GÜSBD 2023; 12(3): 910 - 917 | **Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi** | **Araştırma Makalesi**
GUJHS 2023; 12(3): 910 – 917 | Gümüşhane University Journal of Health Sciences | Original Article

In Silico Binding Affinities of the Molecules in Camellia Sinensis Teas to NLRP3 NACHT Domain

Camellia Sinensis Çaylarındaki Moleküllerin NLRP3 NACHT Domainine In Siliko Bağlanma Afiniteleri

Zekeriya DÜZGÜN[1], Birgül Vanizor KURAL[2]

ABSTRACT

NLRP3 inflammasome secretes proinflammatory cytokines in response to microbial infection and cellular damage, induces pyroptotic cell death, and triggers many pathological conditions. For this reason, it is important to determine the products that can inhibit the NLRP3 protein. In this study, the affinities of 27 molecules in *Camellia sinensis* tea species to ADP and inhibitor cavities in the NACHT domain of NLRP3 were analyzed *in silico* using molecular docking, molecular dynamics simulation, and free energy calculation method MM/GBSA. Among the components, theaflavic acid, (-)-epicatechin gallate and (-)-epigallocatechin gallate gave better binding affinities. It was concluded that it would be beneficial to conduct advanced studies on whether these three compounds contribute to the preventability of NLRP3-mediated inflammatory diseases.

Keywords: (-)-Epicatechin gallate, (-)-Epigallocatechin gallate, NLRP3, NACHT, Theaflavic acid,

ÖZ

NLRP3 inflamazomu, mikrobiyal enfeksiyona ve hücresel hasara yanıt olarak proinflamatuar sitokinleri salgılar, piroptotik hücre ölümüne neden olur ve birçok patolojik durumu tetikler. Bu nedenle NLRP3 proteinini inhibe edebilen ürünlerin belirlenmesi önemlidir. Bu çalışmada, *Camellia sinensis* çay türünde bulunan 27 molekülün, NLRP3'ün NACHT domainindeki ADP ve inhibitör kavitelere afiniteleri, moleküler yerleştirme, moleküler dinamik simülasyonu ve serbest enerji hesaplama yöntemi MM/GBSA kullanılarak *in siliko* olarak analiz edildi. Bileşenler arasında tiflavik asit, (-)-epikateşin gallat ve (-)-epigallokateşin gallat daha iyi bağlanma afiniteleri verdi. Bu üç bileşiğin NLRP3 aracılı inflamatuvar hastalıkların önlenebilirliğine katkı sağlayıp sağlamadığına yönelik ileri çalışmaların yapılmasının faydalı olacağı kanısına varıldı.

Anahtar Kelimeler: (-)-Epikateşin gallat, (-)-Epigallokateşin gallat, NLRP3, NACHT, Tiflavik asit

The numerical calculations expressed in this paper were performed at TUBITAK ULAKBIM, High Performance, and Grid Computing Center (TRUBA resources).

[1] Dr. Öğr. Üyesi Zekeriya DÜZGÜN, Medical Biology, Giresun University, Department of Medical Biology, zekeriya.duzgun@giresun.edu.tr, 0000-0001-6420-6292
[2] Prof. Dr. Birgül Vanizor KURAL, Medical Biochemistry, Karadeniz Technical University, Department of Medical Biochemistry, bvanizorkural@ktu.edu.tr, 0000-0003-0730-9660

İletişim / Corresponding Author: Birgül Vanizor KURAL
e-posta/e-mail: bvanizorkural@ktu.edu.tr

Geliş Tarihi / Received: 30.03.2023
Kabul Tarihi/Accepted: 20.09.2023

INTRODUCTION

The nucleotide-binding oligomerization domain (NOD, also known as NACHT), leucin rich repeated (LRR), and pyrin domain (PYD)-containing protein 3 (NOD-like receptor family pyrin domain containing 3 or NLRP3) is a cytoplasmic multidomain protein that its inflammasome functions in the immune system and thereby contribute in pathogenesis of inflammatory disorders including neurodegenerative diseases (i.e. Alzheimer's disease), cardiovascular diseases (i.e. atherosclerosis), metabolic diseases (i.e. diabetes) and cancers (i.e. lung cancer).[1-3] The level of NLRP3 is not sufficient to activate the inflammasome in normal cells.[4] Following infection or tissue injury, the LRR domain interacts with the NACHT domain containing the ATP binding site, promoting NLRP3 to oligomerize.[5] The NLRP3 inflammasome is composed of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1. Inhibition of NLRP3 protein or NLRP3 inflammasome will contribute to the prevention or treatment of inflammatory diseases by reducing the clinical process.[3] The Researches into its effective inhibitors is ongoing.

According to the manufacturing process, different types of *Camellia sinensis* plant teas as white tea, yellow tea, green tea, black tea, dark tea, and oolong tea can be produced.[6] These teas (mainly green tea) are widely preferred and consumed non-alcoholic beverages by the world's population because of their attractive tastes, aromas and beneficial effects on health, thanks to containing phytochemicals such as catechins, theaflavins, thearubigins, caffeine, amino acids, vitamins, polysaccharides (Table 1).[6-8] Inhibitory effects of some of these tea components such as caffeine[1,9-11], theophylline[12], (-)-epicatechin[13], epigallocatechin-3-gallate[14], quercetin[4,15], on NLRP3 inflammasome have been reported before.[1,4,9-11,12-15]

The present study aimed to identify the affinities of important tea components to the NLRP3 NACHT domain by an *in silico* study, and to compare them. Thus, the identification of possible effective inhibitors may be shed light on further in vivo and in vitro studies on NLRP3-related diseases.

METHOD

Homology Modelling and Validation

The 7ALV-encoded XRAY crystal structure of the human NLRP3 NACHT Domain was obtained from the Protein DATA Bank, 153-163th, 178-200th, 213-216th, 452-462th, 496-497th, 513-515th, 539-554th and 589th non-terminal missing residues were remodeled using the MODELS 10.2 tool in the Chimera software with reference to the human NLRP3 NACHT domain sequence.[16,17] Structural quality after modeling and minimization was evaluated with ERRAT and ProSA. Overall Quality Factor was 91.6179 according to ERRAT and Z-Score: -8.72 according to ProSA. These scores are included in the quality standard determined by the tools.[18,19]

Molecular Docking

Autodock Vina 1.2 software was preferred as the molecular docking algorithm to investigate the binding interaction of 27 tea components on the human NLRP3 NACHT domain.[20] Molecules found in high amounts in the teas[6-8] were included in the present study. The structures of these compounds were obtained from the PubChem database.[21] Optimization of the compounds and automation of the docking process was performed with the POAP tool.[22,23] In the optimization phase of the compounds, the obconformer method with 100 minimization steps was used to transition the compounds in the 2d structure to the 3d structure.[22] For the minimization of the compounds, the 1000-step MMFF94 force field algorithm was preferred according to the steepest-descent

algorithm.[24] Interactions and 2d plots were visualized with UCSF ChimeraX and BIOVIA Discovery Studio Visualizer 2021 San Diego: Dassault Systèmes.[25]

Molecular Dynamic Simulations

Using Vina, a molecular docking tool, protein-compound complexes were generated separately for both ADP and inhibitor binding sites of the NLRP3 NACHT Domain.[20] Molecular docked tea compounds and NLRP3 NACHT Domain were combined using Chimera software, The topological data of the compound conformations obtained after docking was generated with the ACPYPE tool.[26,27] The AM1-BCC quasi-experimental quantum computing method with the ACPYPE tool was preferred.[28] All simulations were run with GROMACS 2021.2 software.[29] "Leap Frog" integration and a 2-fs time step were applied in all simulations. The Amber99SB-ildn force field and the "TIP3P" water model were used.[30] A system in the form of "Rhombic Dodecahedron" was created under periodic boundary conditions (PBC).[31] Neutralization of the system was carried out with 0.15 mM Na-Cl. The size of the system was adjusted to be at least 1.2 nm from the corner of the protein-compound complex. Energy minimization was carried out by the steepest descent algorithm with 50,000 steps. In the system equilibrium stage, NVT and NPT simulations were performed respectively. The NVT stage was run at 300 ps and the NPT stage at 1000 ps. In the NVT stage, bonds and atoms were restricted, and in the NPT stage, only the bonds were restricted with the LINCS restriction algorithm.[32] In the NVT stage, Berendsen termostat was preferred as the temperature coupling algorithm and the temperature was set to 310 K, and as the pressure coupling algorithm in the NPT stage.[33] In the NPT stage, the V-rescale temperature coupling algorithm was used. The pressure was set to one atmosphere. In the production phase, V-rescale was used as the temperature coupling algorithm and Parrinello-Rahman with isothermal compressibility was used as the pressure matching algorithm.[34] The simulation length was determined as 100 ns and the time step was 2fs. Verlet was used as the cutting scheme. All restrictions were removed during the production phase. The "Particle-mesh-Ewald" algorithm was preferred for long-range electrostatic interactions.[35] The cut-off value was determined as 10 Å in the Van Der Waals interactions. All MD simulations were performed in TUBITAK TRUBA clusters.

Binding Free Energy Calculations

After investigating the binding interaction of 42 tea components and the human NLRP3 NACHT domain by molecular docking method, the binding free energy was calculated according to the MM/GBSA approach for compounds that scored better than ADP and inhibitor (1-[4-chloranyl-2,6-di(propane-2-yl)phenyl]-3-[4-(2-oxidanylpropan-2-yl) furan-2-yl] sulfonyl-urea)).[36] MM/GBSA calculations were made with the gmx_MMPBSA tool.[37] Binding free energies were calculated by making 100 measurements at 100 ps intervals during the last 10 ns of the 100 ns MD simulation. In the vacuum electrostatic calculation, the dissolved dielectric constant was calculated as 2, the salt concentration was 0.15 M, and the temperature was 310 K. Entropy was not included in the calculation due to the high computational cost.

RESULTS AND DISCUSSION

Molecular Docking

The molecular docking method was applied to investigate the interaction between ADP and inhibitor binding sites of the human NLRP3 NACHT domain and 27 tea compounds. The score was calculated as -10.1 as the ADP compound was re-docked at the ADP binding site. In the molecular docking study with other compounds, it was observed that 3 compounds scored better than ADP. These compounds are theaflavic acid, (-)-Epicatechin gallate and (-)-epigallocatechin gallate. Their scores were calculated as -11.1,

-10.6, and -10.6 respectively. The other 24 compounds scored lower (Table 1).

The ADP RMSD value was calculated as 1.390.[38] In examination of the interaction of epicatechin gallate and protein-ADP cavity, it was observed that Glu152 and Gln509 made hydrogen bonds with the phenyl ring of the compound. It was determined that Pro412, Leu413, and Ile151 made pi-alkyl bonds, while Trp416 made pi-pi bonds. When the interaction of epigallocatechin gallate protein-ADP cavity was considered, it was observed that Gln509 made hydrogen bonds with the phenyl ring at two sites, while Ile521 made a single hydrogen bond. In addition, Ile151 and Leu413 showed pi-Alkyl interaction while Pro412 and Trp416 interacted with pi-pi. When the theaflavic acid protein-ADP cavity interaction was analyzed, it was observed that Gln509, Arg262, Ile230, and Gly229 formed hydrogen bonds, while Leu413 and Ile234 formed pi-alkyl bonds (Figure 1).

The inhibitory re-docking score in the inhibitor binding domain of the NLRP3-NACHT was calculated as 0.705. The inhibitor interacted with the protein inhibitor domain by hydrogen bonding in Arg578, Glu629, and Gln624, and by many pi-alkyl bonds (Figure 2). Epicatechin gallate interacted with Ala227, Ser626, Gln624, and Arg578 via hydrogen bonding and with Pro352 and Leu628 via pi-alkyl bond in the protein inhibitor cavity. It was seen that Tyr632 showed pi-pi interaction. As the interaction of epigallocatechin gallate protein inhibitor binding cavity was examined, it was observed that Glu629, Arg578, and Ala228 made hydrogen bonds, while Pro352, Val353, Ile411 and Ala227 made pi-alkyl bonds. It was determined that theaflavic acid interacted with Asp662, Glu629 and Arg351 through hydrogen bonding, and with Phe575, Val414, Ile411 and Ala227 via pi-alkyl bonds in the inhibitor cavity (Figure 2).

Table 1. Docking scores of human NLRP3 NACHT domain ADP and inhibitor binding sites with tea compounds.

Ligand Name	PubChem CID	Score for ADP cavity (kcal/mol)	Score for inhibitor cavity (kcal/mol)
Theaflavic acid	6178836	-11.1	-9.3
(-)-Epicatechin gallate	107905	-10.6	-9.5
(-)-Epigallocatechin gallate	65064	-10.6	-9.2
ADP.ligand		-10.1	-8.4
(-)-Theaflavin	169167	-10.1	-2.0
Inhibitor.ligand		-9.6	-9.4
Quercetin	5280343	-9.6	-8.6
Myricetin	5281672	-9.4	-8.3
Kaempferol	5280863	-9.1	-8.0
Chlorogenic acid	1794427	-9.0	-8.5
(-)-Epicatechin	72276	-8.8	-8.1
(+)-Catechin	9064	-8.7	-8.1
(-)-Epigallocatechin	72277	-8.7	-8.0
(-)-Catechin	73160	-8.6	-8.1
Theaflavin-3,3'-digallate	21146795	-8.1	9.8
Coumarin	323	-6.9	-5.6
Caffeine	2519	-6.7	-5.7
Theobromine	5429	-6.5	-5.6
Theophylline	2153	-6.5	-5.6
Theaflavin_3-gallate	136825044	-6.4	-8.2
Theaflavin-3'-O-gallate	71307578	-6.4	-1.8
Gallic_acid	370	-6.3	-5.7
Thearubigin	76182283	-6.2	1.0
L-Theanine	439378	-5.6	-5.2
DL-Glutamine	738	-5.5	-4.5
Glutamic acid	33032	-5.4	-4.6
DL-Histidine	773	-5.3	-4.8
L-Glutamic acid	33032	-5.3	-4.5
DL-Asparagine	236	-5.1	-4.3

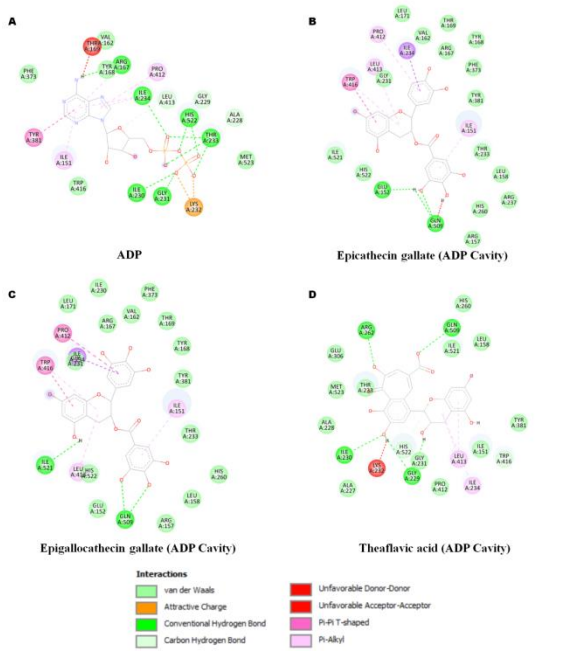

Fig. 1 2D diagrams of human NLRP3 NACHT domain ADP cavity residues interactions with compounds after molecular docking.

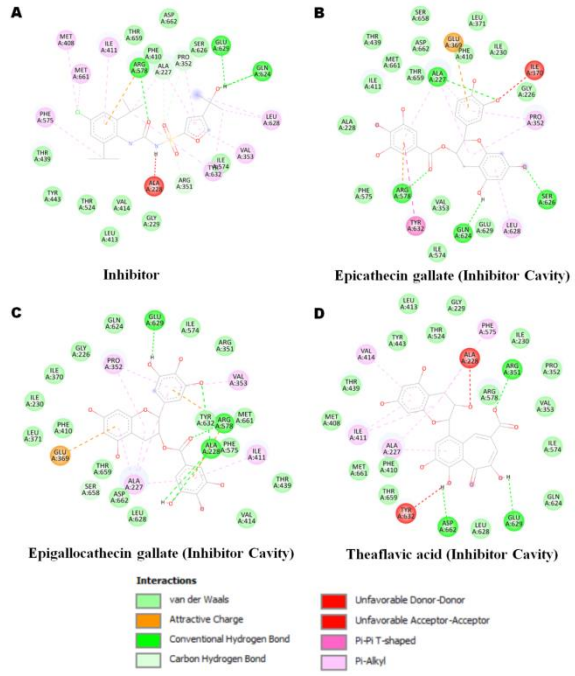

Fig. 2 2D diagrams of the interaction of human NLRP3 NACHT domain inhibitor cavity residues with compounds after molecular docking

Molecular Dynamics Simulation

After molecular docking calculations, 100 ns MD simulations of theaflavic acid, (-)-epicatechin gallate and (-)-epigallocatechin gallate complexes, which had better scores than ADP and inhibitor, were compared with ADP and inhibitor. As the RMSD results in Figure 3 were examined, it was observed that ADP and the inhibitor were very stably bound to the cavity as expected, small conformational fluctuations occurred in ADP and the conformational structure of the protein remained stable. According to the RMSD graph evaluations, in the theaflavic acid-ADP cavity interaction, the conformation of the compound remained constant under 2 Angstroms, but in the interaction of the compound with the Inhibitor cavity, the stability was below 1 angstrom. On the other hand, protein stability was impaired in the ADP cavity interaction. In addition, a slight fluctuation was observed when approaching the 100th ns in the inhibitor cavity interaction. In the RMSD plot of the interaction of epicatechin gallate with ADP and the inhibitory cavity, the conformation of the compound remained stable around 2 Angstroms in both cavities. On the other hand, the protein conformation fluctuated after 85 ns in the simulation where it was bound by the ADP cavity. No drastic fluctuation was observed in the protein in the interaction with the inhibitor cavity. However, the RMSD value gradually increased with time. As the RMSD graph of the interaction of epigallocatechin gallate with ADP and the inhibitor cavity was examined, it was observed that the compound did not remain stable in the ADP cavity and the protein stability was also impaired. In the inhibitor cavity, on the other hand, protein stability was achieved after 45 ns and the conformational stability of the compound was very good (Figure 3).

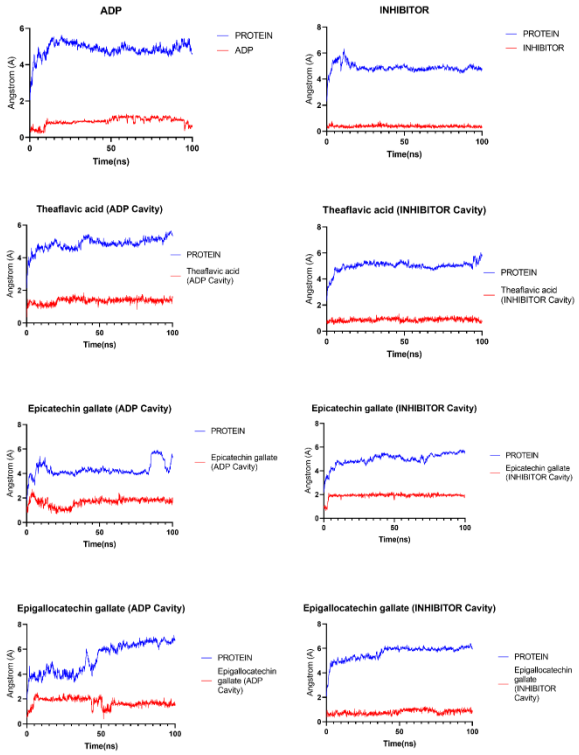

Fig. 3 RMSD plots of the interactions of selected compounds with ADP and inhibitor cavities.

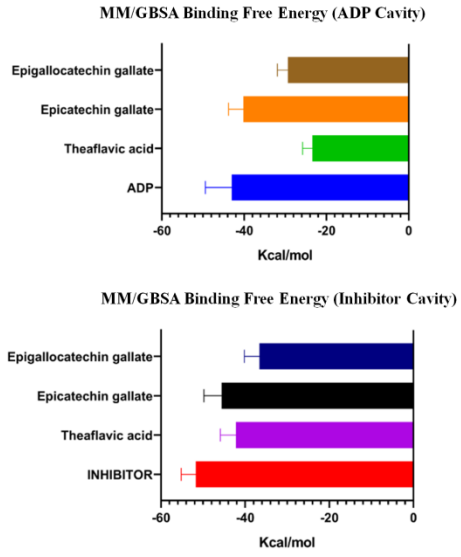

Fig. 4 Binding free energies of selected compounds on human NLRP3 NACHT Domain in comparison with ADP and inhibitor.

Binding Free Energy

After the molecular docking calculations, the binding free energies of theaflavic_acid, (-)-epicatechin gallate and (-)-epigallocatechin gallate, which are 3 compounds with better scores than ADP and inhibitor, were calculated by MM/GBSA method in comparison with ADP and inhibitor.

As seen in Figure 4, the binding free energies of the inhibitor and ADP to their cavities were calculated as -51.71 and -43.03 kcal/mol, respectively, as the best binding compounds. As the binding free energies of the other compounds were calculated, it was seen that epicatechin gallate stood out as the best binding compound in both cavities.

In the present study, the affinities of 27 molecules, found in the teas produced from *Camellia sinensis*, to ADP and inhibitor cavities in the NACHT domain of NLRP3 were determined by using molecular docking, molecular dynamics simulation and free energy calculation methods.

Of these molecules, theaflavic acid showed highest affinity but DL-asparagine lowest. When we compare the tea-specific molecules, theaflavin acid, (-)-epicatechin gallate, (-)-epigallocatechin gallate and (-)-theaflavin exhibited high affinities, while theobromine, theophylline, theaflavin_3-gallate, theaflavin-3'-O-gallate, thearubigin and L-theanine showed low affinities. The binding scores of (-)-epicatechin, (+)-catechin, (-)-epigallocatechin, (-)-catechin and theaflavin-3,3'-digallate were moderate. As far as we researched there was no study on the effect of theaflavic acid on NLRP3 protein.

Although there are limited studies on the effects of a few of these molecules on NLRP3, some have been focused on. Most studies on tea components' effect on the NLRP3 inflammasome have been related to epigallocathecin gallate. Jena et al.[14] found the binding affinity of epigallocatechin-3-gallate to NLRP3 as -9.6 by the interactions with van der Waals in Ala165, Ser120, Glu228, Lys163, Tyr202, Glu1005, Ile123, Ser234,

Gln233, Tyr237, Val1029, Leu1001, Gly1002, Ser973, Leu1003, Arg918, Leu974, Glu945, Gly975, Tyr201 and Ser1033, with conventional in Val1028, Asn1000, Ser1004 and Pro1032, with hydrogen bonds in Asp947, with carbon-hydrogen bond in Pro164, with pi-pi T-shaped in PHE1030, with pi-alkyl Lys124 and Lys127 residues.

One study showed that epigallocatechin-3-gallate suppressed NLRP3 inflammasome activation.[39] Another study reported that epi-gallocatechin-3-gallate improved NLRP3 inflammasome activation in diabetic rats' kidneys. [40]

We found docking scores of NLRP3 NACHT domain ADP and inhibitor binding sites with (-)-epicatechin as 8.8 and 8.1 respectively. Supportingly, Wu et al.[13] reported the inhibition effect of this molecule on NLRP3 inflammasome in expreminetal-induced acute gouty arthritis. Although we found lower affinity for cathecin than ADP or inhibitör ligand, some authors reported that catechin inhibited the NLRP inflammasome.[4,9] Inhibitory effect of caffeine on NLRP3 inflammasome was also reported by some researchers.[10,11]

CONCLUSION AND RECOMMENDATIONS

According to this *in silico* study that investigates the interactions of some molecules of the drinking teas (such as black and green teas) with the NLRP3 NACTH domain, theaflavic acid, (-)-epicatechin gallate and (-)-epigallocatechin gallate gave better binding scores than ADP and inhibitor. Therefore, it can be said that these components may be effective to suppress NLRP3 inflammasome activation. More studies are needed to be made about the effects of tea molecules on all domains of NLRP3 protein to identify inhibitor molecule which may be a drug candidate.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

The numerical calculations epxressed in this paper were performed at TUBITAK ULAKBIM, High Performance, and Grid Computing Center (TRUBA resources).

REFERENCES

1. Fan, F. S. (2022). "Inhibition of NLRP3 inflammasome activation by caffeine might be a potential mechanism to reduce the risk of squamous cell carcinoma of the oral cavity and oropharynx with coffee drinking". Frontiers in Oral Health, 3(1), 1-4.
2. Xu, J. and Núñez, G. (2022). "The NLRP3 inflammasome: activation and regulation". Trends in Biochemical Sciences, 48(4), 331-344.
3. Zheng, Y, Xu, L, Dong, N. and Li, F. (2022). "NLRP3 inflammasome: The rising star in cardiovascular diseases". Frontiers in Cardiovascular Medicine, 9(1), 1-20.
4. El-Sayed, S, Freeman, S. and Bryce, R. A. (2022). "A Selective Review and Virtual Screening Analysis of Natural Product Inhibitors of the NLRP3 Inflammasome". Molecules, 27(19), 6213.
5. Toldo, S, Mezzaroma, E, Buckley, L. F, Potere, N, Di Nisio, M, Biondi-Zoccai, G, Van Tassell, B. W. and Abbate, A. (2022). "Targeting the NLRP3 inflammasome in cardiovascular diseases". Pharmacology & therapeutics, 236(1), 1-20.
6. Zhao, T, Li, C, Wang, S. and Song, X. (2022). "Green tea (Camellia sinensis): A review of its phytochemistry, pharmacology, and toxicology". Molecules, 27(12), 3909.
7. Abudureheman, B, Yu, X, Fang, D. and Zhang, H. (2022). "Enzymatic oxidation of tea catechins and its mechanism". Molecules, 27(3), 942.
8. Bag, S, Mondal, A, Majumder, A. and Banik, A. (2022). "Tea and its phytochemicals: Hidden health benefits & modulation of signaling cascade by phytochemicals". Food Chemistry, 371(1), 1-13.
9. Jhang, J.-J, Lu, C.-C, Ho, C.-Y, Cheng, Y.-T. and Yen, G.-C. (2015). "Protective effects of catechin against monosodium urate-induced inflammation through the modulation of NLRP3 inflammasome activation". Journal of Agricultural and Food Chemistry, 63(33), 7343-7352.

10. Vargas-Pozada, E. E, Ramos-Tovar, E, Rodriguez-Callejas, J. D, Cardoso-Lezama, I, Galindo-Gómez, S, Talamás-Lara, D, Vásquez-Garzón, V. R, Arellanes-Robledo, J, Tsutsumi, V. and Villa-Treviño, S. (2022). "Caffeine inhibits NLRP3 inflammasome activation by downregulating TLR4/MAPK/NF-κB signaling pathway in an experimental NASH model". International Journal of Molecular Sciences, 23(17), 9954.

11. Zhao, W, Ma, L, Cai, C. and Gong, X. (2019). "Caffeine inhibits NLRP3 inflammasome activation by suppressing MAPK/NF-κB and A2aR signaling in LPS-Induced THP-1 macrophages". International Journal of Biological Sciences, 15(8), 1571.

12. Jiao, P, Li, W, Shen, L, Li, Y, Yu, L. and Liu, Z. (2020). "The protective effect of doxofylline against lipopolysaccharides (LPS)-induced activation of NLRP3 inflammasome is mediated by SIRT1 in human pulmonary bronchial epithelial cells". Artificial cells, nanomedicine, and biotechnology, 48(1), 687-694.

13. Wu, C, Li, F, Zhang, X, Xu, W, Wang, Y, Yao, Y, Han, Z. and Xia, D. (2022). "(−)-Epicatechin ameliorates monosodium urate-induced acute gouty arthritis through inhibiting NLRP3 inflammasome and the NF-κB signaling pathway". Frontiers in Pharmacology, 13

14. Jena, A. B, Dash, U. C. and Duttaroy, A. K. (2022). "An *in silic*o investigation on the interactions of curcumin and epigallocatechin-3-gallate with NLRP3 inflammasome complex". Biomedicine and Pharmacotherapy, 156113890.

15. El-Shaer, N. O, Hegazy, A. M. and Muhammad, M. H. (2023). "Protective effect of quercetin on pulmonary dysfunction in streptozotocin-induced diabetic rats via inhibition of NLRP3 signaling pathway". Environmental Science and Pollution Research1-9.

16. Burley, S. K, Bhikadiya, C, Bi, C, Bittrich, S, Chen, L, Crichlow, G. V, Christie, C. H, Dalenberg, K, Di Costanzo, L. and Duarte, J. M. (2021). "RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences". Nucleic Acids Research, 49(D1), 437-451.

17. Dekker, C, Mattes, H, Wright, M, Boettcher, A, Hinniger, A, Hughes, N, Kapps-Fouthier, S, Eder, J, Erbel, P. and Stiefl, N. (2021). "Crystal structure of NLRP3 NACHT domain with an inhibitor defines mechanism of inflammasome inhibition". Journal of Molecular Biology, 433(24), 167309.

18. Colovos, C. and Yeates, T. O. (1993). "Verification of protein structures: patterns of nonbonded atomic interactions". Protein Science, 2(9), 1511-1519.

19. Wiederstein, M. and Sippl, M. J. (2007). "ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins". Nucleic Acids Research, 35(suppl_2), W407-W410.

20. Trott, O. and Olson, A. J. (2010). "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading". Journal of Computational Chemistry, 31(2), 455-461.

21. Kim, S, Chen, J, Cheng, T, Gindulyte, A, He, J, He, S, Li, Q, Shoemaker, B. A, Thiessen, P. A. and Yu, B. (2023). "PubChem 2023 update". Nucleic Acids Research, 51(D1), D1373-D1380.

22. O'Boyle, N. M, Banck, M, James, C. A, Morley, C, Vandermeersch, T. and Hutchison, G. R. (2011). "Open Babel: An open chemical toolbox". Journal of Cheminformatics, 3(1), 1-14.

23. Samdani, A. and Vetrivel, U. (2018). "POAP: A GNU parallel based multithreaded pipeline of open babel and AutoDock suite for boosted high throughput virtual screening". Computational Biology and Chemistry, 74, 39-48.

24. Halgren, T. A. (1996). "Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94". Journal of Computational Chemistry, 17(5-6), 490-519.

25. Pettersen, E. F, Goddard, T. D, Huang, C. C, Meng, E. C, Couch, G. S, Croll, T. I, Morris, J. H. and Ferrin, T. E. (2021). "UCSF ChimeraX: Structure visualization for researchers, educators, and developers". Protein science, 30(1), 70-82.

26. Pettersen, E. F, Goddard, T. D, Huang, C. C, Couch, G. S, Greenblatt, D. M, Meng, E. C. and Ferrin, T. E. (2004). "UCSF Chimera-A visualization system for exploratory research and analysis". Journal of Computational Chemistry, 25(13), 1605-1612.

27. Sousa da Silva, A. W. and Vranken, W. F. (2012). "ACPYPE-Antechamber python parser interface". BMC Research Notes, 51-8.

28. Jakalian, A, Bush, B. L, Jack, D. B. and Bayly, C. I. (2000). "Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method". Journal of computational chemistry, 21(2), 132-146.

29. Abraham, M. J, Murtola, T, Schulz, R, Páll, S, Smith, J. C, Hess, B. and Lindahl, E. (2015). "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers". SoftwareX, 1, 119-25.

30. Lindorff-Larsen, K, Piana, S, Palmo, K, Maragakis, P, Klepeis, J. L, Dror, R. O. and Shaw, D. E. (2010). "Improved side-chain torsion potentials for the Amber ff99SB protein force field". Proteins: Structure, Function, and Bioinformatics, 78(8), 1950-1958.

31. Makov, G. and Payne, M. (1995). "Periodic boundary conditions in ab initio calculations". Physical Review B Condens Matter, 51(7), 4014-4022.

32. Hess, B, Bekker, H, Berendsen, H. J. and Fraaije, J. G. (1997). "LINCS: A linear constraint solver for molecular simulations". Journal of Computational Chemistry, 18(12), 1463-1472.

33. Berendsen, H. J, Postma, J. v, Van Gunsteren, W. F, DiNola, A. and Haak, J. R. (1984). "Molecular dynamics with coupling to an external bath". The Journal of chemical physics, 81(8), 3684-3690.

34. Martoňák, R, Laio, A. and Parrinello, M. (2003). "Predicting crystal structures: the Parrinello-Rahman method revisited". Physical Review Letters, 90(7), 075503.

35. Darden, T, York, D. and Pedersen, L. (1993). "Particle mesh Ewald: An N· log (N) method for Ewald sums in large systems". The Journal of chemical physics, 98(12), 10089-10092.

36. Genheden, S. and Ryde, U. (2015). "The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities". Expert Opinion on Drug Discovery, 10(5), 449-461.

37. Valdés-Tresanco, M. S, Valdés-Tresanco, M. E, Valiente, P. A. and Moreno, E. (2021). "gmx_MMPBSA: a new tool to perform end-state free energy calculations with GROMACS". Journal of Chemical Theory and Computation, 17(10), 6281-6291.

38. Bell, E. W. and Zhang, Y. (2019). "DockRMSD: an open-source tool for atom mapping and RMSD calculation of symmetric molecules through graph isomorphism". Journal of Cheminformatics, 11(1), 1-9.

39. Di, M, Zhang, Q, Wang, J, Xiao, X, Huang, J, Ma, Y, Yang, H. and Li, M. (2022). "Epigallocatechin-3-gallate (EGCG) attenuates inflammatory responses and oxidative stress in lipopolysaccharide (LPS)-induced endometritis via silent information regulator transcript-1 (SIRT1)/nucleotide oligomerization domain (NOD)-like receptor pyrin domain-containing 3 (NLRP3) pathway". Journal of Biochemical and Molecular Toxicology, 36(12), e23203.

40. Yang, R, Chen, J, Jia, Q, Yang, X. and Mehmood, S. (2022). "Epigallocatechin-3-gallate ameliorates renal endoplasmic reticulum stress-mediated inflammation in type 2 diabetic rats". Experimental Biology and Medicine, 247(16), 1410-1419.