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In Silico Analysis and Molecular Docking Studies of Alzheimer's Disease Biomarkers Using Kaempferol

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A B S T R A C T

Kaempferol is a flavonoid which is present in many fruits and vegetables. It has various health benefits due to its anti neurotoxic and antioxidant role. The current research explores the possible reasons and mechanisms for its neurotoxic activity through the procedure of molecular docking. Molecular docking was performed using the molecular modeling software "auto dock". The studies were done to evaluate the binding affinity characteristics of kaempferol with the proteins associated with Alzheimer's disease (AD) Amyloid beta 42(Aβ42) and tau, antioxidant markers such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione S transferase (GST), and pro-inflammatory markers like tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6), in an attempt to understand the mechanism of action of kaempferol. The docking energy and hydrogen bonds were tabulated. Docking Scores indicated the application of kaempferol as a potential, natural therapeutic agent for the Alzheimer's disease (AD).

Introduction

Flavonoids are large family of biological polyphenolic plant compounds that are present in various vegetables and fruits. Flavonoids are classified into six major subclasses such as flavon-3-ols, flavonols, flavanones, flavones and isoflavones (Kumar *et al.*, 2007). Studies have reported that flavonoids are potent anti-oxidant and anti-inflammatory substances. The general intake of flavonoids in the daily diet should be around 1-2g/day (Durga *et al.*, 2014)

Kaempferol is a member of flavonol subclass of flavonoids, which is commonly present in tea, broccoli, grapefruit, and apple and is said to have strong anti-oxidant and anti-inflammatory properties (Zuk *et al.*, 2011). Kaempferol has a structure of diphenyl propane and is synthesized by the condensation of 4-coumaroyl-COA with three molecules of malonylCOA (Brenda Winkel-Shirley., 2002). Kaempferol has anti-oxidant, anti-cancer, anti-inflammatory

and anti-microbial activities. Consumption of kaempferol rich foods can reduce the risk factors of cardiovascular diseases and cancers. *In vitro* and *in vivo studies* suggested that kaempferol can be used to treat diseases such as neurodegenerative diseases, diabetes, allergies and inflammatory disorders (Calderon *et al.,* 2011).

The Blood-Brain-Barrier (BBB), also known as neurovascular unit, is a complex gateway that regulates the movement of molecules and cells between the blood and central nervous system (CNS).The BBB consists of endothelial cells that are inter-related to one another through multiprotein tight junction (TJ) complexes (Abbott *et al.,* 2002). Previous studies demonstrate that Kaempferol has the ability to cross the BBB (Kuresh *et al.,* 2004).

Kaempferol has a neuro protective effect and it is associated with the capacity to reduce the metalloproteinase activation. It is used to prevent protein nitro-tyrosines accumulation and protect against cell death which is caused by oxidative stress (Lopez *et al.,* 2007). Kaempferol has efficiently blocked the increasing reactive oxygen species (ROS) related to the oxidative stress caused by glutamate in the mouse hippocampal cell line HT-22 (Giuseppe *et al.,* 2010). Administration of kaempferol gave an efficient protection against NPA-induced neurodegeneration in wistar rats (Brouillet *et al.,* 2005).

The human brain is highly functional. The left hemisphere is responsible for language and motor control and the right hemisphere is responsible for visuospatial and attentional function (Todd *et al.,* 2012). The basic functional unit of the brain is the neurons. They are the information processing cells in the brain. Neurons are of different types which also differ in their size, shape and function. Neurons connect with other neurons to form a network. The mature human brain consists of more than 100 billion neurons (Joan *et al.,* 2010). Neurodegenerative disease like Parkinson's, Alzheimer's and Huntington's disease have the progressive loss of the structure and function of neurons. Such diseases are incurable and results in neuronal cell death (Johannes *et al.,* 2013).

Alzheimer's disease (AD) is a chronic neurodegenerative disease that usually starts slowly and worsens over time (Awanish *et al.,* 2015). Accumulation of amyloid beta protein 40, 42 and tau protein are the major reasons for AD (John *et al.,* 2002). Amyloid Precursor Protein (APP) is a family of type I membrane protein. It is abundantly expressed in the brain. APP generates the beta amyloid peptides (Huizheng *et al.,* 2006). Amyloid beta 42 (Aβ42) plays an important role in the formation of plaques in the brain. The Aβ42 residue contains a significant amount of isomerized and racemized aspartyl residues predominate in dense plaques (Alex *et al.,* 1993). In Alzheimer's Aβ42 production will be increased when compared to the less aggregation prone Aβ40 isoform (Martin *et al.,* 2010). Tau proteins belong to the family of the microtubule associated proteins. They are found in neurons and it is important to form a network of neuronal microtubules (Luc bee *et al.,* 2000) (Fig 1).

In this context, insilco investigations continue to be a great promise for evaluating the interaction energies between a known compound and a target ligand. In view of the above, the present study merits in evaluating the potential role of kaempferol as an anti-neurotoxic agent based on the binding pattern, hydrogen bond formation and energy values.

Materials and Methods

UNIPROT

The Universal Protein Resource (UniProt) is a free accessible database. It contains a wide range of information about protein sequence and annotation data (www.uniprot.org).

The primary sequence of amyloid beta 42 (Aβ 42) (P05067), tau protein (P10636), antioxidant proteins such as Superoxide dismutase (SOD) (P00441), Catalase (CAT) (P04040), Glutathione S-transferase (GST) (P09211), anti-inflammatory proteins such as Tumor necrosis factor (TNFα) (P01375) and Interleukin 6 (IL-6) (P05231) were retrieved from UniProt

Protein Structure Preparation

Structures of *Homo sapiens* brain amyloid beta 42 (Aβ 42), tau protein, antioxidant protein such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione S-transferase(GST), Anti-inflammatory protein such as tumor necrosis factor (TNFα) and Interleukin 6 (IL-6) were retrieved from the database Protein Data Bank (http://www.rcsb.org/pdb).

The proteins were prepared by deleting the water molecules. The PDB ID of Aβ 42, tau protein, SOD, CAT, GST, TNFα, IL-6 are as follows 1MWP, 4FL5, 1AP5, 1F4J, 1TNF, 1ALU.

PUBCHEM

PubChem is a free accessible database which contains about chemical molecules and their activities against biological assays. It is maintained by National Centre for Biotechnology Information (NCBI) (https://pubchem.ncbi.nlm.nih.gov/)

ACD/CHEMSKETCH

ACD/Chemsketch is the powerful chemical drawing and graphics package from ACD/Labs software, which will draw molecular structures, reactions and calculate chemical properties very quickly and easily. The three-dimensional structures of carbazole alkaloids were drawn by chem sketch.

OPEN BABEL

Open Babel is a software used to interconvert chemical file formats. It's an open collaborative project allows to search, convert, analyze or store data.

Ligand Structure Preparation

In this study for the ligand kaempferol the 2D structure and chemical formula were obtained from PubChem. The PubChem ID of kaempferol is 5280863. The structure of kaempferol was drawn using chem sketch software (chemically intelligent drawing interface freeware).This was followed by ligand construction using chem sketch draw mode. 3D structure optimization were computed and finally, the ligand was saved in "MOL" file format and then "MOL" file was converted to "PDB" format using open babel molecular converter. The protein and ligand files which are prepared were then taken for docking.

Docking Methodology

The binding mode and interaction of Aβ 42, Tau protein, SOD, GST, CAT, TNFα and IL-6 with kaempferol were studied using software "AutoDock". Docking calculation in auto dock was performed using refined protein and desired ligand in "PDB" format. The torsion parameter was analyzed and finally, the ligand structure was saved as

"PDBQT" file format. The Grid box size (126*126*126) was set and saved as "GPF" file format. This was followed by docking calculation which involves search parameter and genetic algorithm. Then the file was saved as "DPF" file format. The interaction between protein and ligands are shown and ligand conformation was retained (Fig5 and 6).

PYMOL

Pymol is an open source tool to visualize molecules available from (www.pymol.org). It runs on windows, Linux and MacOS. It has excellent capabilities in creating high-quality images from 3D structures.

Visualization and analyzing docking results

Once the target ligand was docked against all proteins of interest the results were visualized for all interactions, binding energy, H-bond formation and few other parameters using "PYMOL" software.

Results and Discussion

In the current research, the possible anti-neurotoxicity properties of the ligand kaempferol were explored by interpretation of the mechanism of its action by molecular docking studies, which was achieved by insilco method, using "AutoDock" software tool (Fig 2a & 2b).

The structure-function relationship of kaempferol was investigated to gain the knowledge of its biological activity against the target proteins such as Aβ42, tau, SOD, CAT, GST, TNF-α and IL-6 using the 3D structure of the protein retrieved from the PDB database (Fig 3and Fig 4).The ligand properties are tabulated in the (Table 1). The docking score demonstrates the binding energy between the protein and the selected compound. Interpretation of the pattern of binding between the target protein and ligand recommended that the binding pattern diversed with the protein character (Durga *et al.,* 2016)

Evaluation, of the pattern of binding between the target protein and ligand, suggested that the binding pattern varied with the protein nature. This was explored based on our observation that kaempferol interacted with protein Aβ 42, with its amino acids Val^{47},$Glu^{79,}$ Arg^{102} (Fig 5a) with tau amino acids at $Asn^{50,}$$Val^{51,}$$Tyr^{18}$,$Asp^{21,}$$Arg^{18}$ (Fig 5b)and antioxidant enzyme SOD with its amino acids $Thr^{190,}$$Gly^{86,}$ $Lys^{90,}$ Asn^{84} (Fig 6a) while with CAT amino acids at $Ser^{538,}$ $Ser^{96,}$ $His^{324,}$ Ile^{330} and Arg113 (Fig 6b), and with GST amino acid at $Gly^{95,}$$Leu^{99,}$$Gln^{125,}$$Lys^{44,}$$Ala^{45}$ (Fig 7a) alone. For the anti-inflammatory protein TNF-α binding of kaempferol was visible at amino acid residues $Gln^{25,}$ $Ala^{134,}$ $Ile^{136,}$ Asn^{46} (Fig 7b) Whereas For IL-6 Binding Was Observed At $Arg^{168,}$ $Leu^{64,}$ $pro^{65,}$ $Ser^{176,}$ Met^{67}respectively (Fig 7c).

These docking data are revealed that ligand kaempferol interacts with target proteins at various amino acid residues and the mechanism of binding pattern differs from protein to protein. The binding nature of the kaempferol was further evident by the number of hydrogen bonds formed between the ligand and target protein (Table 2). Neurotoxic parameters such as Aβ42 and tau formed 2 hydrogen bonds each respectively. Whereas, antioxidant enzymes such as SOD, CAT, and GST formed 2, 1and 2 hydrogen bonds respectively. In contrast to this, the anti-inflammatory cytokines formed 3(TNF-α) and 2(IL-6) hydrogen bonds with the ligand kaempferol.

Fig.1 Alzheimer's disease pathology (http://www.shehjar.com)

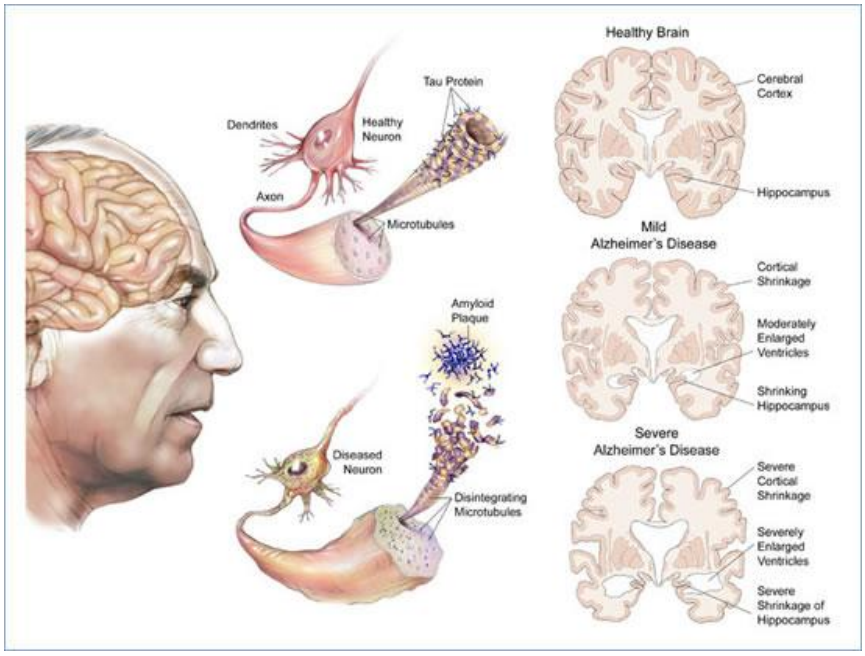
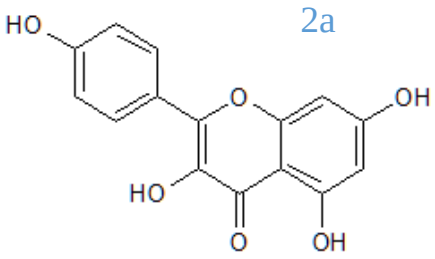


Fig.2 (a & b) 2D and 3D structures of ligand molecule (Kaempferol)



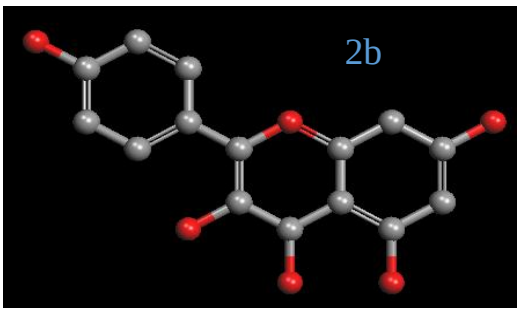
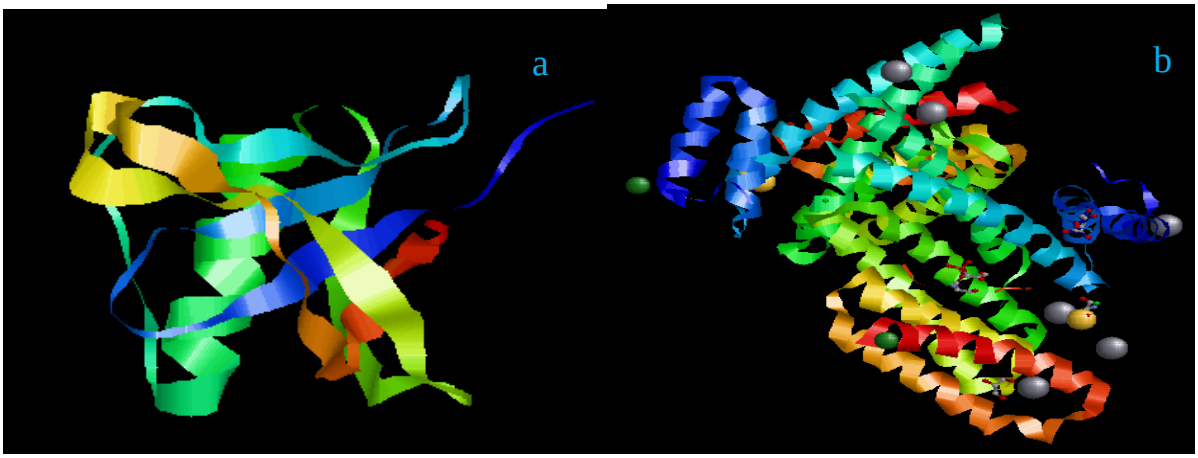


Fig.3 Structure of target protein molecules. 'a' represents Aβ42, 'b' represents tau, 'c' represents antioxidant SOD and 'd' represents antioxidant CAT



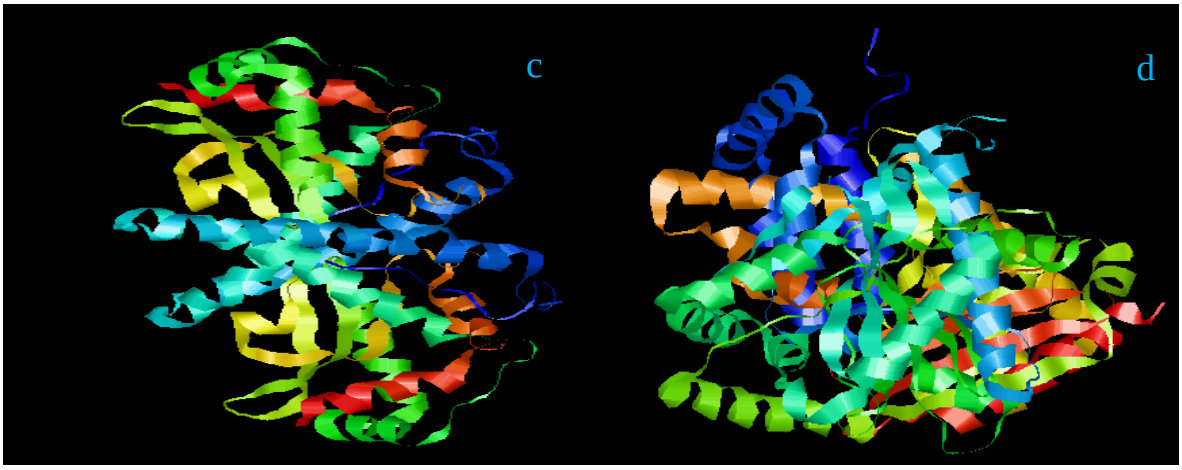


Fig.4 Structure of target protein molecules. 'a' represents GST, 'b' represents TNF- α and 'c' represents IL-6

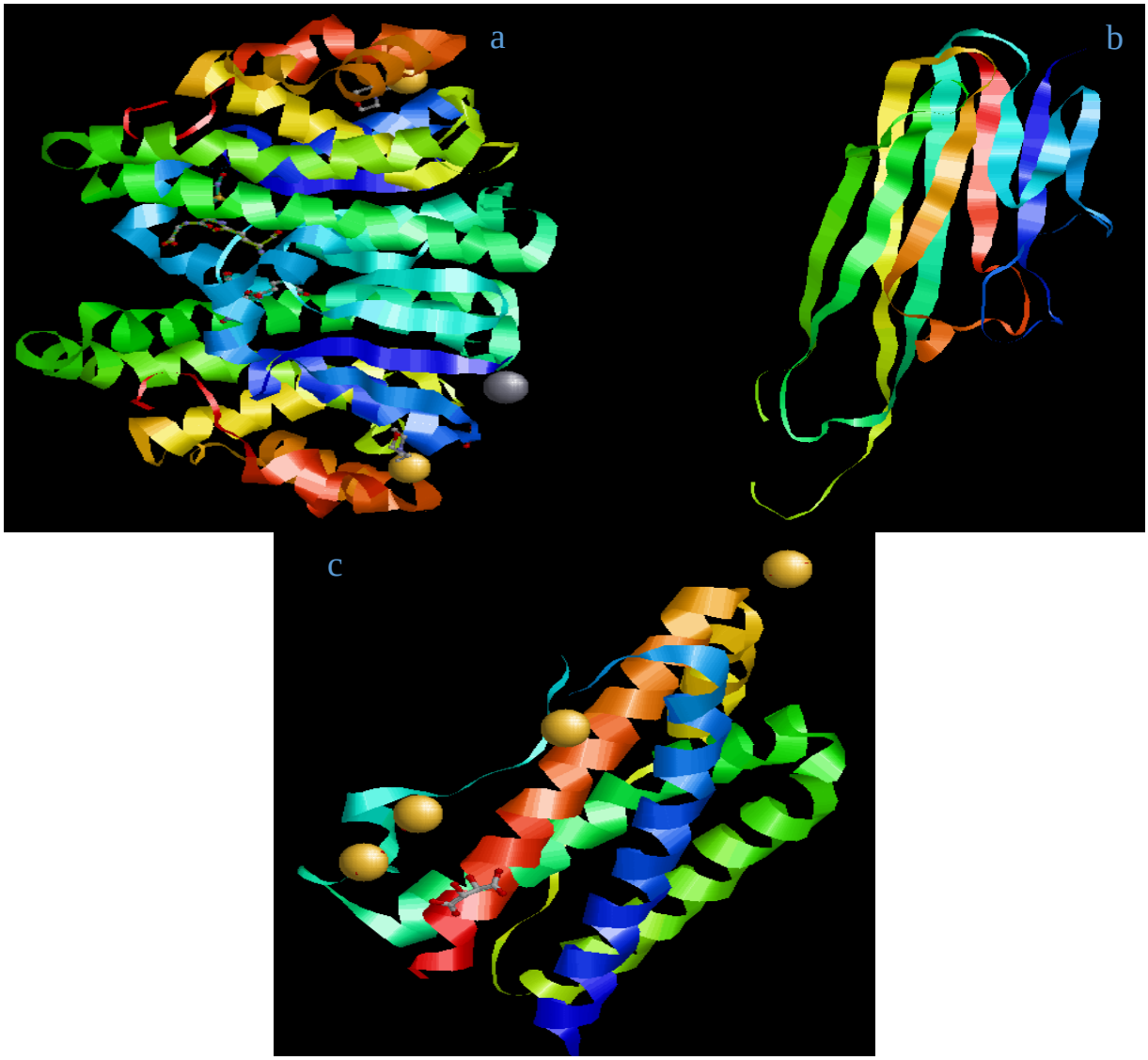


Fig.5 (a & b) Binding pattern of ligand molecule (kaempferol) with target proteins 'a' represents 'Aβ42' and 'b' represents 'tau'

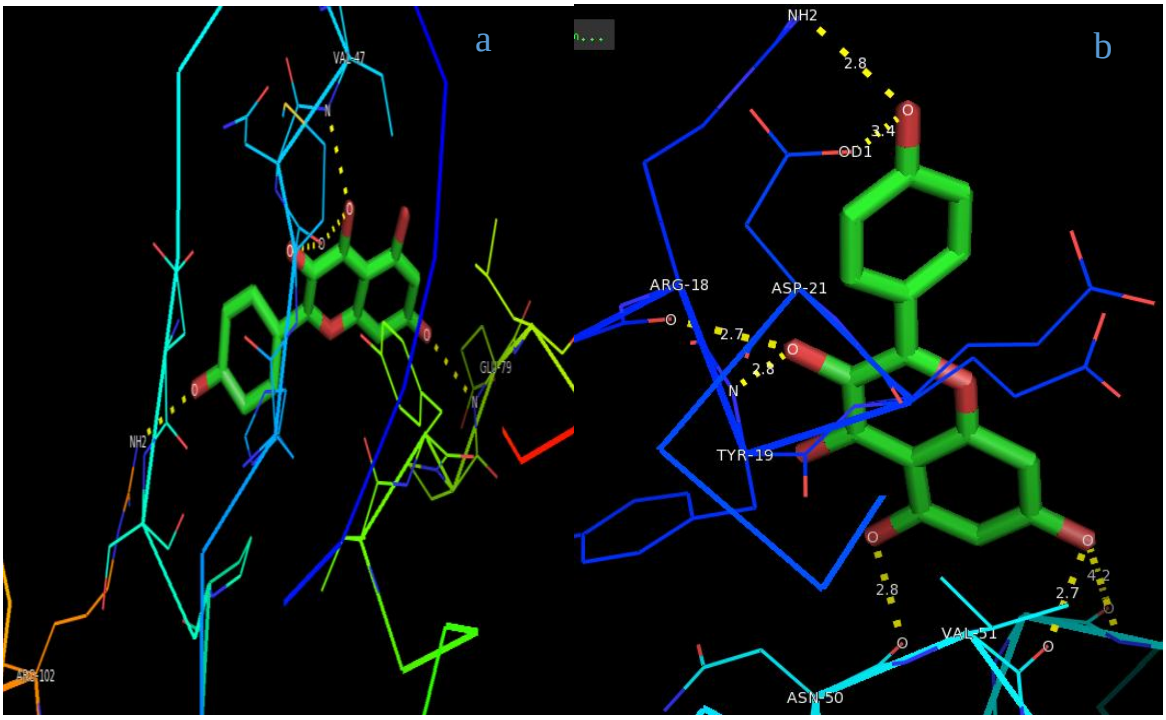


Fig.6 (a & b) Binding pattern of ligand molecule (kaempferol) with target anti-oxidant proteins. 'c' represents 'SOD' and 'd' represents 'CAT'

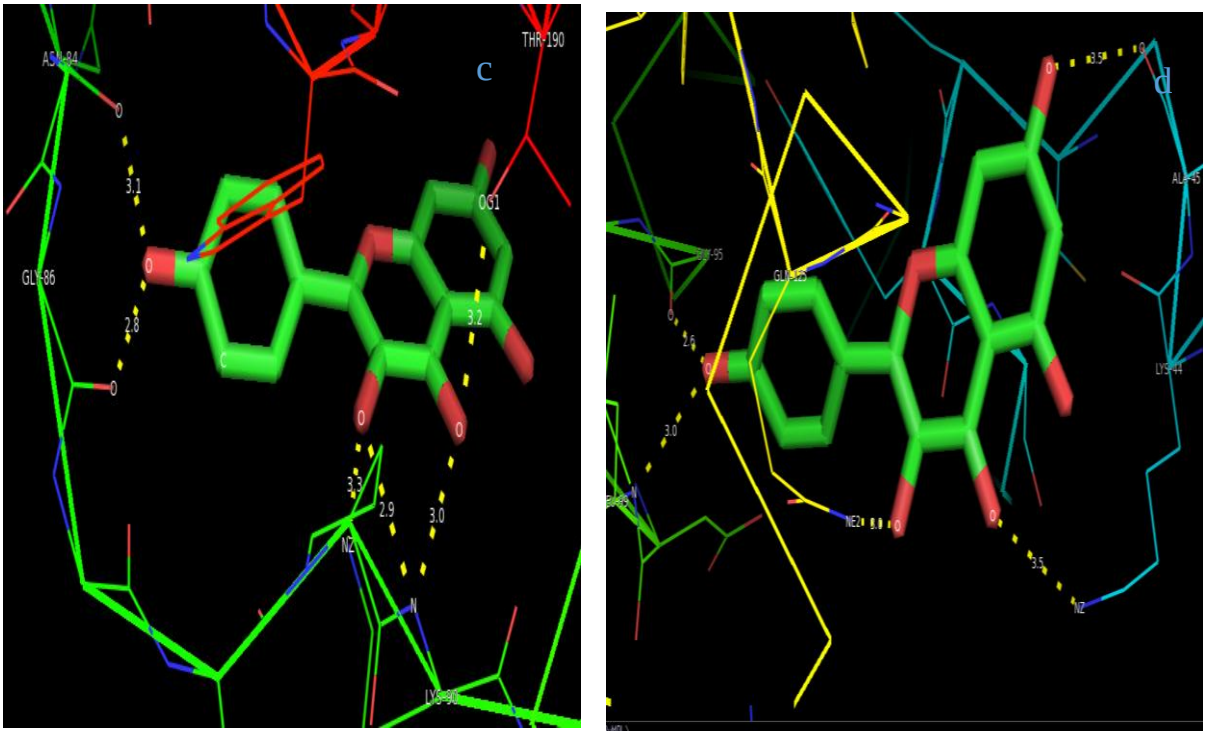


Fig.7 (a, b & c) Binding pattern of ligand molecule (kaempferol) with proteins. 'a' represents 'GST', 'b' represents 'TNF α', 'c' represents 'IL-6'

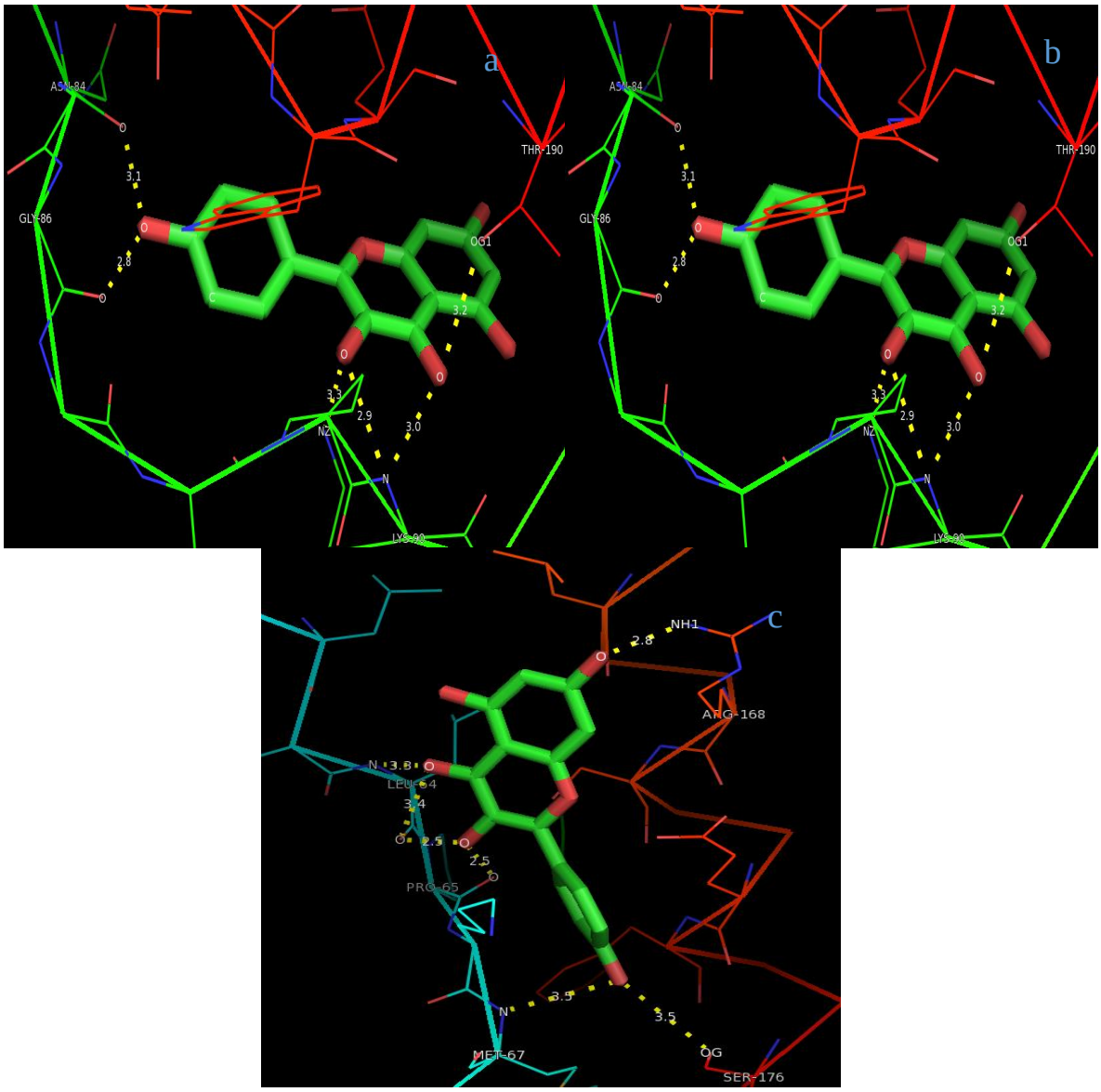


Table.1 Kaempferol compound details

Name of the compound	Alternative name	Molecular weight	Molecular formula	H-Bond donor	H-Bond acceptor	description
Kaempferol	Kaempferol; Kaempherol; Kaempferol; Populnetin; Rhamnolutein	286.239 g/mol	C15H10O6	4	6	Kaempferol is a natural flavonol, a type of flavonoid widely present in all plants

Table.2 Docking score and number of hydrogen bonds formed between the proteins and kaempferol compound

S. No	PROTEINS	KEY RESIDUES	DOCKING ENERGY (Kcal/Mol)	H-BOND
1	Aβ42	Val^{47},Glu^{79}, Arg^{102}	-7.48	2
2	TAU	Asn^{50},Val^{51},Tyr^{18},Asp^{21},Arg^{18}	-8.40	2
3	SOD	Thr^{190},Gly^{86}, Lys^{90}, Asn^{84}	-6.89	2
4	CAT	Ser^{538}, Ser^{96}, His^{324}, Ile^{330}	-9.05	1
5	GST	Gly^{95},Leu^{99},Gln^{125},Lys^{44},Ala^{45}	-7.99	2
6	TNF-α	Gln^{25}, Ala^{134}, Ile^{136}, Asn^{46}	-6.77	3
7	IL-6	Arg^{168}, Leu^{64}, pro^{65}, Ser^{176}, Met^{67}	-7.93	2

In correspondence to the above data the docking score was measured and viewed considering the electrostatic and steric properties. The data showed that the binding pattern differs with the protein and thus also influences the docking score value. Higher fitness score of -6.89 and -7.99 were observed for the antioxidant enzymes SOD and GST. In contrast to this fitness score of -7.48 and -8.4 were observed for the Aβ42 and tau proteins. The fitness score of CAT were observed as -7.52.Whereas for TNF-α and IL-6 the fitness score of -6.77 and -7.93 were observed. Thus based upon docking studies, kaempferol can be used as a potential anti-neurotoxic agent.

In the current study, different target proteins were docked with the ligand (kaempferol) to study the mechanism of ligand interaction and binding energy. The docking score of kaempferol was calculated using "auto-dock" software. Though the pattern of binding between the ligand and each protein varied, docking scores indicate that kaempferol has potential anti-neurotoxic effect.

References

Abbott NJ Astrocyte-Endothelial Interactions And Blood– Brain Barrier Permeability. J Anat,629 – 638,2002

Alex E. Roher*T, Jonathan D. Lowensont, Steven Clarket, Amina S. Woods§, Robert J. Cotter.Eric Gowing*, And Melvyn J. Ball B-Amyloid(1-42) Is A Major Component Of Cerebrovascular Amyloid Deposits: Implications For The Pathology Of Alzheimer Diseaseproc. Natl. Acad. Sci. USA Vol. 90, 1993.

Ashwini Kumar A, Chaluveelaveedu Murleedharan Nisha A, Chitrangda Silakari A, Isha Sharma A, Kanukanti Anusha A,Nityasha Gupta A, Prateek Nair A, Timir Tripathi B,Awanish Kumar A,* Current And Novel Therapeutic Moleculesand Targets In Alzheimer's Disease Journal Of Formosan Medical Association 2015

Brenda Winkel-Shirley Biosynthesis Of Flavonoids And Effects Of Stress Research Gate, 2002.

Carmen López-Sáncheza, Francisco Javier Martín-Romerob, Fei Sunc, Laura Luisc,Alejandro K. Samhan-Ariasb, Virginio García-Martínezа, Carlos Gutiérrez-Merinob Blood Micromolar Concentrations Of Kaempferol Afford Protection Against Ischemia/Reperfusion-Induced Damage In Rat Brain Research Gate, 2007.

Durga.M, Nathiya.S, Devasena.T* "Immunomodulatory And Antioxidant Actions Of Dietary Flavonoids" International Journal Of Pharmacy And Pharmaceutical Sciences Vol 6 Issue 2, 2014

Giuseppe Filomenia,1, Ilaria Graziania,1, Daniela De Zioa,B, Luciana Dinic, Diego Centonzeb,D,Giuseppe Rotilioa,E, Maria R. Cirioloa,E,*.Neuroprotection Of Kaempferol By Autophagy In Models Of Rotenone-Mediated Acute Toxicity: Possible Implications For Parkinson's Disease ISSN- 0975-1491 Vol 6 Issue 2, 2014

Hui Zheng*1, 2 And Edward H Koo*1,2 The Amyloid Precursor Protein: Beyond Amyloid Molecular Neurodegeneration 2006.

J.M. Calderón-Montaño, E. Burgos-Morón, C. Pérez-Guerrero And M. López-Lázaro* A Review On The Dietary Flavonoid Kaempferol Mini-Reviews In Medicinal Chemistry, 2011, 11, 298-344.

Joan Stiles & Terry L. Jernigan The Basics Of Brain Development Neuropsychol Rev 2010

Johannes C.M. Schlachetzki,1 Soraya Wilke Saliba,2 Antonio Carlos Pinheiro De Oliveira2 Studying Neurodegenerative Diseases In Culture Models Revista Brasileira De Psiquiatria 2013

Johnhardy 1 And Dennis J. Selkoe2* The Amyloid Hypothesis Of Alzheimer's Disease:Progress And Problems On The Road To Therapeutics *Science* 297, 2002.

Kumar S, Pandey AK. Chemistry And Biological Activities Of Flavonoids: An Overview Scientific world journal. 2013; 162750(Pubmed)

Kuresh A. Youdim,* Barbara Shukitt-Hale, And James A. Josephy Flavonoids And The Brain: Interactions At The Blood–Brain Barrier And Their Physiological Effects On The Central Nervous System Free Radical Biology & Medicine, Vol. 37, 2004.

Luc Bue´Ea,*,1, Thierry Bussie`Rec,1, Vale´Rie Bue´E-Scherrerb, Andre´ Delacourtea,Patrick R. Hof*,C,D,E Tau Protein Isoforms, Phosphorylation And Role In Neurodegenerative1 Disorders Brain Research Reviews 33 (2000)

Magdalena Żuk1,5 *, Anna Kulma1, Lucyna Dymińska2, Katarzyna Szołtysek2, Anna Prescha3, Jerzy Hanuza2,4,Jan Szopa1,5 Flavonoid Engineering Of Flax Potentiate Its Biotechnological Application BMC Biotechnology 2011.

Martin Citron Alzheimer's Disease: Strategies For Disease Modification Nature Reviews Volume 9 2010.

Todd M. Preuss The Human Brain: Rewired And Running Hot Ann N Y Acad Sci. 2011

http://www.shehjar.com

www.uniprot.org

http://www.rcsb.org/pdb

https://pubchem.ncbi.nlm.nih.gov/

www.pymol.org

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