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Case Study……!!!

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MOLECULAR DOCKING STUDIES OF CURCUMIN AS ANTI-INFLAMMATORY AGENT: A CASE STUDY

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ABSTRACT

Inflammation is a key etiological factor for several disease conditions such as hypersensitivity, asthma, Inflammatory Bowel Disease (IBD), rheumatoid arthritis and many others. Most of the currently marketed therapeutic drugs are associated with adverse side effects and are not suitable for chronic therapies and so some of them were withdrawn from the markets. For instance, Non-Steroidal Anti-Inflammatory (NSAID's) drugs are reported to have adverse drug interactions and hence are not prescribed along with warfarin, antihypertensives and diuretics. Thus, treatment of these inflammatory disorders still remains a growing health concern and has become a major challenge to the health professionals. The anti-inflammatory, anti-oxidant, chemopreventive and chemotherapeutic activities of curcumin have been demonstrated both in cultured cells and in animal models. Though curcumin is well documented and reported to have anti-inflammatory activity, the exact mechanism of action and efficient in vivo dosage required for potential anti-inflammatory activity of curcumin anti-inflammatory activity and to identify the possible, safe and effective dose requirement for the anti-inflammatory activity in vitro and in vivo. The objective of the current study is to understand and establish the role of curcumin in the treatment of inflammatory condition. The specificity and binding affinity of curcumin to major inflammatory mediators such as, cytokines/chemokines, signaling proteins and transcription factors were evaluated using molecular docking. To find the interaction between drug molecule and receptor by performing docking studies.

1. INTRODUCTION[1,2,3]

Turmeric (the common name for *Curcuma longa*) is an Indian spice derived from the rhizomes of the plant and has a long history of use in Ayurvedic medicine as a treatment for inflammatory conditions. *C.longa* is a perennial member of the *Zingiberaceae* family and is cultivated in India and other parts of Southeast Asia. The primary active constituent of turmeric and the one responsible for its vibrant yellow colour is curcumin, first identified in 1910 by Lampe and Milobedzka. While curcumin has been attributed numerous pharmacological activities, including antioxidant and antimicrobial properties, this article focuses on one of the best-explored actions, the anti-inflammatory effects of curcumin. Based on early research conducted with cell cultures and animal models, pilot and clinical trials indicate curcumin may have potential as a therapeutic agent in diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis, as well as certain types of cancer. Numerous clinical trials are currently in progress that, over the next few years, will provide an even deeper understanding of the therapeutic potential of curcumin.

Turmeric is comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. The curcuminoid complex is also known as Indian saffron. Curcumin is a lipophilic polyphenol that is nearly insoluble in water but is quite stable in the acidic pH of the stomach.

Figure no 1-Turmeric field

Figure no 2- Turmeric plant

1.1 USES OF TURMERIC

- It is used as Spices Condiment, Flavouring Agent, Colouring Agent.
- It is used in Eczema, Chicken Pox, Shingles, Allergy and Scabies.

- It is used As Anti-Inflammatory, Antioxidant, Antitumour, Antibacterial, Antifungal and Antiviral activities, which indicate potential in clinical medicine.

Inflammation is a complex host (systemic/local) response to a wide range of tissue injury and infection, generally marked by increased levels of cytokines, cytokine receptors, adhesion molecules, immunoregulatory factors and several other mediators. Histamine, a biogenic amine known to regulate the secretion of proinflammatory cytokines like IL-1α, IL-1β, IL-6 and chemokines like RANTES or IL-8(Vannier and Dinarello, 1993; Meretey *et al*., 1991; Jeannin *et al*., 1994 ; Bayram *et al*., 1999). Hisatmine is a crusical player both in inflammatory and allergic response and proposed to mediate immediate hypersenstivity (Tripathi *et al*., 2010). Expression of histamine receptors on the endothelial cells is regulated by histamine and thereby effects the entire inflammatory reaction (Schaefer *et al*., 1999). Recent animal studies have shown that histamine released from non-mast cells contribute more towards angiogenesis and generation of inflammatory granulation (Ghosh *et al*., 2002). β- hexosaminidase an acid exoglycosidase produced, stored in rat mast cells granules and is released from the mast cells in parallel with histamine upon stimulation (Lynch *et al*., 1978 Lagunoff *et al*., 1970; Lagunoff, 1972).

It is evidenced that at least 86% of the mast cell content of β -hexosaminidase is available for immunologic release from the secretory granules along with histamine released from the activated mast cells (Schwartz *et al*., 1979). Therefore, the release of β-Hexosaminidase from the activated immune cells has been considered as a potent degranulation marker for histamine release (Schwartz and Austen, 1980; Ozawa *et al*., 1993). Thus β- Hexosaminidase and histamine are ideal markers to evaluate the inflammatory response *in vitro* and *in vivo*.

Inflammation is a key etiological factor for several disease conditions such as hypersensitivity, asthma, Inflammatory Bowel Disease (IBD), rheumatoid arthritis and many others. Most of the currently marketed therapeutic drugs are associated with adverse side effects and are not suitable for chronic therapies and so some of them were withdrawn from the markets. For instance, Non-Steroidal Anti-Inflammatory (NSAID's) drugs are reported to have adverse drug interactions and hence are not prescribed along with warfarin, antihypertensives and diuretics. Thus, treatment of these inflammatory disorders still remains a growing health concern and has become a major challenge to the health professionals.

Among the alternative compounds screened for anti-inflammatory property, curcumin was most widely screened and well established for its anti-inflammatory properties (Menon and Sudheer, 2007). Primary source of curcumin is Curcuma longa (turmeric), which is one of the common dietary supplements with long history of use in traditional medicines of India and China. Curcumin is regarded as a multi target drug candidate with several in-intro and in-vivo studies proposing its anti-cancer, anti-viral, anti-arthritis, anti-oxidant, antiamyloid and anti-inflammatory activities (Vorlaphim *et al*., 2011).

Major constituents of *Curcuma longa* are identified to be curcumin and curcuminoids such as demethoxycurcumin, bis-demethoxycurcumin and cyclocurcumin. The anti-inflammatory, anti-oxidant, chemopreventive and chemotherapeutic activities of curcumin have been demonstrated both in cultured cells and in animal models.

The anti-inflammatory and antioxidant activities have been well documented and recently reviewed (Hatcher *et al*., 2008). Additionally, curcumin which is the major and reportedly the most active component of the traditional herbal remedy is effective in various disease conditions and essentially mediates its therapeutic effects mainly via its anti-inflammatory and anti-oxidant properties (Hatcher *et al*., 2008).

Though curcumin is well documented and reported to have anti-inflammatory activity, the exact mechanism of action and efficient in vivo dosage required for potential anti-inflammatory activity of curcumin anti-inflammatory activity and to identify the possible, safe and effective dose requirement for the anti-inflammatory activity in vitro and in vivo. In silico docking studies and in vitro cell line studies have been performed to elucidate the mechanism of action followed by in vivo studies to evaluate the dose dependent efficacy of curcumin against carrageenan induced paw edema and to arrive at the median Effective Dose (ED50) upon oral administration. curcumin is yet to be determined.

1.2 CHEMICAL PROPERTIES

Curcumin, the most active component of turmeric, makes up 2–5% of this spice. The yellow color of the turmeric is due to the curcumin compound. Curcumin (C21H20O6) was first described in 1910 by Lampe and Milobedeska and shown to be a diferuloylmethane, 1,7-bis (4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione, and is practically insoluble in water. Curcumin is a bis-α-β-unsaturated β-diketone; under acidic and neutral conditions, the bis-keto formof the compound predominates, and at pH above 8, the enolate form is generally found

Hence at pH 3–7, it acts as an extraordinarily potent H-atom donor and above pH 8, it acts mainly as an electron donor, a mechanism more suitable to the scavenging or antioxidant properties of curcumin. Curcumin is quite unstable at basic pH and degrades within 30 minutes. Human blood or antioxidants such as ascorbic acid, or the presence of 10% fetal bovine serum in the culture media prevents this degradation.

Curcumin has a molecular weight of 368.7 and the commercial grade curcumin contains curcuminoids, 10–20% desmethoxycurcuminand less than 5% bisdesmethoxycurcumin. The commercial grade curcumin is just as effective as pure curcuminin preclinical models of carcinogenesis.

Figure no 3- Rhizome of Turmeric

2. MATERIAL AND METHODS

2.1 Target Identification

Three dimensional structures of the Ligand Binding Domain Structure of PPAR receptor 3PRG and the Crystal Structure of TNF-TNFR2 complex receptor 3ALQ was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). Retrieved the data from biological databases like Protein Data Bank (PDB), Chem draw, PubChem. In silico studies were carried out using softwares and online tools like Swiss–Pdb Viewer (SPDBV) Osiris Property Calculator.

Protein Data Bank (PDB) is a repository for the 3-D structural data of large biomolecules, such as protein, DNA and RNA . NCBI PubChem is a chemical compound database that provides information on biological activities of small molecules. Swiss PDB viewer (SPDBV) is a molecular modeling and structure analysis tool.

2.2 Need and Objective [3, 4, 5]

In order to know the binding conformations, the molecules exhibit geometric and complementarily, both of which are essential for successful drug activity by means of computational process of searching for a ligand that is able to fit both geometrically and

energetically in to binding site of a protein. The objective of the current study is to understand and establish the role of curcumin in the treatment of inflammatory condition. The specificity and binding affinity of curcumin to major inflammatory mediators such as, cytokines/chemokines, signaling proteins and transcription factors were evaluated using molecular docking. To find the interaction between drug molecule and receptor by performing docking studies

2.3 Plan of work [7, 8]

Drug designing is a process of designing a drug molecule that can interact and bind to a target. Receptors are molecules which can be seen on the surface of the cell transmit signals upon binding by a small molecule triggering a cellular process. In an unbounded state receptor, functionalities of the receptor remain silent. Hence this definition says that receptor binds specifically to a particular ligand or vice versa, but in some cases high concentrations of ligands will bind to multiple receptor sites.

Drug design, sometimes referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. Drug design involves the design of small molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design (CADD). Computational Chemistry/CADD is the chemistry whose major goals are to create efficient mathematical approximations and computer programs that calculate the properties of future drug molecules and thus helping in the process of drug design and discovery.

3. PURPOSE OF CADD

Drug Discovery today are facing a serious challenge because of the increased cost and enormous amount of time taken to discover a new drug, and also because of rigorous competition amongst different pharmaceutical companies.

3.1 GOALS OF CADD

- Define a target structure
- And/ or its binding site
- And/ or its active ligand (possibly bound to protein)
- Find a new molecule that changes the target's activity

3.2 MODERN DRUG DISCOVERY PROCESS

- Target identification
- Target validation
- Lead identification
- Lead optimization
- Preclinical phase
- Drug discovery

Drug receptors usually remain without endogenous ligand. The receptors for these drugs molecules can be enzyme, ion channel, protein, nucleic acids etc. Hence the drug molecule will go and cross link the DNA and stops DNA replication. It is used to treat malignant tumors. Receptors for endogenous regulatory ligands are hormones, neurotransmitters, autacoids, growth factors, cytokines etc. Hence the function of these receptors is to sense the ligands and initiate the response. For example, Aspirin is a small pain killer drug molecule which contains nine carbon atoms, eight hydrogen atoms and four oxygen atoms. Design of the molecules should be complementary in shape and charge to the target.

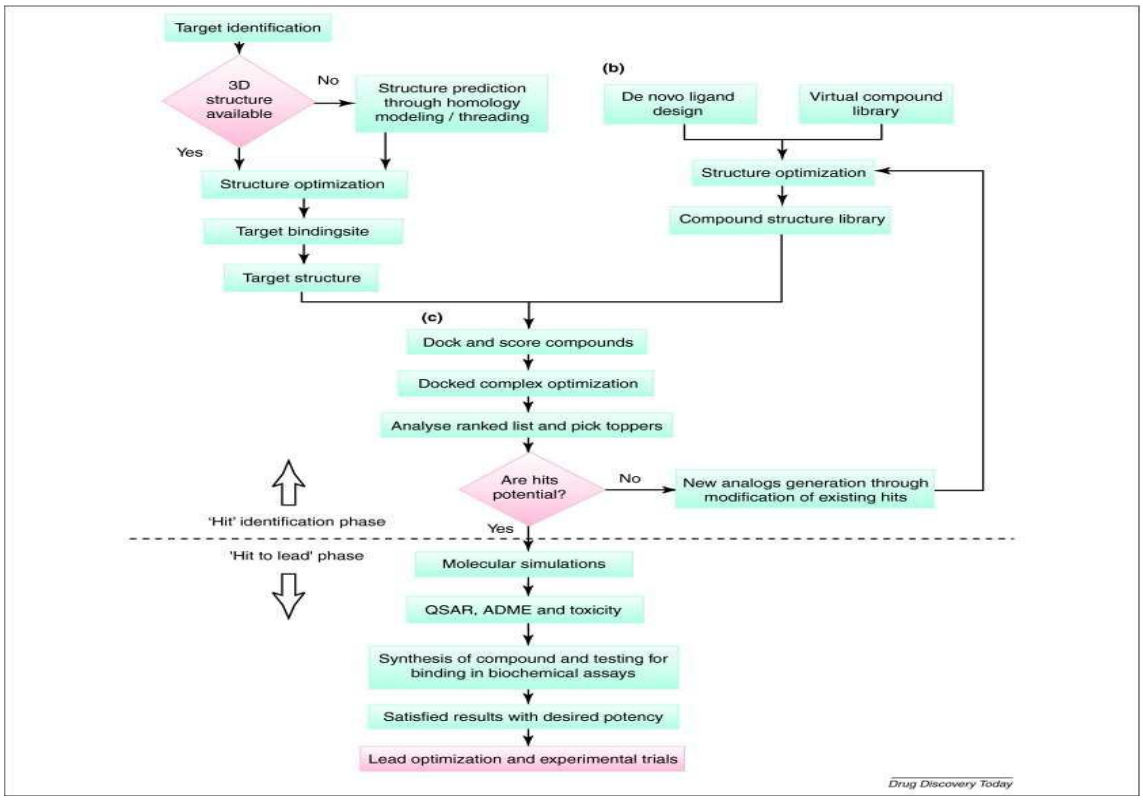

Figure no 4 -The Computer-Aided Drug Discovery Process

3.3 DRUG TARGETS

- Enzyme – inhibitors
- Receptors - agonists or antagonists
- Ion channel – blockers
- Transporter –inhibitors
- DNA – blockers

Drug molecule mediates signal transmission through a molecule that is complementary which is essential for a biological process. The evolution of the receptor functions depends on the development of specific sites which are designed to bind drug molecules. Drug molecule binding capacity is important for the regulation of biological functions. Drug-receptor interactions occur through the molecular mechanics involving the conformational changes among low affinity and high affinity states. Drug molecule binding interactions changes the receptor state and receptor function.

The application of computational methods to study the formation of intermolecular complexes is a subject of intensive research. Drug exerts its biological activity by binding to the pocket of receptor molecule (usually protein). In their binding conformations, the molecules exhibit geometric and chemical complementarily, both of which are essential for successful drug activity. The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking.

Molecular docking helps in studying drug/ ligand or receptor/ protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand - receptor complex and calculating the energy of interaction for different ligands to design more effective ligands.

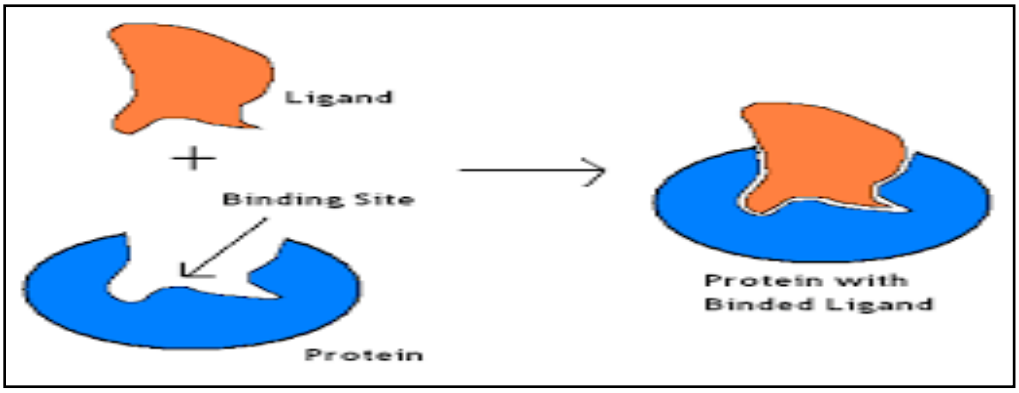

Figure no 5-Ligand Protein Binding Interaction

Molecular modeling has become a valuable and essential tool to medicinal chemists in the drug design process. Molecular modeling describes the generation, manipulation or representation of three-dimensional structures of molecules and associated physico-chemical properties. It involves a range of computerized techniques based on theoretical chemistry methods and experimental data to predict molecular and biological properties. Depending on the context and the rigor, the subject is often referred to as 'molecular graphics', 'molecular visualizations', 'computational chemistry' or 'computational quantum chemistry'. The molecular modeling techniques are derived from the concepts of molecular orbitals of Huckel, Mullikan and 'classical mechanical programs' of Westheimer, Wiberg and Boyd.

The techniques are used in the fields of computational chemistry, drug design, computational biology and materials science for studying molecular systems ranging from small chemical systems to large biological molecules and material assemblies. The common feature of molecular modeling techniques is the atomistic level description of the molecular systems. Most molecular modeling studies involve three stages.

- In the first stage, a model is selected to describe the intra- and inter- molecular interactions in the system. The two most common models that are used in molecular modeling are quantum mechanics and molecular mechanics. These models enable the energy of any arrangement of the atoms and molecules in the system to be calculated, and allow the modeler to determine how the energy of the system varies as the positions of the atoms and molecules change.
- The second stage of a molecular modeling study is the calculation itself, such as an energy minimization, a molecular dynamics or Monte Carlo simulation, or a conformational search.
- Finally, the calculation must be analyzed, not only to calculate properties but also to check that it has been performed properly.

4. MOLECULAR MODELING STRATEGIES

i) Direct drug design

In the direct approach, the three-dimensional features of the known receptor site are determined from X-ray crystallography to design a lead molecule. In direct design, the receptor site geometry is known; the problem is to find a molecule that satisfies some geometric constraints and is also a good chemical match. After finding good candidates according to these criteria, a docking step with energy minimization can be used to predict binding strength.

ii) Indirect drug design

The indirect drug design approach involves comparative analysis of structural features of known active and inactive molecules that are complementary with a hypothetical receptor site. If the site geometry is not known, as is often the case, the designer must base the design on other ligand molecules that bind well to the site.

4.1 HOMOLOGY MODELING SOFTWARE

- **Abalone:** is designed for macromolecular simulations (proteins, DNA). It supports both explicit and implicit solvent models. In contrast to Ascalaph, tailored to the simulation of small molecules, Abalone is focused on molecular dynamics modeling of biopolymers. It supports such effective methods as the Replica Exchange and hybrid Monte Carlo.
- **Ascalaph:** is general purpose molecular modeling software that performs quantum mechanics calculations for initial molecular model development, molecular mechanics and dynamics simulations in the gas or in condensed phase. It can interact with external molecular modeling packages (MDynaMix, NWChem, CP2K, PC GAMESS/Firefly and Delphi).
- **Yasara:** is a molecular-graphics, modeling and simulation package for Linux and Windows. Yasara is powered by PVL (Portable Vector Language), a new development framework. PVL allows you to visualize even the largest proteins and enables true interactive real-time simulations with highly accurate force fields on standard PCs.
- **RasMol*:*** is a molecular graphics program developed at the University of Edinburgh. The software is intended for the visualization of proteins, nucleic acids and small molecules. The program has the ability to read in PDB as well as several other formats. Coloring schemes including atom type, temperature factor and hydrophobicity.
- **MacroModel:** is a computer program for molecular modelling of organic compounds and biopolymers. It features various force fields coupled with energy minimization algorithms for the prediction of geometry and relative conformational energies of molecules. Macro Model also has the ability to perform molecular dynamics simulations to model systems at finite temperatures using stochastic dynamics and mixed Monte Carlo algorithms.
- **SYBYL-X:** provides capabilities for crucial small molecular modeling and simulation, includng structure-activity relationship modeling, pharmacophore hypothesis generation,

molecular alignment, conformational searching, homology modeling, sequence alignment, and other key tasks required to understand and model the static and dynamic 3D structural properties of proteins and other biological macromolecules.

- **Amber:** is a suite of programs for molecular simulation and analysis of proteins, nucleic acids, lipids, carbohydrates. Amber" refers to two things: a set of molecular mechanical force fields for the simulation of biomolecules (which are in the public domain, and are used in a variety of simulation programs); and a package of molecular simulation programs which includes source code and demos.
- **MOE:** internal representation of organic chemical structures and flexible architecture provide a solid foundation for molecular modeling and computational chemistry.

4.2 CATEGORIES OF DOCKING

1. Protein-Protein Docking:
    - Both molecules are rigid.
    - Interaction produces no change in conformation.
    - Similar to lock and key model.
2. Protein-Ligand Docking:
    - Ligand is flexible but the receptor protein is rigid.
    - Interaction produces conformational changes in ligand.

4.3 DOCKING SCORE

- To place a ligand (small molecule) into the binding site of a receptor in the manners appropriate for optimal interactions with a receptor.
- To evaluate the ligand-receptor interactions in a way that may discriminate the experimentally observed mode from others and estimate the binding affinity.

4.4 APPLICATIONS OF HOMOLOGY MODELING:

- Studying the effect of mutations.
- Identifying active and binding sites on protein (useful for ligand design).
- Searching for ligands of a given binding site (database mining).
- Designing novel ligands of a given binding site.
- Modeling substrate specificity.
- Predicting antigenic epitopes.

- Protein–protein docking simulations.
- Molecular replacement in X-ray structure refinement.
- Rationalizing known experimental observations.
- Planning new computational experiments with the provided models.

TURMERIC[2,3,4]

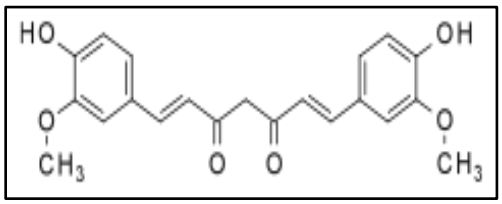

Figure no 6- Keto form of Curcumin

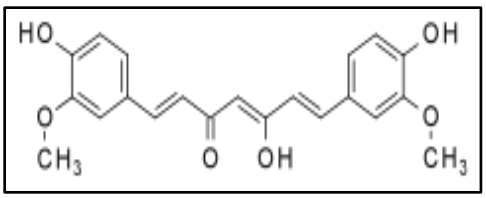

Figure no 7- Enol form of Curcumin

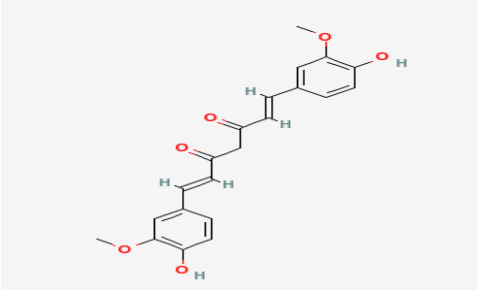

Figure no 8-2D Structure of Curcumin

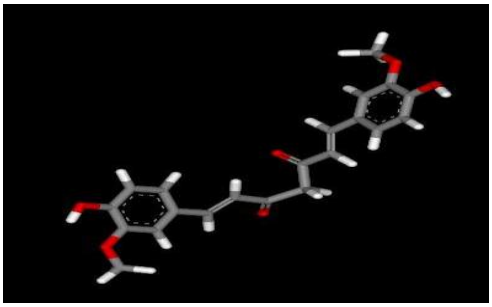

Figure no 9-3D Structure of Curcumin

4.5 POTENTIAL MECHANISMS (PPAR-γ) [3,4,10]

The mechanism by which Curcumin induces its Anti-inflammatory effects is yet to be elucidated. Studies have shown that peroxisome proliferator-activated receptor gamma (PPAR-γ) has been associated with anti inflammatory effects. PPARs belong to the superfamily of nuclear receptors consisting of three genes that give rise to three different subtypes, PPAR-α, PPAR-δ, and PPAR-γ. Among them, PPAR-γ is the most widely studied form. Upon ligand binding, PPAR-γ forms heterodimers with the retinoid X receptor and binds to a peroxisome proliferation response element (PPRE) in a gene promoter leading to regulation of gene transcription. Recent evidence suggests that PPAR- γ ligands exert their effects in HT-29 colon cancer cells by phosphorylation of the PPAR-γ by the extracellular signal regulated kinase 1/2, thereby causing a physical interaction with the p65 subunit of the NF-κB preventing the activation of the NF-κB pathway.

The inhibition of cell signaling pathways, Akt, NF-κB, AP-1, or JNK, has been implicated as the mechanism responsible for apoptosis induction by curcumin.

A recent study reported that curcumin potentiates the antitumor effect of gemcitabine in pancreatic cancer by suppressing proliferation, angiogenesis, and down regulating NF-κB and NF-κB-regulated gene products. However, it is plausible that curcumin induced anti-inflammatory effect caused by the up regulation of PPAR-γ is associated with the NF-κB pathway. Analogs of curcumin (Cur), such as demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), tetrahydrocurcumin (THC) and turmerones, modulate inflammatory signaling and cell proliferation signaling to same extent as curcumin was investigated. The results indicate that the relative potency for suppression of tumor necrosis factor (TNF)-induced nuclear factor-κB (NF-κB) activation was Cur > DMC > BDMC; thus suggesting the critical role of methoxy groups on the phenyl ring. THC, which lacks the conjugated bonds in the central seven-carbon chain, was completely inactive for suppression of the transcription factor. Turmerones also failed to inhibit TNF-induced NF-κB activation. The suppression of NF-κB activity correlated with inhibition of NF-κB reporter activity and with down-regulation of cyclooxygenase-2, cyclin D1 and vascular endothelial growth factor, all regulated by NF-κB. In contrast to NF-κB activity, the suppression of proliferation of various tumor cell lines by Cur, DMC and BDMC was found to be comparable; indicating the methoxy groups play minimum role in the growth-modulatory effects of curcumin. THC and turmerones were also found to be active in suppression of cell growth but to a much lesser extent than curcumin, DMC and BDMC. Whether suppression of NF-κB or cell proliferation, no relationship of any of the curcuminoid was found with reactive oxygen species (ROS) production. Overall, our results demonstrated that different analogs of curcumin present in turmeric exhibit variable anti-inflammatory and anti-proliferative activities, which do not correlate with their ability to modulate the ROS status.

TNFα-Activation

Tumour necrosis factor (TNF), formerly known as TNFα or TNF alpha is the best-known member of this class. TNF is amonocyte-derived cytotoxin that has been implicated in tumor regression, septic shock, and cachexia. It is a potent pyrogen, causing fever by direct action or by stimulation of interleukin-1 secretion; it can stimulate cell proliferation and induce cell differentiation under certain conditions. Major steps in the TNF-mediated cytotoxicity cascade include G protein-coupled activation of phospholipases, generation of free radicals, and damage to nuclear DNA by endonucleases.

4.6 MOLECULAR DOCKING USING VLifeMDS

VLifeMDS provides a facility to dock different ligands in protein binding sites chosen by the user. VLifeMDS provides both rigid (no torsional flexibility for protein as well as ligand) and flexible (torsional flexibility to ligand with rigid protein) docking of the molecules.

The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand - receptor complexes having minimum interaction energy based on different scoring functions viz. only electrostatics, sum of steric and electrostatic (parameters from MMFF force field) and Dock Score. This utility allows one to screen a set of compounds for lead optimization. VLifeMDS uses genetic algorithm (GA), Piecewise Linear Pairwise Potential (PLP) and Grid algorithms to minimize the interaction energy between ligand - receptor.

Distinction of good or bad docked conformation is based on scoring or fitness function. (MDS uses fitness functions on only electrostatic and both steric and electrostatic interactions between receptor-ligand as well as Dock Score scoring function). The Dock score or X-Cscore as it is called compute binding affinity of a given protein ligand complex with known 3-D structure. Dock/X-Cscore scoring function include terms for vander walls interaction, hydrogen bonding, deformation penalty, hydrophobic effects.

V life has provides following functions:

- Building polypeptides using V Life MDS, Molecular Docking using V Life MDS.
- Homology modeling using Biopredicta.
- Protein complex optimization using V Life MDS, Using alignment method in V Life MDS.
- Building molecules using V Life MDS.
- Conformational search using V Life MDS.
- Optimizing Molecules using V Life MDS, Using miscellaneous utilities in V Life MDS.
- QSAR using V Life MDS.

Steps of Docking

- 2D structures of ligands were drawn in ChemDraw.
- 2D Structures were converted to 3D.
- Conformers were generated and optimized.
- Lowest energy conformer was selected and used for docking.
- Docking was done by GA based docking.
- Cavity 7 was selected for docking.
- Dock score was calculated.
- Docked Complex was optimised.

4.7 PREPARATION OF PROTEIN

- The Ligand Binding Domain Structure of PPAR receptor 3PRG used in this study was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). The PDB files were energy minimized using VLifeMDS and it was used for further studies.

Figure no 10- Ligand Binding Domain structure of PPAR

- The Crystal Structure of TNF-TNFR2 complex receptor 3ALQ used in this study was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). The PDB files were energy minimized using VLifeMDS and it was used for further studies.

Figure no 11- Crystal Structure of TNF-TNFR2 complex

Docking studies (Interaction of Curcumin with PPAR-*γ* & TNFα):

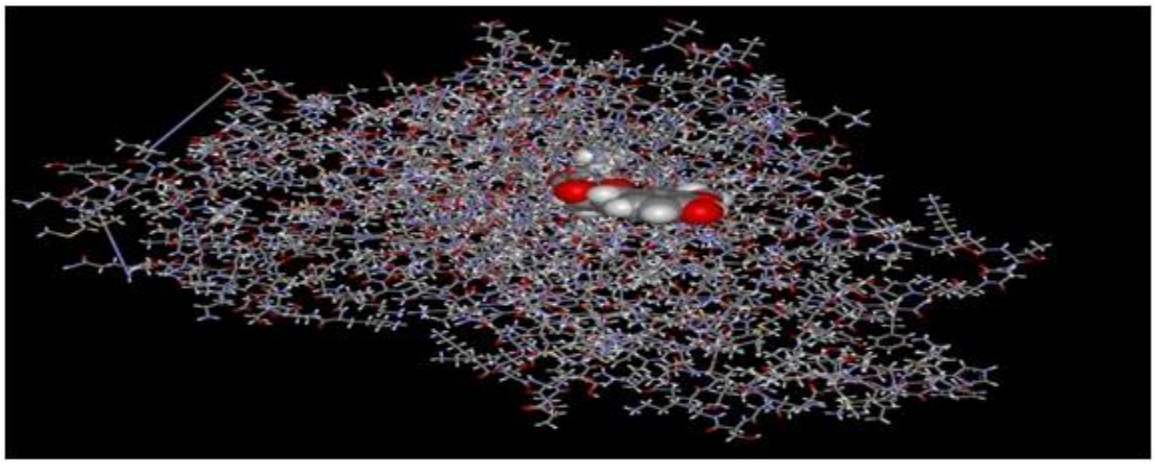

Figure no 12 - Docked image of target molecule PPAR-*γ* (2PRG)

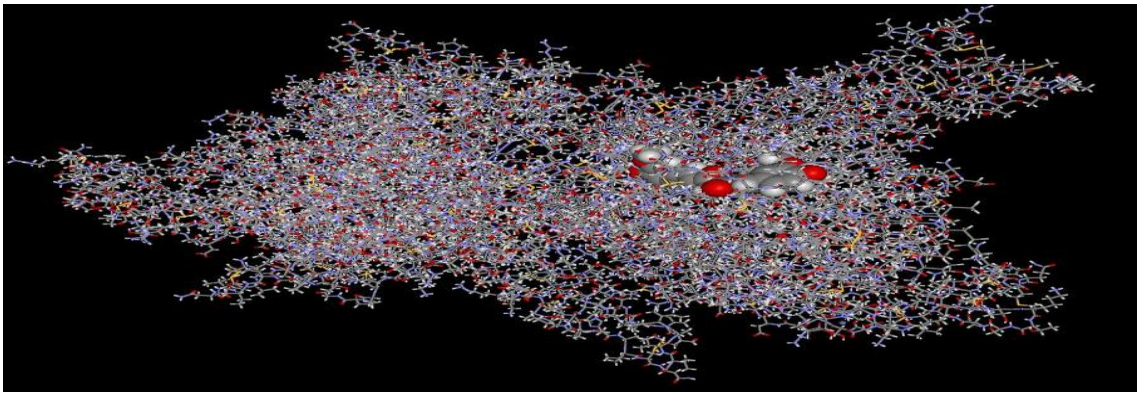

Figure no 13- Docked image of target molecule TNF- α (3ALQ)

5. RESULT AND DISCUSSION

SR.NO	NAME OF RECEPTORS	DOCK SCORE
1.	PPAR-γ(2PRG)Ligand binding domain of human PPAR	-4.08
2.	TNF- α(3ALQ)Crystal structure of TNF-TNFR2 complex	-4.95

- This studies shows some representative runs in these docking methodologies employed by VLifeMDS. It should be noted that docking method is iterative and has to be repeated changing different input parameters till user gets the best possible scoring value (whichever scoring function user chooses) within the desired range. It is up to the user to choose which docking method suits best for a given dataset. VLifeMDS also provides different scoring functions for each of Grid and GA based docking methods.
- Docking studies: The binding mode and the interactions of PPAR- γ(2PRG), TNF-α(3ALQ) with curcumin were studied using VLife MDS. By compairing the interactions of Curcumin with PPAR-γ(2PRG) and TNF- α(3ALQ),Among this both interactions, the PPAR-γ(2PRG)has more binding capabilities with curcumin because its docking score is -4.08kcal/mol as compare with the TNF- α(3ALQ) it has docking score -4.95kcal/mol which shows good result. VLife MDS results indicate that these ligands interact with crucial residues in the active site region.

6. CONCLUSION

It can be concluded that the study was to identify the best binding capabilities of receptors (PPAR-γ(2PRG) & TNF- α(3ALQ)with ligand (Curcumin) as Anti-inflammatory that show Anti-inflammatory action/effect of Curcumin and activates this both receptors. These 2 receptors were selected for the studies. These receptors were retrieved from pubchem and their properties were calculated using online tools EXPLORER, CS ChemDraw Ultra 7.01, VLifeMDS.Based on these 2 receptors PPAR-γ(2PRG), TNF- α(3ALQ) were found to be suitable for docking studies.

Docking studies were performed with PPAR-γ(2PRG) & TNF- α(3ALQ) receptors using CS ChemDraw Ultra 7.01, VLifeMDS. From CS ChemDraw Ultra 7.01, VLifeMDS dock studies the best pose was obtained with least energy value. The interaction with active site indicates that these two receptors can be considered as binding sites of Curcumin. Also, we can try to compare the different docking (stabilizing) receptor - ligand interactions (e.g. charged, hydrogen bonding, pi-stacking and other interactions) as reported in literature and also in the final docked complex for a given receptor - ligand set, as obtained with VLifeMDS to ascertain their role in overall docking stabilization of the complex.

Overall results suggest that, curcumin mediates its anti-inflammatory activity by its direct effect on multitarget inflammatory mediators while others were mediated by the downstream effects of curcumin.

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