In silico approach for exploration of Anti-inflammatory activity of *cis-9,trans-11* Conjugated linoleic acid

Rashmi H.K[1], Sujana Ghanta Vemuri[2] and Uma Maheswari Devi P[1*]

Abstract— The Cycloxygenase-2 (COX-2) and inducible Nitric oxide synthase (iNOS) are the hallmark of chronic inflammation, which are over expressed in many cancers, and are key targets for the treatment of many inflammatory related diseases. Several synthetic and biological Nonsteroidal anti-inflammatory drugs have been studied for their inhibitory activities. Conjugated linoleic acids (CLA) are family of Linoleic acid isomers which have potential health benefits. The CLA isomers *cis-9, trans-11* and *trans-10, cis-12* are the most abundant isomers found in the meat and dairy products derived from ruminants. The present study is focused to evaluate anti-inflammatory activity of *cis-9, trans-11* CLA isomer against pro-inflammatory genes like COX-2 and iNOS using *in silico* docking studies. Molecular docking experiments were carried out using Discovery studio *v*3.5 to determine their probable binding levels, Interaction energy, Hydrogen bonding and Intermolecular distance. The CLA isomer *cis-9, trans-11* was docked into active binding sites of COX-2 and iNOS individually, the binding energies with COX-2 and iNOS–were -48.7 and -43.0 Kcal/mol respectively. These results reflect the anti-inflammatory potential of *cis-9, trans-11* CLA isomer.

Index Terms— **Anti-Inflammatory, Conjugated linoleic acid, Cyclooxygenase-2, Diclofenac, Hydrogen bonds, inducible Nitric oxide synthase, Molecular modeling, molecular docking,**

---

1 Introduction

Prostaglandins are regulatory compounds that play important roles in many physiological processes in the humans and disturbance in prostaglandin synthesis/metabolism leads to various patho-physiological processes like pain, fever, inflammation, cancer and cardiovascular events [1]. Cyclooxygenases (COX) known as prostaglandin-endoperoxide synthase (PTGS) are enzymes that catalyze the conversion of arachidonic acid and oxygen to prostaglandin H2, which is further converted to other prostaglandins, prostacyclin and thromboxanes [2, 3]. Cyclooxygenase exists in two isoforms COX-1 and COX-2, which are differentially regulated and expressed. COX-1 is a housekeeping enzyme which is expressed constitutively in many tissues whereas COX-2 is non constitutive and expressed in most tissues and is rapidly induced in many cell types by cytokines, mitogens and endotoxins in cells during inflammation and facilitates inflammatory response [4, 5]. Over expression of COX-2 in response to growth factors, oncogenes and tumor promoters lead to chronic inflammation [6]. The inhibitors of COX-2 are effective for the relief of chronic pain in elderly patients with rheumatoid arthritis and osteoarthritis [7]. Nitric oxide synthases (NOS) are enzymes catalyzing the production of Nitric oxide (NO) a short lived important cell signaling pleiotropic regulatory molecule from L-arginine. Nitric oxide plays a diverse variety of roles like angiogenesis, neural development, platelet aggregation, leukocyte adhesion and as a neurotransmitter [8]. There are three isoenzymes of NOS endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) which convert L-arginine to L-citrulline yielding NO in presence of oxygen [9]. The endothelial NOS and the neuronal NOS are expressed constitutively, which are Ca2+and calmodulin-dependent isoforms, which function to produce low levels of NO predominantly for blood pressure regulation and nerve function [10]. The inducible NOS (iNOS), is Ca2 independent and expressed in response to inflammatory signals. Only iNOS produces a definite amount of NO in a micromolar range [11]. Sustained induction of iNOS leads to formation of reactive intermediates of NO which may cause damage to DNA, inhibit DNA repair mechanism modify cell signaling, and promote proinflammatory and angiogenic activities of the cell [12]. Over production of NO in brain cells leads to development of Parkinson's disease [13]. The two remarkable key enzymes namely Cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS) are important mediators of inflammatory process. The NSAIDS, which target the COX-2 and iNOS, down regulates the proinflammatory genes, and also decreases the incidences of many cancers including breast and colorectal [14].

2 Conjugated Linoleic acid *cis-9,tarns-11 isomer*

Conjugated Linoleic acids are the isomers of Linoleic acid (C18:2 n-6) and are formed by the hydrogenation of unsaturated fatty acid by ruminal microorganism and are predomi-

- *Rashmi H.K, Research scholar, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh- India. 517502. E-mail:hkrspmvv2011@gmail.com.*
- *Prof. P Uma Maheswari Devi, Head, Dept of Applied Microbiology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh- India. 517502. Ph 9848894900 E-mail:umadevi66@yahoo.co.in*

nant in meat, fat, milk and milk products of ruminants [15]. The active biological CLA isomers are *cis-9,trans-11* (*c9,t11*) CLA and *trans-10,cis-12* (t10,c12) CLA. The isomer *c9,t11* CLA is abundant in the diet (90% of dietary CLA is *c9,t11* CLA and 10% is *t10,c12* CLA) [16] [17]. Research reports suggest the beneficial effects of CLA isomers on different immune functions [18, 20]. Thus in silico approach was used to understand the anti-inflammatory properties of CLA isomer through docking analysis.

3 MATERIALS AND METHODS

3.1 Homology modeling of COX-2 protein

Accelrys Discovery Studio *v*3.5 was employed to create homology models. The 3D homology models were built using Discovery Studio MODELLER by considering the bound ligand (anti-inflammatory inhibitors) from template, optimizing side chains of proteins and setting high optimization levels. The validity of the 3D structure was also assessed by observing, "verify score" value closest to "verify expected high score" and it was considered as the best model .

3.2 Ligand structures

The 3D structures of *cis-9,trans-11* CLA (Pubchem ID: 5280644) (Fig.1A) and Diclofenac (DIF) (Fig. 1B) was retrieved from Pubchem database. The structure was additionally checked against Zinc database [21].

3.3 Protein structures

The 3D crystal structure of Inducible nitric oxide synthase (iNOS, PDB_ID: 4NOS performed by X-RAY Diffraction) was retrieved from the Protein Data Bank (www.rcsb.org).

3.4 Protein and Ligand preparation

Protein was prepared with CHARMm force field using prepare protein option on the protein by keeping the ligands and removing water. Ligand was prepared with gradient ionization ranging from pH 6.5 to pH 8.5 and prepare ligand option of CHARMm was used to fix bad valences generating 3D coordinates of isomers and tautomers.

3.5 Molecular Docking

Receptor-ligand docking was performed using CHARMm-based molecular docking algorithm, CDOCKER (Accelrys Discovery Studio *v*3.5 package from Biosystems Technologies, San Diego, CA, USA) on Z800 workstation (Hewlett Packard, Palo Alto, California, United States). CDOCKER was used to dock each ligand to generate 10 conformations starting each with 1000 dynamic steps at target temperature of 1000K along with including electrostatic interactions and refined by simulated annealing. CHARMm force field was applied along with a grid extension of 8 Å. The partial charges for ligands were set up by Momany-Rone partial charge's method. Docking against multiple binding pockets was identified from receptor cavities. COX-2, active sites were defined around the bound inhibitor DIF which was copied from template structures during modeling. *Cis-9,trans-11* CLA and DIF were docked with COX-2 individually in the site spheres of DIF. Docking with iNOS was performed against multiple binding pockets which were identified from receptor cavities using DS Catalyst SBP (structure based pharmacophore) which provides tools for fast and easy creation of SBP models from the putative binding site in a protein. Binding pockets/sites were defined as active sites and the ligands were docked into the active sites. CLA isomer *cis-9,trans-11* and DIF were docked with iNOS individually at the site spheres created around active sites.

4 RESULTS AND DISCUSSION

In silico approach was used to determine the anti-inflammatory activity of *cis-9,trans-11* CLA isomer on comparison with DIF against COX- 2(Fig. 2A) and iNOS(Fig. 2B) proteins using Discovery Studio *v*3.5. The homology model of COX-2 (Fig 2A) was built based on template 1PXX by comparative analysis, which showed the presence of well conserved structural elements. The geometry of the built model was evaluated with Ramachandran plot (Fig. 3), calculations computed with SWISS PDB Viewer (http://spdbv.vital-it.ch). The RMSD value of the COX-2 homology model was 0.52 Å thus validating the modeled structure. In the present study, the ligand *cis-9,trans-11* CLA isomer and DIF were docked with COX-2 at the site sphere of DIF, and iNOS at the active site identified from receptor cavities of the protein. The docking energy of *cis-9,trans-11* CLA (Fig. 4A) is -48.7 Kcal/mol and showed 0.8 fold higher value compared to DIF whose docking energy was -38.0 Kcal/mol (Fig. 4B) (Table 1). The complex of *cis-9, trans-11* CLA isomer with COX-2 showed one hydrogen bond with SER530 (Fig. 5A). The docking of DIF with COX-2 showed three hydrogen bonds with SER530 (Fig. 5B). Upon docking of *cis-9,trans-11* CLA and DIF with iNOS resulted in -43 and -33.8 Kcal/mol energies respectively (Fig. 6A and 6B). *cis-9,trans-11* CLA docked with iNOS showed 2 hydrogen bonds, with LYS103 (Fig. 7A), whereas DIF showed 2 hydrogen bonds with SER2097 and LYS2098 (Fig. 7B). Literature from X-ray crystallographic studies of arachidonic acid with COX-2 showed that the action of NSAIDS is through the coordination of carboxylate with TYR385 or SER530 and have a structural and functional role in the chelation of ligand with the receptor [22]. The ligand *cis-9,trans-11* CLA possessed high shape complementarity with the active pocket of COX-2 enzyme and able to form one hydrogen bond with SER530 with a bond length of 2.22274 Å which is significant for exhibiting protein-ligand interaction and may indicate the mportance of *cis-9,trans-11* CLA as novel inhibitor of COX-2 enzymes.

4.1 Figures

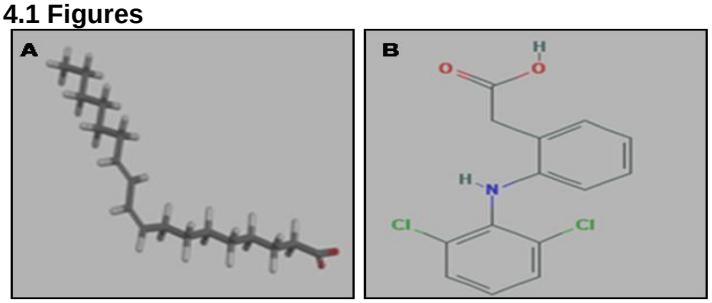

Figure1. A) 3D structure of *cis-9, trans-11* CLA and B) DIF

International Journal of Scientific & Engineering Research Volume 6, Issue 2 , February- 2015
ISSN 2229- 5518

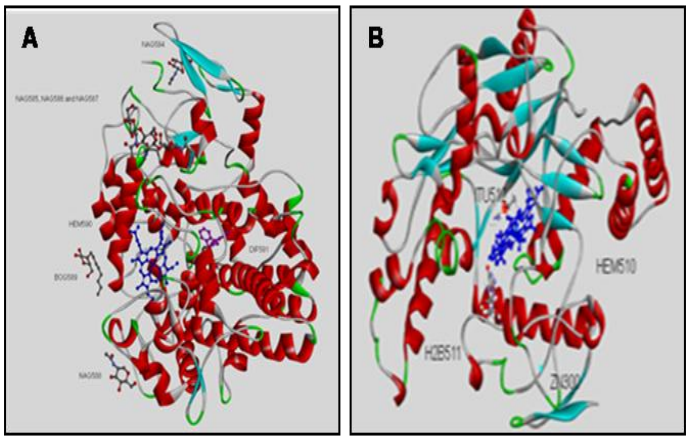

Figure 2. A) The 3D ribbon structure of human COX-2 homology model created against 1PXX as the template. B) Prepared 3D ribbon structure of iNOS (PDB_ID:4NOS), chain A.

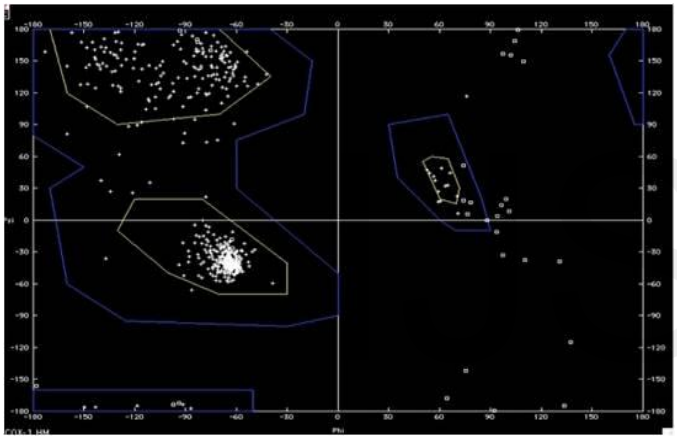

Figure 3. Ramachandran Plot of COX-2 Homology model.

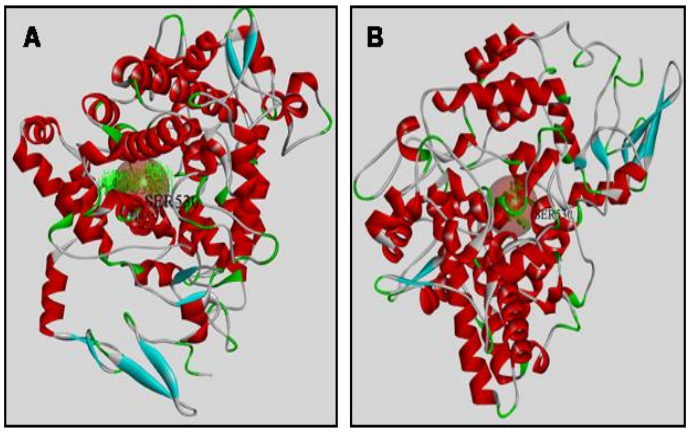

Figure 4. **Docking of *cis-9,trans-11* with COX-2.**
A) *cis-9,trans-11* CLA isomer and B) DIF with COX-2 in active site, red spheres.

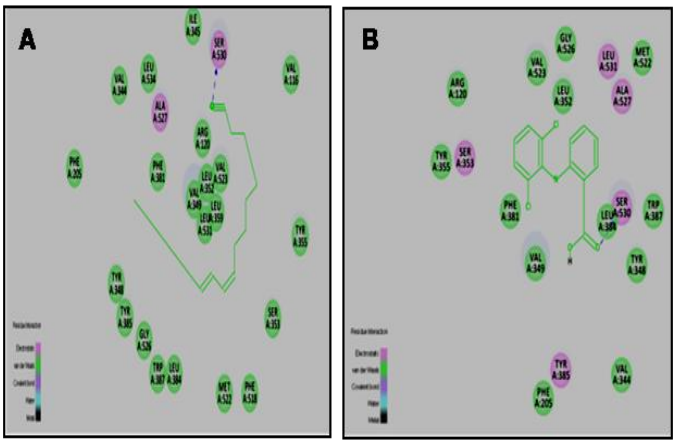

Figure 5. The 2D diagram of Receptor-Ligand Hydrogen bonding with COX-2. A*) cis-9,trans-11* CLA isomer and B) DIF with COX-2.

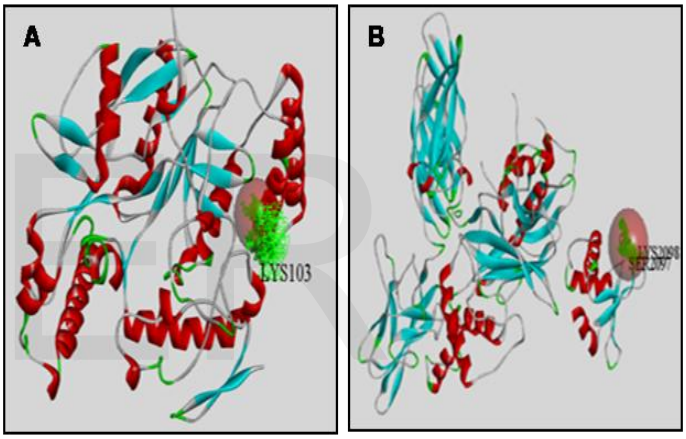

Figure 6. **The Docking of *cis-9,trans-11* CLA isomer with iNOS**
A) *cis-9,trans-11* isomer and B) DIF in the active sphere (Red) of iNOS .

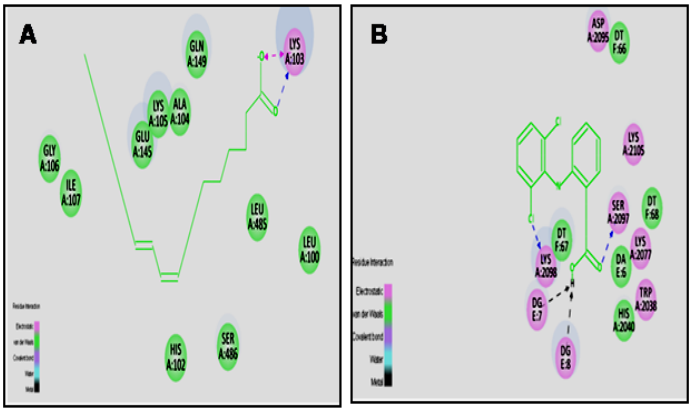

Figure 7. The 2D diagram of Receptor-Ligand Hydrogen bonding with iNOS. A*) cis-9,trans-11* CLA isomer and B) DIF with iNOS(4NOS)

Table 1. Docking affinity scores of CLA isomer *cis-9,trans-11* and DIF with COX-2 and iNOS, Hydrogen bonds and their distance.

LIGAND	PROTEIN	-CDOCKER interaction enery(Kcal/ mol)	HYDROGEN BONDS	DIS-TANCE (Å)
CLA somer cis-,trans-11	COX-2	48.7	A:SER530:HG - CLA9E,11Z:O20	2.22274
	iNOS	43.0	A:LYS103:HZ1-CLA9E,11Z:O19 A:LYS103:HZ1-CLA9E,11Z:O20	2.00725 1.89857
Diclofenac	COX-2	38.0	A:SER530:HG - DIF:O18 A:SER530:HG - DIF:O18 DIF:H30 - A:SER530:OG	2.02645 2.06074 2.35744
	iNOS	33.8	A:SER2097:HG-DIF:O18 A:LYS2098:HZ3-DIF:C13	1.82296 2.36656

5 ACKNOWLEDGEMENT

The authors wish to thank the authors/ editors/ publishers of all the articles, journals and books from where the literature for this article has been reviewed and discussed. Department of Bioinformatics, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh, India for providing Research facilitiy.

6 REFERENCES

[1] Demers, L.M., Prostaglandins in human disease. Clin Lab Med, 1984. 4(4): p. 787-801.
[2] DeWitt, D.L., Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim Biophys Acta, 1991. 1083(2): p. 121-34.
[3] Smith, W.L., R.M. Garavito, and D.L. DeWitt, Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem, 1996. 271(52): p. 33157-60.
[4] Miller, S.B., Prostaglandins in health and disease: an overview. Semin Arthritis Rheum, 2006. 36(1): p. 37-49.
[5] Kurumbail, R.G., et al., Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature, 1996. 384(6610): p. 644-8.
[6] Marnett, L.J., et al., Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem, 1999. 274(33): p. 22903-6.
[7] Savage, R., Cyclo-oxygenase-2 inhibitors: when should they be used in the elderly? Drugs Aging, 2005. 22(3): p. 185-200.
[8] Smith, W.L., D.L. DeWitt, and R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem, 2000. 69: p. 145-82.
[9] Luiking, Y.C., M.P. Engelen, and N.E. Deutz, Regulation of nitric oxide production in health and disease. Curr Opin Clin Nutr Metab Care. 13(1): p. 97-104.
[10] Marletta, M.A., Nitric oxide synthase structure and mechanism. J Biol Chem, 1993. 268(17): p. 12231-4.
[11] Forstermann, U. and H. Kleinert, Nitric oxide synthase: expression and expressional control of the three isoforms. Naunyn Schmiedebergs Arch Pharmacol, 1995. 352(4): p. 351-64.
[12] Lirk, P., G. Hoffmann, and J. Rieder, Inducible nitric oxide synthase--time for reappraisal. Curr Drug Targets Inflamm Allergy, 2002. 1(1): p. 89-108.
[13] Beckman, J.S., et al., Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A, 1990. 87(4): p. 1620-4.
[14] Gupta, R.A. and R.N. Dubois, Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer, 2001. 1(1): p. 11-21.
[15] Aktan, F., iNOS-mediated nitric oxide production and its regulation. Life Sci, 2004. 75(6): p. 639-53.
[16] Kepler, C.R., et al., Intermediates and products of the biohydrogenation of linoleic acid by Butyrinvibrio fibrisolvens. J Biol Chem, 1966. 241(6): p. 1350-4.
[17] Fritsche, J. and H. Steinhart, Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. 1998. Lebensm. Unters. F. A 1998, 206, 77-82.
[18] O'Shea, M., J. Bassaganya-Riera, and I.C. Mohede, Immunomodulatory prop erties of conjugated linoleic acid. Am J Clin Nutr, 2004. 79(6 Suppl): p. 1199S-1206S.
[19] Wijga, A.H., et al., Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. Thorax, 2003. 58(7): p. 567-72.
[20] Chin, S., et al., Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Comp Anal 1992(5:185-97).
[21] Irwin, J.J. and B.K. Shoichet, ZINC--a free database of commercially available compounds for virtual screening. J Chem Inf Model, 2005. 45(1): p. 177-82.
[22] Garofalo, A., et al., The FAR protein family of the nematode Caenorhabditis elegans. Differential lipid binding properties, structural characteristics, and developmental regulation. J Biol Chem, 2003. 278(10): p. 8065-74.