B-4917
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RESEARCH ARTICLE Indian Journal of Animal Research

Exploring Inhibitory Potential of Curcumin against Multiple Targets Involved in the Cancer Progression, Metastasis and Apoptosis Pathways by *in silico* Molecular Docking

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10.18805/IJAR.B-4917

ABSTRACT

Background: Cancer is a multifactorial disease characterized by altered gene expression and one of the most leading causes of death in the world. Curcumin obtained from *Curcuma longa* known to exhibit anti-inflammatory, antioxidant as well as anti-cancer properties. The present study was undertaken to know different targets through which curcumin acts to elicit the antitumor effect.

Methods: In this present study *in silico* interaction of curcumin with different cancer targets were analyzed by molecular docking using Biovia Discovery studio 4.0. Proteins were selected based on their role in cancer pathways which include transcription factor Nf-kB p50 and p65 subunit, Matrix metalloproteinase involved in invasion and metastasis, cell cycle regulator cyclin D and apoptotic factor Bax.

Result: Curcumin found to interact with all the selected proteins with high dock score of 125.428, 94.778, 114.5,111.731,98.6844 with Nf-kB p50, p65 subunits, MMP9, Cyclin D1 and Bax proteins, respectively. Amino acid residues to which curcumin interact, number of hydrogen bonds and hydrogen bond length also predicted. RMSD (Root Mean Square Deviation) value was found to be one in all the interactions. Based on the molecular interaction studies and ADME (Absorption Distribution Metabolism Excretion) predictions it was found that curcumin can act as potent inhibitor of many cancer targets and can be used as a starting molecule for the design of anticancerous drug.

Key words: Curcumin, Cyclin D, *In silico,* MMP9, Nf-Kb, Bax.

INTRODUCTION

Cancer is a multifactorial disease involving modulation of various pathways and targets which is a second most common cause of death following cardiovascular diseases. It was estimated that worldwide around 19.3 million cases and almost 10 million cancer deaths occurred in 2020 (Sung *et al*., 2020). Several research works are going on worldwide to identify the suitable chemopreventive and chemotherapeutic agents that can act on multiple signaling pathways. Identifying pharmacologically safe phytochemicals having multiple target effects is a hotspot in cancer research.

Curcumin is a major active principle obtained from the rhizome of *Curcuma longa* commonly known as turmeric. It is potentially known for its anti-inflammatory, anti-oxidant, antiseptic, antiplatelet, hepatoprotective and immune protective effects. Curcumin also found to have anticancer property by regulating different levels involved in cellular growth and apoptosis. Also, it acts on various transcription factors, oncogenes and signaling proteins and various stages of carcinogenesis, thus making it a potential chemotherapeutic agent for many human cancers (Mahajanakatti *et al*., 2014).

In silico methods are used along with *in vitro* data to create the model and to test it. Such models are used for the discovery and optimization of novel molecules with the affinity to target (Ekins *et al*., 2007). It is having significant role in cancer biology to find out targets in metastatic cancers (Kumari *et al*., 2013). Chemically curcumin is (1E, 6E)-1,7-bis(4-hydroxy-methoxyphenyl)-1,6- heptadiene-3,5-dione in which two phenyl hydrophobic domains are linked by flexible linker. Structurally curcumin exist in keta and enol tautomeric forms due to intramolecular hydrogen bonding. Molecular docking studies have proved that curcumin has the ability to adopt different conformations so that it can maximize the hydrophobic interactions with the protein to which it is bound. The phenolic and carbonyl functional groups located at the ends and center can involve in hydrogen bonding with the target molecule (Hobani *et al*.,2017). In the present

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How to cite this article: Veni, P.K., Thangapandiyan, M., Raja, P. and Rao, G.V.S. (2022). Exploring Inhibitory Potential of Curcumin against Multiple Targets Involved in the Cancer Progression, Metastasis and Apoptosis Pathways by *in silico* Molecular Docking. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4917.

Submitted: 13-04-2022 **Accepted:** 02-08-2022 **Online:** 24-08-2022

communication we aimed at studying the inhibitory potential of curcumin with various cancer targets.

MATERIALS AND METHODS

Selection of proteins

The proteins were selected based on their role in different pathways associated with cancer. The receptors selected were given in Table 1. The three-dimensional protein structure of these proteins was available in Protein Data Bank database (www. https://www.rcsb.org/).

Ligand preparation

The 3D structure of curcumin was downloaded from the chemical database "PubChem" (https://pubchem.ncbi.nlm.nih.gov/) which is a repository of chemicals. The identified lead compound was subjected to drug likeness, ADME profiles and toxicity analysis using bioinformatics online tool Pre-ADMET.

Molecular docking

The molecular docking between the ligand and proteins was done using the commercial software Biovia Discovery Studio 4.0 version in Bioinformatics Centre, Madras Veterinary College, TANUVAS, Chennai. The protocol for receptor ligand interaction namely Libdock was used for molecular docking. The dock score, relative energy and RMSD value were observed for analysis. The protein ligand interaction was visualized using discovery studio visualizer.

RESULTS AND DISCUSSION

Selection of proteins

The proteins were selected based on their function in cancer development and progression. Five different proteins (Nf-kB p50 subunit, Nf-kB p65 subunit, MMP 9, Cyclin D1 and Bax) were selected based on its functional role in cancer pathways.

Nuclear factor-kappa B is a transcriptional factor belonging to *rel* family that usually exist as dimeric form either as homodimer (p50/p50) or heteromer (p50/p65) in the cytoplasm. In case of tumour cells Nf-kB become active and undergo nuclear translocation where it will activate target genes. It is involved in many immune, inflammatory and apoptotic responses. p50 subunit binds to the DNA while the p65 subunit is responsible for the transcriptional activation (Nail Besli *et al*., 2020).

Invasion and metastasis are mainly responsible for the cancer associated deaths. Several proteolytic enzymes take part in the degradation of tumour microenvironment such as extracellular matrix and basement membrane. MMPs are zinc dependent endopeptidase involved in ECM proteolytic process (Egeblad *et al*., 2002).

Cancer can be due to the overexpression of proteins associated with cell cycle which can act either as positive or negative regulators. Cyclin proteins (Cyclin D, E, A and B) along with protein kinase group (CDK 4, 6 and 2) act as positive regulators and leads to acceleration of cell cycle (Sumirtanudin *et al*., 2020).

Most of the cancer therapy aimed at induction of apoptosis which can be achieved either by intrinsic or extrinsic pathway. The extrinsic pathway is mostly associated in controlling the cell turnover and elimination of mutant cells while intrinsic pathway is involved in antineoplastic drug action. Bax protein is an important mediator in intrinsic pathway which will get activated by DNA fragmentation and in turn activates a cascade of reactions by releasing 'cytochrome c' from mitochondria that helps in activation of caspases and ultimately leads to cell death (Kulsoom *et al*., 2018).

Ligand preparation

According to Lipinski rule of five (Lipinski *et al*.,1997) the compound was filtered as given in Table 2. Drug likeness, ADME and toxicity predictions of curcumin were performed. The pharmacokinetic features of curcumin were satisfactory with human intestinal absorption value of 94.403394, MDCK cell permeability value about 99.9895, skin permeability value of -2. 33227, pure water solubility 10.8005 mg/L, Plasma protein binding 88.030378 and blood barrier penetration value 0.0913545. The toxicity studies showed that curcumin is a non-mutagen as well as non-carcinogen predicted by Ames test and carcinogenicity mice model test, respectively. Drug likeness predictions

Table 1: Selected drug targets.

Protein	PDB 1D	Role in cancer
Nf-kB p50	1NFK	Transcription factor, regulate various other proteins involved in apoptosis, invasion and metastasis
Nf-kB p65	6QHL	
Cyclin D1	2W96	Involved in cell cycle
MMP 9	1L6J	Involved in invasion, metastasis and angiogenesis
Bax	4S0O	Pro apoptotic protein

Table 2: Identity of compound.

Compound name	Compound ID	Molecular formula	Molecular weight	H-acceptors and donors	Log P-Value
Curcumin	CID 969516	$C_{12}H_{20}O_6$	368.4	6,2	3.2

showed that curcumin has better drug like properties and fulfilling all the five rules.

Molecular docking

Molecular docking between curcumin and various receptors were performed to analyze the inhibitory action of curcumin. Best docking conformations were selected based on docking score and relative energy. The results on molecular docking are summarized in Table 3.The present study showed that curcumin has best binding properties to all receptors screened. Curcumin found to interact with p50 subunit of Nf-kB with a dock sore of 125.428 and binding energy 6.93 (Fig 1). They found to interact with LYS 241 and LEU22 (Fig 2). Kumar and Bora. (2012) earlier reported similar interactions between curcumin and its derivatives to LYS 241 which was found to be a key residue involved in the binding of Nf-kB with DNA at the consensus sequences through hydrogen bonding. Curcumin also found to bind with p65 subunit of Nf-kB subunit with high affinity (Fig 3 and 4). Nf-kB is a nuclear factor that act on various signaling pathways, involved in cell proliferation and cell survival thereby involve

Table 3: Molecular docking interaction of curcumin with different target proteins.

Protein	Active sites	Ligand	Dock score	Relative energy (kcal/mol)	H bond length(A°)	Amino acids	RMSD value
Nf-kB p50 subunit	23	Curcumin	125.428	6.93	62 bonds 0.526 to 3.07	LYS 241ARG 54	1
Nf-kB p65 subunit	2	curcumin	94.778	7.9331	41 bonds 0.532 to 3.19	LYS 49LEU222	1
MMP 9	6	Curcumin	114.5	2.4262	50 bonds 1.44 to 3.07	LEU 39ARG 51ASP 182ARG 95	1
Cyclin D1	18	Curcumin	111.731	6.9197	27 bonds 1.03 to 3.85	ARG 26CYS 8ALA133 HIS 68VAL 27	1
Bax	10	Curcumin	98.6844	10.9832	52 bonds 0.22 to 3.09	PRO 49ALA 46LEU 47	1

Fig 1: Curcumin and Nf-kB p50 subunit.

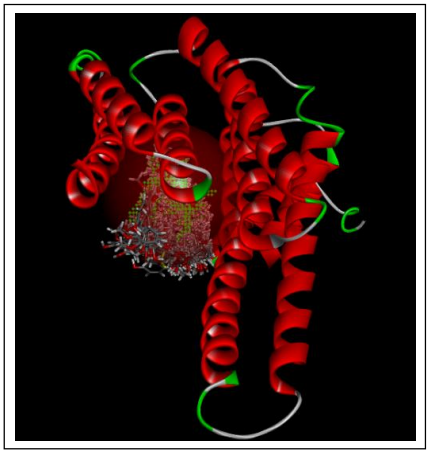

Fig 3: Curcumin and p65 subunit.

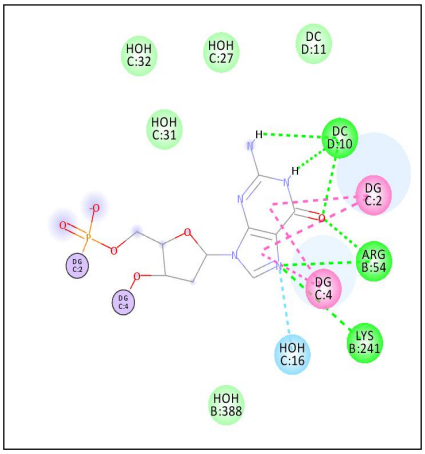

Fig 2: 2D diagram of curcumin and Nf-kBp50.

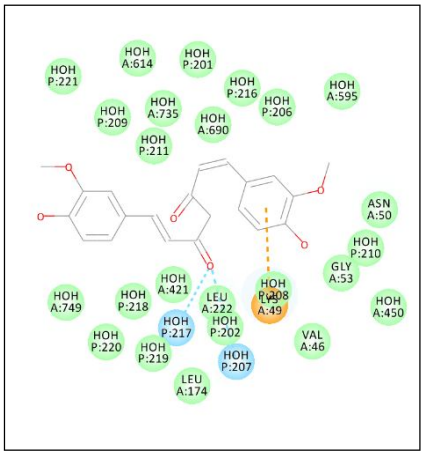

Fig 4: 2D diagram of curcumin and p65 subunit.

in pathogenesis of cancer. So, inhibitors of Nf-kB likely to have benefits in the treatment of cancer. The present study revealed that curcumin has the ability to bind to both the subunits of Nf-kB with good binding affinity.

MMPs are mostly associated with functioning and migration of cancer cells. The result of present study indicates that curcumin can bind with MMP-9 with a dock score of 114.5 (Fig 5) and it was found that they interact with LEU 39 and ARG 51 with hydrogen bond distance of 1.47 and 1.48 A° (Fig 6). Jerah *et al*. (2015) reported that curcumin can bind with MMP-3 with similar binding affinity as that of its known inhibitors and suggested that curcumin can act as a potential starting molecule for the design of anticancer drugs that target MMP enzymes.

Fig 5: Curcumin and MMP9.

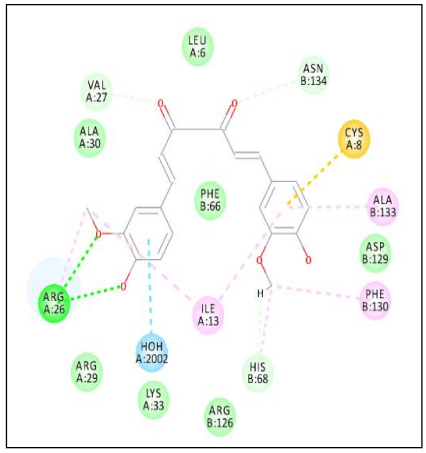

Fig 8: 2D diagram of curcumin and cyclin D.

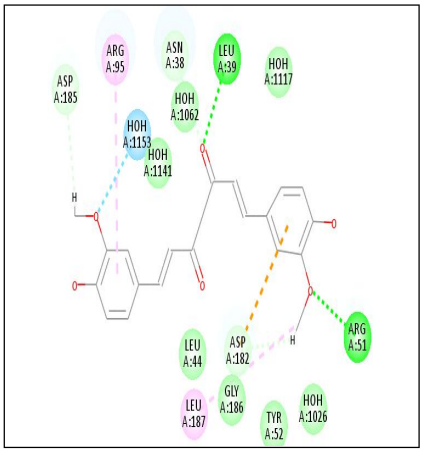

Fig 6: 2D diagram of curcumin and MMP9.

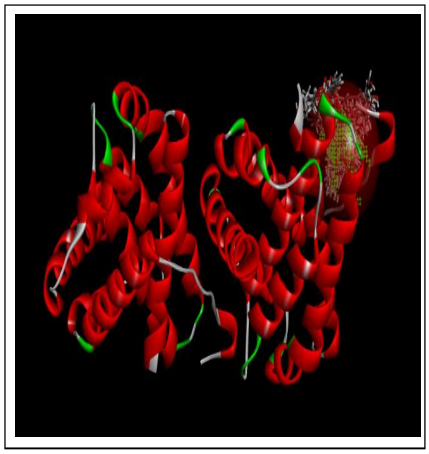

Fig 9: Curcumin and Bax interaction.

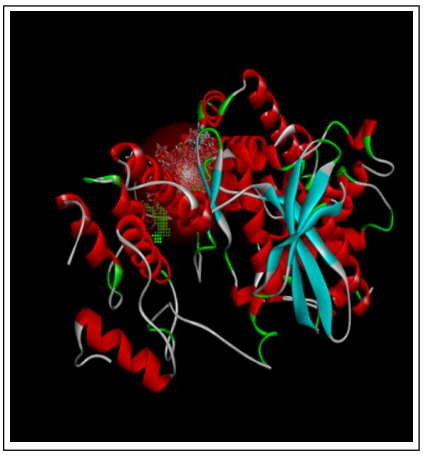

Fig 7: Curcumin and Cyclin D1 interaction.

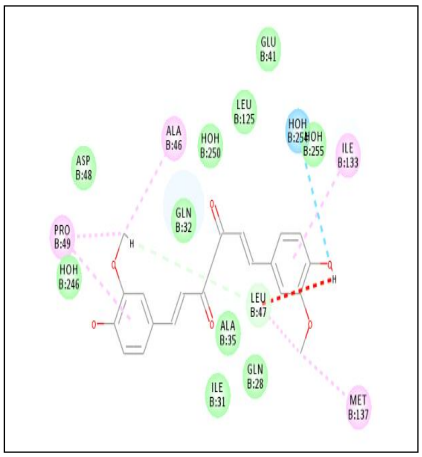

Fig 10: 2D diagram of Curcumin and Bax.

The present study results suggested that curcumin can regulate cell cycle and apoptosis by binding with Cyclin D1 and Bax proteins. Curcumin found to interact with ARG26 of cyclin D1 with a hydrogen bond distance of about 1.07 A° (Fig 7 and 8). Curcumin interacts with Bax with a docking score of 98.6844 and binding energy 10.9384 (Fig 9 and 10). The present results also suggested that curcumin can act as a potential starting molecule for the design of anticancerous drug by interacting with multiple targets in the signaling pathways and apoptosis.

CONCLUSION

Cancer is one of the leading causes of death worldwide and still researches are going on to find out the suitable chemotherapeutic agents having wide range of anti tumor effects. This *in silico* molecular docking study showed that curcumin act as a potential inhibitor of multiple cancer targets involved in cancer progression, metastasis and apoptosis. Our study results concluded that curcumin can act as suitable drug candidate against various cancers.

Conflict of interest: None.

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