Journal of Molecular Structure 1258 (2022) 132668

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

Genistein: A novel inhibitor of IL-6/IL-6R interface of the Interleukin-6–mediated STAT3 dependent pathway of carcinogenesis

Saurabh Sharma[a,b], Lakshay Malhotra[a], Prakarsh Yadav[a], Vandana Mishra[b,*], Radhey Shyam Sharma[b,c,*], Ethayathulla Abdul Samath[a,*]

[a] Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India
[b] Bioresources and Environmental Biotechnology Laboratory, Department of Environmental Studies, University of Delhi, Delhi-110 007, India
[c] Delhi School of Climate Change & Sustainability, Institute of Eminence, University of Delhi, Delhi-110007, India

ARTICLE INFO

Article history:
Received 6 July 2021
Revised 17 February 2022
Accepted 19 February 2022
Available online 21 February 2022

Keywords:
Breast cancer
IL-6/STAT3 signaling pathway
Molecular docking
Molecular dynamics simulation
Cytotoxicity assay

ABSTRACT

Phytotherapy for breast cancer, the second most common cancer diagnosed among women and a leading cause of their death globally, may serve as an adjuvant therapy to combat adverse chemo-and radiotherapy challenges. IL-6/STAT3 signaling pathway, governing breast cancer progression, remains a favourite therapeutic target in developing phytotherapy. Therefore, identifying phytocompounds with novel targets influencing the IL-6/STAT3 pathway would help to develop phytotherapy for breast cancer. In this study, we demonstrate the novel binding site, i.e., the interface of IL-6 and IL-6Rα of the IL-6/STAT3 signaling pathway of cancer progression. Ten potent anticancer phytocompounds were analyzed for their binding with IL-6/IL-6Rα complex using molecular docking and molecular dynamics simulations. Among all, genistein showed excellent binding energy (-10.59 kcal/mol) with the IL-6/IL-6Rα interface of the STAT3 pathway. In cytotoxicity experiment, Genistein showed IC_{50} of 38 µM and 18 µM for 24 and 48 h, respectively, whereas quercetin showed IC_{50} of more than 50 µM and 35 µM at 24 h and 48 h, respectively on MDA-MB-453 breast cancer cell line. Based on bioinformatic analyses and cellular toxicity assay, we propose genistein as a potential therapeutic phytocompound to inhibit the IL-6/STAT3 pathway by blocking the interaction of IL-6 with the IL-6 receptor.

1. Introduction

Interleukin 6 (IL-6), a pro-inflammatory cytokine, plays a primary role in expanding and differentiating tumor cells. IL-6 mediates tumorigenesis by regulating key processes such as apoptosis, cell proliferation, survival, angiogenesis, and metastasis [1,2]. Increased expression of IL-6 in plasma and tumor tissues has been reported in breast and other types of cancer [3]. Breast cancer (BC), with an estimated 2 million new cases annually, remains the second most common cancer of women diagnosed globally and causes 6,27,000 deaths [4]. An aberrant STAT3 (signal transducer and activator of transcription 3) signaling pathway acts as one of the factors for the pathogenesis of BC and initiation of malignancy [5–7]. The IL-6 mediated STAT3 pathway is initiated by the binding of IL-6 to IL-6Rα along with GP130 to form a hexameric complex of IL-6/IL-6Rα/GP130 hetero-trimers. The IL-6 has three conserved epitopes site I, II, and III. At the site I interface, the IL-6Rα domain specifically binds and at site III GP130 binds to IL-6. Whereas, at site II both IL-6Rα and GP130 form common interface interactions with IL-6. In the site I interface residues Phe74, Lys171, Gln176, Arg178, Arg179 from helices A and D interact with D3 domain of the IL-6Rα receptor. At the same time, IL-6 site II involves the interaction of IL-6Rα and GP130 in two distinct interfaces, IIa and IIb. The interface IIa consists of IL-6 helices A and C interacting with a small hinge region between the periphery of D2 and D3 domains of GP130. On the other hand, interface IIb covers the D3 domain of IL-6Rα and Domain D3 of GP130. Thus, two heterotrimers assemble to form a functional hexameric signaling complex through site III [8,9]. Once the complex is formed it triggers the STAT3 phosphorylation, cascade dimerization, nuclear translocation, and DNA binding [10]. IL-6 is also reported as a predictive marker for assessing advancing breast cancer [11] where the IL-6/STAT3 signaling induces angiogenesis and epithelial-to-mesenchymal transition and cancer progression.

Abbreviations: IL-6, Interleukin 6; IL-6R, Interleukin 6 Receptor; STAT-3, Signal transducer and activator of transcription 3; MD simulations, Molecular Dynamics simulations; gp130, Glycoprotein 130.

* Corresponding authors.
E-mail addresses: mistletoe_h@hotmail.com (V. Mishra), rads26@hotmail.com (R.S. Sharma), ethayathulla@gmail.com (E. Abdul Samath).

https://doi.org/10.1016/j.molstruc.2022.132668
0022-2860/

Though IL-6 and STAT3 are attractive therapeutic/pharmacological targets [12], targeting STAT3 remains challenging as it is localized intracellular and lacks its enzymatic activity [13]. IL-6 also acts as a pro-tumorigenic agent [14], besides serving as an activator of the STAT3-dependent inflammatory pathway [7]. Though inhibiting translocation of STAT3 from cell membrane to nucleus controls cancer initiation, progression and metastasis, IL-6 remains a realistic therapeutic target as it activates STAT3 intracellularly [15,16]. Several STAT3 inhibitors with varying mechanisms have been reported; however, only a few have crossed the preclinical evaluation phase to the clinical trials phase [17].

In contrast to synthetic chemical inhibitors, phytocompounds are considered safe and more potent in controlling cancer during chemotherapy [18,19]. In addition, different phytocompounds from medicinal herbs exhibit antiproliferative and apoptosis-inducing activities on different breast cancer cell lines [20,21]. Available drugs, such as madindoline A (MDL-A), raloxifene and bazedoxifene significantly reduce the risk of BC and improved patient survival by inhibiting the STAT3 signaling pathway [10]. However, drug resistance, low response rates and drug reactions are major issues of the current cure [22]. Also, a lack of effective therapy to prevent metastasis and recurrence of BC warrants designing and identifying novel chemotherapeutic agents for effective treatments with fewer side effects. Therefore, the discovery of new drugs and target molecules is still required. Hence, phytocompounds from traditional medicinal plants that regulate the IL-6/STAT3 cancer signaling pathway would serve as a novel strategy to control breast cancer and increase the acceptance of phytotherapy in the treatment.

Phytocompound-mediated regulation of different breast cancer signaling pathways has been suggested as a promising adjuvant therapy for cancer treatment [23,24]. Several plant preparations and their active ingredients have been reported to suppress the progression and development of cancer. Kapinova et al. (2018) has reported that more than 5000 individual phytocompounds were identified from plant-derived foods, such as fruits, vegetables, and grains [25]. In a recent review, Levitsky et al. (2015) highlighted the ten most therapeutic drugs such as genistein (polyphenols), quercetin (polyphenols), parthenolide (sesquiterpene lactone), xanthatin (sesquiterpene lactone), Rosmanol (diterpene), carnosol (diterpene), resveratrol (stilbenoid), rohitukine (alkaloid) and curcumin (diarylheptanoid) as the potent anticancer phytocompounds [23] but their roles in the IL-6/STAT3 pathway remain unknown. Therefore, using molecular docking, MD simulations and cell-based assay we tried to identify the phytocompounds which can inhibit the IL-6-mediated STAT3 pathway by inhibiting the binding of IL-6/IL-6Rα to identify the novel therapeutic compound for breast cancer treatment. Our study showed genistein as a potent therapeutic phytocompound that inhibits IL-6 and IL-6Rα, interactions and prevents the cascade of the IL-6/STAT3 pathway. Therefore, we propose genistein as a potential phytocompound beneficial in combination therapy to reduce the severity of breast cancer.

2. Materials and methods

2.1. Molecular docking

Molecular docking of ten known Phytocompounds (Genistein, Parthenolide, Rosmanol, Xanthatin, Quercetin, Carnosol, Resveratrol, Raloxifene, Rohitukine, and Curcumin) was performed with the crystal structure of human IL-6 in complex with IL-6R/gp130 (PDB ID: 1P9M) [8] using AutoDock4.2.6. For our docking studies, the monomeric IL-6 and IL-6Rα complex was used. Before docking the solvent molecules in the crystal structure were removed and energy minimized. Similarly, the coordinates of the phytocompounds retrieved from http://zinc.docking.org/ were energy minimized before docking. The site I interface in the IL-6/IL-6Rα complex was defined as the docking/binding site as described in the previous studies [26]. Out of 10 docked poses, the conformation with the best free energy of binding was selected as the best-docked pose for all 10 different phytocompounds. Pymol software was used to analyze the docked ligand.

2.2. Molecular dynamics

The IL-6/IL-6Rα complex both in the presence and absence of docked phytocompounds were subjected to 50 ns MD simulations using GROMACS-5.1.3. Before the MD run, the models were energy minimized using GROMOS 54a7 force field [26,27]. Both protein and the protein-ligand complex were placed in a cubic box extended to 10 Å from the edges in each direction and solvated with water model SPC216. The solvated complexes were again energy minimized using the steepest descent algorithm by convergent criteria of $\leq$1000.0 KJ/mol/nm to remove the van der Waal clashes between the solute and solvents. Before subjecting the complexes for 50 ns MD simulations, the system was equilibrated at 1 atm pressure and 300 K for 100 ps using Berendsen thermostat and Parrinello-Rahman barostat in Periodic boundary conditions. The leapfrog dynamics integrator was performed to integrate the position and velocity of atoms at the step size of 1fs, LINCS algorithm was used to constrain all the bonds [28]. PME (Particle Mesh Ewald) algorithm was used for the calculation of the Long-range electrostatic interactions, whereas the Verlet scheme for neighbor searching was used for calculating the short-range interactions [29]. For the MD simulations of the IL-6 receptor and IL-6 only in the absence of any phytocompounds, the same protocol was used for comparative study [30].

MM/PBSA calculations: The Molecular Mechanics/Poisson Boltzmann Surface Area (MM/PBSA) was used to calculate the free binding energy of the IL-6 and IL-6 receptor phytocompound complexes using the single trajectory method by using the default parameters. The g_mmpbsa GROMACS module was used for binding free energy estimation [31].

2.3. Cell culture

The Human metastatic breast cancer cell line MDA-MB-453 was purchased from NCCS (National Center for Cell Science) Pune, India. Cells were frozen in LN_2 in multiple vials and cultured for less than 15 population doubling for this study. The cells were grown in DMEM media (Dulbecco's Modified Eagle's medium) (Gibco, Thermo Fisher Scientific) with 10% FBS (Fetal Bovine Serum) South American origin (Gibco, Thermo Fisher Scientific) and with 1% Antibiotic- Antimycotic 100X (Gibco). Thermo-scientific humified incubator was used for maintaining the cells at 37 °C with 5% CO_2. Trypsin 0.25% (1X) and 0.2% EDTA in Dulbecco's phosphate-buffered saline (Himedia) was used for cell dissociation during cell passaging.

2.4. Cell cytotoxicity

To demonstrate the antiproliferative effect of genistein and quercetin on the MDA-MB-453 breast cancer cell line, MTT assay was performed in a 96 well plate using MTT (3-(4,5-Dimethylthiazol-2-yl)−2,5-Diphenyltetrazolium Bromide) (Molecular Probes, Invitrogen) reagent. 8000 cells were seeded in DMEM complete media for 24 h and 48 h and then challenged with different concentrations of either genistein or quercetin ranging from 0 to 50 µM. To estimate the cell cytotoxicity (%) in MDA-MB-453 at 24 h after the treatment, the cells were incubated with 0.5 mg/mL MTT (prepared in PBS) reagent for 4 h at 37 °C in 5% CO_2. After

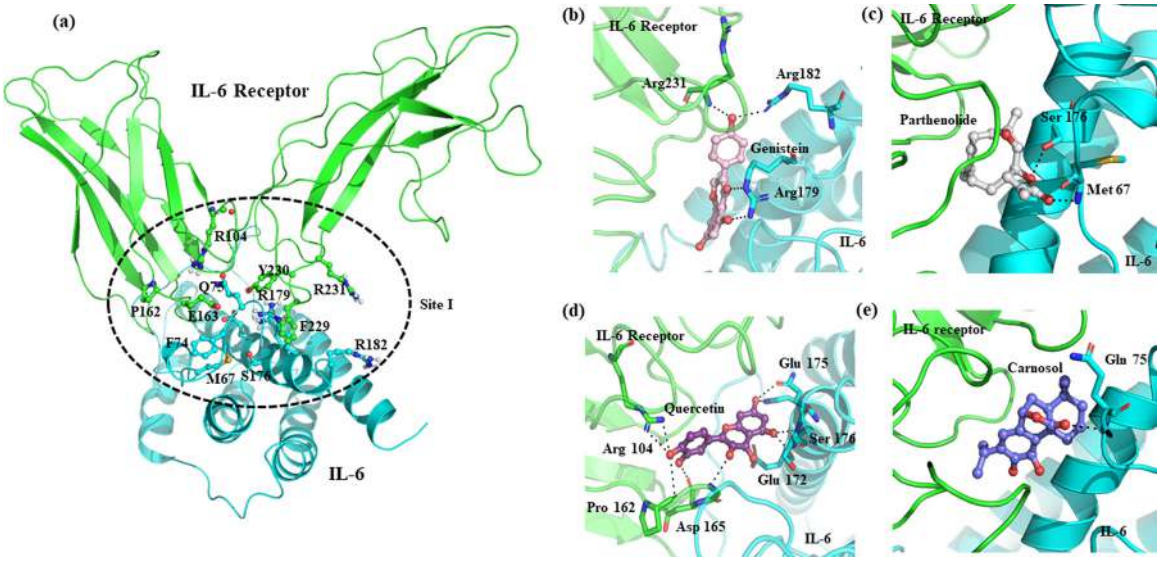

Fig. 1. Docking of phytocompounds (higher binding energy) with IL-6/IL-6Rα complex. (a) Residues forming the site-I binding pocket in IL-6 and IL-6R are shown in ball and stick. (b-e) The hydrogen-bonded, and electrostatic interactions formed between phytocompounds (b) genistein, (c) parthenolide, (d) quercetin, and (e) carnosol and IL-6 (cyan) /IL-6Rα (green) complex are shown in dotted lines.

that, the existing solution was replaced with 80 µL DMSO to dissolve formazan crystals, followed by measurement at 575 nm using Spectramax i3x. The assay was performed in triplicates, and the IC_{50} and the graphs were made using Sigma plot 14.0. The following equation was used for calculating the cytotoxicity percentage [26].

$$Cytotoxicity\ Percentage = \left(\frac{1 - Absorbance\ of\ sample}{Absorbance\ of\ negative\ control}\right)100$$

The absorbance of negative control refers to "the absorbance of the untreated cells" and absorbance of the sample refers to "the absorbance given by the cells treated with different concentrations of compounds" used in the MTT assay and the readings were taken at 575 nm.

3. Results

3.1. Molecular docking

The docking of all plant-derived phytocompounds; Genistein, Parthenolide, Quercetin, Carnosol, (Fig. 1) Curcumin, Raloxifene, Rosmanol, Rohitukine, Vinblastine, and Xanthatin (Fig. 2) were performed at the interacting interface between IL-6 and IL-6Rα (site I) in the crystal structure of IL-6/IL-6R/GP130 complex (PDB ID: 1P9M). Out of these ten compounds, genistein showed comparatively higher binding energy (−10.59 kcal/mol) with IL-6 and IL-6Rα complex. The binding energy of other compounds varied from −9.88 kcal/mol to −5.64 kcal/mol shown in Table 1. Ki values were calculated from the docking energy using AutoDock4.2.6. For further MD simulations analysis, compounds with binding energy greater than −8 kcal/mol (Genistein, Quercetin, Carnosol, and Parthenolide) were selected.

3.2. Molecular dynamics

The molecular dynamics of IL-6 and IL-6 receptor (alpha subunit) were performed both in the presence and absence of ligands to analyze the binding of phytocompounds to the complex. The RMSD, SASA, Rg and RMSF plots showed protein (IL-6/IL-6Rα) was stable throughout MD simulations (Fig. 3a-d). In absence of ligands, the IL-6 and IL-6Rα were stabilized by hydrogen-bonded and hydrophobic interactions throughout the MD simulations. Upon analysis of MD trajectories of protein, three hydrogen bonds were constantly observed throughout the 50 ns of MD simulations along with other varying hydrogen bonds between IL-6 and IL-6Rα complex (Table S1). In the protein-ligand complex, the MD trajectories showed many stable hydrogen-bonded interactions formed between IL-6 and IL-6Rα were lost due to the binding of phytocompounds at site I (Table S1). Although the RMSD, SASA, and Rg plots showed that the protein-ligand was stable throughout MD simulations. The RMSF plot showed in the protein-ligand complexes, the region from 100 to 135 and145 to 176 showed large fluctuations in the IL-6/IL-6Rα which imparts instability. In the genistein complex, the loop regions in the IL-6Rα between 220th - 240th residues and 250th - 260th showed higher RMSF. A similar shift was observed in the quercetin complex. The average RMSD of the ligands Quercetin and Genistein is approximately 0.4 nm. In carnosol and parthenolide complexes, no major change in the displacement of the receptor-protein complex was observed (Fig. 3d). Genistein and quercetin formed stable H-bonded interactions with IL-6/IL-6Rα after 50 ns MD simulations (Fig. 3e and f).

Table 1
Binding energy and inhibition constant of phytocompounds with IL-6/IL-6Rα complex.

S. No.	Anticancer compounds	B. E. (kcal/mol)	Ki
1.	Genistein	−10.59	17.38 nM
2.	Quercetin	−9.88	56.82 nM
3.	Carnosol	−9.40	128.21 nM
4.	Parthenolide	−8.16	1.05 µM
5.	Xanthatin	−7.43	3.57 µM
6.	Rosmanol	−7.33	4.25 µM
7.	Rohitukine	−7.12	6.01 µM
8.	Raloxifene	−6.75	11.20 µM
9.	Curcumin	−6.54	16.00 µM
10.	Vinblastine	−5.64	73.54 µM

3.3. H-bond occupancy

The occupancy of hydrogen bonds formed between ligand and protein complex was calculated using GROMACS for the entire 50 ns MD simulations. Among all, quercetin formed as many as

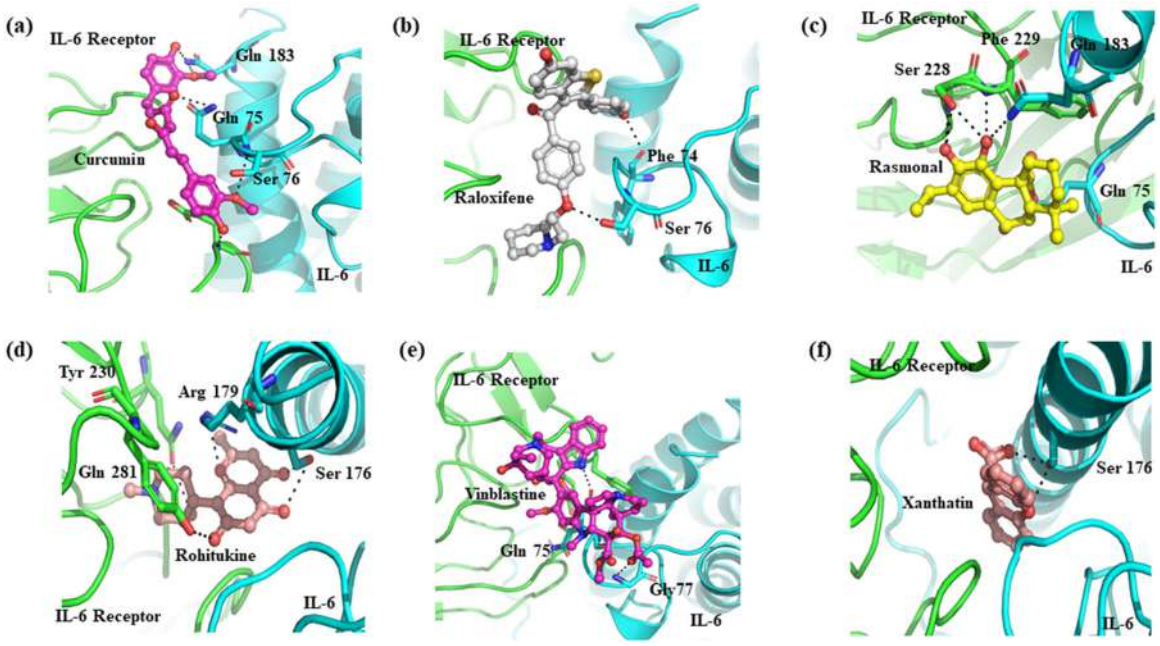

Fig. 2. Docking of phytocompounds (low binding energy) with IL-6/IL-6Rα complex. Interactions formed by phytocompounds (a) curcumin, (b) raloxifene, (c) rasmonal, (d) rohitukine, (e) vinblastine, and (f) xanthatin with IL-6 (cyan) /IL-6Rα (green) complex are shown in dotted lines.

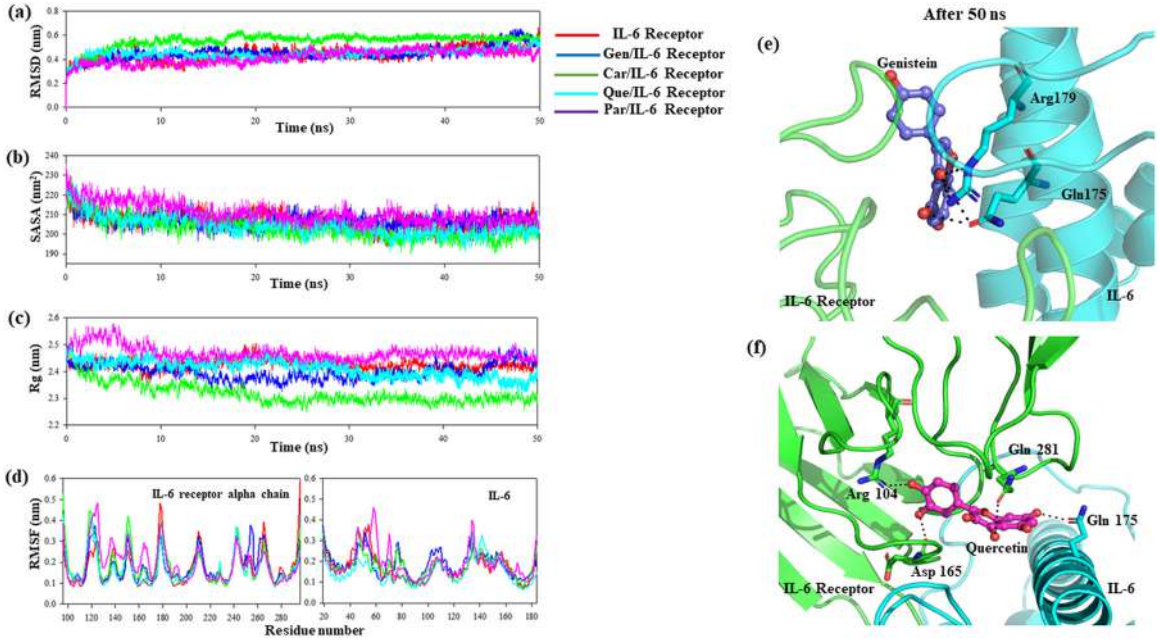

Fig. 3. Molecular dynamic simulations of IL-6/IL-6Rα complex with phytocompounds: The IL-6/IL-6Rα with the ligands like Gen, Car, Que, and Par are shown in Blue, Green, Cyan and magenta color respectively, and the IL-6/IL-6Rα alone is shown in red color (a) RMSD: The Root Mean Square Deviation (RMSD) graph shows that the complex was generally stable. (b) SASA: the solvent-accessible surface area upon ligand binding, (c) Radius of Gyration: Rad. of Gyr. plot showing the compactness of protein compounds complex during molecular dynamics, and (d) RMSF plot shows the flexibility of the polypeptide chain during molecular dynamics. (e) and (f) shows the interactions formed by genistein and quercetin with IL-6/IL-6Rα after 50 ns MD simulations.

9 H-bond during MD simulation with a 5–6 average H-bonds. The residues, Asp165 and Gly164 from IL-6Rα showed the highest H-bond occupancy with quercetin throughout 50 ns MD simulations. Next to quercetin, genistein formed a maximum of 5 hydrogen bonds with 2 H-bonds as an average. In the genistein complex, Arg179 of IL-6 molecule showed the highest H-bond occupancy compared to Arg182 of IL-6 and Gln281 of the IL-6Rα. After genistein, carnosol and parthenolide formed a maximum of 3 and 1 H-bonds, respectively, with an average of 2 and 1 (Fig. S1 e-h). The residues Ser76 and Gln75 from IL-6 predominantly form the H-bond with carnosol with the highest H-bond occupancy. While in the case of parthenolide, Met67 of IL-6 showed 11.3% as the highest occupancy in 50 ns MD simulations. The H-bond occupancy showed quercetin and genistein have more stable interactions (Table 2).

Apart from the hydrogen-bonded interactions quercetin and genistein also formed stable van der Waals and hydrophobic interactions with IL-6 and IL-6Rα (Fig. S1). Upon structure analysis of 50 ns trajectories of genistein and quercetin complexes, a significant displacement was observed in the binary complex (Fig. 4b and d) indicating the destabilization of the IL-6 and IL-6Rα in comparison to IL-6/IL-6Rα Apo protein (Fig. 4a). On the other

Table 2
Hydrogen-bond occupancy and MM/PBSA total binding energy of ligands with IL-6/IL-6Rα complex.

Phytocompound	IL-6/IL-6R	Occupancy (%)	MM/PBSATotal Energy (kJ/mol)
Genistein	Gen (OAQ) - 179 ARG HE (IL-6)	38.3	−80.560 ± 0.106
	Gen (OAQ) −179 ARG H21 (IL-6)	21.2	
	Gen (OAI) - 281 GLN E21(IL-6R)	19.2	
	Gen (OAT)- 182 ARG H11(IL-6)	14.0	
	Gen (OAQ) – 179 ARG H11(IL-6)	11.0	
Quercetin	Que (HAV)- 165 ASP O (IL-6R)	86.4	
	Que (OAR) - 164 GLY H (IL-6R)	83.4	
	Que (HAT)- 175 GLN OE1(IL-6)	76.4	
	Que (OAR) - 230 TYR HH (IL-6R)	67.4	
	Que (OAU)- 104 ARG H21(IL-6)	58.8	
	Que (OAS) - 176 SER HG (IL-6R)	33.9	−63.061 ± 0.108
	Que (HAU)- 162 PRO O (IL-6R)	29.2	
	Que (HAU)- 191 GLY O (IL-6R)	26.8	
	Que (OAU)- 104 ARG H21 (IL-6)	10.6	
Carnosol	Car (OAB)- 75 GLN H(IL-6)	31.9	
	Car (OAB)- 76 SER H(IL-6)	24.6	−50.991 ± 0.089
	Car (OAB)- 75 GLN E21(IL-6)	22.4	
Parthenolide	Par (OAA)- 67 MET H (IL-6)	11.3	−44.744 ± 0.132

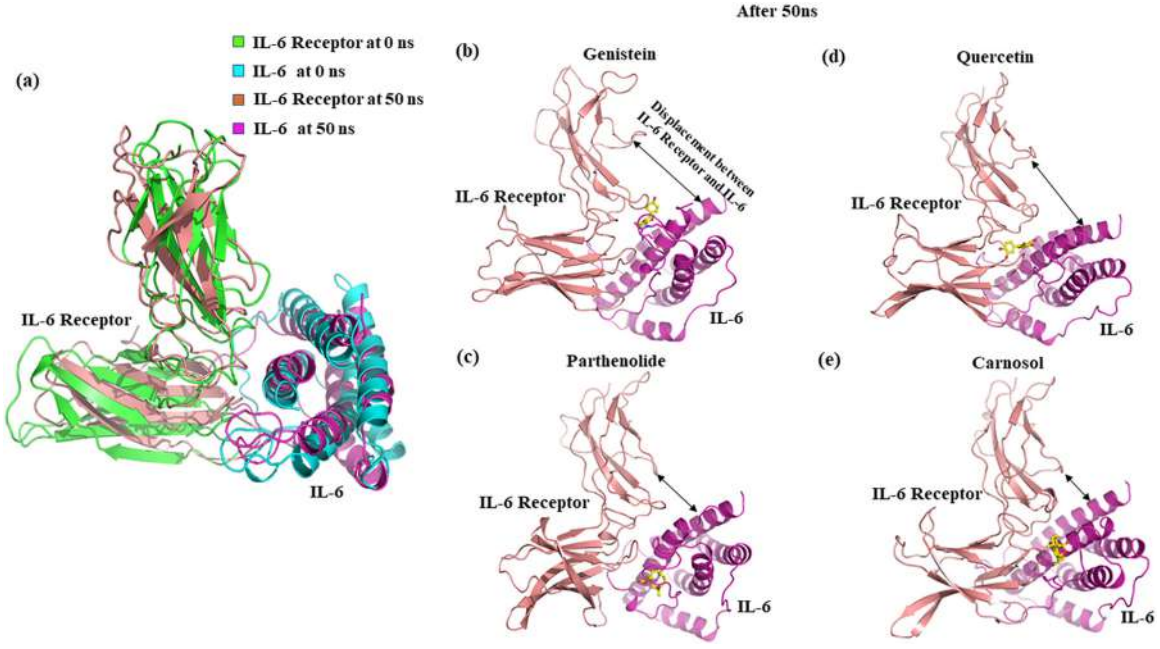

Fig. 4. IL-6 and IL-6 receptor complex before and after 50 ns Molecular dynamics simulations. (a) The superposition of IL-6-IL-6Rα complex before (0 ns) and after 50 ns MD simulations without ligand. The displacement between IL-6 and IL-6 receptor after MD simulations with (b) genistein (c) parthenolide (d) quercetin, and (e) carnosol.

hand, no significant variations were observed in parthenolide and carnosol complexes (Fig. 4c and e). Apart from the interactions, no significant change in RMSF and displacement were observed in the parthenolide or carnosol complexes. The MM/PBSA analysis was also performed for all IL-6 and IL-6R phytocompound complexes. The analysis showed that Genistein (−80.56 KJ/mol) and quercetin (−63.06 KJ/mol) have higher binding energy in comparison to carnosol (−50.99 KJ/mol), and parthenolide (−44.74 KJ/mol) (Table 2) (Fig. S2). Hence, based on these analyses both genistein and quercetin were selected for further MTT cell-based assay in MDA-MB-453 cell lines.

3.4. MTT assay

The cytotoxicity of genistein and quercetin in MDA-MB-453 breast cancer cells was assessed using the MTT assay. In the presence of genistein, a significant decrease in cell proliferation was observed in MDA-MB-453 cells at 24 and 48 h of drug treatment in both dose and time-dependent manners. Genistein showed a comparatively low IC_{50} of 38 µM at 24 h and 18 µM at 48 h, while quercetin showed IC_{50} greater than 50 µM at 24 h and 35 µM at 48 h of drug treatment (Fig. 5a-d).

4. Discussion

The IL-6 mediated STAT3 pathway plays a pivotal role in tumor growth and metastasis. IL-6 is a pleiotropic cytokine that can either promote or inhibit tumor growth [32]. In human physiology, it plays a central role in the activation of different inflammatory pathways [33]. Clinical studies have shown that an increase in IL-6 expression leads to tumor progression at different stages of breast cancer [34,35]. IL-6 is also reported as a marker for assessing ad-

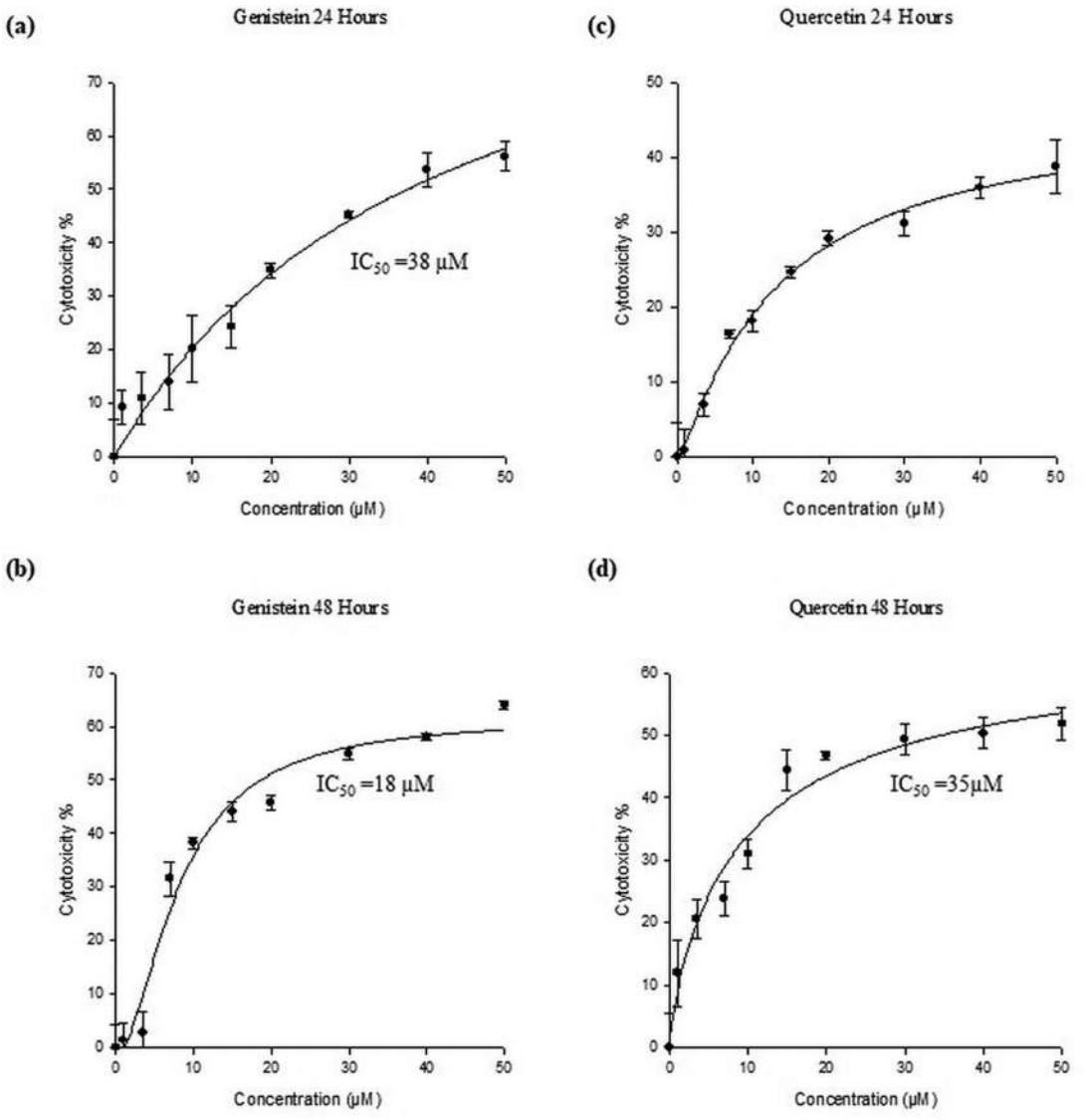

Fig. 5. IC_{50} of genistein and quercetin on MDA-MB-453 breast cancer cell line. Genistein showed relatively low IC_{50} at (a) 24 h (38 µM) and (b) at 48 h (18 µM) in comparison with quercetin with IC_{50} (c) at 24 h (>50 µM) and (d) at 48 h (35 µM).

vanced stages of breast cancer [11]. In addition, the IL-6 mediated STAT3 signaling is also reported to play an important role in cancer progression by angiogenesis and inducing a transition from epithelial to mesenchymal. Thus, in preclinical and clinical studies, the STAT3 signaling pathway remains an attractive therapeutic target [17,36,37]. To initiate the cascade of STAT3 signaling pathway proteins, IL-6/IL-6R/GP130 needs to form a hexameric complex. To form the hexameric complex, IL-6 initially forms interactions IL-6R and GP130 at epitopes site I, II and III (Fig. S3). Upon interactions, two heterotrimers of IL-6/IL-6R/GP130 are assembled to form a functional hexamer [8,9]. Once the complex is formed it triggers the STAT3 phosphorylation, which leads to the inhibition of IL-6 mediated cascade [10].

To halt the IL-6 mediated STAT3 pathway in cancer, inhibitors were designed to target the IL-6 epitopes site I, II, and III. The compound XZH-5 is targeted at site II and site III to inhibit IL-6 mediated STAT3 phosphorylation. Similarly, raloxifene, bazedoxifene and natural inhibitor MDL-A target site II at IL-6/GP130 interface [10]. The antibody Olokizumab binds at site III to block IL-6 mediated gp130 interaction [38]. In this study, we targeted site I of IL-6 and IL-6Rα binding site using potent phytocompounds. Our docking and MD simulations results showed that the natural compounds genistein and quercetin formed stable interactions at site I of the IL-6/IL-6Rα interface.

The cytotoxicity assay also showed genistein significantly decreased cell proliferation of MDA-MB-453 breast cancer cells with IC_{50} of 38 µM at 24 h and 18 µM at 48 h. Similarly, quercetin also showed IC_{50} more than 50 µM at 24 h and IC_{50} value of 35 µM for 48 h. Based on our results, we propose a mechanism by which genistein inhibits IL-6 mediated STAT3 signaling pathway in cancer cells (Fig. 6). The IL-6/STAT3 pathway is initiated by binding of IL-6 receptor to IL-6 molecule at site I interface which induces a conformational change in IL-6 and increases its affinity towards the binding of GP130 at site III interface to form a functional hexamer. Binding of genistein at site I prevent the binding of IL-6 receptor and thereby receptor-induced conformational change in IL-6 is lost which is required for binding of GP130 (Fig. S4). As a result, the whole heterotrimeric complex could not be formed to initiate further downstream STAT3 phosphorylation and cascade of the signal pathway.

Our results corroborate with the epidemiological studies where it has been shown that the genistein in soy-derived food helps

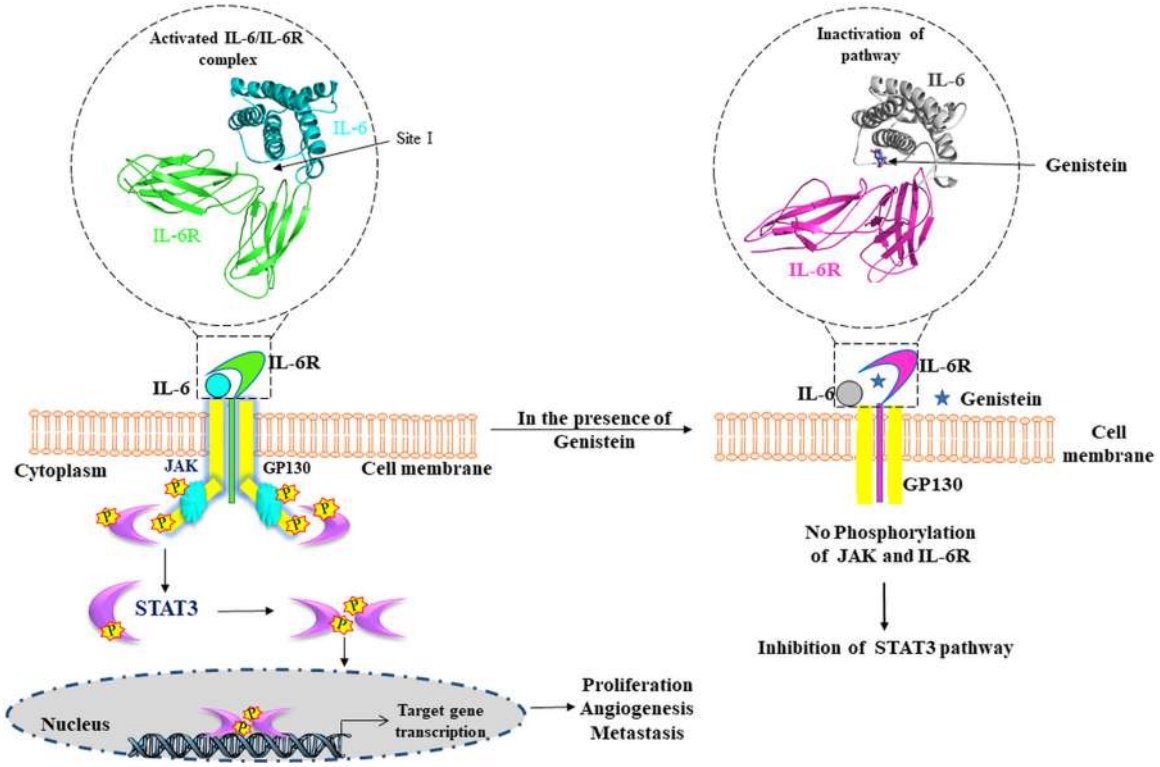

Fig. 6. Proposed mechanism for the inhibition of STAT3 pathway by genistein: (a) Shows the IL-6 mediated activation of STAT3 signaling and their subsequent downstream signaling pathway initiated by IL-6/IL-6Rα/GP130 complex. (b) Shows the binding of genistein at the site-I interface between IL-6 and IL-6Rα destabilize IL-6/IL-6Rα/GP130 complex formation leading to the inactivation of the IL-6 mediated STAT3 signaling pathway.

in prognosis with a reduction in breast cancer recurrence [39,40]. Similarly, in the MCF-7 breast cancer cell line, it has been reported that genistein inhibits cell growth at 20 nM genistein concentration [41], although the underlying mechanism of dose-dependent differential action needs further investigation. Other studies reported in SK-OV-3 ovarian cancer and colon cancer also shown that genistein at 90 μM and 200 μmol/L concentration inhibit cell proliferation [42-44]. Together with other studies, our proposed mechanism demonstrates that the phytocompound genistein can be taken forward for further studies in the field of breast cancer therapeutics.

5. Conclusion

In conclusion, molecular docking, and MD simulation-based analyses showed that genistein inhibits the binding of IL-6 with IL-6Rα to prevent the IL-6 mediated STAT3 dependent carcinogenesis. Furthermore, cytotoxicity of genistein with low IC_{50} (38 μM) at MDA-MB-453 metastatic breast cancer cell line indicates that this phytocompound can be considered as a potential therapeutic compound against cancer cell lines. Therefore, bioinformatics-guided cell culture assays on phytochemicals would increase the acceptance of phytotherapy as adjuvant therapy for breast cancer treatment.

Ethical approval

The article does not involve any study with human participants or animals to be performed by any of the authors.

Declaration of Competing Interest

The authors have no substantial financial or commercial conflicts of interest with the current work or its publication.

CRediT authorship contribution statement

Saurabh Sharma: Data curation, Writing – original draft, Visualization, Investigation, Software, Validation. **Lakshay Malhotra:** Visualization, Investigation, Software, Validation. **Prakarsh Yadav:** Visualization, Investigation, Software, Validation. **Vandana Mishra:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Radhey Shyam Sharma:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Ethayathulla Abdul Samath:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Acknowledgments

VM thanks Institute of Eminence, University of Delhi for Faculty Research Grant-Projects (IoE/FRP/LS2020/27 dated 31.12.2020). SS thank the Indian Council of Medical Research (ICMR), New Delhi, and All India Institute of Medical Sciences, New Delhi for the research associate fellowship. LM thank DBT for the fellowship.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.132668.

References

[1] J. Bromberg, T.C. Wang, Inflammation and cancer: IL-6 and STAT3 complete the link, Cancer Cell 15 (2009) 79–80, doi:10.1016/j.ccr.2009.01.009.
[2] A. Masjedi, V. Hashemi, M. Hojjat-Farsangi, G. Ghalamfarsa, G. Azizi, M. Yousefi, F. Jadidi-Niaragh, The significant role of interleukin-6 and its signaling pathway in the immunopathogenesis and treatment of breast cancer, Biomed. Pharmacother. 108 (2018) 1415–1424, doi:10.1016/j.biopha.2018.09.177.
[3] K. Nakamura, T. Nakamura, T. Iino, T. Hagi, K. Kita, K. Asanuma, A. Sudo, Expression of interleukin-6 and the interleukin-6 receptor predicts the clinical

outcomes of patients with soft tissue sarcomas, Cancers (Basel) 12 (2020), doi:10.3390/cancers12030585.
[4] A. Omar, A. Bakr, N. Ibrahim, Female medical students' awareness, attitudes, and knowledge about early detection of breast cancer in Syrian Private University, Syria, Heliyon 6 (2020), doi:10.1016/j.heliyon.2020.e03819.
[5] C.-.Y. Loh, A. Arya, A.F. Naema, W.F. Wong, G. Sethi, C.Y. Looi, Signal transducer and activator of transcription (STATs) proteins in cancer and inflammation: functions and therapeutic implication, Front. Oncol. 9 (2019) 48, doi:10.3389/fonc.2019.00048.
[6] I. Segatto, G. Baldassarre, B. Belletti, STAT3 in breast cancer onset and progression: a matter of time and context, Int. J. Mol. Sci. 19 (2018) 2818, doi:10.3390/ijms19092818.
[7] H. Yu, D. Pardoll, R. Jove, STATs in cancer inflammation and immunity: a leading role for STAT3, Nat. Rev. Cancer 9 (2009) 798–809, doi:10.1038/nrc2734.
[8] M.J. Boulanger, D. Chow, E.E. Brevnova, K.C. Garcia, Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex, Science 300 (2003) 2101–2104, doi:10.1126/science.1083901.
[9] J.N. Varghese, R.L. Moritz, M.-.Z. Lou, A. van Donkelaar, H. Ji, N. Ivancic, K.M. Branson, N.E. Hall, R.J. Simpson, Structure of the extracellular domains of the human interleukin-6 receptor -chain, Proc. Natl. Acad. Sci. 99 (2002) 15959–15964, doi:10.1073/pnas.232432399.
[10] H. Li, H. Xiao, L. Lin, D. Jou, V. Kumari, J. Lin, C. Li, Drug design targeting protein-protein interactions (PPIs) using multiple ligand simultaneous docking (MLSD) and drug repositioning: discovery of raloxifene and bazedoxifene as novel inhibitors of IL-6/GP130 interface, J. Med. Chem. 57 (2014) 632–641, doi:10.1021/jm401144z.
[11] N. Vainer, C. Dehlendorff, J.S. Johansen, Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer, Oncotarget 9 (2018) 29820–29841, doi:10.18632/oncotarget.25661.
[12] H. Kitamura, Y. Ohno, Y. Toyoshima, J. Ohtake, S. Homma, H. Kawamura, N. Takahashi, A. Taketomi, Interleukin-6/STAT3 signaling as a promising target to improve the efficacy of cancer immunotherapy, Cancer Sci. 108 (2017) 1947–1952, doi:10.1111/cas.13332.
[13] M. Liu, C. Xiao, M. Sun, M. Tan, L. Hu, Q. Yu, Xanthatin inhibits STAT3 and NF-κB signalling by covalently binding to JAK and IKK kinases, J. Cell. Mol. Med. 23 (2019) 4301–4312, doi:10.1111/jcmm.14322.
[14] S.-.W. Wang, Y.-.M. Sun, The IL-6/JAK/STAT3 pathway: potential therapeutic strategies in treating colorectal cancer (Review), Int. J. Oncol. 44 (2014) 1032–1040, doi:10.3892/ijo.2014.2259.
[15] K.S. Siveen, S. Sikka, R. Surana, X. Dai, J. Zhang, A.P. Kumar, B.K.H. Tan, G. Sethi, A. Bishayee, Targeting the STAT3 signaling pathway in cancer: role of synthetic and natural inhibitors, Biochimica et Biophysica Acta (BBA) 1845 (2014) 136–154, doi:10.1016/j.bbcan.2013.12.005.
[16] A.J. West, V. Tsui, S.S. Stylli, H.P.T. Nguyen, A.P. Morokoff, A.H. Kaye, R.B. Luwor, The role of interleukin-6-STAT3 signalling in glioblastoma, Oncol. Lett. 16 (2018) 4095–4104, doi:10.3892/ol.2018.9227.
[17] A.L.A. Wong, J.L. Hirpara, S. Pervaiz, J.-.Q. Eu, G. Sethi, B.-.C. Goh, Do STAT3 inhibitors have potential in the future for cancer therapy? Expert Opin. Investig. Drug. 26 (2017) 883–887, doi:10.1080/13543784.2017.1351941.
[18] M.S. Abu-Darwish, T. Efferth, Medicinal plants from near east for cancer therapy, Front. Pharmacol. 9 (2018), doi:10.3389/fphar.2018.00056.
[19] R. Mishra, S. Sharma, R.S. Sharma, S. Singh, M.M. Sardesai, S. Sharma, V. Mishra, Viscum articulatum Burm. f. aqueous extract exerts antiproliferative effect and induces cell cycle arrest and apoptosis in leukemia cells, J. Ethnopharmacol. 219 (2018) 91–102, doi:10.1016/j.jep.2018.03.005.
[20] A.A. Al-Fatlawi, A.A. Al-Fatlawi, M. Irshad, null Rahisuddin, A. Ahmad, Effect of parthenolide on growth and apoptosis regulatory genes of human cancer cell lines, Pharm. Biol. 53 (2015) 104–109, doi:10.3109/13880209.2014.911919.
[21] A.V. Mavrogiannis, I. Kokkinopoulou, C.K. Kontos, D.C. Sideris, Effect of vinca alkaloids on the expression levels of microRNAs targeting apoptosis-related genes in breast cancer cell lines, Curr. Pharm. Biotechnol. 19 (2018) 1076–1086, doi:10.2174/1389201019666181112103204.
[22] X. Wang, H. Zhang, X. Chen, Drug resistance and combating drug resistance in cancer, Cancer Drug Resist. 2 (2019) 141–160, doi:10.20517/cdr.2019.10.
[23] D.O. Levitsky, V.M. Dembitsky, Anti-breast cancer agents derived from plants, Nat. Prod. Bioprospect. 5 (2015) 1–16, doi:10.1007/s13659-014-0048-9.
[24] M. Younas, C. Hano, N. Giglioli-Guivarc'h, B. Haider Abbasi, Mechanistic evaluation of phytochemicals in breast cancer remedy: current understanding and future perspectives, RSC Adv. 8 (2018) 29714–29744, doi:10.1039/C8RA04879G.
[25] A. Kapinova, P. Kubatka, O. Golubnitschaja, M. Kello, P. Zubor, P. Solar, M. Pec, Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention, Environ. Health Prev. Med. 23 (2018) 36, doi:10.1186/s12199-018-0724-1.
[26] L. Malhotra, H.K.V. Goyal, S. Jhuria, K. Dev, S. Kumar, M. Kumar, P. Kaur, A.S. Ethayathulla, Curcumin rescue p53Y220C in BxPC-3 pancreatic adenocarcinomas cell line: evidence-based on computational, biophysical, and in vivo studies, Biochimica et Biophysica Acta (BBA) 1865 (2021) 129807, doi:10.1016/j.bbagen.2020.129807.
[27] M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, E. Lindahl, GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers, SoftwareX 1-2 (2015) 19–25, doi:10.1016/j.softx.2015.06.001.
[28] B. Hess, H. Bekker, H.J.C. Berendsen, J.G.E.M. Fraaije, LINCS: A linear constraint solver for molecular simulations, J. Comput. Chem. 18 (1997) 1463–1472. doi:10.1002/(SICI)1096-987X(199709)18:12⟨1463::AID-JCC4⟩3.0.CO;2-H
[29] M. Kawata, M. Mikami, U. Nagashima, Rapid calculation of the Coulomb component of the stress tensor for three-dimensional systems with two-dimensional periodicity, J. Chem. Phys. 115 (2001) 4457–4462, doi:10.1063/1.1395564.
[30] P. Yadav, M. Kumar, R. Bansal, P. Kaur, A.S. Ethayathulla, Structure model of ferrochelatase from Salmonella Typhi elucidating metalation mechanism, Int. J. Biol. Macromol. 127 (2019) 585–593, doi:10.1016/j.ijbiomac.2019.01.066.
[31] R. Kumari, R. Kumar, O.S.D.D. Consortium, A. Lynn, g_mmpbsa—A GROMACS Tool for High-Throughput MM-PBSA Calculations, ACS Publications, 2014, doi:10.1021/ci500020m.
[32] D.T. Fisher, M.M. Appenheimer, S.S. Evans, The two faces of IL-6 in the tumor microenvironment, Semin. Immunol. 26 (2014) 38–47, doi:10.1016/j.smim.2014.01.008.
[33] C. Gabay, Interleukin-6 and chronic inflammation, Arthritis Res. Ther. 8 (2006) S3, doi:10.1186/ar1917.
[34] O. Kornasiewicz, M. Grąt, K. Dudek, Z. Lewandowski, Z. Gorski, K. Zieniewicz, M. Krawczyk, Serum levels of HGF, IL-6, and TGF-α after benign liver tumor resection, Adv Med Sci 60 (2015) 173–177, doi:10.1016/j.advms.2015.01.008.
[35] Y. Ma, Y. Ren, Z.-.J. Dai, C.-.J. Wu, Y.-.H. Ji, J. Xu, IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients, Adv. Clin. Exp. Med. 26 (2017) 421–426, doi:10.17219/acem/62120.
[36] J. Huynh, A. Chand, D. Gough, M. Ernst, Therapeutically exploiting STAT3 activity in cancer — using tissue repair as a road map, Nat. Rev. Cancer 19 (2019) 82–96, doi:10.1038/s41568-018-0090-8.
[37] R. Siersbæk, V. Scabia, S. Nagarajan, I. Chernukhin, E.K. Papachristou, R. Broome, S.J. Johnston, S.E.P. Joosten, A.R. Green, S. Kumar, J. Jones, S. Omarjee, R. Alvarez-Fernandez, S. Glont, S.J. Aitken, K. Kishore, D. Cheeseman, E.A. Rakha, C. D'Santos, W. Zwart, A. Russell, C. Brisken, J.S. Carroll, IL6/STAT3 signaling hijacks ER enhancers to drive breast cancer metastasis, Cancer Cell 38 (2020) 412–423, doi:10.1016/j.ccell.2020.06.007.
[38] S. Shaw, T. Bourne, C. Meier, B. Carrington, R. Gelinas, A. Henry, A. Popplewell, R. Adams, T. Baker, S. Rapecki, D. Marshall, A. Moore, H. Neale, A. Lawson, Discovery and characterization of olokizumab, MAbs 6 (2014) 773–781, doi:10.4161/mabs.28612.
[39] T.A. Mace, M.B. Ware, S.A. King, S. Loftus, M.R. Farren, E. McMichael, S. Scoville, C. Geraghty, G. Young, W.E. Carson, S.K. Clinton, G.B. Lesinski, Soy isoflavones and their metabolites modulate cytokine-induced natural killer cell function, Sci. Rep. 9 (2019) 5068, doi:10.1038/s41598-019-41687-z.
[40] S. Poschner, A. Maier-Salamon, M. Zehl, J. Wackerlig, D. Dobusch, B. Pachmann, K.L. Sterlini, W. Jäger, The impacts of genistein and daidzein on estrogen conjugations in human breast cancer cells: a targeted metabolomics approach, Front. Pharmacol 8 (2017) 699, doi:10.3389/fphar.2017.00699.
[41] N.C. Lucki, M.B. Sewer, Genistein stimulates MCF-7 breast cancer cell growth by inducing acid ceramidase (ASAH1) gene expression, J. Biol. Chem. 286 (2011) 19399–19409, doi:10.1074/jbc.M110.195826.
[42] A. Antosiak, K. Milowska, K. Maczynska, S. Rozalska, T. Gabryelak, Cytotoxic activity of genistein-8-C-glucoside form Lupinus luteus L. and genistein against human SK-OV-3 ovarian carcinoma cell line, Med. Chem. Res. 26 (2017) 64–73, doi:10.1007/s00044-016-1725-5.
[43] P. Zhou, C. Wang, Z. Hu, W. Chen, W. Qi, A. Li, Genistein induces apoptosis of colon cancer cells by reversal of epithelial-to-mesenchymal via a Notch1/NF-κB/slug/E-cadherin pathway, BMC Cancer 17 (2017) 813, doi:10.1186/s12885-017-3829-9.
[44] W. F. Van Gunsteren & H. J. C. Berendsen, (1988), A Leap-frog Algorithm for Stochastic Dynamics, Molecular Simulation, 1:3, 173-185, doi:10.1080/08927028808080941.