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KVERSETIN HOSILALARI SINTEZI VA ULARNING TIMOTSIT HUJAYRALARIGA TA'SIRI

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Annotatsiya: Hozirgi vaqtda flavonoidlar turli xil oziq-ovqat, farmatsevtika, tibbiyot va kosmetika sanoatida ajralmas komponenti sifatida qaraladi. Dunyoning yetakchi ilmiy tadqiqot markazlarida o'simlik flavonoidlarining turli xil biologik va farmakologik xususiyatlari ustida jadal ilmiy izlanishlar olib borilmoqda. Yuqoridagilardan kelib chiqqan holda, ushu tadqiqot ishida kversetinning atsillangan hamda metillangan hosilalari sintez qilindi. Hujayra yorug'lik o'tkazuvchanligi usuli yordamida gipoosmotik stress sharoitda hujayra hajm boshqarilishiga kversetin va hosilalari konsentratsiyaga bog'liq ta'siri o'rganildi.

Kalit so'zlar: Yuqori samarali suyuqlik xromatografiyasi (YuSSX), kversetin, pentaatsilkversetin, pentametilkversetin

Kirish

Kversetin kundalik iste'mol qilinadigan oziq-ovqat mahsulotlarida jumladan, piyoz [1], brokkoli kabi sabzavotlar, mango [2], olma kabi mevalar, choy, rezavorlar bundan tashqari ko'plab o'simlik urug'lari, gullari va barglarida keng tarqalgan. O'simliklarda nafaqat aglikon (kversetin), balki uning uglevodlar bilan konyugatsiyalangan glikozid hosilalari, shuningdek, metil efirlari holida ham mavjud. Uzoq yillar mobaynida olib borilgan polifenol moddalarning tadqiqi shuni ko'rsatdiki polifenol moddalari keng farmakologik ta'sirga ega. Bunday tabiiy fenol moddalardan biri bo'lgan kversetin o'zining antioksidant [3], antikonserogen [4], antibakterial [5] hamda semizlikka [6], virusga [7] va yallig'lanishga qarshi ta'sirlari [8] tufayli tobora ko'proq e'tiborni tortmoqda. Ma'lumotlarga ko'ra, kversetin turli xil saraton hujayralarini (masalan, yo'g'on ichak, oshqozon osti bezi [9], ko'krak, o'pka [10], tuxumdon, prostata bezi [11], jigar saratonlari hamda leykemiya [12]) modulyatsiya qilish va ularning o'sishini cheklash orqali (sitostatik ta'sir ko'rsatib) ko'payishini oldini olishi mumkin [13-16]. Bundan tashqari kversetinning samarali antioksidant ekanligi ham isbotlangan bo'lib, antioksidant faolligi quyidagilarga bog'liq [17-18]: (a) B halqasi - katexol guruhi; (b) C halqasidagi 2,3 uglerodlar orasidagi konyigurlangan qo'shbog'; va (c) A halqadagi 5- va 7-holatlardagi -OH guruhi. Kversetinning yallig'lanishga qarshi ta'siri esa uning antioksidant va erkin radikallarni neytrallash xususiyatlari bilan bog'liq [19-20]. Ba'zi tadqiqot natijalarida kversetinning yurak-qon tomir kasalliklarini oldini olishini ham ko'rsatdi. Inson salomatligi uchun potentsial foydalari tufayli kversetin oziq-ovqat va farmatsevtika sanoatida ozuqaviy tarkibiy qism sifatida foydalanish markaziga aylandi.

Material va metodlar

O'zbekiston hududida manzarali daraxt sifatida o'stiriladigan Yapon saforasining (*Sophora japonica L.*) g'unchalaridan iyun-iyul oylarida yig'ib olindi va ular quyosh nuridan himoyalangan xonada doimiy massaga kelgunga qadar quritildi. Quritilgan o'simlik g'unchalarini maydalab, 1:15 nisbatda suv bilan 3 soat mobaynida 100°C haroratda ekstraksiya qilindi. Suvli ekstrakt filtrlanib, sovutkichda 24 soatga qoldirildi. Cho'kmaga tushgan texnik rutin filtrlab olindi va etanolda qayta kristallab tozalandi. Tozalangan rutin 1:100 nisbatda 5% li

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H_2SO_4 bilan gidroliz qilib texnik kversetin olindi. So'ngra, etanolda qayta kristallab toza kversetin olindi. Kversetinning quruq xom ashyoga nisbatan o'rtacha unumi 5,3% ni tashkil qildi.

Yorqin sariq rangli amorf kukun T_{suyuq}= 310±2ºC; R_f= 0,55 (sis: B), R_f=0,67 (sis: C). YuSSX bo'yicha asosiy modda miqdori 83±2% tashkil etdi.

Pentaatsilkversetin sintezi: Mexanik aralashtirgich bilan jihozlangan hajmi 100 ml bo'lgan tagi yumaloq kolbada kversetin (7g) va sirka angidridi (43,8 ml) 130ºC haroratda 1 soat davomida qizdirildi. So'ng aralashma sovutilib sovuq suv bilan suyultirildi va vakumli bug'latgichda filtrlandi. Sirka hidi yo'qolgunicha suv bilan yuvildi. Olingan mahsulot atseton-metanolda qayta kristallandi.

Oq rangli amorf kukun. Unum 10,07g (84,9%). T_{suyuq}=195±2ºC; R_f=0,37 (sis: C). YuSSX bo'yicha asosiy modda miqdori 94±2% tashkil etdi.

Monometiltetraatsilkversetin (Ramnetin tetraatsetat) sintezi: Mexanik aralashtirgich va teskari sovutgich bilan jihozlangan hajmi 50 ml bo'lgan yumaloq tubli kolbada kversetin pentaatsetat (2g), suvsiz atsetonda (30 ml) eritilib, kukun holidagi K_2CO_3 (5,2g), CH_3I (4ml) solib, havo namligidan himoyalangan holda 20 soat davomida 56ºC haroratda qizdirildi. So'ng erimagan kaliy tuzlari filtrlandi va filtrat bug'latildi. Olingan mahsulot atseton-metanolda qayta kristallandi.

Sariq rangli amorf kukun. Unum 1,16g (61,62%). T_{suyuq}= 250±2ºC R_f=0,38 (sis: C). YuSSX bo'yicha asosiy modda miqdori 85±2% tashkil etdi.

Pentametilkversetin sintezi: Mexanik aralashtirgich bilan jihozlangan hajmi 100 ml bo'lgan tagi yassi kolbada 8 ml DMSO dagi 1,66 g KOH kukini suspenziyasiga sekinlik bilan harorat 10 ºC oshib ketmasligi nazorat qilib turilgan holda 1g kversetin, so'ng 2,5 ml Me_2SO_4 qo'shildi. Hosil bo'lgan to'q jigarrang aralashma xona haroratida yorqin jigarrangga kirgunicha 2 soat davomida aralashtirildi. So'ng H_2O (80 ml) bilan suyultirildi. Hosil bo'lgan suspenziya etilatsetat (50/20/20) bilan ekstraktsiya qilindi. Etilatsetatli qism 5% li NaOH (10ml x 4), H_2O (10 ml x 3), hamda sho'r suv (10 ml) bilan yuvildi va Na_2SO_4 bilan quritilib, vakumda bug'latildi. Olingan mahsulot MeOH da qayta kristallandi.

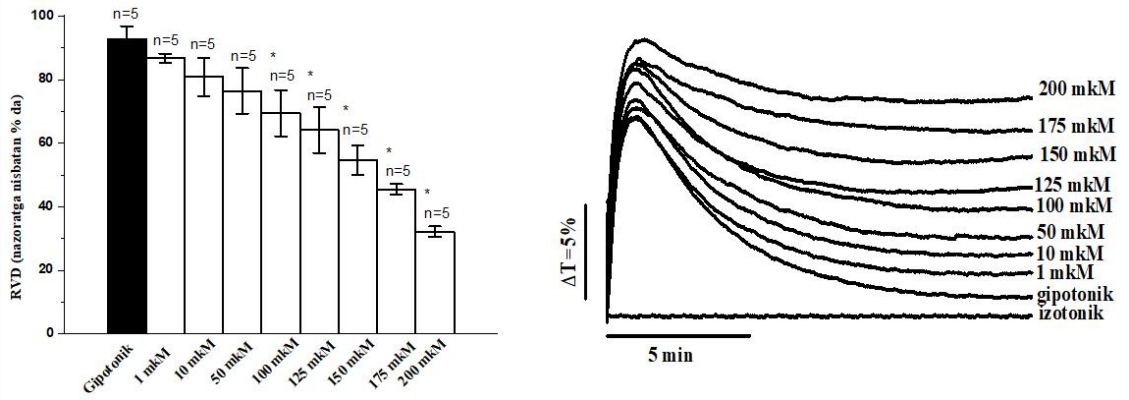
Oq rangli amorf kukun. Unum 1,03g (83,73%). T_{suyuq}=150±2 ºC R_f= 0,07 (sis: C). YuSSX bo'yicha asosiy modda miqdori 97±2% tashkil etdi.

Olingan natijalar tahlili

Hujayra hajmining statsionarligiga plazmatik membrana orqali suv va osmolitlarning o'tishini tartibga solish orqali erishiladi. Izotonik muhitda (normal Ringer eritmasi) timotsitlar hajmi 15 minut davomida o'zgarmasdan saqlandi. Timotsitlar gipotonik muhitga tushganda dastlab tezda shishib, keyin izotonok hajmga yaqin darajagacha hajmini tikladi. Ushbu jarayon regulyator hajm kamayishi *(RVD – regulatory volume decrese)* deyiladi. Timotsitlarning gipoosmotik stress sharoitida o'z hajmini boshqara olish qobiliyatini aniqlash uchun (***RVD*** = ***((*** T_{max}***-*** T_o***) /(*** T_{max}***-*** T_{15}***)) x 100%***) formula orqali ifodalangan *RVD* parametridan foydalanildi.

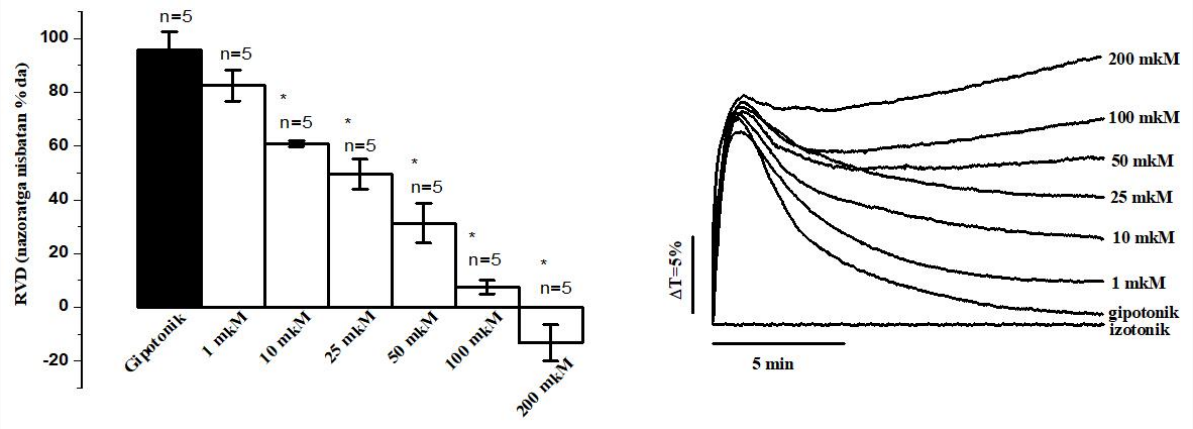
Dastlabki tajribalarimizda kversetinning gipoosmotik stress sharoitida timotsit hujayralari hajm boshqarilishi tizimiga ta'siri o'rganildi. Nazoratda gipoosmotik stress sharoitida (ya'ni 147±2 mOsm/kgH_2O osmotik bosimda) timotsit hujayra hajm kamayishi o'rtacha 92,8±4,1% (n=5) ga teng bo'ldi. Shu muhitda timotsit hujayralari hajm boshqarilishiga kversetinning turli kontsentratsiyalardagi eritmalari ta'sir ettirildi (1-rasm). Olingan natijalarda moddaning 100 mkM kontsentratsiyasidan boshlab *RVD*ga ta'siri kuzatildi va bu 69,5±7,3% (n=5); 125 mkM da 64,2±7,3% (n=5); 150 mkM da 54,6±4,7% (n=5); 175 mkM da 45,5±1,6% (n=5); 200 mkM da 32,2±1,6% (n=5) ga teng bo'ldi. Kversetin 100 mkM konsentratsiyadan boshlab timotsitlar hajm boshqarilishiga ingibirlovchi ta'sir ko'rsatdi. Natijalarning statistik tahlili yarim maksimal effekt $C_{(50\%)}$=135,3±14,2 mkMga Xill koeffitsienti esa 6,2±2,6 ga teng ekanligi aniqlandi. Olingan natijalardan kversetin kontsentratsiyaga bog'liq holda timotsit hujayralarida *RVD* jarayonini sezilarli darajada ingibirlashi aniqlandi.

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1-rasm. Gipoosmotik stress sharoitda hujayra hajm boshqarilishiga kversetinning konsentratsiyaga bog'liq ta'siri. A) Hujayralar suspenziyasining yorug'lik o'tkazuvchanligi o'zgarishi registratsiyasining original yozuvi ko'rsatilgan; B) Ordinata o'qida – hujayra hajmining boshqarilishi % hisobida ko'rsatilgan, absissa o'qida – muhitda kversetinning kontsentratsiyasi mkM da ifodalangan, n – tajribalar soni. Nazoratdan statistik ravishda farq $P<0,05$ yulduzchalar (*) bilan belgilangan.

Tajribalarimizni kversetinning pentaatsillangan hosilasi kversetinpenta-atsetatni gipoosmotik stress sharoitida timotsit hujayralari hajm boshqarilishi tizimiga ta'siri o'rganish bilan davom ettirdik. Nazoratda gipoosmotik stress sharoitida hujayra hajm kamayishi o'rtacha 95,9±6,6% (n=5)ga teng bo'ldi. Shu muhitda kversetinpentaatsetatni turli kontsentratsiyalarini timotsit hujayralariga ta'sir ettirdik (2-rasm). Olingan natijalarda moddaning 10 mkM kontsentratsiyasidan boshlab *RVD*ga ta'siri kuzatildi va bu 60,9±1,04% (n=5); 25 mkMda 49,6±5,5% (n=5); 50 mkMda 31,4±7,4% (n=5); 100 mkMda 7,6±2,5% (n=5); 200 mkMda 13,2±6,8% (n=5) ga teng bo'ldi. Natijalarning statistik tahlili yarim maksimal effekt $C_{(50\%)}$=22,7±1,9 mkMga Xill koeffitsienti 0,9±0,1 ga teng ekanligi aniqlandi. Olingan natijalardan kversetin hosilasi kversetinpentaatsetat kontsentratsiyaga bog'liq holda 200 mkMda timotsit hujayralarida *RVD* jarayonini to'liq ingibirlashi aniqlandi.



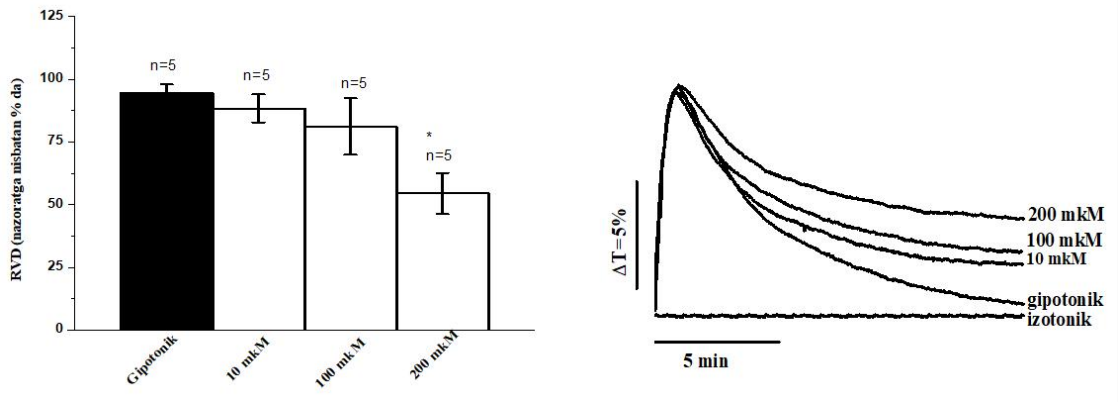
2-rasm. Gipoosmotik stress sharoitda hujayra hajm boshqarilishiga kversetinpentaatsetatning konsentratsiyaga bog'liq ta'siri. A) Hujayralar suspenziyasining yorug'lik o'tkazuvchanligi o'zgarishi registratsiyasining original yozuvi ko'rsatilgan; B) Ordinata o'qida – hujayra hajmining boshqarilishi % hisobida ko'rsatilgan, absissa o'qida – muhitda kversetinpentaatsetatning kontsentratsiyasi mkM da ifodalangan, n – tajribalar soni. Nazoratdan statistik ravishda farq $P<0,05$ yulduzchalar (*) bilan belgilangan.

Keyingi tajribalarimizni pentametilkversetinni gipoosmotik stress sharoitida kalamush timotsit hujayralari hajm boshqarilishi tizimiga ta'siri o'rganish bilan davom ettirdik. Nazoratda gipoosmotik stress sharoitida hujayra hajm kamayishi o'rtacha 94,6±3,3% (n=5)ga teng bo'ldi.

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Shu muhitda pentametilkversetinni turli kontsentratsiyalarini timotsit hujayralariga ta'sir ettirdik. Olingan natijalarda moddaning faqatgina 200 mkM kontsentratsiyasida *RVD*ga ta'siri kuzatildi va bu 54,4±8,2% (n=5) ga teng bo'ldi. Natijalarning statistik tahlili yarim maksimal effekt $C_{(50\%)}$=100,2±3,8 mkMga Xill koeffitsienti esa 23,03±4,3 ga teng ekanligi aniqlandi.

Olingan natijalardan pentametilkversetin kontsentratsiyaga bog'liq holda timotsit hujayralarida RVD jarayoniga juda kam ta'sir etishi aniqlandi.



3-rasm. Gipoosmotik stress sharoitda hujayra hajm boshqarilishiga pentametil-kversetinning konsentratsiyaga bog'liq ta'siri. A) Hujayralar suspenziyasining yorug'lik o'tkazuvchanligi o'zgarishi registratsiyasining original yozuvi ko'rsatilgan; B) Ordinata o'qida – hujayra hajmining boshqarilishi % hisobida ko'rsatilgan, absissa o'qida – muhitda pentametilkversetinning kontsentratsiyasi mkM da ifodalangan, n – tajribalar soni. Nazoratdan statistik ravishda farq P<0,05 yulduzchalar (*) bilan belgilangan.

Xulosa

Gipoosmotik stress sharoitda hujayra hajm boshqarilishiga kversetin va hosilalari konsentratsiyaga bog'liq ta'siri tekshirilganda, kversetin 100 mkM, pentaatsilkversetin 10 mkM dan boshlab ta'sir etishi kuzatildi. Pentametilkversetin esa faqat eng yuqori konsentratsiyada ya'ni 200 mkM dagina hujayra hajm boshqarilishiga ta'sir etishi aniqlandi. Bunda pentaatsilkversetin eng kuchli ingibirlovchi ta'airga ega deb xulosa qilishimiz mumkin.

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