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Molecular docking interaction between superoxide dismutase (receptor) and phytochemicals (ligand) from *Heliotropium indicum* Linn for detection of potential phytoconstituents: New drug design for releasing oxidative stress condition/inflammation of osteoarthritis patients

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Abstract
Antioxidant properties of medicinal plants play a very important role in different inflammatory, pain and oxidative stress related diseases. Osteoarthritis is most common musculoskeletal joint disease among middle aged and older people. The present study was attempted to detect potential Phyto-constituents in *Heliotropium indicum* against inflammation and pain. Superoxide dismutase (SOD) is an anti-oxidative enzyme and it was increased due to free radical generation or oxidative stress condition. This present study was focused at assessing the target level analysis of antioxidant activity of these (Lupeol and β amyrin) Phyto-constituents through molecular docking studies. The interaction between SOD and phytochemicals from *H. indicum* was carried out by using PyRx software to compare energy value and binding site of phytochemicals in reference to established synthetic non-steroidal anti-inflammatory drugs (NSAIDs). This result show that both phytoconstituents (lupeol and β amyrin) possessed potent capacity and is capable of activating SOD and showed better affinity towards SOD. Based on molecular docking we found few phytochemicals of *H. indicum* that can be used as lead compounds in future drug development as anti-inflammatory agent at low cost. It is also suggested to carry out functional assay of predicted compounds to validate suitability of this lead.

Keywords: Antioxidant, molecular docking, *H. indicum*, phytochemicals, SOD, Anti-inflammation

1. Introduction
Osteoarthritis is the most common form of musculoskeletal joint disease and is among the most frequent health problems for idle aged and older people. It is a degenerative joint disease and is characterized by articular cartilage destruction [1, 2]. Osteoarthritis processes not only affect the articular cartilage but involve the entire joint, including subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles. Ultimately the articular cartilage degenerates with fibrillation, ulceration and full thickness loss of the joint surface. Clinically it is characterized by pain, inflammation, stiffness, limitation of movement [3].
'Oxidative stress' is a physiological condition or to the situation of a serious imbalance between production of reactive oxygen species/reactive nitrogen species (ROS/RNS) and antioxidant defense. Free radicals are produced from normal cell metabolism in situ or from external sources. When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates an oxidative stress condition. Oxidative stress plays an important role in the development of chronic and degenerative illness or this oxidative damage is caused by oxygen free radicals, is a serious mechanism in the Pathogeny of many diseases. [4, 5, 6].

Antioxidants are a group of substance which present at low concentration in relation to oxidized substances, significantly inhibit or delay oxidation processes. ROS are produced continuously as by-products of various metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria, and peroxisomes [7, 8]. Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a vaiety of enzymatic scavengers such as superoxide dismutase (SOD), Glutathione reductase (GR), Glutathione peroxidise (GPx) and catalase [9]. Antioxidant compounds play an important role as a health protecting factor.

These antioxidants reduce the risk for chronic diseases. Most of the antioxidant compounds are derived from plant sources and its ability to trap free radicals. This plant derived antioxidants play important role in alleviating problems related to oxidative stress.

Superoxide dismutase (SOD) is an oxidative stress marker or antioxidant enzyme and it increases due to free radical generation to form reactive oxygen species during oxidative stress and cause osteoarthritis [10, 11, 12, 13]. SOD catalyzes the superoxide into molecular oxygen and hydrogen peroxide.

Inflammation is a complex host (systemic/local) response to a wide range of tissue injury and infection, generally marked by increased levels of cytokines, cytokine receptor, adhesion molecules, immune-regulatory factors and several other mediators [14, 15, 16, 17]. Inflammation is a basic way in which the body reacts to infection, irritation or other injury. It is mediated by molecules called prostaglandins. Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids. Both pro inflammatory cytokines and anti inflammatory activities have been documented [18, 19, 20, 21]. It is a key etiological factor for osteoarthritis. Thus, treatment of this inflammatory and oxidative stress related disorder still remains a growing health concern and has become a major challenge to the health professionals.

Medicinal plants always play an important role in the treatment of many diseases world wise. The traditional systems of medicine of all the countries have used plants and their products for the treatment of various ailments. Among several medicinal plants, *Heliotropium indicum*, commonly known as hatisur and an annual 'Indian heliotrope' belonging to Boraginaceae family. The whole is traditionally used as an herbal medicine for treating inflammatory diseases, pain etc. because it has antioxidant and anti-inflammatory properties. [22-25].

Molecular docking' is the process that involves placing a molecule/compound (ligand) in appropriate configuration to interact a protein (receptors). This interaction study describes proteins (receptors) are the main molecular targets to detect drug action easily. Several compounds (ligands) either synthetic drugs or phytochemicals, bind to the protein targets to show the allosteric or inhibitory effects, which help in new and efficient drug development as a lead molecule. The virtual screening reveals large libraries of drug like compounds, which are commercially available, computationally screened against targets of recognized structure and that are predicted to bind properly in an experimental assay [26, 27, 28].

The objective of the present study was to identify potential lead compounds by knowing binding affinity and energy value of SOD towards different phytochemicals present in *H. indicum* in reference to established common NSAIDs through molecular docking approaches. The analysis between these phytochemicals (ligands) with SOD (receptor) have been probed by using computional prediction for new drug design for oxidative stress and inflammatory related disorders.

2. Methodology

2.1 Selection of Receptor

The crystal structure of protein SOD was retrieved from the protein data bank in Euroe. (http://www.ebi.ac.uk/pdbe/). The 3-D ribbon structure was exhibited in Fig.1 after visualizing in AutoDock Tool developed by the Scripps Research Institute [29]. According to Perry *et al*. (2010), the active site found in each CuZnSOD subunit containing one copper ion joined by three histidine residues, which shows side chains for all reside outside of the β-barrel.

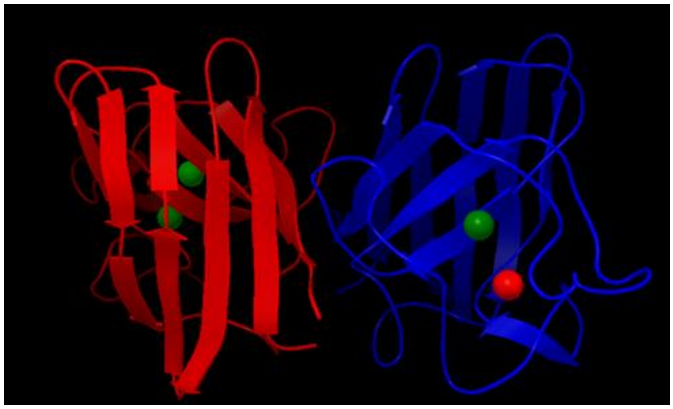
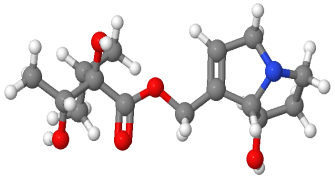
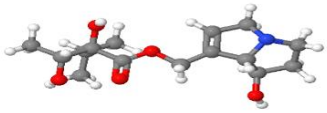
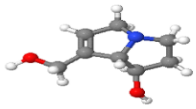
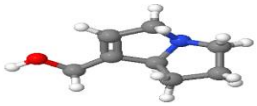
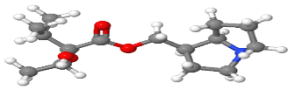
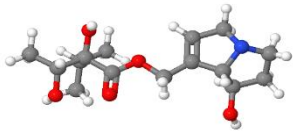
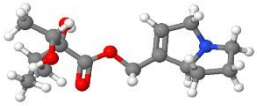
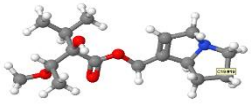
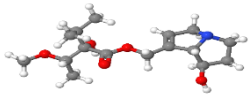
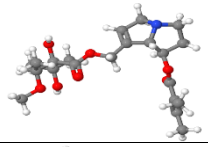
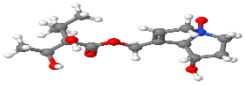
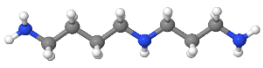
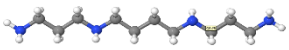
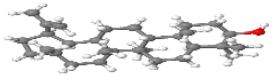


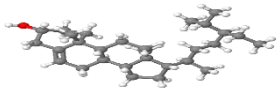
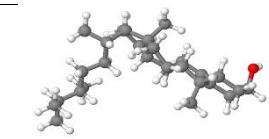
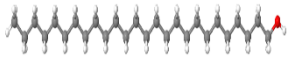
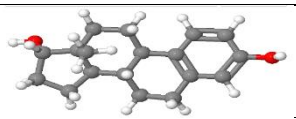
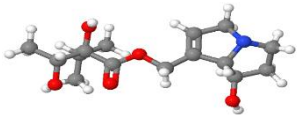
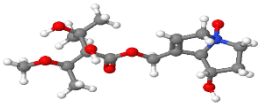
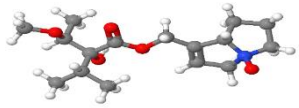
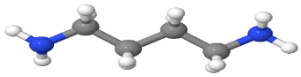
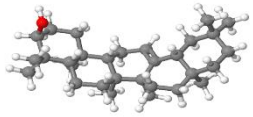
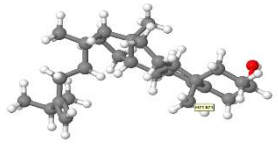
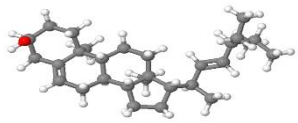
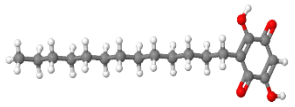
Fig 1: Three-dimensional (3D) ribbon structure of superoxide dismutase (PDB ID: 1CB4) [Chain A = red, attached two copper ions (green ball) at 152 and 153 position and Chain B = blue, attached with copper and zinc ion (green and red ball) 152 and 153 position]

2.2 Selection of ligands

The selection of ligands (phytochemicals) of *H. indicum* were done from the literatures investigated by researchers. (30, 31) In the present study, established 27 phytochemicals were taken such as Echinatine, Rinderine, Lindelofidine, Retronecine, Supinidine, Trachelanthamine, Indicine, Supinine, Heleurine, Heliotrine, Lasiocarpine, Indicine-n-oxide, Spermidine, Spermine, Lupeol, β-sitosterol, Campesterol, Hexacosan-1-ol, Estradiol, Lycopsamine, Europine-N-oxide, Heleurine-N-oxide, Putrescine, β-amyrin, Chalinasterol, Stigmasterol and Rapanone**.** The CAS no and Canonical SMILES of these compounds were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and tabulated in Table 1. The three-dimensional (3-D) structure and.pdb file of each phytochemical was obtained from CORINA online server after inserting SMILES string in appropriate place.

Table 1: Established phytochemicals of *Heliotropium indicum* L.

Sl. No.	Phytochemicals	CAS No.*	Canonical SMILES*	Structure in 3D
1.	Echinatine	480-83-1	CCCC(CCO)(C(=O)OCC1=CCN2C1C(CC2)O)O	
2	Rinderine	6029-84-1	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1C(CC2)O)O	
3.	Lindelofidine	18929-90-3	C1CC2C(CCN2C1)CO	
4.	Retronecine	480-85-3	C1CN2CC=C(C2C1O)CO	
5.	Supinidine	551-59-7	C1CC2C(=CCN2C1)CO	
6.	Trachelanthamine	14140-18-2	CC(C)C(C(C)O)(C(=O)OCC1CCN2C1CCC2)O	
7.	Indicine	480-82-0	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1C(CC2)O)O	
8.	Supinine	551-58-6	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1CCC2)O	
9.	Heleurine	488-00-6	CC(C)C(C(C)OC)(C(=O)OCC1=CCN2C1CCC2)O	
10.	Heliotrine	303-33-3	CC(C)C(C(C)OC)(C(=O)OCC1=CCN2C1C(CC2)O)O	
11.	Lasiocarpine	303-34-4	CC=C(C)C(=O)OC1CCN2C1C(=CC2)COC(=O)C(C(C)OC)(C(C)(C)O)O	
12.	Indicine-n-oxide	41708-76-3	CC(C)C(C(C)O)(C(=O)OCC1=CC[N+]2(C1C(CC2)O)[O-])O	
13.	Spermidine	124-20-9	C(CCNCCCN)CN	
14.	Spermine	71-44-3	C(CCNCCCN)CNCCCN	
15.	Lupeol	545-47-1	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)C	

16.	β-sitosterol	68555-08-8	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
17.	Campesterol	474-62-4	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C	
18.	Hexacosan-1-ol	506-52-5	CCCCCCCCCCCCCCCCCCCCCCCCCCO	
19.	Estradiol	50-28-2	CC12CCC3C(C1CCC2O)CCC4=C3C=CC(=C4)O	
20.	Lycopsamine	10285-07-1	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1C(CC2)O)O	
21.	Europine-N-oxide	65582-53-8	CC(C(C(=O)OCC1=CC[N+]2(C1C(CC2)O)[O-])(C(C)(C)O)O)OC	
22.	Heleurine-N-oxide	.....	CC(C)C(C(C)OC)(C(=O)OCC1=CC[N+]2(C1CCC2)[O-])O	
23.	Putrescine	110-60-1	C(CCN)CN	
24.	β-amyrin	559-70-6	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C)C	
25.	Chalinasterol	474-63-5	CC(C)C(=C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C	
26.	Stigmasterol	83-48-7	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
27	Rapanone	573-40-0	CCCCCCCCCCCCCC1=C(C(=O)C=C(C1=O)O)O	

*Data retrieved from Pub Chem Database, CAS No.= Chemical Abstract Service Registry Number; SMILES = Simplified molecular-input line-entry system

2.3 Virtual screening/molecular docking and interaction

The molecular docking was carried out by a virtual screening method through PyRx software (virtual screening Tool, Version 0.8) developed by Trott and Olson. (32) This molecular docking was visualized the output. Pdbqt file by using AutoDock Vina Software, developed by Morris *et al.* (33) and the results of three dimentional structure were rendered by using MGL Tools. The PyRx software is an easy virtual screening with minimum steps and time to obtain docking output file.

This software is combination of AutoDock vina, AutoDock 4.2, Mayavi, Open Babel and Python tools. It is also non-commercial, less time consuming docking program that basically predict receptor ligand interactions along with providing energy value for each test compound. Docking of 27 phytochemicals with SOD (PDB ID:1CB4) was analysed for the docking of phytoconstituents (ligands) and the SOD (receptor) to identify the residues involved in the study of receptor-ligand interactions. All the ligands and receptor file were as.pdb file and each file taken prior converted to. pdbqt file format by made macromolecule and ligand in PyRx tool. The docking site on this target protein was expressed by forming a grid box with the dimensions of X: 40.91, Y:61.77, Z:42.02 Å, with a grid spacing of 0.375 Å, centered on X:16.17, Y:69.88, Z:15.33 Å. The present tool predicts docking result by obtaining energy value for each ligand. Finally, all the 27 ligands were analysed to detect binding position and energy value. The resultant structural complexes of the individual ligand/receptor binding were finally observed in AutoDock Vina software to determine some specific contacts between the atoms of the test compounds and amino acids of the SOD.

3. Results

The present results clearly revealed or indicate that the interaction of the established phytochemicals (ligands) present in the different parts of *Heliotropium indicum* with the target protein superoxide dismutase (receptor), data were energetically favourable. In table 2, the binding energy values of each test compounds were obtained. It was observed that among those 27 phytochemicals, lupeol (-9.2 kcal/mol) and β amyrin (-9.0 kcal/mol) have lowest energy values where putrescine (-3.4 kcal/mol) has highest energy values respectively. In case of lowest energy value means highest binding affinity. Among these two phytochemicals (lupeol and β amyrin), β amyrin is more stronger than lupeol because it has one no. hydrogen contact in the docking interaction that is VAL 7.

The hydrophobic contacts residues were found GLY54, LYS9, VAL146, ASN51, VAL146, LYS9, VAL7 for Lupeol while LYS9, ASP11, GLY54, ASN51, GLY145, LYS9, CYS144 for β-amyrin. No hydrogen bond contact in Lupeol but one hydrogen bond contact with VAL7 residue for β-amyrin was observed (Table 2). 3-D binding pose of Lupeol and β-amyrin and their interaction are exhibited in Fig 2 and 3. Other 26 phytochemicals were also studied to check binding energy values, hydrogen bond contacts and hydrophobic contacts residues (Table 2) but these two phytocompounds (ligands) were observed suitable binding energies against the SOD receptor. The binding position of these two ligands were found opposite side of the active site of SOD, which may act as an allosteric activator (ligand binding occurs other site than active site of receptor or protein).

Table 2: Receptor-ligand binding energy value and interaction

Sl. No.	Phytochemicals	Binding energy (Kcal/mol)	Hydrogen no. and contact	Hydrophobic contact
1.	Lupeol	-9.2	-----	GLY54, LYS9, VAL146, ASN51, VAL146, LYS9, VAL7
2.	β-amyrin	-9.0	1 and VAL7	LYS9, ASP11, GLY54, ASN51, GLY145, LYS9, CYS144
3.	Stigmasterol	-8.2	-----	GLY106, ALA1, ILE140, ARG113, SER109, ILE111, ILE111, ILE149, SER109, ARG113
4.	Campesterol	-8.0	-----	GLY145, VAL146, VAL146, VAL7, GLY145, VAL7, LYS89, ASN61, LYS9
5.	Estradiol	-8.0	1 and VAL146	LYS9, VAL7, VAL146, ASN51, LYS9
6.	Chalinasterol	-7.9	-----	VAL7, LYS9, GLY145, VAL146, ASN51, VAL146, GLY145, VAL7, LYS9, ASN51
7.	β-sitosterol	-7.6	-----	GLY145, VAL146, ASN51, LYS9,
8.	Indicine-n-oxide	-6.9	1 and VAL146	VAL17, ASN51, VAL146, LYS9, ASN51, VAL146, AP11, GLY10, LYS9, VAL7
9.	Echinatine	-6.7	1 and VAL146	Val146, ASN51, GLY145, Cys144
10.	Rinderine	-6.7	1 and VAL146	VAL 146, ASN 51, GLY54, CYS144, GLY145
11.	Trachelanthamine	-6.7	3 and VAL7, VAL146, AL146	ASN51, GLY145
12.	Indicine	-6.7	2 and VAL146, ASN 51	GLY54, ASN51, VAL146
13.	Lycopsamine	-6.7	2 and VAL146, CYS144	GLY54, ASN51, GLY145, VAL146
14.	Europine-n-oxide	-6.7	1 and VAL146	LYS9, VL17, GLY145, VAL146, LYS9, GLY 145
15.	Heliotrine	-6.5	2 and VAL146, VAL146	VAL7, GLY54, ASN51, ASN51
16.	Heleurine-n-oxide	-6.3	1 and VAL146	LYS9, GLY145, VAL7, VAL146, VAL146, GLY145, LYS9
17.	Supinine	-6.2	1 and VAL7	CYS6, GLY145, VAL146, VAL146
18.	Heleurine	-6.1	1 and SER109	GLY106, SER109, ARG113, GLY106, ILE111, ILE111, ILE149, ALA1
19.	Lasiocarpine	-6.1	2 and SER109, SER 109	GLY106, SER109, ARG113, ILE111, ILE 111, GLY106, SER106
20.	Rapanone	-5.7	----------	SER105, ALA1, GLY106, SER109, LEU104, ILE111, ALA1, ILE149, ILE111, ARG113, SER109
21.	Retronecine	-5.4	3 and VAL7, VAL7, VAL146	GLY145
22.	Lindelofidine	-5.2	1 and VAL7	GLY145, VAL146, VAL146, GLY145, ASN51
23.	Supinidine	-5.2	1 and VAL7	VAL146, VAL146, GLY145, CYS 6
24.	Hexacosan-1-ol	-4.7	1 and ASN51	LYS9, ASN51, VLA146, VAL146, LYS9
25.	Spermine	-4.3	1 and ASN51	VAL7, VAL146, VAL146, GLY145, VAL7, ASN51, GLY49
26.	Spermidine	-4.2	1 and GLY49	VAL7, CYS6, VAL146, ASN51, VAL146, GLY146, VAL7
27.	Putrescine	-3.4	1 and VAL7	VAL 146, ASN 51, GLY 49

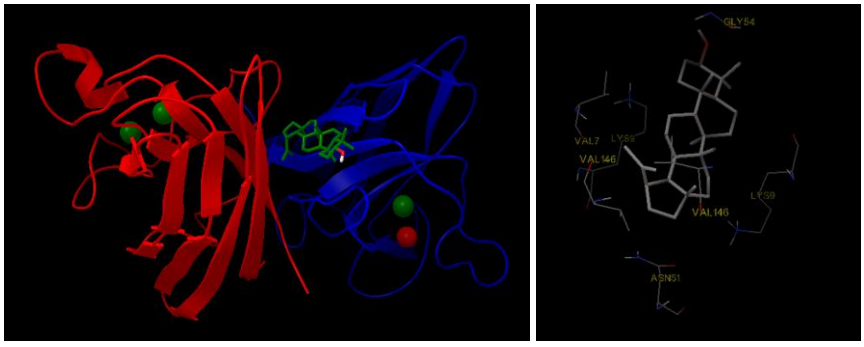


Fig 2: Three-dimensional (3-D) docking pose and molecular interactions of Lupeol docking interaction.

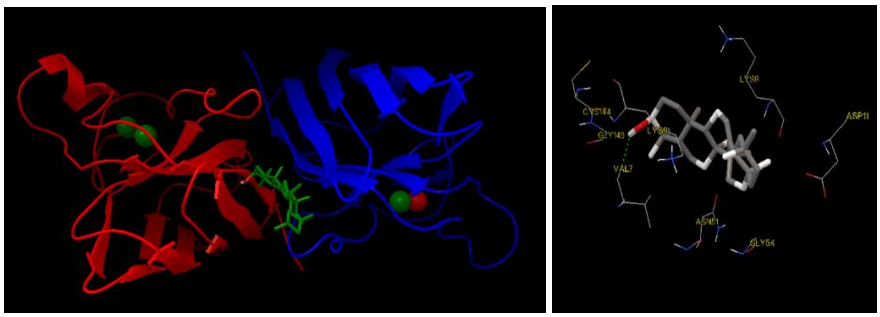


Fig 3: Three-dimensional (3-D) docking pose and molecular interaction s of β amyrin docking interaction.

4. Discussion

Discovery of active compounds from natural products have gained enormous importance in the field of drug discovery. Drug discovery from plants involves a multidisciplinary approach combining botanical ethnobotanical, phytochemical and biological techniques. Drug discovery typically starts with an analysis of binding sites in target proteins or an identification of structural motifs common to active compounds. According to Brooijmans *et al*. [34] in silico molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure based drug design. Molecular docking continues to hold great promise in the field of computer based drug designing which screens small molecules by orienting and scoring them in the binding site of a protein. This docking process involves the prediction of ligand confirmation and orientation (posing) within targeted binding site.

Superoxide dismutase (SOD) is known as anti-oxidant enzyme. The main function of SOD is to decompose superoxide radicals into molecular oxygen and hydrogen peroxide inside the cells, and prevent superoxide toxicity. The SOD activity was increased due to oxidative stress condition and acts as the causative factor for many diseases [35, 36]. It was observed that crude extract of *Heliotropium indicum* and other medicinal plants induced SOD activity after topical application in the OA patients. From decade, *Heliotropium indicum* plant used as an herbal medicine for treating inflammatory diseases, pain, and having antioxidant properties [37, 38, 39].

The earlier study made to detect the exact compounds among several established phytochemicals having potent capacity to increase the SOD activity through molecular docking interaction study. But in the present result, two phytochemicals such as lupeol and β amyrin showed lowest energy values and highest binding affinity. In this interaction study, these two compounds (lupeol and β amyrin) were ligated at opposite side of the active site of the receptor and act as allosteric activator. This allosteric activator helps the SODenzyme activity and prevent disease through antioxidant enzymatic system [40, 41, 42, 43]. This drug designing emphasizes an analysis of binding sites in a target protein [44] and this silico molecular docking help to identify novel ligand for receptor in which structure based drug designing can easily be achieved [45]. This computer based drug designing molecular docking interaction determine how closely the lowest energy (highest binding affinity) poses predicted by the docking score. We analyzed the hydrogen bond interaction of the receptor with these two phytoconstituents. A close view of the binding interaction of the receptor with β amyrin was shown fig 3. As shown in fig 3 there are one hydrogen bond (green dotted line) fromed between the receptor and β amyrin. The residue involved in forming hydrogen bond was VAL7. Lupeol did not form any hydrogen bond interaction with the receptor. This hydrogen bond interaction makes important contributions to the interaction between ligand and the receptor.

5. Conclusion

An approach to virtual screening under computational biology along with receptor ligand binding affinity can be an easy screening method prior to identify the efficacy of exact lead compounds that have potent therapeutic efficacies without any side effects. From this docking studies we concluded that binding of these two phytochemicals (lupeol and β amyrin) to the domain of the SOD receptor may lead to increase its activity and reduce oxidative stress. These phytochemicals from *H. indicum* can be used in further drug designing and development as anti-inflammatory and pain relieving phytomedicine at low cost.

6. References

1. Namazi, H, Majd, Z. Botulinum toxin as a novel addition to anti-arthritis armamentarium. Am. J Immunol. 2005; 1:94-95. DOI:10:3844/ajisp.2005.94.95.
2. Mazzetti I, Grigolo B, Pulsatelli L, Dolzani p, Silvestri T, Roseti L *et al*. Differential roles of nitric oxide and oxygen radicals in chondrocytes affected by osteoarthritis and rheumatoid arthritis, Clin Sci. 2001; 101:593-9
3. Conaghan, Phillip. Osteoarthritis- National clinical guideline for care and management in adults, 2008.
4. Lien Ai Pham – Huy, Hua Hc., Chong Phy-Huy. Free Radicals, Antioxidants in Disease and Health, Review Article, Int. J Biomed Sci. 2008; 4(2):89-96.
5. Humad S, Zarling EJ, Skosey J. Lipid peroxidation in rheumatoid Artritis: measurement of pentane in breath samples by gas chromatography [Abstract]. Clin Res., 1985; 33:919A.
6. Plachta H, Bartnikowska E, Obara A. Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries. Clin Chim Acta., 1992; 211:101-2.
7. Del Rio A, Sandalio LM, Corpas FJ, Palma JM, Barroso JB. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling, Plant Physiol. 2006; 141:330e335.
8. Navrot N, Rouhier N, Gelhaye E, Jaquot JP. Reactive oxygen species generation and antioxidant systems in plant mitochondria, Physiol. Plant. 2007; 129:185e195.
9. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants, Trends Plant Sci. 2004; 9:490e498.
10. Maneesh M, Jayalekshmi H, Suma T, Chatterjee S, Chakrabarti A, Singh TA, Evidence for oxidative stress in osteoarthritis, Indian J Clin. Bio Chem., 2005; 20(1):129-130.

11. Vijayakumar D, Suresh K, Manoharan S. Lipid peroxidation and antioxidant status in blood of rheumatoid arthritis patients. Indian Journal of Clinical Biochemistry. 2006; 21(1):104-108.
12. Daisy P, Suveena. S, Latha RCR. Target level analysis of antioxidant activity of costunolide and eremanthin isolated from *Costus speciosus*. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4):32-35.
13. Amzoiu DC, Pisoschi C, Stoian AM, Rau G. Blood glutathione peroxidase activity in patients with osteoarthritis treated with oxicams. Current Health Sciences Journal. 2016; 42 (1):29-34.
14. Venkata M, Sripathy R, Anjana D, Omashekara NS, Krishnaraju A, SKrishanu *et al*. American Journal of Infectious Diseases. 2012, 8(1):26-33.
15. Tomlinson A *et al*. Cyclo-oxygenase and nitric oxide synthase isoforms in rat anti-inflaminduced pleurisy, Br. J Pharmacol. 1994; 113:693-698.
16. Parihar G *et al*. Anti-inflammatory effect of *Calotropis procera* root bark extract, Asian Journal of Pharmacy and Life Science. 2011; 1(1):29-44.
17. Edwards SH. Chemicals mediators of inflammation, in: Anti-inflammatory agents. The Merck Veterinary Manual, 2014. Available: http://www.merckvetmanual.com/mvm/pharmacology/anti-inflammatory_agents/chemical_mediators_of_inflammation.html#v3337363.
18. Black S, Kushner I, Samols D. C-reactive protein, J Biol. Chem. 2044; 279(47):48487-48490.
19. Denarello CA. Proinflammatory cytokines, Chest. 2000; 118(2):503-508.
20. Zhang JM, An J. Cytokines, inflammation and pain, Int. Anesthesiol. Clin. 2007 45(2):27-37.
21. Bretscher P *et al*. Phospholipid oxidation generates potent anti-inflammatory lipid mediators that mimic structurally related pro-resolving eicosanoids by activating Nrf2, EMBO Mol. Med. 2015; 7:593-607.
22. Srinivas K, Rao ME, Rao SS. Anti-inflammatory activity of *Heliotropium indicum* Linn. And *Leucas aspera* Spreng. In albino rats. Indian Journal of Pharmacology. 2000; 32:37-38.
23. Boye A, Koffuor GA, Ameyaw EO, Abaitey AK. Analgesic activity and safety assessment of *Heliotropium indicum* Linn. (Boraginaceae) in rodents, International Journal of Pharmacology. 2012; 8(2):91-100.
24. Dash GK, Abdullah MS. A review of *Heliotropium indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research. 2013; 4(4):1253-1258.
25. Roy A. Pharmacological activities of Indian heliotrope (*Heliotropium indicum* L.): A review. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):101-104.
26. Reddy AS *et al*. Virtual screening in drug discovery - A computational perspective, Curr. Pro. Pept. Sci. 2007; 8(4):329-351.
27. Lavecchia A, Di Giovanni C. Virtual screening strategies in drug discovery: A critical review, Curr. Med. Chem. 2013; 20(23):2839-2860.
28. Lionta E *et al*., Structure-based virtual screening for drug discovery: Principles, applications and recent advances, Curr. Top. Med. Chem. 2014; 14:1923-1938.
29. Morris GM *et al*. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998; 19:1639-1662.
30. Dash GK, Abdullah MS. A review of *Heliotropium indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research. 2013; 4(4):1253-1258.
31. Roy A. Pharmacological activities of Indian heliotrope (*Heliotropium indicum* L.): A review. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):101-104.
32. Trott O, Olson AJ, Auto Dock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J Comput. Chem. 2010; 31:455-461.
33. Morris GM *et al*., Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J Comput. Chem. 1998; 19:1639-1662.
34. Brooijmans N, Kuntz ID. Molecular recognition and docking algorithms. Annu Rev. Biophys Biomol Struct. 2003; 32:335-373.
35. Amzoiu DC, Pisoschi C, Stanciulescu C, Benza M, Popescu F, Rau G. Blood superoxide dismutase activity in patients with knee osteoarthritis treated with oxicam. Farmacia. 2014; 62(3):460-466.
36. Amzoiu DC, Pisoschi C, Stoian AM, Rau G. Blood glutathione peroxidase activity in patients with osteoarthritis treated with oxicams. Current Health Sciences Journal. 2016; 42 (1):29-34.
37. Boye A, Koffuor GA, Ameyaw EO, Abaitey AK. Analgesic activity and safety assessment of *Heliotropium indicum* Linn. (Boraginaceae) in rodents, International Journal of Pharmacology. 2012; 8(2):91-100.
38. Dash GK, Abdullah MS. A review of *Heliotropium indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research. 2013; 4(4):1253-1258.
39. Roy A. Pharmacological activities of Indian heliotrope (*Heliotropium indicum* L.): A review. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):101-104.
40. 40. Puglisi A, Tabbi` G, Vecchio G. Bio conjugates of Cyclodextrins of manganese salen-type ligand with superoxide dismutase activity. Journal of Inorganic Biochemistry. 2004; 98:969-976.
41. Valdivia A, Pérez Y, Ramírez H, Cao R, Villalonga R. Improved pharmacological properties for superoxide dismutase modified with β-cyclodextrin–carboxymethylcellulose polymer. Biotechnology Letters. 2006; 28:1465-1470.
42. Xueshan Y, Xiaopu Y, Wenli F. Effect of carbohydrates on activities of superoxide dismutase extracted from sheep blood. Food Science. 2011; 32:100–103.
43. Ma X, Deng D, Chen W. Enzyme Inhibitors and Activators: Inhibitors and Activators of SOD, GSH-Px, and CAT. Intech Chapter. 2017; 9:207-224 (doi.org/10.5772/65936).
44. Daisy P, Suveena S, Latha RCR. Target level analysis of antioxidant activity of costunolide and eremanthin isolated from *Costus speciosus*. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4):32-35.
45. Brooijmans N, Kuntz ID. Molecular recognition and docking algorithms. Annu Rev. Biophys Biomol Struct. 2003; 32:335-373.