УДК 575.112:004.94
https://doi.org/10.20538/1682-0363-2026-2-115-120

Computer Simulation of Xanthotoxin Interaction with Caspase 3

Chasovskikh N.Yu., Shestakova E.E., Novitsky D.E.

Siberian State Medical University
2 Moskovsky trakt, 634050 Tomsk, Russian Federation

ABSTRACT

Aim. To perform computer modeling and evaluate the interactions between caspase 3 and xanthotoxin.

Materials and methods. The structures of caspase 3 and xanthotoxin were extracted from the PDB protein database (ID 2J31) and the PubChem chemical compounds and mixtures database (ID 4114), respectively. PyMOL v. 2.5 was used to prepare the caspase 3 structure for docking, and OpenBabel was used to convert xanthotoxin to .pdb format. Molecular docking was performed in AutoDock Vina. Molecular dynamics was performed using GROMACS 2023.3. The CHARMM 36 force field was used to generate the protein topology, and the CHARMM General Force Field (CGenFF) server was used for the ligand topology. Xmgrace and PyMOL were used to analyze the results.

Results. Molecular docking of caspase 3 and xanthotoxin resulted in a stable complex with binding energy of -4.83 kcal/mol. The amino acid residues of caspase 3 involved in intermolecular interaction were identified: tyrosine 195 (TYR195), valine 266 (VAL266), lysine 137 (LYS137). The results of the molecular dynamics simulation confirmed the stability of the complex obtained as a result of docking.

Conclusion. The data obtained indicate the possible role of the studied furocoumarin in the implementation of programmed cell death and can be used for further experimental studies.

Keywords: docking, molecular dynamics, caspase 3, xanthotoxin

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors declare that they received no funding for the study.

For citation: Chasovskikh N.Yu., Shestakova E.E., Novitsky D.E. Computer Simulation of Xanthotoxin Interaction with Caspase 3. *Bulletin of Siberian Medicine.* 2026;25(2):115–120. https://doi.org/10.20538/1682-0363-2026-2-115-120.

Компьютерное моделирование взаимодействия ксантотоксина с каспазой 3

Часовских Н.Ю., Шестакова Е.Е., Новицкий Д.Е.

Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель – провести компьютерное моделирование и оценить взаимодействие каспазы 3 с ксантотоксином.

Материалы и методы. Структуры каспазы 3 и ксантотоксина извлечены из банка данных белков PDB (ID 2J31) и базы данных химических соединений и смесей PubChem (ID 4114) соответственно. Для подготовки структуры каспазы 3 к докингу применяли PyMOL v. 2.5, для конвертации ксантотоксина в формат .pdb использовали OpenBabel. Докинг проводили в AutoDock Vina. Молекулярно-динамическое моделирование выполняли с использованием GROMACS 2023.3. Для генерации топологии белка использовали силовое поле CHARMM 36, для топологии лиганда – сервер CHARMM General Force Field (CGenFF). Для анализа результатов применяли Xmgrace и PyMOL.

✉ *Shestakova Evgeniia E.,* evgenika06@gmail.com

Результаты. В результате проведенного молекулярного докинга каспазы 3 и ксантотоксина получен стабильный комплекс с энергией связывания –4,83 ккал/моль. Выявлены аминокислотные остатки каспазы 3, участвующие в межмолекулярном взаимодействии: тирозин 195 (TYR195), валин 266 (VAL266), лизин 137 (LYS137). Результаты проведенной симуляции молекулярной динамики подтвердили устойчивость комплекса, полученного в результате докинга.

Заключение. Полученные данные свидетельствуют о возможной роли изучаемого фурокумарина в реализации программируемой гибели клеток и могут быть использованы для дальнейших экспериментальных исследований.

Ключевые слова: докинг, молекулярная динамика, каспаза 3, ксантотоксин, апоптоз

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Часовских Н.Ю., Шестакова Е.Е., Новицкий Д.Е. Компьютерное моделирование взаимодействия ксантотоксина с каспазой 3. *Бюллетень сибирской медицины.* 2026;25(2):115–120. https://doi.org/10.20538/1682-0363-2026-2-115-120.

INTRODUCTION

Socially sensitive diseases, particularly cancer, are the leading causes of disability and mortality in Russia. According to the Rosstat data for 2023, malignant tumors account for 16.4% of all deaths in Russia [1]. One of the key factors in the development of these pathologies is the dysregulation of apoptosis, which involves the activation or suppression of the cell death program [2].

Apoptosis is a form of cell death that eliminates excess or functionally abnormal cells to maintain tissue homeostasis in the body [3]. The main pathways that initiate apoptosis are the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway. Additionally, there are granzyme B-dependent, endoplasmic reticulum (ER) stress, perforin/granzyme, and nuclear apoptosis pathways. The induction of apoptosis and the activation of pro-apoptotic proteins trigger a cascade of caspases [4]. At the first stage of each pathway, an initiator caspase is activated (caspase 8 in the extrinsic pathway, caspase 9 in the intrinsic pathway, and caspase 10 in the perforin – granzyme pathway), which subsequently activates effector caspase 3. In the extrinsic (receptor) pathway, following the binding of a ligand to the corresponding receptor (a molecule from of the TNFα receptor superfamily) on the cell surface, a cascade of intracellular reactions is activated, resulting in the activation of caspases 8 and 10. These caspases then activate caspases 3, 6, and 7, which facilitate cell death [5].

In the intrinsic (mitochondrial) pathway, in response to stressors (such as DNA damage or a lack of growth factors), the mitochondrial protein p53 is activated. In turn, p53 suppresses Bcl-2 and activates Bax, whose dimeric form activates the serine protease Ich-1, leading to the induction of apoptosis and activation of effector caspases [5]. This process ultimately results in the cleavage of caspase 3, causing DNA fragmentation, degradation of cytoskeletal and nuclear proteins, formation of apoptotic bodies, and their subsequent engulfment by phagocytic cells [4].

Caspase 3 is one of the main effectors of apoptosis, making molecules that can modify its activity particularly interesting due to their potential therapeutic properties. Currently, furocoumarins, especially xanthotoxin (8-methoxypsoralen), are actively being studied as such molecules [6–8]. Xanthotoxin exhibits photosensitizing properties and can induce apoptosis in various tumor cell lines [9, 10]. It is hypothesized that xanthotoxin can activate caspase 3; however, the exact mechanism remains unknown [11, 12].

To predict and evaluate interactions between proteins and molecules with potential therapeutic properties (including those that can be valuable for studying apoptosis mechanisms), *in silico* methods are currently being used extensively [13, 14].

Given this context, the aim of this study was to conduct computer simulation and evaluate the interaction between caspase 3 and xanthotoxin.

MATERIALS AND METHODS

The experimental three-dimensional (3D) structure of caspase 3 (PDB identifier: 2J31) was extracted from the Protein Data Bank (PDB) [15]. The 3D structure of xanthotoxin (PubChem identifier: 4114) was retrieved from the PubChem Substance and Compound database [16].

The 3D structure of caspase 3 was cleaned of all water molecules and ligands, analyzed, and prepared for further simulation using PyMOL v. 2.5 [17].

The 3D structure of xanthotoxin from the PubChem database was retrieved in .sdf format and converted to .pdb format for molecular docking using the OpenBabel converter [18].

AutoDock Vina [19] was used to conduct the molecular docking of caspase 3 and xanthotoxin. AutoDock Vina employs an empirical and knowledge-based function to predict the binding affinity of compounds [19]. The dimensions of the calculation grid for caspase 3 were set to 25 x 25 x 25 Å, centered at the coordinates (31.0, 21.0, 55.0).

Molecular dynamics (MD) simulation of the caspase – xanthotoxin complex was performed using GROMACS 2023.3 [20]. The protein topology was generated using the CHARMM36 force field, while the ligand topology was created using the CHARMM General Force Field (CGenFF) server [21]. The complex was solvated in a cubic box with a dimension of 1.0 nanometer (nm) using the TIP3P water model, and neutralization was achieved with Cl^- ions. Energy minimization was conducted using the steepest descent method [22]. Equilibrium state simulations lasting 100 picoseconds (ps) were carried out using NVT (canonical ensemble, where N is the number of particles, V is the volume, and T is the temperature) and NPT (isothermal – isobaric ensemble, where N is the number of particles, P is the pressure, and T is the temperature) ensembles. The parameters for these ensembles were optimized for a temperature of 300 K and a pressure of 1 bar. The main phase of MD simulation was conducted over 50 nanoseconds (ns) with a time step of 2 femtoseconds (fs). The Xmgrace software was used to generate graphs illustrating the simulation results [23].

For further analysis of the obtained results, we evaluated the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) [24].

RESULTS AND DISCUSSION

Molecular docking of caspase 3 and xanthotoxin resulted in the most stable complex with a binding energy of –4.83 kcal/mol. The residues involved in intermolecular interaction were identified: tyrosine 195 (TYR195), valine 266 (VAL266), and lysine 137 (LYS137) (Fig. 1).

The obtained complex is stabilized due to several types of interactions: π-stacking (or π-π interactions) between the ligand and TYR195, π-cation interactions between the ligand and VAL266, and hydrophobic interactions between the ligand and both TYR195 and VAL266. π-stacking refers to the non-covalent interaction between aromatic systems (benzene rings) that occurs due to the overlap of their π-electron clouds. π-cation interactions represent a non-covalent bond between an aromatic system and a positively charged amino group of the protein. Hydrophobic interactions are characterized by the tendency of non-polar molecules to cluster together in an aqueous environment to minimize the contact area of their non-polar surfaces with water.

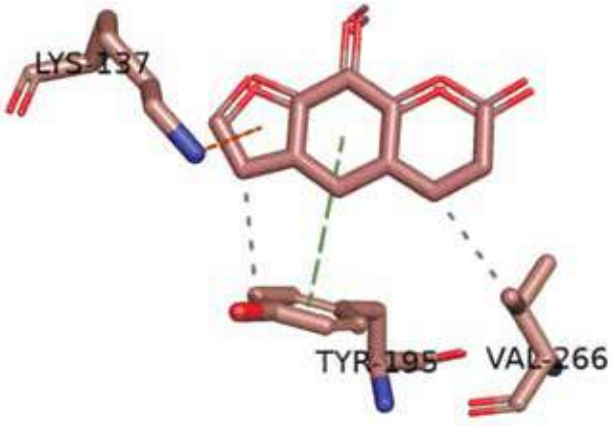

Fig. 1. This figure illustrates the amino acids of caspase 3 that are involved in interactions with xanthotoxin. Dashed lines indicate the following types of interactions: gray dashed lines represent hydrophobic interactions, green dashed lines indicate π-stacking, and orange dashed lines denote π-cation interactions

Subsequently, MD simulations were conducted, and their characteristics are described below.

RMSD is a commonly used metric for assessing the stability and conformational changes of a protein or protein – ligand complex during MD simulations. Constant RMSD values throughout the simulation process indicate that the initial conformation and structural stability of the system are maintained [25].

In our case, there was a sharp increase in RMSD during the first 10 ns, followed by values remaining within the range of 0.2–0.3 nm from 10 to 40 ns, indicating structural stability (Fig. 2).

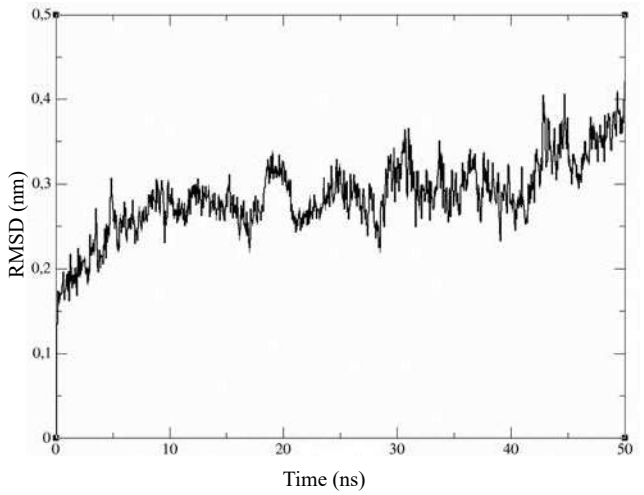

Fig. 2. The root mean square deviation of caspase 3 in complex with xanthotoxin

The RMSF metric is used to evaluate the mobility of amino acid residues in a protein and specific regions of the polypeptide chain. For the caspase 3 and xanthotoxin complex, the maximum RMSF value of 1.0 nm was observed in the first 30 residues, indicating high mobility in this region (Fig. 3). Beyond this point, RMSF values remained at around 0.05–0.15 nm, suggesting stability among these residues and indicating the presence of secondary structural elements of the protein (α-helices and β-sheets) in this zone. Peaks of approximately 0.3 nm were noted in the regions of amino acid residues 160–190, 200–220, and 250–270, indicating flexible loops.

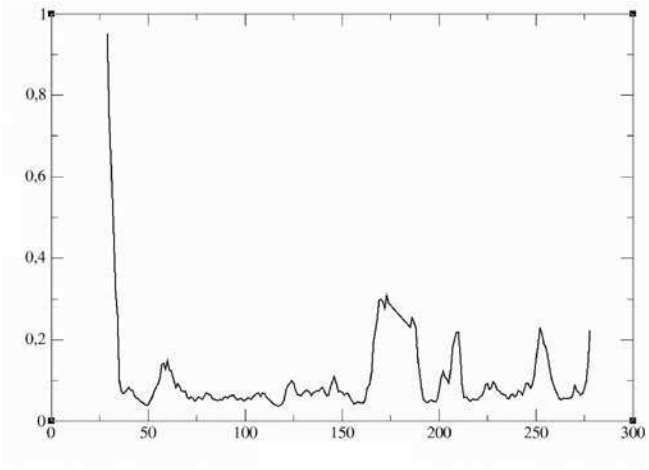

Fig. 3. Root mean square fluctuation (RMSF) of caspase 3

The radius of gyration is used to assess the compactness of the protein – ligand complex during simulation. A loss of compactness can impact the stability of the complex by introducing weak intermolecular interactions.

According to the findings, the radius of gyration ranged from 1.78 to 1.83 nm, indicating stability and compactness of the structure (Fig. 4). During the first 10 ns, the protein adopted a stable, compact form; the radius of gyration fluctuated between 1.74 and 1.78 nm without significant variations.

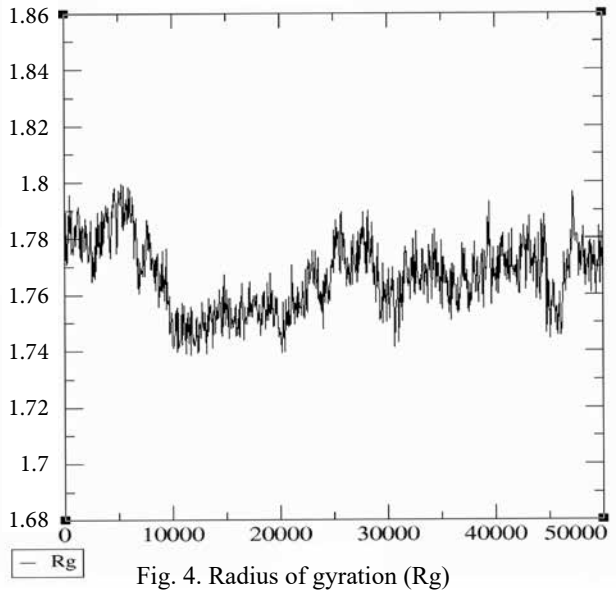

Fig. 4. Radius of gyration (Rg)

The findings suggest that a stable xanthotoxin – caspase 3 complex can form. It can be hypothesized that this interaction may alter the activity of caspase 3, which is involved in programmed cell death processes.

Experimental data indicate that programmed cell death in tumor cells is associated with the activation of caspase 3 in the presence of xanthotoxin. Caspase 3 is essential for transmitting apoptotic signals following exposure to DNA-damaging agents [26]. A study by G. Viola et al. shows that caspase 3 is activated after irradiation in the presence of xanthotoxin [12]. Additionally, apoptosis induction was noted in HepG2 cell line (a liver cancer cell line) after treatment with xanthotoxin [11]. However, the molecular mechanisms underlying this phenomenon remain unexplored.

CONCLUSION

The computer simulation of the interaction between caspase 3 and xanthotoxin, conducted through molecular docking followed by verification

using molecular dynamics demonstrated the presence of a stable intermolecular interaction. The resulting complex features a binding energy of -4.83 kcal/mol and several types of chemical bonds: π-stacking and π-π interactions between the ligand and TYR195, π-cation interactions between the ligand and VAL266, and hydrophobic interactions between the ligand and both TYR195 and VAL266. The results of molecular dynamics simulation confirmed the stability of the complex obtained from docking.

Thus, the findings of this study suggest that xanthotoxin has the potential to interact with caspase 3, forming a stable complex. These data indicate a possible role of the studied furocoumarin in the process of programmed cell death. Further experimental studies are necessary to validate the results of the computer simulation.

REFERENCES

1. Natural Movement of the Population of the Russian Federation for 2023 (Statistical Bulletin). Moscow: Rosstat, 2024. (In Russ.).
2. Glukhov A.I., Gryzunova G.K., Usay L.I., Aleynikova T.L., Chernikova N.V., Burt A.Yu. The Role of Apoptosis in the Pathogenesis of Some Critical Conditions. *General Reanimatology*. 2019;15(2):79-98. (In Russ.). DOI:10.15360/1813-9779-2019-2-79-98
3. Chasovskikh N.Yu. et al. Apoptosis and Oxidative Stress. Tomsk: Pechatnaya Manufaktura, 2009:148. (In Russ.).
4. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516. DOI: 10.1080/01926230701320337
5. Sokolov D.I. Immunological Mechanisms of Apoptosis in Placental Development. *Medical Immunology (Russia)*. 2008;10(2–3):125–138. (In Russ.). DOI:10.15789/1563-0625-2008-2-3-125-138
6. Fu K, Zhang J., Wang L., Zhao X., Luo Y. Xanthotoxin induced photoactivated toxicity, oxidative stress and cellular apoptosis in Caenorhabditis elegans under ultraviolet A. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*. 2022;251:109217. DOI: 10.1016/j.cbpc.2021.109217.
7. Hähnel V., Brosig A.M., Ehrenschwender M., Burkhardt R., Offner R., Ahrens N. Apoptosis induction by extracorporeal photopheresis is enhanced by increasing the 8-methoxypsoralen concentration and by replacing plasma with saline. *Transfusion*. 2021;61(10):2991-2999. DOI: 10.1111/trf.16634.
8. Abdel Hafez O.M., Amin K.M., Abdel-Latif N.A., Mohamed T.K., Ahmed E.Y., Maher T. Synthesis and antitumor activity of some new xanthotoxin derivatives. *Eur. J. Med. Chem*. 2009;44(7):2967–2974. DOI: 10.1016/j.ejmech.2009.01.006
9. Yang H., Xiong J., Luo W., Yang J., Xi T. 8-Methoxypsoralen Induces Intrinsic Apoptosis in HepG2 Cells: Involvement of Reactive Oxygen Species Generation and ERK1/2 Pathway Inhibition. *Cell Physiol. Biochem*. 2015;37(1):361–374. DOI: 10.1159/000430360.
10. Choi E.K., Kim H.D., Park E.J., Song S.Y., Phan T.T., Nam M. et al. 8-Methoxypsoralen Induces Apoptosis by Upregulating p53 and Inhibits Metastasis by Downregulating MMP-2 and MMP-9 in Human Gastric Cancer Cells. *Biomol. Ther. (Seoul)*. 2023;31(2):219-226. DOI: 10.4062/biomolther.2023.004.
11. Kurita M., Shimauchi T., Kobayashi M., Atarashi K., Mori K., Tokura Y. Induction of keratinocyte apoptosis by photosensitizing chemicals plus UVA. *J. Dermatol. Sci*. 2007;45(2):105-12. DOI: 10.1016/j.jdermsci.2006.10.010.
12. Viola G., Fortunato E., Cecconet L., Disarò S., Basso G. Induction of apoptosis in Jurkat cells by photoexcited psoralen derivatives: Implication of mitochondrial dysfunctions and caspases activation. *Toxicol. In Vitro*. 2007;21(2):211-6. DOI: 10.1016/j.tiv.2006.09.016.
13. Issa M.Y., Elshal M.F., Fathallah N., Abdelkawy M.A., Bishr M., Salama O. et al. Potential Anticancer Activity of the Furanocoumarin Derivative Xanthotoxin Isolated from Ammi majus L. Fruits: In Vitro and In Silico Studies. *Molecules*. 2022;27(3):943. DOI: 10.3390/molecules27030943.
14. Ismail N.Z., Mohamed W.A.S., Ab Rahim N., Hashim N.M., Adebayo I.A., Mohamad Zain N.N., Arsad H. Molecular docking and molecular dynamic simulations of apoptosis proteins with potential anticancer compounds present in Clinacanthus nutans extract using gas chromatography-mass spectrometry. *J. Biomol. Struct. Dyn*. 2023;41(13):6104-6120. DOI: 10.1080/07391102.2022.2101530.
15. Berman H.M., Westbrook J., Feng Z., Gilliland G., Bhat T.N., Weissig H. et al. The Protein Data Bank. *Nucleic. Acids. Res*. 2000;28(1):235–242. DOI: 10.1093/nar/28.1.235.
16. Kim S., Chen J., Cheng T., et al. PubChem 2025 update. *Nucleic Acids Res*. 2025;53(D1):D1516-D1525. DOI:10.1093/nar/gkae1059
17. Schrödinger L., DeLano W. PyMOL. 2020. URL: http://www.pymol.org/pymol
18. O'Boyle N.M., Banck M., James C.A., Morley C., Vandermeersch T., Hutchison G.R.. Open Babel: An open chemical toolbox. *J. Cheminform*. 2011;3:33. DOI: 10.1186/1758-2946-3-33.
19. Trott O., Olson A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*. 2010;31:455–461. DOI: 10.1002/jcc.21334.
20. Abraham, M.J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B. et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1–2:19–25. DOI: 10.1016/j.softx.2015.06.001.
21. Vanommeslaeghe K., Hatcher E., Acharya C. et al. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem*. 2010;31(4):671–690. DOI: 10.1002/jcc.21367.
22. Groningen Biomolecular Sciences and Biotechnology Institute. GROMACS 4.5 Manual. 2010.URL:https://becksteinlab.physics.asu.edu/pages/courses/2013/SimBioNano/14/gromacs_manual-4.5.pdf

23. Vaught A. Graphing with Gnuplot and Xmgr: Two graphing packages available under Linux. *Linux J*. 1996;1996:7.
24. Cheng X., Ivanov I. Molecular dynamics. *Methods Mol. Biol*. 2012;929:243–285. DOI: 10.1007/978-1-62703-050-2_11.
25. Liu K., Watanabe E., Kokubo H. Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations. *J. Comput. Aided Mol. Des.* 2017;31(2):201–211. DOI: 10.1007/s10822-016-0005-2.
26. Oleinick N.L., Morris R.L., Belichenko I. The role of apoptosis in response to photodynamic therapy: what, where, why, and how. *Photochem. Photobiol. Sci*. 2002;1(1):1–21. DOI: 10.1039/B108586G.

Author Information

Chasovskikh Nataliya Yu. – Dr. Sci. (Med.), Associate Professor, Head of the Medical and Biological Cybernetics Division, Siberian State Medical University, Tomsk, chasovskih.ny@ssmu.ru, https://orcid.org/0000-0001-6077-0347

Shestakova Evgeniia E. – Assistant of the Medical and Biological Cybernetics Division, Siberian State Medical University, Tomsk, chizik.ee@ssmu.ru, http://orcid.org/ 0000-0003-4731-8449

Novitsky Dmitry E. – 6th-year Student, Siberian State Medical University, Tomsk, novitskydima07@mail.ru

(✉) **Shestakova Evgeniia E.,** chizik.ee@ssmu.ru

Received on June 23, 2025;
approved after peer review on July 4, 2025;
accepted on September 09, 2025