Middle East Research Journal of Pharmaceutical Sciences
ISSN: **2789-7702 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: 10.36348/merjps.2022.v02i01.002

KUWAIT SCHOLARS PUBLISHER
Website: http://www.kspublisher.com/
Email: office.kspublisher@gmail.com

Chlorogenic Acid a Potent Anti-inflammatory Agent: *In-Silico* Molecular Docking approach

Shayara Bano[1*], Jitender K. Malik[1]
[1]Institutes of Pharmacy, P.K. University, Shivpuri (M.P.), India

ABSTRACT: Chlorogenic acid (5-O-caffeoylquinic acid) is a phenolic compound of the hydroxycinnamic acid family. This polyphenol has many health-enhancing properties, most of which are relevant for the treatment of metabolic syndrome, including antioxidant, anti-inflammatory, antilipidemic, antidiabetic, and antihypertensive effects. In addition, chlorogenic acid has antioxidant properties, especially against lipid oxidation. Protective properties against degradation and prebiotic activity of other bioactive compounds present in foods. In addition, chlorogenic acid has antioxidant properties, especially against lipid oxidation. Protective properties against degradation and prebiotic activity of other bioactive compounds present in foods. In addition, chlorogenic acid has antioxidant properties, especially against lipid oxidation. Protective properties against degradation of other bioactive compounds present in food and prebiotic activity. ***Methods:*** Molecular docking of COX2, NF-κB inducing kinase (NIK) & PhospholipaseA2 (PLA2) with chlorogenic acid was carried out by AutoDock. ***Result:*** The molecular docking result revealed that chlorogenic acid showed encouraging docking score. The docking score found to be -6.71, -6.31 & -4.43 kcal mol^{-1} respectively.

Keywords: Chlorogenic acid, docking score, molecular docking, COX2, NF-κβ inducing kinase (NIK) & PhospholipaseA2 (PLA2).

RESEARCH PAPER

***Corresponding Author:**
Shayara Bano
Institutes of Pharmacy, P.K. University, Shivpuri, India

How to cite this paper:
Shayara Bano & J. K. Malik.; "Chlorogenic Acid a Potent Anti-inflammatory Agent: *In-Silico* Molecular Docking". Middle East Res J. Pharm. Sci., 2022 Jan-Feb 2(1): 10-20.

Article History:
| Submit: 16.01.2022 |
| Accepted: 07.02.2022 |
| Published: 27.02.2022 |

INTRODUCTION

Inflammation is a biological response to a series of chemical reactions whose main function is to protect against infection and repair tissue damage caused by injury. Several mediators are released during the inflammatory process. Activation of the phospholipase-A2 (PLA2) family of inflammatory lipid mediators, platelet-activating factor, cyclooxyginase-2, leukotrienes, nerve growth factors, inducible nitric oxide synthase, bradykinin, cytokines, and adhesion molecules [1]. Most of the essential components of the inflammatory process reside in the circulatory system, and most of the early mediators (facilitators) of inflammation increase the movement of plasma and blood cells from the circulatory system to the tissues surrounding the injury. Collectively known as exudates, these substances protect the host from infection and promote tissue repair and healing.

Cardinal Sign of inflammation [2]:

Redness	Heat	Swelling	Pain	Loss of Function
Caused by dilation of arterioles/ increased blood flow	Increased chemical activity & increased blood flow to skin surface	Caused by accumulation of blood & damaged tissue cells	Direct injury of nerve fibers, pressure of hematoma on n. endings Chemical irritants – bradykinin, histamine, prostaglandin	Increased pain/ swelling

Types of inflammation [3]

Acute inflammation	Chronic inflammation
The duration of inflammation is relatively short, lasting minutes, hours, or a day or two. Its main features are exudation of fluid and plasma proteins (edema) and migration of leukocytes, mainly neutrophils.	It is generally long lasting and histologically associated with the presence of lymphocytes and macrophages and proliferation of small blood vessels and fibroblasts.

Molecular targets of anti-inflammatory agents

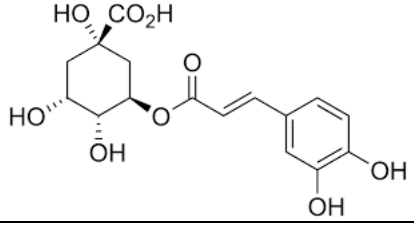
COX enzymes (COX-1 and COX-2) catalyze the biosynthesis of prostaglandins, prostacilins, and thromboxanes from arachidonic acid. COX-1 is constitutively expressed in most tissues, whereas COX-2 is expressed in specific tissues and is induced by cytokines and growth hormone. COX-1 has regulatory effects on platelet aggregation and gastric mucosal biogenesis, and COX-2 is involved in pathological conditions such as inflammation, pain, and fever. NSAIDs exert their anti-inflammatory activity by inhibiting COX-1 and COX-2. Long-term inhibition of COX-1 in the gastrointestinal system causes damage to the gastrointestinal tract through ulceration and gastric bleeding. Coxibs, selective COX-2 inhibitors, are designed to inhibit COX-2 over COX-1 to achieve desired anti-inflammatory activity with minimal gastrotoxic side effects [4]. COX-1 and COX-2 were nearly identical despite residues Ile434, His513, and Ile523 in COX-1, but Val434, Arg513, and Val523 in COX-2. These differences lead to increased volume of active COX-2 sites and additional side pockets away from the main channel. The structure of coxibene consists of a diarylheterocycle with a sulfonamide or methylsulfone moiety attached to the side pocket of COX-2, providing isoform-selective inhibition.

The phospholipase A2 (PLA2) enzyme is required to increase arachidonic acid levels for eicosanoid metabolism and biosynthesis under physiological conditions and for activation of inflammatory cells. The PLA2 superfamily consists of cytosolic calcium-dependent PLA2 (cPLA2), cytosolic calcium-independent PLA2 (iPLA2), and secreted PLA2 (sPLA2). iPLA2 constitutively produces low levels of free fatty acids with relatively low specificity for certain esterified fatty acids. cPLA2 hydrolyzes phospholipids containing arachidonic acid to produce pro-inflammatory eicosanoids. sPLA2 is an inducible enzyme that potentiates cPLA2 function and controls the magnitude and duration of elevation of free fatty acid levels, including arachidonic acid [5].

Nuclear factor (NF)-κB is a group of eukaryotic transcription factors that regulate the expression of genes important for immune responses. NF-κB-inducing kinase (NIK) activates NF-κB2 by promoting the proteolytic processing of target genes and the generation of NF-κB transcripts. NIK is also required for signaling pathways triggered by other cytokines. NIK regulates both inflammatory and tumor-associated angiogenesis. NIK is highly expressed in endothelial cells of inflammatory rheumatoid arthritis tumor tissue and synovial tissue [6].

Chlorogenic acid is a phenolic compound from the hydroxycinnamic acid family. The compound's chemical structure consists of a caffeic acid moiety and a quinic acid moiety; therefore, it is also known as 5-O-caffeoylquinic acid (5-CQA) [7].

Description of Chlorogenic acid [8]

IUPAC Name	3-(3,4-Dihydroxycinnamoyl)quinic acid 3-O-Caffeoylquinic acid
Structure	
Mol. Wt.	354.31
Mol. Formula	$C_{16}H_{18}O_9$
M.P.	205 - 209 °C
Appearance	solid
Taste	Astringent, sweet, and sour tastes, which change with the concentration.
Class	Polyphenolic

Chlorogenic acid is reported to be beneficial in hypertension, hyperglycemia, antimicrobial, antitumor, memory enhancer, weight management [9].

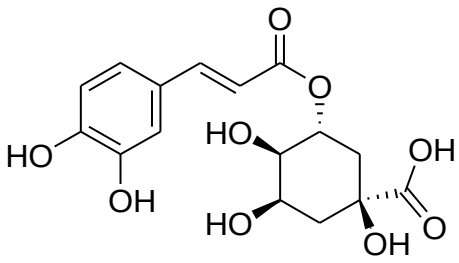
The present research work was planned to design the molecular docking of Chlorogenic acid as dual inhibitors COX2, NF-κB inducing kinase (NIK) & PhospholipaseA2 (PLA2) followed by evaluation of their anti-inflammatory activity and *in-silico* docking studies.

Experimental Work

Molecular docking studies

Ligand Preparation:

2D Structure of ligand Chlorogenic acid was drawn by using ChemDraw [10]. The two-dimensional structures of ligand was converted into 3-D structures with optimized 3D geometry by using Chem3D software. The optimized structure was saved in PDB format for AutoDock compatibility [11].



Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is given in Table 1 [12].

Table 1: The grid-coordinates of the grid-box used in the current study

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
5ikr	46	44	46	0.375	38.042	2.131	61.28
4idv	46	44	46	0.375	16.134	13.917	87.361
3elo	40	40	40	0.442	-15.237	-31.016	-7.715

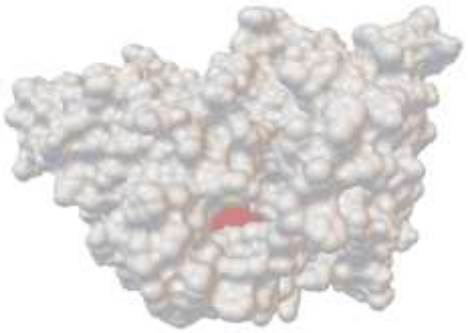


Figure 1: Grid box covering all active sites in COX2 enzyme (5ikr)

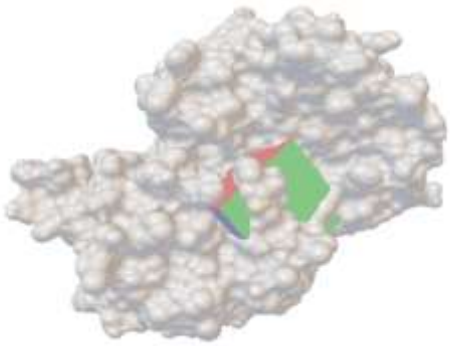


Figure 2: Grid box covering all active sites in NF-κβ inducing kinase enzyme (4idv)

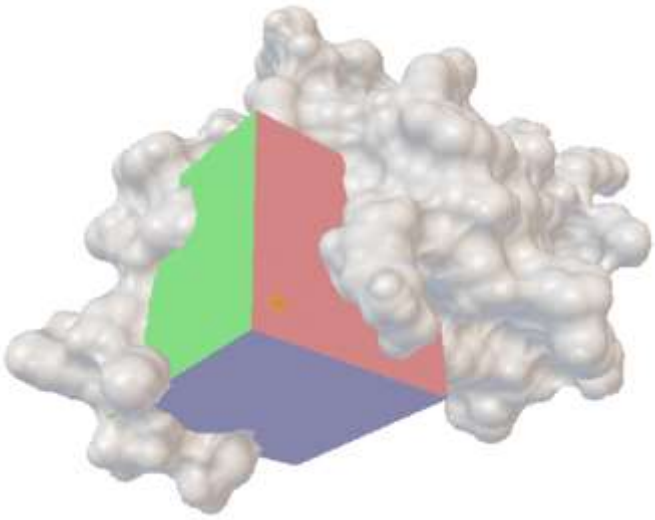


Figure 3: Grid box covering all active sites in phospholipase A2enzyme (3elo)

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13].

Macromolecular structure

Cycloxygenase-2 (COX2)

The crystal structure of the COX2enzyme consisting of macromolecular receptor associated with bound endogenous ligand mefenamic acid is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5ikr.pdb) registered in the Protein data bank was used [14].

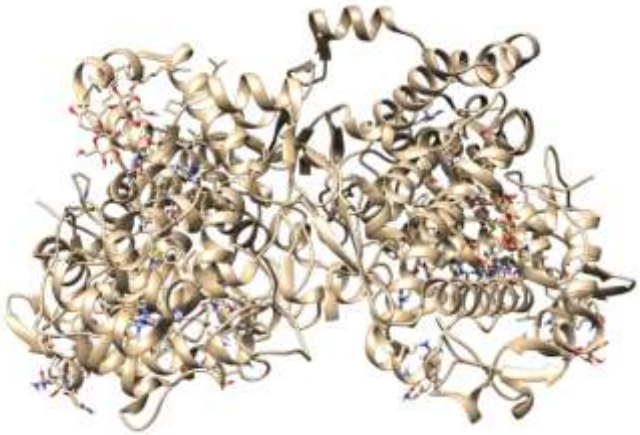


Figure 4: Crystal structure of COX2enzyme with bound ligand mefenamic acid (PDB ID-5ikr)

NF-κβ inducing kinase

The crystal structure of the NF-κβ inducing kinase enzyme consisting of macromolecular receptor associated with bound ligand 13V is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (4idv.pdb) registered in the Protein data bank was used [15].

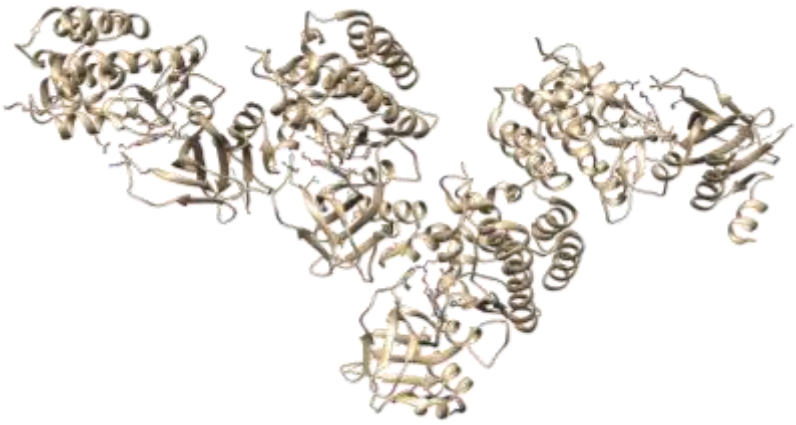


Figure 5: Crystal structure of NF-κβ inducing kinase enzyme with bound ligand13V (PDB ID-4idv)

Phospholipase A2

The crystal structure of the Phospholipase A2enzyme consisting of macromolecular receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (3elo.pdb) registered in the Protein data bank was used [16].

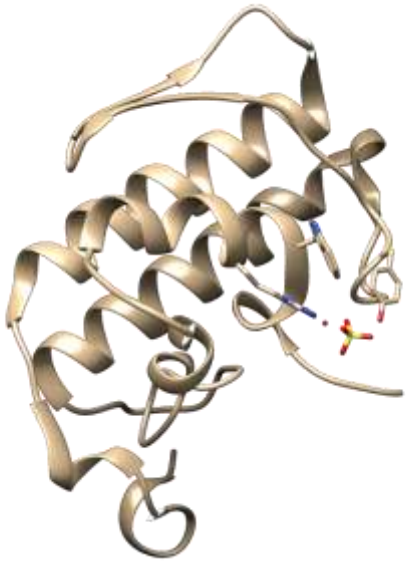


Figure 6: Crystal structure of Phospholipase A2enzyme (PDB ID-3elo)

Molecular Docking Simulation Studies

Docking of ligand Chlorogenic acid was performed against COX2 enzyme, NF-κβ inducing kinase enzyme, and Phospholipase A2 enzyme was performed by Autodock to establish its probable mechanism of action for their lipid lowering effect. All the bonds of ligand Chlorogenic acid were kept flexible, while no residues in receptor were made flexible [17].

Toxicity & ADME-T Studies

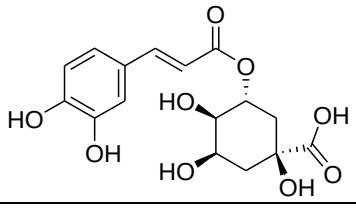
The pharmacokinetics of ligand molecule was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME-T properties [18].

RESULTS AND DISCUSSION

Docking studies of COX2 enzyme, NF-κβ inducing kinase enzyme, and Phospholipase A2 enzyme against Chlorogenic acid ligand was tabulated in table 2.Interaction of Chlorogenic acid with COX2 enzyme, NF-κβ inducing kinase enzyme, and Phospholipase A2 enzyme showed in fig.7-15. The molecular docking result revealed that chlorogenic acid showed encouraging docking score. Chlorogenic acid binding

with COX2 enzyme, NF-κβ inducing kinase enzyme & Phospholipase A2 enzyme showed binding energy-6.71,-6.31 & -4.43 kcalmol^{-1}respectively.Binding interaction of ligand with inflammatory mediators enzyme showed that chlorogenic acid binds with NF-κβ inducing kinase at active site covalently Leu472, Gly475, Asp534, Asn520, Cys533, Leu522, Arg416, Arg408, Glu413, Ser410, Asp519, Ala427, Leu47. The pharmacokinetic profiling of the Chlorogenic acid ligand has revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like mutagenic, reproductive effects, irritant effect, and tumorogenic properties. The pharmacokinetic and toxicity profiling results of Chlorogenic acid was shown in Figure 16.

Table 2: Result of docking of COX2 enzyme, NF-κβ inducing kinase enzyme, and Phospholipase A2 enzyme

S. No	CompoundName	Structure	Binding Energy		
			COX2 (5ikr)	NF-κβ inducing kinase (4idv)	Phospholipase A2 (3elo)
1	Chlorogenic acid		-6.71	-6.31	-4.43
	Interacting Residues		Leu334, Ile335, Arg333, Ser332, Gln327, Leu316	Leu472, Gly475, Asp534, Asn520, Cys533, Leu522, Arg416, Arg408, Glu413, Ser410, Asp519, Ala427, Leu471	Asn24, Thr120, Leu118, Gly26, Tyr25, Cys29, Cys124, Ser34, Gly30, Asp119

Interactions

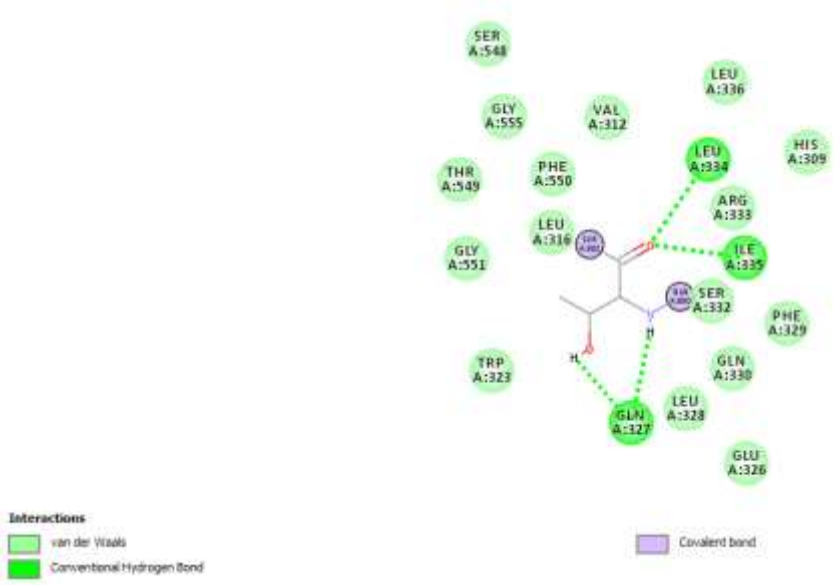


Figure 7: Two-dimensional binding interaction of Chlorogenic acid with COX2 enzyme

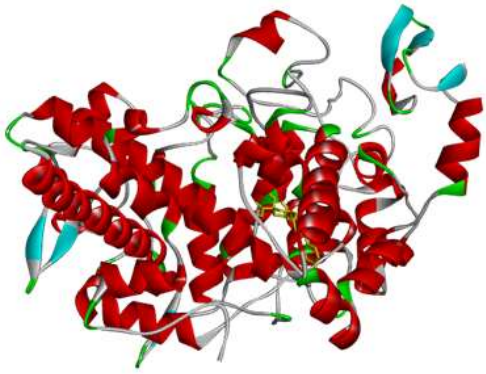


Figure 8: Three-dimensional binding interaction of Chlorogenic acid with COX2 enzyme

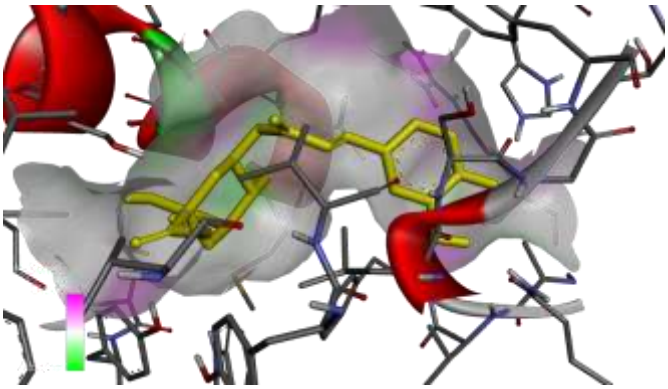


Figure 9: Binding conformation of ligand Chlorogenic acid with COX2 enzyme

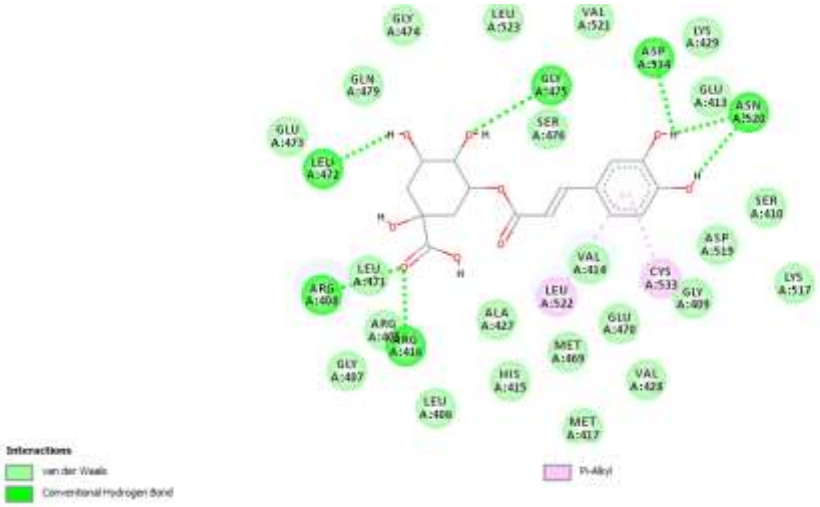


Figure 10: Two-dimensional binding interaction of Chlorogenic acid with NF-κβ inducing kinase enzyme

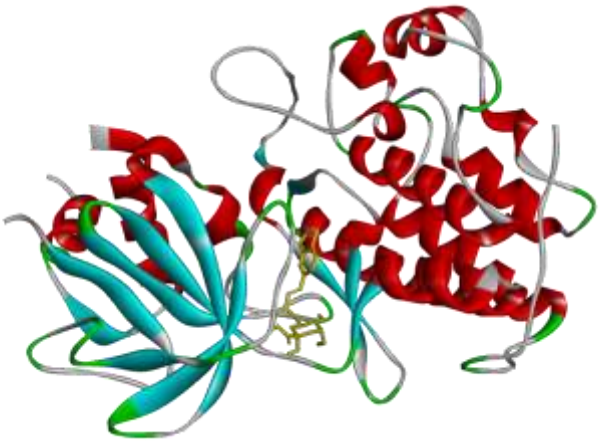


Figure 11: Three-dimensional binding interaction of Chlorogenic acid with NF-κβ inducing kinase enzyme

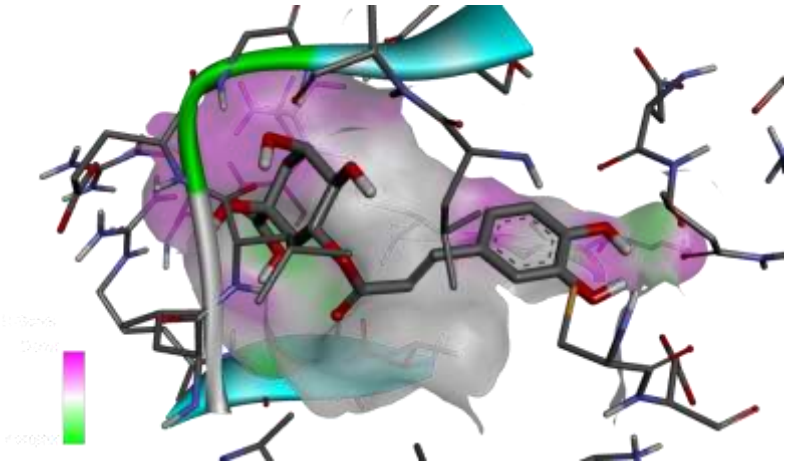


Figure 12: Binding conformation of ligand Chlorogenic acid with NF-κβ inducing kinase enzyme

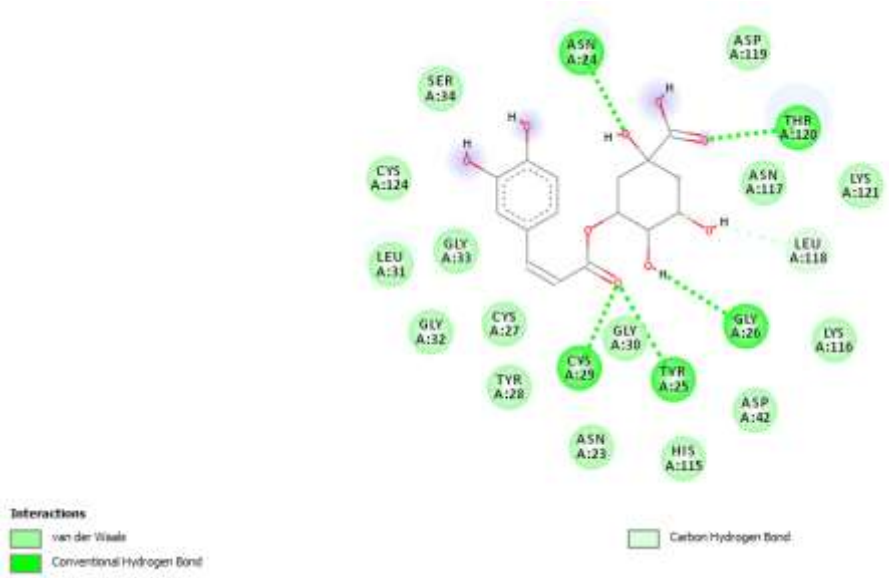


Figure 13: Two-dimensional binding interaction of Chlorogenic acid with Phospholipase A2enzyme

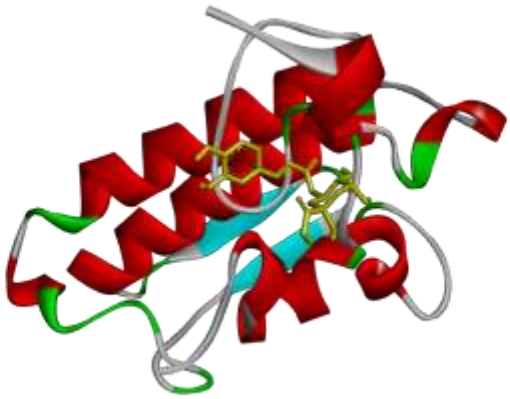


Figure 14: Three-dimensional binding interaction of Chlorogenic acid with Phospholipase A2enzyme

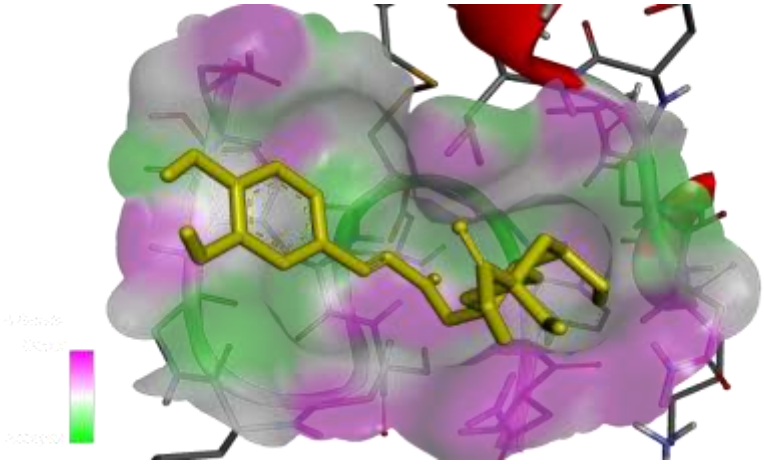


Figure 15: Binding conformation of ligand Chlorogenic acid with Phospholipase A2enzyme

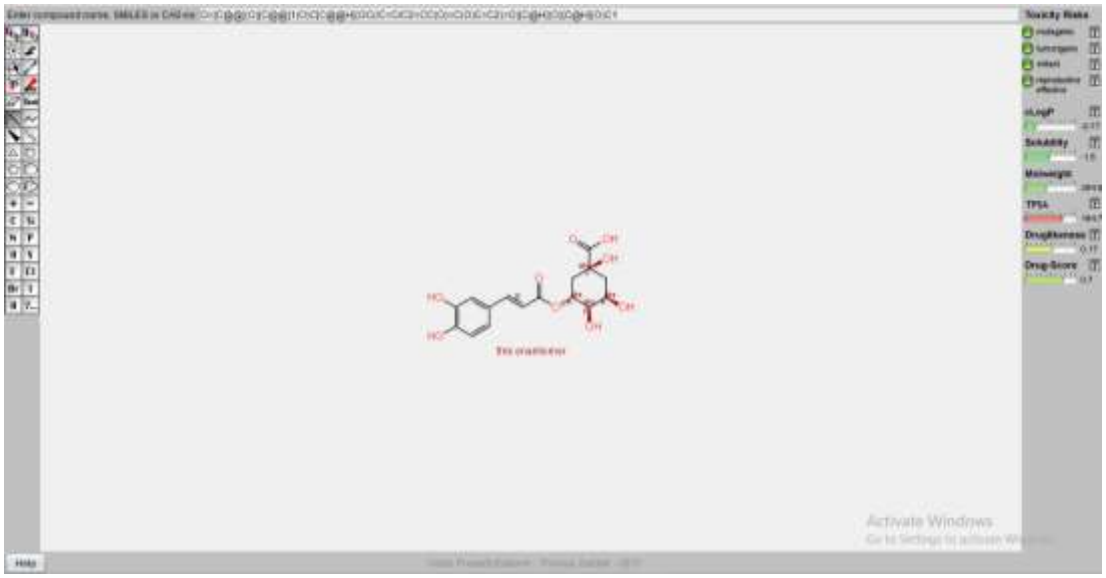


Figure 16: Pharmacokinetic and toxicity profiling of Chlorogenic acid

CONCLUSION

In-Silico molecular docking studied carried out for elucidation of proposed mechanism of chlorogenic acid. The exact mechanism of action for the anti-inflammatory action of chlorogenic acid was still not revealed. With intent to propose the most probable mechanism of action of chlorogenic acid the docking based computational analysis has been performed against the inflammatory drug targets like COX2 enzyme, NF-κβ inducing kinase enzyme, and Phospholipase A2 enzyme. The docking analysis, chemical interactions, followed by the physicochemical based pharmacokinetic profiling has revealed that the chlorogenic acid is executing its anti-inflammatory action *via* dual inhibiting the COX2 and NF-κβ inducing kinase enzyme.

REFERENCE

1. Prabhakar M C, Rajnarayana K, Reddy M S, Krishna D R, Krishna D H. Inflammation and novel therapeutic approaches for its management. Indian Journal of Pharmaceutical Science. 2003; 65(6): 565-575.
2. Dey NC and Dey TK. A Text Book of Pathology. IIIrd edition, Calcutta, Messrs Allied Agency; 1970.
3. Himesh Soni etal. Antimicrobial and Anti-inflammatory Activity of the Hydrogels Containing Rutin Delivery. Asian Journal of Chemistry; 25(15),(2013), 8371-8373.
4. Orlando BJ, Malkowski MG. Substrate-selective inhibition of cyclooxygenase-2 by fenamic acid derivatives is dependent on peroxide tone. Journal of Biological Chemistry. 2016;291(29):15069-15081. DOI: 10.1074/jbc.M116.725713
5. Dennis EA, Norris P. Eicosanoid storm in infection and inflammation. Nature Reviews Immunology. 2015. DOI: 10.1038/nri385
6. Li K, McGee LR, Fisher B, Sudom A, Liu J, Rubenstein SM, et al. Inhibiting NF-κB-inducing kinase (NIK): Discovery, structure-based design, synthesis, structure–activity relationship, and co-crystal structures. Bioorganic & Medicinal Chemistry Letters 2013;(23):1238-1244. DOI: 10.1016/j.bmcl.2013.01.012
7. Santana-Gálvez J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Chlorogenic Acid: Recent Advances on Its Dual Role as a Food Additive and a Nutraceutical against Metabolic Syndrome. Molecules. 2017 Feb 26;22(3):358.
8. https://pubchem.ncbi.nlm.nih.gov/compound/Chlorogenic-acid.
9. Rajnish Kumar etal. Therapeutic Promises of Chlorogenic Acid with Special Emphasis on its Anti-Obesity Property.2020;13:1;7-16.
10. Himesh Soni etal. (2022). Silibin as Potent Inhibitor of COVID -19 Main Protease: In-Silico Docking Approach. Journal of Volume-4, Issue-1 (January-June, 2022)Molecular Pharmaceuticals and Regulatory Affairs.1-7.
11. Himesh Soni, Satish Sarankar, Sarvesh Sharma & Jitender K Malik. Hydroxychloroquine as Potent Inhibitor of COVID -19 Main Protease : Grid Based Docking Approach. EJMO 2020;4(3):219–226.
12. Himesh Soni, Dr. V.K. Gautam, Sarvesh Sharma, Jitender K Malik. Rifampicin as Potent Inhibitor of COVID - 19 Main Protease: In-Silico Docking Approach. Saudi J Med Pharm Sci, September, 2020; 6(9): 588-593.
13. T. Sander, J. Freyss, M. von Korff, J.R. Reich, C. Rufener, OSIRIS, an entirely in-house developed drug discovery informatics system, J Chem Inf Model, 49 (2009) 232-246.
14. Kciuk, M., Mujwar, S., Szymanowska, A., Marciniak, B., Bukowski, K., Mojzych, M., &Kontek, R. (2022). Preparation of Novel Pyrazolo [4, 3-e] tetrazolo [1, 5-b][1, 2, 4] triazine Sulfonamides and Their Experimental and Computational Biological Studies. International Journal of Molecular Sciences, 23(11), 5892.
15. Mujwar, S., Sun, L., &Fidan, O. (2022). In silico evaluation of food-derived carotenoids against SARS-CoV-2 drug targets: Crocin is a promising dietary supplement candidate for COVID-19. Journal of Food Biochemistry, e14219..
16. Fidan, O., Mujwar, S., &Kciuk, M. (2022). Discovery of adapalene and dihydrotachysterol as antiviral agents for the Omicron variant of SARS-CoV-2 through computational drug repurposing. Molecular Diversity, 1-13.
17. Shah, K., & Mujwar, S. (2022). Delineation of a Novel Non-Steroidal Anti-Inflammatory Drugs Derivative Using Molecular Docking and Pharmacological Assessment. Indian Journal of Pharmaceutical Sciences, 84(3), 642-653.
18. Kciuk, M., Gielecińska, A., Mujwar, S., Mojzych, M., Marciniak, B., Drozda, R., &Kontek, R. (2022). Targeting carbonic anhydrase IX and XII

isoforms with small molecule inhibitors and monoclonal antibodies. Journal of Enzyme Inhibition and Medicinal Chemistry, 37(1), 1278-1298.