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Full-Length Research Article

Angiotensin-converting Enzyme Inhibitor Captopril Prevents Neuronal Overexpression of Amyloid-beta and alpha-synuclein in *Drosophila melanogaster* Genetic Models of Neurodegenerative Diseases

Ishola I.O.[a], Afolayan O.O.[b], Badru W.A.[c], John N.[a], Adeyemi O.O.[a]

[a]*Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria*

[b]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria*

[c]*Department of Pharmacology, Lagos State University, College of Medicine, Ikeja, Lagos State, Nigeria*

[d]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria*

Summary: Parkinson disease (PD) and Alzheimer's disease (AD) are progressive neurodegenerative disorders characterized by loss of selective neurons in discreet part of the brain. The peptide angiotensin II (Ang II) plays significant role in hippocampal and striatal neurons degeneration through the generation of reactive oxygen species. Blockade of the angiotensin converting enzyme or ATI receptors provides protection in animal models of neurodegenerative diseases. In the present study, the neuroprotective effect of captopril was investigated in *Drosophila melanogaster* model using the UAS-GAL4 system to express the *syn*uclein and Aβ42 peptide in the flies' neurons. The disease causing human Aβ42 peptide or α-syn was expressed pan-neuronally (elav-GAL4) or dopamine neuron (DDC-GAL4) using the UAS-GAL4 system. Flies were either grown in food media with or without captopril (1, 5, or 10μM). This was followed by fecundity, larva motility, negative geotaxis assay (climbing) and lifespan as a measure of neurodegeneration. Elav-Gal4<Aβ or DDC-GAL4<α-syn flies displayed a significant decrease in larva motility when compared with normal control (w1118) which was reversed by the supplementation of the media with captopril (5 or 10 mM) indicative of neuroprotection. Interestingly, supplementation of flies' media with captopril improved climbing activity in Elav-Gal4<Aβ or DDC-GAL4<α-syn flies when compared with vehicle-treated only. Moreover, flies grown on captopril caused no significant change in lifespan. Findings from this study confirmed the neuroprotective action of captopril in genetic or familial forms of neurodegeneration.

Keywords: *Alzheimer's disease; captopril; Parkinson's disease; neurodegeneration; Drosophila melanogaster; UAS-GAL4 system*

**Address for correspondence: oishola@cmul.edu.ng; +2348033018908*

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INTRODUCTION

Neurodegenerative diseases represent a major threat to human health. These age-dependent disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years (Wyss-Coray, 2016). Extracellular deposits of Aβ and intraneuronal tau inclusions denote AD while intracellular inclusions of α-synuclein make up the Lewy pathology of PD (Jaiswal et al., 2012). Overexpression of these mutant proteins can give rise to disease-associated phenotypes (Jaiswal et al., 2012). Better elucidation of the cellular mechanisms for clearance of abnormal/misfolded proteins and of the effects of toxic protein accumulation on neuronal survival may allow the development of effective treatment for these disorders (Abeliovich and Gitler, 2016).

Various animal and cell culture models of PD and AD have been developed and have substantially contributed to the understanding of disease pathogenesis, however, none of the existing models fully recapitulates all pathological features of PD and AD, and each model provides opportunities to investigate certain aspects of the disease. Albeit, the UAS-Gal4 system allows for the definition of which cells are expressing a particular gene or its product and is a useful tool in investigating PD and AD. *Drosophila melanogaster* which have been genetically engineered to express the Gal4 gene in a particular section of tissue can be used in the study of PD and AD (Duffy, 2002; Bier, 2005. The fly genome is smaller with substantially less gene redundancy than that of mammals, while it contains homologs of approximately 70% of human disease-related genes. In this study, we characterized the physiological effect of overexpression of amyloid-β or α-synuclein in the nervous system of Drosophila melanogaster using the upstream activation sequence (UAS)/yeast transcriptional activator binding to UAS (GAL4) system (Duffy, 2002;

Ishola et al., 2021). The transgenic flies over-expressing amyloid-β or α-synuclein in the nervous system showed a moderate decrease in locomotor activity and median lifespan of adult flies. Amyloid-β protein precursor (AβPP) and the microtubule-associated protein tau (MAPT) are the two key players involved in Alzheimer's disease (AD) and are associated with amyloid plaques and neurofibrillary tangles respectively, two key hallmarks of the disease (Jucker and Walker, 2013). Drosophila models have been widely used to understand the complex events leading to AD in relation to aging (Ishola et al., 2021). Similarly, genetic and environmental risk factors are associated with PD. The genetic factors includes about 20 genes, such as SNCA, parkin, PTEN-induced kinase1 (pink1), leucine-rich repeat kinase 2 (LRRK2), ATP13A2, MAPT, VPS35, and DJ-1, whereas the environmental factors consist of oxidative stress-induced toxins (Adams et al., 2000; Abeliovich and Gitler, 2016). Drosophila melanogaster has emerged as an excellent model organism to study both environmental and genetic factors associated with PD, Thus, provide insights to the pathways relevant for AD and PD pathogenesis, facilitating development of therapeutic strategies.

The brain RAS is implicated in neurodegenerative disorders including PD and AD (Liao et al., 2014). In humans, Angiotensin converting enzyme activity is increased in the cerebrospinal fluid of PD and AD patients which is thought to reflect a response to increased brain inflammation (Schulz and Heusch, 2006). A genetic polymorphism in the ACE gene is associated with increased risk of PD (Huo et al., 2017). Moreover, inhibition of the angiotensin-converting enzyme (ACE) by a centrally acting ACE inhibitor retards symptoms of neurodegeneration, Abeta plaque formation and tau hyperphosphorylation in experimental models of AD (Quitterer and AbdAlla, 2020). Blockade of ACE, using captopril has been shown to exert neuroprotective effects in the striatum (Sonsalla et al., 2013). The purpose of the present study was to examine the neuroprotective effects of the ACE inhibitor captopril in an acute as well as a progressive neurodegeneration in *Drosophila melanogaster* model of PD and AD.

MATERIALS AND METHODS

Drugs and chemicals: Captopril (Alpa Laboratories ltd. India), diethyl-ether (Guangdong Guangbua sci. Tech CO. Ltd. China), sugar, corn flour (Benchmark foods and Spices Ltd, Lagos state, Nigeria), yeast (STK industries Ltd, China), agar (Himedia Laboratoies Pvt. Ltd, Mumbai, India), malt (Sigma Aldrich, Germany), propanoic acid (LOBA Chemie Laboatory Reagents & fine chemicals , Mumbai, India), methyl-p-hydroxy benzoate (LOBA Chemic Laboratory Reagents & fine chemicals , Mumbai, India), orthophosphoric acid (Thermo Fischer Scientific India pvt, Ltd, Mumbai, India), phosphate buffered saline (Gibco Technologies,USA).

Fly Stocks and Culture: Wild type W118, *ELAV-gal4/FM*, UAS-Aβ42/TM3, *Ddc-Gal4/TM3, UAS-α-synuclein/CyO,* and Caxton S (Cs) flies were obtained from Dr. Rakesh Mishra *Drosophila* Laboratory, Centre for Cellular and Molecular Biology, Hydrabad. India. Flies strains were maintained on standard cornflour, malt, yeast, sugar, orthophosphoric acid, propionic acid and agar at 26±2°C with 60-75% relative humidity and a 12h light/dark cycle

Construction of Aβ42 and *α-synuclein* transgenic *Drosophila* models: To construct the *UAS-Aβ42/TM3* or *UAS-α-synuclein/CyO* transgenic *Drosophila*, respectively, with Elav-GAL4/FM or *Ddc-Gal4/TM3* genetic background. Virgin females of Elav-GAL4/FM or *Ddc-Gal4/TM3* were mated with UAS-Aβ42 or *UAS-α-synuclein* transgenic *Drosophila*, respectively. *Elav-Aβ42* and *Ddc-α-synuclein Drosophila* were collected within 48 hours for lifespan or behavioural assays (Ishola et al., 2021).

Fecundity assay: Fecundity, also known as *reproductive rate* is a measure of the number of offspring produced by an organism over time. Assessment of fecundity allows monitoring of the effect of captopril on reproductive activity. Parameters recorded include, the number of eggs laid, number of dark pupa and the number of flies that emerged from the dark pupa through the course of the study. Cs fly strain was used; male and female mated at a ratio of 1:2 (5 males to 10 females) n=3 vials per group. The canton-special (CS) strain was used for fecundity studies. The fecundity assay began at 24 hours after mating. The number of eggs laid was counted every 24/48h by viewing under the stereomicroscope until the dark pupa as well as the number of flies that emerged from each vial were recorded.

Larval Motility: Third instar larvae were removed from their individual medium, after a quick wash in distilled water, twenty larvae from each group, comprising media only, media supplemented with captopril (1, 5, or 10µM) (n=3 vials) were gently placed on 2%$^{w}/_{v}$ agar slabs in 245mm×245mm square petri-dishes using a small paint brush; all on the surface of a 2B sheet with partitioned square boxes measuring 0.5cm by 0.5cm. The larvae was allowed to acclimatize for one minute and the number on line crosses in one minute was recorded.

Collection of female virgins: Collection of female virgins is easy. Females remain virgins for only 8-10 hours after eclosure and must be collected within this time frame. To obtain virgin flies, flies were anaesthetized using diethyl ether, after which they were placed under the stereomicroscope and the female virgins separated and collected.

Larval Motility Assay: The desired number of individual larva were collected, washed in phosphate buffered saline and transferred to a petri dish containing freshly prepared 2 % Agar (previously poured and allowed to solidify) and placed over a 2B paper with a 0.1cm^2 grid. Flies were left to acclimatize for a minute, until the desired number of larva has been counted. Female virgin flies of the DDC-Gal4/+ strain were crossed with male flies of the UAS-*syn* strain in the ratio 2:1, also female virgins of the Elav-Gal4/+ strain were crossed with UAS-Aβ in the ratio 2:1. On the 4th or 5th day of crossing, larva assessment was taken. The fly crossings used to larva motility are Elav<Aβ and DDC<*syn*, with Elav<W1118, DDC<W1118 as negative control (Ishola et al., 2021).

Negative Geotaxis Assay: To assess climbing behavior, 20 adult flies were placed at the bottom of a clean vial and second identical vial placed above. The bottom vial was labeled per centimeter. After 8 seconds, the number of flies that passed the 8cm mark was compared over time with the proportion of the control flies. The climbing behavior for a cohort of flies was followed periodically for 28days. The crossings used to assess this stringent assay were the Elav<Aβ and DDC<*syn*, with Elav<W1118, DDC<W1118 as negative control (Ishola et al., 2021).

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 as *Drosophila melanogaster* survival throughout its lifespan. Twenty (20) male/ female flies expressing the gene of interest were placed on standard fly meal at regulated temperature (18 -23ºC). The flies were counted every 2 days and transferred to new vials. At each time point, the number of flies that were observed to die and those that were lost to follow-up (for example, flies that escaped or were accidentally killed) were counted until the last fly in the vial died (Ishola et al., 2021)s.

Statistical Analysis

Data analyses were performed using GraphPad Prism software (GraphPad Software, Inc.). Results were expressed as mean and standard deviation values. One-way ANOVA was adopted for column analysis, while the grouped data were analyzed with the Two-way ANOVA. The results were analyzed by Tukey's *Post hoc* multiple comparison test.

RESULTS

Fecundity Assay

A ratio of 1:2 of Canton S strain of flies (5 females: 10 males) were grown on normal feed and captopril supplemented feed media (1, 5 and 10 mM). *Post hoc* analysis revealed significant decrease in the number of eggs laid [$F(3,20)=16.79, P<0.001$] (Fig. 1a), dark pupa [$F(3,20)=5.15, P<0.01$] (Fig. 1b) and eclosion [$F(3,20)=4.34, P<0.05$] (Fig. 1c) by captopril 10mM.

Larva Motility Assay

Effect of captopril on larva motility in *syn*uclein expressing flies.: One way ANOVA revealed significant effect of treatment [$F(4,42)=3.84$; $P < 0.01$]. moreover, Post hoc analysis showed that DDC-GAL4 > α-syn flies grown on normal food media showed significant deficit in locomotor activity. However, supplementation of media with captopril (5 or 10 mM) produced significant increase in larva locomotion activity in comparison with food media only (figure 2).

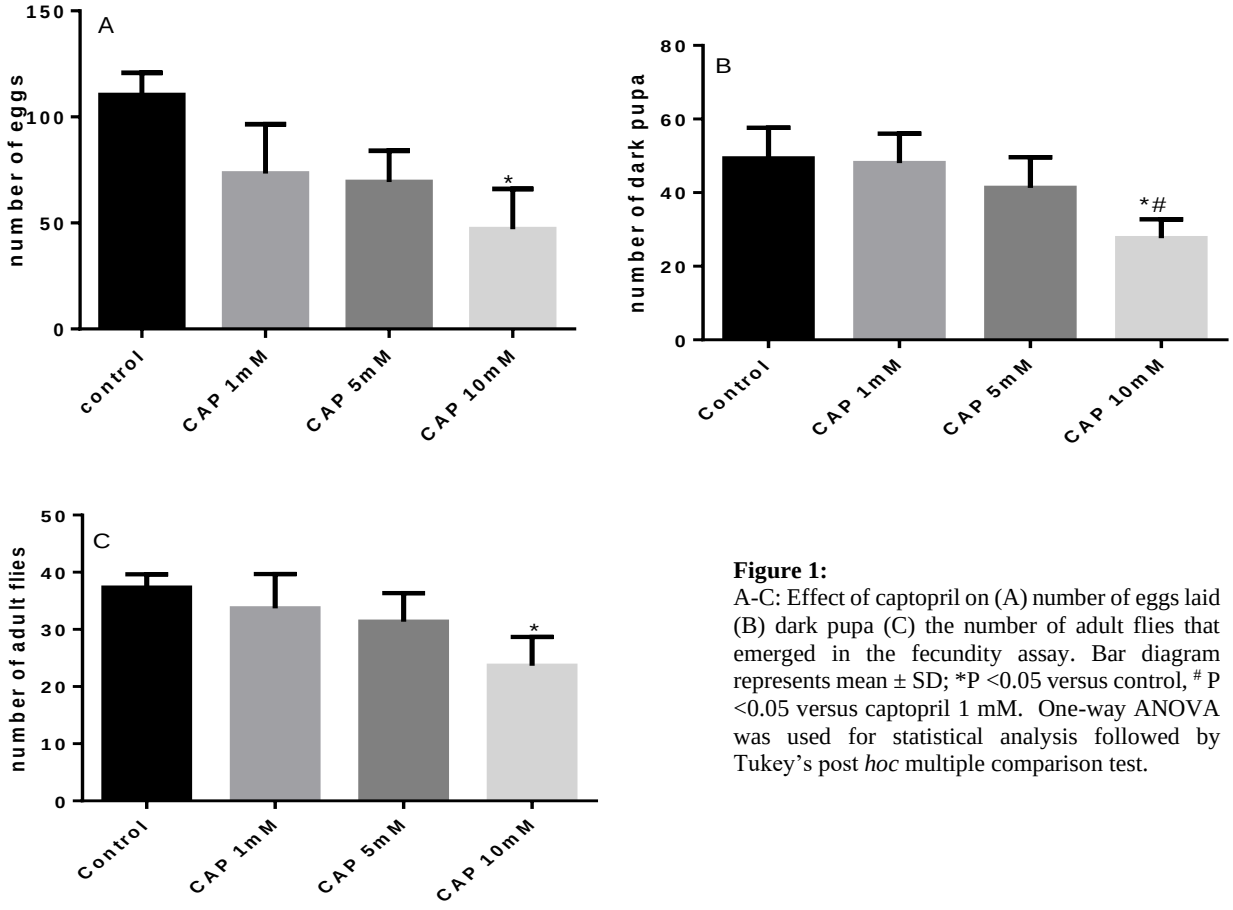


Figure 1:
A-C: Effect of captopril on (A) number of eggs laid (B) dark pupa (C) the number of adult flies that emerged in the fecundity assay. Bar diagram represents mean ± SD; *P <0.05 versus control, # P <0.05 versus captopril 1 mM. One-way ANOVA was used for statistical analysis followed by Tukey's post *hoc* multiple comparison test.

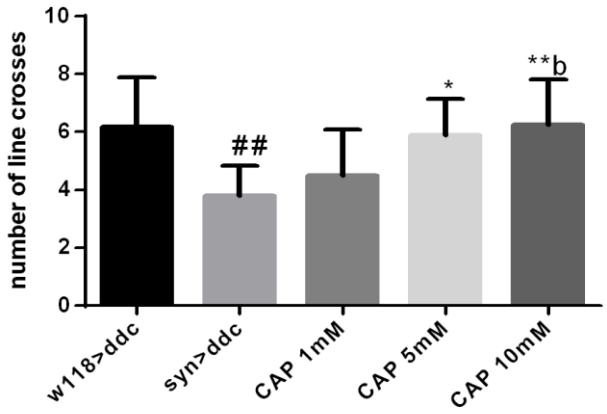


Figure 2:
Effect of captopril on the number of line crosses in UAS-*syn* < DDC-Gal4 larva. Values are expressed as mean ± SD. ##P<0.01 versus w1118; *P<0.01 versus *SYN*×DDC control; **P<0.01 versus *SYN*×DDC; bP<0.01 versus 1mM captopril. Statistical level of significant analysis by one-way ANOVA, followed by *Tukey's* multiple comparison test.

Effect of captopril on larva motility of Aβ expressing flies: Post hoc analysis showed that there was no significant change in the number of line crosses in UAS-Aβ < elav-Gal4 larva compared to control. Moreso, there was no significant difference in the number of line crossings in larva cultured on captopril supplemented feed media (1mM and 5mM) in comparison with the control. However, captopril (10mM) caused significant increase [F (4,45)=3.11; P<0.01] in number of line crosses in UAS-Aβ < elav-Gal4 larva (AD model) compared to those cultured in vehicle media (Fig. 3).

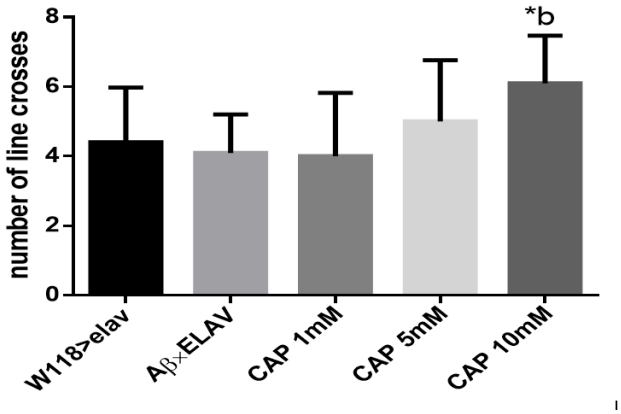


Fig 3: Effect of captopril on number of line crosses in UAS-Aβ < elav-Gal4 larva. values are expressed as mean ± SD.*p<0.05 versus Aβ×Elav control, bP<0.05 versus captopril 1Mm. Analysis was done by one-way ANOVA followed by Tukey's multiple comparison test.

Climbing Assay

Effect of captopril on climbing in Aβ expressing flies: Two-way ANOVA revealed significant effect of treatments on climbing activity [F(3,40)=30.56,p<0.001] (Fig.4). Post hoc analysis showed time course decrease in climbing activity with peak effect at day 28. The decline in climbing activity induced by overexpression of Aβ pan-neuronally was ameliorated in Aβ×Elav flies grown in media supplemented with captopril (5 and 10mM) (Fig. 4).

Effect of captopril on climbing in *syn*uclein expressing flies: Two-way ANOVA revealed significant effect of treatments on climbing activity [F(3,40)=31.16,p<0.001] (Fig.5). Post hoc analysis showed time course decrease in climbing activity with peak effect at day 28. The decline in climbing activity induced by overexpression of synuclein in dopamine neuron was ameliorated in syn×DDC flies grown in captopril (5 and 10mM) supplemented media (Fig. 5).

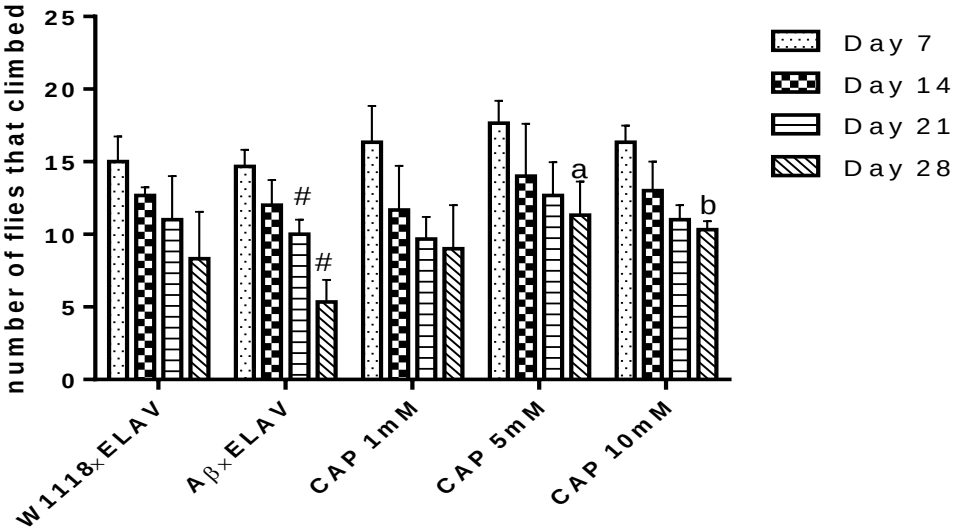


Figure 4:
Effect of captopril on the climbing ability of Aβ expressing flies. Bar chart represents mean ± SD; (aP <0.05, bP<0.05) versus Aβ × elav control. Data were analysed by two-way ANOVA followed by Tukey's *post hoc* multiple comparison test.

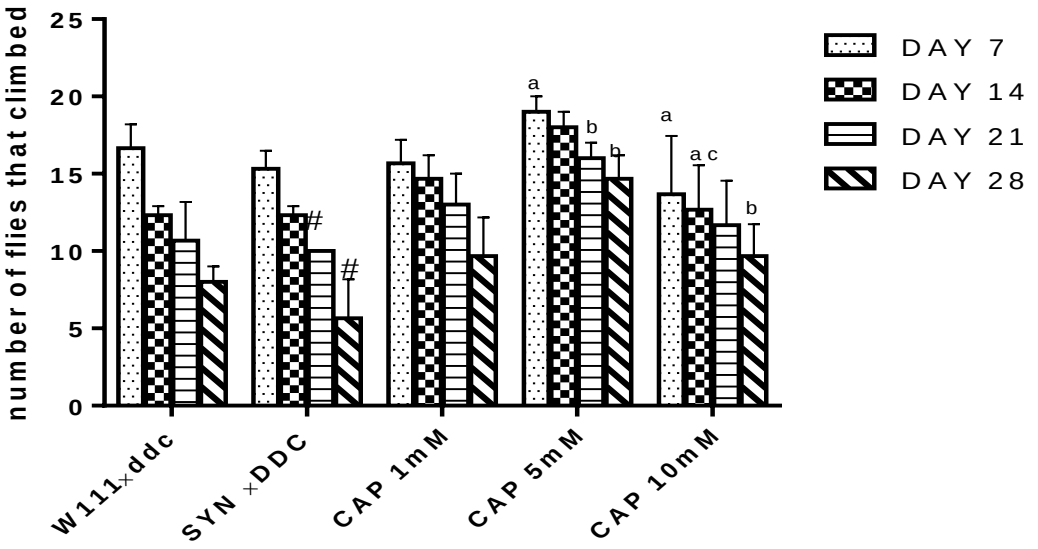
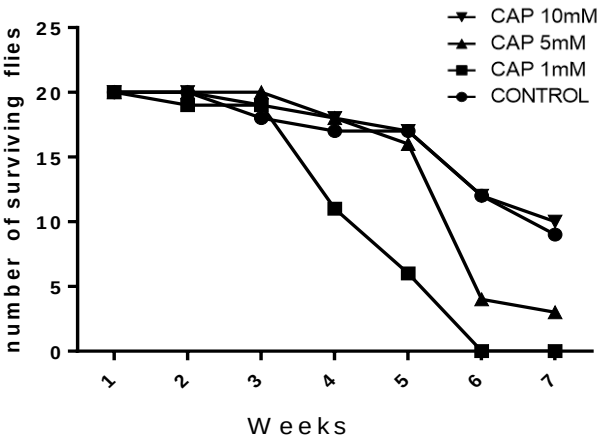


Figure 5:
Effect of captopril on the climbing ability following *syn*uclein overexpression in dopamine neuron in flies. Bar chart represents mean ± SD; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus day 7 in syn×ddc control; $^{a}P<0.05$; $^{b}P < 0.01$, $^{c}P < 0.001$versus respective days in *Syn*× ddc control. Data was analysed by two-way ANOVA followed by *Tukey*'s multiple comparison test.

Longevity assay

Two-way ANOVA revealed significant effect of treatments on lifespan [F(3,18)=5.95,P<0.0] (Fig. 6) in Cs flies. CS flies grown on feed media supplemented with captopril 5mM concentration showed a slight increase in the number of surviving flies while flies cultured on 10mM feed media showed a significant improvement in the number of surviving flies when compared to CS flies cultured on normal feed (Fig. 6).



DISCUSSION

In this study, *syn*uclein expressing flies replicated several features of human PD, including locomotor dysfunction, lewy body like formation and age-dependent loss of dopaminergic neurons. Treatment of *syn*uclein expressing flies with captopril (1mM, 5 mM and 10 mM) for a period of 28 days showed a dose- dependent increase in the number of line crosses in the larva motility assay when compared to the pathologic model (synuclein expressing flies), and thus, indicating the potential neuroprotective role of captopril in PD. The mechanism by which ACE inhibitors provide neuroprotection in PD may involve an attenuation of AngII effect on ATI receptors. Considerable evidence suggests that reducing AngII actions on ATI receptors reduces oxidative stress, neuronal loss and neuroinflammation by inhibition of ATI receptors.

One of the distinguishing characteristic features in Parkinson's disease is the loss of dopaminergic neuron and the aggregation of lewy bodies (Spillantini *et al.*,1998) in the substantia nigra while that of Alzheimer's disease is the aggregation of Aβ peptides at specific brain regions which have been implicated in the pathogenesis of AD (Selkoe, 2011). These proteins are neurotoxic proteins and can result in the loss of brain neurons which are involved in motor coordination. Several studies have shown association between ACE-I and a decrease in the incidence of PD (Gao et al., 2017; Perez-Lloret et al., 2017). Moreso, in vitro studies revealed that Angiotensin II (Ang II) induced apoptosis of dopaminergic neurons through its type 1 receptor (AT1R) (Gao et al., 2017). Similarly, captopril ameliorate MPTP-induced PD in mice (Yazdani *et al.*, 2006; Sonsalla *et al.*, 2012), thus confirms the neuroprotective effect of captopril provides neuroprotection.

An increase or decrease in *drosophila* fertility could be due to several reasons, including temperature variations or other regulating factors which could result in increase or decrease in; number of eggs laid by mated female flies, hatchability and/ or mortality of laid eggs during development (Ram and Wolfner, 2007). Fecundity assay is a test that provides a knowledge of flies' potential to reproduce. Examination of fecundity in CS strain cultured on normal and those on feed supplemented with captopril showed that there was a decrease in the number of eggs as the concentration increased from 1mM to 10mM, though

this decrease was not significantly different from that of control. However, at the highest concentration (10mM), there was a significant decrease in the number of eggs laid, dark pupa and eclosion suggestive of possible impact of captopril on flies' fertility.

Several studies have also demonstrated an association between ACE-I and a decrease in the incidence of AD (Zou *et al.,* 2007; Abdalla *et al.,* 2013; Bernstein *et al.,* 2014). However, despite the beneficial effects of ACE-I on AD, studies on the effect of ACE-I remain inconsistent and unclear. Since flies do not endogenously produce Aβ peptides, Aβ is produced by transgene expression thus allowing the exploration of the amyloidogenic pathway through which captopril acts. In this present study, Aβ expressing flies showed significantly impaired motor function. Treatment of Aβ expressing flies with various concentrations of captopril showed more improvement in the larva motility compared to control. This result suggests that captopril may play an important therapeutic role.

Aging is a phenomenon that results in steady physiological deterioration in nearly all organisms in which it has been examined. Individual aging is manifest at the population level as an increase in age-dependent mortality, which is often measured by observing life span in age-matched flies. Life span assay in *Drosophila* provides the link between insect ACE and longevity (Liao *et al.,* 2014; Kumar *et al.,* 2016) and depends on strict regulation of nutritional and environmental conditions. Measurement of lifespan in Canton S (CS) fly strain cultured on normal feed and those on feed supplemented with captopril showed that there was a slight increase in the lifespan at the 10mM concentration of captopril but there was no significant change in lifespan when compared to control.

In conclusion, the present study showed that captopril improves locomotor activity and confers a neuroprotective effect in Drosophila possibly through inhibition of amyloid beta or alpha synuclein protein aggregation. Thus, could be a potential adjunct in the management of neurodegenerative diseases.

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REFERENCES

AbdAlla, S., Langer, A., Fu, X., & Quitterer, U (2013). ACE Inhibition with Captopril Retards the Development of Signs of Neurodegeneration in an Animal Model of Alzheimer's Disease. International Journal of Molecular Sciences, 14(8), 16917–16942.

Abeliovich A. and Gitler A. D. (2016). Defects in trafficking bridge Parkinson's disease pathology and genetics. Nature 539, 207-216.

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Venter, J. C. (2000). The genome sequence of Drosophila melanogaster. Science (New York, N.Y.), 287(5461), 2185–2195.

Bier E (2005). Drosophila, the golden bug, emerges as a tool for human genetics. Nat Rev Genet 6: 9-23. 21.

Duffy, J. D. (2002). GAL4 System in Drosophila: A Fly Geneticist's Swiss Army Knife. Genesis, 34, 1-15.

Fischer JA, Giniger E, Maniatis T, Ptashne M (1988). GAL4 activates transcription in Drosophila. Indian journal of pharmacology; 332:853-856.

Gao Q, Ou Z, Jiang T, Tian YY, Zhou JS, Wu L, Shi JQ, Zhang YD. Azilsartan ameliorates apoptosis of dopaminergic neurons and rescues characteristic parkinsonian behaviors in a rat model of Parkinson's disease. Oncotarget. 2017;8(15):24099-24109.

Huo Z, Luo X, Zhan X, Chu Q, Xu Q, Yao J, Pang H. Genetic analysis of indel markers in three loci associated with Parkinson's disease. PLoS One. 2017;12(9):e0184269.

Ishola I.O, Badru A, Ofi E.O, Akinleye M.O, Adeyemi O.O (2021) Ameliorative influence of atorvastatin in transgenic Drosophila Melanogaster model of neurodegenerative diseases Nigerian Journal of Pharmacy 55 (1) 40-45.

Jaiswal M, Sandoval H, Zhang K, Bayat V, Bellen HJ. Probing mechanisms that underlie human neurodegenerative diseases in Drosophila. Annu Rev Genet. 2012;46:371-96. doi: 10.1146/annurev-genet-110711-155456.

Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature. 2013 Sep 5;501(7465):45-51.

Kumar, S., Dietrich, N., & Kornfeld, K. (2016). Angiotensin Converting Enzyme (ACE) Inhibitor Extends Caenorhabditis elegans Life Span. PLOS Genet, 12(2), e1005866.

Liao, F.-T., Chang, C.-Y., Su, M.-T., & Kuo, W.-C. (2014). Necessity of angiotensin-converting enzyme-related gene for cardiac functions and longevity of Drosophila melanogaster assessed by optical coherence tomography. Journal of Biomedical Optics, 19(1), 11014.

Munoz A, Rey P, Guerra MJ, Mendez-Alvarez E, Soto-Otero R, Labandeira-Garcia JL (2006). Reduction of dopaminergic degeneration and oxidative stress by inhibition of angiotensin converting enzyme in a MPTP model of parkinsonism. Neuropharmacology. 51:112–120.

Perez-Lloret S, Otero-Losada M, Toblli JE, Capani F. Renin-angiotensin system as a potential target for new therapeutic approaches in Parkinson's disease. Expert Opin Investig Drugs. 2017;26(10):1163-1173.

Quitterer U, AbdAlla S. Improvements of symptoms of Alzheimer`s disease by inhibition of the angiotensin system. Pharmacol Res. 2020 Apr;154:104230. doi: 10.1016/j.phrs.2019.04.014.

Ram KR, Wolfner MF. Sustained post-mating response in Drosophila melanogaster requires multiple seminal fluid proteins. PLoS Genet. 2007;3(12):e238.

Sanchez-Lemus E, Benicky J, Pavel J, Saavedra JM (2009). In vivo Angiotensin II AT1 receptor blockade selectively inhibits LPS-induced innate immune response and ACTH release in rat pituitary gland. Brain, Behavior, & Immunity. 23:945–957.

Schulz R, Heusch G (2006). Angiotensin II type 1 receptors in cerebral ischaemia-reperfusion: initiation of inflammation. Journal of Hypertension - Supplement. 24:S123–129.

Selkoe, D (2011). Resolving controversies on the path to Alzheimer's therapeutics. Nat Med 17, 1060–1065.

Sonsalla PK, Coleman C, Wong LY, Harris SL, Richardson JR, Gadad BS, Li W, German DC. The angiotensin converting enzyme inhibitor captopril protects nigrostriatal dopamine neurons in animal models of parkinsonism. Exp Neurol. 2013;250:376-83.

Sonsalla PK, Wong L-Y, Harris SL, Richardson JR, Khobahy I, Li W, Shrikanth B, German DG (2012). Caffeine treatment prevents nigral dopamine neuron loss in a progressive rat model of Parkinson's disease. Experimental Neurology 234:482–487.

Spillantini MG, Crowther RA, Jakes R, et al (1998). Alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proceedings of the National Academy of Sciences of the United States of America;95:6469-6473.

Von Bohlen und Halbach O., & Albrecht D (2006). The CNS renin-angiotensin system. Cell and Tissue Research, 326(2), 599–616.

Wyss-Coray T. (2016). Ageing, neurodegeneration and brain rejuvenation. Nature 539, 180-186.

Zou, K., Yamaguchi, H., Akatsu, H., Sakamoto, T., Ko, M., Mizoguchi, K., Michikawa, M. (2007). Angiotensin-converting enzyme converts amyloid beta-protein 1-42 (Abeta(142)) to Abeta(1-40), and its inhibition enhances brain Abeta deposition. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 27(32), 8628–8635.

Conte, F.P., Ferraris, F.K., Costa, T.E., Pacheco, P., Seito, L.N., Verri Jr, W.A., Fernando, Q., Cunha, F.Q., Penido, C., Henriques, M.G. (2015). Effect of Gedunin on Acute Articular Inflammation and Hypernociception in Mice. Molecules 20:2636-2657.

D'Amour, F.E. and Smith, D.L. (1941). A method for determining loss of pain sensation. J Pharmacol Exp Ther. 72 (1): 74-79.

Dollo, G., Thompson, D., Le Corre, P., Chevanne, F., Le Verge, R. (1998). Inclusion complexation of amide-type local anesthetics with β-cyclodextrin and derivates: III. Biopharmaceutics of bupivacaine-HP β-CD complex following percutaneous sciatic nerve administration in rabbits. Int J Pharm. 164:11–9.

Dollo, G., Le Corre, P., Freville, J.C., Chevanne, F., Le Verge, R. (2000). Biopharmaceutics of local anesthetic cyclodextrin complexes following loco-regional administration. Ann Pharm Fr. 58:425–32.

Eddy, N.B. and Leimbach, D.J. (1953). Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther. 107:385-393.

El-Feky, G.S., El-banna, S.T., Khalil, S.K.H. (2013). Preparation, in vitro and in vivo evaluation of oral indomethacin-HP-β-cyclodextrin loaded chitosan nanoparticles. Int J Pharm Pharm Sci. 5(4):638-645.

Esmat, A., Al-Abbasi, F.A., Algandaby, M.M., Moussa, A.Y., Labib, R.M., Ayoub, N.A., Abdel-Naim, A.B. (2012). Anti-Inflammatory Activity of Pistacia khinjuk in Different Experimental Models: Isolation and Characterization of Its Flavonoids and Galloylated Sugars. J Med Food. 15(3): 278–287.

Franco de Lima, R.A., de Jesus, M.B., Saia Cereda, C.M., Tofoli, G.R., Cabeça, L.F., Mazzaro, I., Fraceto, L.F., de Paula, E. (2012). Improvement of tetracaine antinociceptive effect by inclusion in cyclodextrins. J. Drug Target. 20(1):85-96. doi: 10.3109/1061186X.2011.622400.

Franzotti, E.M., Santos, C.V., Rodrigues, H.M., Mourão, R.H., Andrade, M.R., Antoniolli, A.R. (2000). Anti-inflammatory, analgesic activity and acute toxicity of Sida cordifolia L. (Malva-branca). J. Ethnopharmacol, 72(1-2):273-7.

Gidwani, B. and Vyas, A. (2015). A Comprehensive review on cyclodextrin-based carriers for delivery of chemotherapeutic cytotoxic anticancer drugs. Biomed Res. Int. 2015:198268. doi: 10.1155/2015/198268.

Gornall, A.G., Bardawill, C.J., David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. J Biol. Chem. 177:751–766.

Hunskaar, S. and Hole, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30(1):103–114.

Jain, M. and Parmar, H.S. (2011). Evaluation of antioxidative and anti-inflammatory potential of hesperidin and naringin on the rat air pouch model of inflammation. Inflamm. Res. ,60:483– 491.

Khalid, S.A., Duddeck, H., Gonzalez-Sierra, M. (1989). Isolation and characterization of an antimalarial agent of the neem tree Azadirachta indica. J. Nat. Prod. 52(5):922-6.

Kim, J.Y., Kim, D.H., Jeong, H.G. (2006). Inhibitory effect of the coffee diterpene kahweol on carrageenan-induced inflammation in rats. Biofactors. 26: 17 -28.

Kirkova, M., Kassabova, T. and Russanov E. (1992). In vivo effects of indomethacin I. Activity of antioxidant enzymes and lipid peroxidation. Gen Pharmacol. 23:503–507.

Koster, R., Anderson, M., De Beer, E.J. (1959). Acetic acid for analgesic screening. Fed Proc. 18:412.

Kothari, N., Keshari, R.S., Bogra, J., Kohli, M., Abass, H., Malik, A., Dikshit, M., Barthwal, M.K. (2011). Increased myeloperoxidase enzyme activity in plasma is an indicator of inflammation and onset of sepsis. J Crit Care. 26: 435.e1-435.e7.

Liao, C.R., Kao, C.P., Peng, W.H., Chang, Y.S., Lai, S.C., Ho, Y.L. (2012). Analgesic and Anti-Inflammatory Activities of Methanol Extract of Ficus pumila L. in Mice. Evid Based Complement Alternat Med 2012:1-9. doi:10.1155/2012/340141.

Miro, A., Quaglia, F., Sorrentino, U., La Rotonda, M.I., Di Villa Bianca, R. D., Sorrentino, R. (2004). Improvement of gliquidone hypoglycaemic effect in rats by cyclodextrin formulations. Eur J Pharm Sci. 23:57–64.

Martin, S.W., Stevens, A.J., Brennan, B.S., Davies, D., Rowland, M., Houston, J.B. (1994). The Six-Day-Old Rat Air Pouch Model of Inflammation: Characterization of the Inflammatory Response to Carrageenan. J Pharmacol Toxicol Methods. 32(3):139 – 147.

Moron, M.S., Depierre, J.W., Mannervick, B. (1979). Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochim Biophys Acta. 582(1):67-78.

Manjavachi, M.N., Quintao, N.L.M., Campos, M.M., Deschamps, I.K., Yunes, R.A., Nunes, R.J., Leal, P.C., Calixto, J.B. (2010.) The effects of the selective and non-peptide CXCR2 receptor antagonist SB225002 on acute and long-lasting models of nociception in mice. Eur J Pain. 14(1):23–31. doi: 10.1016/j.ejpain.2009.01.007.

Ohkawa, H., Ohishi N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.

Okhale, S.E., Amupitan, J.O., Ndukwe, I.G., Oladosu, P.O., Okogun, J.I. (2012). Synthetic modification of gedunin and comparative antibacterial activity of gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt. Afr. J Pure Appl. Chem. 6(14):183-189.

Ologe, M.O., Adegoke, A.O., Iwalewa, E.O., Ademowo, O.G. (2016). Spectrophotometric studies of a novel Gedunin-2-Hydroxypropyl-β-cyclodextrin binary complex. Afr J Med Med Sci. 45:159-169.

Ologe, M.O., Tella, A.C., Atolani, O., Adegoke, O.A., Ademowo, O.G. (2021). Inclusion complex of gedunin-2-hydroxypropyl-β-cyclodextrin prepared by kneading and

freeze-drying methods: synthesis and structural characterization. Acta Biol Marisiensis 4(1): 83-97.

Otero-Espinar, F.J., Torres-Labandeira, J.J., Alvarez-Lorenzo, C., Blanco-Méndez, J. (2010). Cyclodextrins in drug delivery systems. J Drug Deliv Sci Technol.20 (4):289-301.

Pulli, B., Ali, M., Forghani, R., Schob, S., Hsieh, K.L.C, Wojtkiewicz, G., Linnoila, J.J., Chen, J.W. (2013). Measuring Myeloperoxidase Activity in Biological Samples. PLoS One. 8(7): e67976. doi:10.1371/journal pone.0067976.

Quintans, J de S.S., Menezes, P.P., Santos, M.R.V., Bonjardim, L.R., Almeida, J.R., Gelain, D.P., Araújo, A.A., Quintans-Júnior, L.J. (2013). Improvement of p-cymene antinociceptive and anti-inflammatory effects by inclusion in β-cyclodextrin. Phytomedicine 20(5):436-40. doi: 10.1016/j.phymed.2012.12.009.

Quintans-Junior, L.J., Barreto, R.S.S., Menezes, P.P., Almeida, J.R.G., Viana, A.S., Rita, C.M., Oliveira, R.C.M., Oliveira, A.P., Daniel, P., Gelain, D.P., de Lucca Junior, W., Araujo, A.A.S. (2013). β-Cyclodextrin-complexed (_)-linalool produces antinociceptive effect superior to that of (_)-linalool in experimental pain protocols. Basic Clin Pharmacol. Toxicol.113: 167–172.

Ravangpai, W., Sommit, D., Teerawatananond, T., Sinpranee, N., Palaga, T., Pengpreecha, S., Muangsin, N., Pudhom, K. (2011). Limonoids from seeds of Thai Xylocarpus moluccensis. Bioorg Med Chem Lett 21(15): 4485-4489.

Ribeiro, R.V., Silva, R.M., Lima, J.C., Martins, D.T. (2010). Antiinflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from Macrosiphonia velame (A. St.-Hil.) M. Arg. in animal models. Braz J Pharm Sci. 46 (3):515 -523.

Rodrigues, P.O., Becker, A.C., Dellagnelo, V.A., Benetti, C.N., Pereira, E.M., Wagner, T.M., Silva, M.A.S. (2014). Pharmacodynamic and Pharmacokinetic Studies of β-Cyclodextrin: Dexamethasone Acetate Complexes in Mice. Braz Arch Biol Technol. 57(6):887-894.

Sensoy, D., Gönüllü, U., Sagirli, O., Yener, G., Altug, T. (2009). Preparation, Characterization and Anti-inflammatory Activity of Celecoxib-β-Cyclodextrin Inclusion Complexes. Asian J. Chem. 21(3):1759-1768.

Sin, Y.M., Pook, S.H., Tan, T.M., Petterssoon, A., Kara, A.U., The, W.F. (1997). Changes in Gluthathione and its Associated Enzymes during Carrageenan-induced Acute Inflammation in Mice. Comp. Biochem. Physiol. 116C:191-195.

Sedgwick, A.D. and Lees, P. (1986). Studies of eicosanoid production in the air pouch model of synovial inflammation. Agents Actions 8:429-438.

Singh, I., Kumar, P., Pahuja, S., Tung, V., Arora, S. (2011). Development and pharmacological evaluation of cyclodextrin complexes of etoricoxib. Acta Pol. Pharm. 68(2):279-284.

Sinha, V.R. and Amita Goel, H. (2010). In vivo bioavailability and therapeutic assessment of host-guest inclusion phenomena for the hydrophobic molecule etodolac: pharmacodynamic and pharmacokinetic evaluation. Sci Pharm.78:103-15.

Tanas, S., Odabasoglu, F., Halici, Z., Cakir, A., Aygun, H., Aslan, A., Suleyman, H. (2010). Evaluation of Anti-Inflammatory and Antioxidant Activities of Peltigera Rufescens Lichen Species In Acute And Chronic Inflammation Models. J Nat Med. 64(1):42–9.

Ueda, K., Higashi, K.K., and Moribe, K. (2021). Amorphous Drug Solubility and Maximum Free Drug Concentrations in Cyclodextrin Solutions: A Quantitative Study Using NMR Diffusometry. Molecular Pharmaceutics 18(7), 2764-2776. DOI: 10.1021/acs.molpharmaceut.1c00311.

Wankar, J., Kotla, N. G., Gera, S., Rasala, S., Pandit, A., Rochev, Y. A. (2020). Recent Advances in Host–Guest Self-Assembled Cyclodextrin Carriers: Implications for Responsive Drug Delivery and Biomedical Engineering. Adv. Funct. Mater. 2020, 30, 1909049. https://doi.org/10.1002/adfm.201909049.

Winter, C.A., Risley, E.A., Nuss, C.W. (1962). Carrageenan–induced oedema in the hind paw of the rat as an assay for anti–inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111:544–547.