INTRODUCTION

Amongst all cancers known to affect mankind, colorectal cancer (CRC) is the third most cause of cancer deaths [1, 2]. There is increasing necessity to find a source of pioneering chemo preventive and chemotherapeutic agents for CRC. The American Cancer Society has estimated the epidemiological data of colon cancer to be 95,270 new cases and 49,190 deaths in 2016. Colorectal cancer represents the third most cancer frequently diagnosed malignancy in the world [3]. Colorectal cancer is the third foremost site of cancer in men and women and is the second leading cause of cancer-related deaths. Although the mortality of colorectal cancer has decreased by about 26% over the decades, only 3% has been due to improved treatment strategies. Suppression of apoptosis is often associated with increased expression of anti-apoptotic proteins and decreased expression of pro-apoptotic proteins [4]. For instance, anti-apoptotic protein Bcl-2 is over-expressed in various cancer cells, contributing to the inhibition of apoptosis. Colon cancers are relatively resistant to most conventional anti-tumor drugs and this resistance is closely linked to loss of apoptosis signaling. One of the important multi-domain pro-apoptotic Bcl2 family proteins essential for initiating apoptotic cell death is BAX and subsets of colon cancers are found to be associated with BAX mutation [5].

Flavonoids have a C6-C3-C6 three-ring skeleton (the rings are termed the A-, B-, and C-rings) and can be divided into several classes in terms of structure; these are the flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids. Flavonoids, the most common polyphenolic compounds of edible plants, exhibit a wide range of biological and pharmacological activities, including anti-inflammatory, antioxidant, and anticancer effects [6]. Several structures-activity relationship studies have revealed that the presence of a 2’-hydroxy group on the B-ring is important in terms of enhancement of antitumor activity [7, 8]. Kaempferitrin is a chemical compound. It can be isolated from the leaves of Hedysarum verticillate [9]. Kaempferitrin induces apoptosis through the sequential activation of caspase-8 and caspase-3 (fig. 1).

![Chemical structure of kaempferitrin](image)

**Fig.1:** Chemical structure of kaempferitrin (molecular weight: 578.523 g/mol)

The Akt signaling is a constitutively activated pathway in the inherited colorectal cancer (FAP) and earn up to 80% of sporadic colorectal cancers (CRC) due to inactivating mutations of the adenomatous polyposis coli (APC) tumor suppressor gene. APC is a component of the β-catenin degradation complex whose mutations are indeed now clearly recognized as early and sufficient events to promote intestinal tumor development. Many chemotherapeutic drugs causing nuclear stress will function independently from tumor suppressor protein p53 and still lead to cell cycle arrest and or apoptosis. Since it is known that most cancers lack functional p53, it is with great interest to explore these molecular mechanisms. It is known that more than 50% of human cancers lack functional p53.
Consequently, drugs triggering cell death in p53-null cells may have great potential in the treatment of many cancers [10]. A number of molecular abnormalities have been associated with CRC, including mutations in K-ras oncoprotein; the inactivation of the tumor suppressor genes APC, p53 and DCC; mutations in the DNA mismatch repair regulators mutL-homolog 1 and mutS alpha or mutS beta; and the dysregulation of DNA methylation, microsatellite stability, and non-coding RNAs [11]. In silico docking study performed here demonstrates the rationale for the different binding activities of kaempferitrin.

MATERIALS AND METHODS

Docking

In the present work two of the best docking programs, AutoDock Vina and MGL tool were used for docking calculations. Both programs require the pdbqt input files and allow for flexibility of all the torsional bonds of small molecules. For AutoDock program, the implemented empirical free energy function and the lamarckian genetic algorithm were used. Gastiger charges and hydrogen atoms were added to small molecules and protein structures. For all docking calculations, the amount of docking runs was set to 250 with 5,000,000 energy evaluations for each run. The size of the box that defines the search space was set at 1 Å around the small molecules. For each 25-DKP derivative, the first result (the lowest energy conformation) of Vina and AutoDock were selected as the docking result. Finally, the pymol program was used to do analysis of the docking results.

Preparation of receptors structures for docking

3D crystallographic structures of proteins were obtained from protein data bank (PDB) (http://www.pdb.rcsb.org), and those of small molecules were retrieved from PubChem compound database (http://www.ncbi.nlm.nih.gov/search). The structure of kaempferitrin was taken from chemspider (Chemsipder code: 4588900) [20]. The 3D structures of proteins were retrieved as follows: BAX (Pdb: 1UNR), Cytochrome P450 (Pdb: 4NZ2), Protein Kinase B (Pdb: 4S0O), Bcl-2, (Pdb: 4MAN), Caspase-3 (Pdb: 5I9B), Cox-2 (Pdb: 1CX2), TNF-α (Pdb: 4TWT) and VEGF (2C7W). Initially, DNA, ligand, and crystallographic water molecules were removed in the 3D structure using Discovery Studio Visualizer; AutoDock Tools assigned polar hydrogen, Kollman United atom charges, salvation structure using Discovery Studio Visualizer; AutoDock Tools and crystallographic water molecules were removed in the 3D structure.

Grid generation and molecular docking

The auto grid was used for the preparation of the grid map using a set of 190 protein-ligand complexes that had been used as a training set for the AutoDock scoring function. Previous studies were proved the binding strength and interaction of amino acids using natural bio active flavonoids [12]. In that way, our study was developed binding energy scoring function of kaempferitrin docked with different types of inflammatory proteins and apoptotic proteins. The computational analysis of this drug was the first time to understand the mechanism of interaction [13] between kaempferitrin and apoptotic proteins.

Table 1: The binding energies of protein kinase B, BAX, Bcl-2, caspase-3, COX-2, TNF-α cytochrome P450 and VEGF with the known small molecule kaempferitrin

| S. No | Ligand     | Target proteins gscore (kcal/mol) | Protein kinase B | BAX | Bcl-2 | Caspase-3 | COX-2 | TNF-α | Cytochrome P450 | VEGF |
|-------|------------|-----------------------------------|------------------|-----|-------|----------|-------|-------|-----------------|------|
| 1     | Kaempferitrin | -6.7                              | -6.9             | -7.2 | -7.3  | -8.8     | -8.0  | -7.4  | -7.2            |      |

BAX: Bcl-2-associated X protein, Bcl-2: B-cell lymphoma-2, COX-2: Cyclooxygenase-2, TNF-α: tumor necrosis factor alpha, VEGF: Vascular endothelial growth factor

Table 2: Apoptotic proteins with their residues

| S. No | Ligand     | Macromolecules | Amino acid residues |
|-------|------------|----------------|--------------------|
| 1     | Kaempferitrin | BAX            | ASP-102, ASN-48, GLN-52, ASP-104 |

Docked results with protein kinase B, BAX, Bcl-2 and caspase 3

To elucidate possible interaction between kaempferitrin and Protein Kinase B, BAX, Bcl-2, and Caspase 3 was carried out. Kaempferitrin docked well into protein kinase B and the docking score was 6.7 kcal/mol (fig. 2A). Nine residues residing in the binding site of protein kinase B ligand had hydrophobic interactions with kaempferitrin ARG-25, LEU-52, ASN-54, PHE-55, GLU-17, LYS-14, ASP-63, GLU-67, GLY-65, LEU-66.

BAX: Bcl-2-associated X protein, Bcl-2: B-cell lymphoma-2, COX: Cyclooxygenase-2, TNF-α: tumor necrosis factor alpha, VEGF: Vascular endothelial growth factor
TRP-22, THR-21 and GLY-16. The first evidence that Akt plays a major role in oncogenesis was produced by the isolation of the transforming retrovirus from an AKR mouse T-cell lymphoma [14], which was subsequently shown to contain transduced sequences of cellular origin [15]. The PI3K/Akt or Protein Kinase B pathway plays a main role in regulating censorious cellular survival, including cell division, apoptosis and metabolism [16].

Previous studies [17,18] proved that Akt docked with different types of compounds which could be interacted with amino acids as well. In our present study, proved that kaempferitrin interacted with various amino acid residues and having more binding energies.

BAX, Bcl-2 and Caspase 3 docked well into kaempferitrin and their docking scores were -6.9, -7.2, and -7.3 kcal/mol respectively. The docking score analysis of BAX interaction with kaempferitrin showed in fig. 2(B) and their residues were ASP-102, ASN-48, GLN-176, ASP-2, SER-249, and hydrogen bond lengths of 1 Å. The interaction of amino acid residues showed in table 1 and table 2 respectively.

Docked results with COX-2 and TNF-α

Analysis of the docking results suggests that COX-2 and TNF-α has a binding affinity with kaempferitrin through the formation of one non-polar hydrogen bond with binding energies of -8.8 and -8.0 kcal/mol and hydrogen bond lengths of 1 Å. The interaction of amino acid residues of kaempferitrin with proteins were shown in fig. 3 and table 2. The docked interaction results and residues involved in van der waals forces are described in Supplementary table 2. Kaempferitrin exhibited more binding affinity or hydrogen bond formation with COX-2 and TNF-α.

These interaction results show that COX-2 and TNF-α interacts more highly with kaempferitrin. Cyclooxygenase (COX), known as prostaglandin (PG) H2 synthase, is the rate limiting enzyme in the conversion of arachidonic acid into PGs. Over expression of COX-2 has been frequently observed in colon tumors and COX-2 plays a major role in colon carcinogenesis. Many studies have revealed that PG2, the metabolite of COX-2 enzyme reaction is an effective mitogen, which contributes to the development of colon cancer [24, 25]. Targeting COX-2 is one of the recent therapeutic methods for the treatment of colon cancer [26, 27]. Previous findings were demonstrated that natural products computationally inhibited the expression of COX-2 [28]. Based on the previous findings, our present docking studies proved that COX-2 has high binding affinity with their compounds. Kaempferitrin binds with COX-2 and have good binding score compared to the previous studies [29]. Tumor necrosis factor-alpha (TNF-α) is a central regulator of inflammation, and TNF-α antagonists may be effective in treating inflammatory disorders in which TNF-α plays an important pathogenetic role [30]. TNF-α also interacted with different types of chemo preventive compounds. In that way, our study demonstrated that kaempferitrin indicated more binding affinity with TNF-α.

Docked results with cytochrome P450

The docked results showed (Supplementary table 1) that kaempferitrin compounds found to have good binding affinity and the interaction of amino acid residues showed in table 2. Fig 4 shows that kaempferitrin interacted with Cytochrome P450 and the amino acid residues of ASP-414, LYS-322, GLU-416, GLU-438, ALA-439, and hydrogen bond lengths of 1 Å. The cytochromes P450 (CYPs) constitute the major enzyme family capable of catalyzing the oxidative biotransformation of most drugs and other lipidphilic xenobiotics and are therefore of particular relevance for clinical pharmacology [31-33]. Previous study showed that drug-drug interaction of Cytochrome P450 and their compounds [34]. Our results demonstrated that amino acid residues of Cytochrome P450 binds with kaempferitrin have high binding energy.

Docked results with VEGF

The docking results are ranked according to the binding energies with kaempferitrin. The docking binding energy of VEGF to kaempferitrin score was -7.2 and their residues were LEU-47, GLN-
CYS-61, CYS-60, ASP-63, GLU-67, GLY-65, LEU-66 (fig. 5). In table 1 and table 2, the binding affinity and the interaction of amino acid residues were showed clearly. Vascular endothelial growth factor is one of the most important factors for de novo-formation of new blood vessels [35]. Besides endothelial cells, it is also produced by a range of other cell types such as fibroblasts [36], neutrophils [37] and macrophages [38]. Previous study [39] screened the docking binding activity of the designed molecule of VEGF was to optimize the maximum binding efficiency. Similarly, our results confirmed that the kaempferitrin bind with VEGF and having maximum binding scores.

Fig. 3: Illustration of docked complexes of COX-2 (A) and TNF-α with kaempferitrin

Fig. 4: Ligand-protein interactions of kaempferitrin with Cytochrome P450

Fig. 5: Illustration of interaction of amino acid residues with kaempferitrin and VEGF
CONCLUSION

The molecular docking simulations performed for the selected active genes BAX, Bcl-2, Caspase-3, COX-2, TNF-α, Cytochrome P450 and VEGF. Our future goal is to optimize this scaffold to enhance the antitumor activity and selectivity against colon cancer. Altogether, molecular docking studies imply that kaempferitrin is an effective inhibitory compound for colon cancer. In conclusion, PI3K is a promising target for anticancer drug design. In our effort to develop novel PI3K inhibitors, we recruited structure-based design and molecular docking to optimize the lead PI3K an inhibitor. Additionally, kaempferitrin showed pronounced broad-spectrum antimicrobial, analgesic and anti-inflammatory, and antidepressant activity. Our future goal is to optimize this scaffold to enhance the antitumor activity and selectivity against colon cancer.

AUTHOR CONTRIBUTION

Mydhili Govindarasu and Manju Vaiyapuri designed the study. Maydhili Govindarasu and Mariyappan Palani collected and analyzed the data. Mariyappan Palani provided computational tools to deliver the docking results. Mydhili Govindarasu wrote the first draft of the manuscript. All authors interpreted the results and approved the final version of the manuscript. All authors are the guarantors.

CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

1. Karthi N, Kalaiyarasu T, Kandakumar S, Mariyappan P, Manju V. Pelargonidin induces apoptosis and cell cycle arrest via mitochondria mediated intrinsic apoptotic pathway in HT29 cells. RSC Adv 2016;6:45 064-76.
2. Ragunath M, Nadanasabapathy S, Manju V. Anti-inflammatory and pro-apoptotic effects of umbelliferone in colon carcinogenesis. Hum Exp Toxicol 2016;35:1-14.
3. Valentina P, Assunta S, Emma M, Roberta B, Raffaella S, Giulia R, Amapina R. 5-FU targets p53 to induce mitochondrial apoptosis via cystathionine-β-synthase in colon cancer cells lacking p53. Oncotarget 2016;7:50333-48.
4. Lin J, Page C, Jin X, Sethi AO, Patel R, Nunez G. Suppression activity of pro-apoptotic gene products in cancer cells, a potential application for cancer gene therapy. Anticancer Res 2001;21:831-9.
5. Kaleigh F, Manabu K. Evading apoptosis in cancer. Trends Cell Biol 2013;23:620-33.
6. Alonso-Castro AJ, Ortiz-Sanchez E, Garcia-Regalado A, Ruiz G, Nunez-Martinez JM, Gonzalez-Sanchez I, et al. Kaempferitrin induces apoptosis via intrinsic pathway in HeLa cells and exerts antitumor effects. J Ethnopharmacol 2013;145:476-89.
7. Rong T. Chemistry and biochemistry of dietary polyphenols. Nutrients 2010;2:1231-46.
8. Kalaiyarasu T, Karthi N, Sharmila Gowri V, Manju V. In vitro assessment of antioxidant and antibacterial activity of green synthesized silver nanoparticles from digitaria radicosa leaves. Asian J Pharm Clin Res 2016;9:297-302.
9. The Human Metabolome Database. Available from: http://www.hmdb.ca/metabolites/HMDB37438. [Last accessed on 01 May 2017].
10. Soussi T, Lozano G. p53 mutation heterogeneity in cancer. Biochem Biophys Res Commun 2005;331:1 834-42.
11. Frederick E, Deelhy G, Roux S. Molecular aspects of colorectal carcinogenesis: a review. J Cancer Biol Res 2015;3:1 057.
12. Mariyappan P, Karthi N, Manju V. Computational studies on different types of apoptotic proteins docked with a dietary flavonoid eriodictyol in colon cancer. Asian J Pharm Clin Res 2017;10:223-6.
13. Ranjan S, Dasgupta N, Chinmappan S, Ramalingam C, Kumar A. A novel approach to evaluate titanium dioxide nanoparticle-protein interaction through docking: an insight into mechanism of action. Proc Natl Acad Sci India B Biol Sci 2015. p. 1-7.
14. Staal SP, Hartley JW, Rowe WP. Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. Proc Natl Acad Sci USA 1977;74:3065-7.
15. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA 1998;95:5034-7.
16. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase-AKT pathway in human cancer. Nat Rev Cancer 2002;2:489-501.
17. Alicia H, Israel V, Rafael C, Fabian L, Ping J, Yongping Y, et al. Docking of protein kinase B inhibitors: implications in the structure-based optimization of a novel scaffold. Chem Biol Drug Des 2010;76:269-76.
18. Sefika KU, Seval A, Elif O. Virtual screening and docking of potential protein kinase B inhibitors. Cell 2012;102:60-1.
19. Debatin KM. Apoptosis pathways in cancer and cancer therapy. Cancer Immunol Immun 2004;53:153-9.
20. Suzuki M, Youle RJ, Tjandra N. Structure of bax: coregulation of dimer formation and intracellular localization. Cell 2000; 103:645-54.
21. Reed JC. Mechanisms of apoptosis. Am J Pathol 2000;157:1415-30.
22. Wong RSY. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 2011;30:87.
23. Gao D, Xu Z, Qiao P, Liu S, Zhang L, He P, et al. Cadmium induces liver cell apoptosis through caspase-3A activation in pursed carp (Cyprinus carpio). Plos One 2013;8:83423.
24. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001;1:11-21.
25. Han JA. Cyclooxygenase-2 promotes cell proliferation, migration and invasion in 2D2S human osteosarcoma cells. Exp Mol Med 2007;39:469-76.
26. Al-Fayez M, Cai H, Tunstall R, Steward WP, Gescher AJ. Differential modulation of cyclooxygenase-mediated prostaglandin production by the putative cancer chemotherapeutic flavonoids tricin, apigenin and quercetin. Cancer Chemother Pharmacol 2001;50:65-71.
27. O’Leary KA, de Pascual-Teresa S, Needs PW, Bao YP, O’Brien NM, Williamson G. Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. Mutat Res 2004;551:245-54.
28. Ismat M, Jumina J, Sofia MH, Mustofa M. In silico molecular docking of xanthone derivatives as cyclooxygenase-2 inhibitor agents. Int J Pharm Pharm Sci 2017;9:98-104.
29. Sara C, Salvatore A, Angola Di C, Giovanna P, Adele P, Manuela S, et al. Synthesis, biological evaluation and docking analysis of a new series of methylbenzyl and sulfamoyl acetamides and ethyl acetates as potent COX-2 inhibitors. Bioorg Med Chem 2015;23:810-20.
30. Esposito E, Cuzzocrea S. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia reperfusion injury and trauma. Curr Med Chem 2009;16:3152-67.
31. Nelson DR. Cytochrome P450 nomenclature. Methods Mol Biol 2004;320:1-10.
32. Guengerich FP. Cytochrome P450 and chemical toxicology. Chem Res Toxicol 2008;21:70-83.
33. Zanger U. The CYP2D Subfamily. In: C Ioannides. Ed. Cytochromes P450: Role in the metabolism and toxicity of drugs and other xenobiotics. Royal Society of Chemistry; 2008. p. 241-75.
34. Xuelin Z, Yan W, Tao H, Penelope MY, John W, Yiu Wa K, et al. Enzyme kinetic and molecular docking studies for the inhibitions of milrinone on major human cytochrome P450 isozymes. Phytomedicine 2013;20:367-74.
35. A. mollis, P. hedges, S. lycopersicum. The role of vascular endothelial growth factor in wound healing. J Surg Res 2009;153:347-58.
36. Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. Am J Pathol 1998;152:1445-52.
37. Gaudry M, Bregerie O, Andrieu V, Benna J, Pocklalo MA, Hakim J. Intracellular pool of vascular endothelial growth factor in human neutrophils. Blood 1997;90:4153-61.
38. Berse B, Brown LF, Van de Water L, Dvorak HF, Senger DR. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 1992;3:211-20.
39. Shamsuddin SK, Amin Malik Shah AM, Muhammad Al, Aman Shah AM, Majed M, Rosenani SM. Designing the angiogenic inhibitor for brain tumor via disruption of VEGF and IL17A expression. Eur J Pharm Sci 2016;93:304-18.
40. Feinstein WP, Brylinski M. Calculating an optimal box size for ligand docking and virtual screening against experimental and predicted binding pockets. J Cheminform 2015;7:18.
41. Mydhili Govindarasu, Mariyappan Palani, Manju Vaiyapuri. In silico docking studies on kaempferitrin with diverse inflammatory and apoptotic proteins functional approach towards the colon cancer. Int J Pharm Pharm Sci 2017;9(9):199-204.

How to cite this article
- Mydhili Govindarasu, Mariyappan Palani, Manju Vaiyapuri. In silico docking studies on kaempferitrin with diverse inflammatory and apoptotic proteins functional approach towards the colon cancer. Int J Pharm Pharm Sci 2017;9(9):199-204.