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Interaction of Oxidative Stress Responsive Genes in Cancer, Diabetes, Heart Stroke and Brain Stroke: A Study to Find Therapeutic Drug Target

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Abstract: *Extensive biomedical research have shown that imbalanced metal metabolism plays undesired role in catalyzing invivo chemical reactions those lead to oxidative stress and cell apoptosis as final cause. Lethal mutations in mitochondrial or endoplasmic reticulum DNA in cells leads to oxidative stress and free radical generation in normal cells. In this present work, gene expression of stress responsive genes in cancer, diabetes, heart stroke and brain stroke were studied. Differentially expressed genes were analyzed in each of the four diseases under study. Based on p-value and LogFC value, the common significant genes were further annotated in terms of their functions and pathways. On many-fold over expression, some drug targets have been proposed. In next phase of this work, Using molecular docking studies, some herbal compounds have been proposed to act as inhibitors to these drug targets that are following ADME and lipiniski rule of five.*

Keywords: *Docking, Inhibitors, Expression, Lipiniski rule, ADMET Properties.*

1. INTRODUCTION

Oxidants are byproducts of key aerobic cellular processes of respiration, metabolism and the mitochondrial electron transport chain and are removed continuously by an array of antioxidant mechanisms. [10] An imbalance between the production of reactive oxygen species (ROS) and the detoxification of their reactive intermediates causes oxidative stress. Cells must respond to this imbalance before the highly reactive molecules damage cellular structures, particularly DNA. Severe and prolonged oxidative stress can trigger apoptosis and necrosis. Numerous pathological conditions have an oxidative stress component, including cardiovascular diseases, neurodegenerative diseases. [18][7]In order to compensate for increased oxidative stress, cancer cells have been identified to garner redox adaptive mechanism that enhances their ability to detoxify ROS; exposure to constant oxidative through a number of mechanisms. [9] The role of Oxidative stress in the Etiology of cancer is supported by multiple lines evidence. although ROS are generally thought of as damaging to cell due to their ability to induce oxidative stress at high concentrations low levels of ROS are actually essential to normal cell function. This is in part due to the fact that ROS can act as second messenger in signaling cascades that are vital for cellular responses to external stimuliation.[8][9][10]

The Human Oxidative Stress and Antioxidant Defense RT² Profiler™ PCR Array profiles the expression of 84 genes related to oxidative stress. Peroxidases are represented on this array including glutathione peroxidases (GPx) and peroxiredoxins (TPx). Also included are the genes involved in reactive oxygen species (ROS) metabolism, such as oxidative stress responsive genes and genes involved in superoxide metabolism such as superoxide dismutases (SOD). Using real-time PCR, you can easily and reliably analyze expression of a focused panel of genes related to oxidative stress with this array. [19][20]

In this present work, Oxidative drug targets have been proposed based on its essentiality to cancer, diabetes, heart stroke and brain stroke were studied. Further, docking and ADMET analysis have been done to report promising lead molecules for these 5 drug targets.

2. MATERIAL AND METHODOLOGY

2.1. Structure Prediction and its Validation

Target proteins G6PD (Glucose-6-phosphate 1-dehydrogenase) with PDBID: 1QKI, GSS (Glutathione synthetase) with PDBID: 2HGS and SRXN1 (Sulfiredoxin-1) with PDBID: 1XW3 was downloaded from PDB database [1]. As the GCLC (Glutamate-cysteine ligase catalytic subunit) and HOMX1 (HMOX1 protein) targets don't have structure till now. We predicted its structure based on homology modeling from Phyre2 [14], CPH [16] and Swiss model [6]. The two structures were optimized from Modloop (Loop refinement) [17] and Spdbv (Energy minimization) [6] and validated in Rampage [13] and Erratplot [15], the former check the stereo chemical properties of modeled structures and latter one analyze the non bonded interactions.

2.2. Ligand Selection

Referring the various literatures and ZINC database [11] we found that probably herbal inhibition of target protein. The ligand library was obtained from the ZINC database [11] were checked which ligand's follows Lipinski's rule [3]. Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules.

In property section, we selected Lipinski's parameters as follows:

MW ------- 0 to 500

Xlogp----- 0 to 5

HBD------0 to 5

HBA-------0 to 10

2.3. High Throughput Docking Studies

Virtual screenings of all Harbal ligands were carried out with oxidative protein in "Molegro Virtual Docker (MVD 4.2.0)."[5] MVD includes three search algorithms for molecular docking namely MolDock Optimizer, MolDock Simplex Evolution (SE), and Iterated Simplex (IS). In this software, MolDock Optimizer algorithm and simulated annealing approach to explore wide range of ligand conformational flexibility and rotational flexibility of selected receptor hydrogens was used for high throughput screening. We find active sites from MVD and cross checked with pocket-finder [4]. It predicts Catalyst generated ligand conformations in the protein Ligand Interaction.

2.4. Molecular Descriptors

The molecular descriptors of screened herbal components were predicted by loading them into an online server, **Pkcsm Pharmacokinetic server.** [2] Molecular descriptors like Water solubility, Skin Permeability, CNS permeability, AMES toxicity and side effects such as mutagenicity, carcinogenicity and teratogenicity were determined. To calculate the overall drug score, **Pkcsm** combined logP, logS, molar mass, drug-likeness and toxicity risks into a single number to predict the molecule's over all drug potential. On the basis of non-bonded and bonded interactions, molecular descriptors properties and scoring functions, best herbal ligands as potent inhibitors of Oxidative proteins were proposed.

3. RESULT AND DISCUSSION

Target proteins G6PD (PDBID: 1QKI), GSS (PDBID: 2HGS) and SRXN1 (PDBID: 1XW3) was downloaded from PDB database. We got partial modeling of GCLC protein and HOMX1 protein structure from Swiss model server. The validation results were in good agreement with an ideal protein structure.70 herbal ligands were collected form ZINC database. On the basis of Lipinski rules 5 the proposed best 10 ligands to inhibit oxidative proteins .Table 1

S.No	Compound ID	Compound Name	Molecular Weight	Log P	H-Bond Acceptors	H-Bond Donors
1	487423	4-Hydroxybenzyl-2,4-dihydroxyphenyl ketone	244.24	2.22	4	3
2	4097179	Cupreine	311.405	2.52	4	3
3	4098984	Retinoyl b-glucuronide	475.558	3.97	8	3
4	13544730	Daidzein-4'-O-sulfate Disodium Salt	333.297	0.15	7	1
5	13909135	7,8-dihydroxy-schizandrin	448.512	3.07	8	2
6	14762534	Xanthohumol D	370.401	3.67	6	4

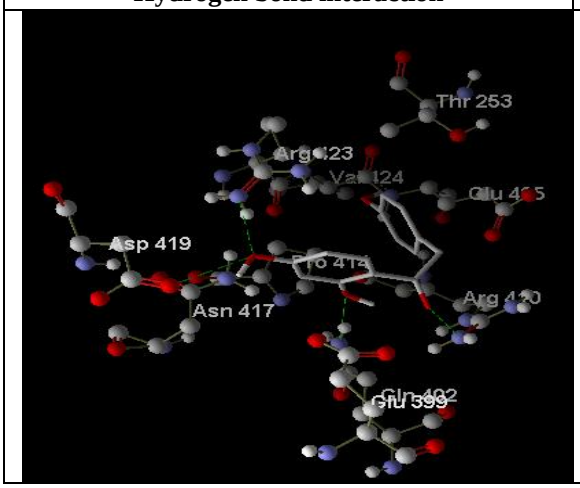
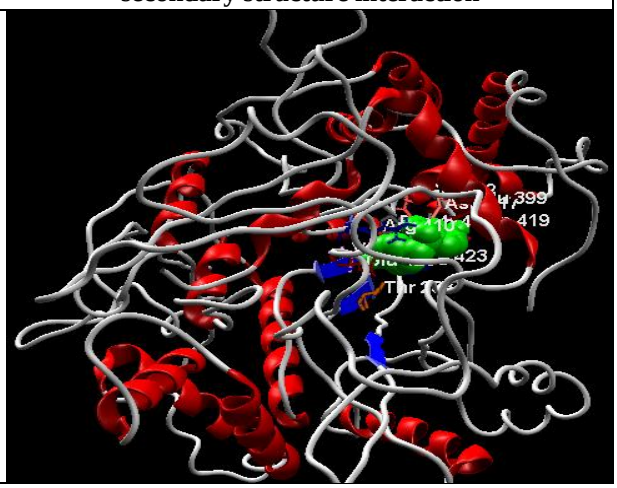
7	14762797	Icaritin	368.385	4.96	6	3
8	35859104	3-O-methy rosmarinic acid	373.337	1.93	8	3
9	71404433	(-) -7 (S) -hydroxymatairesinol	374.389	0.92	7	3
10	13109239	15-Hydroxydehydroabietic acid	315.433	4.31	3	1

10 ligands were proposed, which follow the lipinski's rule of five. After ligands validation we performed Molecular docking studies of oxidative proteins. On the basis of scoring function and hydrogen bond interactions of ligand-protein we selected 4 ligands compound which interact with target proteins. Molecular Docking Score are given in Table 2.

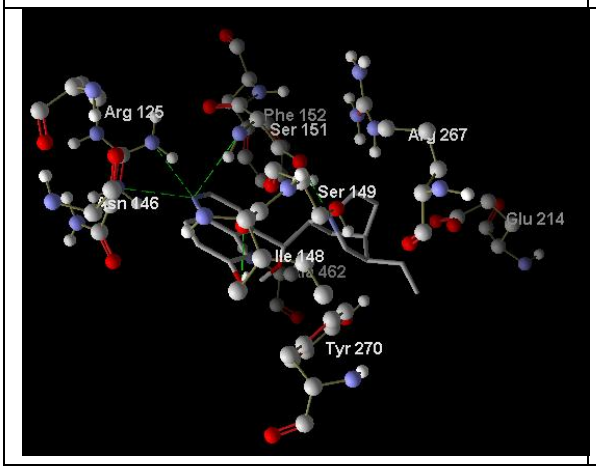
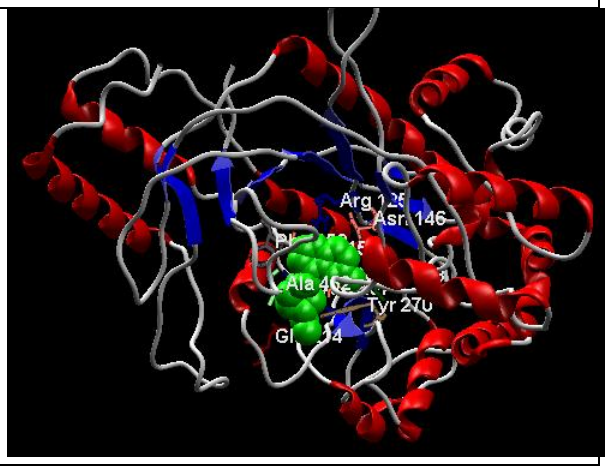
S.No	Protein(Gene ID)	Compound ID	RMSD	MOLDOCKSCORE	Interaction
1	GCLC	487423	0.15	-88.15	4
2	GSS	4097179	1.7	-98.50	6
3	HOMX1	4098984	2.6	-120.10	5
4	SRXN1	13544730	0.11	-66.5	8
5	G6PD	4098984	2.0	-149.5	5

It is shows the favorable interactions between ligand-protein. The following diagram labels are used in Molegro Virtual Docker (MVD 4.0.2).

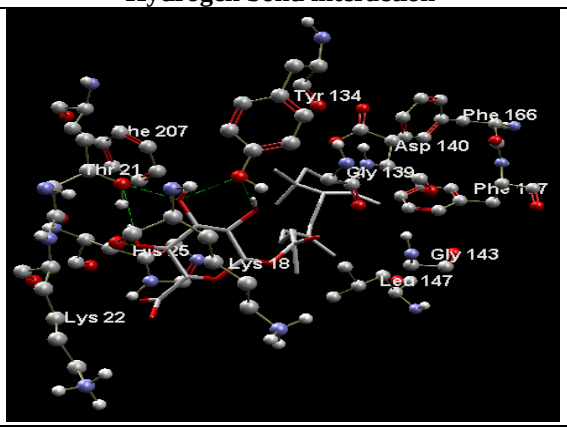
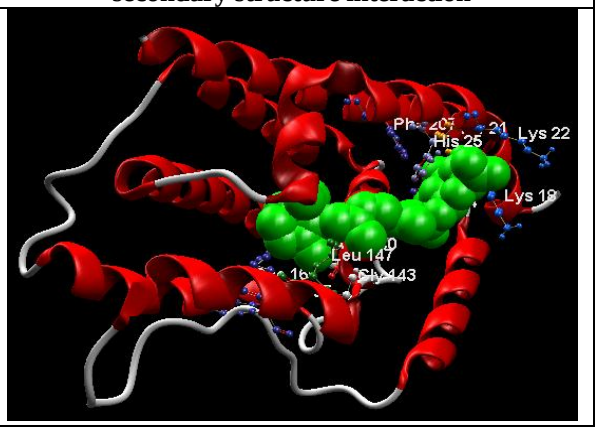
These Diagrams show Hydrogen bond interaction, secondary structure interaction of 487423 (4-Hydroxybenzyl-2, 4-dihydroxyphenyl ketone) ligand with GCLC protein.

Hydrogen bond interaction	secondary structure interaction
	

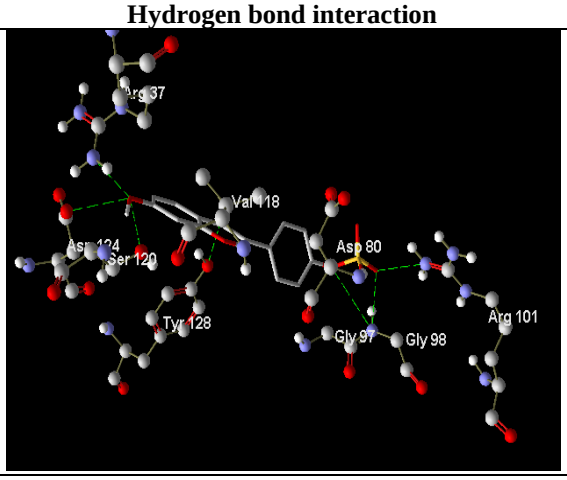
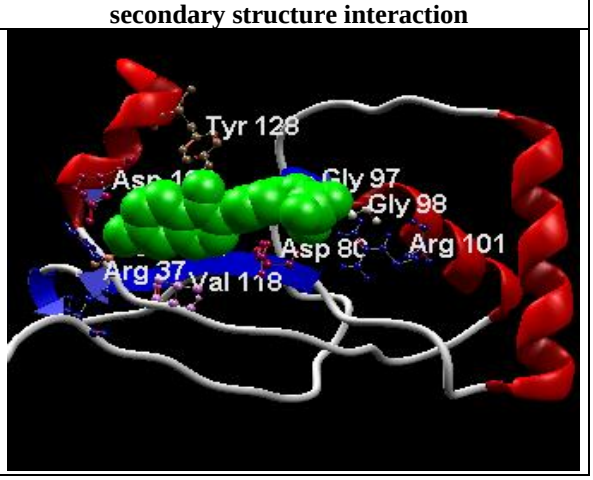
These Diagrams show Hydrogen bond interaction, secondary structure interaction of 4097179 (Cupreine) ligand with GSS protein.

Hydrogen bond interaction	secondary structure interaction
	

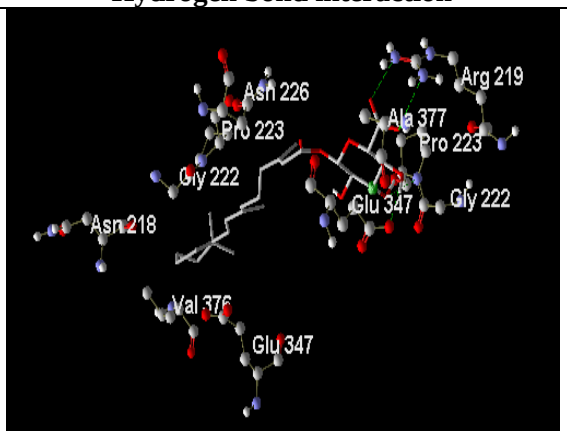
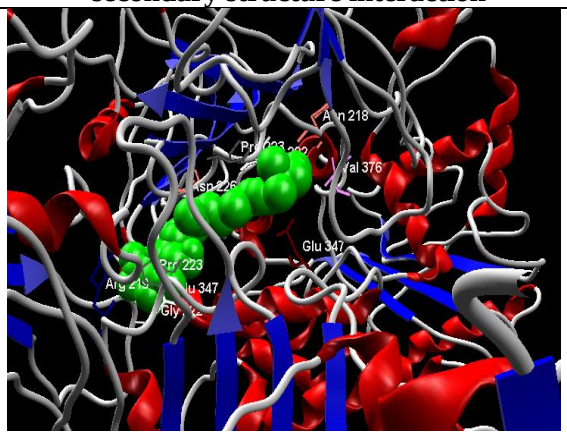
These Diagrams show Hydrogen bond interaction, secondary structure interaction of 4098984 (Retinoyl b-glucuronide) ligand with HOMX protein.

Hydrogen bond interaction	secondary structure interaction
	

These Diagrams show Hydrogen bond interaction, secondary structure interaction of 13544730 (Daidzein-4'-O-sulfate Disodium Salt)ligand with SRXN1 protein.

Hydrogen bond interaction	secondary structure interaction
	

These Diagrams show Hydrogen bond interaction, secondary structure interaction of 4098984 (Retinoyl b-glucuronide) ligand with G6PD protein.

Hydrogen bond interaction	secondary structure interaction
	

These four ligands were checked in ADMET study and found that they follow Molecular Descriptors properties very well. Table 3

Property	Molecular Descriptors	487423	4097179	4098984	13544730
Absorption	Water solubility (log mol/L)	-2.672	-2.226	-3.798	-3.925
	Caco2 permeability (log Papp in 10-6cm/s)	1.029	1.184	0.119	0.162
	Intestinal absorption (human) (% Absorbed)	91.034	95.465	45.179	64.909
	Skin Permeability (log Kp)	-3.357	-3.772	-2.913	-2.791
	P-glycoprotein substrate (Yes/No)	Yes	Yes	Yes	Yes
	P-glycoprotein I inhibitor (Yes/No)	No	No	Yes	No

	P-glycoprotein II inhibitor (Yes/No)	No	No	No	No
Distribution	VDss (human) (log L/kg)	-0.578	0.792	-0.664	-1.685
	Fraction unbound(human) (Fu)	0.252	0.401	0.336	0.178
	CNS permeability (log PS)	-2.319	-2.541	-3.567	-2.417
Metabolism	CYP2D6 substrate (Yes/No)	No	No	No	No
	CYP3A4 substrate (Yes/No)	N0	Yes	Yes	Yes
	CYP1A2 inhibitior (Yes/No)	Yes	Yes	No	Yes
	CYP2C19 inhibitior (Yes/No)	No	No	No	No
	CYP2C9 inhibitior (Yes/No)	No	No	No	No
	CYP2D6 inhibitior (Yes/No)	No	No	No	No
	CYP3A4 inhibitior (Yes/No)	No	No	No	No
Excretion	Total Clearance (log ml/min/kg)	0.184	1.261	1.16	0.528
	Renal OCT2 substrate (Yes/No)	No	No	No	No
Toxicity	AMES toxicity (Yes/No)	Yes	Yes	No	Yes
	Max. tolerated dose (human) (log mg/kg/day)	1.518	0.286	-0.147	0.902
	Oral Rat Acute Toxicity (LD50) (mol/kg)	1.958	2.447	1.73	2.263
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	2.406	2.494	1.99	1.979
	Hepatotoxicity(Yes/No)	No	No	Yes	No
	Skin Sensitisation(Yes/No)	No	No	No	No
	Minnow toxicity(log mM)	1.294	1.764	0.694	1.206
	T.Pyriformis toxicity(log ug/L)	1.019	0.708	0.362	0.607

As these four proposed ligands have no Lipinski failures, they are bioactive compounds and following ADMET properties .They are showing good interaction with of **oxidative proteins**, so they can be used as a potent and active inhibitors to these proteins.

4. CONCLUSION

As per interaction studies of these 10 herbal compounds with oxidative proteins, only four ligands were found to be most energetically stable on the basis of moldock score and also found promising in protein-ligand interactions. Out of these four screened ligands, each is quite promising at all ADMET properties. So we may conclude that 4 ligand can work as oxidative inhibitor and thus could be useful for controlling the diseases.

ACKNOWLEDGMENT

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