**Effect of myricetin on cognitive impairments in the transgenic *Drosophila* model of Parkinson’s Disease**

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Parkinson’s Disease (PD) is a progressive neurodegenerative disorder involving the loss of dopaminergic neurons. Despite the availability of many drugs to ease the life of PD patients, there is no permanent cure until now. Now-a-days, there has been a considerable attention towards the use of herbal products to treat PD patients worldwide due to less side effects. In this context, here we investigated myricetin, a common plant derived flavonoid, on the cognitive impairments exhibited by the transgenic *Drosophila* expressing human α-synuclein in the neurons. The PD flies were allowed to feed on the diet having 10, 20 and 40 µM of myricetin for 24 days and then assayed for cognitive impairments. The exposure of myricetin showed a dose dependent significant delay in the cognitive impairments. Molecular docking studies showed the positive interaction between myricetin and α-synuclein. The results suggest a protective effect of myricetin against the cognitive impairments.

**Keywords:** Molecular docking, Neurodegenerative disorder, α-Synuclein

Parkinson’s Disease (PD) is a progressive neurodegenerative disease which results mainly due to the loss of dopaminergic neurons in the mid brain. It is mainly characterized by the resting tremor, bradykinesia and rigidity. The drug therapy in PD is effective only in the early stages of the disease and is also associated with a number of side effects. Substantial evidences suggest that the aggregation of alpha synuclein and the formation of Lewy bodies (LBs) is the crucial step in the progression of the disease. To date, there is no preventive therapy for PD. A recent study on herbal resources to treat PD suggests that the compounds which could slow down or prevent aggregation and fibrillation of α-synuclein could act as a possible therapeutic agent.

Myricetin belongs to the flavonoid class of polyphenolic compounds, which possess antioxidant properties. It is commonly found in vegetables, fruits, nuts, berries, tea and is also a part of red wine. Myricetin exhibit structural similarity with fisetin, luteolin, and quercetin and is reported to have similar functions as other members of the flavonol class of flavonoids. In the past few years, small organic molecules, especially certain polyphenolic compounds, have been extensively tested for their ability to inhibit fibril formation *in vitro*. Here, we docked ligand myricetin and α-synuclein and analyzed for synthesizing possible therapeutic drug for the treatment of PD.

**Materials and Methods**

Molecular docking studies of myricetin and α-synuclein fibrils [6FLT]

The rigid molecular docking studies were performed using HEX 8.0.0 software, which is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein, enzymes and DNA molecule. The structure of the ligand Myricetin was sketched by CHEMSKETCH (http://www.acdlabs.com) and converted to pdb format from mol format by OPENBABEL (http://www.vcclab.org/lab/babel/). The crystal structure of the α-synuclein fibrils (protein) [PDB ID: 6FLT] was downloaded from the protein data bank (http://www.rcsb.org/pdb). It consists of chains (A, B, C, D, E, F, G, H, I, J) having sequence length of 121 which is found in *Homo sapiens*. Visualization of the docked pose was done using CHIMERA (www.cgl.ucsf.edu/chimera) and PyMol (http://pymol.sourceforge.net/) molecular graphics program.
**Drosophila culture and crosses**

The flies were cultured on standard *Drosophila* food containing agar, corn meal, sugar and yeast at 25°C⁸⁻⁹. Crosses were set up using six virgin females of UAS-Hsap/SNCA.F5B and mated to three males of GAL4-elav. The progeny expressed human αS in the neurons and the flies were referred as PD flies¹⁰. The PD flies were exposed to 10, 20 and 40 µM of myricetin mixed in the culture medium for 24 days. As negative control, the PD flies were allowed to feed on the diet supplemented with 10⁻³ M of L-dopamine. The control flies (UAS-Hsap/SNCA.F) were also exposed to the doses 10, 20 and 40 µM of myricetin. *Drosophila* is a well established research model that does not require ethical clearance.

**Open-field assay**

Open-field assay (OFA) was performed according to the method described by Hirth¹¹. Accordingly, three flies from each group were kept in an arena divided by squares (1 cm X 1 cm) measuring 9 cm of diameter, which can be covered by Petri dish. The number of squares crossed by each fly, during a given time-window (30 s), was counted. The values were expressed as mean of five independent experiments.

**Courtship assay**

The assay was performed by the method of Nichols *et al.*¹². Newly eclosed virgin male and female flies (PD and Control) were separated and kept in different diet vials for 24 days at 25°C under 12 h light/dark. On the day of experiment one pair was transferred into a mating chamber and observed. Observations were made till successful copulation noting the time of each behavior (orientation, male song, chasing and licking) and the total time of courtship behaviors until mating. The courtship index (C.I.) was calculated by dividing the time of courtship by the total time until copulation.

**Odor choice index**

The assay was performed by the method of Simonnet *et al.*¹³. The test flies were starved for 16-18 h at 25°C before the experiment. Two small filters paper dipped in propionic acid and distilled water, respectively, were kept in the two tubes of the Y maze. The number of flies entering both the tubes was counted. Five replicates per group and 20 flies per group were used in the assay. The odor choice index (OCI) was calculated as:

\[ \text{OCI} = \frac{\text{Number of flies in tube 1} - \text{Number of flies in tube 2}}{\text{Total number of flies}} \]

**Statistical analysis**

The statistical analysis was done by performing one-way ANOVA using SPSS 16 and the level of significance was kept at *P* <0.05.

**Results**

Atomic resolution structure and cavities of α-synuclein are illustrated in Fig. 1 (A and B). The docked model of ligand and protein is illustrated in Fig. 2. One can observe that the ligand myricetin snugly fits into the energetically favoured pose into the curved contour of the coil of 6FLT exhibiting proper interaction of ligand and protein as shown through various docked pose in Fig. 3 (A and B). Spherical and stick representation of docked ligand and protein is shown in Fig. 4. Hydrogen bonding, energy and hydrophobic interactions between ligand and protein for various conformations of docked pose were analyzed as illustrated in Fig. 5. The results of molecular docking revealed that the drug binds efficiently with the protein receptor and exhibit free energy of binding (FEB) values of -283.76 kcal mol⁻¹. The ligand exhibited stabilization through hydrophobic and Vander Waals interactions with nearby nucleotides as illustrated in Table 1.

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![Fig. 1](image1.jpg) — (A) Atomic resolution structure of α-synuclein (solvent excluded); and (B) cavities shown therein

![Fig. 2](image2.jpg) — Docking of flavonoid myricetin with alpha-synuclein (6FLT) resulting in docked model
hydrogen bonds and Vander Waals interactions can be witnessed between ligand and protein residue as glycine (GLY 73.I HA2…1.het O 14.752) binds to oxygen of the flavonoid myricetin and (GLY 73.I C…1.het C 14.266) shows interaction between C to carbon of the myricetin. Likewise, many intermolecular and intramolecular noncovalent interactions were observed in the protein-flavonoid docked model. Few such interactions are seen between protein residue threonine, (an amino acid) binds to oxygen of the myricetin (THR 75.I HG1…1.het O 13.410) through its HG1 residue, another residue of threonine binds through its HG21 to ligand hydrogen (THR 59.A HG21…1.het C 7.718), while another threonine (THR 72.I HB…1.het H 16.693) bind to hydrogen of ligand through its HB residue. Similarly, (THR 59. A C…1.het O 8.541) this threonine residue bind through C residue to carbon of the myricetin and and another oxygen of the ligand is bound to HG22 of the threonine as (THR 75.I HG22…1.het O 13.349). Another amino acid, glutamic acid has its bond with the flavonoid through its hydrogen part (GLU 61.C OE1…1.het H 4.934). Other H-bonds and noncovalent interactions are seen between amino acid alanine as (ALA 56.A N…1.het O 10.858) through its N to oxygen of myricetin and lysine (LYS 58.A H…1.het O 15.269) shows H-bond H of lysine and oxygen of flavonoid myricetin. Amongst the distances depicted here, threonine, lysine and glycine have the longest noncovalent interactions while the shortest is depicted through glutamic acid. Threonine is seen to possess all types of noncovalent interactions short and long range both. The H-bonds and noncovalent interactions are depicted in Table 1. Apart from these there are numerous interactions which are present between the docked flavonoid and 6FLT protein.

A significant reduction in the activity of 8.37 fold was observed in PD flies compared to control flies (Fig. 6A; P <0.05). The flies exposed to 10, 20 and 40 µM of myricetin showed a dose dependent significant increase of 2, 2.25 and 3.31 folds in the activity compared to PD flies (Fig. 6A; P <0.05). The PD flies exposed to 10⁻³ M of dopamine showed a significant increase of 7.93 fold in the activity compared to PD flies (Fig. 6A; P <0.05). The PD flies showed a reduction of 2.19 fold in CI compared to control flies (Fig. 6B; P <0.05). The PD flies exposed to 10, 20 and 40 µM of myricetin showed a significant increase of 1.23, 1.33 and 1.47 folds in CI compared to unexposed PD flies (Fig. 6B; P <0.05). The PD flies exposed to 10⁻³ M of L-dopa showed a significant increase of 2.16 fold in CI compared to PD flies (Fig. 6B; P <0.05). The PD flies showed a significant

| Table 1 — H-bonds and non-covalent interactions between amino acid residue and myricetin |
|-----------------|-----------------|-----------------|
| ATOM 1          | ATOM 2          | Distance (Å)    |
| GLY 73.I HA2    | 1.het O         | 14.752          |
| THR 75.I HG1    | 1.het O         | 13.410          |
| GLU 61.C OE1    | 1.het H         | 4.934           |
| THR 59.A HG21   | 1.het C         | 7.718           |
| ALA 56.A N      | 1.het O         | 10.858          |
| LYS 58.A H      | 1.het H         | 15.269          |
| THR 72.I HB     | 1.het H         | 16.693          |
| THR 59.A C      | 1.het O         | 8.541           |
| GLY 73.I C      | 1.het C         | 14.266          |
| THR 75.I HG22   | 1.het O         | 13.349          |
decrease of 16.8 fold in OCI compared to control flies (Fig. 6C; \(P < 0.05\)). The PD flies exposed to 10, 20 and 40 \(\mu\)M of myricetin showed a significant increase of 2.4, 3.6 and 5.2 folds in OCI compared to unexposed PD flies (Fig. 6C; \(P < 0.05\)). The PD flies exposed to \(10^{-3}\) M of L-dopa showed a significant increase of 16 folds in OCI compared to unexposed PD flies (Fig. 6C; \(P < 0.05\)).

**Discussion**

The results of the present study reveal that the exposure of PD flies to myricetin prevents the loss of cognitive impairments. In our earlier study, the exposure of myricetin to PD flies delayed the loss of climbing ability of the PD flies (expressing human \(\alpha\)-synuclein in the brain)\(^{14}\). In open field assay, the PD flies exposed to myricetin showed improved activity compared to the unexposed PD flies.

Dopamine signaling in *Drosophila* has been shown to mediate voluntary movements besides other functions\(^{10}\). Dopamine in *Drosophila* is synthesized in a similar way as in mammals by the action of tyrosine hydroxylase on tyrosine\(^{15}\). In our earlier study, myricetin showed the protective effect against the loss of dopaminergic neurons. The antioxidant potential of myricetin was attributed for its protective effect\(^ {16}\). This protection results in maintaining the dopamine level as the dopaminergic neurons are the main site of dopamine production\(^ {10}\).

The effect of PD on sexual behaviour has not been properly understood. Hence, we decided to study the effect of myricetin on Courtship Index (CI) exhibited by PD flies. Several neuromodulators such as dopamine and serotonin regulates the male sexual behaviour in mammals and in *Drosophila* dopamine has been reported to enhance mating\(^ {17,18}\). In our study, the PD flies showed a significant decline in the CI, this is due to the loss of dopaminergic neurons responsible for producing dopamine. A reduction in the dopaminergic neurons seems to be responsible for the reduction in CI. The exposure of myricetin to PD flies results in an increase in CI which supports the protection of dopaminergic neurons by the myricetin. PD patients are reported to have olfactory deficits and the olfactory impairment gets severe as the disease progresses\(^ {19}\). Olfactory impairments have been considered as one of the earlier non-motor traits of PD\(^ {20}\). Dopaminergic neurons are involved in different types of signaling necessary for olfaction\(^ {21}\). It has been suggested that calyx is involved in the process of odour learning in *Drosophila*\(^ {22}\). A number of *in vitro* and *in vitro* studies suggested that the dopaminergic neurons in the olfactory bulb are involved in modulating information and behavioural odour discrimination. It has been reported that flavonoids are concentrated in the brain and activate ERK-CREB and Akt-CREB mediated memory, and thus could play a promising role for enhancing memory\(^ {24}\). The inhibition of tyrosine hydroxylase by thimerosal leads decreased dopamine level which in turn causes dopamine related behavioural alterations in *Drosophila melanogaster*. The flies showed reduced locomotion, climbing ability and increased oxidative stress\(^ {25}\). In our study, PD flies exposed to myricetin showed dose dependent increase in OCI. This is possibly due to the protection of dopaminergic neurons by myricetin as is evident by our earlier published work\(^ {16}\). These transgenic flies mimicked the cognitive impairments associated with the PD, hence any improvement in the cognitive impairment after
being exposed to flavonoids (in the present study myricetin) supports its therapeutic effects against the PD symptoms.

PD is also characterized by inflammation mitochondrial dysfunction, iron accumulation and oxidative stress. In the present fly model based on Gal4-UAS system, the human α-synuclein is expressed and leads to the formation of Lewy bodies (LBs) resulting in the death of dopaminergic neurons. A number of natural plant products have been reported to curtail the progression of the disease either by reducing the oxidative stress or by providing a protection against the damage of dopaminergic neurons. The neuroprotective effects of natural plant products have been attributed to the inhibition of monoamine oxidases and to modulate the content of neurotransmitters such as dopamine, norepinephrine and serotonin in the substantia nigra.

Besides having the antioxidant potential and free radical scavenging activity, number of flavonoids can also interact with α-synuclein and inhibit α-synuclein fibrillation. The pathogenesis of PD involves the propensity for αS to aggregate and form fibrils which leads to the formation of LBs which further damage the dopaminergic neurons. Caruna et al. have studied the effects of 14 phenolic compounds including myricetin on the inhibition of αS aggregation oligomers as well as disaggregation. They found that the compounds were effective in reducing the formation of αS aggregates as well as disaggregates already formed oligomers. Myricetin has been reported as one of the most promising agents against the post ischemic brain neurodegeneration due to its powerful antioxidant and antifibrillary tangles. Zhu et al. have shown that baicalein not only inhibits the formation of α-synuclein fibrils but also disaggregates the existing fibrils. Myricetin has shown antifibrillogenic and fibril destabilizing effects for α-synuclein fibrils in vitro.

Molecular docking has proved to be an important tool to computationally/theoretically calculate and rationalize the design and various conformations of upcoming chemotherapeutic drugs. The design of molecules that can recognize specific sequences and structures of nucleic acids plays an important role both for understanding nucleic acid molecular recognition. Interaction between myricetin and α-synuclein fibrils (6FLT), were taken into consideration to study the possible docking modes between the flavonoid and the protein. In order to investigate its pharmaceutical significance, myricetin was docked with 6FLT and various interactions were explored. The positive interaction between myricetin and α-synuclein suggests that it is potent in inhibiting the aggregation of α-synuclein. The molecular structural requirements that appear necessary to provide a flavonoid the ability to inhibit α-synuclein fibrillation were determined to be a vicinal dihydroxy-phenyl moiety. The presence of this moiety suggests that the myricetin was effective in inhibiting the α-synuclein fibrillation. This is also supported by docking studies and the interaction between myricetin and α-synuclein exhibit free energy of binding value of -283.76 kcal mol. It has been reported that physiological concentration of αS is about 1 μM in normal human brain and 70 pM in cerebrospinal fluid. The concentration of the flavonoid or its metabolites may reach up to 10 μM.

Most of the flavonoids are able to cross blood brain barrier (BBB) and the protective effect is attributed to their antioxidant potential due to the presence of aromatic ring by which they can performing chelating action. The study on rats showed that myricetin reduced the toxic effects of 6-OHDA in the substantia nigra due to reduction in the oxidative stress via iron chelating properties. The extract of Bacopa monnieri and Macuna pruriens (having myricetin as one of the component) showed a significant increase in spontaneous locomotor activity and grip strength test in MPTP model of PD. Myricetin exert neuroprotective effect in Drosophila exposed to rotenone by reducing oxidative stress, protecting the loss of dopaminergic neurons and by improving the climbing ability of flies.

Conclusion

The results of our present study showed that the myricetin interact with α-synuclein and forms energetically favoured complex which may be helpful in reducing the formation of αS aggregates thereby preventing the formation of Lewy bodies (LBs). It also reduced the cognitive impairments which may also be attributed to the neuroprotective property of myricetin as it is a powerful antioxidant. Overall, our results here have shown that myricetin is effective in reducing the cognitive impairments exhibited in transgenic Drosophila model of PD.
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Conflict of interest
Authors declare no competing interests.

References


