

Efficacy of Quercetin as a Therapeutic for Colorectal Cancer: an *In-silico* analysis

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Abstract

A lot of evidence suggests that cancer stem cells within a solid tumour initiate and sustain tumour growth and are quiescent even after therapeutic intervention by anti-cancer drugs. Several flavonoids interfere in cancer-stem cell-related pathways and therefore offer a promising approach for prevention. Quercetin is one such flavonoid with wide spectrum therapeutic effects. Potential mechanisms for quercetin in preventing cancer stem cells include antioxidant, anti-inflammation as well as the modulation of multiple molecular events involved in carcinogenesis. Synthesis of quercetin and peptide-based nanoparticles to further increase the efficacy of a promising nutraceutical. It can be used as a chemical template for combinatorial synthesis. Molecular targets can be identified. With the molecular targets of quercetin being known, it may be possible to develop more refined chemicals that specifically target those commonly shared sites. Synergistic effect: with the understanding of the molecular action of quercetin, the possible synergistic effects on chemo-prevention by using quercetin. In the present study, an attempt was made to synthesize quercetin encapsulated nanoparticle for target drug delivery systems and to try against various cancer stem cell lines.

Keywords: Cancer-stem cells, Quercetin, Flavonoid, Antioxidant, Nutraceutical, Nanoparticle, Synergistic effect.

1. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumours, which do not spread while the malignant tumours spread to other part of our body. There are over 200 types of cancer are available and all these are happened generally due to the causative agents like toxic or chemical compound exposure, ionizing radiations, pathogens, and or due to human genetics[1] It is also greatly influenced by Lifestyle including the environmental and dietary exposure [2-3].

Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat and avoiding too much sunlight exposure. Early detection through screening is useful for cervical and colorectal cancer. The benefits of screening in breast cancer are controversial. Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy[4]. Pain and symptom management are an important part of care. Palliative care is particularly important in people with advanced disease. The chance of survival depends on the type of cancer and extent of disease at the start of treatment. In children under 15 at diagnosis, the five-year survival rate in the developed world is on average 80%. For cancer in the United States, the average five-year survival rate is 66% [5].

Colorectal cancer (CRC) is one of the leading causes of mortality and morbidity in the world[6].It is the third most common malignancy and the fourth leading cause of cancer-related deaths worldwide, accounting for approximately 1,400,000 new cases and about 700,000 deaths worldwide[7]. In recent decades there has been a significant increase in the incidence of CRC; in particular, the number of newly diagnosed CRC cases has increased from 783,000 in 1990 to 1,361,000 in 2012 and counting. Despite the prevalence of CRC in both sexes it was higher in men than in women [8].Moreover, the risk of developing colorectal cancer increases with age and the 90% patients who are diagnosed are over the age of 50[9].

Colorectal cancer evolves through a stepwise accumulation of genetic and epigenetic alterations, leading to the transformation of normal colonic mucosa into invasive cancer[10]. Different molecular pathways have been identified to be deregulated in colorectal carcinogenesis which demonstrates the heterogeneous nature of CRC. Approximately 70-85% of the CRC develop via chromosomal instability (CIN) pathway, characterized by sequential accumulation of genetic aberrations which leads to loss of heterozygosity (LOH) and chromosomal abnormalities[11].The second most common phenomenon occurring in CRC is the dysfunction of DNA Mismatch Repair (MMR) system which results in microsatellite instability (MSI). This contributes about 15% of the sporadic CRC [12]. The third most mechanism by which CRC progresses is the CpG island methylator phenotype (CIMP) pathway. It consists of the aberrant hypermethylation of CpG dinucleotide sequences localized in the promoter regions of genes involved in cell cycle regulation, apoptosis, angiogenesis, DNA repair, invasion, and adhesion. This leads to the loss of gene expression [13].

Currently, there is an urgent need for the discovery and development of novel preventive and therapeutic strategies to combat the severity of the colon cancer. In silico docking study performed with various deregulated protein demonstrates the rationale for the different binding activities of quercetin.

Nutraceuticals are products, which other than nutrition are also used as medicine. A nutraceutical product may be defined as a substance, which has physiological benefit or provides protection against chronic disease. Nutraceuticals may be used to improve health, delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of the body. Nowadays, nutraceuticals have received considerable interest due to potential nutritional, safety, and therapeutic effects. Recent studies have shown promising results for these compounds in various complications. In the present review much effort has been devoted to present new concepts about nutraceuticals based on their diseases modifying indications[14]. Emphasis has been made to present herbal nutraceuticals effective on hard curative disorders related to oxidative stress including allergy, Alzheimer, cardiovascular, cancer, diabetes, eye, immune, inflammatory and Parkinson's diseases as well as obesity. The recently published papers about different aspects of nutraceuticals as alternative for pharmaceuticals were searched using scientific sites such as Medline, PubMed, and Google Scholar. The used terms included nutraceutical and allergy, Alzheimer, cardiovascular, cancer, diabetes, eye, immune, inflammatory or Parkinson[15].

Quercetin is a plant pigment (flavonoid). It is found in many plants and foods, such as red wine, onions, green tea, apples, berries, Ginkgo biloba, St. John's wort, American elder, and others. Buckwheat tea has a large amount of quercetin. People use quercetin as a medicine[16].

Quercetin is most taken by mouth to treat conditions of the heart and blood vessels and prevent cancer. It is also used for arthritis, bladder infections, and diabetes. But there is limited scientific evidence to support these uses. Quercetin has antioxidant and anti-inflammatory effects which might help reduce inflammation, kill cancer cells, control blood sugar, and help prevent heart disease[17]. One population. Some research suggests that eating high amounts of quercetin in the diet might reduce the chance of developing pancreatic cancer, especially in men who smoke and study found no link between quercetin intake from the diet and the chance of ovarian cancer[18].

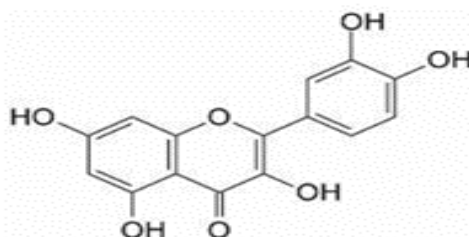


Fig 1: Structure of Quercetin

Oral drug delivery certainly is the most convenient route of drug administration because of the ease of administration. But there are several challenges in achieving oral delivery such as varying pH (highly acidic stomach), the presence of enzymes, first-pass effect in the liver and the intestinal barrier to drug absorption[19-21]. The above challenges limit the drug from entering the systemic circulation thereby reducing oral bioavailability. Nanoparticle technology is an increasingly exploited formulation technique to overcome the limitations of oral drug delivery.

2. Materials and Methods

2.1 Software:

AutoDockTools (ADT) and AutoDockVina was downloaded from www.scripps.edu. PyMol, DS Visualizer and T.E.S.T Software was downloaded from <https://pymol.org>, <http://accelrys.com> and <https://www.epa.gov>.

2.2 Ligand preparation for docking:

The 3D structure of the ligand Quercetin (CID-5281647) was downloaded from PubChem Open Chemistry Database (<https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin>) and was optimized using AutoDockTools.

2.3 Preparation of proteins for docking:

PDB file of target proteins was obtained from RCSB protein data bank of whose PDB ID is given in Table 2. The original structure of proteins was reduced to a unimolecular receptor by using PyMol and modification was brought about by adding polar hydrogens. All the torsional bonds of ligands were set free by Ligand module and gasteiger charges were computed using Auto Dock Tools (ADT).

2.4 Docking Studies of Quercetin with Proteins:

Molecular docking simulations were carried out using AutoDockVina program to study the interaction between the Quercetin and the proteins. The grid size of and the grid centres of XYZ points with a grid spacing of 1 Å are listed in Table 3.4.1 The docking parameter file were set up in text document. The binding mode was predicted by using the Lamarckian genetic algorithm and the results were analysed using the binding energies. Default settings were used for all other parameters.

Table 1: Grid Map of Target Proteins

Sl No.	PROTEIN RECEPTORS	PDB ID	GRID SIZE			GRID CENTER		
			X	Y	Z	X	Y	Z
1	APC	3NMZ	74	80	76	39.464	-35.473	-15.899
2	BUBR1	3SI5	119	106	83	30.683	5.024	22.005
3	CDK8	3RGF	83	77	83	-2.890	4.499	12.674
4	CK2a	3WAR	83	68	81	-9.187	-5.094	9.013
5	FABP6	5L8I	88	56	82	50.866	-3.069	79.121
6	KRas	4OBE	61	64	94	-8.687	-25.161	29.411
7	SPINDLE ASSEMBLY CHECKPOINT PROTEIN HUMAN MAD2	1DUJ				-12.439	27.627	-3.726
8	Bcl-x _L	1MAZ	48	45	60	2.495	23.599	41.558
9	Bcl-2	2XA0	93	76	88	53.738	17.134	-20.970
10	COX-2	1CX2	135	103	151	-38.815	-31.433	-32.898
11	CYTOCHROME P450	4NZ2	113	106	95	-40.780	-62.099	-32.838
12	PRTOEIN KINASE B	1UNR	46	40	155	12.356	-2.332	-0.006
13	TNF α	4TWT	82	85	99	-5.076	84.96	237.20
14	NFKB	1VKX	94	108	116	8.442	40.60	50.57

3. RESULTS AND DISCUSSION

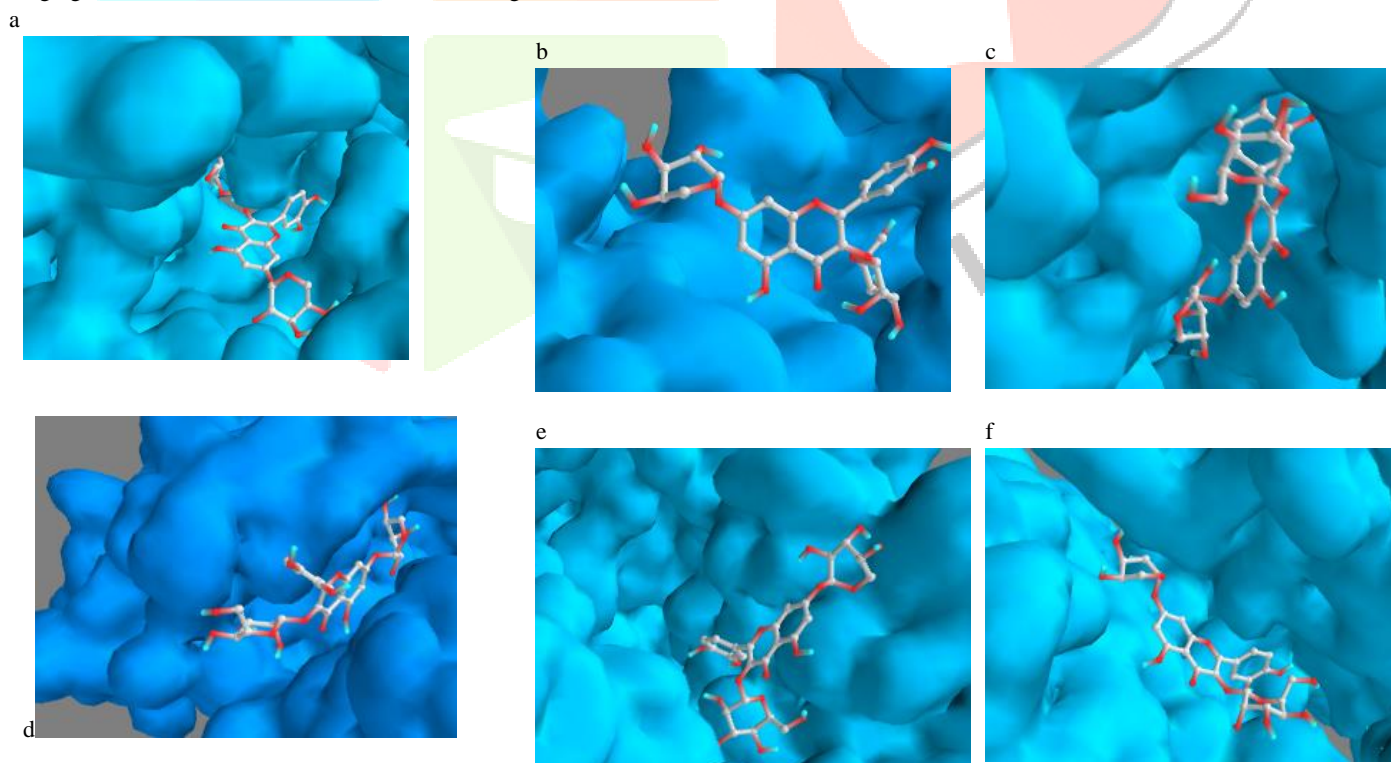
3.1 Molecular docking:

It is a novel approach to determine the Quercetin–protein interaction by using molecular docking. In our study binding energy scoring function of Quercetin with different types of inflammatory and anti-apoptotic proteins was developed and the computational analysis of the compound for the first time to understand its mechanism of interaction in CRC was done. The docking scores of Quercetin with anti-apoptotic and inflammatory proteins are summarized in Table 2.

Table 2: Docking results for the Quercetin-protein interaction analysed

SI No.	PROTEIN RECEPTORS	PDB ID	BINDING ENERGY(kcal/mol)
1	APC	3NMZ	-9.3
2	BUBR1	3SI5	-7.9
3	CDK8	3RGF	-8.5
4	CK2 α	3WAR	-8.9
5	FABP6	5L8I	-6.8
6	K-Ras	4OBE	-8.9
7	SPINDLE ASSEMBLY CHECKPOINT PROTEIN HUMAN MAD2	1DUJ	-8.1
8	Bcl-xL	1MAZ	-8.7
9	Bcl-2	2XA0	-9.2
10	COX-2	1CX2	-10.2
11	CYTOCHROME P450	4NZ2	-8.9
12	PRTOEIN KINASE B	1UNR	-7.3
13	TNF α	4TWT	-8.7
14	NFKB	1VKX	-9.1

The negative binding energy of Quercetin with protein show the efficiency in the formation of Quercetin-protein complexes. Quercetin makes most stable docked complex with COX-2 (PDB ID 1CX2) as it has most binding energy of -10.2. Quercetin makes hydrogen bonds and other non-interactive bonds in the active pockets of above listed proteins (Fig.4.1.1). All the proteins selected for this study showed good binding energy ranging from -10.2 to -6.8 which confirm the binding.



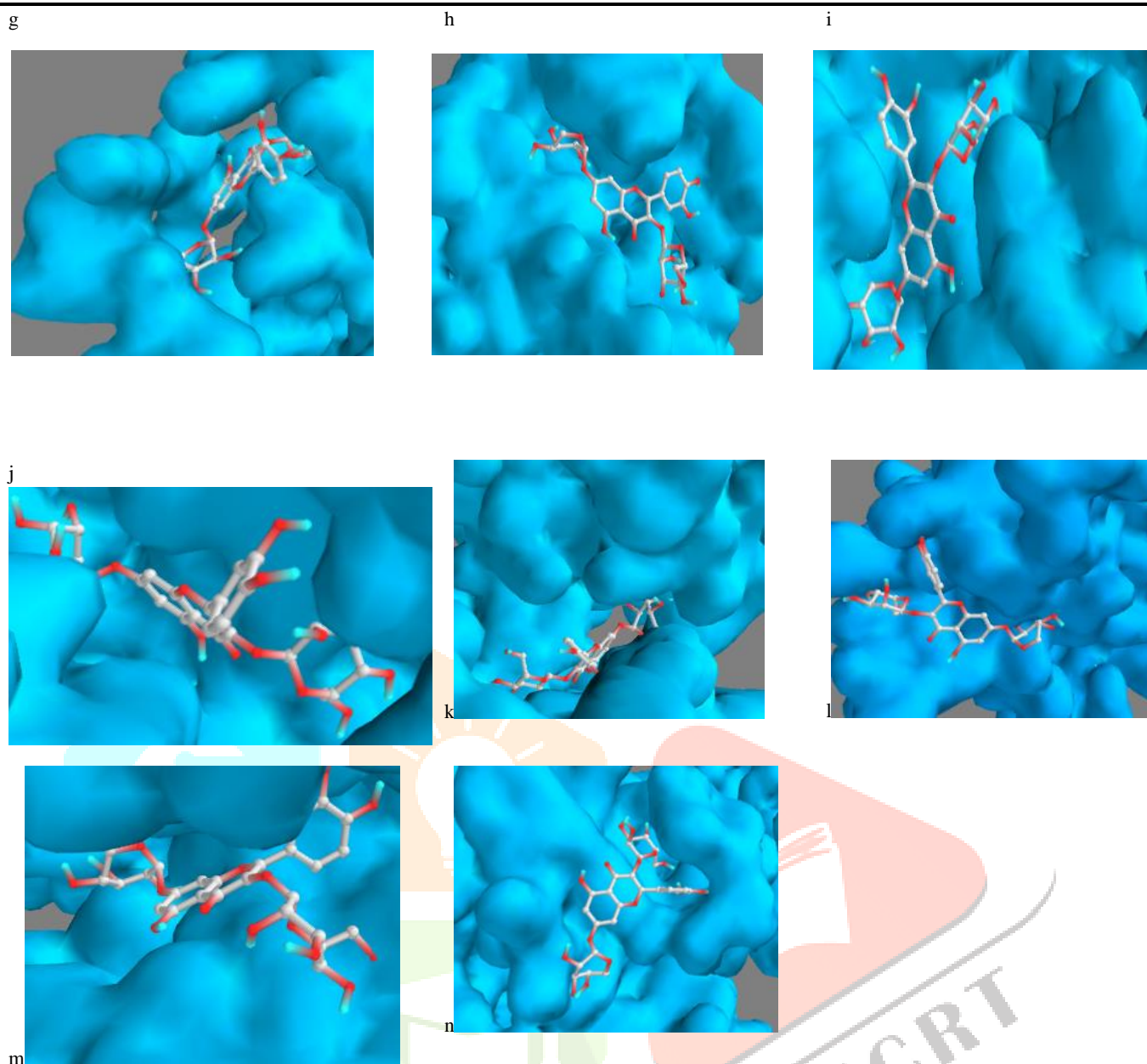


Figure 2: Interaction of Quercetin with: a) APC protein; b) BUBR1 protein; c) CDK8 protein; d) CK2a protein; e) FABP6 protein; f) K-Ras protein; g) SPINDLE ASSEMBLY CHECKPOINT PROTEIN HUMAN MAD2 protein; h) Bcl-xL protein; i) BCL 2 protein; j) COX-2 protein; k) CYTOCHROME P450 protein; l) PRTOEIN KINASE B protein; m) TNF α protein; n) NF- κ B protein

3.1.1 Wnt/ β catenin pathway

APC: Approximately 70-85% of CRCs develop via the CIN pathway where there is increased expression of Wnt/ β catenin pathway. This occurs mainly due to the mutation associated with APC gene. APC protein is an important regulator which regulates the degradation of cytoplasmic β catenin, so once the APC is mutated, cytoplasmic β -catenin accumulates and binds to the Tcf family of transcription factors, altering the expression of various genes affecting proliferation, differentiation, migration, and apoptosis leading to CRC [13]; Galiatsatos, Foulkes, & Ph, 2006). So in our in silico studies we found that the Quercetin binds with various amino acid residues (ASN627(2), HIS672(2), THR628, SER172, LEU629(2), GLU633, TYR175(2), TRP242(2)) of APC protein with favourable binding energy of -9.3Kcal/mol. **Cyclin-Dependent Kinase (CDK) CDK8:** CDK8 mediates transcriptional control of Wnt/ β catenin pathway by mediating the mechanism by which β -catenin activates gene transcription in the nucleus [19]. Abreaction in the gene coding for CDK 8 has been observed in a substantial fraction of colon cancers and in a study conducted by Firestein *et al* (2008) it was found that the suppression of CDK8 expression inhibited proliferation in colon cancer cells. So in our studies when we docked Quercetin with CDK8 we found that Quercetin form a stable interaction with amino acid residues (TRP6(2), ARG157(3), TYR162, GLN161, PRO158(2), PHE195(3)).

KRAS: KRAS is a protooncogene which is most frequently mutated (30-60%) in colorectal cancer. The mutation is mainly the gain of function which upregulates the signalling of Wnt/ β catenin pathway leading to formation of polyps in colon [20] gain of function of KRAS is usually the activation of KRAS by the attachment of GTP and lots of research is done to prevent the formation of active KRAS-GTP [20]

3.1.2 Akt Pathway

Protein Kinase B (Akt kinase): AKT protein kinase transduces signals from growth factors and oncogenes to downstream targets that control crucial elements in tumour development. The AKT pathway is one of the most frequently hyperactivated signalling pathways in colon cancers [33]. So, inhibitors of PI3K/Akt signalling have been suggested as potential therapeutic agents and in our studies, we found that Quercetin docks with Protein kinase B with a favourable energy of -7.7Kcal/mol meaning that might block the hyperactivation of Akt signalling pathway by preventing the phosphorylation of Akt kinase.

3.1.3. Arachidonic acid (AA) metabolism - COX Pathway

Cyclooxygenase-2 (COX-2) Pathway: Cyclooxygenase-2 (COX-2) is an inducible enzyme that regulates prostaglandin synthesis and is overexpressed at sites of inflammation and in colon cancer. This enzyme is involved in the regulation of apoptosis, angiogenesis, and tumour cell invasiveness [11]. Selective inhibitors of COX-2 are used to regress colorectal polyps in CRC patients. So there is need for the development of novel agents which could suppress the overexpressed activity of COX 2 enzyme [12]. In our docking study we found that Quercetin bind to COX 2 enzyme with favourable binding energy of -10.2 Kcal/mol which shows that w the possibility of using Quercetin as a potential therapeutic drug in the treatment of CRC. And Quercetin can be a novel agent in inhibiting the COX-2 enzyme.

3.1.4. NF- κ B Pathway

Nuclear factor- κ B: Nuclear factor- κ B (NF- κ B) transcription factors controls the expression of genes involved in many physiological processes such as innate and adaptive immune responses, cell proliferation, cell death, and inflammation. It has become clear that aberrant regulation of NF- κ B and the signalling pathways leads to cancer development and progression. Constitutively activated NF- κ B have been observed in 66% of CRC cell lines and 40% of human CRCs. So there is a urgent need for the development of anti NF- κ B therapies [15]. In our studies we found that Quercetin interacts with amino acids(HIS405, SER471, ASN403, LEU467(2), SER471, ILE493, ALA497(3)) of NF- κ B protein with binding energy of -9.1 Kcal/mol. This shows that the Quercetin can be a potential candidate for the development of anti NF- κ B therapies for the treatment of CRC.

3.1.5. TNF Signalling Pathway

Tumour Necrosis Factor- α (TNF α): Tumour necrosis factor- α (TNF α) is a pro-inflammatory cytokine predominantly produced by macrophages as well as tumour cells. It acts as the inducer for the production of colony-stimulating factor (CSF)-1 which mediates a role in wide biological activities such as inflammation, apoptosis, cell proliferation and differentiation [14]. In addition, TNF α mediates a key role in regulating the inflammatory processes in tumour progression. Several studies reported that there is an increased expression of TNF α in the serum of CRC patients which shows that TNF α might be a promising candidate for the development of novel therapies for CRC [19]. In this aspect our docking study showed a promising result as Quercetin docked with TNF α with a favourable binding energy of -8.7 Kcal/mol.

3.1.6. Cytochrome P450 Pathway

Cytochrome P450s (CYPs): Cytochrome P450s represent a large class of heme-containing enzymes that catalyse central role in the oxidative metabolism of a wide range of xenobiotics and biologically active endogenous compounds [20]. Recently it was found that P450s play a major role in tumour development via their metabolism of many carcinogens - many of the carcinogens require metabolic activation by P450s before exerting their genotoxic effect [21]. So the development of P450 inhibitors which might inhibit the metabolic activation of carcinogens would suppress the tumour progression [22]. In this aspect our study showed that Quercetin can be a potential candidate for P450 inhibition as it showed a favourable binding score of -8.9 Kcal/mol.

3.1.7. Apoptosis Pathway

B-cell lymphoma 2 (Bcl-2) and Bcl-xL proteins: These are anti-apoptotic proteins which play pivotal role in the regulation of the mitochondrial death pathway. They inhibit the activity of pro apoptotic proteins such as BAX and Bak under normal physiological conditions. In cancer cells these proteins are overexpressed and are responsible for dysregulation of apoptosis [9]. Inhibition of these protein might increase the rate of cancer cells undergoing apoptosis and reduce the burden of tumour growth as shown in the experiment. In our docking studies we found that Quercetin docks with amino acids of Bcl-2 and Bcl-xL with a binding energy of -8.6 and -9.2 Kcal/mol, which infers that it might restrict the activity of anti-apoptotic proteins thus favouring the apoptosis pathway.

3.1.8 Chromosome Instability Pathway

BUBR1 and MAD 2: BUBR1 and MAD2 are one of the key elements of the mitotic checkpoint complex that monitors the mitotic spindle checkpoint by blocking the onset of anaphase until all chromosomes properly attach to spindles. Heterozygous mutations in BUB1B encoding BUBR1 and MAD2 result in premature chromatid separation leading to cancer formation. It was found that there is an altered expression of BUBR1 and MAD2 in CRC cell lines (Li & Zhang, 2004). So these proteins can be considered as potential target for the development of novel therapies for the treatment of CRC [32]. By considering this aspect, we docked Quercetin with BURB1 and MAD2 protein and found s favourable binding score of -7.9 Kcal/mol and -8.1 Kcal/mol which shows the possibility of using Quercetin to treat CRC by means of targeting proteins involved in Spindle Assembly Checkpoint (SAC). *ck2 α :* CK2 α is a highly conserved, ubiquitous protein serine/threonine kinase which participates in the regulation of various cell cycle stages, presumably through phosphorylation of the proteins associated with cell cycle progression [13]. In Zouet al(2011) experiment they found that there is an increased expression of CK2 α in colorectal cancer and the suppression of this protein kinase inhibited the proliferation of CRC cells. So, in our study we found that Quercetin forms a stable interaction with ck2 α indicating that it might possibility inhibit the activity of CK2 α protein kinase.

3.1.9 Fatty Acid Binding Protein 6(FABP6)

FABP6 is a bile acid binding protein found in the distal portion of the small intestine and has been shown to be important in maintaining bile acid homeostasis [22]. High levels of bile acids induce oxidative damage, inflammation and hyperproliferation of CRC and the key transporter of bile acid to ileal epithelial cells is FABP6. In an experiment it was found that this protein is overexpressed and blocking of this transporter might reduce the tumour proliferatio [23]. By considering this hypothesis, we docked Quercetin with FABP6 and found that it has a favourable binding score of -6.8Kcal/mol indicating that Quercetin might act as inhibitor in the transport of bile acid to colon mucosa hereby restricting the progression of CRC.

Conclusion

Molecular docking of Quercetin with various target protein – APC, BUBR1, CDK8, CK2 α , FABP6, KRas, SPINDLE ASSEMBLY CHECKPOINT PROTEIN HUMAN MAD2, BCL -XL, BCL 2, COX 2, CYTOCHROME P450, PRTOEIN KINASE B, TNF α , NFKB, ck2 α and Fatty Acid Binding Protein 6(FABP6) was successfully performed using Auto dock Vina. Out of the 14 proteins docked, APC, K-ras and CDK8 are involved in Wnt/ β catenin pathway, and the docking result shows that Quercetin is a potential candidate in inhibiting the hyper activated Wnt/ β catenin pathway. Similar results were obtained when Quercetin was docked with proteins involved in the apoptotic pathway (Bcl -2 & BclxL.). In addition to this, Quercetin also docked with proteins involved in chromosome instability pathway - Spindle Assembly Checkpoint Protein Human MAD2 and BURB1. Based on docking analysis it was found that Quercetin has higher affinity towards enzymes involved in Arachidonic acid (AA) metabolism as the binding score was maximum for COX2(-10.2 Kcal/mol). Cytochrome P450 enzyme belongs to a class of enzymes which are involved metabolism of food and drug. It was found that these enzymes are involved in the progression of CRC via activation

of procarcinogen to carcinogen in colon. The docking result shows that Quercetin can control the activation of this enzyme, in other words Quercetin can control the food and drug metabolism taking place in colon. FABP6 protein was docked with Quercetin and result showed that Quercetin might regulate activity of FABP6, which shows the potential of Quercetin in controlling the transportation of bile acid. Akt, TNF α and NF κ B pathways are overexpressed in most of the cancer and the docking analysis shows that Quercetin might suppress the signalling of these pathways by inhibiting various proteins involved in the pathways. CK2 α is an important enzyme which act as connecting link between multiple pathways such as Akt and Wnt/ β catenin pathways and are found to be overexpressed. The docking result shows that Quercetin might restrict the signal transduction involved these pathways via regulating the activation of CK2 α . In silico analysis of Quercetin with these target proteins shows that Quercetin is a potential candidate in the development of novel drug for the treatment of cancer especially CRC. Future studies both in vitro and in vivo are required to evaluate the properties of Quercetin and its potential as an anticancer drug.

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