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Research Article

Effect of Imperatorin in Neuropathology of Parkinson's Disease: An *In Silico* Study

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ABSTRACT

Parkinson's disease (PD) is an age related neurodegenerative disorder characterized by the dopaminergic neurons loss in the midbrain. Even though there are some drugs in the market to ease parkinsonian symptoms, an accurate drug to prevent or cure the disease is still unknown. This study is an attempt to estimate *in silico;* a bioactive plant fucocoumarin Imperatorin; for its ability as an anti-PD drug, using Autodock 4.2, Pre-ADMET and molinspiration tools against the antioxidants involved in neuropathology of PD, keeping amantadine as a positive control. The molecules selected for the study are Cyclo- Oxygenase 1 (COX-1), Homo-Oxygenase-1(HO-1), NRF2-Keap1, Lipo-Oxygenase 1(LOX-1), Phospholipase A2 (pA2), DJ-1 and superoxide dismutase (SOD). The reliability of the 3 Dimensional (3-D) structures generated were confirmed using WHATIF Server. The study predicted Imperatorin as a potent anti-PD drug, being good inhibitors of COX-1, HO-1 and LOX-1, having less human toxicity and better ability to cross Blood Brain-Barrier (BBB).

Keywords: Imperatorin, Antioxidants, Autodock 4.2, WHAT IF, Pre-ADMET, molinspiration.

INTRODUCTION

Parkinson's disease (PD) is a movement disorder that impairs the patient's motor skills and other functions due to neurodegenerative dopaminergic loss in the substantia nigra. The PD incidence of 13.4 per 100,000 were identified world-wide, which rapidly increased over 60 years of age. Its epidemiology in India varied as 6 to 53 per 100000 heads[1].Till now, certain bioactive plant secondary compounds like flavonoids, polyphenols and coumarins which are at primary stages of research and some synthetic compounds[2] are used as drugs in clinics to treat PD symptoms while there is no exact curative drug for the disease.

Most of the neurological disorders, such as ischemia, spinal cord injury, Alzheimer's disease (AD), multiple sclerosis and epilepsy are characterized by inflammatory reactions and oxidative stress, followed by altered phospholipid metabolism and accumulation of lipid peroxides resulting increased phospholipase A_2 (pA2) activity[3]. Excessive Reactive Oxygen Species (ROS), one of the several implications in the pathogenesis of PD, causes DNA breakage, oxidation of enzymes and lipid peroxidation, resulting in irreversible damage to the cells. Therefore, the regulation of ROS and apoptosis has become a research target for the prevention and treatment of neurodegenerative diseases, especially PD[2].

DJ-1 is a multifunctional protein participating in mitochondrial regulation anti-oxidative stress reaction and chaperone, protease and transcriptional regulation. DJ-1 is located in the cytoplasm, nucleus, and mitochondria in cells, and secreted DJ-1 has been observed in various cultured cells and tissues, including cancer cells, tissues and astrocytes. Moreover, DJ-1 is essential for Nrf2 stabilization by alarming Nrf2 association with Keap1, an inhibitor protein that encourages the ubiquitination and Nrf2 degradation. These findings incriminate DJ-1's effects on Nrf2 in the progress of Parkinson's disease[4]. Astrocytic Nrf2 is neuroprotective to MPTP neurotoxicity in mice. The consequence is of considerable interest in regard to understanding the mechanisms of astrocyte-mediated protection against neurodegeneration. Thus, astrocytic Nrf2 modulation holds great potential for the neuroprotective or therapeutic strategies to treat PD[5]. DJ-1 stimulates the expression of superoxide dismutase (SOD) and glutathione ligase genes by an unknown mechanism to reduce ROS level. DJ-1 protects dopaminergic neurons from oxidative stress through up-regulation of glutathione synthesis and from the toxic consequences of mutant human α-synuclein through increased expression of heat shock protein 70. In view of the above, DJ-1 has multiple specific mechanisms for protecting dopamine neurons from cell death[6].

Idiopathic PD is hallmarked for cytoplasmic constituent of Lewy bodies having Homo Oxygenase-1(HO-1). Moderate HO-1 immunoreactivity was consistently observed in neuromelanin-containing (dopaminergic) neurons in the substantia nigra (SN) of PD patients. This can help to develop biomarkers for monitoring degenerating Dopamine (DA) neurons in PD[7].

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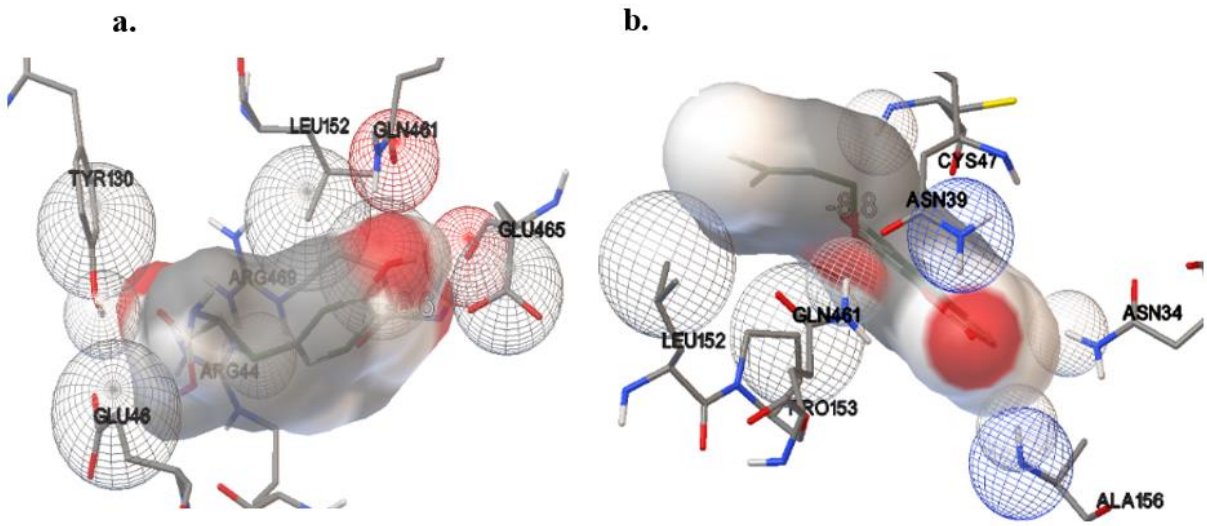

Figure 1: Interactions between a. Amantadine and Cyclo-oxygenase- 1. b. Imperatorin and Cyclo-oxygenase- 1.

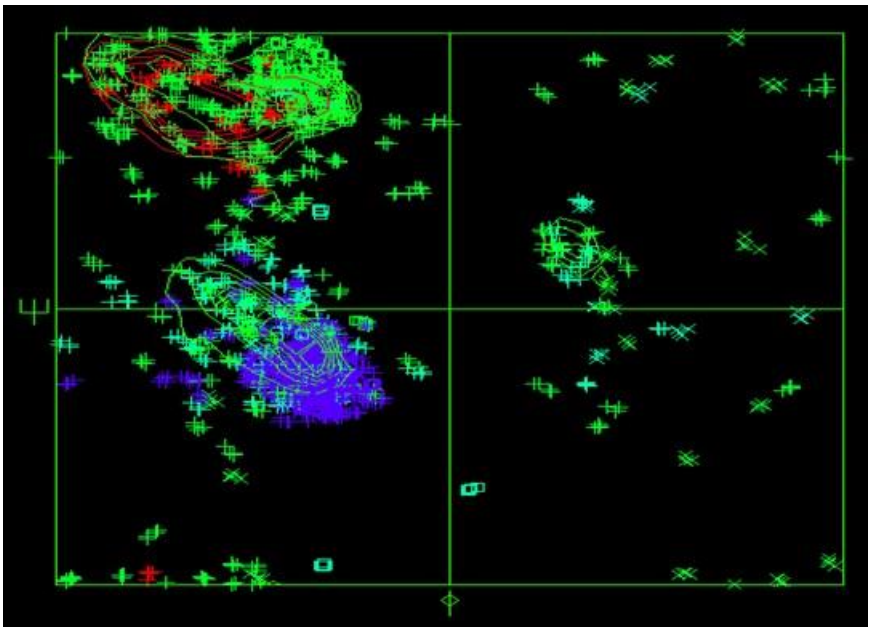

Figure 2: Ramachandran plot Analysis of human Cyclo-oxygenase 1.

During neurodegenerative processes in PD, different pro-inflammatory activities were observed like of Cyclooxygenase (COX) expression in the brain. Many COX inhibitors, protect dopaminergic neurons from microglia toxicity and neuro inflammatory processes. This may result in suspending the inception or slow down progression of parkinsonian symptoms. According to[8] three specific recombinant human 12/15-LOX inhibitors *in vitro* were able to rescue both neuronal as well as oligo-dendro-glial cells from cell death induced by oxidative stress. Elevated activities of pA2 and generation of lipid mediators may be involved in oxidative stress and neuro inflammation associated with neurological disorders. Several pA2 inhibitors have been recently discovered and used for the treatment of ischemia and other neurological diseases in cell culture and animal models. At this time, very little is known about *in vivo* neurochemical effects, mechanism of action, or toxicity of pA2 inhibitors in human or animal models of neurological disorders. The pA2 inhibitors, quinacrine and chloroquine, arachidonyl trifluoromethyl ketone, bromoenol lactone, cytidine 5-diphosphoamines, and vitamin E, also prevent neurodegeneration and its associated immunoreactivity suggesting that pA2 is involved in the neurodegenerative process. This also suggests that pA2 inhibitors can be used as neuroprotectants and anti-inflammatory agents against neurodegenerative processes in PD[3].

Imperatorin, a plant secondary compound, specifically a fucocoumarin, is a commercial bioactive compound famous for its antioxidant properties[9].Amantadine is well known for its therapeutic effects on depression. This antiparkinsonian drug is precisely recognized for its ability to block the neuromuscular transmission influencing the synthesis and excretion of dopamine. Amantadine, is a selective blocker of serotonin-activated ion channel, nicotine-acetylcholine ion channel receptors, N-methyl-D-

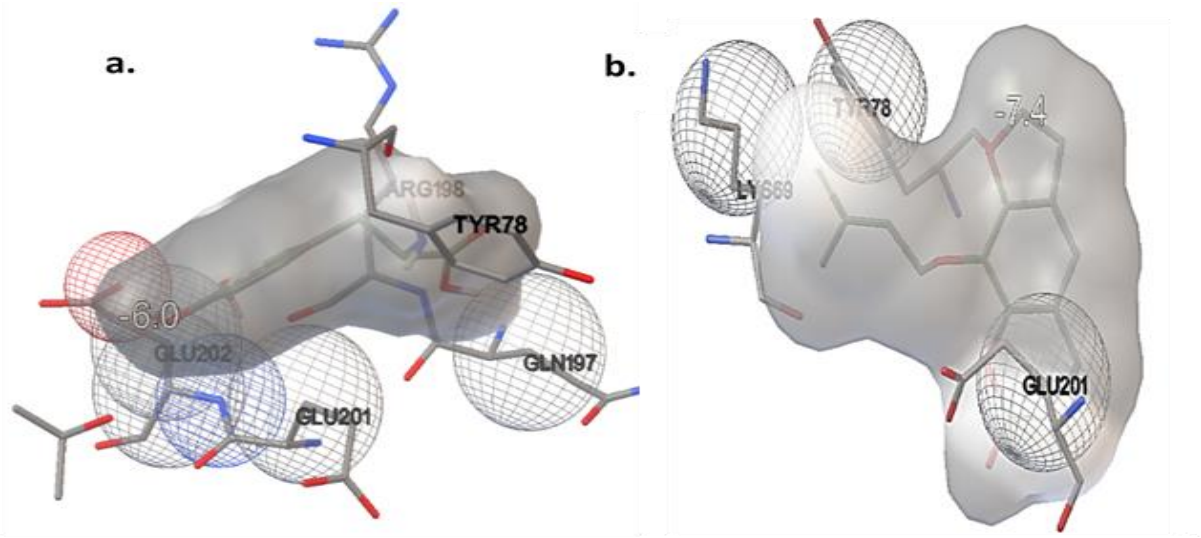

Figure 3: Interaction between a. Amantadine and Homo-Oxygenase-1. b. Imperatorin and Homo-Oxygenase-1.

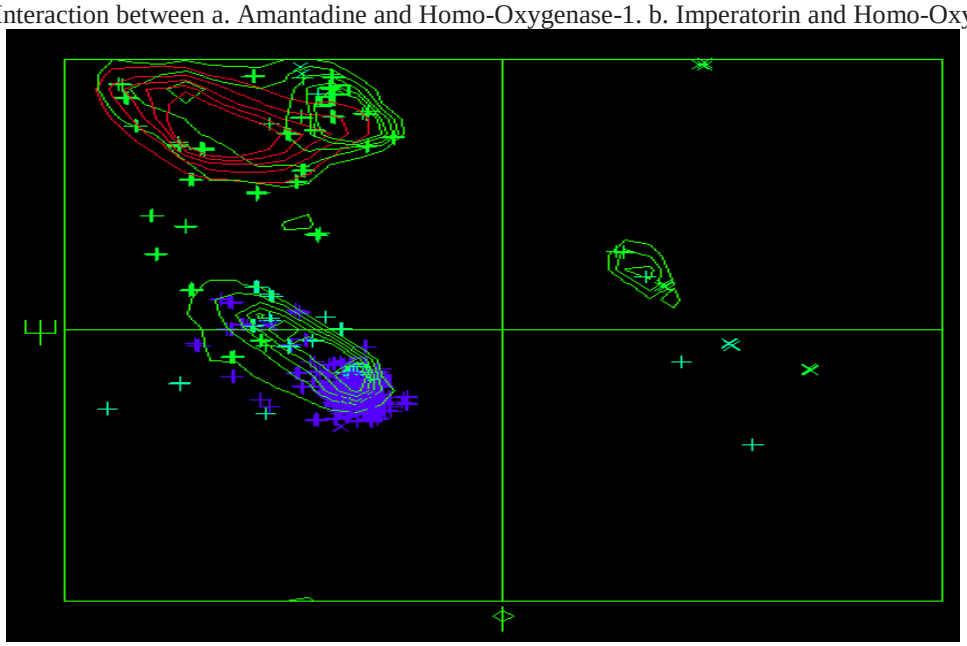

Figure 4: Ramachandran plot Analysis of human Homo Oxygenase 1.

Table 1: Calculation of Molecular Physiochemical Properties.

Compound	Amantadine	Imperatorin
N atoms	11	20
Molecular weight	151.25	270.28
TPSA	26.02	52.59
mi log P	2.65	+3.95
nRtb	0	3
nOH	1	4
nOHNH	2	0
Rule of violation	0	0
BBB Permeability score	+0.203	+0.361

n=number, TPSA=Total Polar Surface Area, Log P=octoanol/water coefficient.

aspartate (NMDA) ion channel receptors, as well. This also upsurge the fluidity of cellular membranes and rise the electrically stimulated and secreted levels of dopamine and serotonin[10].This is an *in silico* study for the analysis of Imperatorin with Amantadine as a positive control, for its antioxidative inhibitory roles against COX-1, HO-1, NRF2-Keap 1, LOX-1, pA2, DJ-1 and SOD, to target Imperatorin as a novel anti-PD agent.

MATERIALS AND METHODS

The *in silico* BBB crossing ability of the ligands were analysed using Cbligand tools. Molinspiration tools and Pre ADMET were used for analyzing ADME and molecular physio-chemical properties and toxicity of Amantadine and Imperatorin. AutoDock tools 1.5.6 and MGL tools 1.5.6 packages (The Scripps Research Institute, Molecular Graphics Laboratory, and 10550 North Torrey Pines Road, CA, 92037) were used to build the receptor models. The 3-D predicted structures of the protein and receptor models were analyzed using WHATIF for its reliability.

ADME and Toxicity Properties estimation

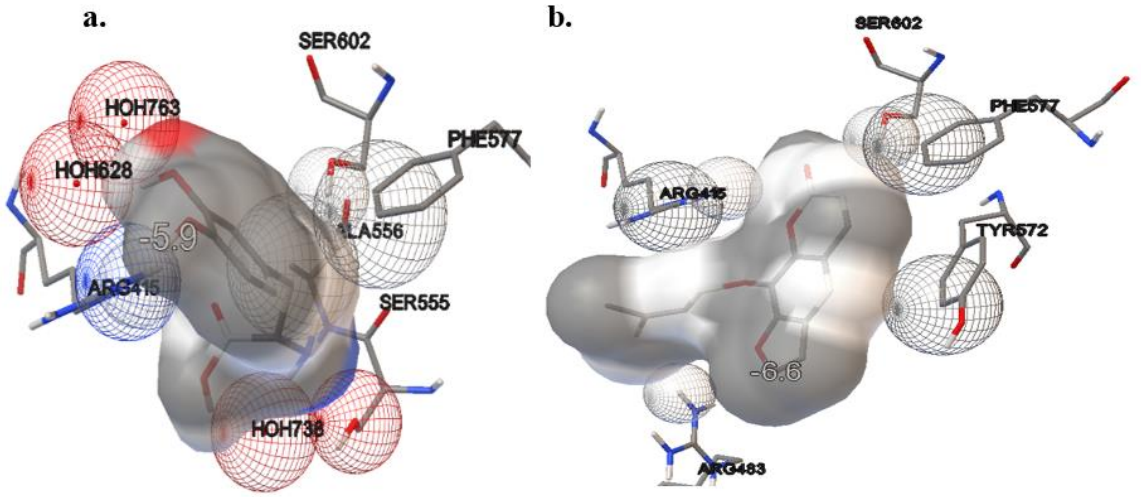

Figure 5: Interactions between a. Amantadine and Nrf2 – Keap1, b. Imperatorin and Nrf2-Keap1.

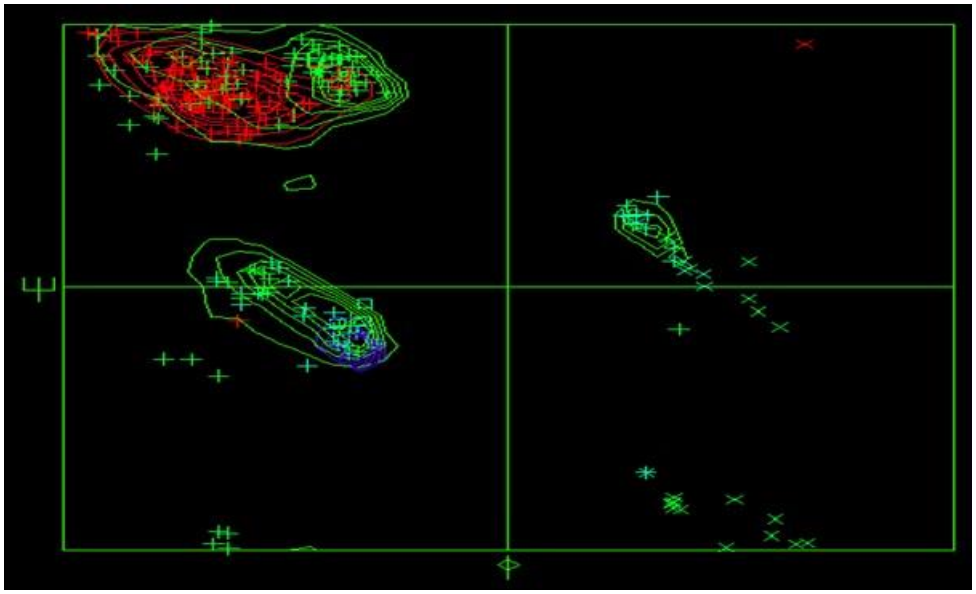

Figure 6: Ramachandran plot Analysis of Nrf2- KEAP 1.

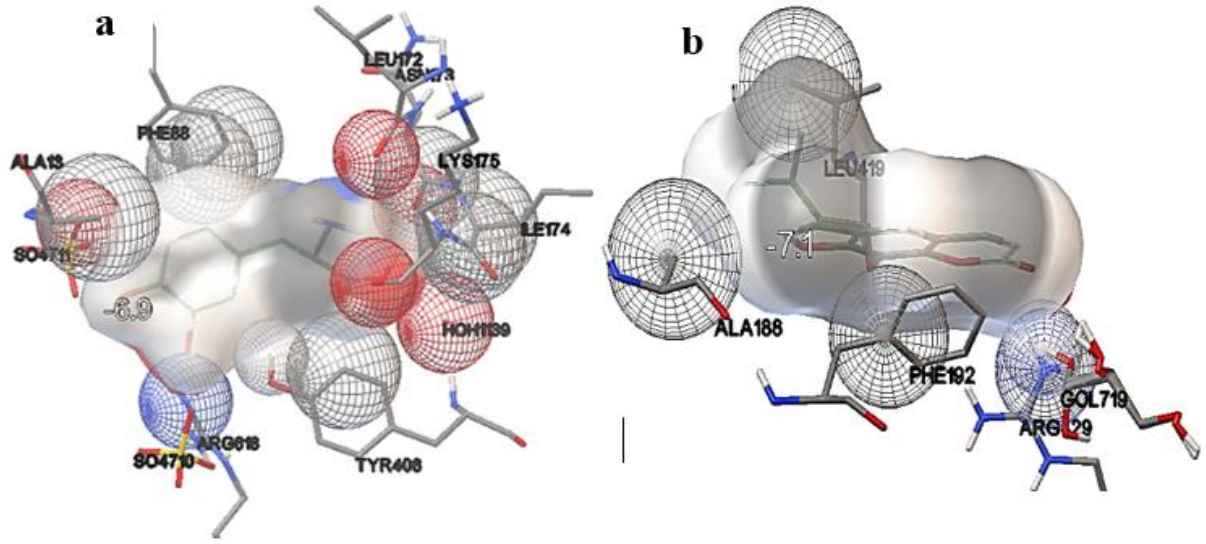

Figure 7: Interactions between a. Amantadine and Lipo- Oxygenase 1 b. Imperatorin and Lipo- Oxygenase 1.

Table 2: Bioactivity predictions using molinspiration tools.

Sl. No.	Bioactive Properties	Bioactivity scores	
		Amantadine	Imperatorin
1	GPCR Ligand	-0.50	-0.37
2	Ion Channel Modulator	-0.07	-0.02
3	Kinase Modulator	-0.85	-0.56
4	Nuclear Receptor Ligand	-1.27	-0.18
5	Protease Inhibitor	-0.54	-0.60
6	Enzyme Inhibitor	-0.40	+0.09

An *in silico* study of the synthesized compounds were performed for the prediction of ADME properties like Total polar surface area (TPSA), miLog P, number of rotatable bonds, number of hydrogen donor and acceptor atoms according to Lipinski's rule of five[11]. A web-based program (www.cbligand.org/BBB) was used for BBB crossing prediction. The bioactivity and toxicity risks of ligands were analysed through Pre ADMET[12].

WHAT IF analysis

The Prepared structures of all the seven receptors were checked for the 3-D structure reliability through WHAT IF server[13]. Ramachandran plot was generated for the molecules selected for AutoDock.

Molinspiration studies

The Physiochemical properties of Imperatorin was compared with the positive control amantadine using molinspiration tools[14].

Molecular docking studies

The molecular docking studies were done according to[15].The 3-D structures of Amantidine (Pub chem ID: 2130) and Imperatorin (Pub chem ID: 10212) were retrieved from PubChem and optimized for docking using Discovery studio. The protein Crystallographic structures of receptors Cyclo- Oxygenase 1 (1CX2), Homo-Oxygenade-1(4WD4), NRF2-Keap 1 (1U6D), Lipo-Oxygense 1 (4NRE), Phospholipase A2 (1UMV) , DJ-1 (4ZGG) and SOD (4BCY) were retrieved from www.rcsb.org was prepared for docking by deleting all heteroatoms, ligands and water molecules and optimized by minimization of energy by using Discovery Studio. The obtained structures were saved and used for the docking studies. 3D structure of proteins was generated after optimization in Discovery studio. Gasteiger charges and rotatable bonds of the ligand were determined automatically by AutoDock tools to generate different conformers for the docking[16].Grid boxes were generated using autogrid4 having 40 × 40 × 40 grid points in xyz with grid spacing of 1.000 Å, taking Lamarckian genetic algorithm with the parameters as the number of runs: 50, population size: 150, number of evaluations: 2,500,000 and number of generations: 27,000 numbers of generations[17]. Finally, Autodock tools 1.5.6 was used to retrieve Protein-Ligand Interactions[18].

RESULTS AND DISCUSSION

PD is a progressive neurological disorder demarcated by a distinctive clinical condition considered by bradykinesia, tremor, rigidity, and postural instability. Parkinsonian degeneration, either inherited or sporadic, are characterized by neuronal loss in selective populations of vulnerable dopaminergic neurons of brain[19].

Over the ages, human have relied on nature for their basic needs like for the production of many medicines, including lifesaving drugs, proving as the basis of numerous sophisticated traditional medicine system that have been in existence since thousands of years back in our country. Most of those medicines in Homeopathy, Allopathy, Ayurveda, Siddha and Unani medical systems are plant secondary compounds. The classes of secondary metabolites used are mainly polyketides, fatty acids, terpenes, steroids, phenylpropanoids, alkaloids, specialized amino acids and carbohydrates. One such group is fucocoumarins to which Imperatorin, also known as 9-[(3-methyl-2-buten-1-yl) oxy] - 7H-furo [3,2-g]chromen-7-one or 8-(1,1-dimethylallyloxy)- psoralen, belongs to. It has been recognized that fucocoumarins are a budding appreciated reserve for the prevention and cure of some Central Nervous System (CNS) diseases. The effect of imperatorin on processes of learning, its anxiolytic effect and anti-epileptic activity were also designated. However, few mechanisms of action were consigned to imperatorin to explain these activities[20]. On the other hand, Amantadine is a synthetic tricyclic amine with antiviral, antiparkinsonian, and antihyperalgesic activities. It employs its antiparkinsonian effect by exciting the dopamine release from striatal dopaminergic nerve terminals and preventing its pre-synaptic reuptake. This agent may also exercise some anticholinergic effect by preventing the stimulation of acetylcholine in N-methyl-D-aspartic acid (NMDA) receptors, causing anti-hyperalgesia[21]. Thus, in this study, Amantadine was considered as the positive control.

Free radicals have been suggested to augment neuronal loss in cerebral ischemia[22], seizure disorders[23], schizophrenia[24], ageing[25], PD[26, 27] and AD[28]. Thus in this study, Autodock studies were performed against various antioxidant molecules involved in PD neuropathology, to know possible interactions between the molecules and Imperatorin, with respect to Amantadine.

The Prostaglandins formation from arachidonic acid is catalyzed by COX enzymes. The electron reduction during the Prostaglandin reaction has been concerned with the leakage of electrons, which in turn could react with cellular oxygen to form ROS. Moreover, carbon-centered radicals are generated in the COX-2/arachidonic acid system which are responsible for the generation of oxidative stress[29]. Thus, inhibiting COX enzymes can reduce the ROS production thus contributing to prevent PD-associated neurodegeneration. Here, Autodock studies revealed that COX-1 was inhibited by Imperatorin with a lesser inhibitory energy score of -8.8 Kcal/mol than Amantadine with a score of -6.6 Kcal/Mol (Figure 1). The Ramachandran plot is the 2D plot of the φ-ψ torsion angles of the protein backbone. It provides a simple view of the conformation of a protein. The φ-ψ angles cluster into

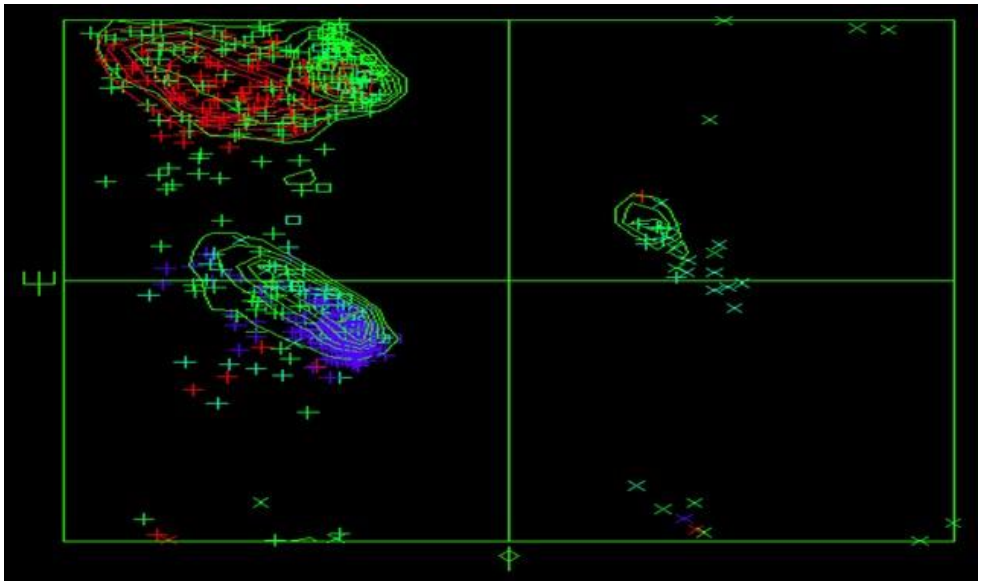

Figure 8: Ramachandran plot Analysis of Lipo Oxygenase 1.

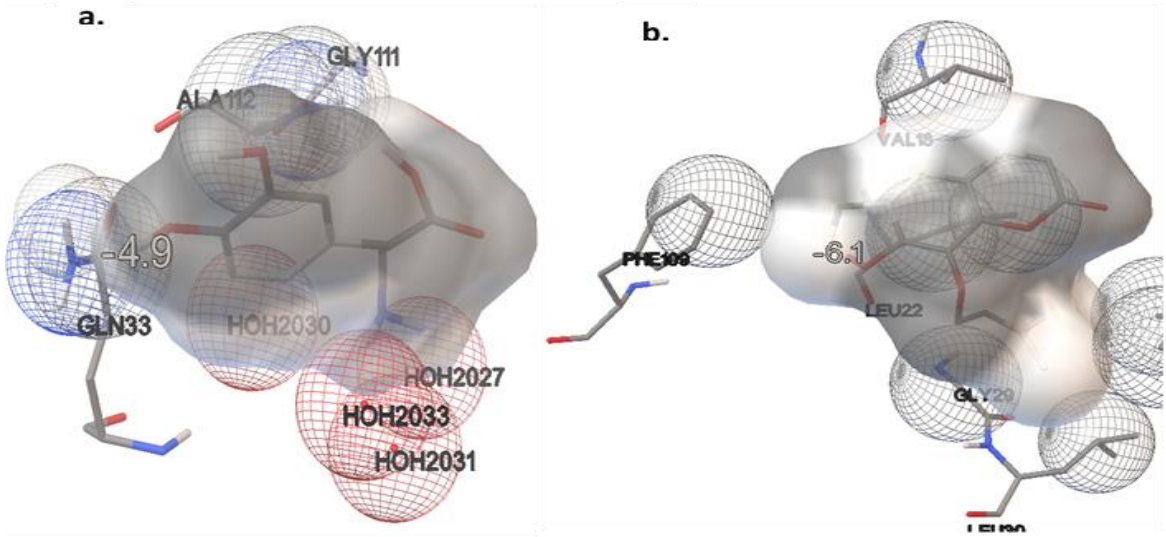

Figure 9: Interactions between a. Amantadine and pA2. b. Imperatorin and pA2.

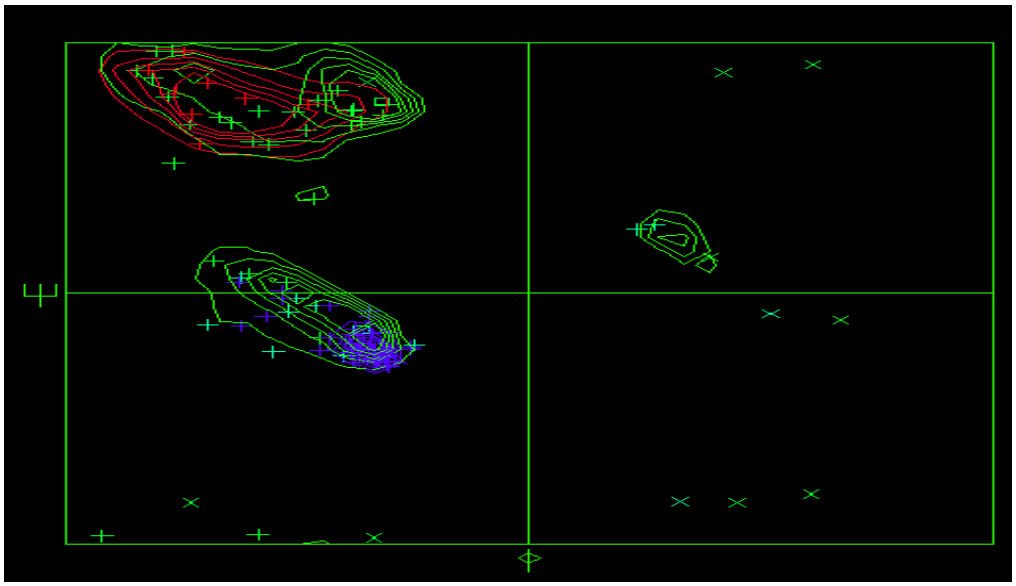

Figure 10: Ramachandran plot Analysis of human Phospholipase A2.

Table 3: ADMET profile of Imperatorin with standard Amantadine drug.

Compound	Amantadine	Imperatorin
Buffer solubility mg/L	1738.91	11.9498
Caco2 permeability	21.3247	56.1038
CYP 2C19 inhibition	Inhibitor	Inhibitor
CYP_2C9_inhibition	Inhibitor	Inhibitor
CYP_2D6_inhibition	Inhibitor	Non
CYP_2D6_substrate	Weakly	Non
CYP_3A4_inhibition	Inhibitor	Inhibitor
CYP_3A4_substrate	Non	Weakly
HIA	100.000000	97.728639
MDCK	68.7766	228.711
Pgp_inhibition	Non	Non
Plasma_Protein_Binding	100.000000	94.717141
Pure_water_solubility_mg_L	622.785	2.09914
Skin_Permeability	-2.78996	-3.16911
SKlogD_value	0.175840	3.849950
SKlogP_value	1.514940	3.849950
SKlogS_buffer	-1.939420	-4.354460
SKlogS_pure	-2.385360	-5.109780
Ames_test	Non-Mutagen	Non-Mutagen
Carcino_Mouse/Rat	Positive	Negative

distinct regions in the Ramachandran (RM) plot where each region corresponds to a particular secondary structure[30].The Figure 2 described the 3D structure reliability of the selected COX enzyme after removal of heteroatoms and water molecules in RM plot.

Figure 2: Ramachandran plot Analysis of human Cyclo-oxygenase 1 The vigorous activity of oxidative metabolic processes in neuronal cells results in the profuse deposition of nueromelanin pigment in nigrostriatal neurons stimulating HO-1 expression, and accelerated aging in nigrostriatal (dopaminergic) perikarya. The consistent oxidative stressors present in blood and cerebrospinal fluid increase the extreme expression of HO-1 in choroid plexus epithelial cells, ependymocytes, and many cerebrovascular endothelial cells in the brain .Since the

Figure 4: Ramachandran plot Analysis of human Homo Oxygenase 1 HO-1 gene is delicately subtle to up-regulation by oxidative stress, the dopaminergic neurons in the human SN gets degenerated[7]. The results showed that Imperatorin inhibits HO-1 with an inhibitory energy of -7.4Kcal/Mol than of amantadine with -6.0 Kcal/Mol (Figure 3). The Figure 4 illustrates the HO-1 enzyme's RM Plot after energy minimization.

The Figure 5 indicates that the inhibition of Nrf2-Keap1 was slightly higher with a lower inhibition energy of -5.9 Kcal/Mol than amantadine with -6.6 Kcal/Mol. The Figure 6 depicts the RM plot of the enzyme. The expression of various cytoprotective genes were reported to be regulated by Nrf2 against the oxidative and inflammatory stress. When treated with microglia conditioned media which induced Nrf2 nuclear translocation, there was an elevation in HO- 1 expression and suppression of interferon-g-induced ROS and NO production[31]. Thus, regulation of Nrf2 by its inhibition partially helps in PD oxidative stress reactions.

Figure 6: Ramachandran plot Analysis of Nrf2- KEAP 1. The 15-LOX-1 in humans is one of the key mediators in neurodegenerative disease implicated in a variety of neurodegenerative diseases; including stroke, AD and PD, because it is triggered by reactive oxygen species (ROS).

Elevated amounts of 12/15-LOX have been found in experimental stroke in mice and in early phases of AD and PD[8]. The Figure 7 and Figure 8 shows the Autodock interaction sites with inhibition energies for the interactions (LOX1 with Imperatorin: -7.1KCAl/Mol; LOX1 with Amantadine: -6.9Kcal/Mol) and RM Plot for LOX1 respectively.

Many disease states and neuronal injury results in elevated pA2 activity and extreme assembly of pro inflammatory mediators, eicosanoids, and platelet activating factors. pA2 contributes to the pathogenesis of the neurological disorders such as ischemia, AD, PD and MS disorders by attacking neural membrane phospholipids, releasing pro

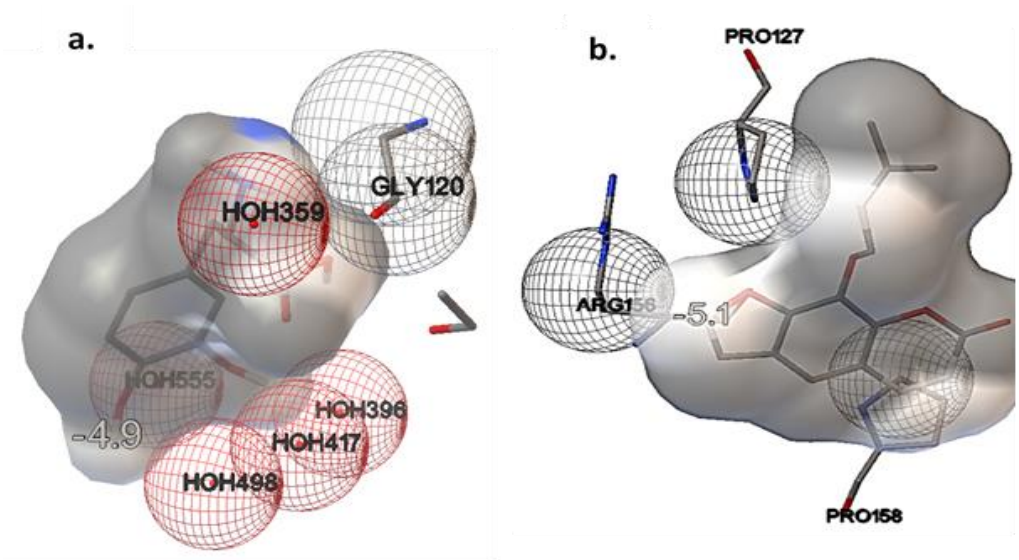

Figure 11: Interactions between a. Amantadine and DJ1 b. Imperatorin and DJ1.

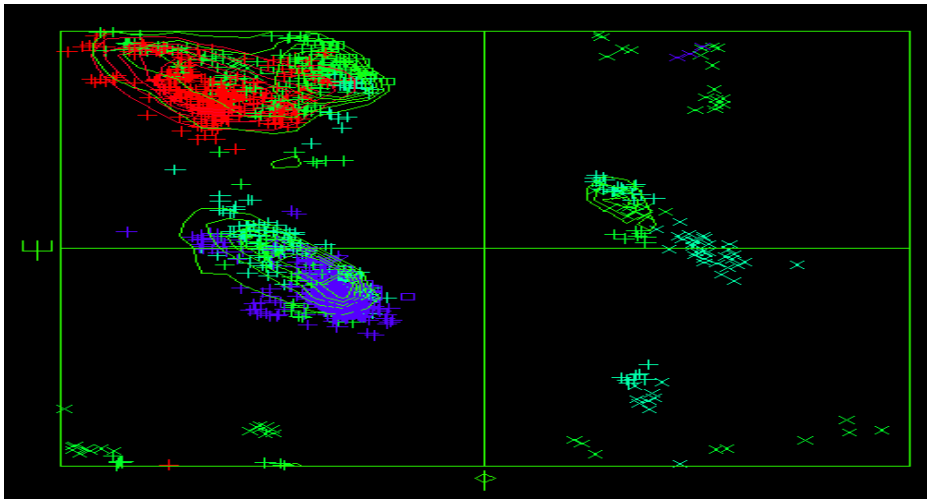

Figure 12: Ramachandran plot Analysis of Protein DJ1.

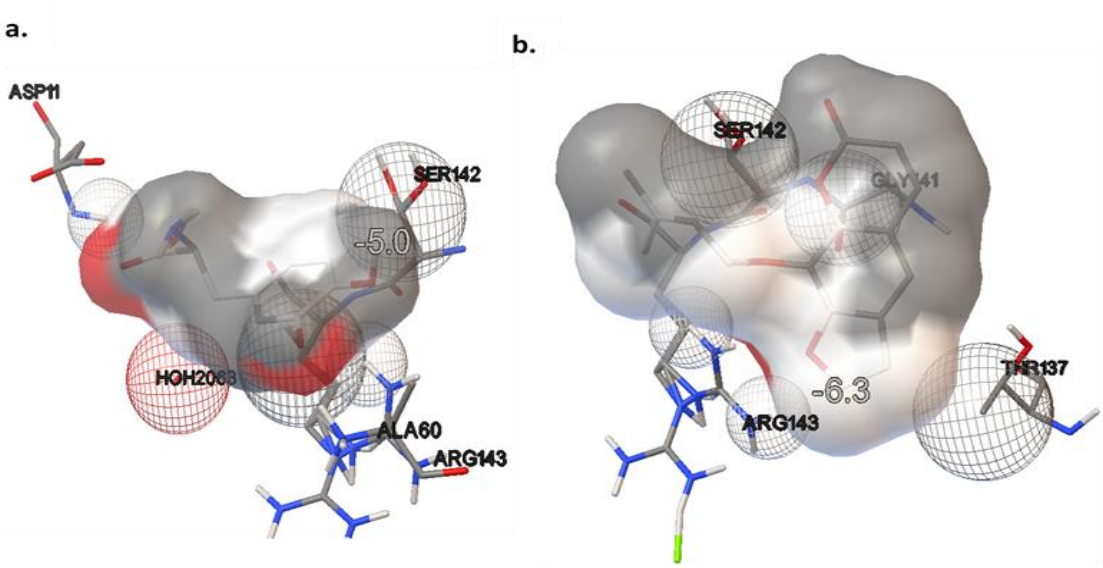

Figure 13: Interactions between a. Amantadine and SOD b. Imperatorin and SOD.

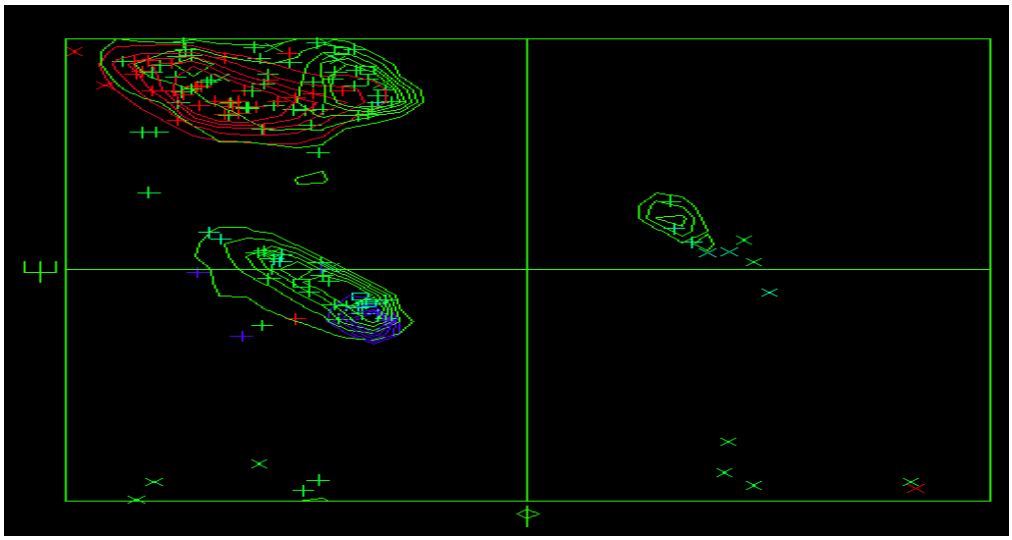

Figure 14: Ramachandran plot Analysis of human Cyclo-oxygenase 1.

inflammatory lipid mediators such as prostaglandins, leukotrienes, and thromboxanes, and PAF, and generating 4-hydroxynonenal (4-HNE). Thus, inhibition of pA2 activity provides an attractive approach for designing

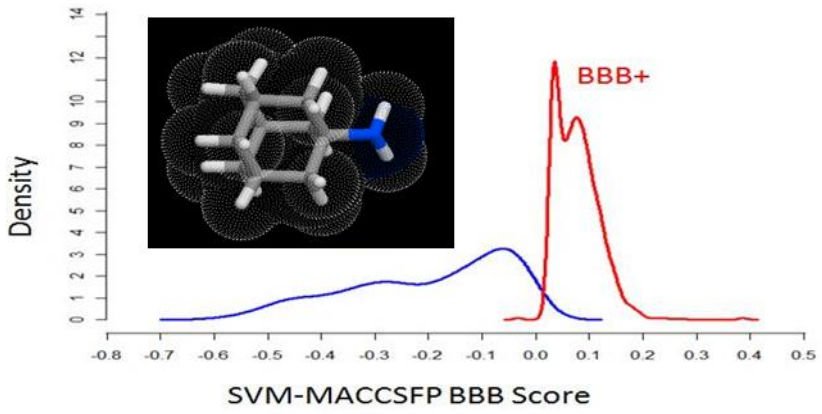

Figure 15: Blood-brain barrier permeability scores for Amantadine.

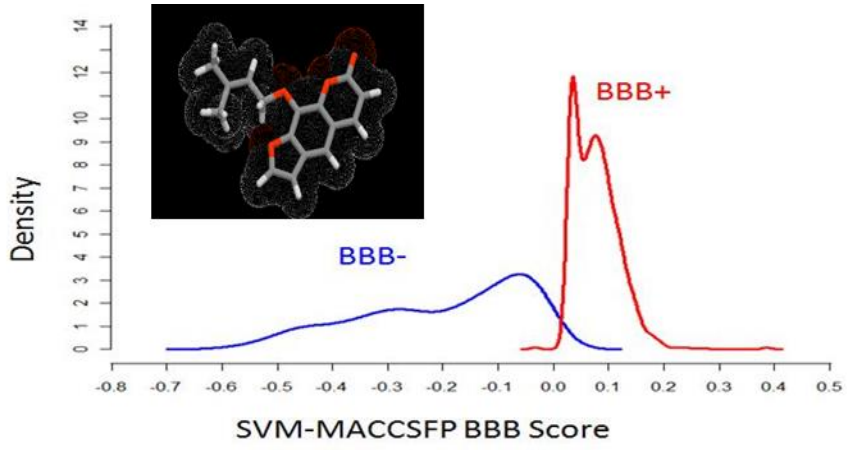

Figure 16: Blood-brain barrier permeability scores for Imperatorin.

novel drugs for the treatment of inflammation and oxidative stress associated with acute neural trauma such as ischemia, spinal cord injury, and head injury and some neurodegenerative disorders such as AD, PD, and MS[3]. The Figure 9 shows the inhibitory energy of Imperatorin to pA2 as -6.1 Kcal/Mol and with Amantadine as -4.9 Kcal/Mol. Moreover Figure 10 depicts pA2 enzyme's RM plot.

Figure 10. Ramachandran plot Analysis of human Phospholipase A2. DJ-1 has been implicated in protection against oxidative stress. Human DJ-1 is a 189-amino-acid multifunctional protein that is widely expressed in both brain and peripheral tissues.

Figure 12.Ramachandran plot Analysis of Protein DJ1. Loss of function of DJ-1 has been linked to autosomal recessive PD and Parkinsonism–dementia–amyotrophic lateral sclerosis complex[32].Thus its inhibition has a great role in designing anti-PD drug. The Figure 11 and Figure 12 describes the Imperatorin (-5.1Kcal/Mol) and Amantadine (-4.9 Kcal/Mol) inhibition energies as well as the DJ1 RM Plot, respectively.

Figure 14. Ramachandran plot Analysis of human Cyclo-oxygenase 1. The superoxide dismutase (SOD) in PD represents the point of contact between mitochondrial respiratory failure and oxidative stress. The activity level of Mn SOD may have a significance in the induction of oxidative stress; both lower-than-normal and higher-than-normal activities could induce oxidative stress[33].The Autodock study revealed that the inhibition energy for SOD with Imperatorin and Amantadine was -6.3 Kcal/Mol and -5.0 Kcal/Mol respectively (Figure 13). Similarly, the RM Plot for SOD was generated (Figure 14).

The molecular physiochemical properties (Table. 1) revealed that Imperatorin have higher Total Polar Surface Area of 52.59 than amantadine with 26.02. The miLog P was positive revealing its lipid soluble nature. Moreover, the Blood Brain-barrier (BBB) permeability was higher (+0.361) than Amantadine (+0.203), stating its better applicability as a neuroprotective agent. This is well in par with[34] proving the ability of Imperatorin to act as anticonvulsant drug by crossing BBB using Chimney test in mice models. The Bioactivity scores (Table. 2, Figure 15, 16) predicted using molinspiration tools revealed that Imperatorin is a better enzyme inhibitor with positive value as 0.09 than amantadine with negative 0.40 value. This can be expected as usage of Imperatorin as a better inhibitor of antioxidant enzymes, contributing to reduction of ROS and thus acting as a better anti-PD agent.

The Pre ADMET studies revealed that all the properties were better for Imperatorin with respect to Amantadine (Table. 3). The amantadine was carcinogenic while Imperatorin was predicted to be non-carcinogenic. Furthermore, Caco2 permeability, CYP 2C19 inhibition, CYP_2C9_inhibition, CYP_2D6_inhibition,

CYP_2D6_substrate, CYP_3A4_inhibition, CYP_3A4_substarte properties were found almost equal to that of Amantadine. Moreover, Imperatorin was proved to be non-mutagenic with higher Human Intestinal Absorption (HIA), and plasma proteins binding which enhances its possibilities as a better drug of choice for clinical trials for PD.

CONCLUSION

The study predicted Imperatorin as a potent anti-PD being good inhibitors of COX-1, HO-1 and LOX-1. The drug was predicted to be BBB positive stating its ability to cross BBB to reach the targeted site and produce the desired effects. The ADMET studies also revealed its ability to act as non-mutagenic as well as non-carcinogenic with minimal toxicity. The physiochemical properties predicted it as a better enzyme inhibitor than Amantadine; the positive control. This *in-silico* analysis predicted its wider dimensions and possibilities as a novel drug of choice against PD. Nevertheless, further experimentation in *in vitro* and *in vivo* models is mandatory to reconfirm its potential mechanism of action and put forward the drug for clinical trials in human.

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CONFLICTS OF INTEREST

The authors have no conflict of interest.

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