

MOLECULAR UNDERSTANDING AND *INSILICO* VALIDATION OF TRADITIONAL MEDICINES FOR PARKINSON'S DISEASE

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ABSTRACT

Neurodegenerative defects are due to oxidative stress and excitotoxicity leading to depletion of neurotransmitters like dopamine and serotonin, abnormal ubiquitination and mitochondrial dysfunction. Parkinson's disease, a neurodegenerative disorder is characterized by pronounced loss of dopamine producing neurons in the substantia nigra compacta. Levodopa (L-Dopa) supplementation is the most effective treatment of Parkinson's disease. The enzyme Catecholamine O- Methyl Transferase (COMT) metabolizes the L- Dopa to 3-O-Methyl Dopa. Inhibition of COMT may prolong the effect of L-Dopa, thereby L-Dopa enters the brain and converted to dopamine by Aromatic l-amino acid decarboxylase and the dosage of L-Dopa can be reduced. The current study was focused to screen the neuroprotective phytochemicals from plants to inhibit COMT using *in silico* studies. The results suggest that the medicinal plant supplements will reduce the severity of Parkinson's disease.

Keywords: Parkinson's disease, phytochemicals, AutoDock, Ginsenosides RB I, Amentoflavone, COM

INTRODUCTION

Neurodegenerative diseases of the human brain encompass a diversity of disorders. The age dependent neurodegenerative diseases include Parkinson's disease and Alzheimer's disease¹, which are caused by genetic and environmental influences² and lead to the accumulation of protein aggregation thereby causing oxidative stress and inflammation³. Parkinson's disease is characterized by progressive loss of dopamine producing neurons in the substantia nigra pars compacta, and results in a drastic depletion of dopamine in the striatum, to which these neurons project⁴.

Uncontrollable tremor, rigidity, akinesia, bradykinesia and postural instability are the primary symptoms⁵ whereas the classical Parkinson's comprises the α -synuclein aggregation and fibrillation, known as Lewy bodies in substantia nigra, which are associated with the nigrostriatal degeneration⁶. The genes responsible for the cause of disease includes α -synuclein (SNCA), Parkin (PARK 2), Leucine-Rich Repeat Kinase 2 (LRRK2), PTEN-Induced Putative Kinase1 (PINK 1), Ubiquitin carboxyl-terminal esterase L1 (UCH-L1) and DJ-1 (PARK7) (7,8).

The dopamine precursor Levodopa (L-Dopa) is proved to be a powerful drug for Parkinson's disease. To increase the action of L-Dopa and to control the catabolism of L-Dopa, the adjuvant like inhibitors of peripheral L-Amino Acid Decarboxylase (AADC), Catechol O-Methyl Transferase (COMT) or Mono Amine Oxidase B (MAO B) can be supplemented⁹.

The characteristic perturbations in the metabolome could be used as the marker for disease diagnosis (10). In order to overcome the side effects of synthetic drugs, phytochemicals can be used as substituents. Arouma *et al.*¹ reported that dietary herbal extracts can significantly contribute to the modulation of the complex mechanisms of neurodegenerative diseases. Therefore, the present study focused to screen phytochemicals for the inhibition of Catecholamine-O-Methyl Transferase (COMT) using molecular docking studies.

MATERIALS AND METHODS

Preparation of ligand structures

The phytochemicals used for docking were selected based on the literature survey from the plants reported to possess Neuroprotection. The neuroprotective compounds are Ginsenosides RB I and Ginsenosides RG I (*Panax ginseng*), Amentoflavone (*Ginkgo biloba*), Emodin (*Rheum emodi*), Cyanidin (*Cayratia carnososa*), Baicalin and Baicalein (*Scutellaria baicalensis*), Stigmasterol (*Centella asiatica*), Wogonin (*Scutellaria baicalensis*), Curcumin (*Curcuma longa*), Eriodictyol (*Citrus aurantifolia*), Daucosterol (*Dioscorea opposita*), alpha Tocotrienol (*Elaeis guineensis*), Orientin

(*Ocimum sanctum*), Apigenin (*Turnera aphrodisiaca*), Resveratrol (*Citrus paradisi*), Tanshinones II A (*Salvia miltiorrhiza*), Naringenin (*Citrus paradisi*), Gingerol (*Zingier officinale*), Kaempferol (*Passiflora incarnata*), Salvianic acid A (*Salvia miltiorrhiza*), Ascorbic acid (*Phyllanthus emblica*), Bacopaside II (*Bacopa monneiri*), Carthamin (*Carthamus tinctorius*), Asiaticoside (*Centella asiatica*) and Saponarin, Saponaretin, Isoorientin, Gynocardin, Scopoletin (*Passiflora incarnata*). The available structures were retrieved from the PubChem structure database and the compounds for which the structure was not available were drawn using ACD/ChemSketch.

Preparation of protein structure

The crystal structure of the protein Catecholamine O-Methyl Transferase complexed with the S-Adenosyl Methionine and Dinitro Catechol (PDB ID: 3BWM) has been retrieved from the Protein Data Bank.

Energy Minimization using Swiss PDB view

The heteroatoms present in the crystal structure were removed from the complex and the total energy was minimized using CHARMM force field.

Molecular docking using AutoDock

Computer simulated automated docking studies were performed using AutoDock versions 4.0 that include Lamarckian Genetic Algorithm search engine and an empirical free energy function for estimation. The protein COMT was prepared for molecular docking by adding all hydrogen atoms using standard procedures. The water molecules and other heteroatoms were deleted except magnesium ion. The binding energy and inhibitory constants were observed for each ligand protein complex.

RESULTS

The selected phytochemicals and the synthetic drug, tolcapone were docked in the active site of optimized and energy minimized COMT and the results were analyzed to identify natural compounds with good inhibitory activity considering the interactions, binding energy and inhibitory constant. Out of the 31 compounds tested in this study, 20 compounds had the binding energy less than -6.0 Kcal/mol and the docked results of first 10 compounds with the binding energy less than -7.0 were tabulated (Table.1). These compounds had very good interaction with active site residues and also low inhibitory constant. The interactions of these natural compounds and synthetic drug with COMT were shown in Fig.2.

Dinitrocatecholamine : COMT complex (PDB ID: 3BWM) exhibited interactions with LEU199, ASP141, ASN170, LYS144 and Mg 300 (Fig.1). Whereas the synthetic drug tolcapone had interaction with

one residue LYS 144 and had very low binding energy (-7.9 Kcal/mol) and inhibitory constant (1.6nM). When docked with COMT, Ginsenoside RB1 had very low binding energy, 4 interactions with 3 important residues and also the inhibitory constant was in millimolar which was still lower than the synthetic drug tolcapone.

had three hydrogen bonds and Stigmasterol and Wagonin had two hydrogen bonds where the binding energy ranged between -7.85 to -7.06 and the inhibitory constant between 1.75 to 6.71mM. All the above mentioned phytochemicals had interactions with important residues (Table 1, Fig.2).

Amentoflavone, Emodin, Cyanidin, Baicalin and Eriodictyol also had 4 hydrogen bonds with the receptor whereas Curcumin and Baicalin

Table.1 Docked Results with binding energy, Number of hydrogen bonds, inhibitory constants and interacting residues

Name of the ligand	Binding energy (kcal/mol)	No. of H- bonds	Inhibitory constant (µm)	Interacting residues
Synthetic drug				
Tolcapone	-7.9	1	1.61	LYS144
Natural compounds				
Ginsenosides RB I	-12.14	4	1.26(nM)	ASP141(2), GLU199, MET40
Amentoflavone	-7.85	4	1.75	LYS144, ASN170, PRO174, GLU199, MG
Emodin	-7.76	4	2.05	ASP141, ASN170, GLU199
Cyanidin	-7.68	4	2.33	GLU90, ASP141, LYS144, ASN170
Baicalin	-7.62	3	2.61	LYS144(2), GLU199,
Stigmasterol	-7.47	2	2.74	LYS144, ASN170
Wogonin	-7.25	2	4.85	ASP141, GLU199
Curcumin	-7.15	3	5.73	LYS144, ASN170
Baicalein	-7.07	4	6.58	ASP141,LYS144, ASN170, GLU199
Eriodictyol	-7.06	4	6.71	LYS144, ASN170, GLU199

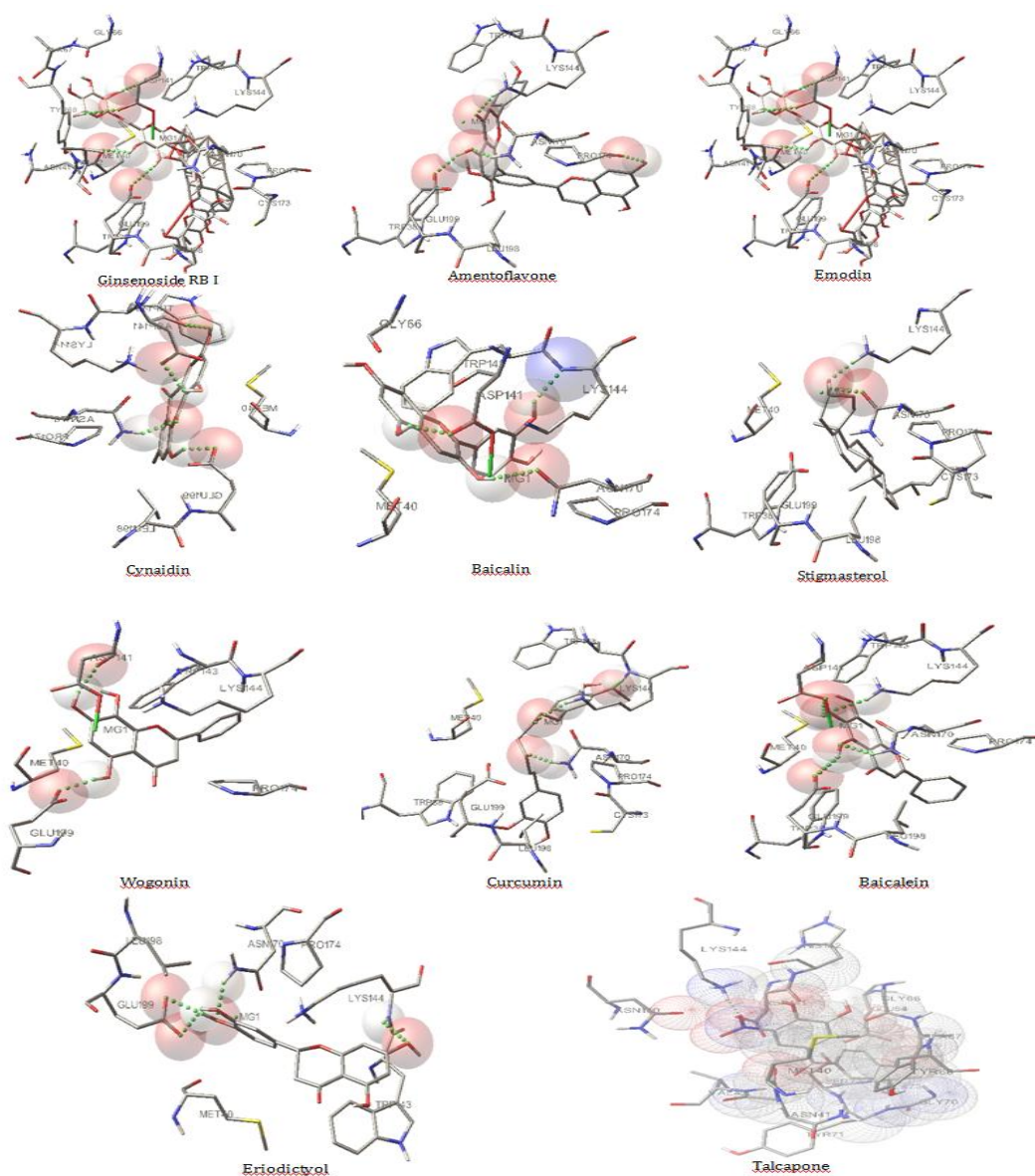


Fig.2 Interaction of phytochemicals and synthetic drug with the receptor COMT

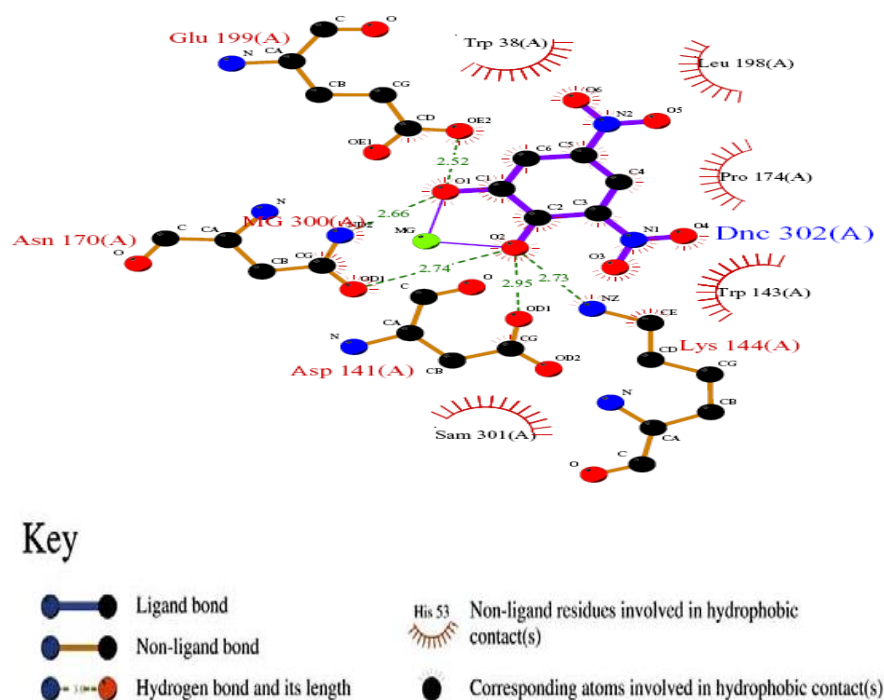


Fig.1 Interaction of Dinitrocatechol with the COMT active site residues

DISCUSSION

Catecholamine O-Methyl Transferase (COMT) is the major enzyme that catalyzes the methylation of catechols. S-adenosylmethionine acts as a methyl donor resulting in the formation of methyl derivatives¹¹. L-Dopa is a precursor of dopamine and a most powerful drug and widely used as anti-parkinsonian agent. The standard oral formulation of L-dopa has relatively short half-life but the administration of L-dopa in combination with COMT inhibitor results in the conversion of L-dopa to 3-O-Methyl Dopa and extends its half life which reduces the risk of motor complications. Synthetic drugs were introduced to enhance the levodopa treatment and tolcapone is one among them. Agid¹² reported that tolcapone may also cause side effects such as nausea, diarrhea, postural hypotension, orange discoloration of urine and abnormalities in liver enzyme levels requiring periodic monitoring of liver function. Hence in the present study, phytochemicals were tried for COMT inhibitors.

The active site of COMT is a shallow groove which is located in the outer surface of the enzyme¹³ which recognizes the catechol by the co-ordination of two hydroxyl oxygen of ligand with magnesium ion located at the bottom of the groove¹⁴. AdoMet-binding domain and the catalytic site together constitute the active site of COMT. Amino acid residues in the catalytic site are important in the binding of substrates, water and Mg^{2+} and for the catalysis of O-methylation. Ado-met is the first one to bind with COMT, then Mg^{2+} that improves the ionization of the hydroxyl groups. LYS 144 acts as a general catalytic base in the nucleophilic methyl transfer reaction and accepts the proton of one of the hydroxyls and is the first step of nucleophilic attack of the catechol oxygen. The other hydroxyl groups should be bonded with the carboxyl oxygen of GLU199¹⁵.

The synthetic drug tolcapone showed the binding energy of -7.9 Kcal/mol, one hydrogen bond and inhibitory constant of 1.61 μ m. Of the selected phytochemicals, Ginsenoside Rb1 obtained from the roots of *Panax ginseng* exhibited very low binding energy (-12.14 Kcal/mol) and very low inhibitory constant (1.26nM) which are still lower than for the drug, tolcapone and the inhibitory constant is in Nano Molar units. Moreover the ligand forms hydrogen bonds with active site residues ASP141 and GLU199 apart from MET40. Rausch *et al.*¹⁶ reported that Ginsenosides are positively effective for neurodegenerative disorders and delay neuronal aging and the

results of the present study may indicate the mode of action of Ginsenoside Rb1 from *Panax ginseng*.

The docked complex of COMT with the flavanoid, Amentoflavone found in the leaf of *Ginkgo biloba* had binding energy and inhibitory constant equal to that of the drug and the hydrogen bond interactions were also as that of the drug with the residues LYS144, ASN170 and GLU199 in addition to PRO174. The Mg^{2+} ion bind in the shallow cleft of the protein surface shows interaction with the compound Amentoflavone, ASP141 and ASN170. Whereas the Ginsenoside Rb1 and other phytochemicals did not show any interaction with the metal ion Mg^{2+} .

From the results of docked complexes of COMT with the phytochemical compounds Emodin, Cyanidin, Baicalin, Stigmasterol, Wogonin, Curcumin, Baicalein and Eriodictyol were energetically favourable with the binding energy around -7 kcal/mol and have interactions with the active site residues LYS144, ASN170, GLU199, ASP141. The above mentioned natural compounds showed the binding energy similar to tolcapone. Whereas the other phytochemicals Daucosterol, Tocotrienol, Orientin, Apigenin, Resveratrol, Tanshinones II A, Saponaretin, Naringenin, Isoorientin, Gynocardin, Ginsenosides RG I, Scopoletin, Gingerol, Kaempferol, Salviatic acid A, Saponarin, Ascorbic acid, Bacopaside II, Carthamin, Asiaticoside exhibited slightly higher binding energy and significantly less interaction with the COMT residues when compared to the drug molecule. So the present study validated that the phytochemical Ginsenoside Rb1 from *Panax ginseng* and Amentoflavone from *Ginkgo biloba* may be considered as very good inhibitors for COMT and suggested as good adjuvants of L-Dopa treatment.

As Ginsenoside Rb1 is isolated from the roots of *Panax ginseng* and Amentoflavone from the leaves of *Ginkgo biloba* which is an endangered plant, the mass production of these compounds can be done through cell suspension culture.

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