UO‘K 574.16

LEONURUS TURKESTANICUS VA RK4 POLIFENOLINING AORTA SILLIQ MUSKULIGA VAZORELAKSANT TA’SIRI FONIDA CA^{2+} KANALLARINING ROLI

G.J. Mirzaalimova, A.E. Zaynabiddinov

Maqolada Leonurus turkestanicus ekstrakti va RK4 polifenolining aorta silliq muskuliga vazorelaksant ta’siri hamda Ca^{2+} kanallarning roli o‘rganildi. Natijalar moddalarning potensialga bog‘liq va retseptor-boshqariluvchi Ca^{2+} oqimlarini susaytirib, tomir bo‘shashuvini yuzaga kelishini ko‘rsatdi. Olingan ma’lumotlar o’simlik birikmalarining farmakologik faolligi va qon tomir tonusini tartibga solish mexanizmlari haqidagi tushunchalarni kengaytiradi.

Kalit so‘zlar: *Leonurus turkestanicus ekstrakti, RK4 polifenoli, vazorelaksant, L-tipidagi Ca^{2+} kanallar, verapamil, fenilefrin, fentolamin, Ca^{2+} ionlari, sarkoplazmatik retikulum*.

В статье изучено вазорелаксантное действие экстракта Leonurus turkestanicus и полифенола RK4 на гладкую мускулатуру аорты, а также роль Ca^{2+}-каналов в механизме их действия. Установлено, что исследуемые вещества вызывают расслабление сосудистой стенки за счёт подавления потенциалзависимых и рецептор-управляемых потоков Ca^{2+}. Полученные данные расширяют представления о фармакологической активности растительных соединений и механизмах регуляции сосудистого тонуса.

Ключевые слова: *экстракт Leonurus turkestanicus, полифенол RK4, вазорелаксант, L-тип Ca^{2+}-каналов, верапамил, фенилэфрин, фентоламин, ионы Ca^{2+}, саркоплазматический ретикулум.*

KIRISH

Qon tomiri tonusi, mayda arteriyalar va arteriolalar devoridagi silliq muskul hujayralarining qisqarish faolligi qon aylanish orqali qon oqimiga qarshilikning asosiy omilidir [1]. Qon tomirlarining tonusi qon bosimini tartibga solishda va qon oqimini tananing to‘qimalari va organlari o‘rtasida taqsimlashda muhim rol o‘ynaydi. Qon aylanish tizimida qon tomiri silliq muskul hujayralarining kontraktil faolligini tartibga solish qon aylanishidagi gormonlar, neyrotransmitterlar, endoteliydan kelib chiqadigan omillar va qon bosimining vazodilatator va vazokonstriktor stimullarining murakkab o‘zaro ta’siriga bog‘liq. Bu signallarning barchasi qon tomiri silliq muskul hujayralarining qisqarish apparati faoliyatini regulyatsiya qiladi. Ion kanallari bu jarayonda muhim rol o‘ynaydi. Barcha muskul hujayralari singari, qon tomir silliq muskul qisqarishi uchun asosiy nishon Ca^{2+} ionlari hisoblanadi. Silliq muskul hujayralari membranasidagi kanallar orqali kalsiy oqimi va hujayra ichidagi depodan (SR) Ca^{2+} ning chiqishi Ca^{2+} ionlarining asosiy manbai hisoblanadi. Bundan tashqari, ionlarning ion kanallari orqali harakatlanishi ko‘p jihatdan membrana potensialini aniqlaydi. Membrana potensiali sitozolik Ca^{2+} konsentratsiyasi bilan birga ion kanallari orqali Ca^{2+} oqimini yuzaga keltiradi, qisqarish mexanizmning Ca^{2+} ga sezgirligini tartibga soladi hamda modulyatsiya qiladi [2].

Qon aylanish tizimida gipoksiyaga javoban vazodilatatsiya asosan silliq muskul hujayralarida $[Ca^{2+}]_{in}$ ning kamayishi bilan bog‘liq, ammo endotelial hujayralar orqali bo‘shashtiruvchi va qisqartiruvchi omillarning o‘zgarishi bilan modulyatsiyalanishi mumkin, ushbu jarayonning borishi ham Ca^{2+} ionlariga bog‘liq bo‘lishi mumkin. Ko‘pgina tadqiqotlar shuni ko‘rsatdiki, qon tomirlaridagi ion kanallari ham Ca^{2+} o‘tkazuvchan, ham Ca^{2+} o‘tkazmaydigan gipoksiya bilan boshqariladi [3]. Arteriya qon tomir silliq muskul hujayralarida olib borilgan elektrofiziologik tadqiqotlar shuni ko‘rsatdiki, gipoksiya potensialga bog‘liq Ca^{2+} kanallarga to‘g‘ridan-to‘g‘ri ta’sir ko‘rsatishi mumkin. Bunda Po_2 ning tez pasayishi va L-tip Ca^{2+} kanallarni inhibirlaydi. Izolyatsiya qilingan inson koronar qon tomir silliq muskul hujayralarida O_2 ning kamayishi potensialga bog‘liq L-tip Ca^{2+} kanallari orqali Ca^{2+} oqimini pasaytiradi va $[Ca^{2+}]_i$ ni kamaytiradi [4]. Tadqiqotlarda gipoksiya sharoitida arteriya qon-to-

mirlarining qisqarishi va bo‘shashishida $[Ca^{2+}]_{in}$ ning o‘zgarishi K^+ kanallari faolligiga yoki hujayra ichidagi pH o‘zgarishiga bog‘liq emas, bu esa gipoksiya bilan bog‘liq bo‘lgan $[Ca^{2+}]_i$ ning individual hujayralarida kuzatilgan o‘zgarishlar to‘g‘ridan-to‘g‘ri Ca^{2+} kanallarini inhibirlashi bilan bog‘liq ekanligi qayd qilingan [5].

MATERIAL VA METODLAR

Aorta qon tomiri preparatini tayyorlash standart usulda amalga oshirildi.

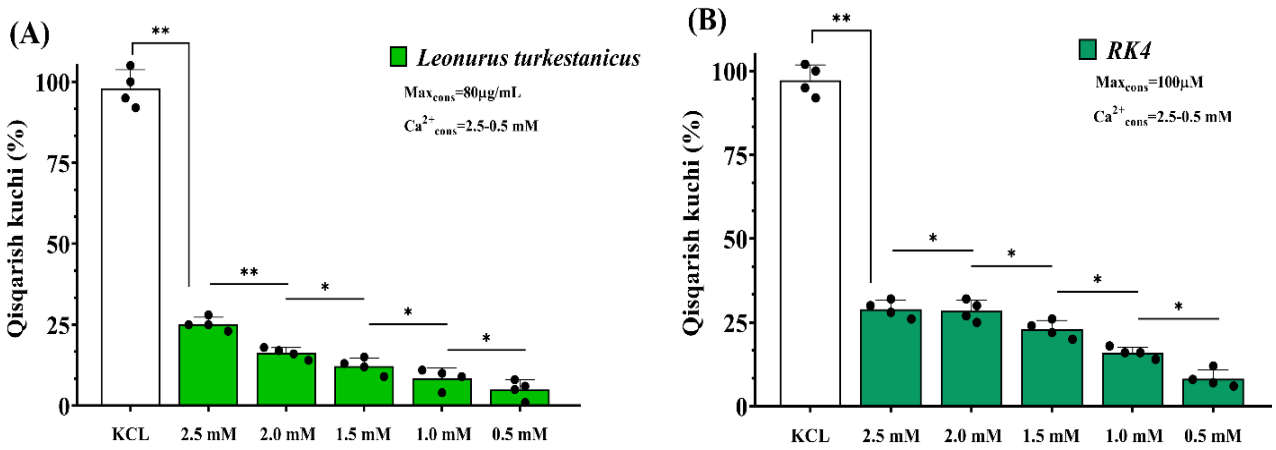
Tajribalar standart ozuqa va suv bilan ta’minlangan sharoitda boqilgan, sog‘lom oq, zotsiz kalamushlarda (150–200 *gr*) amalga oshirildi. Tajriba hayvonlari servikal dislokatsiya usulida jonsizlantirilgandan keyin jarrohlik usulida aorta qon tomiri ajratib olindi va halqasimon segmentlar (l=2–4 mm; ø=1–2 mm) shaklida kesildi. Tajribalarda quyidagi kimyoviy tarkibga ega bo‘lgan Krebs-Xenzeleyt fiziologik eritmasidan foydalanildi (mM hisobida): (mM): NaCl – 120,4; KCl – 5; $NaHCO_3$ – 15,5; NaH_2PO_4 – 1,2; $MgCl_2$ – 1,2; $CaCl_2$ – 2,5; $C_6N_{12}O_6$ – 11,5; (pH=7,4) [6]. Fiziologik eritma karbogen (O_2–95% va CO_2–5%) bilan aeratsiyalandi, harorat doimiyligi (t=+37±0,5°C) ultratermostat (U–8, Bolgariya) yordamida ta’minlandi. Aorta qon tomiri preparatining qisqarish faolligi izometrik sharoitda FT–03 (“Grass Instrument Co.”, AQSh) kuch sensori, signal kuchaytirgich qurilma (“Grass Instrument”, AQSh) orqali Endim 621.02 samopisesida (Chexiya) standart uslub (mexanografiya) yordamida qayd qilindi. Tajribalarda verapamil gidroxlorid [7, 8] (“Sigma–Aldrich”, Germaniya), $NaHCO_3$, $CaCl_2$, $MgSO_4$, glyukoza, NaCl, KCl, NaH_2PO_4 (Rossiya) reaktivlaridan foydalanildi. Tajribada o‘rganilgan polifenolning aorta muskuli qisqarishiga ta’sirida Na^+/Ca^{2+}-almashinuvchi tizim ishtrokini aniqlashda yurak glikozidi – ouabaindan foydalanildi. Ouabain 20 mkM konsentratsiyada aorta muskulida maksimal qisqarishni yuzaga keltirishi aniqlandi. Tajribada tashqaridan Ca^{2+} ionlarining potensialga bog‘liq Ca^{2+}-kanallar orqali kirishini yo‘q qilish maqsadida uning blokatori – verapamildan foydalanildi. Shuningdek, tajribada polifenolning aorta muskul qisqarishiga ta’sirida Na^+/Ca^{2+}-almashinuvchi tizim rolini aniqlashda uning blokatori – KB-R7943dan foydalanildi. KB-R7943 ouabain yordamida chaqirilgan aorta muskuli qisqarishiga dozaga bog‘liq holda ta’sir ko‘rsatib, 30 mkM konsentratsiyada uni maksimal darajada kamaytiradi.

Olingan natijalar “OriginPro v. 8.5 SR1” (EULA, Northampton, MA 01060–4401, AQSh) maxsus dastur paketi yordamida statistik qayta ishlandi. Natijalar G.F.Lakin (1990) tomonidan keltirilgan uslublar yordamida matematik–statistik qayta ishlandi [9]. *n* marta takror amalga oshirilgan tajribalar natijalari M±m shaklida keltirilgan bo‘lib, M o‘rtacha arifmetik qiymatni va m standart xatolik qiymatini ifodalaydi. Shuningdek, tajriba natijalari guruhlar o‘rtasidagi qiymatlarning statistik ishonchlilik darajasi Styudent t-mezoni asosida hisoblandi va $p<0,05$, $p<0,01$ qiymatlarda statistik ishonchli deb baholandi. Dastlab kalamush aorta preparati 1 *gr* (9,8~10 mN) kuchlanish berish asosida, ~45–60 daqiqa davomida me’yoriy elektromexanik faollik qayd qilinishga qadar inkubatsiyalandi.

NATIJALAR VA ULARNING TAHLILI

Tadqiqotda Leonurus turkestanicus va RK4 polifenolining aorta muskuli qisqarish faolligiga vazorelaksant ta’siri, xususan, potensialga bog‘liq Ca^{2+} kanallari orqali namoyon bo‘lishi o‘rganildi. Bu maqsadda 50 mM KCl yordamida potensialga bog‘liq Ca^{2+} kanallarining faollashuvi chaqirildi. Tajribalarda kalsiysiz (Ca^{2+} = 0) muhitga $CaCl_2$ ni kumulyativ tarzda qo‘shish orqali yuzaga keladigan aorta qisqarishlariga moddalarning ta’siri baholandi.

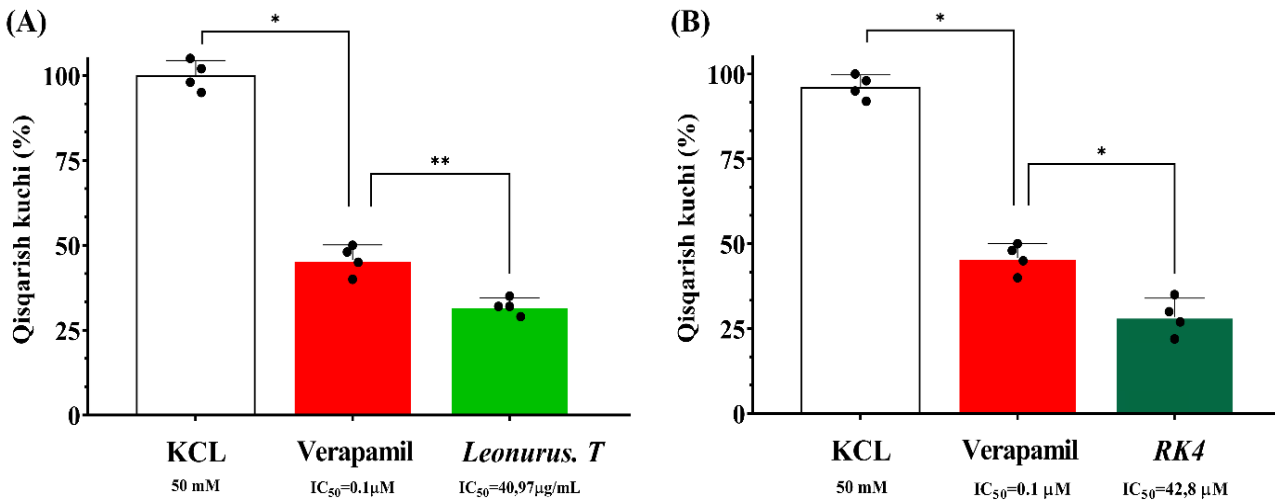
Natijalarga ko‘ra, $CaCl_2$ konsentratsiyasini 0,5 mM dan 2,5 mM gacha oshirish L-tip Ca^{2+} kanallari orqali ionlarning kirishini kuchaytirib, aorta muskuli qisqarishlarining bosqichma-bosqich ortishiga olib kelishi aniqlandi (1-rasm, A). Shu bilan birga, inkubatsiya muhitida avvaldan tekshirilayotgan gidrolizlanuvchi taninlar mavjud bo‘lganda, $CaCl_2$ ni qo‘shish natijasida hosil bo‘lgan qisqarish kuchining ushbu pog‘onali ortishi mutanosib ravishda kamayib borishi kuzatildi (1-rasm, B).



1-rasm. Leonurus turkestanicus (A) va RK4 (B) polifenolining aorta muskuli preparatiga vazorelaksant ta'sirining muhitda $[Ca^{2+}]_0$ konsentratsiyasi o'zgarishlariga bog'liqligi. Ordinata o'qida 50 mM KCl yordamida chaqirilgan aorta qisqarish kuchi 100% deb olingan. Absissa o'qida $CaCl_2$ning konsentratsiyasi (mM) ifodalangan *(barcha holatlarda ishonchlilik ko'rsatkichi *p<0,05; n=5).*

O'tkazilgan tajriba natijalari tahliliga asoslanib aytish mumkinki, Leonurus turkestanicus ekstrakti va RK4 polifenolining vazorelaksant ta'siri aorta silliq muskul hujayralari plazmolemmasida joylashgan L-tip Ca^{2+} kanallari orqali Ca^{2+} ionlarining kirishini kamaytirishi bilan bog'liq bo'lib, buning natijasida hujayra ichki $[Ca^{2+}]$ darajasi pasayadi.

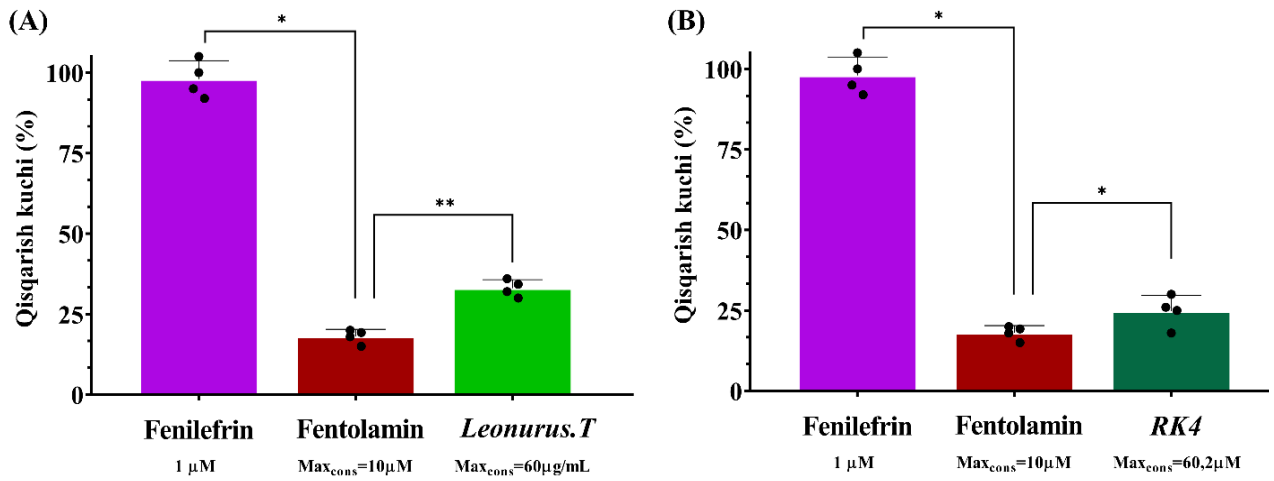
Keyingi bosqichda ushbu moddalarning vazorelaksant ta'sirida Ca^{2+} kanallari ishtirokini aniqlash uchun L-tip Ca^{2+} kanallarining maxsus blokatori – verapamil qo'llandi. Olingan natijalarga ko'ra, verapamil aorta silliq muskul preparatlarida dozaga bog'liq ta'sir ko'rsatib, 0,1 μM konsentratsiyada qisqarish kuchini 45,75±2,9% ga kamaytirdi. Shu bilan birga, verapamilning yarim maksimal samarali konsentratsiyasi (IC_{50}) 0,1 μM ekani aniqlandi. Shuningdek, verapamilning 0,1 μM konsentratsiyasi mavjud sharoitda Leonurus turkestanicus (IC_{50} = 40,97 μM) va RK4 polifenoli (IC_{50} = 42,8 μM) qo'shimcha vazorelaksant ta'sir ko'rsatib, qisqarish kuchini mos ravishda 13,75±3,1% (nazoratga nisbatan 45,75±2,9%) va 17,25±2,4% (nazoratga nisbatan 48,05±2,6%) ga kamaytirdi (2-rasm, A va B).



2-rasm. Leonurus turkestanicus (A) va RK4 (B) polifenolining kalamush aorta qon tomiri silliq muskul preparatida muhitda L-tip Ca^{2+}-kanalining spetsifik blokatori – verapamil (0,1 mkM) mavjud sharoitda 50 mM KCl yordamida chaqirilgan qisqarishga vazorelaksant ta'siri. Ordinata o'qida KCl (50 mM) yordamida chaqirilgan qisqarish kuchi nazorat sifatida 100% deb olingan *(barcha holatlarda ishonchlilik ko'rsatkichi *p<0,05; n=4-5).*

Olingan natijalar tahlili shuni ko‘rsatadiki, Leonurus turkestanicus ekstrakti va RK4 polifenolining vazorelaksant ta’siri bevosita L-tip Ca^{2+} kanallarining bloklanishi bilan bog‘liq. Ushbu moddalarning L-tip Ca^{2+} kanallarini susaytirishi natijasida aorta silliq muskul hujayralariga Ca^{2+} ionlarining kirishi kamayadi, buning oqibatida hujayra ichki [Ca^{2+}] darajasi pasayib, qisqarish faolligi sezilarli darajada susayadi. Bundan tashqari, Leonurus turkestanicus va RK4 polifenolining verapamil mavjud sharoitda ham aorta qisqarishini qo‘shimcha ravishda kamaytirishi, ularning faolligi faqat Ca^{2+} kanallari blokadasi bilan cheklanmasdan, balki boshqa ion transport tizimlariga ham ta’sir qilishi mumkinligini ko‘rsatadi.

Keyingi bosqich tajribalarda Leonurus turkestanicus va RK4 polifenolining verapamil mavjud sharoitda FE bilan chaqirilgan aorta silliq muskuli qisqarishlariga vazorelaksant ta’sirini kuzatdik. Mazkur kuzatuvlarni to‘ldirish va yanada aniqroq tushuntirish maqsadida biz retseptor-boshqariluvchi Ca^{2+} kanallari ishtirokini baholash uchun α_1–adrenoretseptorlarning blokatori – fentolamin (FA)dan foydalanib qo‘shimcha tadqiqotlar o‘tkazdik. Natijalarga ko‘ra, muhitda 10 μM fentolamin mavjud bo‘lganda Leonurus turkestanicus (60,0 μM) va RK4 polifenoli (60,2 μM)ning vazorelaksant ta’siri fentolaminsiz sharoitga nisbatan mos ravishda 33,07±2,7% va 24,75±2,9% ga kamaygani aniqlandi (3-rasm, A va B).



3-rasm. Leonurus turkestanicus (A) va RK4 (B) polifenolining vazorelaksant ta’siriga fentolaminning ta’siri. Ordinata o‘qida FE (1 mkM) yordamida chaqirilgan aorta silliq muskuli qisqarish kuchi 100% deb olingan *(barcha holatlarda ishonchlilik ko‘rsatkichi *p< 0,05; n=4).*

XULOSA

Olingan natijalar tahlili Leonurus turkestanicus ekstrakti va RK4 polifenolining vazorelaksant ta’siri aorta silliq muskul hujayralari plazmolemmasida joylashgan L-tip Ca^{2+} kanallari orqali Ca^{2+} ionlarining kirishini kamaytirishi bilan bog‘liq bo‘lib, buning natijasida hujayra ichki [Ca^{2+}] darajasi pasayadi. Shuningdek, ularning vazorelaksant ta’siri bevosita L-tip Ca^{2+} kanallarining bloklanishi bilan bog‘liq. Ushbu moddalarning L-tip Ca^{2+} kanallarini susaytirishi natijasida aorta silliq muskul hujayralariga Ca^{2+} ionlarining kirishi kamayadi, buning oqibatida hujayra ichki [Ca^{2+}] darajasi pasayib, qisqarish faolligi sezilarli darajada susayadi.

Tajribalardan olingan natijalar asosida shuni xulosa qilish mumkinki, o‘rganilayotgan moddalarning vazorelaksant ta’siri faqat L-tip Ca^{2+} kanallari blokadasi bilan emas, balki retseptor-boshqariluvchi Ca^{2+} kanallarining ham bloklanishi bilan bog‘liq. Ushbu kompleks ta’sir natijasida aorta silliq muskul hujayralarida hujayra ichki [Ca^{2+}] darajasi keskin pasayadi, bu esa moddalarning vazorelaksant samaradorligini yanada kuchaytiradi.

ROLE OF CA^{2+} CHANNELS IN THE VASORELAXANT EFFECT OF LEONURUS TURKESTANICUS AND RK4 POLYPHENOL ON AORTIC SMOOTH MUSCLE

Gulkhayoxon J. Mirzaalimova[1], Anvar E. Zaynabiddinov[1]

Ilmiy xabarnoma. Seriya: Biologik tadqiqotlar – Scientific Bulletin. Series: Biological Research. 2025. 8(92). 86-91.

[1]Andijan State University, Andijan, 170100, str. University, 129 (Uzbekistan). E-mail: agsu_info@edu.uz

Keywords: *Leonurus turkestanicus extract, RK4 polyphenol, vasorelaxant, L-type Ca^{2+} channels, verapamil, phenylephrine, fentolamine, contraction force, Ca^{2+} ions, sarcoplasmic reticulum.*

This study investigated the vasorelaxant effects of *Leonurus turkestanicus* extract and RK4 polyphenol on rat aortic smooth muscle. Since vascular tone is largely regulated by intracellular Ca^{2+} concentration, the role of Ca^{2+} influx pathways in smooth muscle contraction was evaluated. Experiments were performed on isolated aortic rings from healthy white rats under isometric conditions using mechanography. The involvement of L-type Ca^{2+} channels, the Na^{+}/Ca^{2+} exchanger, and receptor-operated Ca^{2+} channels was assessed using verapamil, KB-R7943, and the α_1-adrenoceptor antagonist fentolamine, respectively.

The results showed that cumulative $CaCl_2$ application induced a concentration-dependent increase in aortic contraction, which was significantly attenuated by both *Leonurus turkestanicus* extract and RK4 polyphenol. Verapamil markedly reduced contractile activity, and its combined application with the tested compounds further enhanced vasorelaxation. In the presence of fentolamine, the vasorelaxant effects were partially reduced, indicating the involvement of receptor-operated Ca^{2+} channels in addition to L-type channels.

Overall, *Leonurus turkestanicus* extract and RK4 polyphenol significantly decrease intracellular Ca^{2+} levels in vascular smooth muscle cells by inhibiting both potential-dependent and receptor-operated Ca^{2+} influx. These findings demonstrate a complex vasorelaxant mechanism and suggest that these natural polyphenolic compounds may have potential therapeutic value in cardiovascular pharmacology.

References:

1. Jackson, F.W. (2021). Myogenic Tone in Peripheral Resistance Arteries and Arterioles: The Pressure Is On! *Frontiers in Physiology*. Vol. 12. Pp. 1-9.

2. Moccia, F., Fiorio Pla, A., Lim, D., Lodola, F., Gerbino, A. (2023). Intracellular Ca^{2+} signalling: unexpected new roles for the usual suspect. *Frontiers in Physiology*. Vol. 14. Pp. 1-16.

3. Shimoda, L.A., Polak, J. (2010). Hypoxia. 4. Hypoxia and ion channel function. *American Journal of Physiology-Cell Physiology*. Vol. 300(5). Pp. 951-967.

4. Dumanska, H., Telka, M., Veselovsk, N. (2023). Inhibition of high-voltage-activated calcium currents by acute hypoxia in cultured retinal ganglion cells. *Frontiers in Cellular Neuroscience*. Vol. 17. Pp. 1-9

5. Calderón-Sánchez, E.M., Fernández-Tenorio, M., Ordóñez, A., López-Barneo J., Ureña, J. (2009). Hypoxia inhibits vasoconstriction induced by metabotropic Ca^{2+} channel-induced Ca^{2+} release in mammalian coronary arteries. *Cardiovascular Research*. Vol. 82(1). Pp. 115-24

6. Kim, B., Jo, C., Choi, H.-Y., Lee, K. (2018). Vasorelaxant and hypotensive effects of cheonwangbosimdan in SD and SHR rats. *Hindawi Evidence–Based Complementary and Alternative Medicine*. Vol. Pp. 1-8.

7. Ozaki, H., Ohyama, T., Sato, K., Karaki, H. (1990). Ca^{2+}–Dependent and independent mechanisms of sustained contraction in vascular smooth muscle of rat aorta. *Japanese Journal of Pharmacology*. Vol. 52(510). Pp. 509-512.

8. Martinsen, A., Baccelli, C., Navarro, I., Abad, A., Quetin-Leclerc, J., Morel, N. (2010). Vascular activity of a natural diterpene isolated from Croton zambesicus and of a structurally similar synthetic trachylobane. *Vascular Pharmacology*. Vol. 52. Pp. 63-69.

9. Cherkaoui-Tangi, K., Israili, Z.H., Lyoussi, B. (2016). Vasorelaxant effect of essential oil isolated from Nigella sativa L. seeds in rat aorta: Proposed mechanism. *Pakistan Journal of Pharmaceutical Sciences*. Vol. 29(1). Pp. 1-8.

Mualliflar haqida ma'lumot:

MIRZAALIMOVA Gulhayoxon Jasurbek qizi – Andijon davlat universiteti Odam fiziologiyasi va hayot faoliyati xavfsizligi kafedrasi tayanch doktoranti. E-mail: mirzaalimovag@mail.ru.

ZAYNABIDDINOV Anvar Erkinjonovich – biologiya fanlari doktori, Andijon davlat universiteti Odam fiziologiyasi va hayot faoliyati xavfsizligi kafedrasi professori.