In-silico docking studies of Copper-Zinc Superoxide Dismutase and plant derivatives to identify potential drugs for the treatment of Amyotrophic Lateral Sclerosis

To be submitted as Major Project Report in partial fulfillment of the requirement for the Degree of

Master of Technology

In

Bioinformatics

Submitted by

Deepak Singh

(2K14/BIO/03)

Under the supervision of

Dr. Pravir Kumar

Department of Biotechnology

Delhi Technological University

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, New Delhi-110042, INDIA

CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"*In-silico* docking studies of Copper-Zinc Superoxide Dismutase and plant derivatives to identify potential drugs for the treatment of Amyotrophic Lateral Sclerosis"**, submitted by Deepak Singh (2K/14/BIO/03) in partial fulfillment of the requirement for the major project during M. Tech. Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him under my guidance.

The information and data enclosed in this dissertation is original work of the candidate and has not been submitted elsewhere for honouring of any other degree.

Date:

Dr. Pravir Kumar

Associate Professor
Department of Bio-Technology
Delhi Technological University
(Formerly Delhi College of
Engineering) Shahbad Daulatpur,
Main Bawana Road,
New Delhi-110042

Prof. D. Kumar

Professor and Head
Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of
Engineering) Shahbad Daulatpur
Main Bawana Road,
New Delhi-110042

DECLARATION

I declare that my major project entitled **"*In-silico* docking studies of Copper-Zinc Superoxide Dismutase and plant derivatives to identify potential drugs for the treatment of Amyotrophic Lateral Sclerosis"**, submitted to Department of Biotechnology, Delhi Technological University as a result of the work carried out by me at "Molecular Neuroscience and Functional Genomics Laboratory", Department of Biotechnology, as Major project.

Date: Deepak Singh

Place: New Delhi

ACKNOWLEDGEMENT

I would like to acknowledge my mentor, Dr. Pravir Kumar for providing this wonderful opportunity to further my education and training. It has been a road filled with many trials and tribulations and Prof. D Kumar has been committed to my success and development as a researcher and scholar. For this, I offer my deepest gratitude.

I would like to thank my all teachers and senior lab members whom played key roles not only for the dissertation project but importantly, in my development as a person and a researcher.

My Friends have been a constant source of support throughout this project and deserve acknowledgement and thanks.

I would also like to thank senior management of Delhi Technological University for constant encouragement and support.

Deepak Singh
2K14/BIO/03

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

In-silico docking studies of Copper-Zinc Superoxide Dismutase and plant derivatives to identify potential drugs for the treatment of Amyotrophic Lateral Sclerosis

deepaksingh199221@gmail.com

Name – Deepak Singh
Roll No.-2K14/BIO/03

1. ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a dynamic and lethal disorder caused by degeneration of motor neurons. Most cases of ALS show up sporadically, however around 15-20% of patients have family histories of ALS. It causes muscle weakness and atrophy in entire body due to degeneration of motor neurons. The patients loose all their voluntary muscle functions as the disease progresses but the cognitive and sensory functions remain intact. In spite of the fact that the exact reason for the dominant part of cases is still obscure, mutations in chromosome 21 encoding copper zinc superoxide dismutase (SOD1) has been found in 12-22% of familial cases of the disease. There are evidences that oxidative stress causes death of motor neurons. This enzyme is a powerful antioxidant that protects the cells from damage caused by superoxide, a toxic free radical generated in mitochondria. Free radicals are highly reactive molecules produced by mitochondria during normal metabolism. Free radicals can accumulate and cause damage to DNA and proteins within cells. Till now, over 110 mutations in *SOD1* have been linked with ALS disorder. When the defense against oxidative stress fails, programmed cell death (apoptosis) is up-regulated and the motor neurons die. Currently there is only one drug riluzole which is available for the treatment of ALS but it just improves the life of patient by two to three months. We are looking for possible plant derivatives such as alkaloids, terpenoids and flavonoids which may be used as drugs for the cure of ALS.

2. INTRODUCTION

Neurodegenerative diseases are a group of diseases which are caused due to disorders in the nervous system. These can be of either sporadic nature or hereditary nature. One of the most common late onset motor neuron diseases is Amyotrophic lateral sclerosis (ALS) (Vance et al, 2009). It is a progressive and deadly disorder characterized by neuro-degeneration of motor neuron cells in the brain stem, motor cortex and spinal cord (Rowland et al 2001). The disorder leads to muscle weakness and atrophy to the entire body due to death of upper and lower motor neurons (Kabashi et al, 2008). The patients afflicted with this disorder usually loose all their control on voluntary muscle movements which initiates generalized weakness, difficulty in swallowing but cognitive responses remain intact till later stages of the disorder.

Eight genes associated with ALS have currently been identified: Cu/Zn superoxide dismutase (Rosen et al, 1993) (SOD1), sarcoma/translated in liposarcoma (FUS/TLS), vesicle-associated membrane protein (VAPB), angiogenin (ANG), alsin (ALS2), Tar DNA binding potein (TDBP), senataxin(STX) and dynactin (Puls I et al 2003) (DCTN1). We have emphasized on SOD1 gene which encodes copper-zinc superoxide dismutase enzyme which is a powerful antioxidant that saves the cells from damage caused by a highly toxic free radical superoxide which is generated in the mitochondria.

COPPER-ZINC SUPEROXIDE DISMUTASE (SOD1) Protein : The gene encoding for copper-zinc superoxide dismutase (SOD1) is present on chromosome 21 short arm. SOD1 protein is a 21 kilo dalton homodimer and forms a β-barrel secondary structure and consists of sites of CU/ZN in each subunit (Halliwell et al, 1999). These sites have copper and zinc ions and are responsible for converting excess amounts of superoxide to hydrogen peroxide and dioxygen. The intra subunit disulphide bonds are between cysteine 57 and cysteine 146 in each subunit (Hart et al 2006). The synthesis of SOD1 takes place in cytosol but it is transferred to intermembrane space

of mitochondria (Sturtz et al, 2001). The SOD1 protein without the metal ions and disulphide bonds is very unstable.

SOD1 binds to zinc and copper ions and its function is to destroy free superoxide radicals in the body. This enzyme is present in cytoplasm and intermembrane space of mitochondria and it is responsible for converting toxic radicals to hydrogen peroxide and molecular oxygen , these are then further reduced by enzyme catalases (Pryor et al, 1995). This brings us to the conclusion that if this protein is mutated it would not be able to neutralize free radicals and due to oxidative stress the cell would die. The normal functions of mitochondria causes formation of reactive oxygen species which are involved in cell signaling (Coyle et al, 1993). Examples of reactive oxygen species are superoxide, hydroxyl radical, peroxides, singlet oxygen etc (Halliwel et al, 1999). ROS are normal by product of cell functioning and are present in cell signaling (Muller F et al, 2000). However, during stress conditions there is overproduction of ROS which may lead to damage to cell and hence this is called oxidative stress. Superoxide, one of the ROS is produced by reduction of molecular oxygen is a precursor for most other reactive oxygen species (Lenaz et al, 1998). SOD1 protein reduces this superoxide into singlet oxygen and hydrogen peroxide. Hydrogen peroxide is further reduced to hydroxyl radical which is further reduced to water (Beckman et al, 1996). The formation of ROS is by two means exogenous and endogenous. Exogenous ROS is caused by pollutants, tobacco, dugs, etc. Endogenous ROS is produced by intracellular factors via multiple mechanisms (Martindale et al, 2002). NADPH oxidase present in mitochondria, cytoplasm etc is responsible for production of ROS (Bedard et al 2007). The mitochondria is the powerhouse of cell and it creates energy for normal cell functioning in the form of ATP. ATP is produced by oxidative phosphorylation by transport of proteins across intramembranes of mitochondria via electron transport chain (Hand et al, 2001). In normal cases the end product is an oxygen molecule by due to some premature reductions a

superoxide free radical is formed, which is reduced by SOD1 protein by breaking it into hydrogen peroxide and singlet oxygen (Pryor et al, 1995). Hydrogen peroxide is further reduced by catalase into hydroxyl radical, which is further reduced to water. Mutation in SOD1 protein raptures this ability of protein and hence leads to oxidative stresses which further leads to death of neurons. Superoxide and other ROS cause the death of cells by damaging the DNA, oxidation of amino acids in protein chains, oxidative deactivation of cofactors in enzymes and lipid peroxidation (Abe et al, 1997).

3. Review of literature

Amyotrophic lateral sclerosis is a neurodegenerative disorders that involves the death of motor neurons (Leigh et al, 2000). In a number of places, the term motor neuron disease (MND) is also used. About 5–10% of cases are inherited from a person's parents. The diagnosis is primarily based on a person's signs and symptoms with tests to strike out other causes. The disease typically starts around the age of 60 to 65 years and in inherited or familial cases around the age of 45 to 50 years. The average survival from onset of ALS to death is around three to four years. Most of the patients die from respiratory failure (Fallat et al, 1987). Throughout world, rates of ALS are unknown.

Individuals suffering from the disorder may finally lose the ability to control and perform all the voluntary movements of the body, although the ocular muscle functions for eye movement are left unchanged until the final stages of the disorder (Moscowitch et al, 1982). Cognitive functions are usually not affected in many individuals, although some develop dementia. A higher proportion of patients (30–50%) experience dementia, degenerative muscle weakness disorder and degenerative bone disorder at the final stages of the disorder. Sensory nerves and the autonomic nervous system are usually unaffected, meaning that the majority of individuals with ALS maintain hearing, sight, touch, smell, taste and other senses (Hamida et al, 1990).

At the start of ALS symptoms are so subtle that they are overlooked. The earliest symptoms which can be observed is muscle weakness and muscle atrophy. Other subtle symptoms are trouble in swallowing, talking, cramping, stiffning of affected muscles (Sutedja et al, 2000). The damage on particular motor neurons determines which part of the body shows signs of Amyotrophic lateral sclerosis (Rowland et al, 2000). Report shows that 75% of the patients

suffering from ALS first experience limb weakness as it weakens either an arm or leg. Straying or deviating from a path may also be taken as a sign of early ALS symptoms (Logroscino et al, 2000). Difficulty with tasks requiring skills of hands such as putting thread in a needle. Normally, the symptoms remain confined to one limb for a long duration the illness, this is medically known as monomelic amyotrophy (Rosen et al, 1993).

Although there is no way to predict the progression of disorder but there is a clinically administered method that gives the report of progression of ALS , a 12- item equipment used as a clinical questionnaire or patient-reported questionnaire that gives a score between 0 (severe problem) and 48(normal) (Chieia et al, 2010).

The accruing difficulty in chewing and swallowing makes ingestion terribly difficult and will increase the risk of choking or of entry of food into the lungs. In later stages, acute pneumonia can develop, and maintaining a healthy body weight can become significantly difficult (Books et al, 2000). As the disease progresses, measures of lung efficiency such as capacity and breath pressure diminish. Most patients with ALS succumb to death because of respiratory failure or pneumonia (Fallt et al, 2000). The eye movements function properly until the later stages owing to the structural differences between ocular muscles and skeletal muscles (Phukan et al, 2007).

Around 5 to 10 % of ALS are familial (Wijisekara et al, 2007). Overall, first-degree relatives of an individual with ALS have 10% risk of developing ALS. A mutation on chromosome 21, which codes for superoxide dismutase, is related with 20% of familial cases of Amyotrophic Lateral Sclerosis and is inherited in dominant nature (Logroscino et al, 2008).

The most prevalent ALS-causing mutation is a mutant SOD1 gene mostly occurring in North America; characterized by an exceptionally fast progression from onset to death. The most common mutation found in Scandinavian countries, D90A-SOD1, is slow progressive than

typical ALS, and people with this type of the disorder survive for an average of 11 years (Anderson et al, 2006). The UBQLN2 gene encodes production of the protein ubiquitin 2 in the cell, which controls the degradation of ubiquitinated proteins (Rosen et al, 1993). The Mutations in this UBQLN2 gene hinders protein degradation, leading to neuro-degeneration and causing dominantly heritable ALS (Yang et al, 2001). The most common form of ALS is A4V mutation in SOD1 gene, this means that the mutation causes alanine to be replaced by valine at position 4 of protein chain which affects most of the population. Copper-Zinc superoxide dismutase is an enzyme which plays an active role in catalysis of free radical superoxide into molecular oxygen and hydrogen peroxide, these are further degraded by catalases (Kirkland et al, 2001). Mutation in SOD1 protein hinders this process and the cell dies (Andersen et al, 2006).

Plant derivatives

Plant derivatives such as flavonoids, terpenoids and alkaloids have displayed potential as drugs against various diseases such as neurodegenerative diseases, cancer and other ailments (Camps et al, 2002). Flavonoids basically have a 15 carbon skeleton structure with two phenyl rings and one heterocyclic ring. In plants flavonoids perform various functions such as pigmentation, symbiotic nitrogen fixation and ultra violet rays filtration. Alkaloids are a huge group of compounds which do not have a particular way of classification but most alkaloids contain oxygen in their molecular structure, oxygen free ones are volatile (Chin et al, 2007). They are further classified into central structure based derivatives such as tropanes piperidines, quinalozine etc (Christian et al, 2002). In plants they are mostly found in leaves and the extraction of alkaloids is mostly done from leaves (Fodor et al, 1971). Terpenoids are compounds which are derived from five carbon isoprene units and modified in many ways (Chen et al, 2003). Terpenoids have aromatic qualities and have been used in herbal medications from a long time. The scent of eucalyptus, menthol, turmeric is all through terpenoids (Cheng et al,

2007). In the plants their synthesis is from mevalonic acid pathway and methyl erythrydol phosphate pathway (Adam et al, 2002).

Luteolin

Luteolin is a flavonoid and has a yellow crystalline structure. Luteolin is mainly isolated from leaves but it can also be isolated from seeds in some species of plants (Fletcher et al, 1996). It has a molecular weight of 286.24g/mol, molecular formula $C_{15}H_{10}O_{8,}$. It has shown potential as a drug against cancer and neurodegenerative diseases.

Citral

Citral is a terpenoid which is derived commonly from lemon grass and is known as a prophylactic and pain relieving drug (Akhar et al, 2000). It has a molecular weight of 152.24 g/mol, molecular formula $C_{10}H_{16}O$.

Eriodictyol

Eriodictyol is a flavonoid which is mostly derived from a plant native to North America named Yerba Santa, it has a taste modifying property (Woo et al, 2012). It has shown potential as it protects endothelial cells against oxidative stress (Alam et al, 2000). It has a molecular Weight of 282.25 g/mol, molecular formula $C_{15}H_{12}O_8$.

Sinapine

Sinapine is a alkaloid found mostly in seeds of oil plants of the family Brassicaceae and it is from phenolic compounds group (Austin et al, 1968). It is derived from rape seed and the bitter

taste of rapeseed is from sinapine (Larsen et al, 2003). It has a molecular mass of 310.37 g/mol, molecular formula $C_{16}H_{24}NO_5$.

Caffiene

Caffiene is found in cocoa beans from which coffee is made and it has shown effectiveness against some neurodegenerative diseases (Paulsen et al, 1988). It is of methylxanthine class of derivatives of alkaloids. Xanthine is a purine base found in living organisms, a number of other stimulants are derived from xanthine such as theobromine (Kalow et al, 1991). It has a molecular mass of 152.11 g/mol, molecular formula $C_8H_{10}N_4O_2$.

Cembrene

Cembrene is a monocyclic diterpene extracted from corals (Dauben et al, 1965). It acts as an attractant for termites and in chemical behavior it is oily and has wax like nature. It has a molecular mass of 272.47 g/mol , molecular formula $C_{20}H_{32}$.

Sclarene

Sclarene is a diterpene which is found in the leaves of *Podocarpus hallii* which is a coniferous tree found in the North America It has been used by various native tribes of America. It has a molecular mass of 272.48 g/mol, molecular formula is $C_{20}H_{32}$

Genistein

Genistein is a compound which belongs to the family of isoflavones. It is an angiogenesis inhibitor and a phytohormone (Cassidy et al, 1994). Its chemical structure is similar to prunetol. It has a molecular mass of 270.42 g/mol, molecular formula $C_{15}H_{20}O_5$.

Mesembrine

Mesembrine is an alkaloid which is derived from a plant native to South Africa Sceletium Tortuosum. It is normally used as an antidepressant (Genicke et al, 2001). It has a molecular mass of 289.38g/mol, molecular formula of $C_{17}H_{23}NO_3$.

Tartaric acid

Tartaric acid is an alkaloid derivative and has shown antioxidant properties found in various citric fruits. It is extracted from citric plants and has various chemical and food industry based uses (Deshpande et al, 2009). It has a molecular mass of 150.087g/mol, its molecular formula is $C_4H_6O_6$.

Molecular Docking

Molecular docking is a method by which a ligand is made to interact with a protein molecule and the computer simulation calculates various orientations of the model and based on the binding energy data the strength of association of protein and ligand can be determined (Nadendla et al, 2004). The interactions of ligands with biomolecules are done for drug designing as the data provided is very efficient and it gives a lead for the development of drug.

4. Materials and Methodology

4.1 Web resources and tools

Protein Data Bank

The protein data bank is an online repository of proteins which are submitted by various researchers. Using this software we can search for our desired protein and download it in PDB format.

ZincDB

ZincDB or zinc database is an online repository of molecules which are available in mol2 format. From here our desired drug molecules which are going to be used for docking studies are downloaded.

NCBI

NCBI is an online depository of genomic sequence or any other biological data which can be downloaded free of cost. It consists of nucleotide database, protein database etc.

CASTp Server

CASTp stands for computed atlas of surface topography of proteins which is used for determining the active sites of a protein on which a ligand can interact. This server is provided by the University of Illinois, USA. It gives information about the functional pockets of a protein.

4.2 Softwares

Pymol

Pymol is a protein molecule visualization software. This software can further be used to edit protein structure by removing unnecessary ligands which are attached to the protein molecule file.

Chemsketch

Chemsketch is software which is used to draw chemical structure and create a file in desired format. This was used to draw chemical structure for molecules whose mol2 format was not available online.

Autodock 4.2

Autodock has been developed by the Scripps research institute and the Oslon laboratory. It is a molecular modeling software which can be used for determining protein ligand interactions .

Chimera

UCSF Chimera developed by biomedical technology research resource under NIH is an extensive visualization software which is used for visualizing docking study related data and generating high quality images and animations.

MEGA6

Mega 6 is a software which can be used for multiple sequence alignment and development of phylogenetic tree based on various algorithms such as heuristic, nearest neighborhood etc.

4.3 Methodology for performing studies

1) **Retrieval of protein structure**

The Binary complex structure of human copper-zinc superoxide dismutase with A4V mutation(PDB ID: 3gzq) was retrieved from Protein Data Bank. Processing of protein structures were carried out by "Protein preparation wizard". Before protein preparation process, all the water molecules and hetero-molecule attached with the structures i.e. Hydrogen atoms were added and the geometry of all the hetero groups was corrected. For optimizing the network of H-bonds, hydrogen bonds assignment tool was implemented.

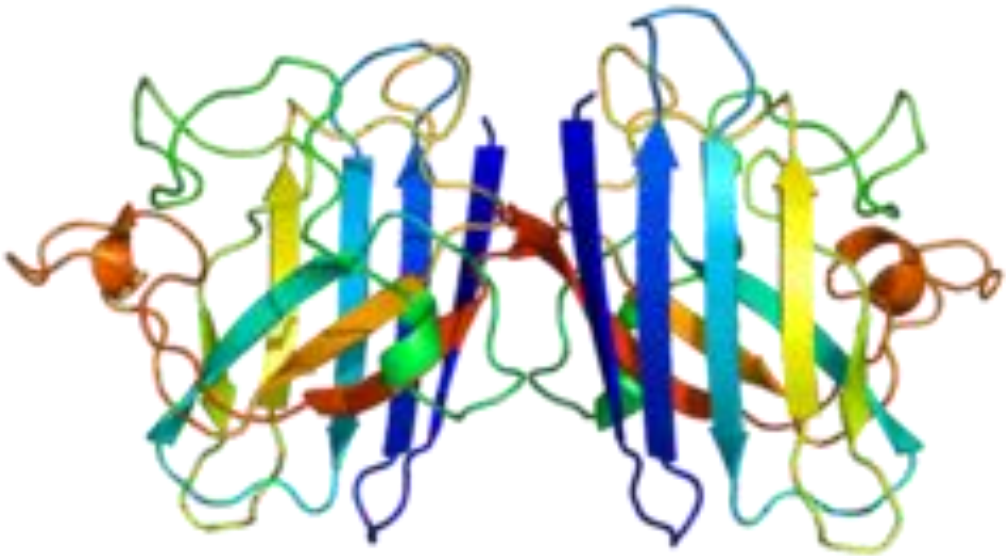

Figure 1. **SOD1 PROTEIN**

2) **Sequence Analysis and Active site Prediction:**

The active site prediction server designed by University of Illinios can be used to determine the active sites. The server computes the cavities in a given protein using the uploaded pdb file. The active sites of copper-zinc superoxide dismutase were identified using CASTp

server. The most essential property produced by CASTp is an overall Site score, which has demonstrated to be successful at distinguishing known binding sites in co-crystallized complexes. Active sites with best site scores were utilized as prerequisite for receptor grid generation with the copper-zinc superoxide dismutase.

3) **Phylogenetic Analysis** -MEGA 6 is an integrated tool for conducting multiple sequence alignment, constructing phylogenetic trees, calculating divergence times, rates of molecular evolution, mining online databases, analysing ancestral sequences and testing evolutionary history.

4) **Preparation of drugs**: Plant derivatives such as flavonoids, terpenoids and alkaloids are selected through literature survey as these drugs are also used in other neurodegenerative diseases. The structure was downloaded from zinc database. Suitability of these compounds as a drug was checked through Lipinski's law of five. Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It checks five properties of drugs of which molecules should comply with 2 or more of the following rules. Molecular mass should be less than 500 dalton, lipophilicity less than 5, hydrogen bond donors less than 5, less than 10 hydrogen bond acceptors, molar refractivity between 40-130.

5) **Docking Studies-** AutoDock 4.2 is a molecular docking software which is used for virtual screening and molecular docking analysis. Its Genetic Algorithm was used to determine the ligand protein interactions .It takes protein molecule in the PDB format and takes the ligand molecule in mol2 format

5. Results and discussion

5.1. Active site prediction

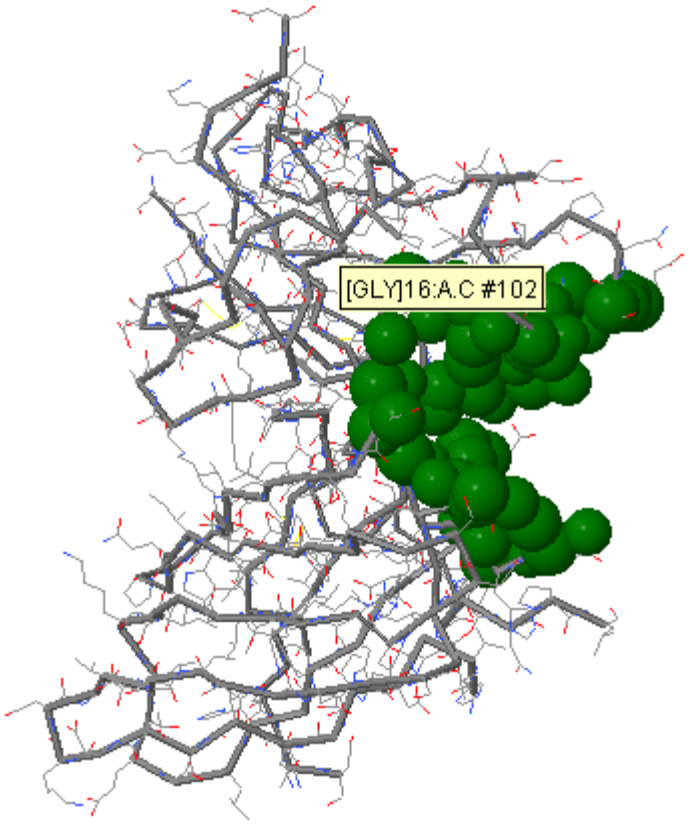

Figure 2: Active site predicted cavities from CASTp server.

The CASTp server gave a pocket for active site in SOD1 protein. The green colour shows the active site present on the SOD1 protein. The sequence of amino acids is ALA^{1}, THR^{2}, LYS^{3}, VAL^{4}, GLN^{22}, GLU^{24}, SER^{25}, PHE^{64}, LEU^{106}, SER^{107}, GLY^{108}, ASP^{109}, CYS^{111}, ILE^{113}, ARG^{115} and ILE^{151}.

5.2. Multiple Sequence alignment

Multiple sequence alignment and phylogenetic tree of copper-zinc superoxide dismutase of eight species namely *Saccharomyces cerevisiae, Homo sapiens, Halobacterium salinarium,*

Thamnophis elegans, Erythrobacteraceae bacterium HL111, Cryptococcus gotti, Saccharomyces cerevisiae s288c and *Idiomarinacea bacterium HL-53* was done to see which regions are conserved and to the phylogenetic tree was created which showed that Saccharomyces cerevisiae shows most close phylogenetic relationship.

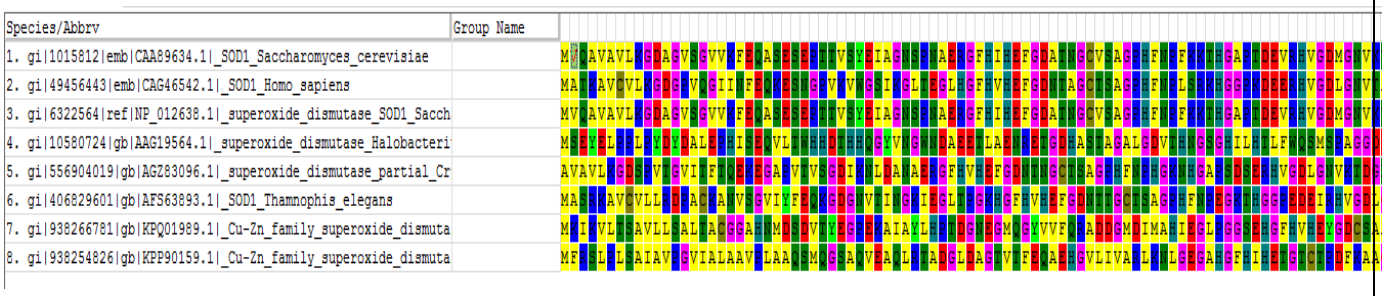

Figure 3: Multiple Sequence Alignment

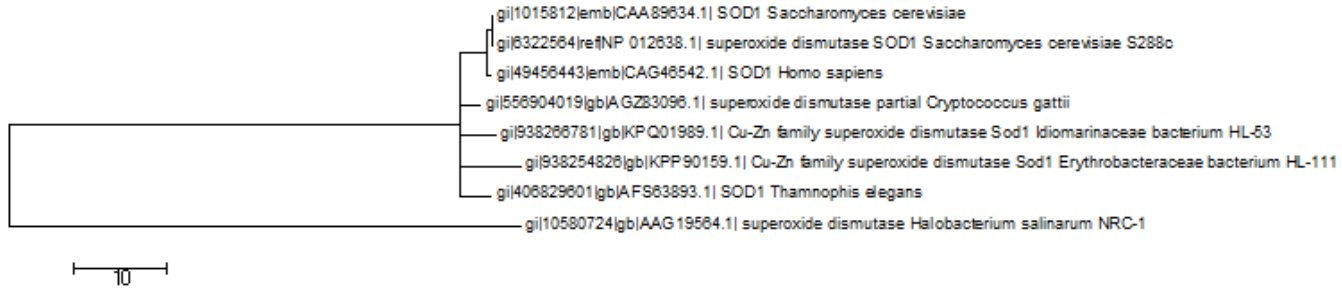

Figure 4: Phylogenetic analysis

5.3. Properties of plant derivatives

Properties based on Lipinski filter were derived and the results are given in table it gives information about molecular mass, lipophilicity, hydrogen bond donors, hydrogen bond acceptors and molar refractivity.

Plant derivative compound	Molecular Mass (Daltons)	Lipophilicity (xlogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Citral	152	2.878	0	1	48.485989
Luteolin	286	2.1252	4	6	72.478676
Sinapine	310	1.672	1	5	84.1031
Caffiene	194	0.06190	0	5	49.100
Eriodictyol	288	2.215499	4	6	71.859680
Cembrene	272	6.617	0	0	91.823967
Genistein	270	2.114	3	5	71.008881
Mesembrine	290	0.9816	1	3	80.240266
Sclarene	272	6.3076	0	0	89.6639
Tartaric acid	148	4.792	2	6	22.027

Table 1: Characteristics of plant derivative drugs used for docking study by Lipinski's filter

5.4. Docking Studies- AutoDock 4.2 is the software used for virtual screening, its docking speed is very fast and effective. Its gives option to select the search function based on Genetic Algorithm, Lamarckian Genetic Algorithm (LGA) .It is an interactive protein docking and molecular superimposition program, which can easily understand protein and DNA structures given in PDB format, and it can also be used analyse SDF files. The protein-ligand interaction is studied using the autodock4.2 software.

Compound Name	Est. Free Energy of Binding (kcal/mol)	Est. Binding Constant (mM)	Est. Intermolecular Energy (kcal/mol)	vdW+Hbond+ desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Est. Internal Energy (kcal/mol)	Torsional Free Energy (kcal/mol)
Citral	-3.39 (kcal/mol)	3.25 mM	-4.59 (kcal/mol)	-4.50 (kcal/mol)	-0.8 (kcal/mol)	-0.27 (kcal/mol)	+1.19 (kcal/mol)
Luteolin	-3.38 (kcal/mol)	3.31 mM	-4.88 (kcal/mol)	-4.43 (kcal/mol)	-0.44 (kcal/mol)	-1.69 (kcal/mol)	+1.49 (kcal/mol)
Sinapine	1.26(kcal/mol)	2.12 mM	-1.43 (kcal/mol)	-1.62 (kcal/mol)	0.19 (kcal/mol)	-1.43 (kcal/mol)	+1.24 (kcal/mol)
Caffiene	-1.38(kcal/mol)	96.8 mM	-1.38 (kcal/mol)	-0.96 (kcal/mol)	-0.43 (kcal/mol)	0 (kcal/mol)	0 (kcal/mol)
Eriodictyol	-2.86 (kcal/mol)	7.98 mM	-4.35 (kcal/mol)	-3.95 (kcal/mol)	-0.41 (kcal/mol)	-1.74 (kcal/mol)	+1.49 (kcal/mol)
Cembrine	-2.89 (kcal/mol)	7.57 mM	-3.19 (kcal/mol)	−3.12 (kcal/mol)	−0.07 (kcal/mol)	−0.23 (kcal/mol)	+0.30(kcal/mol)
Genistein	-3.92 (kcal/mol)	1.34 mM	-5.11 (kcal/mol)	-4.94 (kcal/mol)	-0.17 (kcal/mol)	-0.88 (kcal/mol)	+1.97 (kcal/mol)
Mesembrine	-2.26 (kcal/mol)	22.18 mM	-3.15(kcal/mol)	-2.84 (kcal/mol)	-0.31 (kcal/mol)	-0.37 (kcal/mol)	+0.89 (kcal/mol)
Sclarene	-5.0 (kcal/mol)	215.63µ M	-6.19 (kcal/mol)	-6.21 (kcal/mol)	+0.02 (kcal/mol)	-0.39 (kcal/mol)	+1.19 (kcal/mol)
Tartaric acid	-2.03 (kcal/mol)	32.59 mM	-3.52 (kcal/mol)	-2.20 (kcal/mol)	-1.32 (kcal/mol)	-2.15 (kcal/mol)	+1.49 (kcal/mol)

Table 2: Docking calculation Results

5.5 Protein Drug Interactions

Luteolin

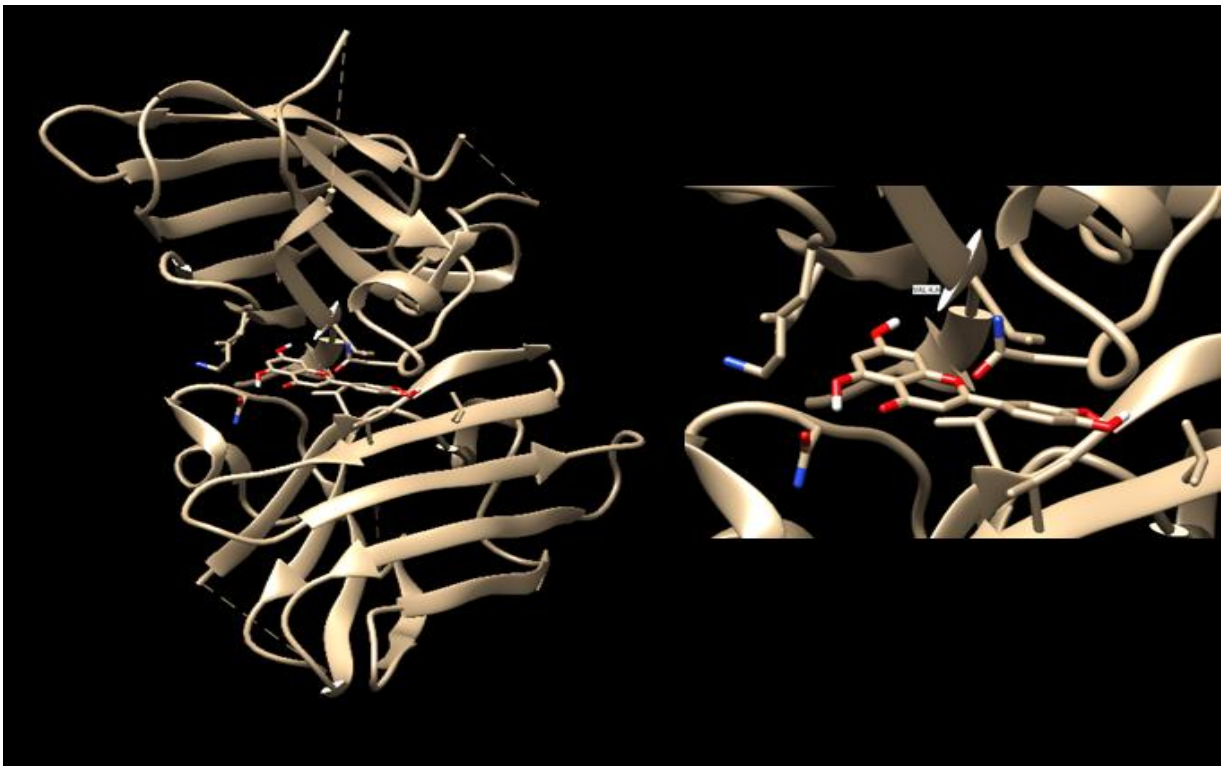

Figure 5(a): Docking of Luteolin with SOD1

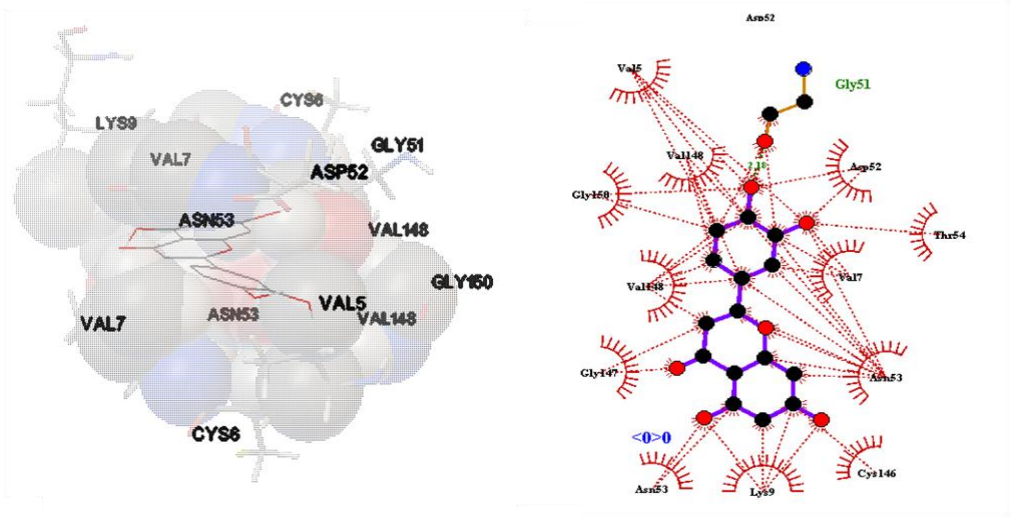

Fig 3D Interactions

Fig 2D Interactions

Figure 5(b): Interaction of Luteolin with SOD1

In this figure docking of Luteolin with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule protein are Asn53, Val7, Val5, Lys9, Cys146, Arg52, Gly51, Thr54, Gly147 and Val148.

Eriodictyol

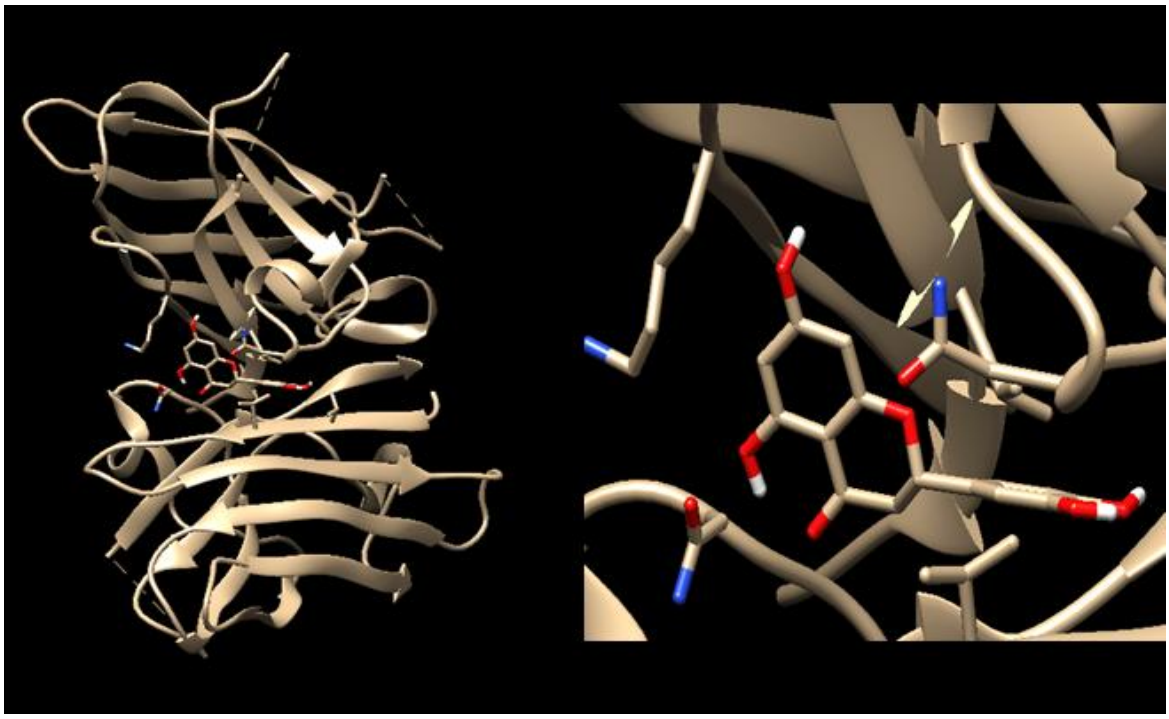

Figure 6(a): Docking of Eriodictyol with SOD1

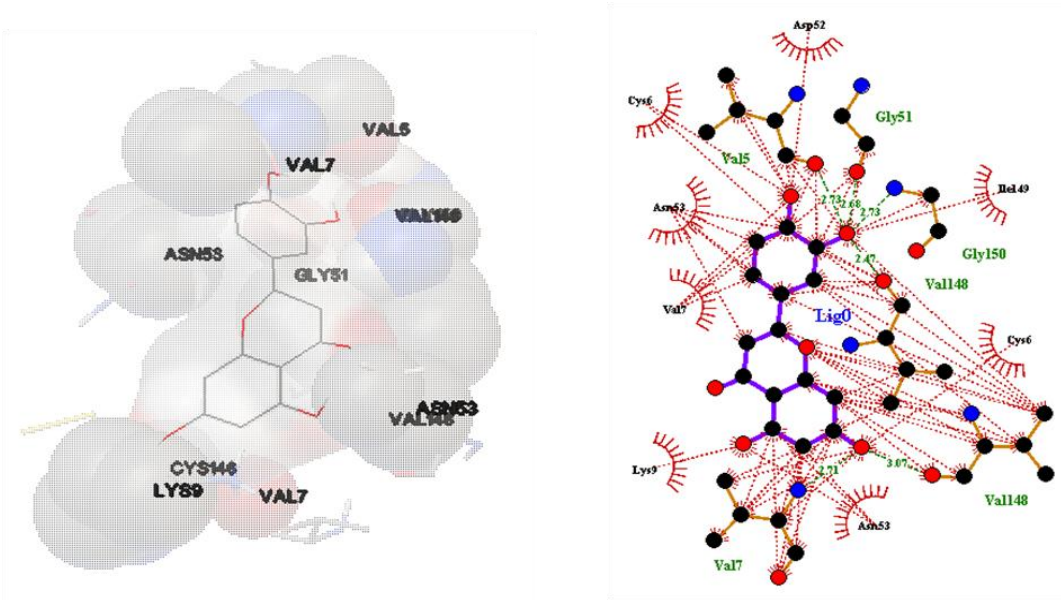

Fig 3D Interactions

Fig 2D Interactions

Figure 6(b): Interaction of Eriodictyol with SOD1

In this figure docking of Eriodictyol with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Val5, Lys9, Cys6, Cys146, Arg52, Gly51, Gly150 and Val148.

Citral

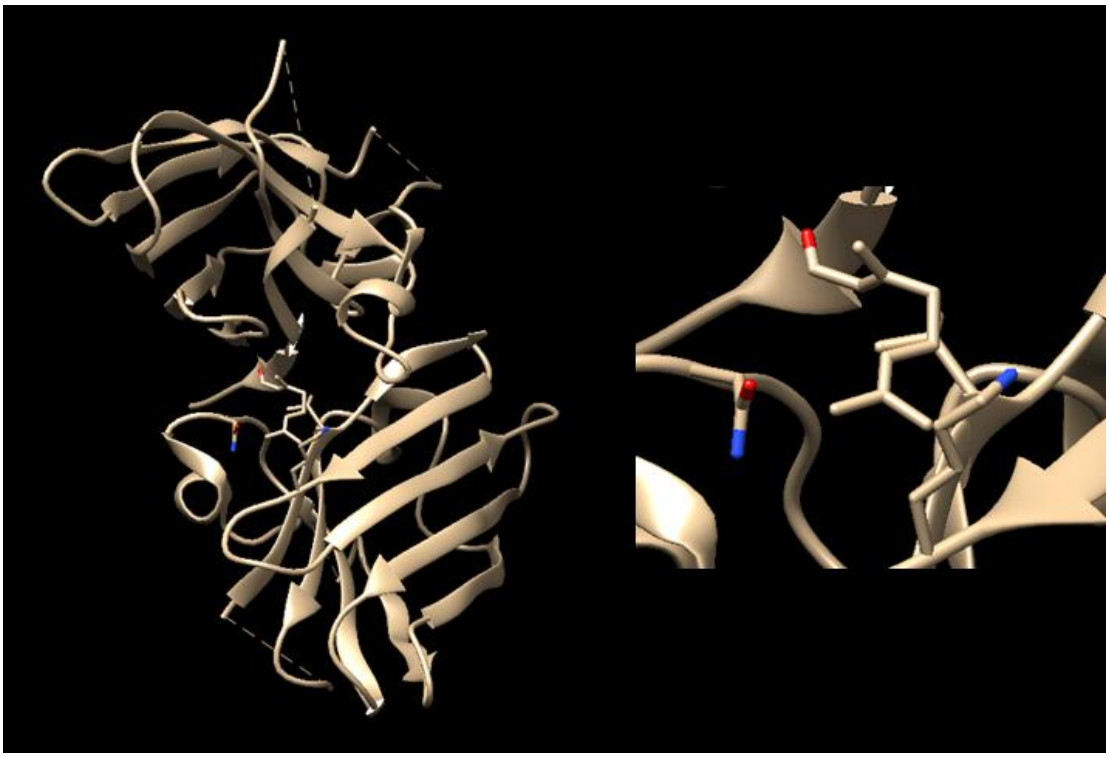

Figure 7(a): Docking of Citral with SOD1

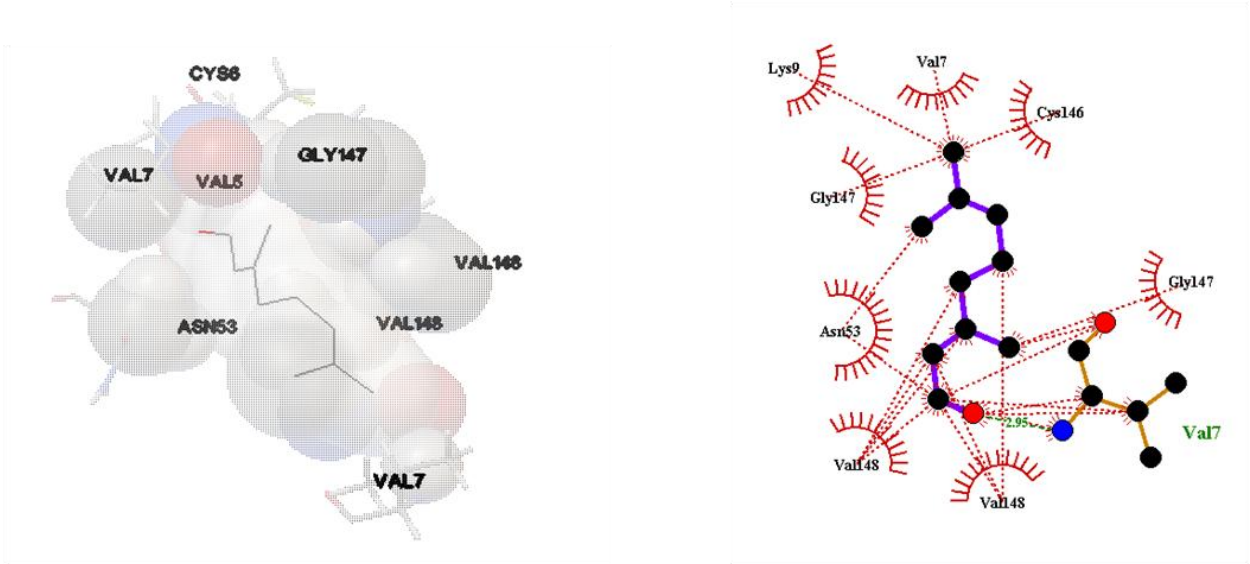

Fig 3D Interactions

Fig 2D Interactions

Figure 7(b): Interaction of Citral with SOD1

In this figure docking of Citral with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Lys9, Cys146, Gly147 and Val148.

Sinapine

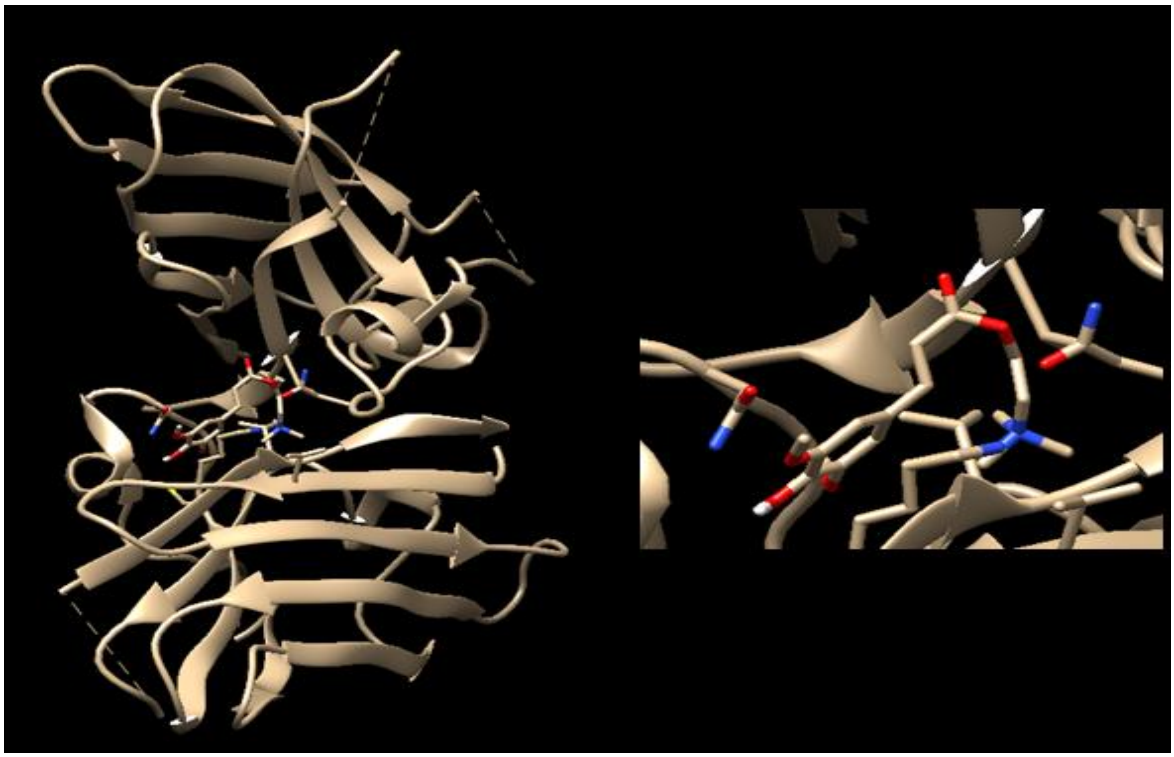

Figure 8(a): Docking of Sinapine with SOD1

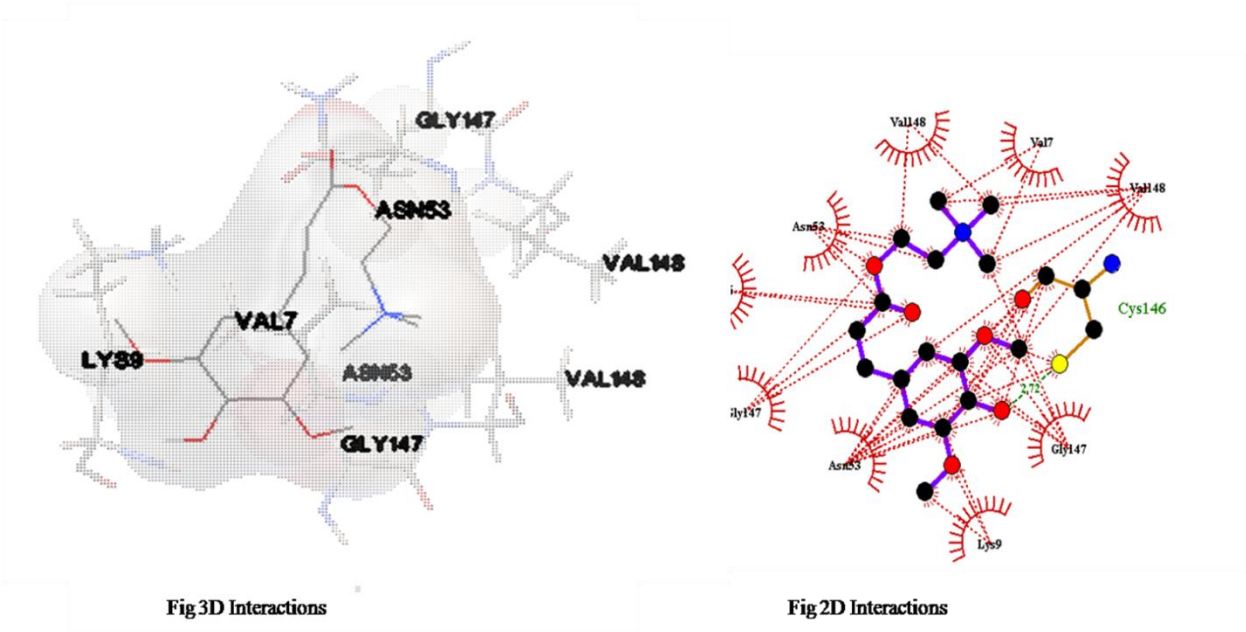

Figure 8(b): Interaction of Sinapine with SOD1

In this figure docking of Sinapine with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Lys9, Cys146, Gly147 and Val148.

Caffiene

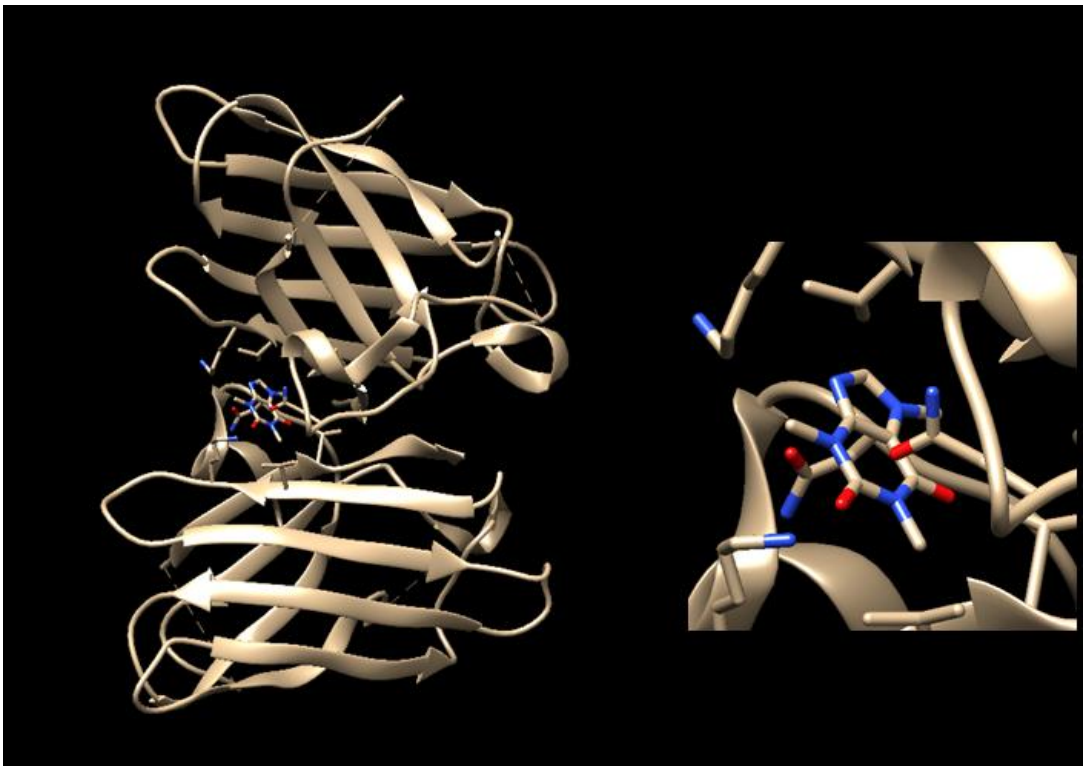

Figure 9(a): Docking of Caffeine with SOD1

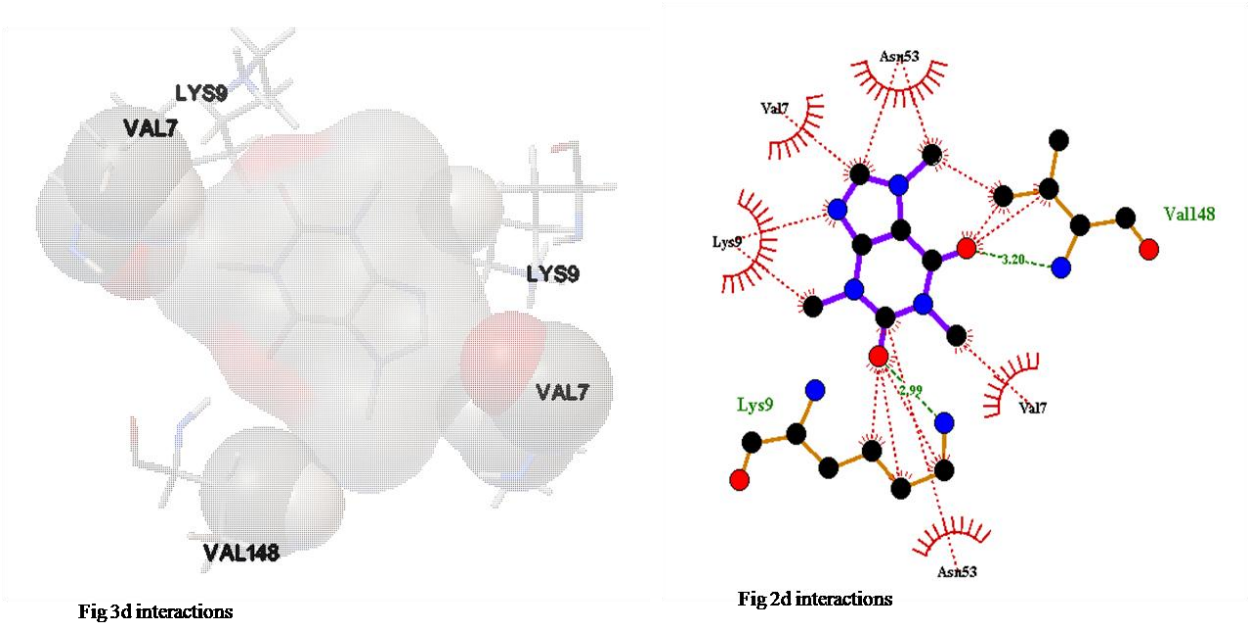

Figure 9(b): Interaction of Caffeine with SOD1

In this figure docking of caffeine with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Lys9 and Val148.

Mesemberine

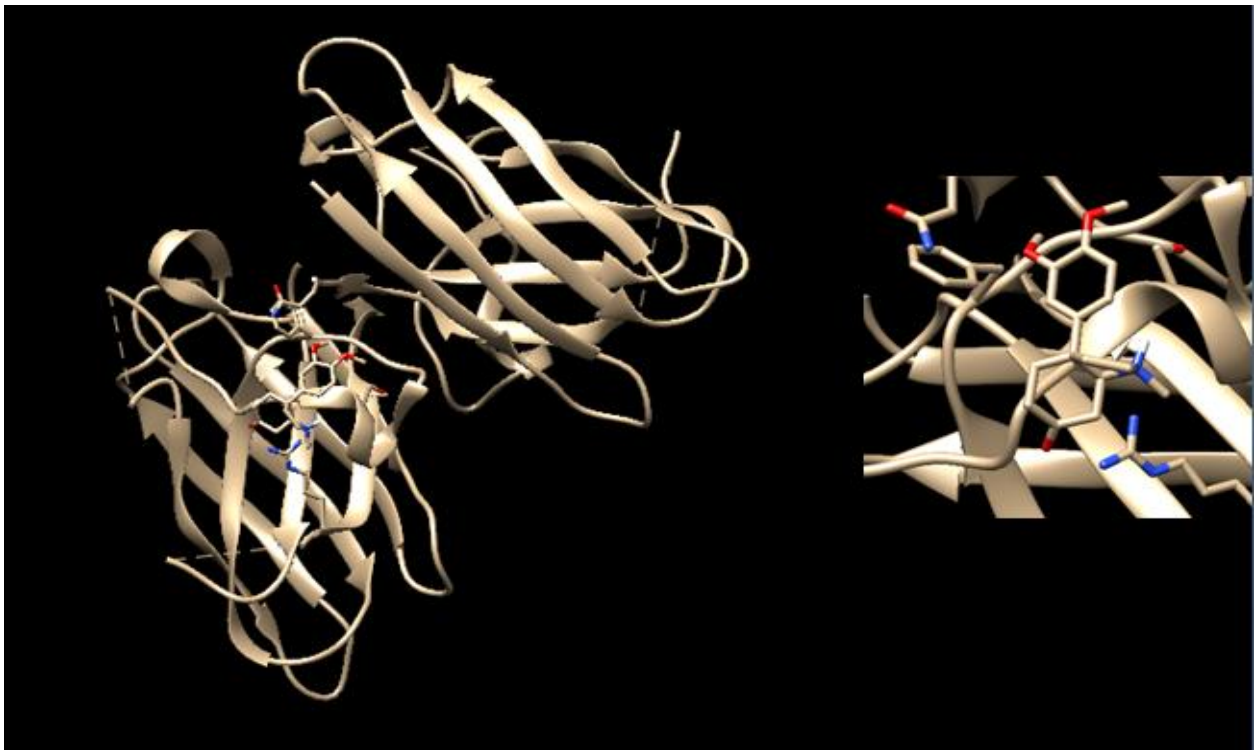

Figure 10(a): Docking of Mesembrine with SOD1

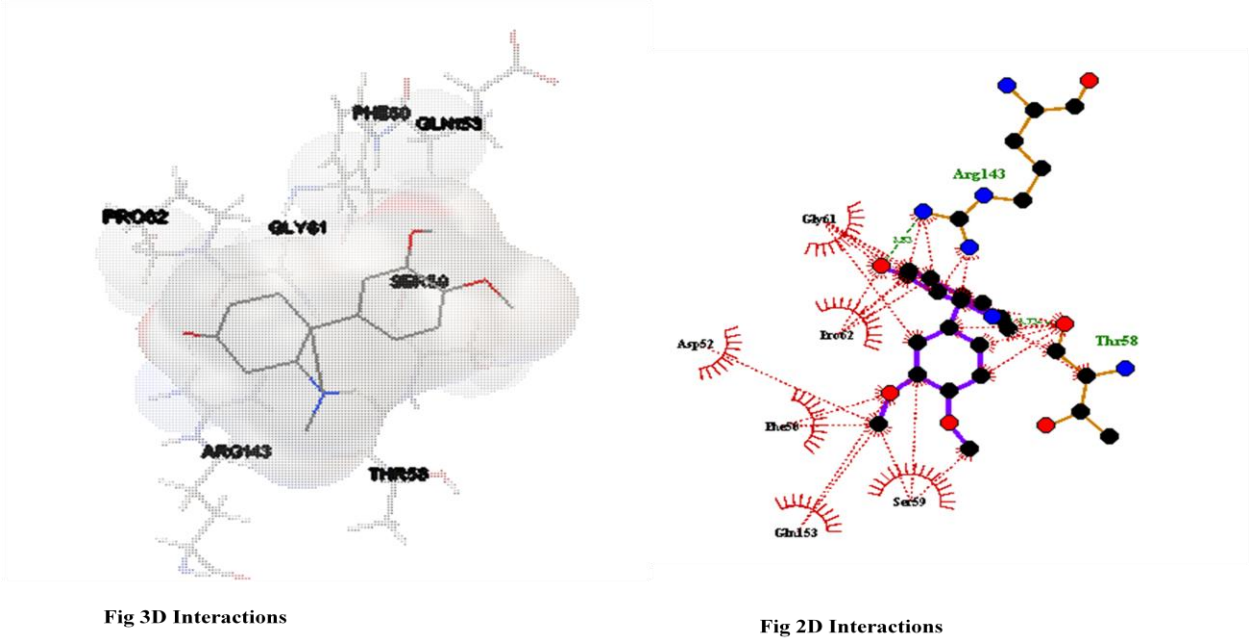

Figure 10(b): Interaction of Mesembrine with SOD1

In this figure docking of Mesembrine with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Arg143, Gly61, Pro62, Thr58, Phe50, Gln153 and Val148.

Tartaric acid

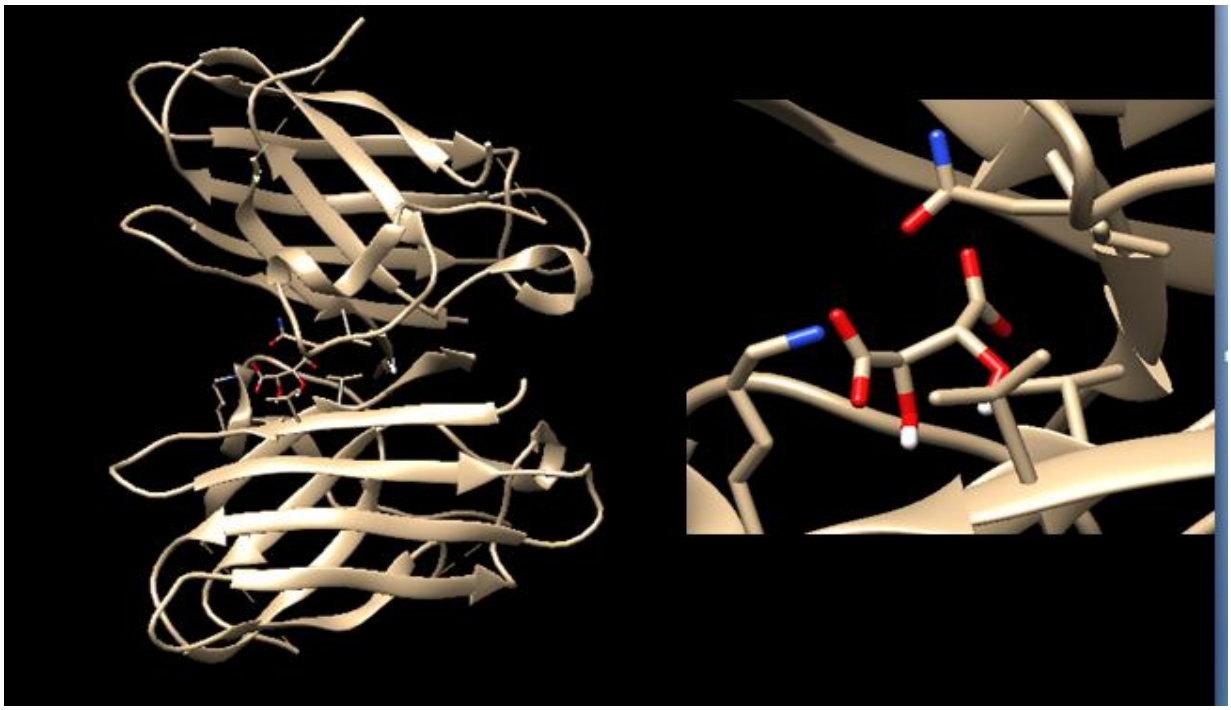

Figure 11(a): Docking of tartaric acid with SOD1

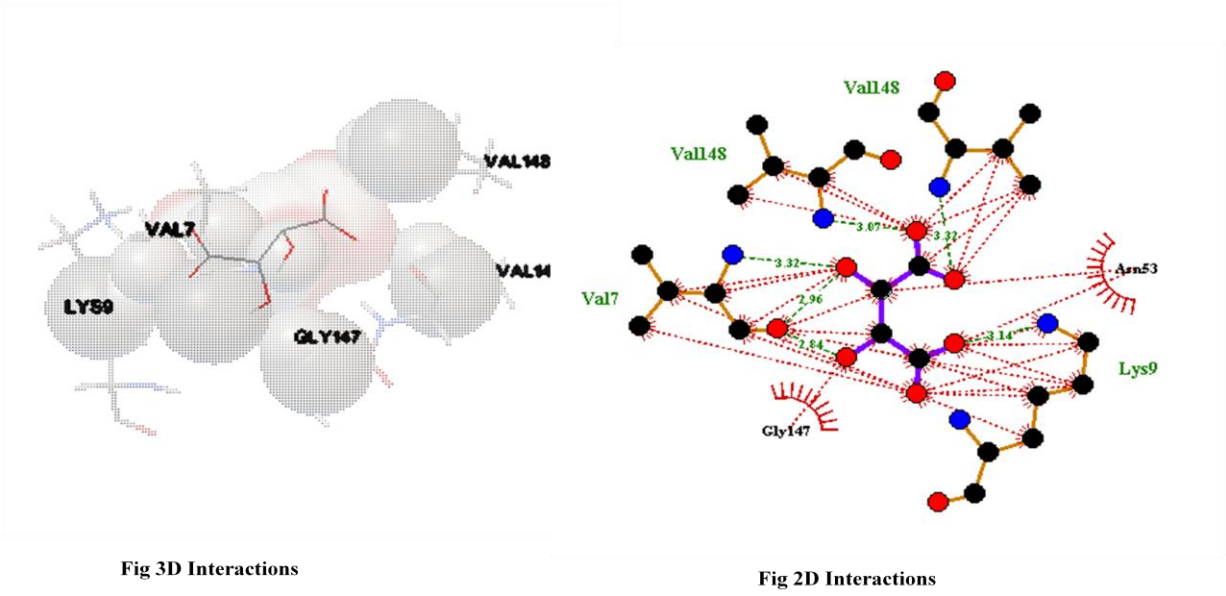

Figure 11(b): Interaction of tartaric acid with SOD1

In this figure docking of tartaric acid with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Gly 147, Val7, Val 14, Lys9 and Val148.

Genistein

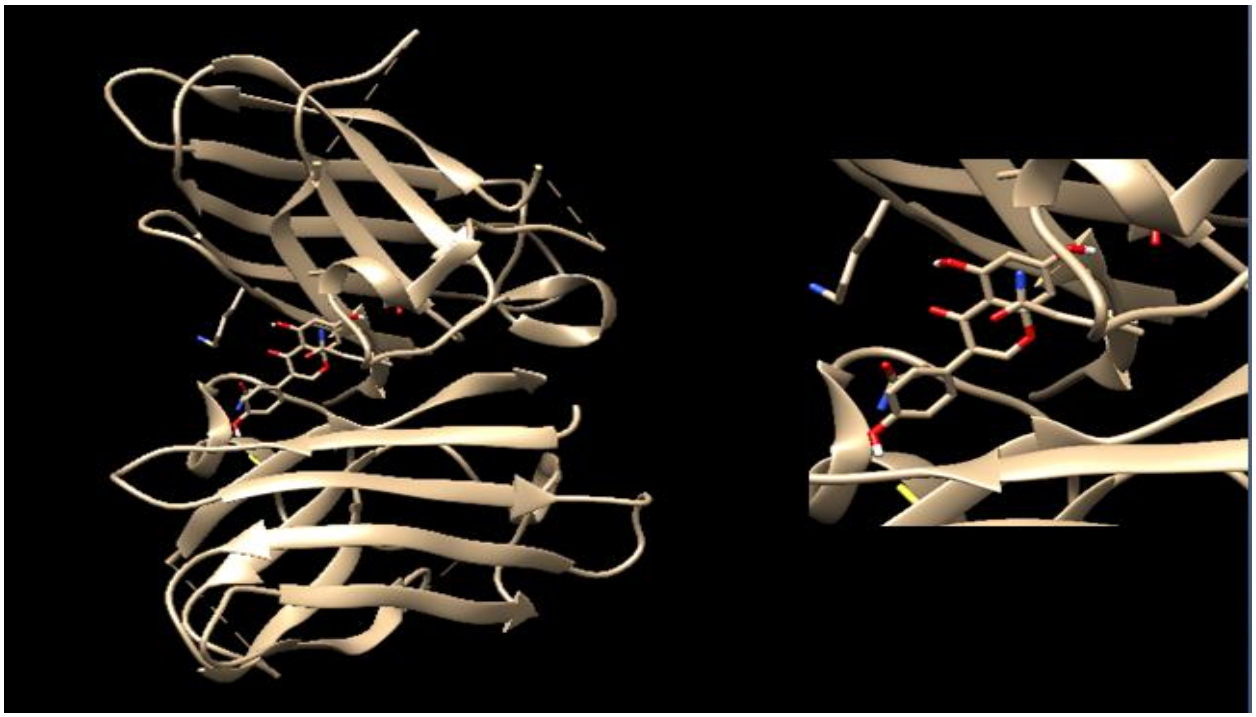

Figure 12(a): Docking of Genistein with SOD1

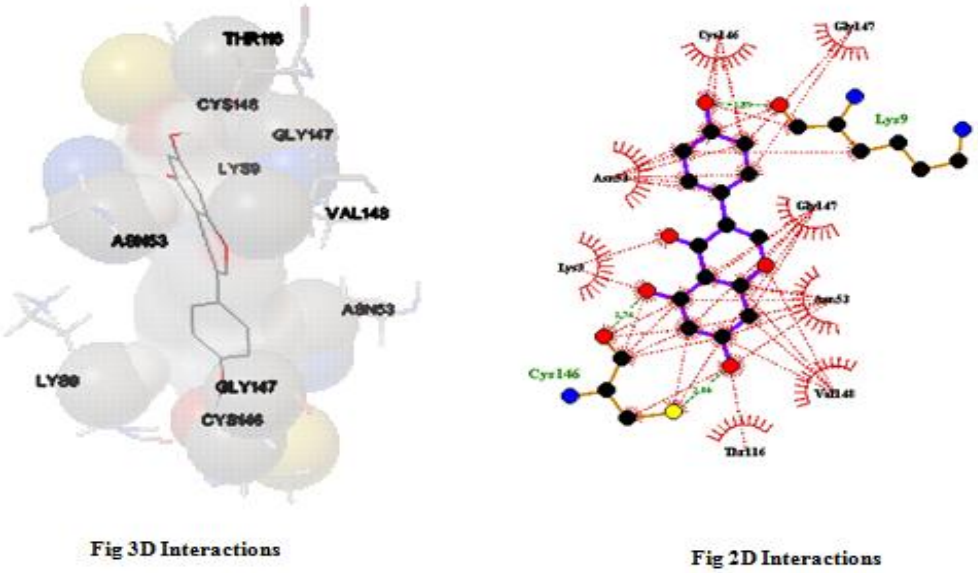

Figure 12(b): Interaction of Genistein with SOD1

In this figure docking of Genistein with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Gly147, Cys146, Lys9 and Val148.

Cembrene

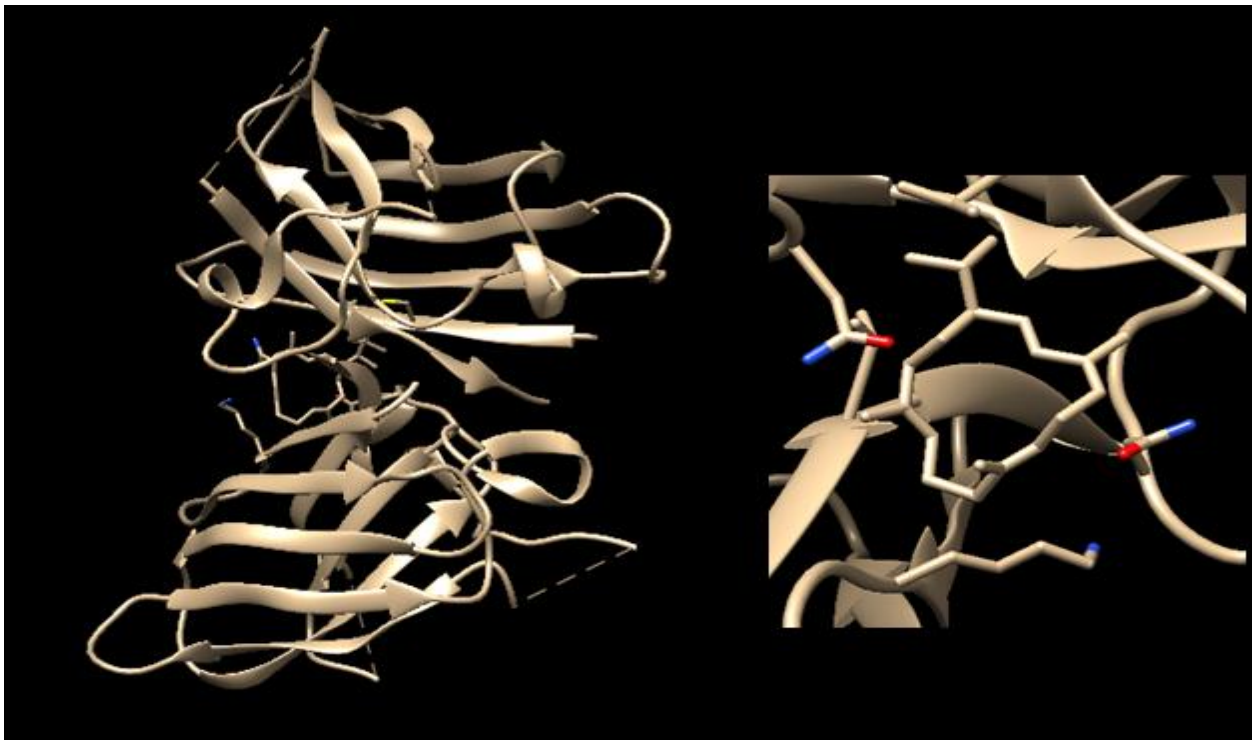

Figure 13(a): Docking of Cembrine with SOD1

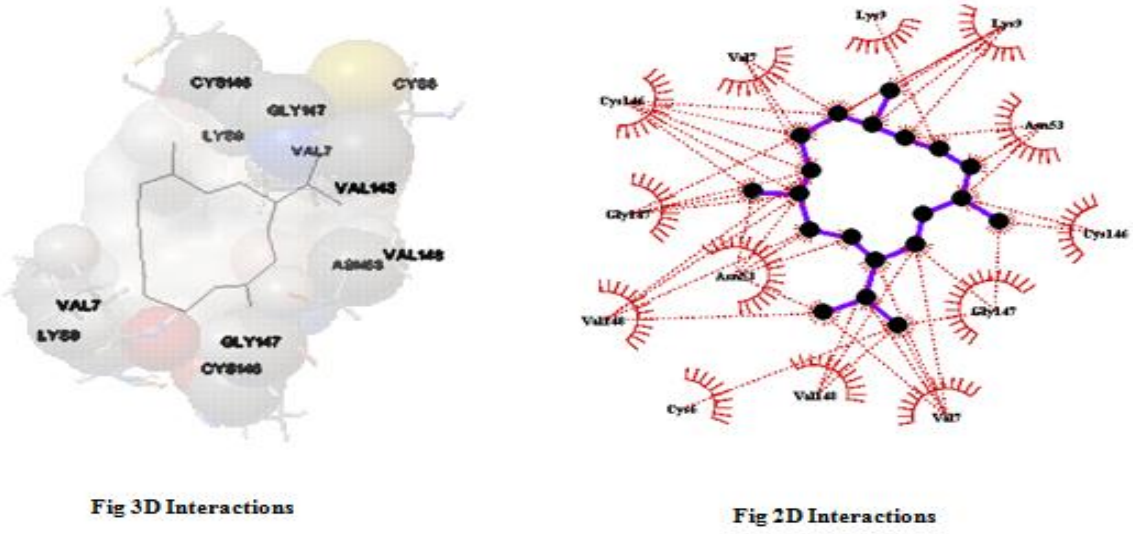

Fig 3D Interactions

Fig 2D Interactions

Figure 13(b): Interaction of Cembrine with SOD1

In this figure docking of Cembrine with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Cys146, Cys6, Lys9 and Val148.

Sclarene

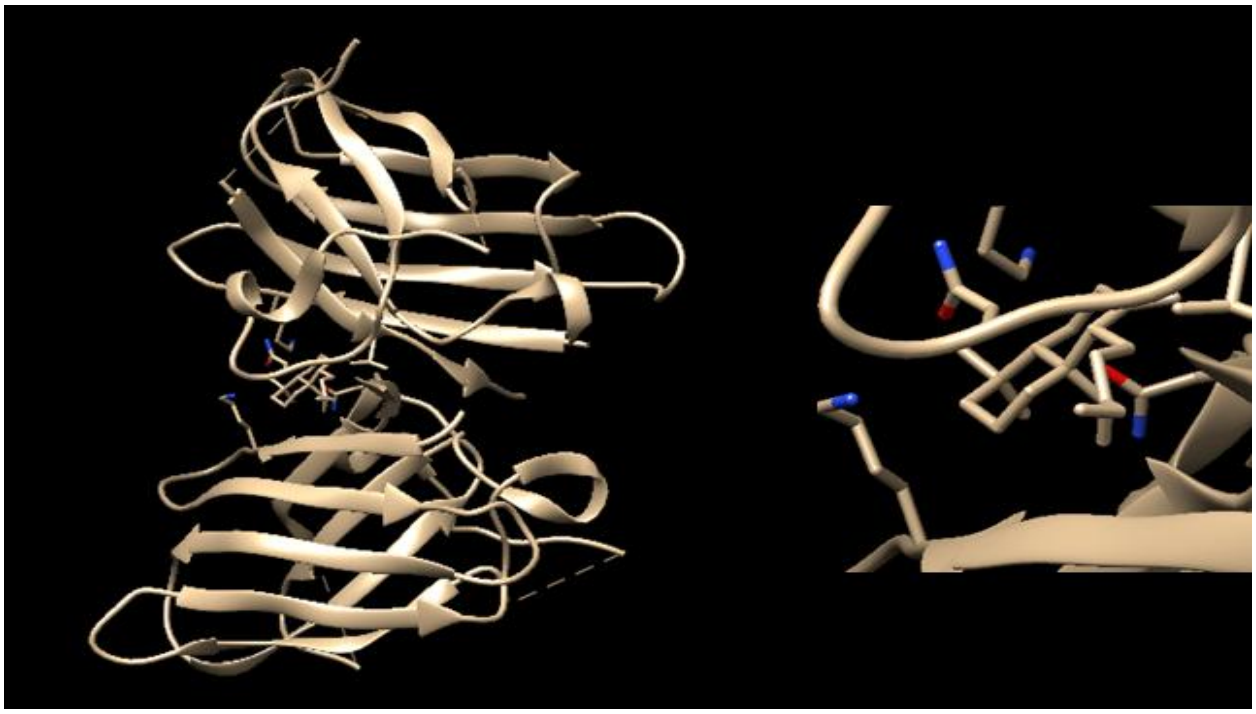

Figure 13(a): Docking of Sclarene with SOD1

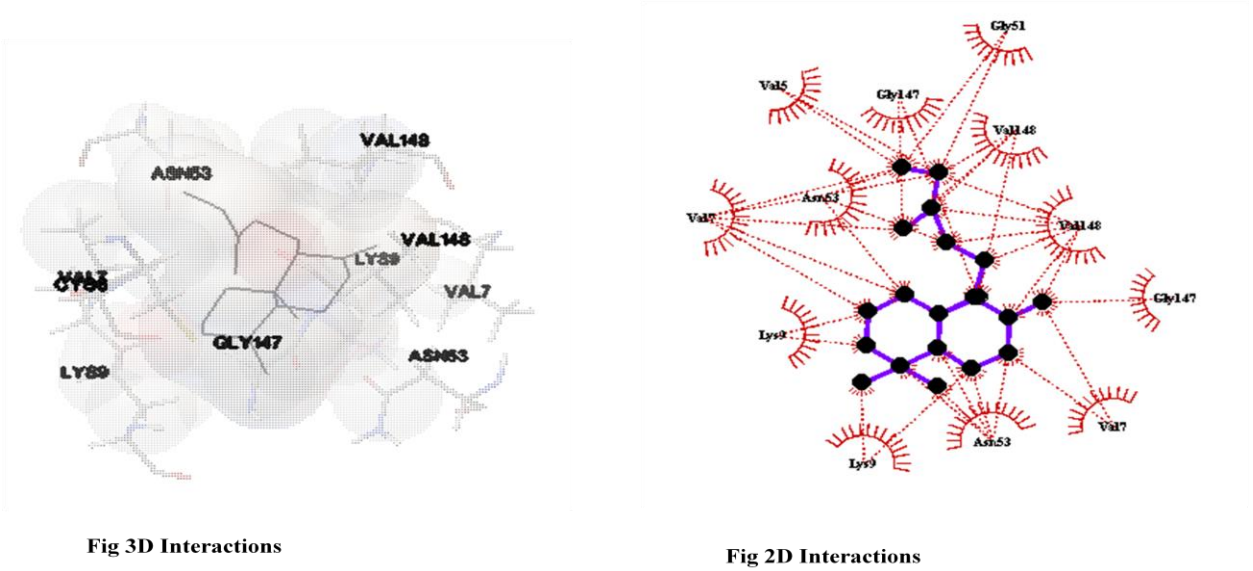

Fig 3D Interactions

Fig 2D Interactions

Figure 13(b): Docking of Sclarene with SOD1

In this figure docking of Sclarene with SOD1 protein is shown. The three dimensional and two dimensional interactions are also shown. The major amino acids which are interacting with drug molecule are Asn53, Val7, Cys6, Gly51, Lys9 and Val148.

6. Conclusion

Recent therapeutics advancement in neurodegenerative diseases reveals that the promising role of plant derivative drugs that has shown potential for the treatment of ALS. As currently there is only one drug riluzole which can just extend the life of patient for 2 months there is an ever increasing need for development of new and effective drugs. Here in *Insilico* investigation, we have successfully searched 10 docking hits of plant derivative drug with SOD1 protein it can be said that there are certain amino acids Val7, Asn53, Gly147 which are interacting with all the drug molecules so it can be said that interaction of these amino acids may play an important role in development of effective drugs .Based on binding energy efficiency sclarene is proving to be the most efficient drug but further study may help in finding better drugs with better binding energy score. Although we have checked the drugs with Lipinski's filter and the in-silico analysis shows hope for finding drugs actual experimental data through experiment would be helpful in validating results.

7. References

Adam P, Hecht S, Eisenreich W, Kaiser J, Grawert T, Arigoni D, Adelbert B, Rohdich F (2002). Biosynthesis of terpenes: Studies on 1-hydroxy-2methyl-2-(E)-butenyl 4-diphosphate reductase. *Proc. Nat. Acad. Sci*.99:12108-12113.

Abe, K.; Pan, L. H.; Watanabe, M.; Konno, H.; Kato, T.; Itoyama, Y (1997). Upregulation of protein-tyrosine nitration in the anterior horn cells of amyotrophic lateral sclerosis. *Neurol. Res.* 19:124–128.

Akhar MS, Iqbal Z, Khan MN, Lateef M (2000). Anthelmintic activity of medicinal plants with particular refence to their use in indo Pakistan subcontinent. *Small Rumin Res*; 38: 99-107.

Alam, J.; Wicks, C.; Stewart, D.; Gong, P.; Touchard, C.; Otterbein, S.; Choi, A.M.; Burow, M.E.; Tou, J (2000). Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. *J. Biol. Chem.*, *275*, 27694–27702.

Andersen P.M (2006). Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr Neurol Neurosci Rep*. 6:37-46

Austin F. L., Wolff I. A., (1968). Sinapine and related esters in seed meal of Crambe abyssinica. *J. agr. Food Chem.*, 16, 132-135.

Beckman, J. S.; Koppenol, W. H (2010). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am. J. Physiol. 271:C1424–1437.

Bedard, K.; Krause, K. H. (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev*. 87:245–313.

Ben Hamida M, Hentati F, Ben Hamida C (1990). Hereditary motor system diseases (chronic juvenile amyotrophic lateral sclerosis). Conditions combining a bilateral pyramidal syndrome with limb and bulbar amyotrophy. *Brain*. ; 13:173-187.

Brooks BR, Miller RG, Swash M, et al (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. P*hysiol, Re.*, 1*:293-9*

Bruijn LI, Miller TM, Cleveland DW (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci*. 27:723-49.

Camps P, Munoz-Torrero D (2002). Cholinergic drugs in pharmacotherapy of Alzheimer's disease. *Mini Rev Med Chem.* 2 11–25.

Cassidy, A., Bingham, S. and Setchell, K. D. (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am. J. Clin. Nutr*. 60, 333–340.

Chieia, Marco A.; Oliveira, Acary S.B.; Silva, Helga C.A.; Gabbai, Alberto Alain (2010). "Amyotrophic lateral sclerosis: considerations on diagnostic criteria". *Arquivos de Neuro-Psiquiatria.* 68, 837–42.

Cheng A, Lou Y, Mao Y, Lu S, Wang L, Chen X (2007). Plant terpenoids: Biosythesis and ecological functions. J. *Integrative Plant Biol*. 49:179-186.

Chen F, Dorothea T, D'Auria JC, Farooq A, Pichersky E, Gershenzon J.(2003). Biosynthesis and emission of terpenoid volatiles from Arabidopsis flowers. *The Plant Cell*. 15: 481-494.

Chin YW, Kinghorn AD, Patil PN (2007). Evaluation of the cholinergic and adrenergic effects of two tropane alkaloids from Erythroxylum pervillei. *Phytother Res*. 21, 1002–1005.

Christen P: Tropane alkaloids: old drugs used in modern medicine (2007). Studies in Natural Products Chemistry, Bioactive Natural Products, *Elsevier Science and Technology* . 22, 717–749.

Coyle, J. T.; Puttfarcken, P (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262:689–695.

Dauben W.G., W.E. Thiessen and P.R. Resnick (1965). Cembrene, a fourteen-membered ring diterpene hydrocarbon. *J. Org. Chem.* 30, 1693-1698

Deshpande, G.R.; Rao, B.M.; Someswararao, N (2009). Quantitative Determination of Tartaric Acid in Tolterodine Tartrate by Ion Chromatography Using Conductivity Detection. *Rasayan J. Chem.* 2, 101–107.

.

Fallat RJ, Norris FH, Holden D, Kandal K, Roggero PC (1987). "Respiratory monitoring and treatment: objective treatments using non- invasive measurements." *Adv Exp Med Biol.* 209:191-200.

Fletcher, D. A., McMeeking, R. F. & Parkin, D. (1996). Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. Agent and Actions. *J. Chem. Inf. Comput.Sci.* 36, 746-749.

Fodor G (1971). The tropane alkaloids. In: The Alkaloids; Chemistry and Physiology, vol. XIII. Ed. *Manske RHF, Academic Press, New York.* 12: 351–396.

Gericke NP, Van Wyk B-E (2001.). Pharmaceutical compositions containing mesembrine and related compounds. *US Patent* 288,104 B1.

Han D, Williams E, Cadenas E (Jan 2001) "Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space". *The Biochemical Journal 353: 411–6.*

Kabashi, E.; Valdmanis, P. N.; Dion, P.; Spiegelman, D.; McConkey, B. J.; Vande Velde, C.; Bouchard, J. P.; Lacomblez, L.; Pochigaeva, K.; Salachas, F.; Pradat, P. F.; Camu, W.; Meininger, V.; Dupre, N.; Rouleau, G. A(2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat. Genet*. 40:572–574.

Kalow W, Tang BK (1991). Use of caffeine metabolite ratios to explore CYP1A1 and xanthine oxidase activities. *Clin. Pharmacol. Ther*. 50, 508.

Kirkland, R.A.; Franklin, J.L (2001). Evidence for redox regulation of cytochrome C release during programmed neuronal death: Antioxidant effects of protein synthesis and caspase inhibition. *J. Neurosci.* 21, 1949–1963.

Larsen L. M., Olsen 0., Ploger A., Sorensen H., (1983). Phenolic choline esters in rapeseed : possible factors affecting nutritive value and quality of rapeseed meal. *Proc. 6th int. Rapeseed Conf*, 1577. Paris, 17-18-19.

Leigh P. N. Amyotrophic lateral sclerosis. In: Eisen, A.A., Shaw, P.J. (2007), Motor Neuron Disorders and Related Diseases. *Elsevier, Edinburgh,pp*. 123 249–278.

Lenaz, G (1998). Role of mitochondria in oxidative stress and ageing. *Biochem. Biophysics.Acta* 1366:53–67.

Logroscino G, Traynor BJ, Hardiman O, et al (2008). Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. *J Neurol Neurosurg Psychiatry*. 79:6-11.

Martindale JL, Holbrook NJ (2002). "Cellular response to oxidative stress: signaling for suicide and survival". *Journal of Cellular Physiology.* 192, 1–15

Moscovitch, M. (1982), 'A neuropsychological approach to perception and memory in normal and pathological aging', in F. I. M. Craik and S. Trehub (eds), Aging and Cognitive Processes, *New York: Plenum Press*.

Muller F (2000). "The nature and mechanism of superoxide production by the electron transport chain: Its relevance to aging". *Journal of the American Aging Association*. 23, 227–253.

Phukan J, Pender NP, Hardiman O (2007). Cognitive impairment in amyotrophic lateral sclerosis. *Lancet Neurol*. 6:994-1003.

Pryor, W. A.; Squadrito, G.L (1995). The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol*. 268: 699–722.

Rosen DR, Siddique T, Patterson D, et al (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature.* 362:59-62.

Rowland LP, Shneider NA (2001). Amyotrophic lateral sclerosis. *N Engl J Med.* 344: 1688-700.

Sturtz, L. A.; Diekert, K.; Jensen, L. T.; Lill, R.; Culotta, V. C. A. (2009). Cu, Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria: a physiological role for SOD1 in guarding against mitochondrial oxidative damage. *J. Biol. Chem.* 276:38084–38089.

Sutedja NA, Fischer K, Veldink JH, et al (2009). What we truly know about occupation as a risk factor for ALS: a critical and systematic review. *Amyotroph Lateral Scler.* 10:295-301

Vance, C.; Rogelj, B.; Hortobagyi, T.; De Vos, K. J.; Nishimura, A. L.; Sreedharan, J.; Hu, X.; Smith, B.; Ruddy, D.; Wright, P.; Ganesalingam, J.; Williams, K. L.; Tripathi, V.; Al-Saraj, S.; Al-Chalabi, A.; Leigh, P. N.; Blair, I. P.; Nicholson, G.; de Belleroche, J.; Gallo, J. M.; Miller, C. C.; Shaw, C. E (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323: 1208–1211.

Wijesekera LC, Leigh PN (2009). Amyotrophic lateral sclerosis. *Orphanet J Rare Dis* 10, 1750-1172.

Woo, J.M.; Shin, D.Y.; Lee, S.J.; Joe, Y.; Zheng, M.; Yim, J.H.; Callaway, Z.; Chung, H.T. (2012). Curcumin protects retinal pigment epithelial cells against oxidative stress via induction of hemeoxygenase-1 expression and reduction of reactive oxygen. *Mol. Vis. 18*, 901–908.

Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, Hung WY, Ouahchi K, Yan J, Azim AC, Cole N, Gascon G, Yagmour A, Ben-Hamida M, Pericak-Vance M, Hentati F, Siddique T (2001). The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet. 29:160-5.