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Ameliorative influence of vildagliptin on genetic model of neurodegenerative diseases in Drosophila melanogaster

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ABSTRACT

Background: Neurodegenerative disorders (ND) are characterized by progressive loss of selectively vulnerable populations of neurons, which contrasts with select static neuronal loss. Self-association of amyloid-beta (Aβ) or α-synuclein peptides into fibrils and/or plaque like aggregates causes neurotoxicity. Hence, identification of specific compounds that either inhibit the formation of Aβ or α-syn-fibrils makes an appealing therapeutic strategy in the development of drugs. In the present study, we investigated the protective effect of vildagliptin (VDG) (oral hypoglycemic agent) on genetic models of ND in *Drosophila melanogaster*.

Methods: The disease causing human Aβ42 peptide or α-syn was expressed pan-neuronally (elav-GAL4) or dopamine neurons (DDC-GAL4) using the UAS-GAL4 system. Flies were either grown in food media with or without vildagliptin (1, 5, or 10μM). This was followed by fecundity, larva motility and negative geotaxis assay (climbing) as a measure of neurodegeneration.

Results: Elav-Gal4<Aβ flies showed significant decrease in larva contraction and motility indicative of neurodegeneration. However, flies grown on vildaglitptin (VDG) showed dose-dependent and significant increase in larva motility in comparison with flies grown on food media only. Interestingly, the treatments did not affect fecundity when compared with normal food control. Moreover, flies grown on VDG showed no significant change in lifespan. DDC-GAL4<α-syn flies showed significant decrease in larva motility and climbing activity which was ameliorated by supplementation of flies food media with VDG suggestive of neuroprotective activity.

Conclusion: Findings from this study showed that vildagliptin is a potential neuroprotective agent in genetic or familial forms of neurodegeneration.

1. Introduction

Gene defects play a major role in the pathogenesis of degenerative disorders of the nervous system[1]. Interestingly, knowledge obtained from genetic studies has allowed the explanation of the molecular mechanisms responsible for the aetio pathogenesis of neurodegenerative disorders, . Neurodegenerative diseases (ND) are complex multifactorial diseases including the inherited, sporadic and transmissible forms while gene-environment interplay could be major predisposing factor[2]. Synucleinopathies such as Parkinson disease (PD) and dementia with Lewy bodies are ND characterized by the abnormal accumulation of aggregates of α-synuclein protein in neurons, nerves or glial cells[3]. However, amyloidopathy such as Alzheimer's disease (AD) comprises of aggregated amyloid-beta (Aβ)

plaques, neurofibrillary tangles (NFT) and cholinergic neuron loss in the brain[4]. Hence, the development of better therapeutic strategies that can halt the pathogenesis of Aβ formation and pathological aggregation. *Drosophila melanogaster* is an attractive model for studying the mechanisms of ND due to its relatively short lifespan, convenient husbandry and facile genetics. The GAL4/upstream activating sequence (UAS) system is widely used for targeted gene expression owing to the ability of the yeast GAL4 transcription factor which activates transcription of its target genes by binding to UAS cis-regulatory sites. In Drosophila, the two components are carried in separate lines allowing for numerous combinatorial possibilities. The driver lines provide tissue-specific GAL4 expression and the responder lines carry the coding sequence for the gene of interest under the control of UAS sites [5]. Thus, genetic form of AD and PD will be generated using the UAS-GAL4 system.

Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor (an oral hypoglycemic agent) has been demonstrated to down-regulate striatal RAGE/NF-κB signaling, TNFα, tumor necrosis factor alpha; ICAM, intracellular adhesion molecule; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase[7], and exerts antioxidant and anti-inflammatory actions[8], cognition enhancement[9] and permeate the blood brain barrier [10]. Thus, this study sought to evaluate the protective influence of vildagliptin on genetic models of neurodegenerative diseases in drosophila.

2. Methods

2.1 Materials and drugs

Vildagliptin (Norvatis Healthcare Pvt. Ltd. South Africa), diethyl-ether (Guangdonng Guangbua Sci. Tech CO. Ltd. China), sugar, corn flour (Latyf food and beverages Ventures Ltd, Ogun State, Nigeria), yeast (STD Industries Ltd, China), agar (Himedia Laboratories Pvt. Ltd, Mumbai, India), malt extract (Sigma Aldrich, Germany), propionic acid, methyl-*p*-hydroxybenzoate (LOBA Chemie, Mumbai, India), orthophosphoric acid (Thermo Fischer Scientific India Pvt. Ltd., Mumbai, India), phosphate buffered saline (Gibco Technologies, USA).

2.2 Fly stock

The strains of *Drosophila melanogaster* used in this study include; *UAS-α-synuclein/CyO; Ddc-Gal4/TM3,* UAS-Aβ42arc/TM3, Elav-GAL4/FM, Canton-Special (CS), and white eye mutant (W1118) were obtained from Dr. Rakesh Mishra Laboratory, Centre for Cellular and Molecular Biology, India. The flies were maintained in the laboratory at a temperature between 18°C – 23°C.

Drosophila media and culture

Virgin *w*1118, elav-Gal4, ddc-Gal4 were mated with either UAS-syn or UAS-Aβ. The crosses were maintained on sugar, cornflour, yeast, malt-agar media treated with propionic acid, orthophosphoric acid and methyl-p-hydroxybenzoate to inhibit microbial growth or vildagliptin (1, 5 and 10 mM) in media. Stocks were maintained on solid media for 2 to 3 weeks before transfer onto new media to re-culture. Stocks were kept at room temperature (22 °C ± 2 °C) while crosses and experiments were carried out at 22 and 26 °C[11].

2.3 Larva motility assay

Ten larva per group in triplicate were collected and transferred to a petri dish containing freshly prepared 2% Agar, placed over a 2B paper with a 0.1cm^2 grid. The larva were left to acclimatize for 60s and number of lines crossed in 60s were recorded for elav-Gal4>UAS-Aβ; ddc-Gal4>UAS-syn, elav-Gal4>w1118, ddc-Gal4>w1118 as negative control.

2.4 Negative geotaxis assay

To assess climbing behaviour, 20 adult flies (in triplicate) were placed at the bottom of a clean vial and a second identical vial was placed above. The bottom vial was labelled per centimeter. After 8 seconds, the number of flies that passed the 8cm mark were recorded for elav-Gal4>UAS-Aβ; ddc-Gal4>UAS-syn, elav-Gal4>w1118, ddc-Gal4>w1118 as negative control. The climbing behaviour for cohorts of flies were recorded periodically for 28 days.

2.5 Fecundity assay

The Canton-special (CS) strain was used for the fecundity assay. The fecundity assay began at 24hours after mating. The number of eggs laid were counted every 24/48h by viewing under the stereomicroscope until the dark pupa emerged. The number of dark pupa as well as the number of flies that emerged from each vial were recorded.

Longevity assay

The longevity of a population of flies provides a robust estimate of their general health. It allows monitoring of the effect of genotype, environment or drug on *Drosophila* survival throughout its lifespan. Twenty (20) male/female flies expressing the gene of interest were placed on standard fly meal at 22±2°C. The flies were counted every 2 days and transferred to new vials. At each time point, the number of flies that died was counted until the last fly in the vial died.

Statistical analysis

Values are expressed as mean±SD. Statistical level of significance were analyzed using one- or two-way ANOVA followed by multiple comparison tests using GraphPad Prism 6 (GraphPad Software, Inc.CA, USA).

3. Results

3.1 Larva motility assay: Effect of vildagliptin on larva motility in Aβ expressing flies

Aβ expressing flies grown on normal food media showed severe locomotion deficit (Fig. 1). One way ANOVA revealed significant effect of treatment [F (4, 70)= 333.30; P < 0.0001]. However, flies grown on food supplemented with vildagliptin (1, 5 and 10mM) showed significant improvement in larva motility, suggestive of its ameliorative effect (Fig. 1).

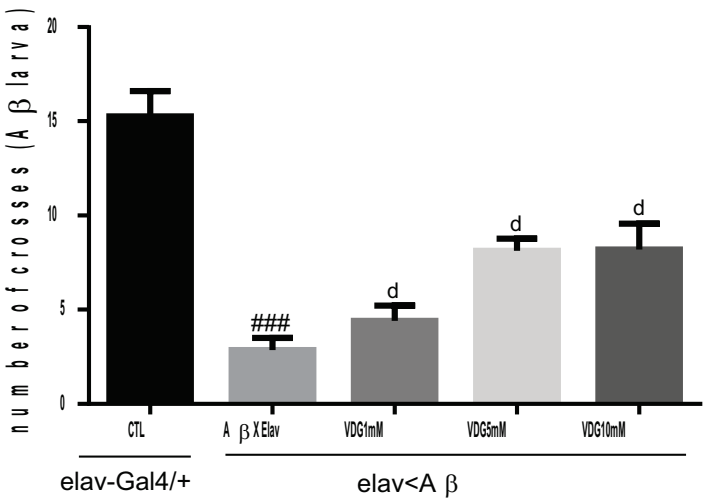

Fig. 1: Effect of vildagliptin on larva motility in Elav-Gal4>UAS-Aβ *Drosophila* transgenic model. Bar chart represents mean ± SD; ###P<0.001 versus normal control; dP<0.0001 elav-Gal4>UAS-Aβ. Analysis by one-way ANOVA, followed by Tukey's multiple comparison test

3.2 Effect of vildagliptin on larva motility of α-synuclein expressing flies

As shown in figure 2, DDC-GAL4 > α-syn flies grown on normal food media showed significant deficit in locomotor activity. Furthermore, one way ANOVA revealed significant effect of treatment [F (4,70)=227.3; P < 0.0001]. Post hoc analysis showed that the flies grown on food supplemented with vildagliptin showed significant increase in larva locomotion activity in comparison with food media only.

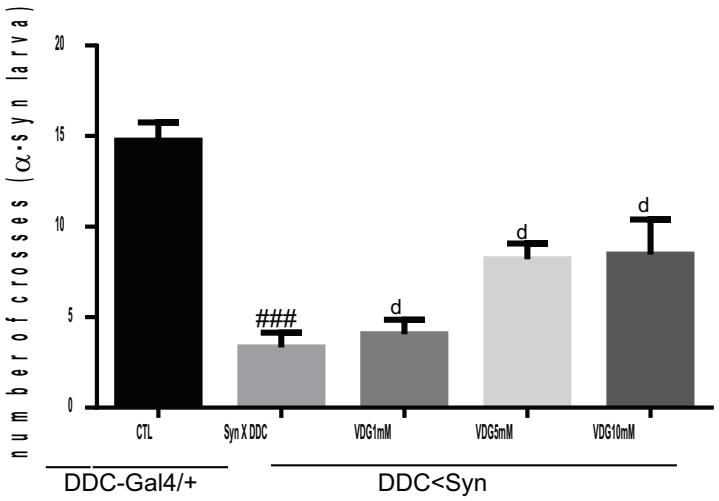

Fig. 2: Effect of vildagliptin on larva motility in ddc-Gal4>UAS-Syn in *Drosophila* transgenic model. Values are expressed as mean±SD; ###P<0.001 versus normal control; dP<0.0001 versus α-syn-DDC control. Analysis by one-way ANOVA, followed by Tukey's *Post hoc* multiple comparison test

3.3 Fecundity assay

Vildagliptin produced no significant effect on the number of eggs counted at all concentrations, relative to the flies grown on normal food media (Fig. 3a). Vildagliptin (1, 5, or 10mM) caused significant decrease in mean number of dark pupa in comparison to normal media (Fig. 3b). The mean number of eclosed flies were significantly reduced ($p< 0.05$, $p< 0.01$) when compared to flies grown on food media only (Fig. 3c).

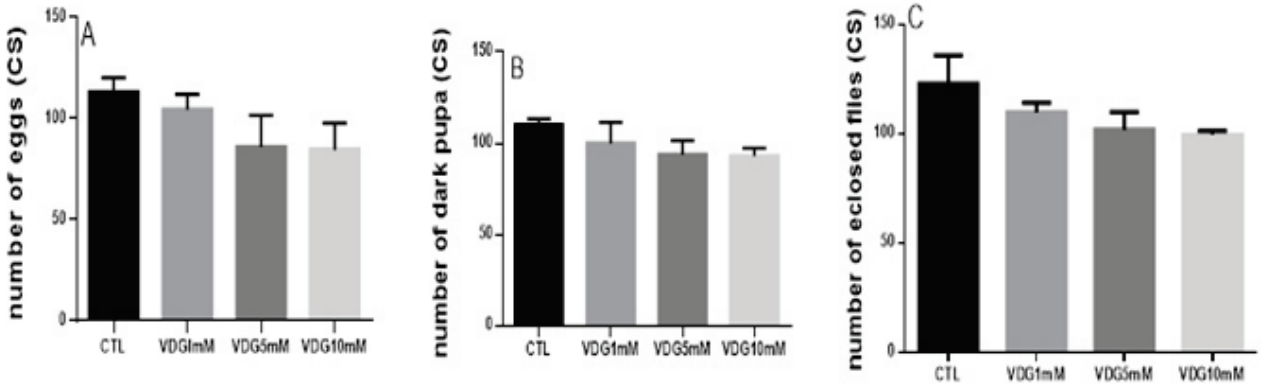

Figure 3A-C: Effect of vildagliptin on (A) number of eggs, (B) number of dark pupa, (C) number of flies based on the fecundity test carried out in *Drosophila*. Bar chart represents mean ± SD; Analysis by one-way ANOVA, followed by Tukey's *post hoc* multiple comparison test.

3.4 Effect of vildagliptin on climbing ability of Elav<Aβ

In order to probe the anti-amyloidogenic potential of vildagliptin in improving the locomotion of the adult flies, time course climbing activity was carried out over a period of 28 days. One-way ANOVA showed a significant ($F (4, 50)$; $P < 0.0001$) improvement in the climbing activity of flies treated with 1mM and 5mM of vildagliptin on day 1, relative to the flies grown on normal feed. However, vildagliptin (10mM) showed no significant effect. The effect of vildagliptin on climbing activity on day 7 showed significant and concentration-dependent ($P < 0.0001$, $P < 0.01$ respectively) increase in climbing activity relative to the untreated group. Results obtained on days 14, 21 and 28 revealed a significant [$F(4,50)=56.56$;$P<0.0001$] increase in performance index, with peak effect produced at a concentration of 10mM, relative to the group fed with normal food media (Fig. 4).

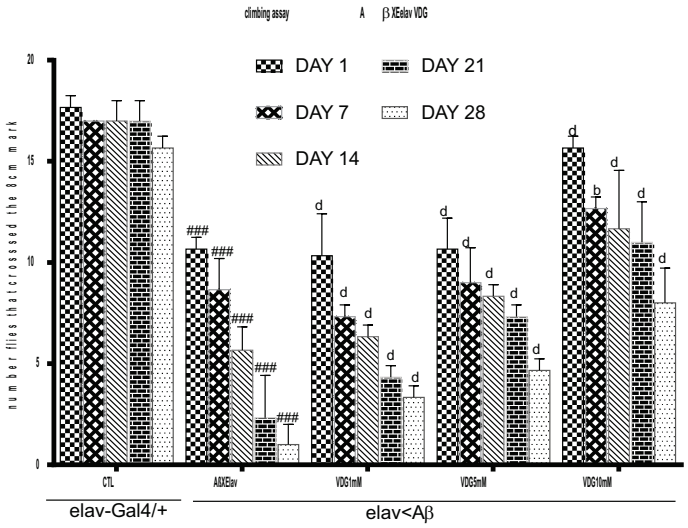

Figure 4: Effect of vildagliptin on the climbing activity of Drosophila transgenic model. Bar chart represents mean ± SD; $^{###}P < 0.0001$ versus control; $^{d}P< 0.0001$ versus elav-Gal4>UAS-Aβ. Analysed by two-way ANOVA followed by Tukey's multiple *post hoc* comparison test.

3.5 Effect of vildagliptin on climbing ability of α-syn flies

Two-way ANOVA showed significant effect of treatments [F(4,50)=141.40;P<0.0001] (Fig. 5). Mating of ddc-Gal4 with UAS-α-syn caused time course decrease in climbing activity of flies grown on normal media only. However, flies grown on media supplemented with vildagliptin caused significant improvement in climbing activity over a period of 28 days when compared with flies grown on normal media.

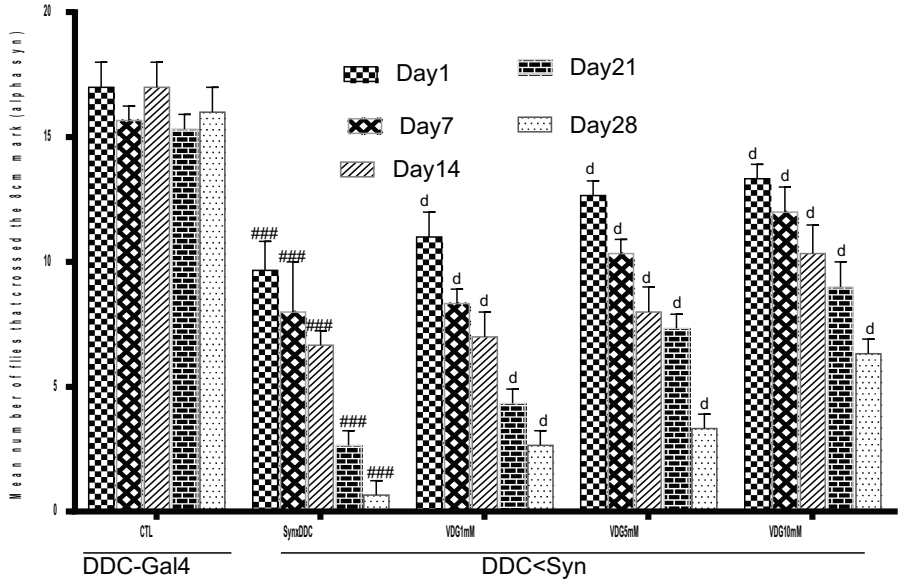

Figure 5: Effect of vildagliptin on the climbing activity in ddc-Gal4>UAS-syn Drosophila. Bar chart represents mean ± SD; ###P < 0.001 versus normal control; dP < 0.0001 versus ddc-Gal4>UAS-syn. Analyzed by two-way ANOVA followed by Tukey's multiple comparison test.

3.6 Effect of vildagliptin on lifespan of Elav-GAL4 strain.

Findings from this study showed that flies grown on food supplemented with vildagliptin (5 mM) caused slight improvement in the lifespan with better outcome at 10mM in comparison to flies grown on normal food (Fig. 6).

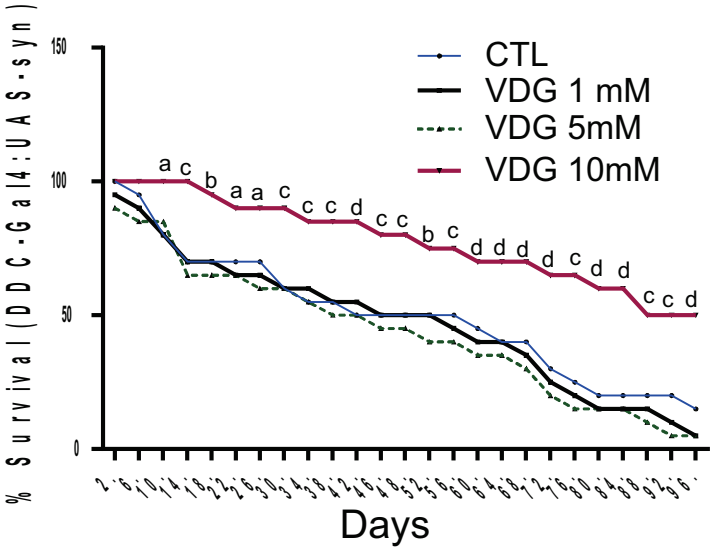

Figure 6: Effect of vildagliptin on lifespan of *ddc-Gal4>UAS-syn*. Values are expressed as mean±SD. aP< 0.05, bP< 0. 01, cP< 0.001, dP< 0.0001 versus control. Analysed by two-way ANOVA followed by Dunnett's multiple comparison test.

4. Discussion

Findings from this study showed that the mating of virgin elav-Gal4 or ddc-Gal4 flies with UAS- Aβ or UAS-syn flies, respectively, increased the expression of Aβ and α-syn in neurons suggestive of AD or PD in *Drosophila* evidenced in reduction of larva motility and climbing activity which were ameliorated by the supplementation of the food media with vildagliptin without affecting its fecundity.

Nds have similar cellular aetiopathogenesis including protein aggregation and inclusion body formation in selected brain regions [12]. Aggregation of soluble oligomers of amyloid beta is well linked to the development of synaptic dysfunction, neuronal apoptosis and through its adverse effect on synaptic structure and plasticity[13] Similarly, alpha-synuclein is a component of Lewy bodies, the pathological hallmark of Parkinson's disease (PD), and is also mutated in familial PD [14]. The toxic properties of α-synuclein are conserved from yeast to man, but the precise underpinnings of the cellular pathologies associated are still elusive, complicating the development of effective therapeutic strategies[15]. In this study, we combined molecular genetics with target-based approaches using the UAS-GAL4 system to establish an age-dependent post-translational modification, enhanced amyloid-beta and α-synuclein toxicity in Drosophila induced motor deficits in larva and adult flies as observed in larva motility and climbing assays, respectively. However, the supplementation of media with vildagliptin reversed the motor phenotype deficit suggestive of reduced aggregation of misfolded protein in the neurons. This findings corroborate earlier reported beneficial effect of vildagliptin in AD and PD models in rodents[16]. Larval Drosophila move up attractive chemical gradients, and down aversive ones. Although their movement is often characterized as a series of runs and directed turns, it can also be modeled as a continuous modulation of turning extent by the detected change in stimulus intensity as the animal moves through the gradient [16]. The climbing test or negative geotaxis on the other had is commonly used to assay the locomotion activity of flies, predictive of neuroprotective activity of new chemical entity. This method offers simplicity, objectivity and robust throughput. In this study, elav-Gal4>UAS-Aβ and ddc-Gal4>UAS-α-syn flies grown on normal food showed motor deficits during development evidenced in significant decrease in larva contraction and crawling ability. Moreover, and reduction in climbing ability from day 20 indicative of neurodegeneration. However, elav-Gal4>UAS-Aβ and ddc-Gal4>UAS-α-syn flies grown on media supplemented with vildagliptin showed significant improvement in larva motility and climbing activity indicative of neuroprotection[17]. In *Drosophila* larvae, intact synaptic transmission from motor neurons results in coordinated peristaltic movement of the larval muscles causing crawling behavior of the larvae. Defects in larval locomotion behavior are often associated with neuronal and synaptic dysfunction [18,19].

In *Drosophila*, a reduction in fertility could be due to several reasons, including fewer eggs being laid by mated females and/or reduced hatchability/increased mortality of the laid eggs during development[20]. Therefore, to determine the cause of reduction in fertility of mates of Canton special (CS) strain, the number of eggs laid, the number of dark pupa which emerged and the resulting number of enclosed flies which emerged were counted periodically. Interestingly, we do not observe any significant change between flies maintained on normal media and vildagliptin supplemented media.

There is a strong interplay between neurodegeneration and reduction in lifespan[21], . Interestingly, degradation of alpha-synuclein by dendritic cell factor has been shown to extends lifespan and ameliorate motor deficit in this study, supplementation of flies' media with vildagliptin confers longevity in flies expressing alpha-synuclein suggestive of its possible anti-aging property.

5. Conclusion

Findings from this study showed that vildagliptin improves motor activity and confers longevity in Drosophila expressing amyloid beta or alpha synuclein suggestive of possible neuroprotective action on genetic or familial forms of neurodegeneration. Thus, could be a potential adjunct in the management of Alzheimer's and Parkinson disease.

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