Protective Effect of Apigenin in Transgenic *Drosophila Melanogaster* Model of Parkinson's Disease

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Summary

Objective: To study the effect of apigenin in transgenic *Drosophila melanogaster* model of Parkinson's disease. **Methods:** In the present study the effect of apigenin at various doses was studied on the climbing ability of the transgenic *Drosophila melanogaster* expressing normal human α-synuclein in the neurons. The selected doses of apigenin were 0.1, 0.5 and 1.0 µl/ml of the culture medium. The Parkinson disease model flies were allowed to feed on the diet supplemented with apigenin for 21 days. **Results:** A significant dose dependent delay in the loss of climbing ability was observed. **Conclusion:** The results suggest that the apigenin is potent in delaying the climbing disability of Parkinson disease model flies and also supports the utility of this model in studying Parkinson's disease symptoms.

Key Words: Apigenin. Parkinson disease. transgenic *Drosophila*. climbing ability.

Introduction

Parkinson's disease (PD) is a common neurodegenerative syndrome characterized by the loss of dopaminergic neurons in the substantia nigra, formation of filamentous intraneuromal inclusions (Lewy bodies) and an extra pyramidal movement disorders [1]. PD is usually classified as a movement disorder, although it also gives rise to several non-motor types of symptoms such as sensory deficits, cognitive difficulties or sleep problems [2]. There are genetic models of PD based on α-synuclein, primarily the transgenic over expression of mutant or wild type forms in mice or flies [3-6]. Over expression of either wild type or mutant α-synuclein in *Drosophila* leads to lewy body like synuclein containing inclusions and loss of dopaminergic neurons as well as a behavioural abnormality that appears to be corrected by levodopa or dopaminergic agonist [1, 7]. Apigenin is one of the several active ingredients found naturally in many fruits and vegetables [8]. Besides having antigenotoxic , anti-inflammatory and free radical scavenging properties it also act as a cell growth inhibitor, anti-carcinogen and enzyme inhibitor [9-12]. In the present study the effect of apigenin was studied on the locomotor ability of Parkinson disease model flies exhibiting α-synuclein in the neurons.

Methods

Drosophila stocks

Transgenic fly lines that expresses wild type human alpha synuclein under UAS control in neurons "(w[*]; P{w[+mC]=USA–Hsap/SNCA.F}"5B and GAL4 "w[*]; P{w[+mC]=GAL4-elavL}3" were obtained from Bloomington Drosophila stock centre (Indiana University, Bloomington, IN). When the males of UAS-Hsap/SNCA.F strains are crossed with the females of GAL4-elav.L (vice-versa) the progeny will express the human α-synuclein in the neurons [1].

Drosophila Culture and Crosses

The flies were cultured on standard *Drosophila* food containing agar, corn meal, sugar and yeast at 25^{o}C (24±1) [13]. Crosses were set up using six virgin females of UAS-Hsap/SNCA.F5B were mated to three males of GAL4-elav. The progeny will express the human α-synuclein in the neurons and the flies were referred as Parkinson disease (PD) flies. First, the climbing assay was performed for the PD flies and UAS-Hsap/SNCA.F (control). The PD flies were exposed to different doses of apigenin mixed in the culture medium. Apigenin was added in the medium at final concentrations of 0.1, 0.5 and 1.0 µl/ml. The UAS-Hsap/SNCA.F act as a control. The vials of PD flies without the apigenin act as a positive control.

Drosophila climbing assay

The climbing assay was performed and described by Pedleton et al. [7]. Ten flies were placed in an empty glass vials (10.5 cm x 2.5 cm). A horizontal line was drawn 8 cm above the bottom of the vial. After the flies had acclimated for 10 min at room temperature, both controls and treated groups were assayed at random, to a total of 10 trials for each. The procedure involved gently tapping the flies down to the bottom of the vials. The number of flies above the mark of the vial was counted after 10 sec of climbing and repeated for 10 times to get the mean number above the mark of flies in this vial. These values were then averaged, and a group mean and standard error were obtained. The mean values of various fly groups were statistically compared using an unpaired group of the student *t*-test. All behavioral studies were performed at 25^{o}C under standard lighting conditions.

Results and discussion

The climbing response of control flies remained essentially unchanged over 21 days in a time course evaluation (Fig. 1). From the day 9 on however, the response of the PD flies were significant lower than those of the control. Based on these results, 21 days as standard duration of treatment was selected for the subsequent treatments with various doses of apigenin. The climbing assay was performed after 21 days of the exposure to various doses of apigenin. The exposure of PD flies to 0.1 µl/ml of apigenin showed a significant delay in the loss of climbing ability. (Fig. 2). Similarly, the exposure of PD flies to 0.5 and 1.0 µl/ml of apigenin in the culture medium significantly delayed the climbing disability in the PD flies (Fig. 2). The results of the present study reveal that the apigenin significantly delayed the climbing disability of the PD flies.

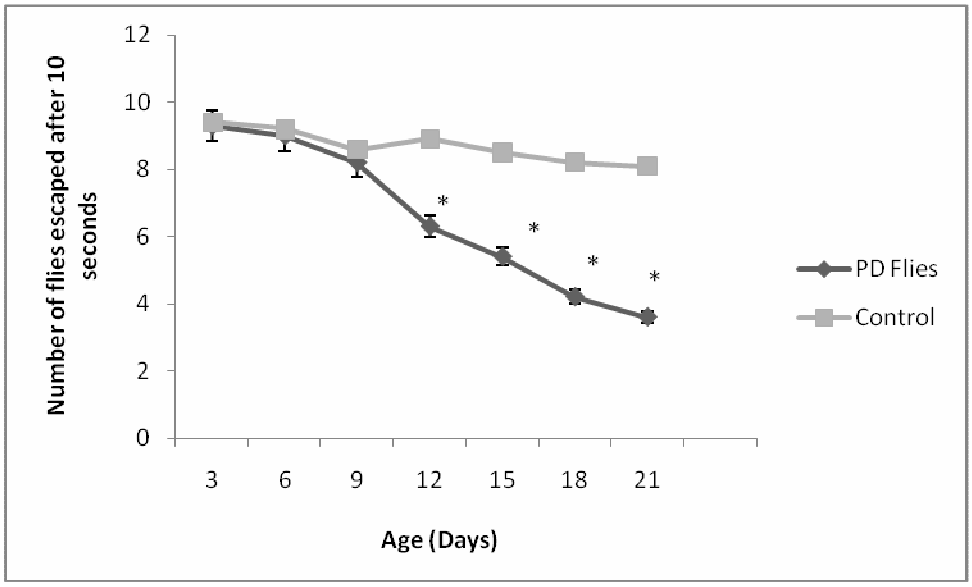

Fig. 1 Climbing ability in Parkinson disease (PD) flies and control for a period of 21 days. The values are the mean of 5 assays (* significant with respect to control $p < 0.01$).

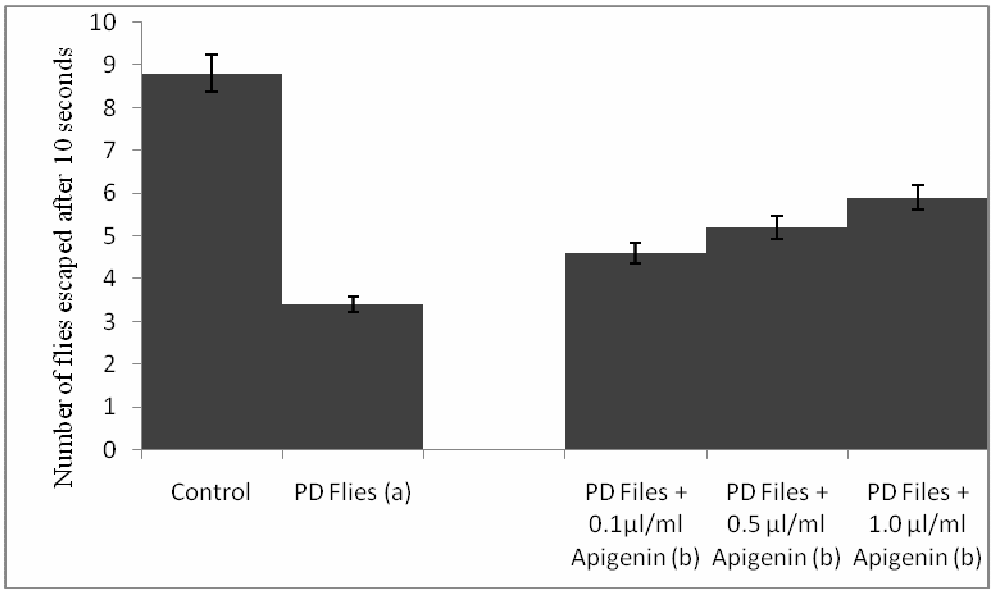

Fig.2 Effects of apigenin on the climbing ability. The flies were allowed to feed on the diet supplemented with apigenin for 21 days and then assayed for climbing ability. The values are the mean of 5 assays (a- Significant with respect to control $p<0.01$; b- significant with respect to PD flies $p< 0.05$).

A time dependent loss of dopaminergic neurons in the dorsomedial group and the intracellular aggregates of α-synuclein (Lewy bodies) were reported by Feany and Bender in the transgenic flies. These changes were followed by the functional loss in climbing ability [1]. PD is characterized by several abnormalities, including inflammation, mitochondrial

dysfunction, iron accumulation, and oxidative stress [7]. Both wild type and mutant α-synuclein form amyloid fibrils resembling those seen in lewy body as well as non-fibrillary oligomers, termed "protofibrills". The accumulation of α-synuclein leads to the toxicity and oxidative stress [14–15]. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates [6]. One hypothesis proposed that the metabolism of the neurons that are being lost in the PD produces endogenous toxins such as hydrogen peroxide, free radicals etc. that leads to the loss of the neurons over time [16]. However, in our present study the treatment of PD flies with apigenin showed the protective effect and reduced the possibility of the loss of climbing ability in PD flies as the age increases. The protection is may be due to the reduction in the oxidative stress or due to the inhibition of the expression of the α-synuclein or preventing the damage of dopaminergic neurons. Oxidative stress has been hypothesized to be linked to both the initiation and the progression of PD. The doses selected for the present study have delayed the end point. Apigenin is not a mutagenic agent [17], but the higher doses have been reported to be genotoxic in cultured human peripheral blood lymphocytes and in Chinese hamster V79 cells as it intercalate DNA molecules [18 – 19]. The present study was on the *Drosophila* model of PD developed by Feany and Bender (2000), expresses the human wild type α-synuclein in the neurons of the fly, with consequent locomotor dysfunction. There are considerable evidences suggesting that mitochondrial dysfunction and oxidative damages may play a role in the pathogenesis of PD. Several natural plant products have been reported to modulate the effects of PD [20]. Flavonoids have been reported to be neuroprotective by scavenging reactive oxygen species [21]. The proteomic analysis of this panneural expression of human wild type α-synuclein in the transgenic flies showed a differential expression of proteins indicating a perturbation of molecular pathways involving metabolism and signaling [22]. Gene expression changes for genes in these molecular pathways have been shown to be greatest in this model at the pre-symptomatic stage, when the potential for neuroprotection is greatest, thus validating this model for identifying potential targets for neuroprotective strategies [23]. The results in the present study suggest that the transgenic fly model mimics the motor impairments associated with PD and a climbing assay can be performed to determine whether or not a variety of compounds or drugs mixed in the fly culture medium prevent the progressive loss of climbing ability [7].

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