



## Computational study of quercetin effect on pre-apoptotic factors of Bad, Bak and Bim

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### ABSTRACT

**Introduction:** Quercetin is an effective compound which is found in many medicinal plants. Quercetin antioxidant properties against cancerous tumors and apoptosis induction have been demonstrated. This study is aimed to investigate the molecular dynamics of quercetin with regard to activating the pre-apoptotic factors such as Bim, Bak, and Bad using simulation software.

**Methods:** In this study, the thermodynamic properties of flavonoid molecule of quercetin on three important factors in apoptotic pathway such as Bim, Bak, and Bad were investigated. After the three-dimensional structure of these molecules was obtained from NCBI and simulation was done by Gromacs software, docking stages were performed by AutoDock software and then the molecular dynamics of the complexes were investigated by Gromacs software.

**Results:** The number of hydrogen bonds between quercetin and Bad was higher than Bak and Bim, which causes Bad to have lower energy than Bak and Bim. Mean root-mean-square deviation at 10 ns of simulation increased for Bad and Bak and decreased for Bim in quercetin presence. Root-mean-square fluctuation investigations indicated that Bim had the highest flexibility in quercetin presence compared to free state.

**Conclusion:** Computational studies indicated that quercetin could have a greater effect on Bad compared to Bak and Bim. However it is necessary to investigate the effect mechanism of quercetin on these three pre-apoptotic factors in experimental studies.

### Implication for health policy/practice/research/medical education

This study indicated that quercetin could have greater thermodynamic effects on Bad compared to Bak and Bim. So we suggest that for cancer research and determination of anticancer effect mechanisms of quercetin through apoptotic pathways, researchers focus more on the role of Bad molecule

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### Introduction

Nowadays medicinal plants and the antioxidant components derived from them are being increasingly used to prevent and treat many of the diseases (1-10). Also the contribution of medicinal plants effective components to treating and preventing various diseases is an issue that has attracted the attention of many researchers (11-16). Relevantly, quercetin is a plant-based flavonoid which is currently used as a food supplement, as well (17). Quercetin (C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>) with IUPAC name of 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one, is considered an important flavonoid component because it exerts the greatest antioxidant activity of all the flavonoids, which is even six times higher than that of vitamin

C (18). It is found in many plants such as onion, apple, green and black tea, red grapes, citrus, broccoli sprouts, and tomato. Several studies have investigated the therapeutic effects of quercetin. For example study of quercetin protective activity against liver diseases has indicated that this flavonoid helps to relieve the complications of alcoholic cirrhosis and significantly reduces the cirrhosis-induced oxidative damage to liver cells (19,20). Several epidemiologic studies have demonstrated the association between quercetin intake and various cardiovascular diseases and asthma and other respiratory disorders (21). Quercetin has also anti-inflammatory, anti-coagulant, antibacterial, hypotensive, and anti-atherogenic properties (22,23), contributes to slowing down the growth of

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certain cancer cells, and can destroy cancer cells through apoptotic pathway and has anticancer effect particularly for hepatocarcinoma and colon cancer (24). Given the role of this flavonoid component in destroying cancer cells, this study investigates the thermodynamics of quercetin molecule on three important factors in apoptotic pathway of cancer cells such as Bim, Bak, and Bad molecules. For this purpose, after the three dimensional structure of the above molecules were obtained from NCBI and simulation was performed by Gromacs software, docking stages were done by AutoDock software and finally molecular dynamics of the complexes were investigated by Gromacs.

### Materials and methods

To develop molecular structures, Protein Data Bank file of Bad, Bak, and Bim proteins (with 1G5JID: 1BXLID: and 1PQIID: respectively) as three important factors in cells apoptosis process were obtained from <http://www.rcsb.org/>. Three-dimensional structure of quercetin was obtained from ChEMBL and converted into PDB file by Mercury 3.6.

Studies of molecular dynamics simulation of the three proteins of interest were done in pure water to have the related structures undergo the variations in temperature and pressure and 140 mMol concentration and reach to equilibrium. Three apoptotic complexes under study (Bad, Bak, and Bim) were simulated in aqueous solvent using Gromacs 4.6.1 software and field of force G43A1. In this study the model SPC216 was used (25). To have the system reach to 140 mMol concentration, an adequate number of Na and Cl ions were introduced instead of solvent.

To perform simulation and to maximize the energy, the algorithm descentminization step (steepest) and integration at 50000 steps were used. Then equilibration steps were performed using NVT and NPT ensembles by means of LINCS algorithm for integration of 50000 steps. Configuration of each system was saved at 0.2 ps intervals. Fixation was done for all bonds. Main sampling was run using NPT ensemble within 30 ns.

To fix temperature and pressure of system at 300 kelvin and one bar, V-rescale thermostat and Parrinello-Rahman barostat were used. The length of bonds including hydrogen atom was fixed by LINCS algorithm. For long distance electrostatic interaction, the particles of 1 nm in diameter and particle mesh Ewald method of integration, Newton movements equations were used by half-step leap and time step of 2 fs. The saved routes in simulation were used as controls to analyze the structural parameters of Bad, Bak, and Bim proteins in presence of ligand (26). Then the output PDB file was simulated in water per the method below molecular docking was done.

To dock the small molecules in macromolecules, AutoDock 4.1 was used. Using this software, we can predict that how a substrate or drug is docked in the receptor and its three-dimensional structure is displayed. Quercetin was docked next to the above pre-apoptotic factors and analyzed by this software.

To attribute the bar to the protein, Kollman bars were used and for ligand Gasteger, bar was used. The used grid to limit docking area included all volume of the protein. To perform docking, genetic algorithm was used and maximum number of evals was considered 200 (27). After docking, the hydrogen and hydrophobic bonds between quercetin and the three pre-apoptotic factors were obtained by Ligplot software. Furthermore, after docking results were derived for the three pre-apoptotic factors, simulation was done according to molecular dynamics simulation in presence of quercetin. Finally, the molecular dynamics simulation of the complex of all three proteins (Bad, Bak, and Bim) with quercetin ligand was done in aqueous solution as per the above method and the saved routes in simulation were used to analyze the structural parameters of complex (25).

### Results

According to docking method, the most possible bond loci for quercetin (Figure 1) drug were obtained on apoptotic factors and then were scored based on energy ranking. Table 1 shows molecular docking results. As shown, the bond energy of quercetin on Bad, Bak, and Bim factors was obtained -70.5, -80.6, and -97.6, respectively. Comparison of energy bonds indicates that quercetin is most effective on Bad and Bak apoptotic factors (with similar bond energies).

To locate the site of hydrogen bonding and hydrophobic bonding of quercetin with Bad, Bak, and Bim molecules, Ligplot software was used. Quercetin molecule creates hydrogen bonding with Thr30, Asp15, Ser18, Val14, and Trp28 amino acids and hydrophobic bonding with Thr30, Asp15, Ser18, Val14, and Trp28 amino acids (Figure 2). These bonds for Bak and Bim molecules are shown in Table 1. The number of amino acids that established hydrogen and hydrophobic bond between quercetin and Bad molecule is higher compared to Bak and Bim molecules. Activation site of the Bad, Bak, and Bim molecules with quercetin exhibited the highest level of energy at docking stage (Figure 3).

In Figure 4, the analysis of total energy of the three pre-apoptotic factors, Bak, Bim, and Bad in simulation in percent water solution and in presence of quercetin is shown with blue and red colors, respectively. Throughout simulation, the total energy of three apoptotic proteins decreased in presence of ligand. This decrease was more pronounced for Bim than the other two molecules. Table 2 indicates variations in energy at different intervals. The rate of

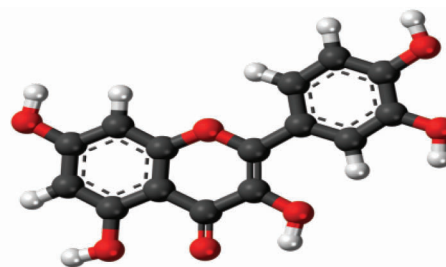


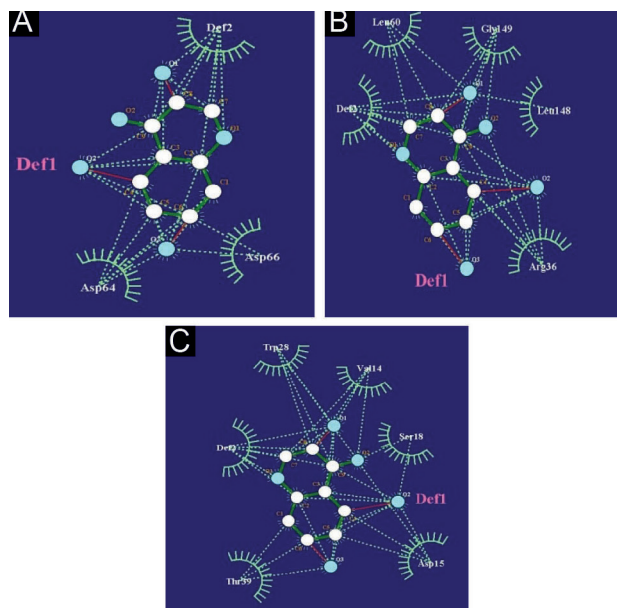
Figure 1. Quercetin compound.

**Table 1.** Molecular docking results and methods of quercetin bonding with apoptotic factors Bad, Bak, and Bim

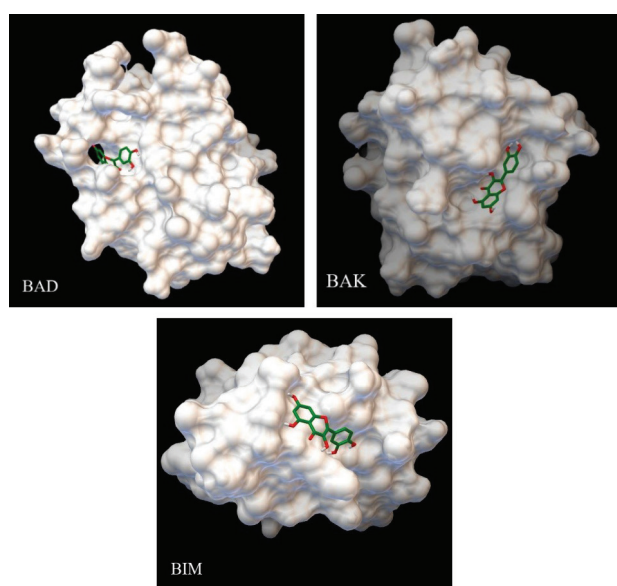
Receptors	BE	FIE	EIC	Interaction bonds	
				Hydrophobic Bonding	Hydrogen Bonding
Bad	-6.97	-8.76	7.76	Trp28, Val14, Ser18, Asp15, Thr30	Trp28, Val14, Ser18, Asp15, Thr30
Bak	-6.80	-8.59	10.37	Gly149, Leu60, Arg36, Leu138	Gly149, Leu60, Arg36, Leu138
Bim	-5.73	-7.52	62.77	Asp66, Asp64	Asp66, Asp64

Abbreviations: BE, binding and Energy (kcal/mol); FIE, final intermolecular energy (kcal/mol); EIC, estimated inhibition constant ( $\mu\text{M}$ ).

variations in radius of gyration for Bad and Bak molecules after simulation in absence of quercetin increased by 0.01



**Figure 2.** Hydrogen bonding and hydrophobic bonding after docking step between quercetin and Bad (A), Bak (B), and Bim (C) apoptotic factors in Ligplot software.



**Figure 3.** Three-dimensional structure of docked molecules, Bad, Bak, and Bim with quercetin.

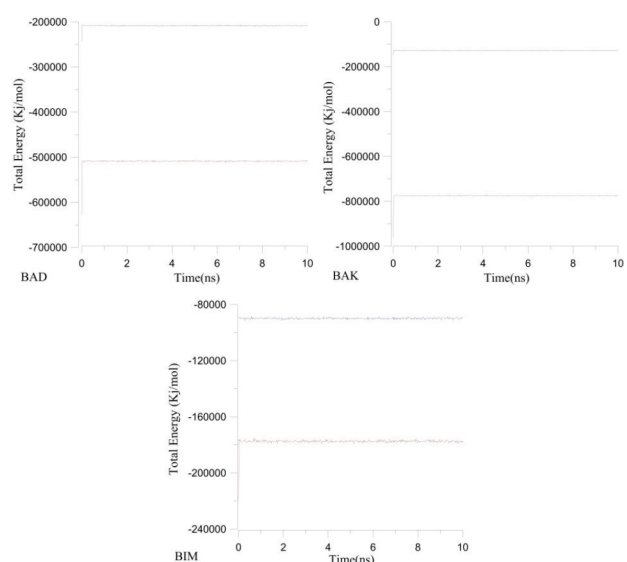
compared to when the quercetin was present, but no variation was seen for Bim molecule. Comparison of mean potential and kinetic energy of Bad, Bak, and Bim molecules between simulations in absence and in presence of quercetin indicated increase in energy level (further negativity) in presence of quercetin (Table 2).

At 10 ns interval, root mean square deviation (RMSD) of Bad, Bak, and Bim molecules was  $0.3 \pm 0.04$ ,  $0.32 \pm 0.03$ , and  $0.21 \pm 0.03$ , respectively, in absence of quercetin and  $0.32 \pm 0.04$ ,  $0.38 \pm 0.04$ , and  $0.19 \pm 0.03$ , respectively, in presence of quercetin (Figure 5).

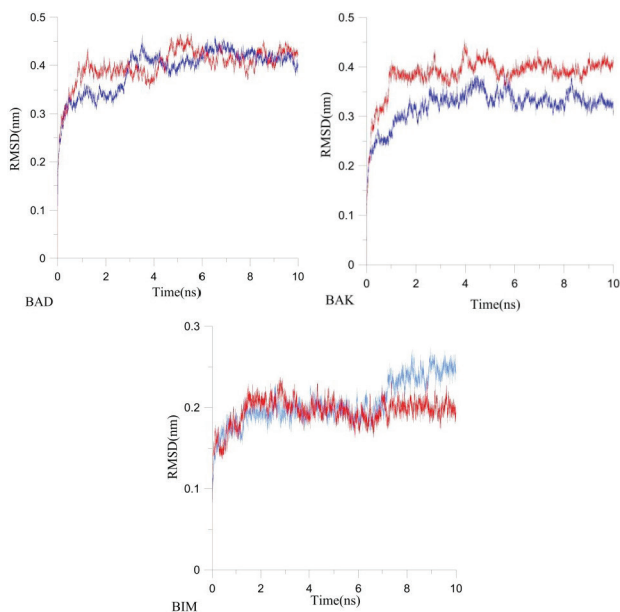
Root mean square fluctuation (RMSF) of Bad, Bak, and Bim molecules was  $0.18 \pm 0.1$ ,  $0.18 \pm 0.09$ , and  $0.15 \pm 0.09$ , respectively, in absence of quercetin and  $0.16 \pm 0.11$ ,  $0.2 \pm 0.13$ , and  $0.11 \pm 0.06$ , respectively, in presence of quercetin (Figure 6).

**Discussion**

Programmed cell death is a process occurring with involvement of many cell factors in the cell to prevent deviation of cell growth and proliferation. Programmed cell death considerably inhibits the process of cells' getting cancerous (28,29). However programmed cell death is introduced within three frameworks, autophagia, necrosis,



**Figure 4.** Variations in total energy of three apoptotic factors Bad, Bak, and Bim alongside quercetin throughout simulation. Blue lines: Simulated pre-apoptotic factors in water and in absence of quercetin; red lines: simulated pre-apoptotic factors in water and in presence of quercetin; total energy of Bad molecule decreased considerably compared to other molecules.



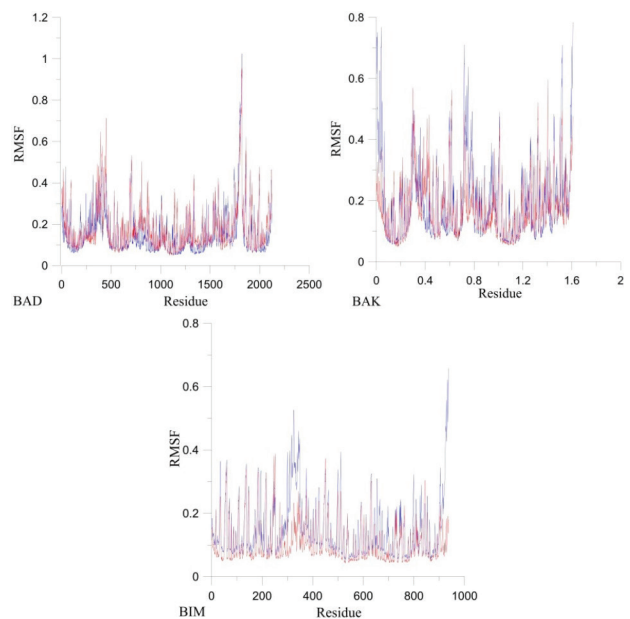
**Figure 5.** Root mean square deviation for Bad, Bak, and Bim apoptotic factors.

Blue lines: Simulated pre-apoptotic factors in water and in absence of quercetin; red lines: simulated pre-apoptotic factors in water and in presence of quercetin; root mean standard deviation of Bim molecule decreased considerably.

and apoptosis (30,31). But very vital factors, especially Bcl-2 family, play an important role in controlling apoptosis and inducing programmed cell death, which inhibits the process of getting cancerous of normal cells in the body (32). When Bcl-2 is activated by anti cancer drugs or in response to apoptotic stimulants, they activate many of the pre-apoptotic factors of their subdivisions such as Bik, Bid, Bax, Bim, Bak, and Bad (33), which affect cell mitochondrial wall and activate cell caspases by activating cytochrome C, comprising a message of death for cell (34,35).

Since the factors involved in cells apoptosis pathway contribute significantly to inducing and/or inhibiting this process, in the present study the molecular dynamics of three pre-apoptotic molecules, Bim, Bak, and Bad, which play a significant role in apoptosis was investigated in presence of quercetin which is a strong flavonoid component. Sambantham et al have already investigated the effect of hesperetin on pre-apoptotic factors using simulation and demonstrated that hesperetin could activate certain effective apoptotic factors such as Bad and Bax and inhibit NF-kB (36).

In this study, analysis of hydrogen and hydrophobic bond



**Figure 6.** Root mean standard fluctuation of three apoptotic proteins, Bad, Bak, and Bim.

Blue lines: Simulated pre-apoptotic factors in water and in absence of quercetin; red lines: simulated pre-apoptotic factors in water and in presence of quercetin; root mean standard deviation of Bim molecule decreased considerably.

between the best locations of quercetin for energy level and three pre-apoptotic factors using Ligplot software indicated that quercetin could efficiently develop hydrophobic and hydrogen bond with the three pre-apoptotic factors, Bim, Bak, and Bad. The highest hydrogen and hydrophobic bonds were seen between Bad molecule and quercetin and the lowest between Bim and quercetin. Since quercetin has the smallest hydrogen and hydrophobic bond with Bim, quercetin is likely to have the least interaction with Bim while exerts the greatest effect on Bad. The higher energy of Bad in presence of quercetin (Table 1) represents a stronger bond between quercetin and Bad than those between quercetin and Bak and Bim molecules. Analysis of total energy of the three pre-apoptotic factors, Bim, Bak, and Bad, indicated that throughout simulation, the total energy of the three pre-apoptotic proteins increased in presence of ligand. This increase in energy suggests that quercetin affects the protein structure, and all complex structures tend to become constant after simulation. This increased energy is lower for Bim than other components. Comparison of stability among three apoptotic complexes in presence and in absence of quercetin by RMSD against the time for proteins indicated that mean RMSD at 10 ns

**Table 2.** Variations in radius of gyration, kinetic, temperature, potential, hydrogen-bonding between of protein and protein

Protein	Rg	TE	AT	AK	AP	AH
G <sub>1</sub> Bad	1.62 ± 0.01	-208343 ± 1697.2	300.0 ± 2.5	48030.7 ± 407.7	-256374 ± 1556.7	161.9 ± 6.8
G <sub>2</sub> Bad	1.62 ± 0.02	-508885 ± 541.8	300.1 ± 1.56	102575.6 ± 534.6	-611460.4 ± 5447.3	168.4 ± 10.4
G <sub>1</sub> Bak	1.43 ± 0.01	-127766 ± 831.3	300.0 ± 5.4	29473.5 ± 533.6	-157240 ± 531.3	113.9 ± 5.7
G <sub>2</sub> Bak	1.43 ± 0.02	-774400 ± 1209.0	300.0 ± 1.3	154963.4 ± 666.5	-929737.1 ± 8477.4	119.3 ± 6.7
G <sub>1</sub> Bim	1.2 ± 0.0	-89910.7 ± 471.3	300.0 ± 4.1	19768.8 ± 267.3	-109679 ± 354.8	73.6 ± 4.2
G <sub>2</sub> Bim	1.2 ± 0.0	-177512 ± 1956.7	300.2 ± 2.8	36045.4 ± 333.7	-213557.8 ± 1938.3	192.9 ± 8.4

Abbreviations: Rg, radius of gyration; TE, total energy; AT, average temperature (K); AK, average kinetic; AP, average potential (KJ/mol); AH, average hydrogen-bonding between of protein and protein (nm); G<sub>1</sub>, factors simulation before docking; G<sub>2</sub>, factors simulation after docking.

of simulation increased for Bad and Bak proteins at quercetin presence while the corresponding mean decreased for Bim molecule in presence of quercetin.

Although as RMSD varies further throughout simulation, the structure deviates further from the primary direction, RMSD investigations indicated that the effect of quercetin molecule on Bim stability was higher than that on Bid and Bak stability. In RMSD, averaging is done on all the particles at any points in time. The more RMSD varies throughout simulation, the more deviation from the primary structure. As RMSD graph of Bim molecule (Figure 5) indicates, RMSD in presence of ligand at 6 ns of simulation is similar to the state when ligand is not present, and RMSD in presence of quercetin decreases considerably at the last 4 ns. Therefore, initially quercetin could competitively affect Bim. Because RMSD is not an appropriate parameter to reflect mobility rate of structural elements, RMSF is used to examine structural flexibility.

By RMSF graph, averaging is performed on total time per each particle. Studies of RMSF indicated that Bim had the highest flexibility in presence of ligand compared to freestate while the RMSF of Bad molecule decreased in presence of ligand, indicating that quercetin decreased flexibility of Bad and Bim molecules and increased that of Bak. The greatest effect of quercetin was seen on Bim molecule.

### Conclusion

Computational studies have shown that quercetin could have a greater effect on Bad compared to Bak and Bim. However docking investigations have demonstrated the lowest effect of quercetin on Bim. This difference is attributed to the dynamicity of molecular dynamics simulation. However the effect mechanism of quercetin on these three apoptotic factors should be investigated by experimental studies.

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### Authors' contributions

This work was carried out in collaboration between all authors. MAS and EHS designed the methodology and coordinated the whole project. JSC performed the software analysis. All authors prepared the draft of manuscript and confirmed the final proof.

### Conflict of interests

Authors declare no conflict of interests.

### Ethical considerations

We considered all ethical issues.

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None.

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