EJMO

DOI: 10.14744/ejmo.2022.39682
EJMO 2022;6(3):251–257

Research Article

The Molecular Dynamics Effects of Rutin on CDKS 2, 4 and 6: In Silico Modelling and Molecular Dynamics

Majid Asadi-Samani,[1] Dhiya Altememy,[2] Javad Saffari-Chaleshtori,[3,*] Korosh Ashrafi-Dehkordi,[4,*] Fatemeh Asadi-Samani[5]

[1]Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Department of Pharmaceutics, College of Pharmacy, Al-Zahraa University for Women, Karbala, Iraq
[3]Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[5]Students Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract

Objectives: This simulated study has mechanistically evaluated the molecular dynamics effects of rutin on CDKs 2, 4, and 6 in cell cycling.

Methods: Protein Data Bank (http://www.rcsb.org) was used to obtain the PDB file of CDK 2, 4, and 6. After simulation of CDKs in Gromacs software, AutoDock 4.2 was used to run 200 stages of molecular docking of CDKs in the presence of the rutin. CDK 2, 4, and 6 were simulated in the presence of rutin after docking.

Results: Rutin had the highest tendency to bind the CDK-2 and CDK-6 via binding 16 and 18 residues in the binding site with hydrogen and hydrophobic bonds (respectively). Also, they had the highest amount of binding energy released. Rutin decreased total energy in CDKs and reduced the radius of gyration in CDK-2 and CDK-6 after docking. The secondary coil structure increased in CDK-2 and decreased in CDK-4 and 6.

Conclusion: Conformational changes in CDK2 and 6 via rutin can inhibit the activity of these proteins and subsequently arrest the cell cycle in phases G1, S, and G2, which can lead the damaged cell to cell repair or Apoptosis.

Keywords: Apoptosis, cell arresting, rutin, molecular dynamic, simulation

Cite This Article: *Samani MA, Altememy D, Chaleshtori JS, Dehkordi KA, Samani FA. The Molecular Dynamics Effects of Rutin on CDKS 2, 4 and 6: In Silico Modelling and Molecular Dynamics. EJMO 2022;6(3):251–257.*

Cell cycle dysregulation leads to aberrant cell proliferation, which is one of the critical hallmarks of cancer. Several factors control the cell cycle to ensure a regular and programmed cell division. One of the most important of these factors is cyclin-dependent kinases (CDKs), whose activity decreases during DNA damage, leading to a temporary cessation of the cell cycle so that the cell has the opportunity to repair and recover. But over activity of CDKs causes cells to grow and reproduce and, in many cases, become cancerous. Targeted inhibition of these regulatory proteins can prevent cell proliferation and tumour formation. Therefore, CDKs are considered attractive targets for the development of anticancer drugs. Most pan-CDK inhibitors, as first-generation CDK inhibitors, have indicated acceptable anticancer effects. However, they have not been approved for clinical (cancer) treatment due to their severe side effects and low specificity. In this regard, optimizing pan-CDK inhibitors or using natural inhibitors could cause some promises and more relevant clinical trials.[1] Studies indicated that flavonoids as natural compounds possessed

***Address for correspondence:** Javad Saffari-Chaleshtori and Korosh Ashrafi-Dehkordi, PhD. Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
Phone: +98 (383)3330061 **E-mail:** j_saffari@yahoo.com; kdashrafi@gmail.com
Submitted Date: August 06, 2022 **Accepted Date:** September 20, 2022 **Available Online Date:** October 16, 2022

inhibitory effects against CDKs and had good selectivity in various proliferative cell lines.[2,3]

Among flavonoids, rutin is a common dietary flavonoid that has been found in numerous foods, beverages, and vegetables. Rutin is also known as quercetin-3-O-rutinoside and vitamin P. To date, it has been reported in more than 70 plant species. Buckwheat from the Polygonaceae family has been introduced as a primary source of natural rutin.[4] Sophora japonica L. (Fabaceae), Eucalyptus spp. (Myrtaceae), and Ruta graveolens L. (Rutaceae) are other primary commercial sources of rutin.[5] In the US, over 860 products containing rutin are marketed. The Dietary Supplement Label Database lists over 860 products containing rutin currently marketed in the US.[6, 7] Various effects of rutin, including antimicrobial, antidiabetic, antioxidant, anti-inflammatory, anticancer, and neuroprotection effects, have been shown in several studies.[6, 8] Also, many studies indicated that it could act as a chemopreventive and chemotherapeutic agent. Its antitumor products are via the suppression of cell proliferation, the induction of autophagy or Apoptosis, and the prevention of metastasis and angiogenesis. Rutin could regulate various molecular targets involved in tumorigeneses, such as reactive oxygen species, mediators of the cell cycle, cellular kinases, transcription factors, inflammatory cytokines, and drug transporters.[9-12] In many human cancer cell lines, rutin has caused cell cycle arrest and apoptosis.[13] Also, Chen et al.[14] indicated that rutin could significantly inhibit the growth of LAN-5 cells via arresting the cell cycle in the G2/M phase and inducing cell apoptosis.

Despite various studies on the anticancer effects of rutin and its role in cell cycle arrest, the molecular pathways of its impact on the cell cycle and Apoptosis have not yet been known. This in silico modelling and molecular dynamics simulation study was designed to evaluate the molecular dynamic effects of rutin on the structure of CDKs 2, 4 and 6. We tried to determine the role of rutin on changes in the structure and function of these regulatory proteins via identification of the effective amino acids at the binding site of these proteins, their degree of affinity to the binding site, and also the number and hydrophobic and hydrogen bonds at the binding sites.

Methods

PDB Files Preparation

The CDK-2 (1AQ1), CDK-4 (2W9Z), and CDK-6 (ID: 5L2T) PDB files were extracted from the protein data bank server (www.rcsb.org) and optimized with Arguslab software after removing their inhibitors. Also, the rutin file (CID: 5280805) was extracted from the Pubchem server and optimized and converted to PDB files by Avogadro software V. 1.2.

Simulation and Molecular Dynamics (MD) of CDKs

Using the Gromacs software, studies on the molecular dynamics simulation of the CDK-2, CDK-4, and CDK-6 molecular structures were first performed in pure water. In this study, the SPC216 model and G43A1 force field were used to reach balance under the changes in temperature and pressure and at 140 mM by adding the calculated Na and Cl values.[15] In the following, the output PDB file is used as a molecular docking input structure to simulate complexes.

Molecular Docking

Molecular docking of rutin on CDK-2, CDK-4, and CDK-6 was done to determine the ligand-receptor's most stable free energy state and find the best binding sites for the ligand-receptor. In this study, we built a Grid Box with suitable (x×y×z nm) parameters (CDK2; 5.2×5.2×3.7 nm, CDK4; 4.6×4.6×3.3 nm, and CDK6; 3.3×3.3×2.3 nm) for each protein. After the production of PDBQ and PDBQT, the rutin file is considered a ligand, and CDKs considered a receptor. We used autogrid4 –p file.gpf –l n.gle Linux order to produce the file.gle text file. After 200 stages of molecular docking running on ligands, we used the Genetic Algorithm and Lamarckian GA parameters. For producing the file.dlg text file used the autodock4 –p n.dpf –l file.dlg Linux order. The data was obtained from the file.dlg files were analyzed.[16]

In this study, we used the LigPlot plus v.2.1 software to specify the number of hydrogen and hydrophobic bonds between CDKs and rutin. The number and type of amino acids present in the binding sites were identified.

Simulation and MD Studies of CDKs and Rutin as an Inhibitor

Following the above method, the simulation of the CDK-2, CDK-4, and CDK-6 protein complexes with rutin was performed in 140 mm of salt and water at the last stage of molecular dynamics simulation. As before, the paths stored in the simulation are used to analyze the structural parameters of the complex. The results of the simulation of the CDKs molecules alone in the absence of ligand and the simulation of the CDKs molecular complexes with rutin using the Grapher 10 software were comparatively analyzed.[17] In this simulation study, the temperature was set to 300K for all the simulation times.

Statistical Analysis

To compare data, statistical analysis was done by Independent Sample T-test. P-values lower than 0.05 were also considered statistically significant.

Results and Discussion

Docking results indicated that rutin in the concentration of 18.48 µM by releasing the 6.46 KJ/mol of BE has the highest tendency to interact with CDK2. While rutin in the concentration of 28.62 mM and releasing the 2.21 KJ/mol of BE has the lowest propensity to interact with CDK4 (Table 1).

The interaction bonds (Hydrogen bonding and hydrophobic bonding) between the residues of CDK 2, 4, and 6 docked with rutin have been shown in Figure 1. The most hydrogen bonds are between the CDK4 and rutin interaction. At the same time, the most hydrophobic bonds are between the CDK6 and rutin interaction. Table 1 and Figure 1 show that rutin has a high interaction binding with CDK4 and CDK6 in the G1 phase and CDK2 in the S and G2 phases of the cell cycle.

The amount of root-mean-square deviation (RMSD) in the simulation of CDK 2, 4, and 6 alone (black colour) and simulation of CDK 2, 4, and 6 in complex with rutin (orange colour) at 10 ns of simulation time has been shown in Figure 2. During the 10 ns of simulation time, the average of RMSD in CDK2-Rutin and CDK4-Rutin complexes have increased ($p<0.001$), while, at this simulation time, the standard of RMSD in CDK6-Rutin complex has decreased ($p<0.001$).

During the 10 ns of simulation time, the average of TE decreased significantly in CDKs that were docked by rutin in comparison with CDKs alone (Fig. 3). The significant decrease of TE after docking the rutin to CDKs shows the high interaction tendency of rutin with them.

During the 10 ns of simulation time, there are variations in RG, especially in CDK4 and CDK 4-Rutin complex. Nevertheless, the mean of Rg after docking the rutin to CDK2 and CDK6 decreased, while the standard of Rg after docking the rutin to CDK increased significantly ($p<0.001$) (Fig. 4).

Table 1. Molecular interaction and the binding energies of CDK 2, 4, and 6 docked with rutin

Receptor	BE kj/mol	FIE kj/mol	EIC	Interaction bonds	
				Hydrogen Bonds	Hydrophobic Bonds
CDK2-Rutin	6.46-	-11.23	18.48 µM	Gly11, Thr14, Glu81, Leu83, Asp145	Val18, Lys9, Ile10, Glu12, Val64, Phe82, Lys129, Gln131, Leu134, Ala144
CDK4-Rutin	-2.11	-6.88	28.62 mM	Glu744, Val710, Ser714, Tyr716, Arg706, Arg664	Met694, Leu703, Gln713, Val701
CDK6-Rutin	-5.26	-10.03	140.46 µM	Asp163, Ala167, Leu166	Glu21, Ala23, Arg44, Arg46, Gly22, Val45, Ala41, Lys43, Val27, Lys29, Gln149, Lys147

EIC: estimated inhibition constant; FIE: final intermolecular energy (kcal/mol); BE: Estimated Free Energy of binding (kcal/mol).

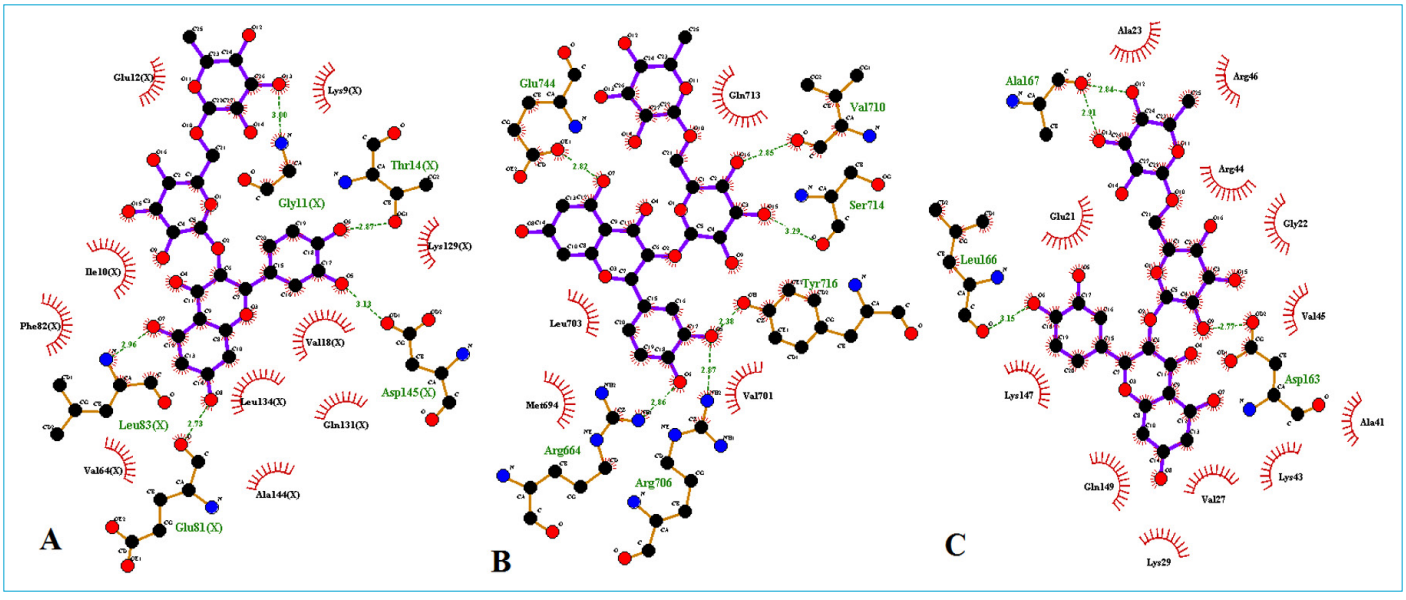

Figure 1. Analyzed protein–ligand interactions and hydrogen bonding and hydrophobic bonding between ligand-receptor **(a)**; CDK2-Rutin, **(b)**; CDK4-Rutin, and **(c)**; CDK6-Rutin.

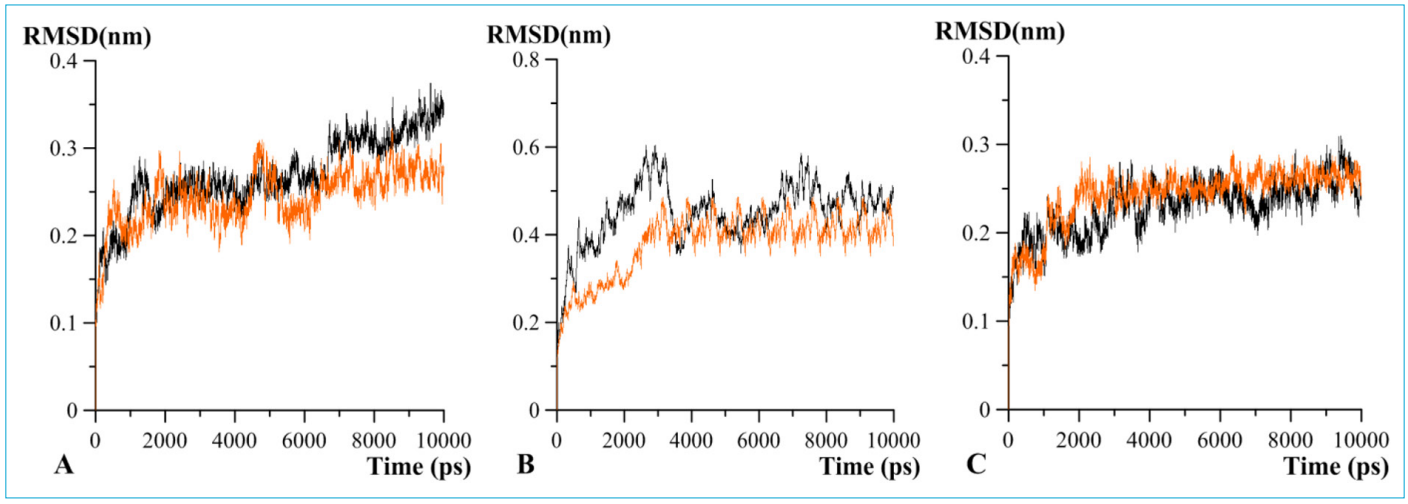

Figure 2. RMSD analysis of CDK 2, 4, and 6 alone (black color) and in complex with rutin (orange color). **(a)**; CDK2 and CDK2-Rutin, **(b)**; CDK4 and CDK4-Rutin, and **(c)**; CDK6 and CDK6-Rutin.

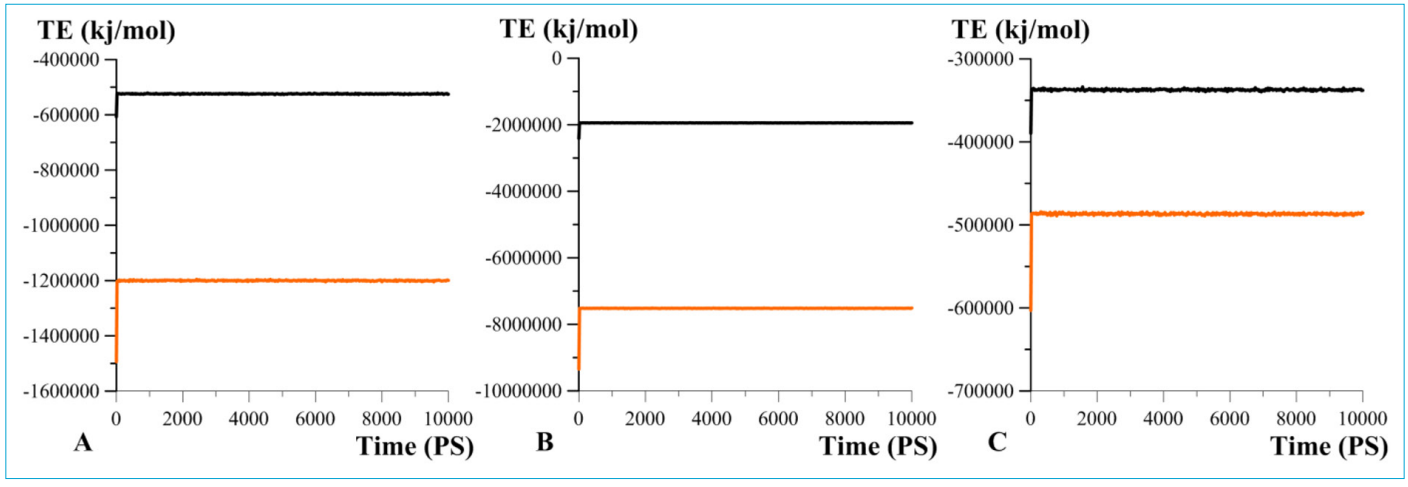

Figure 3. Total Energy (TE) of CDK 2, 4, and 6 alone (black color) and in complex with rutin (orange color). **(a)**; CDK2 and CDK2-Rutin, **(b)**; CDK4 and CDK4-Rutin, and **(c)**; CDK6 and CDK6-Rutin.

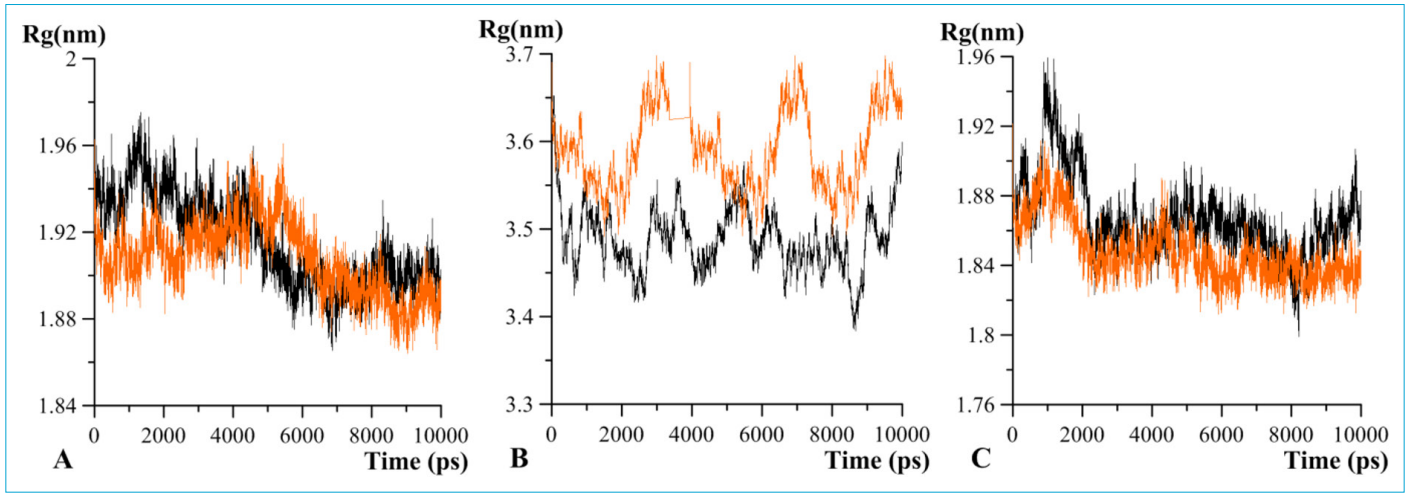

Figure 4. Radius of gyration (Rg) for CDK 2, 4, and 6 alone (black color) and in complex with rutin (orange color). **(a)**; CDK2 and CDK2-Rutin, **(b)**; CDK4 and CDK4-rutin, and **(c)**; CDK6 and CDK6-rutin.

Molecular dynamic parameters including TE, RG, and RMSD in the simulation of CDK 2, 4, and 6 alone (black colour) and simulation of CDK 2, 4, and 6 in complex with rutin (orange colour) at 10 ns of simulation have also been shown in table 2.

The evaluation of the secondary structure variations of CDK2, 4, and 6 alone in comparison with CDK2, 4, and 6 docked to rutin indicated that the secondary structures including α-Helix, β-Sheet, Coil, Turn, and Bend in the CDKs have been changed significantly ($p<0.001$) by docking the rutin (Table 3).

In this in silico modelling and molecular dynamics simulation study that was designed to mechanistically evaluate the molecular dynamics effects of rutin on CDKs 2, 4, and 6 in cell cycling, rutin as a natural active ingredient that produces antioxidant effects indicated a very high tendency for specific binding to CDKs. Rutin exhibited an increased tendency to bind to CDK2 by releasing 6.46 KJ/mol and attach to CDK6 by releasing 5.26 KJ/mol. Sunil et al by showed that some CDK2 inhibitors such as 3,5-diaminoindazoles, imidazo(1,2-b)pyridazines, and triazolo(1,5-a) pyridazines can bind to CDK2 by releasing the various amount of binding energy and inhibiting the CDK2.[18] Compared to the tendency of these anticancer compounds to bind to CDK6, it seems that rutin, as an active plant ingredient, has a relatively high propensity to bind to CDK6 as well as CDK2 and CDK4. Research, however, has shown that the biggest challenge in the clinical application of pan-CDK inhibitors is their significant side effects and low specificity on normal cells. One of the favourite features of rutin is its minimal concentration, which has led to the establishment of these bindings. At least 18.48 μM of rutin was required for the interaction between CDK2 and rutin. Moreover, at least 140.46 μM of rutin was needed for the interaction between CDK6 and rutin. These results indicate that rutin at very low concentrations can bind to CDKs and affect CDK6 in the G1 phase of the cell cycle and CDK2 in the S and G2 phases of the cell cycle.

Table 2. Molecular dynamic parameters of simulation of CDKs

Complexes	TE (kj/mol)	RG (nm)	RMSD (nm)
CDK2	-524298 (3867)	1.92 (0.02)	0.27 (0.04)
CDK2-Rutin	-1200662 (13201)*	1.91 (0.02)*	0.24 (0.03)*
CDK4	-1945589 (20956)	3.49 (0.04)	0.45 (0.07)
CDK4-Rutin	-7529012 (140167)*	3.59 (0.05)*	0.38 (0.07)*
CDK6	-337336 (2430)	1.87 (0.02)	0.23 (0.03)
CDK6-Rutin	-486950 (5291)*	1.85 (0.02)*	0.24 (0.03)*

RMSD: Root mean-square deviation; RG: Radius of gyration; TE: Total Energy; Statistical analysis was done by Independent Sample T test. Each point represents mean±SD. *$p<0.001$ compared with CDKs alone.

Previous studies have revealed that standard oral doses of rutin are 500 to 2000 mg daily, and these doses can be safely continued for long periods, up to 6 months.[19] The molecular docking results in this study have shown that rutin can bind to CDK2 amino acid residues by 10 hydrophobic bonds and 5 hydrogen bonds at the binding site. Rutin also binds to CDK6 amino acid residues with 12 hydrophobic bonds and 3 hydrogen bonds at the binding site. The high tendency of rutin to bind to CDKs and the establishment of hydrogen and hydrophobic interactions between rutin and CDKs indicate the effect of this substance on CDKs in the cell cycle.

The molecular dynamics simulation results also showed that the number of RMSD fluctuations reached stability within 10 nanoseconds of the simulation; at the same time, the mean RMSD for CDK2-rutin complex decreased compared to CDK2, and also the mean RMSD for CDK4-rutin complex decreased compared to CDK4. This means RMSD can be associated with reducing mean protein skeletal fluctuations. It appears that rutin's binding to these CDKs induces changes in protein structure that could affect protein function.[20] On the other hand, with rutin's binding to CDK2 and CDK6, the mean Rg decreased significantly. This binding led to an increase in the rotational Radius of CDK4. These changes in the rotational radius of proteins can alter the spatial structure of the protein so that it affects the availability of active protein sites for interaction with other functional proteins.[21] The changes in the second structure of CDK proteins caused by rutin ducting indicate the effect of rutin on the spatial structure of these proteins. A sharp decrease in the secondary structures of α-helix and β-sheet and a significant increase in the secondary structures of coil and bend following rutin docking to CDK2 indicate that rutin's binding to CDK2 reduces the structural regions of α-helix and β-sheet, and simultaneously increases the functional regions of bend and coil. The coil, turn, and bend regions are functionally active proteins, giving the protein a high degree of flexibility for biological functions. The binding of the protein to CDK2 can lead to inhibition of this protein and disruption of the S and G2 phases of the cell cycle. Besides that, significant changes were made in the secondary structure of CDK4 and CDK6 proteins after rutin's docking so that the secondary bend structure of CDK6 protein significantly increased after rutin's docking. These structural changes can affect the spatial structure of the protein and prevent the phosphorylation of these tyrosine kinase proteins, thereby may inhibit their activity. Inhibition of CDK2 inhibits the cell cycle in the G2 phase and provides an opportunity to repair damaged DNA in the cell genome. At the same time, inhibition of CDK6 leads to the cessation of the G1 phase of the cell cycle, allowing

apoptotic factors to show the damaged cell or cancer cell to programmed death and apoptosis.[22, 23]

CDK-4 and CDK-6 play important controlling roles in inhibiting cell growth at the beginning of the cell cycle and the G0 and G1 phases. So, inhibiting these proteins can serve as a target for the anticancer drugs to prevent cell proliferation.[24] Moreover, activation of the CDK-2 promotes the S/G2 transition. At the same time, inhibition in the G2 phase of the cell cycle causes activation of the DNA repair system.[25] Given the role of rutin's specific inhibitors on CDKs 2 and 6, it can be used as a pan-CDK inhibitor in developing anticancer drugs.

In evaluating inventions on rutin's applications in the prevention and treatment of cancer, no invention was found to examine its effects mainly. However, some inventions have mentioned rutin as an anticancer compound. In an invention called Compounds, composition, methods, and targets for cancer therapy, rutin was reported to serve as an anticancer flavonoid.[26] In an invention called Stimulation of immunity to tumour-specific and endothelial-specific proteins by in vivo dc attraction and maturation, it has been mentioned as an antioxidant compound to stimulate the immune system in cancers.[27] Besides that, in an invention that introduced flavone derivatives as cyclin-dependent kinase inhibitors, rutin was reported as a constituent of a cyclin-dependent kinase inhibitor formulation.[28] In some other inventions, methods of increasing the solubility and stability of rutin have been addressed so that the use of rutin in the fat phase and the rutin's acylated ester has been taken into consideration.[29, 30]

Conclusion

Rutin, a natural antioxidant compound found in many plants, shows a high tendency to interact with CDKs, especially CDK2 and CDK6. This simulation study showed that rutin's binding to CDK2 and CDK6 can lead to inhibition the activity of these two tyrosine kinase proteins. Inhibition of these CDKs can stop the cell cycle by providing an opportunity to repair the damaged cell genome or lead the damaged cell towards Apoptosis. This study indicated the possibility effects of rutin inhibitor molecular dynamics on how it can inhibit CDKs and stops the cell cycle; therefore, since rutin is a natural antioxidant and has far fewer side effects than synthetic drugs, this compound can be used to develop new drugs for the treatment of numerous types of cancers after extensive clinical research on it.

Disclosures

Acknowledgements: The authors are thankful to the Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, for providing necessary laboratory facilities.

Ethics Committee Approval: The protocol of this research has been approved by the Ethical Committee of Shahrekord University of Medical Sciences (ethics code: IR.SKUMS.REC.1397.236).

Funding: This study has been funded by Shahrekord University of Medical Sciences, Shahrekord, Iran: Grant no. 3853.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.A.S., K.A.D.; Design – D.A., J.S.C.; Supervision – K.A.D.; Data collection &/or processing – F.A.S., J.S.C.; Analysis and/or interpretation – J.S.C.; Writing – M.A.S., D.A., J.S.C., K.A.D., F.A.S.; Review and final revision approval – M.A.S., D.A., J.S.C., K.A.D., F.A.S.

References

1. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, et al. CDK inhibitors in cancer therapy, an overview of recent development. Am J Cancer Res 2021;11:1913.
2. Liu HL, Jiang WB, Xie MX. Flavonoids: recent advances as anticancer drugs. Recent Recent Pat Anticancer Drug Discov 2010;5:152–64. [CrossRef]
3. Ponte LGS, Pavan ICB, Mancini MCS, da Silva LGS, Morelli AP, Severino MB, et al. The hallmarks of flavonoids in cancer. Molecules 2021;26:2029. [CrossRef]
4. Kim KH, Lee KW, Kim DY, Park HH, Kwon IB, Lee HJ. Optimal recovery of high-purity rutin crystals from the whole plant of Fagopyrum esculentum Moench (buckwheat) by extraction, fractionation, and recrystallization. Biores Technol 2005;96:1709e1712. [CrossRef]
5. Chua LS. A review on plant-based rutin extraction methods and its pharmacological activities. J Ethnopharmacol 2013;150:805e817. [CrossRef]
6. Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. Trends Food Sci Technol 2017;67:220–35. [CrossRef]
7. DSLD. Dietary supplement Label Database; 2016. Available at: https://dsld.nlm.nih.gov/dsld/. Accessed Oct 25, 2016.
8. Hosseinzadeh H, Nassiri-Asl M. Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. J Endocrinol Invest 2014;37:783–8. [CrossRef]
9. Farha AK, Gan RY, Li HB, Wu DT, Atanasov AG, Gul K, et al. The anticancer potential of the dietary polyphenol rutin: Current status, challenges, and perspectives. Crit Rev Food Sci Nutr 2022;62:832–59. [CrossRef]
10. Marmé D. Tumor angiogenesis: a key target for cancer therapy. Oncol Res Treat 2018;41:164. [CrossRef]
11. Teleanu R, Chircov C, Grumezescu A, Teleanu D. Tumor angiogenesis and anti-angiogenic strategies for cancer treatment. J Clin Med 2020;9:84. [CrossRef]
12. Lin J-P, Yang J-S, Lin J-J, Lai K-Ch, Lu H-F, Ma Ch-Y, et al. Rutin inhibits human leukemia tumor growth in a murine xenograft

model in vivo. Environ Toxicol 2012;27:480–4 [CrossRef]
13. Perk AA, Shatynska-Mytsyk I, Gerçek YC, Boztas K, Yazgan M, Fayyaz S, et al. Rutin mediated targeting of signaling machinery in cancer cells. Cancer Cell Int 2014;14:124. [CrossRef]
14. Chen H, Miao Q, Geng M, Liu J, Hu Y, Tian L, et al. Anti-tumor effect of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. Sci World J 2013;2013:269165. [CrossRef]
15. Project E, Nachliel E, Gutman M. Force field dependant structural divergence revealed during long time simulations of Calbindin d9k. J Comput Chem 2010;31:1864–72. [CrossRef]
16. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998;19:1639–62. [CrossRef]
17. Rather MA, Dutta S, Guttula PK, Dhandare BC, Yusufzai SI, Zafar MI. Structural analysis, molecular docking and molecular dynamics simulations of G-protein-coupled receptor (kisspeptin) in fish. J Biomol Struct Dyn 2020;38:2422–39. [CrossRef]
18. Tripathi SK, Muttineni R, Singh SK. Extra precision docking, free energy calculation and molecular dynamics simulation studies of CDK2 inhibitors. J Theorl Biol 2013;334:87–100.
19. Incandela L, Cesarone MR, DeSanctis MT, Belcaro G, Dugall M, Acerbi G. Treatment of diabetic microangiopathy and edema with HR (Paroven, Venoruton; 0-(beta-hydroxyethyl)-rutosides): a prospective, placebo-controlled, randomized study. J Cardiovasc Pharmacol Ther 2002;7:S11–5. [CrossRef]
20. Brüschweiler R. Efficient RMSD measures for the comparison of two molecular ensembles. Proteins 2003;50:26–34. [CrossRef]
21. Lobanov MY, Bogatyreva N, Galzitskaya O. Radius of gyration as an indicator of protein structure compactness. Mol Biol 2008;42:623–8. [CrossRef]
22. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolifer 2003;36:131–49. [CrossRef]
23. Arellano M, Moreno S. Regulation of CDK/cyclin complexes during the cell cycle. Int J Biochem Cell Biol 1997;29:559–73.
24. Bortolozzi R, Mattiuzzo E, Trentin L, Accordi B, Basso G, Viola G. Ribociclib, a Cdk4/Cdk6 kinase inhibitor, enhances glucocorticoid sensitivity in B-acute lymphoblastic leukemia (B-All). Biochem Pharmacol 2018;153:230–41. [CrossRef]
25. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. Trends in Biochem Sci 2005;30:630–41. [CrossRef]
26. Yu M. Compounds, composition, methods, targets for cancer therapy. 2014; United States patent US 8, 680,129.
27. Ichim T, Koos D. Regen BioPharma Inc, assignee. Stimulation of immunity to tumor specific and endothelial specific proteins by in vivo dc attraction and maturation. 2016; United States patent application US 14/854,136.
28. Lal B, Joshi KS, Kulkarni SA, Mascarenhas M, Kamble SG, Rathos MJ, Joshi RD. Nicholas Piramal India Limited, assignee. Flavone derivatives as inhibitors of cyclin-dependent kinases. 2015; EP037539112004.
29. Perrier E, Mariotte AM, Boumendjel A, Bresson-Rival D. Flavonoide esters and their use notably in cosmetics. 2001; US 6,235,294 B1.
30. Li S, Liang X, Li D. Preparation method of trihydroxyethyl rutoside. Jinan Xinlite Technology Co., Ltd. 2016; US 2016/0096860 A1.