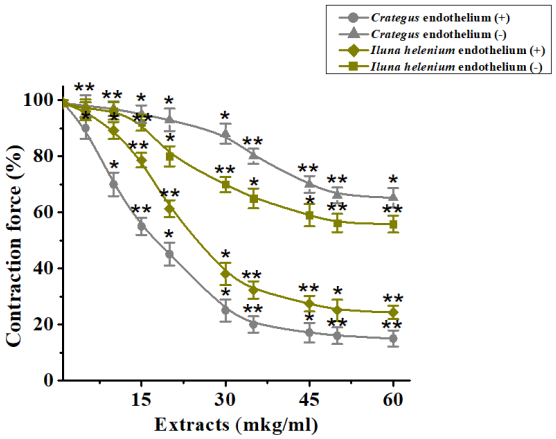

Figure 8 Relaxant effects of *Crategus Rosaceae* and *Inula helenium* extracts on the contraction induced by 1 µM Phe in the presence (+) and absence (–) of the rat aortic endothelial layer. The contraction force induced by 1 µM Phe was considered as 100 % of the control. In all cases $p < 0.05$; n = 5.

Our experiments showed that the extracts' effects on aortic preparations were significantly different when the endothelial layer was absent, suggesting that the substances might directly interact with the endothelium. To confirm this, we conducted additional experiments using L-NAME (100 µM), an inhibitor of endothelial nitric oxide synthase (eNOS) [24]. We observed that the relaxant effects of both *Crategus Rosaceae* and *Inula helenium* extracts were significantly reduced in aortic preparations treated with L-NAME. Specifically, *Crategus Rosaceae* extract reduced the contraction force induced by phenylephrine (Phe) by 69.2 ± 3.5 %, and Inula helenium extract reduced it by 62.5 ± 2.8 % in the presence of L-NAME. Importantly, this reduction in relaxant effect was even more pronounced when the endothelial layer was absent, decreasing by 15 ± 2.9 and 24.3 ± 2.4 % for *Crategus Rosaceae* and *Inula helenium* extracts, respectively (**Figure 9**). These results strongly suggest that the relaxant effects of both *Crategus Rosaceae* and *Inula helenium* extracts are at least partially mediated by the release of nitric oxide (NO) from the endothelium. This is evident because inhibiting eNOS with L-NAME significantly reduced the relaxant effects, and this reduction was even greater in the absence of the endothelium.

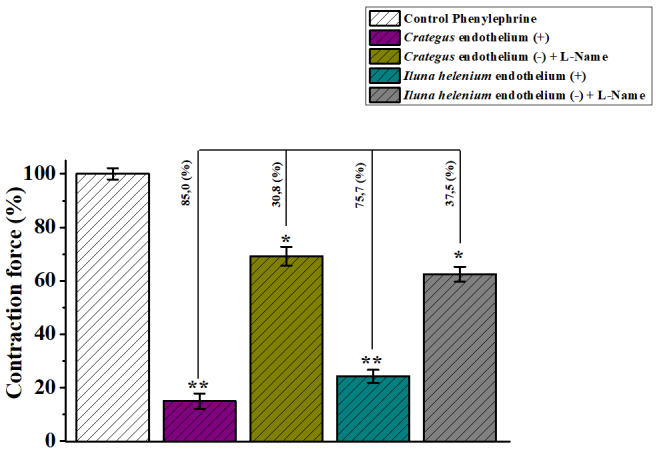

Figure 9 Relaxant effects of *Crategus Rosaceae* and *Inula helenium* extracts, and the eNOS blocker L-NAME (100 µM) on the contraction of rat aortic preparations under incubation conditions. The contraction force induced by 1 µM Phe was taken as 100 % of the control. In all cases $p < 0.05$; n = 6.

Our experimental findings suggest that the extracts exhibit a strong relaxant effect that relies heavily on the presence of the endothelium. This effect is significantly reduced when the endothelium is removed or when nitric oxide synthase (NOS) is inhibited by L-NAME, indicating a crucial role for nitric oxide (NO) in mediating the relaxation [25]. The extracts likely promote relaxation by activating the NOS-cyclic GMP (cGMP)-protein kinase G (PKG) signaling pathway. This pathway works by reducing calcium influx through both L-type and receptor-operated calcium channels (Ca^{2+} L and Ca^{2+} R) in the plasma membrane, as well as inhibiting the release of calcium from the sarcoplasmic reticulum (SR). As a result, intracellular calcium levels ((Ca^{2+})i) decrease in smooth muscle cells (SMCs), leading to muscle relaxation.

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

The blood pressure of rats was measured *in vivo* using the tail cuff method. The rats were divided into 3 groups, each receiving different doses of the *Crategus Rosaceae* extract: 50, 100 and 200 µg/mL. Initially, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of healthy rats were recorded before the induction of hypertension using adrenaline chloride [26].

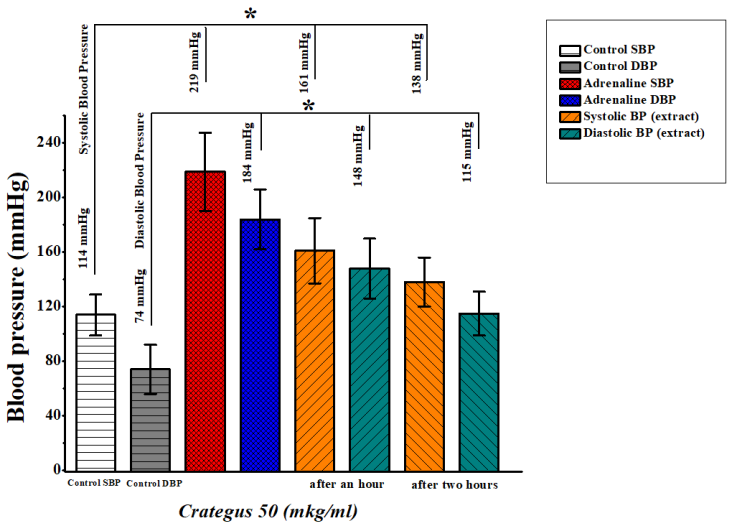

Figure 10 Hypotensive effects of *Crategus Rosaceae* extract (50 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($p < 0.05$; n = 5).

In the first group, the baseline SBP was 114 mmHg, and the DBP was 74 mmHg. After inducing hypertension with adrenaline, the SBP increased to 219 mmHg and the DBP to 184 mmHg. The experiments were continued with *Inula helenium*. In the first group of rats, after administering 50 µg/mL of *Crategus Rosaceae* extract orally, the SBP decreased to 161 mmHg and the DBP to 148 mmHg within the first hour (**Figure 10**). After 2 h, the SBP further decreased to 138 mmHg and the DBP to 115 mmHg [27].

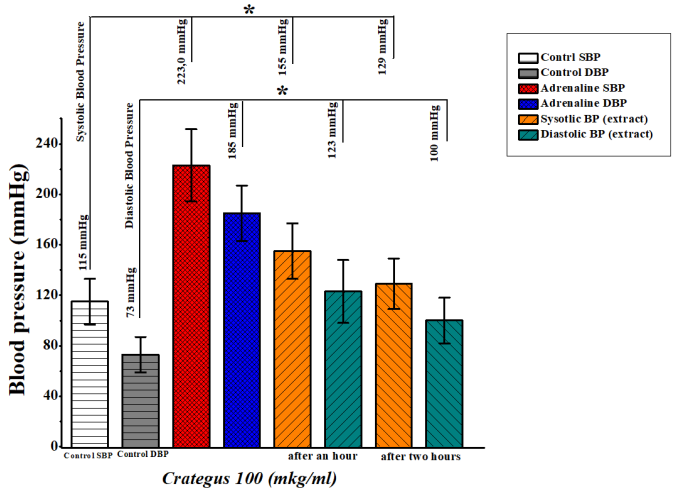

Figure 11 Hypotensive effects of *Crategus Rosaceae* extract (100 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($p < 0.05$; n = 5).

In the second group, the baseline SBP was 115 mmHg, and the DBP was 73 mmHg. After the administration of adrenaline chloride, the SBP rose to 223 mmHg and the DBP to 185 mmHg. Following the oral administration of 100 µg/mL *Crategus Rosaceae* extract, the SBP reduced to 155 mmHg and the DBP to 123 mmHg after 1 h. After 2 h, the SBP and DBP further decreased to 129 and 100 mmHg, respectively (**Figure 11**).

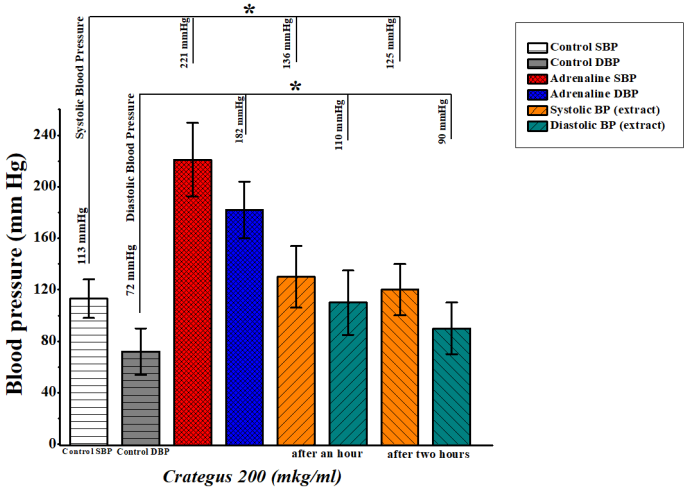

Figure 12 Hypotensive effects of *Crategus Rosaceae* extract (200 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($p < 0.05$; n = 5).

In the third group, the baseline SBP was 113 mmHg, and the DBP was 72 mmHg. After adrenaline-induced hypertension, the SBP increased to 221 mmHg and the DBP to 182 mmHg. The oral administration of 200 µg/mL of the extract resulted in a decrease in SBP to 136 mmHg and DBP to 110 mmHg after 1 h (**Figure 12**). After 2 h, the SBP and DBP further reduced to 125 and 90 mmHg, respectively [28].

The blood pressure of rats was measured *in vivo* using the tail cuff method. The rats were divided into 3 groups, each receiving different doses of *Inula helenium* extract: 50, 100 and 200 µg/mL. Initially, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of healthy rats were recorded before inducing hypertension with adrenaline chloride.

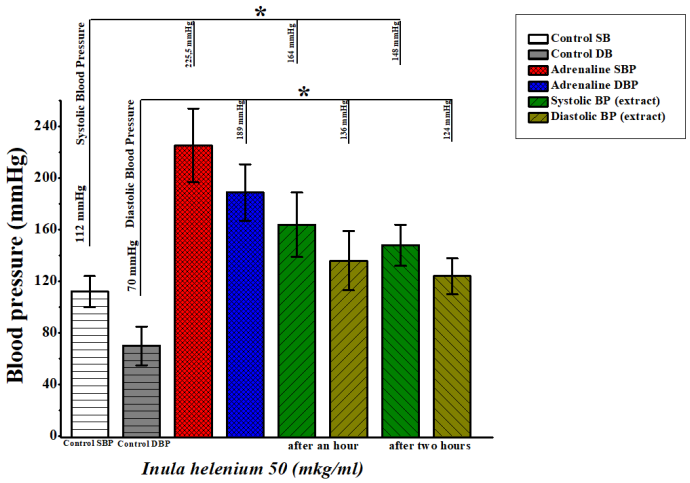

Figure 13 Hypotensive effects of *Inula helenium* extract (50 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($^{*}p < 0.05$; n = 5).

In the first group, the baseline SBP was 112 mmHg, and a DBP was 70 mmHg. After adrenaline-induced hypertension, SBP increased to 225.5 mmHg, and DBP to 189 mmHg (**Figure 13**). Upon administering 50 µg/mL of *Inula helenium* extract orally, the SBP dropped to 164 mmHg, and DBP to 136 mmHg within 1 h. After 2 h, SBP further declined to 148 mmHg, and a DBP to 124 mmHg [29].

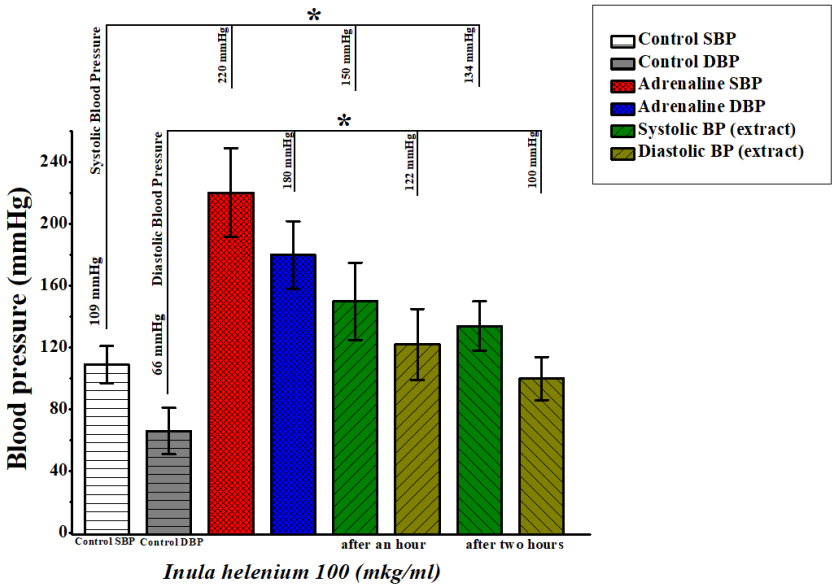

Figure 14 Hypotensive effects of *Inula helenium* extract (100 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($p < 0.05$; n = 5).

In the second group, the initial SBP was 109 mmHg, and a DBP was 66 mmHg. Following adrenaline administration, SBP rose to 220.4 mmHg, and DBP to 180 mmHg [30]. After oral administration of 100 µg/mL of the extract, SBP decreased to 150 mmHg, and DBP to 122 mmHg after 1 h. Two h later, SBP dropped to 134 mmHg, and a DBP to 100 mmHg (**Figure 14**).

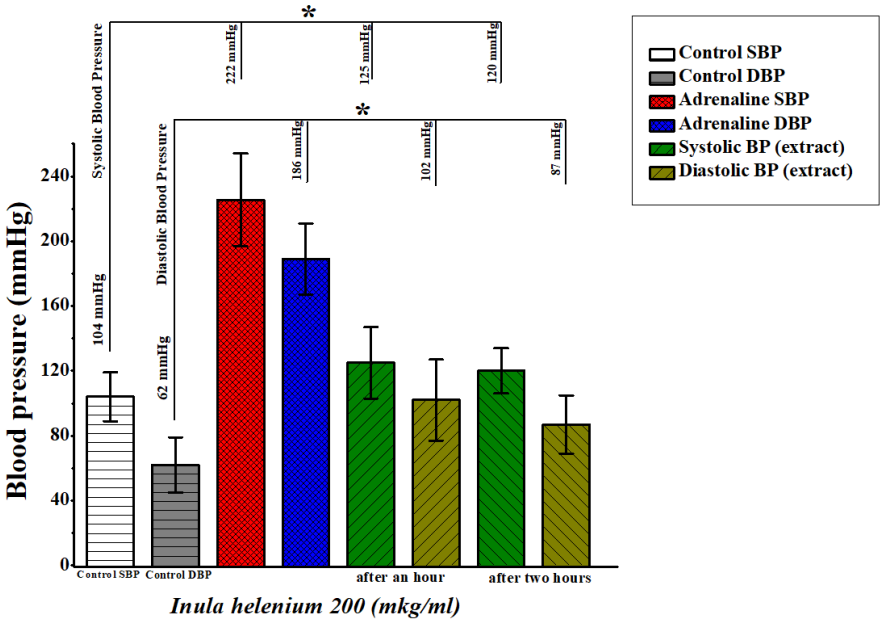

Figure 15 Hypotensive effects of *Inula helenium* extract (200 µg/mL) on systolic and diastolic blood pressure after adrenaline-induced hypertension in rats. Data represent blood pressure changes over time ($p < 0.05$; n = 5).

In the third group, the baseline SBP was 104 mmHg, and a DBP was 62 mmHg. After hypertension induction, SBP rose to 222.0 mmHg, and DBP to 186 mmHg. One h after administering 200 µg/mL of the extract orally, SBP fell to 125 mmHg, and DBP to 102 mmHg. Two h later, SBP decreased to 120 mmHg, and DBP to 87 mmHg [31]. These results indicate that the extract's hypotensive effect requires a prolonged period to manifest its full action (**Figure 15**).

Conclusions

In this study, we investigated the relaxant effects of *Crategus Rosaceae* and *Inula helenium* extracts on rat aortic smooth muscle and their potential mechanisms of action. The *in vitro* experiments demonstrated that both extracts significantly reduced KCl (50 mM)-induced contraction in a dose-dependent manner. This effect was attributed to the inhibition of L-type Ca^{2+} channels, as confirmed by the use of a Ca^{2+}-free Krebs solution and the L-type Ca^{2+} channel blocker verapamil. The extracts also displayed strong relaxant effects on phenylephrine-induced contraction, suggesting additional involvement of receptor-operated Ca^{2+} channels.

Moreover, the experiments showed that the endothelial layer played a critical role in mediating the extracts' vasodilatory effects. The removal of the endothelium or inhibition of nitric oxide synthase (eNOS) with L-NAME significantly reduced the relaxant action of both extracts, indicating that nitric oxide (NO) release from the endothelium is a key component of their mechanism. These findings support the hypothesis that the relaxant effects are mediated by the NOS-cGMP-PKG pathway, which reduces intracellular calcium levels by inhibiting both voltage-dependent and receptor-operated calcium channels in smooth muscle cells.

The results from the *in vivo* experiments using the tail cuff method confirmed that Crataegus extract effectively reduced blood pressure in hypertensive rats, further underscoring its potential antihypertensive properties.

Acknowledgement

The work was supported by the Applied Research Program of the Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan (project A-FA-2021-372 "Creation of medicine with effective control of the cardiovascular system based on *Herba leonuri, Gnaphalii uliginosi herba, Chamomillae recutie flores,* and *Crataegi flores* medicinal plants").

Funding source

Innovative Development Agency under the Ministry of Higher Education, Science and Innovation, grant number is A-FA-2021-372.

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