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Research Article

Antiproliferative Efficacy of Kaempferol on Cultured Daudi Cells: An *In Silico* and *In Vitro* Study

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There is always a constant need to develop alternative or synergistic anticancer drugs with minimal side effects. One important strategy to develop effective anticancer agents is to investigate potent derived compounds from natural sources. The present study was designed to determine antiproliferative activity of Kaempferol using *in silico* as well as *in vitro* study. Docking was performed using human GCN5 (hGCN5) protein involved with cell cycle, apoptosis, and glucose metabolism. Cell viability and cytotoxicity on Daudi cells were evaluated by trypan blue and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in a dose and time dependent manner, respectively. The compound inhibited the proliferation and growth of the Daudi cells, through induced cell death. The pure compound proved lead inhibitors of cell proliferation, thus manifesting significant antiproliferative activity. The docking results revealed that Kaempferol exhibited binding interaction to hGCN5 protein. Further, molecular dynamics using the dock pose of hGCN5-Kaempferol complex were performed to understand the basic structural unit which lead to inefficiency in binding and, therefore, pronounced instability and its possible consequences of reduced binding affinity. The data obtained in this study indicates that Kaempferol is a promising compound leading to inhibition of Daudi cell growth and proliferation.

1. Introduction

Cancer, also called malignant tumor or malignant neoplasm, is characterized by uncontrolled proliferation of the cells with the potential to invade or spread to other parts of the body [1, 2]. The continuous multiplication of cancer cells spreads into tumor and travels through the circulatory system to other organs of the body resulting in metastasis. Cancer is caused by external and internal factors, which may act together or in a sequence to cause cancer [3]. Cancer is a leading cause of disease worldwide. In 2012 about 14.1 million new cancer cases occurred with 8.2 million deaths [4]. Treatment for cancer involves surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, hormonal therapy, and so forth which depends on type, location, and grade of cancer. Under normal physiological conditions, the uncontrolled growth of damaged cells is restricted by apoptosis. However, these cells can escape the regulatory mechanisms of apoptosis as a result of secondary mutations in genes that regulate apoptosis. Cancer chemoprevention is defined as pharmacological interference with synthetic or naturally occurring compounds that may prevent, inhibit, or reverse carcinogenesis or prevent development of invasive cancer.

In this investigation the target molecule selected for docking studies is the hGCN5 protein, which plays a key role in cell cycle and apoptotic regulatory mechanism in a cell. It has been established that hGCN5 protein, which catalyzes the transfer of the acetyl group from the cofactor acetyl coenzyme A (acetyl-coA) to the side chain amino group of lysine residues in the N-terminus of core histone proteins,

is classified as a HAT enzyme [5, 6]. hGCN5 is also known to carry out the acetylation of nonhistone proteins, such as p53—the tumor suppressor molecule [7], the oncoprotein (c-MYC) [8], and the metabolic coactivator (PGC-lα) [9]. The evolutionary links investigated reveal that it was part of the ancestral form of metazoan GCN5 since it shows the presence of the N-terminal domain in human homologs [10]. The first human homolog of yGCN5 is hGCN5 (called hGCN5-L), which is highly homologous to yeast GCN5 but contains an extended amino-terminal domain [6, 10]. These researchers have also explained that hGCN5 exists in two forms resulting from alternative splicing [6, 10]. The longer form (813 amino acids) shares strong homologies with P/CAF. The shorter form (476 amino acids) exhibits a size similar to yeast GCN5 and corresponds to the C-terminal part of the long hGCN5. Less theoretical studies have been performed on the structure and function of hGCN5 which reflects its significance on disease related researches. In addition, crystallographic structure analysis of hGCN5 reveals that this protein has strategically oriented units that work to facilitate a continuous proton transfer mechanism. The location of glutamic residue is beyond the proton transfer distance, while one strictly conserved water molecule among different GCN5 structures could form a continuous proton transfer pathway by hydrogen bonds called as proton wire, which has been also demonstrated in certain other biological systems [11–15]. These basic structural details of the target molecule form the fundamental basis for the docking studies carried out.

Flavonoids, a large group of low molecular weight naturally occurring polyphenolic compounds found in a large variety of fruits and vegetables [16], have numerous biological properties. Flavonoids are commonly used to prevent cardiovascular disease. Moreover, previous studies have suggested that dietary flavonoids are considered to have anticancer potential against various human cancers, such as breast cancer [17], prostate cancer [18], and colorectal cancer [19]. Furthermore, there is evidence that flavonoids inhibit cell growth and proliferation and induces cellular toxicity in cancer cells. Kaempferol, (3,4′,5,7-tetrahydroxyflavone) a natural polyphenol belonging to the flavonoid group, is synthesized at high levels in fruits and vegetables and possesses various important biological properties including antioxidant, antimicrobial, cardiovascular, and antitumor activities. It promotes apoptosis and cell cycle arrest in various cancer cell lines, including lung cancer cells [20], breast cancer cells [21], and colon cancer cells [22]. Moreover, it also inhibits the migration and invasiveness of glioma cells [23]. Kaempferol is therefore selected in this study due to its immense potential.

The present study consequently employs molecular docking which is a frequently used bioinformatic tool, used here specifically to evaluate how micromolecules called ligands (flavonoids) and the target macromolecules (protein) bind and interact together [24, 25]. Such *in silico* modalities help to identify drug targets via computer-based tools, which aid in evaluation of target structures to visualize relative active sites, generate possible molecules which recognize the target, assess their relative binding affinities, and further optimize molecules to improve binding characteristics. One such program employed is autodock tools (ADT), a package of automated docking tools and design to predict how small molecules bind to a target protein of known 3D structure. In this investigation therefore, our focus was mainly to determine optimum binding energies of the docked molecules and in addition visualize the position of the ligand in the protein binding site [26]. The outcome would provide important leads for identifying and generating potential candidates for drug interactions and also understand the molecular details of such binding. The objective of the present work is to study the *in silico* inhibitory activity of commercially available flavonoid Kaempferol against the hGCN5 protein.

In the present study, we have explored molecular interaction of Kaempferol into hGCN5 by molecular docking as well as molecular dynamics simulation (*in silico*) and assayed the cytotoxic activity of Kaempferol on Daudi lymphoma cells by MTT method (*in vitro*). This research has its focus directed towards developing Kaempferol as a lead compound.

2. Materials and Methods

2.1. Chemicals. Kaempferol was purchased from MP Biomedicals (France) whereas all other chemicals were purchased from Hi-Media Laboratories (India).

2.2. Culture of Lymphoma Cells. For cancer cell culture, Daudi cell line was obtained from the National Centre for Cell Science (NCCS), Pune. Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) and antibiotic antimycotic solution. Cell cultures were maintained in a CO_2 incubator at 5% CO_2 and 37°C for this investigation.

2.3. Cell Viability Assay by Trypan Blue Dye Exclusion Technique. Any compound, which is cytotoxic to cells, inhibits the cell growth proliferation and kills the cells. Trypan blue is a supravital dye, used to estimate the number of cells present in the population [27]. It has the ability to penetrate dead cells and give it a blue color. This method gives a score of dead and viable cells [28].

Cellular cytotoxicity induced by the Kaempferol was measured with trypan blue exclusion assay. Sterility was maintained throughout the procedure. For this study 2×10^6 cells were seeded into 24-well plates and treated with or without (as control) compound (12.5–400 μM) for 24, 48, and 72 h. After the incubation period, the cultures were harvested and washed twice with Phosphate Buffered Saline (PBS). The cell pellet was then resuspended with 0.5 mL PBS. Then, 20 μL of cells was mixed with equal volume of 0.4% trypan blue and was counted using a Neubauer haemocytometer by clear field microscopy. Viable and nonviable cells were counted. The percentage cytotoxicity was calculated using the equation shown below:

$$\% \text{ Viability} = \frac{\text{Live cell count}}{\text{Total cell count}} \times 100. \quad (1)$$

2.4. In Vitro Cytotoxicity Determination by MTT Assay. The ability of cells to survive a toxic insult is the basis of most cytotoxic assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. It is

described by the modified method of Mosmann and Wilson [29, 30].

The assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria. 2×10^6 viable cells/mL were plated into the 96-well cell culture plate. The compound was added with the concentrations with 12.5–400 μM, respectively, for 24, 48, and 72 h and incubated at 37°C. After incubation, the supernatants were removed and incubated with MTT (0.5 v/v) in RPMI 1640 without FBS for 4 h in a humidified atmosphere at 37°C and 5% CO_2 incubator. The absorbance (A) of the colored solution was quantified at 540 nm wavelengths by an enzyme linked immunoabsorbent assay reader (ELISA READER). Kaempferol and control were assayed in triplicate in three independent experiments. Percent growth inhibition of cells exposed to treatments was calculated as follows:

$$\% \text{ Inhibition} = \left(100 - \left(\frac{\text{Corrected mean Absorbance of sample}}{\text{Corrected mean Absorbance of control}}\right)\right) \times 100. \quad (2)$$

2.5. Preparation of Protein Target Structure and Ligand. The X-ray crystal structure of Daudi cell protein hGCN5 was retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) (http://www.pdb.org/) protein data bank under the PDB ID: 1Z4R. The structure was subjected to energy minimization with root mean square deviation (RMSD) value of 1.4795 Å. Ligand structure of Kaempferol (3D structure) was retrieved in structure data format (SDF) from PubChem (CID-5280863).

2.6. Molecular Docking. YASARA Structure 31 is used for the molecular docking simulations through the Autodock Vina 1.0. 32. The preparation of receptor and ligand files contains the removal of waters, addition of polar hydrogens, and authentication of active site residues for the docking simulations using YASARA Structure 31. Three-dimensional crystal structure of hGCN5 having resolution 1.74 Å includes 168-amino-acid chain length. The active side amino acid residues were decisive which is being used to dock molecules for automated docking and scoring. The active site residues of the hGCN5 are Cys 579, Ala 580, Val 581, Gln 586, Val 587, Lys 588, Gly 589, Tyr 590, Gly 591, Thr 592, Ala 681, Tyr 621, Phe 622, and Lys 624. According to the YASARA energy parameters assembled through docking poses, docking energy (kcal/mol), and root mean squared deviation (RMSD). The optimum docked pose was identified with relative lower binding energy for further analysis. Better binding of ligands shows better binding affinity through higher positive energies whereas no binding is recognized by negative energies mean. The docking energy was calculated by the following equation:

$$\Delta G = \Delta G_{\text{vdw}} + \Delta G_{\text{Hbond}} + \Delta G_{\text{elec}} + \Delta G_{\text{tor}} + \Delta G_{\text{desolv}}, \quad (3)$$

where

ΔG_{vdw} is van der Waals term for docking energy,

ΔG_{Hbond} is H bonding term for docking energy,

ΔG_{elec} is electrostatic term for docking energy,

ΔG_{tor} is transitional free energy term for ligand when the ligand transits from unbounded to bounded state,

ΔG_{desolv} is desolvation term for docking energy.

The crystallographic protein structure of hGCN5 with Kaempferol was selected for docking procedure validation by redocking approach and also to know the standard docking energy and binding site. The native ligand of cocrystalized complex was first extracted and redocked to its corresponding binding site. The docking of the ligand with the protein was performed for two times and the average of consecutive result was taken as final binding/docking energy. Docking studies on compound prepared through LigPrep were carried out in the active site of protein.

2.7. Molecular Dynamics Simulations. Molecular dynamics simulations have been carried out for the conformational changes and binding stability of the designed ligand in complex with hGCN5 protein. The removal of water molecules and optimization have been performed using (Y) AMBER force field [31], acid dissociation constant (pKa), and density 0.997 g L^{-1} set as per the YASARA Structure software to neutralize the system and subjected to energy minimization using steepest gradient approach (100 cycles). As per the software parameters force constant has been kept at 1000 kJ mol^{-1} nm^{-2}, while number of atoms N, pressure P, and temperature T were stored to standard level including temperature of 298 K (physiological condition, pH = 7.4) and pressure of 1 bar using Berendsen thermostat [32] and barostat [33], respectively. The selected complex was simulated for 10000 ps (production period) with frame capture at every 2500 ps step to analyze the trajectory by various evaluative quantities including root mean squared deviation (RMSD) and root mean squared fluctuation (RMSF). The protein-ligand interaction patterns obtained from the averaged conformations were graphically illustrated using Protein-Ligand Interaction Profiler 1.2.0 program.

2.8. Statistical Analysis. Each parameter was performed in triplicate and the results were expressed as mean ± standard error. The data was statistically analyzed by Student's "t-" test and the values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Cell Viability Assay. The cell viability assay conducted by trypan blue dye exclusion method showed that there was a highly significant ($p < 0.001$) decrease in viability with an increase in time and concentration in compound treated Daudi cells as compared to untreated controlled cells (Figure 1).

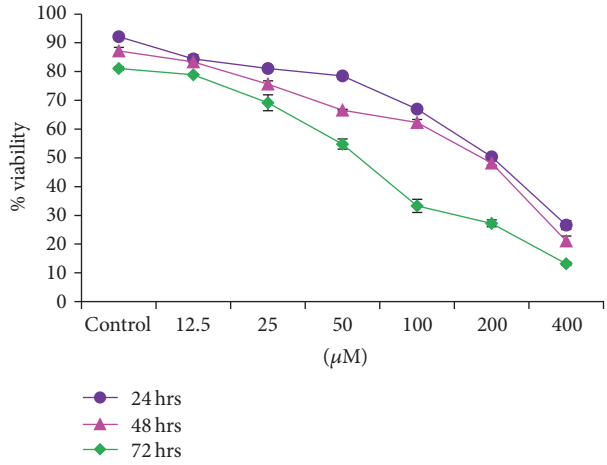


FIGURE 1: Effect of Kaempferol on percentage viability on Daudi cells. Values are mean ± S.E. for three individual experiments.

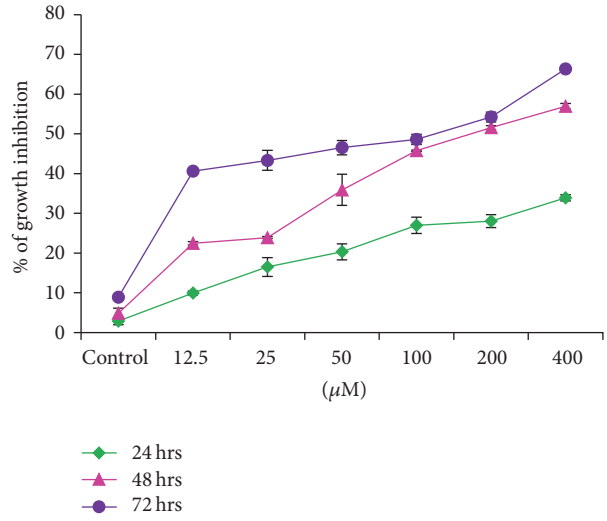


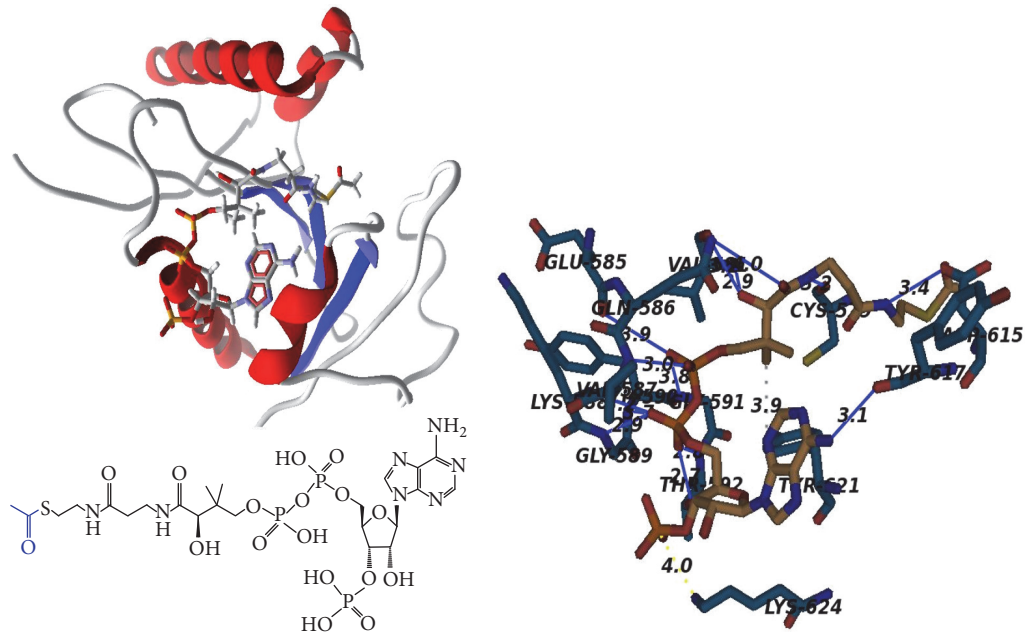
FIGURE 2: Effect of Kaempferol on the percentage decrease of growth proliferation in Daudi cells. Values are mean ± S.E. for three individual experiments. Highly significant ($p < 0.001$) increase in growth inhibition was observed in all the treated samples.

3.2. MTT Assay. Daudi cells were grown in 96 well plates for 24, 48, and 72 h along with different concentrations (12.5–400 μM) of Kaempferol. The formazan crystals were formed, following the reduction of MTT by metabolically active (viable) cells. The percentage decrease of proliferation after treatment with the pure compound is given in Figure 2 compared to control. The IC_{50} value was calculated by plotting the logarithmic graph in Microsoft Excel and was found to be 53.18 μM at 72 h. There was a significant increase in percentage of inhibition of growth proliferation with increased dose and time duration as compared to untreated control cells ($p < 0.001$).

3.3. Docking Analysis. In the molecular docking study native ligand-acetyl-CoA of hGCN5 protein has been redocked for the docking conformation [Figures 3(a) and 3(b)]. The native ligand shows the binding affinity of 8.54 kcal/mol with representing various types of interactions including hydrogen bonding interactions, hydrophobic interaction, water bridge, halogen bonds, and π-π interactions; and Gln 530, Leu 531, Met 534, Val 577, Phe 578, Cys 579, Ala 580, Val 581, Glu 585, Gln 586, Val 587, Lys 588, Gly 589, Tyr 590, Gly 591, Thr 592, Tyr 613, Ala 614, Asp 615, Tyr 617, Ala 618, Gly 620, Tyr 621, Phe 622, Lys 624, and Tyr 645 were found as responsible key residues. After the conformation the selected phytochemical, Kaempferol, has been taken for the docking analysis [Figures 4(a) and 4(b)]. It has shown 7.62 kcal/mol binding affinity with hGCN5 protein which is nearby the native ligand binding energy. There were 7 hydrogen bonds found with 1 hydrophobic interaction and π-stacking. The contacting amino acids residues were Cys 579, Ala 580, Val 581, Gln 586, Val 587, Lys 588, Gly 589, Tyr 590, Gly 591, Thr 592, Ala 681, Tyr 621, Phe 622, and Lys 624. The hydrophobic interaction and π-stacking were observed on Tyr 621 with 3.65 Å and 4.38 Å, respectively [Table 1].

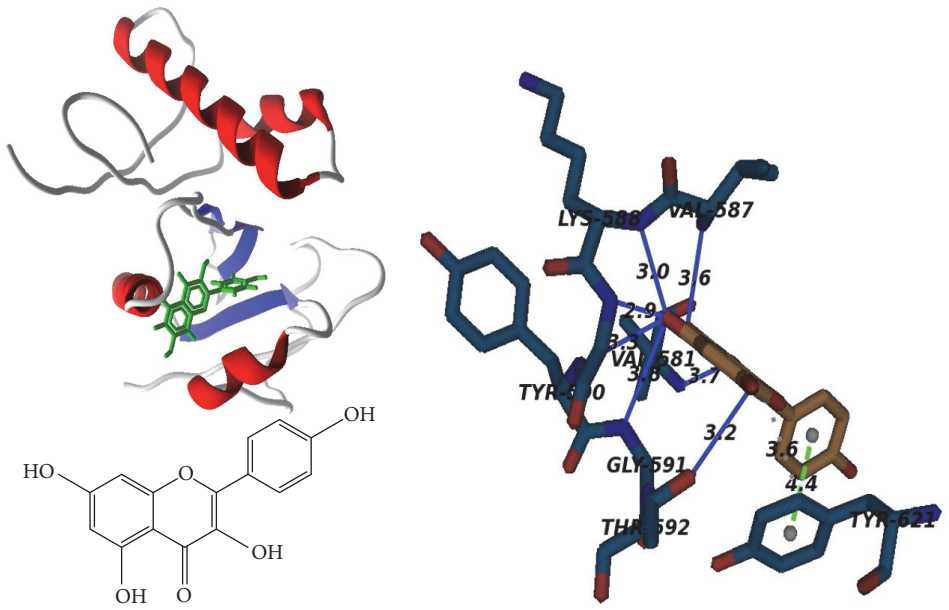
3.4. Molecular Dynamics. To understand the stability of the modeled hGCN5-Kaempferol complex with the aim to reveal its ability to penetrate through the biomembrane, we have carried out 10 ns of molecular dynamics simulations including various parameters which have been analyzed throughout the MD trajectory, especially root mean square deviation (RMSD), energy parameters [Figure 5], and total number of intra- and intermolecular hydrogen bonds with steepest descent function of MD simulation. Statistically significant results of the simulation are presented in Table 2. The average energy of the protein-ligand complex calculated over the simulation trajectory showed that hGCN5 developed effective interactions with the complete ligand dataset as their energies were in the range of −110407.838 kJ mol^{-1} to −83345.139 kJ mol^{-1}. Various energy results were measured during molecular dynamics simulations: −110407.838 mol^{-1} retrieved at initial start and −83345.139 kJ mol^{-1} at 10 ns time trajectory, and the average has been recognized with −83761.006 kJ mol^{-1}. Different types of interactions were formed after the end of the dynamics simulation.

The simulation was analyzed from initial (0 ns) to final (10 ns) conformations; RMSD of common target trajectories highlighted the importance of H bonds and hydrophobic interactions conferred by hot spot residues [Figures 6–11]. The protein-ligand interaction maps generated using Protein-Ligand Interaction Profiler showed that the dominance of the Val 587, Gly 589, and Tyr 621 exhibited less structural motions and acted as anchoring sites for ligand binding at initial level of dynamics simulation. The diverse patterns of H bonding with main and side chains of experimental hot spot residues (Val 518 and Cys 579) were obtained after the completion of the 10 ns target trajectories. The corresponding RMSD values for the Val 518, Cys 579, Gly 589, and Tyr 621 residues represent large fluctuations with more than 2 Å and may act as ligand binding determinants. Similarly we have analyzed the results for the various time intervals periods as shown in Table 1.



(a) (b)

Figure 3: (a) Redock pose of hGCN5 with native ligand-acetyl-CoA in 2D form. (b) Protein-ligand interaction from Protein-Ligand Interaction Profiler.



(a) (b)

Figure 4: (a) Dock pose of hGCN5 with ligand-Kaempferol in 2D form. (b) Protein-ligand interaction from Protein-Ligand Interaction Profiler (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line).

4. Discussion

The greatest challenge for phytochemical and pharmacological studies involves the identification of the specific compounds that are responsible for the beneficial effects and their modes of action, thereby delineating their useful functions as therapeutic drugs [34]. Flavonoids, commonly found in fruits and vegetables, are well known for their outstanding anticarcinogenic properties [35]. Kaempferol is a member of the flavonoid family and exhibits potent antiproliferative activity. Previous studies have demonstrated that Kaempferol inhibits growth and proliferation of cancer cells by various

Table 1: Docking score, binding energy, hydrogen bonds, dissociation constant, and contacting receptor.

Ligand	Binding energy [Kcal/mol]	Number of hydrogen bonds	Dissoc. constant	Contacting receptor residues
Control (native ligand-acetyl-CoA)	8.544	15	545965.187	Gln 530, Leu 531, Met 534, Val 577, Phe 578, Cys 579, Ala 580, Val 581, Glu 585, Gln 586, Val 587, Lys 588, Gly 589, Tyr 590, Gly 591, Thr 592, Tyr 613, Ala 614, Asp 615, Tyr 617, Ala 618, Gly 620, Tyr 621, Phe 622, Lys 624, Tyr 645
Kaempferol	7.6290	7	2557813.25	Cys 579, Ala 580, Val 581, Gln 586, Val 587, Lys 588, Gly 589, Tyr 590, Gly 591, Thr 592, Ala 681, Tyr 621, Phe 622, Lys 624

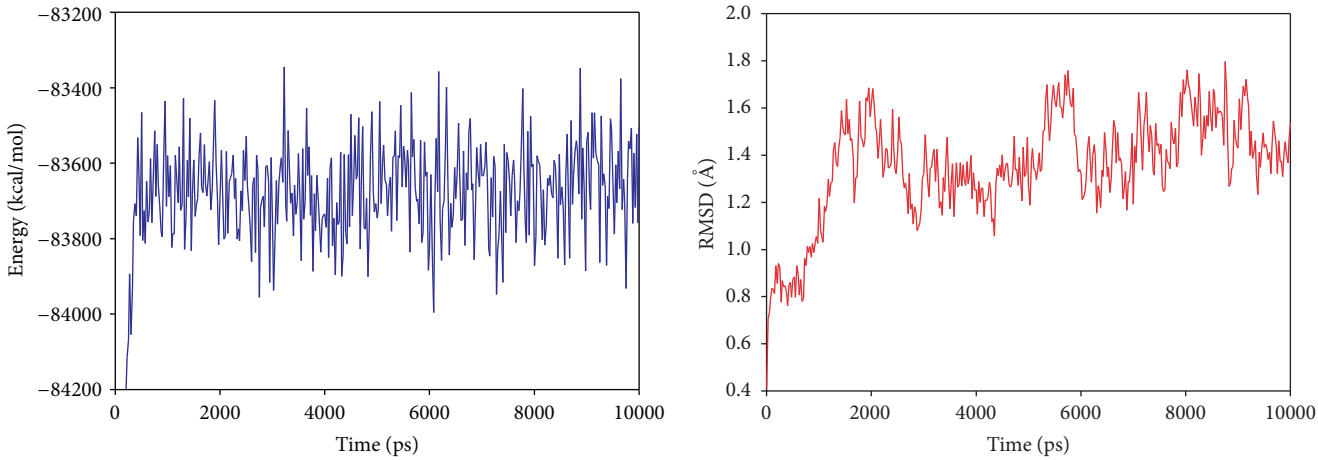


Figure 5: (a) Energy plots and (b) RMSD produced from MD trajectories of prioritized target: hGCN5-Kaempferol complex.

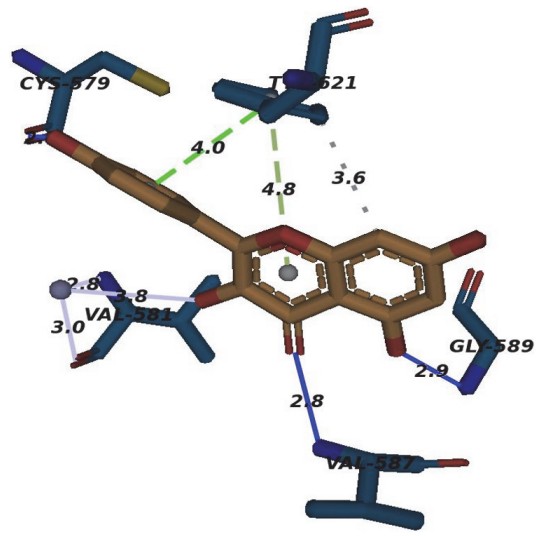


Figure 6: The protein-ligand interaction maps developed from initial molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

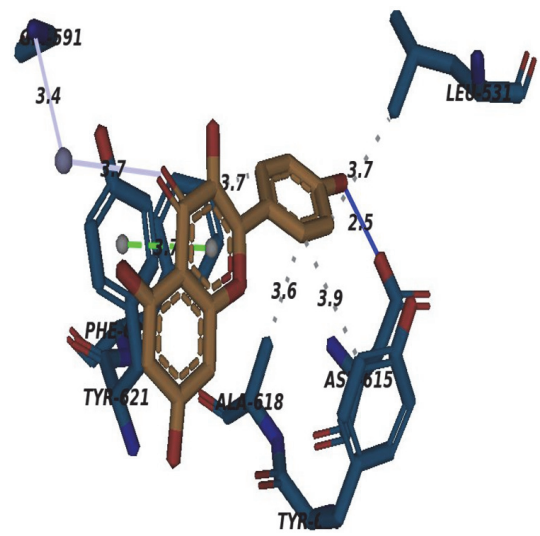


Figure 7: The protein-ligand interaction maps developed from 2.5 ns molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

Table 2: Protein-Ligand Interaction Profiler results of different time intervals of MD simulation of hGCN5-Kaempferol complex.

Time intervals (ps)	Hydrophobic interactions		Hydrogen bonds		Water bridges		π-stacking	
	Amino acids residue	Distance	Amino acids residue	Distance	Amino acids residue	Distance	Amino acids residue	Distance
0	Tyr 621	3.65	Cys 579	1.80	Val 581	3.76	Tyr 621	3.95
	Val 587	1.89	Val 518	3.00	Tyr 621	4.81		
	Gly 589	2.11						
100	Leu 531	3.72	Asp 615	2.16	Gly 591	3.72	Tyr 621	3.74
	Tyr 617	3.89	Asp 615	1.59				
	Ala 618	3.58						
	Phe 622	3.68						
200	Leu 531	3.21	Asp 615	2.11	Val 581	3.17	Tyr 621	4.02
	Tyr 617	3.94	Asp 615	2.54	Gln 586	2.76		
	Ala 618	3.67	Tyr 617	3.30				
	Phe 622	3.94						
300	Ala 618	3.36	Cys 579	1.75	Arg 533	3.42	Tyr 621	6.39
	Tyr 617	3.70	Asp 615	2.00	Cys 579	3.43	Phe 622	5.30
	Phe 622	3.65	Asp 615	1.56	Val 581	3.71		
					Val 581	3.59		
400	Asp 615	3.90	Cys 579	3.30	Val 581	3.66	Tyr 621	3.85
	Ala 618	3.36	Cys 579	1.65				
	Phe 622	3.58	Asp 615	2.72				
			Asp 615	1.60				
Average	Ala 618	3.26	Asp 615	3.16			Tyr 621	3.78
	Tyr 621	3.63	Asp 615	2.13			Tyr 621	3.73
	Phe 622	3.75	Asp 615	2.25				

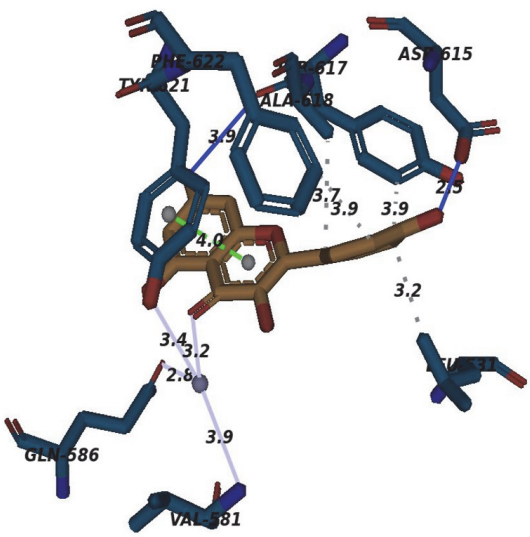


Figure 8: The protein-ligand interaction maps developed from 5 ns molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

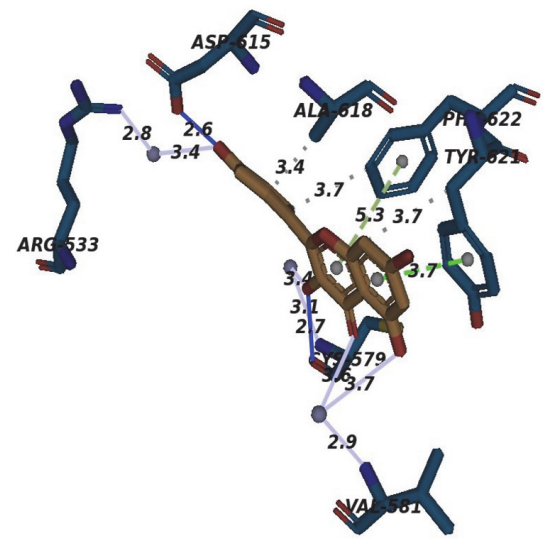


Figure 9: The protein-ligand interaction maps developed from 7.5 ns molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

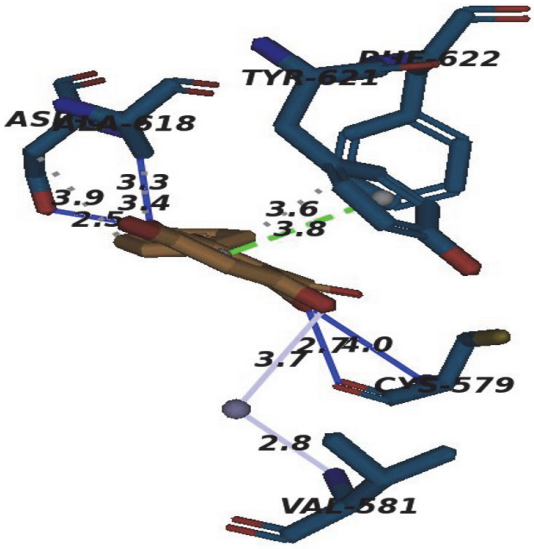


Figure 10: The protein-ligand interaction maps developed from 10 ns molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

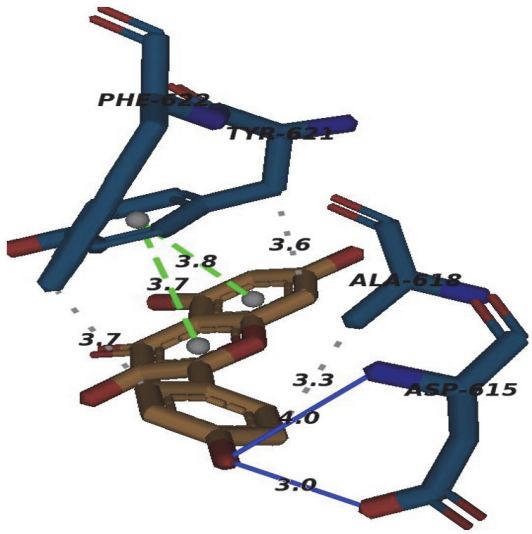


Figure 11: The protein-ligand interaction maps developed from average molecular dynamics (MD) conformations of prioritized common protein target: hGCN5-Kaempferol complex (hydrogen: blue color, hydrophobic interactions: gray color with dotted spots, and π-stacking: green color with dashed line) developed from Protein-Ligand Interaction Profiler server.

mechanisms including apoptosis [36], cell cycle arrest [37], and inhibition of tyrosine phosphorylation [38].

hGCN5 is also known as histone acetyltransferase GCN5. The protein is classified as a HAT enzyme. Kikuchi et al. [39] had mentioned that hGCN5 act as a supervisor in the normal cell cycle progression having comprehensive control over expressions of the cell cycle related genes as well as apoptosis related genes. Xu et al. [40] reported that loss of the GCN5 gene leads to increased apoptosis during mouse development. According to Inche and La Thangue [41] GCN5 is a critical regulator of cell cycle and it has potential role in cancer.

Currently the used synthetic drugs, which are known to protect against cancer, have their adverse side effects. Molecular simulation study is considered to be an important vehicle to investigate the mode of interaction of ligands against its target protein that also makes us understand their binding or inhibition mechanism. Our *in silico* investigation is a novel approach to identify the molecular target involved in inhibition of hGCN5 activity by Kaempferol. Further, molecular dynamics studies exhibiting interaction energy estimates of Kaempferol within the active site of the hGCN5 protein showed that compound is more selective towards hGCN5.

In this study Kaempferol was the lead compound in inhibiting the proliferation of Daudi cells. This effect was dose and time dependent with IC_{50} value of 53.18 μM. To assess the efficacy of Kaempferol in the Burkitt lymphoma malignancy progression, Daudi cells were treated with different concentrations of Kaempferol for 24, 48, and 72 hours. Kaempferol treatment caused a significant loss of viability of cells as measured by this assay in a dose and time dependent manner, respectively. In this study, Kaempferol was used to evaluate their possible anticancer activity. Kaempferol could suppress the proliferation of Daudi cells effectively, which might be related with induction of apoptosis in a dose dependent manner. Docking simulation was carried out to confirm the confirmations of low energy and favorable binding with Kaempferol showed good binding compatibility against hGCN5 protein. Finally the docked result and MD simulations proved that Kaempferol proved to have better binding orientation, RMSD, and potential energy and manifested vital pharmacological properties. Therefore, based on our *in vitro* and *in silico* results, we suggest that the inhibitory activity of Daudi cells might be because of the synergistic effect of this compound. It therefore displays a lead compound for designing a drug molecule against Daudi cells.

5. Conclusion

Treatment with Kaempferol showed antiproliferative effects against Daudi lymphoma cell line which lead to antiproliferation and loss of cell viability. Docking simulation technique was used for preliminary investigation of the potential molecular target for the reported anticancer agent. The analysis of the docked ligand permitted us to establish the binding mode of compound involved in this study and confirm the role as anticancer agent. Though further role of the compound and its exact mechanism of action remain to be explored, it can be suggested that compound can be considered as a possible therapeutic agent against human lymphoma. Hence from the molecular docking through *in silico* analysis of Kaempferol against hGCN5 protein and *in vitro* study, it was concluded that Kaempferol could be considered as a lead compound

for the proliferation of Daudi cells. Further investigations are necessary to develop chemical entity for the prevention and treatment of lymphoma malignancy.

Competing Interests

The authors have declared no conflict of interests.

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