BIOLOGICAL SCIENCES

UDK 591.1.(575.1)

DETERMINATION OF THE RELAXANT EFFECT OF SOME HYDROLYZABLE TANNINS ISOLATED FROM THE PLANT SPECIES E. SANESCENS (L.)

Mutalipov A.,
Andijan State University
Karimjonov H.,
Andijan State University
Zaynabiddinov A.,
Andijan Institute of Economics and Construction
Usmanov P.,
Head of the Cell Biophysics Laboratory of the Institute of Biophysics and Biochemistry under UzMU
Omonturdiev S.,
Institute of Bioorganic Chemistry of the Russian Academy of Sciences
Rakhimov R.
Institute of Bioorganic Chemistry of the Russian Academy of Sciences
https://doi.org/10.5281/zenodo.10450873

Abstract

In studies, 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucose The vasorelaxant effect of - hydrolyzable tannins on the contractile activity of the rat aortic blood vessel preparation was studied depending on their structure. In our experiments, it was determined that the vasorelaxant effect of hydrolyzable tannins depends on their structure and applied doses, and it was determined that it is related to the blockade of Ca^{2+} channels of vascular smooth muscle cells and sarcoplasmic reticulum.

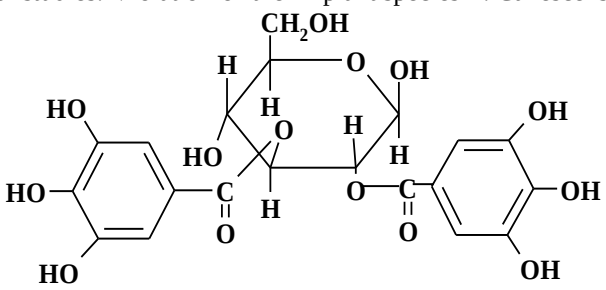
Keywords: Tannin, blood vessel, relaxant effect, aorta, muscle, Ca^{2+}-channel, dose, relaxation.

Abstract. Currently, one of the urgent problems of modern medicine and pharmacology is the creation of highly effective drugs with a targeted effect and no negative side effects. Another fact today is that many diseases, especially cardiovascular diseases, are caused by disruption of the functions of the ion-transport system in cells. Dysfunction of this system causes and/or causes many diseases, including neurodegenerative, cardiovascular and other diseases.

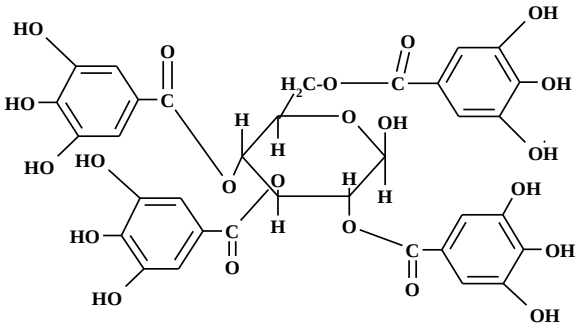
The main function of smooth muscles in blood vessels is to maintain blood vessel tone and arterial blood pressure. Diseases occurring in the cardiovascular system are detected by studying the functioning of smooth muscles [3; pp. 165–168]. In this case, the function of ion channels involved in vasoconstriction/relaxation is studied using control studies. Violation of the function of the channels causes various cardiovascular diseases.

Analysis of the current literature shows that biologically active substances with effective effects on the cardiovascular system have been isolated from plants, including secondary metabolites in plants such as alkaloids, flavonoids, and tannins [4; 11 p.]. These compounds have a wide range of biological activity, and it is reported in the literature that they have antioxidant, antiviral, interferon-inducing, anticancer, and immunosuppressive properties [5; 230 pp.].

Taking into account the above, the purpose of this study is to scientifically substantiate the vasorelaxant effect depending on the chemical structure and dose of certain hydrolyzable tannins (Fig. 1) isolated from the plant species E. Canescens(L.).



1) 2,3-di-O-galloyl- β -D-glucose



2) 1,2,3,4-tetra-O-galloyl- β -D-glucose
Figure 1. Chemical formula of hydrolyzable tannins

Material and methods. Experiments were conducted in the laboratory of experimental innovative research of the Department of Human Physiology and Safety of Life Activities of Andijan State University and were carried out on healthy white, inbred male rats (150-200 gr.) bred in the vivarium under conditions provided with standard food and water.

The preparation of the aortic vascular preparation is carried out using a standard methodincreased [12; p. 110].

After the animals were euthanized by cervical dislocation, the chest was surgically opened, the aorta was isolated, and the connective tissue was cleaned in Krebs-Henseleit saline medium, and annular segments (*l*=2–4 mm; ø=1–2 mm) was cut. Krebs-Henseleit physiological solution with the following chemical composition was continuously circulated in the experimental cell (5 ml): (mM): NaCl – 120.4; KSl – 5; NaHCO3 – 15.5; NaH_2PO_4 – 1.2; $MgCl_2$ – 1.2; $CaCl_2$ – 2.5; $S_6N_{12}O_6$ – 11.5 (pN=7.4). Physiological solution was aerated with carbogen (O2–95% and CO_2–5%), temperature constant (t=+37±0.5°C) was ensured using an ultrathermostat (U–8; Bulgaria). The contractile activity of the aortic vascular preparation was recorded in isometric conditions using a standard method (mechanography) using an Isometric force transducer and amplifier force sensor, a signal amplifier device (Grass Instrument, USA) on an Endim 621.02 samopiset (Czech Republic)was done [13; 195-p; 143; p. 110].Initially, the rat aorta preparation was incubated with a voltage of 1 g (9.8~10 mN) for ~45–60 min until normal electromechanical activity was recorded.

The obtained results and their analysis. 2,3-di-O-galloyl-β-D-glucose in experiments (10–200 μM) and 1,2,3,4-tetra-O-galloyl- β-D-glucose(5–50 μM) of hydrolyzable tannins was found to have a significant vasorelaxant effect on isometric contraction activity (in vitro) of rat aorta blood vessels induced by KCl (50 μM). Including 2,3-di-O-galloyl-β-D-glucosehydrolyzable tannin At a concentration of 30 μM, it was found to reduce the contraction force by 6.17±3.1% compared to the control, and at a maximum concentration of 200 μM, it decreased by 74.8±4.5% (n=4–6). Also 1,2,3,4-tetra-O-galloyl-β-D-glucose hydrolyzable tanninIt was found to reduce the contraction force by 12.5±4.7% compared to the control at a concentration of 10 μM and 85.2±3.2% at a maximum concentration of 50 μM. In this 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucose(IC_{50}) value was found to be – 90 μM and 26.8 μM, respectively (Fig. 2 A and B)

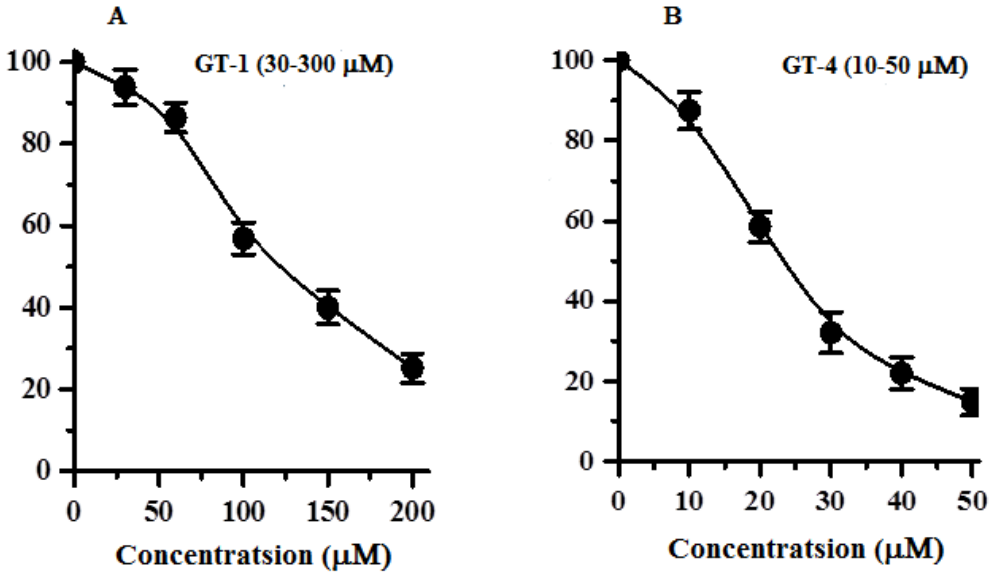


Figure 2.
2,3-di-O-galloyl-β-D-glucose (GT-1) and 1,2,3,4-tetra-O-galloyl-β-D-glucose (GT-4) hydrolyzable tannin of theirconcentration-dependent vasorelaxant effect on contraction induced by KCl (50 mM) in aortic preparation. On the ordinate axis - the contraction force of the aorta is taken as 100%. The abscissa axis shows the concentration of hydrolyzable tannins in μM. In all cases, the reliability index (– p< 0.05; ** p< 0.01; n=4–6).*

During our research, this,2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucose of hydrolyzable tannins KCl depends on the potential of the relaxant effect in conditions of contracture induced by To test the involvement of Ca^{2+} channels A specific blocker of the Ca^{2+} $_L$-channel - verapamil (0.01-1 μM) was tested and further analyzed in the experiments.

Verapamil (IC_{50} =0.1 μM)under the conditions of incubation, the contraction strength of the aortic smooth muscle preparation was observed to decrease by 50±3.4% compared to the control, and under these conditionsof the cup (IC_{50}=90 μM)In addition, the strength of the contraction of the aortic smooth muscle drug under the influence of alkaloids- up to 6.4±3.4%, a decrease of 60.6±4.2% compared to the control was observed. alsoverapamil in incubation conditions1,2,3,4-tetra-O-galloyl-β-D-glucose(IC_{50}(=26.8 μM) aortic smooth muscle preparation reduced contraction force by 9.3±3.1% compared to the condition with additional verapamil and 59.8±4.8% compared to the control.(Figure 3).

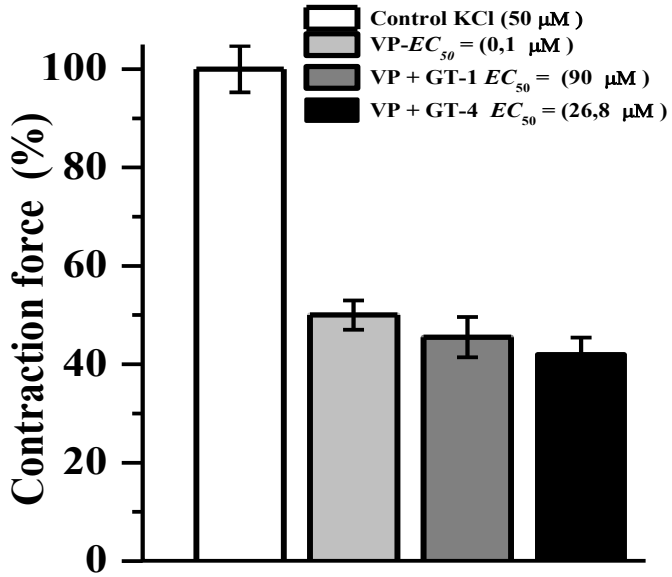


*Figure 3. 2,3-di-O-galloyl-β-D-glucose (GT-1) and 1,2,3,4-tetra-O-galloyl-β-D-glucose (GT-4)Relaxant effect of hydrolyzable tannins depends on the concentration of [Ca2+]o and the state of potential-dependent L-type Ca2+ channels.2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucosethe effect of On the ordinate axis, the contraction force evoked by aortic contraction force of 50 mM KCl is taken as 100%. The abscissa axis shows the concentration of CaCl2. 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β- Relaxant effect of D-glucose (reliability index in all cases *p<0.05; **p<0.01; n=4–6).*

Based on the obtained results, it can be said that 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucosethe relaxant effect of hydrolyzable tannins is based on the blockade of L- Ca^{2+} channels. However, in the presence of verapamil, partial preservation of the relaxant effect of studied hydrolyzable tannins, blockade of L- Ca^{2+} channels, as well as reduction of transport of Ca^{2+} -ions in smooth muscle cells, may be activated by other mechanisms.

In addition to voltage-dependent Ca^{2+} channels, receptor controlled Ca^{2+} channels also play an important role in the control of Ca^{2+} homeostasis in smooth muscle cells [6]. In order to evaluate the effects of hydrolyzable tannins on receptor-controlled Ca^{2+}-channels, aortic muscle contraction induced by α_1-adrenoceptor agonist phenylephrine (FE) is mainly supported by Ca^{2+}-ions entering through receptor-controlled Ca^{2+}-channels [10].

In our experiments, the effect of hydrolyzable tannins on aortic contraction force evoked by FE in the presence of L-type Ca^{2+}-channel blocker-verapamil was studied. Under these conditions, FE-evoked muscle contraction was observed to be 13.7±2.8% lower than control compared to the condition without verapamil. Under these conditions, 2,3-di-O-galloyl-β-D-glucose (100 μM) and 1,2,3,4-tetra-O-galloyl-□-D-glucose (35 μM) hydrolyzable tannins maximally reduced FE-evoked aortic contraction by 74.8±3.4% and 80.4±4.3% compared to control (Fig. 4 A and B).

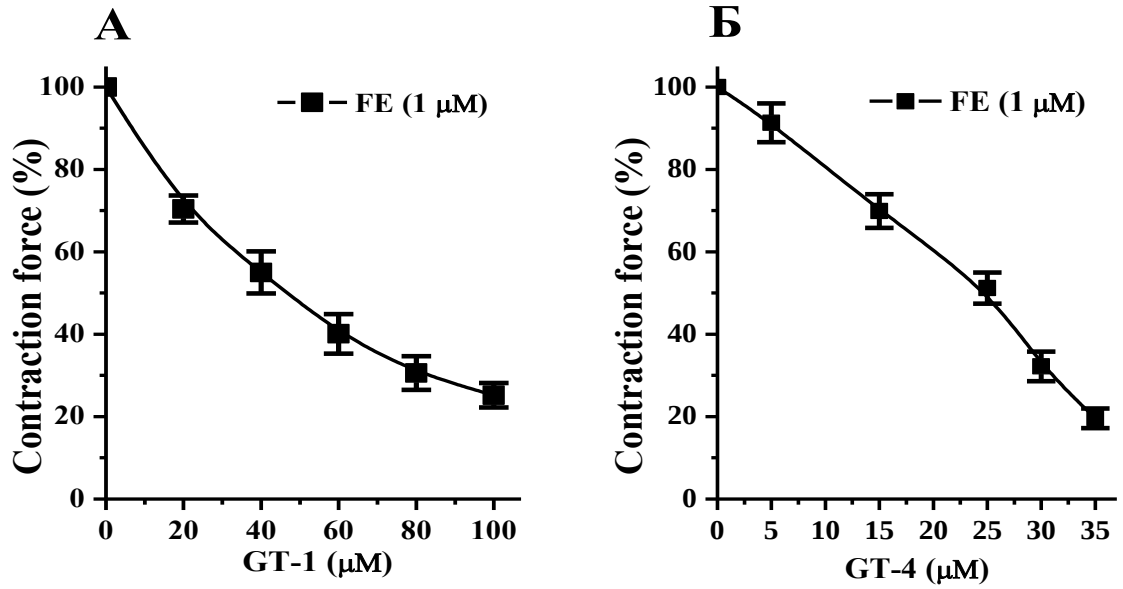


Figure 4.
*2,3-di-O-galloyl-β-D-glucose (A) and 1,2,3,4-tetra-O-galloyl-β-D-glucose(B) Effects of hydrolyzable tannins on phenylephrine-induced rat aortic contraction. 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucose Dose-dependent effect of 1 μM FE-induced rat aortic contraction. On the ordinate axis, aortic contraction force is taken as 100% of aortic contraction force evoked using 1 μM FE. On the abscissa axis is the concentration of hydrolyzable tannins. (in all cases the reliability indicator * – p<0.05; **p<0.01; n=4–6).*

In these experiments, 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucose EC_{50} values were 52.6 μM and 19.4 μM, respectively. The obtained results show that the observed effect of studied hydrolyzable tannins can be predicted to occur with blockade of receptor-controlled Ca^{2+}-channels. The effects of the studied hydrolyzable tannins on receptor-controlled Ca^{2+} channels can be explained by the experiments conducted with α-adrenoceptor blocker-phentolamine (FA) as an additional confirmation. In our experiments, 2,3-di-O-galloyl-β-D-glucose (100 μM) and 1,2,3,4-tetra-O-galloyl-β-D-glucoseRelaxant effect of (35 μM) hydrolyzable tannins in the presence of 10 μM phentolamine was observed to decrease the FE reduction by 55.2±2.3 and 48.4±3.2 compared to the condition without phentolamine (Fig. 5).

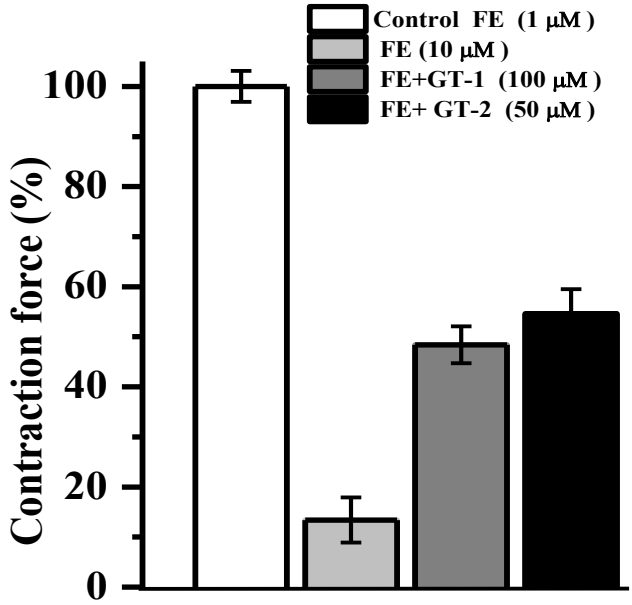


Figure 5.
2,3-di-O-galloyl-β-D-glucoseand1,2,3,4-tetra-O-galloyl-β-D-glucose effect of phentolamine (10 μM) on the relaxant effect of hydrolyzable tannins.On the ordinate axis, aortic contraction force is taken as 100% of aortic contraction force evoked using 1 μM FE. (in all cases the reliability indicator (p<0.05; **p<0.01; n=4–6).*

These results indicate that the relaxant effect of studied hydrolyzable tannins can be related to the blockade of receptor controlled Ca^{2+}-channels.

Conclusions

Hydrolyzable tannins - 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl- β-D-glucosehas a relaxant effect and effectively relaxes the contraction of rat aorta pre-challenged with hyperkalemia and phenylephrine.

Based on the analysis of literature data and experimental results, 2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucoseThe vasorelaxant effect of hydrolyzable tannins on the isometric contraction activity of the rat aortic blood vessel preparation in vitro may be mainly related to the blockade of the L-type Ca^{2+} channel.

2,3-di-O-galloyl-β-D-glucose and 1,2,3,4-tetra-O-galloyl-β-D-glucoseit can be seen that the basis of hydrolyzable tannins is the same. But the difference between them is that with increasing number of galloyl radicals, 1,2,3,4-tetra-O-galloyl-β-D-glucosecauses a significant increase in the relaxant activity of hydrolyzable tannin.

The scientific/experimental results obtained in this research work can be used as a theoretical basis for the development of antihypertensive pharmacological preparations based on hydrolyzable tannins.

References:

1. Gasser T. Ch. Vascular biomechanics: concepts, models and applications // eBook. - 2022. - P. 353

2. RC Webb, Smooth muscle contraction and relaxation // Adv. Physiol. Educ. -2003. - 27. - S. 201–206

3. Orlov S.N., Koltsova S.V., Anfinogenova Ya.D., Kapilevich L.V., Husakova S.V., Smagliy L.V., Baskakov M.B., Medvedev M.A. sodium-potassium-chlorine-cotransport and regulation of myogenic tone of vessels // Bulletin of Siberian Medicine. – 2014. – T.13. No. 6. - S. 165-173.

4. Gayibov U.G., Tilyabaev K.Z., Tukfatullina I.I., Salakhutdinov B.A. Membranoactive properties of gossypola and ego symmetrical and asymmetrical production // Uzbek biological journal. - 2011. – No. 5. – S. 11-15.

5. Kyle JAM, Duthie GG Flavonoids in foods. In: Flavonoids; chemistry, biochemistry and health implications. - 2006. - N. York, Taylor and Francis Group. - R. 219-263.

6. JD Humphrey, ER Dufresne, MA Schwartz, Mechanotransduction and extracellular matrix homeostasis // Nat. Rev. Mol. Cell Bio. – 2014. - No. 15. – R. 802–812.

7. JE Wagenseil, RP Mecham, Vascular extracellular matrix and arterial mechanics // Physiol. Rev. - 2009. - No. 89. - R. 957–989

8. Y. Castier, RP Brandes, G. Leseche, A. Tedgui, S. Lehoux, p47phox-dependent NADPH oxidase regulates flow-induced vascular remodeling // Circ. Res. - 2005. - #97. - R. 533–540.

9. RJ Guzman, K. Abe, CK Zarins, Flow-induced arterial enlargement is inhibited by suppression of nitric oxide synthase activity in vivo // Surgery. - 1997. - #122. - R. 273–279.

10. T. Matsumoto, K. Hayashi, Stress and strain distribution in hypertensive and normotensive rat aorta considering residual strain // J. Biomech. - 1996. - #118. - R. 62–73.

11. H. Wolinsky, Effects of hypertension and its reversal on the thoracic aorta of male and female rats // Circ. Res. - 1971. - #28. - R. 622–637.

12. RL Gleason, E. Wilson, JD Humphrey, Biaxial biomechanical adaptations of mouse carotid arteries cultured at altered axial extension // J. Biomech. - 2007. - #40. - R. 766–776.

13. ZS Jackson, AI Gotlieb, BL Langille, Wall tissue remodeling regulates longitudinal tension in arteries // Circ. Res. - 2002. - #90. - R. 918–925.

14. G. Martufi, M. Lindquist Liljeqvist, N. Sakalihasan, G. Panuccio, R. Hultgren, J. Roy, TC Gasser, Local diameter, wall stress and thrombus thickness influence the local growth of abdominal aortic aneurysms // J. Endovas. Ther. – 2016. - #23. - R. 957–966

15. A. Nchimi, J.-P. Cheramy-Bien, TC Gasser, G. Namur, P. Gomez, A. Albert, L. Seidel, JO Defraigne, N. Labropoulos, N. Sakalihasan, Multifactorial relationship between 18Ffluoro-deoxy-glucose positron emission tomography signaling and biomechanical properties in unruptured aortic aneurysms // Circ. Cardiovasc. Imaging. - 2014. - #7. - R. 82–91.