Borneo International Journal of Biotechnology (BIJB)
Vol. 4 (December 2024), 34 – 50

e-ISSN 2716-697X

Screening of coumarin derivatives as a potential Alzheimer's Disease treatment drugs on *Drosophila melanogaster*

Nurul Akmar Hussin[1] Abdul Ashraf Rasid[2], Ooi Hui Min[3], Ghows Azzam[3] and Mardani Abdul Halim[2*]

[1]Institute for Tropical Biology and Conservation,
Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[2]Biotechnology Research Institute, Universiti Malaysia Sabah,
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[3]Malaysia Genome and Vaccine Institute, National Institutes of Biotechnology Malaysia, Jalan Bangi,
43000 Kajang, Selangor, Malaysia.
*Corresponding email: mardaniccb@gmail.com
https://doi.org/10.51200/bijb.v4i.6004
Received: 4 August 2023 | Accepted: 18 December 2024 | Published: 31 December 2024

ABSTRACT

Alzheimer's disease (AD) stands as the most prevalent form of neurodegenerative ailment worldwide, characterized by the accumulation of amyloid beta (Aβ) plaques. Unfortunately, there is currently no effective cure for this condition. To investigate potential treatment options, researchers have turned to *Drosophila melanogaster* as an ideal animal model for studying AD. In this context, coumarin, a naturally occurring phytochemical initially discovered in tonka bean, and its derivatives have garnered significant attention for their diverse beneficial biological properties. The present study aimed to explore the efficacy of coumarin derivatives in mitigating the adverse effects of Aβ aggregation. Using the *Drosophila* model expressing human Aβ42, researchers observed a rough eye phenotype (REP) and decreased lifespan. To evaluate the neuroprotective effects of coumarin derivatives, the treated groups' eye morphology was compared with both positive and negative control groups. Encouragingly, the group treated with S5-44 exhibited the most favourable eye morphology, closely resembling that of the positive control group compared to other coumarin derivatives. Moreover, the group treated with S3-18 displayed a longer lifespan in comparison to the negative

control group. In summary, most coumarin derivatives utilized in this study partially restored the REP, while one derivative even extended the lifespan of *Drosophila*. These promising findings suggest that coumarin derivatives have the potential to serve as neuroprotective drugs for the treatment of AD. Further research and development in this area may open new avenues for combating this debilitating disease.

Keywords: Alzheimer's disease, amyloid beta, *Drosophila melanogaster*

INTRODUCTION

Alzheimer's disease (AD) was initially documented by Alois Alzheimer in 1907. It is a progressive and irreversible neurodegenerative disorder characterized by the gradual loss of synapses and neuronal cells in the brain. As the most prevalent form of dementia, AD accounts for 50 to 70% of all neurodegenerative dementia cases globally (Winblad et al., 2016). Disturbingly, it stands as the seventh leading cause of death globally (Collaborators, 2021). Remarkable advancements in medical science and technology have undoubtedly improved the quality of life and increased life expectancy. However, this increased longevity comes with a simultaneous rise in age-related diseases, including AD. As our population ages, it becomes crucial to address the challenges posed by Alzheimer's disease and actively seek ways to prevent, manage, and treat this condition to alleviate its impact on individuals and their families.

AD is characterized by the presence of amyloid beta (Aβ) plaques and neurofibrillary hyperphosphorylated tau tangles. Abnormal processing and accumulation of Aβ peptides, derived from the amyloid precursor protein (APP) through sequential cleavage by β- and γ-secretase, represent a significant hallmark of the disease (Wang et al., 2017; Selkoe & Hardy, 2016). The aggregation of misfolded Aβ42 polypeptides gives rise to amyloid clumps, triggering neurodegeneration through the activation of abnormal signalling pathways (Fernandez-Funez et al., 2013; Sarkar et al., 2016; Selkoe & Hardy, 2016; Shankar et al., 2008). Ultimately, these plaques disrupt neuron transmission at synapses, leading to a failure in information transfer and culminating in neuronal cell death (Hardy & Selkoe, 2002; Tanzi & Bertram, 2005; Blennow, 2006).

D. melanogaster has emerged as a widely preferred model for AD research, as evidenced by studies conducted by Iijima et al. (2004), and Wittmann et al. (2001). A notable advantage of using *Drosophila* as a model is that approximately 75% of human disease genes have orthologs in their genome (Rubin, 2000). Employing *Drosophila* as a model organism offers several benefits, including modest dietary and spatial requirements, easy observation and manipulation at various developmental stages, high reproductive capacity, and resilience against environmental challenges such as plagues and pathogens (Stocker & Gallant, 2008). The availability of fully known genome sequences, the simplicity of genetic manipulation, and an extensive collection of available mutants contribute to *Drosophila's* well-established status as a system that facilitates a deeper understanding of human diseases at the molecular level (Botas, 2007).

As projected by the U.S. Alzheimer's Association, the number of affected individuals worldwide is expected to surpass 22 million by 2025. Regrettably, a cure for AD remains elusive. Addressing this critical need, there is a pressing demand for more effective drugs to combat AD, which necessitates the exploration of compound screening using animal models to unlock potential cures. Adopting alternative approaches, such as compound screening, offers a promising avenue for discovering neuroprotective drugs to treat AD. The objective of this study was 1) to investigate the capability of coumarin derivatives in mitigating Aβ aggregation within the *D. melanogaster* model and 2) to assess and compare the neuroprotective effects of various coumarin derivatives for treating AD in the *D. melanogaster* model.

MATERIALS AND METHODS

Preparation of solid fly food

The ingredients for preparing fly food in solid form are shown in Table 1. A pot containing 1 litre of water was brought to a boil. Polenta and yeast were added to the boiling water while ensuring thorough mixing to prevent clumping. In a separate container, corn flour was dissolved in 100 mL of water. Pieces of agar were introduced into the boiling pot and mixed until they dissolved completely. Subsequently, the corn starch solution was added to the mixture. If the mixture appeared too thick, 100 mL of tap water was gradually incorporated into the mixture to achieve the desired consistency. Slowly and while stirring, brown sugar was added to the mixture. Afterwards, the mixture was allowed to cool at room temperature for a few minutes. Finally, nipagin and propionic acid were added to complete the preparation process.

Table 1 The ingredients for preparing fly food in solid form

Ingredients	Volume/Weight
Water	1L
Corn flour	40 g
Polenta	50 g
Brown sugar	100 g
Agar	7 g
Yeast	50 g
Propionic acid	5 mL
Nipagin	30 mL

The coumarin derivatives used in this study were sourced from the School of Chemical Sciences, USM. An amount of 100 mg of the compound was dissolved in 100 µL of 0.3% Dimethyl sulfoxide (DMSO) by thorough mixing. Subsequently, the resulting mixture was combined with 1 mL of solid fly food (Table 2).

Table 2 Preparation of 10 % of compound in the solid fly food

Ingredients	Volume/Weight
Fly food	1 mL
Compound	100 mg
0.3 % DMSO	100 µL

Fly stock and maintenance

The flies (as shown in Table 3) were raised and maintained at a temperature of 25 °C. After the parents were crossed, they were removed from the vial after 6 days, leaving only the larvae and pupae. After 10 days, the virgin female flies were collected for further experiments. Before conducting the experiments, the flies were anesthetized and placed on a CO_2 pad (specifically, the FlyStuff Flypad from Genesee Scientific), where a continuous supply of carbon dioxide was provided. To distinguish between virgin female flies and non-virgin female flies, the researchers relied on differences in their body coloration (lighter color for virgin flies and tan color for non-virgin flies). Additionally, the presence of meconium, a greenish spot visible at the abdomen of virgin flies, served as another key characteristic to differentiate them during the sorting process.

Table 3 Drosophila melanogaster strains

Source	Stock number	Genotype	Description
Kyoto Stock Center	107294	Oregon-R-P2	Wild-type
Bloomington *Drosophila* Stock Center	1104	w[*];P{w[+mC]=GAL4-ninaE.GMR}12	Express GAL4 in the eye under the control of the glass enhancer. Provides strong expression in all cells behind the morphogenetic furrow.
Kyoto Stock Center	107727	y[1] w[*]; P{w[+mC]=Act5C-GAL4}25FO1 / CyO, y[+]	Actin5C-GAL4 driver
Bloomington *Drosophila* Stock Center	33769	w[1118];P{w[+mC]=UAS-APP.Abeta42.B}m26a	Expresses the human Aβ42 fragment of APP under the control of UAS.

Compound screening using Rough Eye Phenotype (REP) assay

The male fly line was crossed with the virgin female fly line, and this cross was carried out in the solid fly food containing the compound (or without the compound). The vials were left undisturbed for 6 days to allow for the completion of the crossing process. After 10 days, the first generation (F1) of flies was collected for further analysis. The eyes of the F1 flies were carefully observed under a motorized stereo microscope (as described in Table 4). Subsequently, images of the fly eyes were captured to document and analyze any observable differences or effects resulting from the cross and the presence or absence of the compound in the solid food.

Table 4 The model of light microscope – Stereo Motorized (cellSens Dimension)

Model	Camera	Software
Olympus SZX16	Olympus DP72	cellSens Dimension version 1.5
(Olympus Optical Co. Ltd. Tokyo, Japan)	(Olympus Corporation, Japan)	(© Olympus Soft Imaging Solutions GmbH 2011)

The images of the F1 flies, obtained after observation under the motorized stereo microscope, were processed using CombineZP software (available at https://combinezp.software.informer.com/). The stacked images allowed for a comprehensive comparison of the eye structures of the treated F1 flies with those in both the positive control and negative control groups. By conducting this comparative analysis, any potential effects resulting from the treatment and the presence or absence of the compound in the solid food could be discerned and evaluated.

Table 5 Parent flies were crossed in three groups: positive control, treated, and negative control, for the REP assay.

Group	Female fly line	Male fly line	Condition
Positive control	Oregon R	GMR-GAL4	Food + DMSO
Treated	UAS-Aβ42	GMR-GAL4	Food + DMSO + compound
Negative control (Untreated)	UAS-Aβ42	GMR-GAL4	Food + DMSO

Capillary Feeder (CAFE) assay

The Capillary Feeder (CAFE) assay, as originally described by William et al. in 2007, was modified in this study by excluding cornmeal and agar and incorporating tryptone. To prepare the modified liquid food (refer to Table 6), 5 g each of yeast extract and glucose were mixed in a 100 mL Schott Duran Bottle. Sterile water was then added up to a volume of 50 mL. Additionally, 1.7 g of tryptone was placed in another 100 mL Schott Duran Bottle, and sterile water was added up to 50 mL. Both bottles were subjected to autoclaving for sterilization. In the bottle containing yeast extract and glucose, 1.5 mL

of nipagin and 0.25 mL of propionic acid were added as preservatives. To create 50 mL of the liquid food, 25 mL of the content from each bottle was carefully extracted and mixed in a new bottle. This resulting liquid food was then utilized for the CAFE assay in the experimental procedure.

Table 6 Preparation of liquid food for CAFE assay

Ingredient	Volume/Weight
Yeast extract	5 g
Glucose	5 g
Tryptone	1.7 g
Propionic acid	0.25 mL
Nipagin	1.5 mL

50 mg of the compound was dissolved in 50 µL of 0.3% Dimethyl sulfoxide (DMSO) through thorough mixing. The resulting mixture was then combined with 450 µL of liquid fly food to achieve a total volume of 500 µL of liquid fly food with the compound (refer to Table 7). During the experimental period, the flies were fed daily with 1 to 2 µL of the prepared liquid food, depending on the number of flies present in each tube. This feeding regimen was followed consistently to ensure that the flies received an appropriate and consistent amount of the liquid fly food with the compound throughout the study.

Table 7 Preparation of 10 % of compound in the liquid food

Ingredient	Volume
Liquid food	450 µL
Compound + 0.3 % DMSO	50 µL
Total	500 µL

Longevity assay

The male fly line was mated with virgin female flies, and this cross was performed in two different conditions: one group with the compound added to the food and another group without the compound. The vials containing the flies were then left undisturbed for 6 days to allow for proper mating and egg-laying. After 10 days, the first generation (F1) flies were collected and utilized in the CAFE assay for further investigation. During the assay, the F1 flies were provided with daily feeding and their housing tubes were changed every two days. Throughout this process, the flies' lifespans were carefully recorded and plotted into graphs for analysis. To ensure robust results, each group was replicated twice, providing two independent replicates for both the positive control

and the treated group. The initial number of flies used in the positive control was 26, while the treated group had 22 flies, and the negative control included 5 flies (Table 8). These numbers represent the total number of flies used at the beginning of the experiment in each respective group.

Table 8 Parent flies were crossed in three groups, namely positive control, treated, and negative control, for the longevity assay

Group	Female fly line	Male fly line	Condition
Positive control	Oregon R	Actin5C-GAL4	Food + DMSO
Treated	Actin5C-GAL4	UAS-Aβ42	Food + DMSO + compound
Negative control (Untreated)	Actin5C-GAL4	UAS-Aβ42	Food + DMSO

RESULT

Rough Eye Phenotype (REP) assay

In studies related to neurodegeneration, the REP (Rough Eye Phenotype) assay is utilized as a method to assess neurotoxicity in *Drosophila*. The REP can be observed in the first generation (F1) flies affected by the expression of the AD gene from their parents, facilitated through the GAL4/UAS system. Flies exhibiting REP display a disrupted hexagonal arrangement of the ommatidia, differing from the organized ommatidia arrangement in the wild-type flies.

To evaluate the neuroprotective effects of coumarin derivatives, the eye morphology of the treated groups receiving various coumarin derivatives (S3-18, S5-52, S3-30, S5-44, S2-12, and S4-34) was compared with both the positive control and negative control groups. To make a comparative analysis, the treated groups were also ranked based on the severity of their REP. The severity of REP in each treated group was determined by comparing their eye morphology with that of the positive control and negative control groups. The coumarin derivative that resulted in an eye phenotype closest to that of the positive control received a higher ranking, while those closer to the negative control had a lower ranking.

The positive control group (GMR-GAL4 × Oregon R) displayed a hexagonal array of organized ommatidia, similar to the characteristics of the wild-type fly eye (Figure 1) In contrast, the treated groups (GMR-GAL4 × UAS-Aβ42) showed a wide range of recovery degrees in their eye morphology (Figure 2 – 7). Visual assessments were made, and the eye phenotype condition was manually ranked from 1 to 6 based on the degree of severity, with rank 1 indicating the closest morphology to that of the wild type and rank 6 for the most severe eye phenotype (Table 9).

Most coumarin derivatives used in the treated groups demonstrated a less severe REP compared to the negative control (GMR-GAL4 × UAS-Aβ42), which displayed defects in the eye phenotype, such as more fused and disorganized ommatidia. This indicates that the majority of the coumarin derivatives used in the study partially restored the REP in the flies affected by AD.

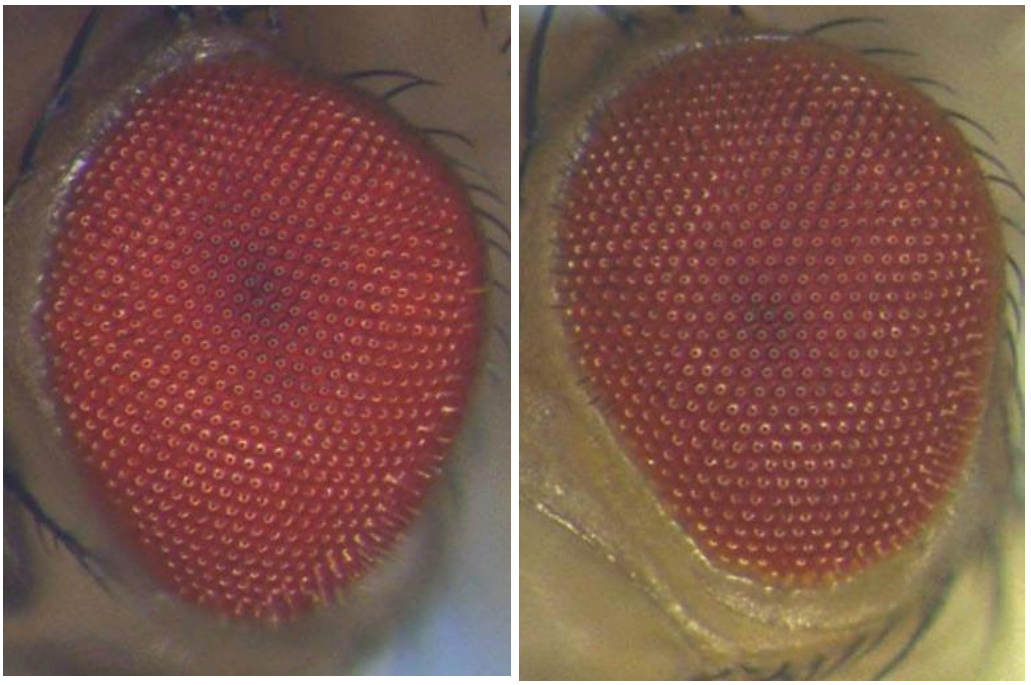

Figure 1 Eye phenotypes are shown for (A) the wild type or Oregon R and (B) the positive control group (GMR-GAL4 × Oregon R)

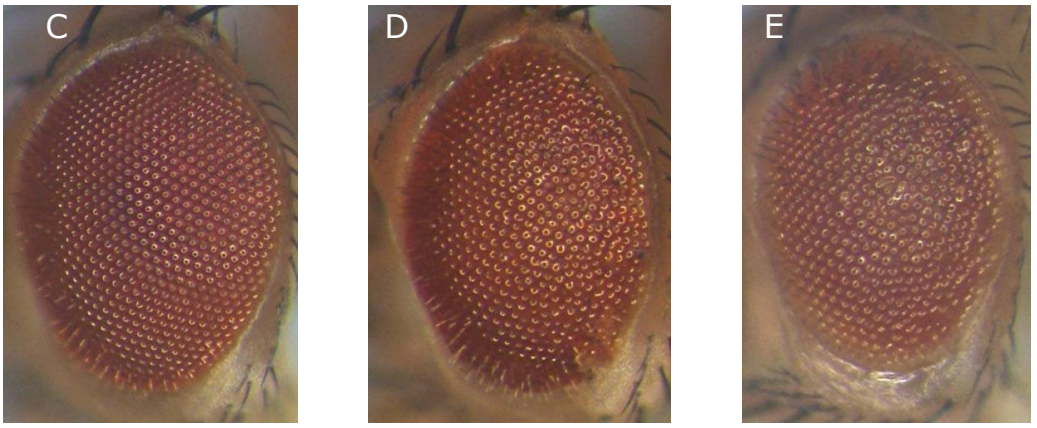

Figure 2 The eye phenotypes are as follows: (C) positive control, (D) treated group with S3-18, and (E) negative control

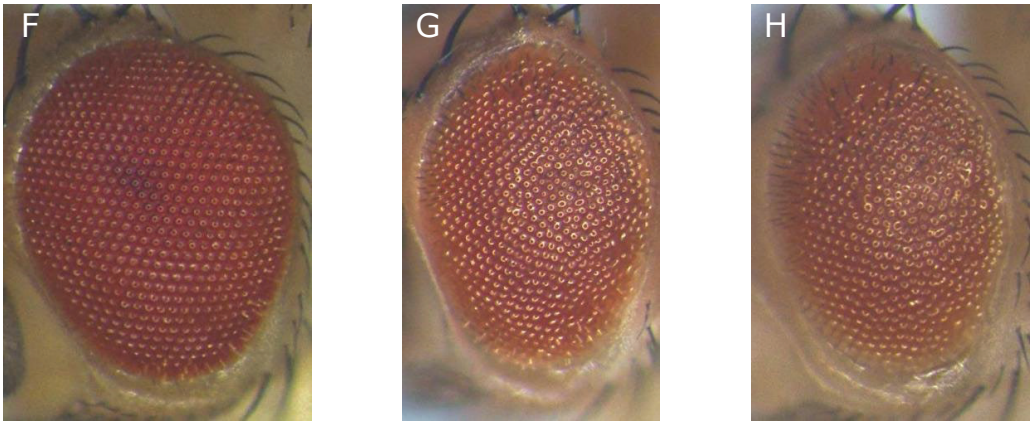

Figure 3 The eye phenotypes observed are as follows:
(F) positive control, (G) treated group with S5-52, and (H) negative control

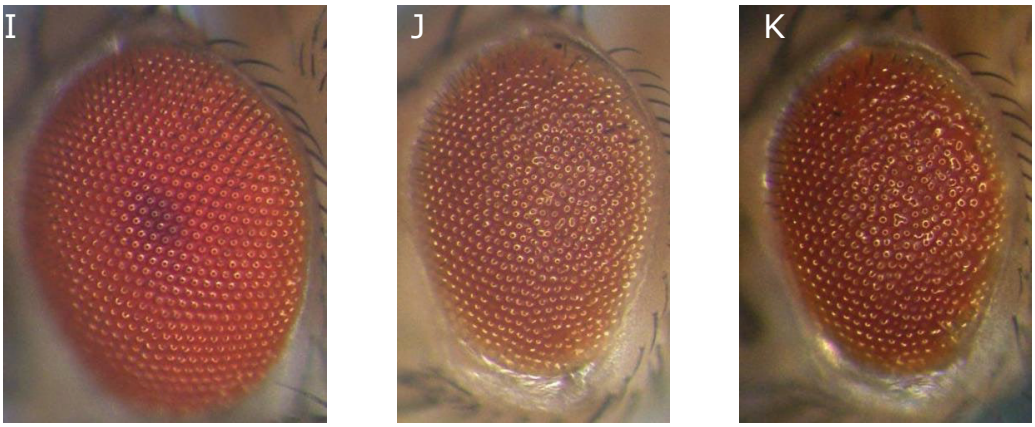

Figure 4 The eye phenotypes are recorded as follows:
(I) positive control, (J) treated group with S3-30, and (K) negative control

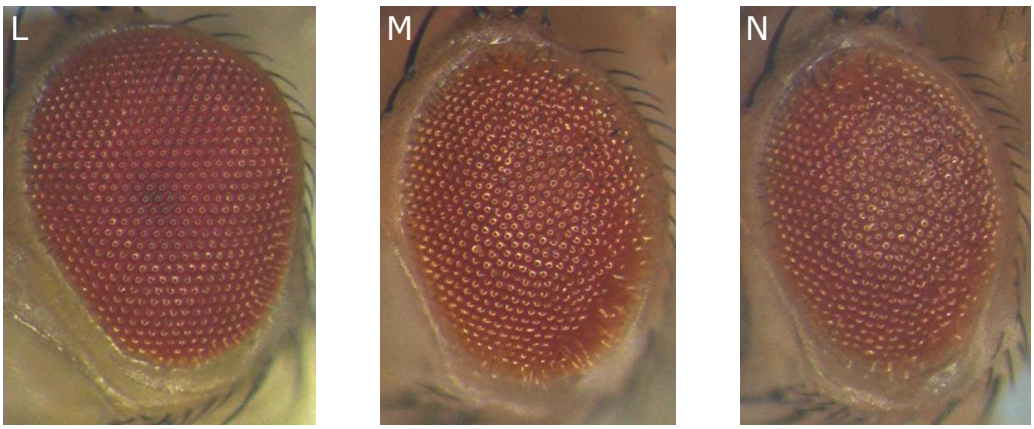

Figure 5 The eye phenotypes observed are as follows:
(L) positive control, (M) treated group with S5-44, and (N) negative control

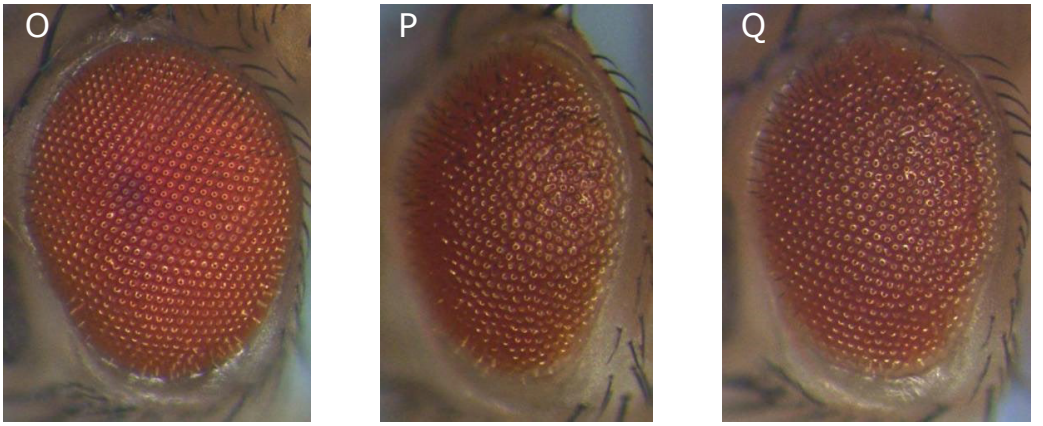

Figure 6 The eye phenotypes are recorded as follows:
(O) positive control, (P) treated group with S2-12, and (Q) negative control

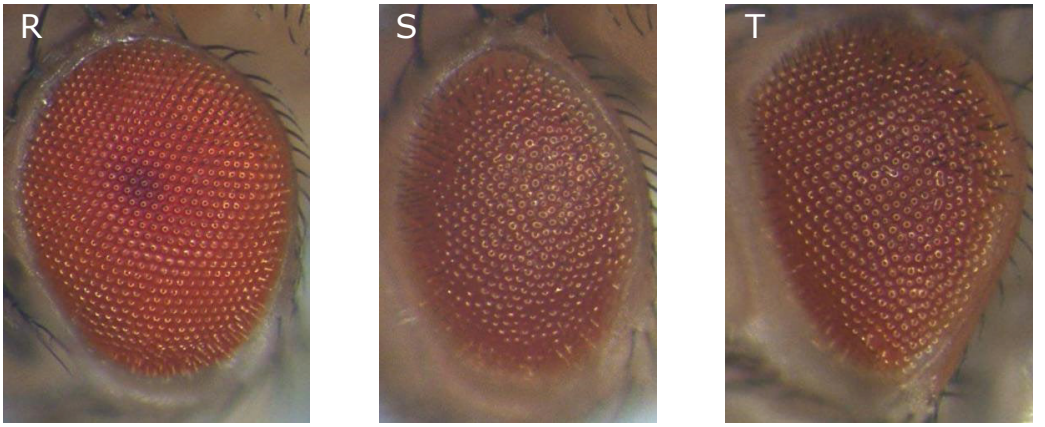

Figure 7 The eye phenotypes observed are as follows:
(R) positive control, (S) treated group with S4-34, and (T) negative control

Table 9 Rank order of the treated groups that treated with coumarin derivatives (S3-18, S5-52, S3-30, S5-44, S2-12 and S4-34)

Coumarin derivatives	Rank order
S3-18	3
S5-52	2
S3-30	4
S5-44	1
S2-12	6
S4-34	5

The group treated with S5-44 exhibited the most similar eye phenotype to that in the positive control, displaying partially organized and hexagonally arranged ommatidia (Figure 5). For S5-52, the eye phenotype showed partially organized ommatidia with only a few fused ommatidia (Figure 3). Flies fed with S3-18 had a moderate severity in eye phenotype, as the ommatidia were neatly arranged, but some fused ommatidia were observed (Figure 2). For S3-30, the eye morphology was almost the same as that in the S3-18 group, but it had more fused ommatidia (Figure 4).

The group treated with S4-34 had disorganized ommatidia, making it challenging to detect a hexagonal arrangement, and the size of the ommatidia was inconsistent (Figure 7). Flies treated with S2-12 had the most severe phenotype, as there was no observable hexagonal arrangement of the ommatidia. Additionally, unequal sizes and fused ommatidia were evident in this treated group (Figure 6).

Lifespan analysis

The lifespans of adult F1 flies, which were raised and kept at a constant temperature of 25 °C, were assessed in this study. The parents were crossed in solid food, with some groups containing the compound and others without it. After 6 days, the first generation (F1) flies emerged from the eggs, and they immediately started consuming the food. A substantial amount of food was consumed during their larval stage, leading to their development into pupae. Once fully developed, the enclosed flies were collected and sorted by sex.

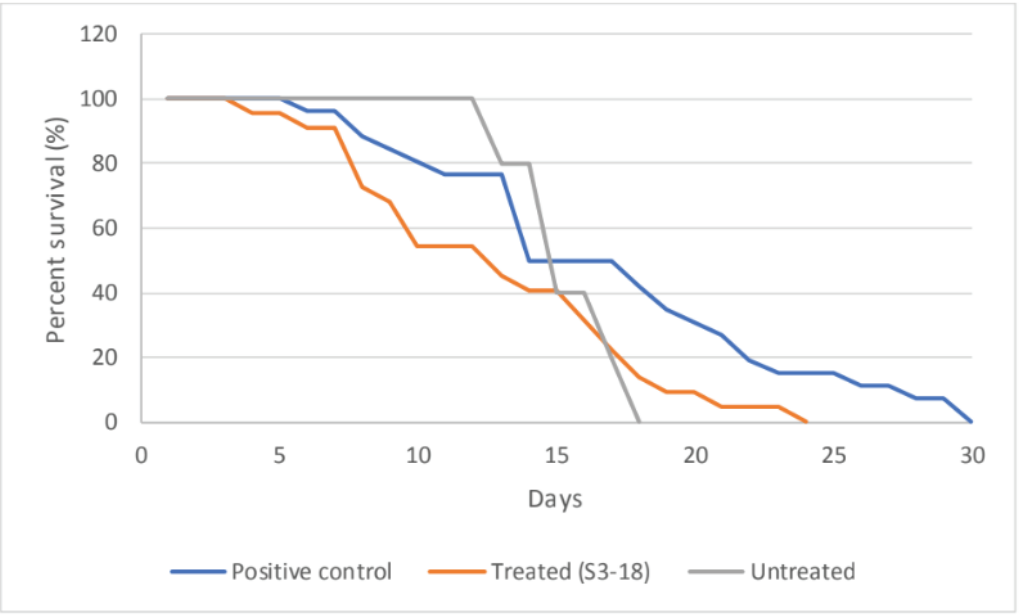

Figure 8 The survival curve shows the results for the positive control (initially 26 flies), the treated group with S3-18 (initially 22 flies), and the negative control (initially 5 flies), with each group having two replicates.

The flies were then maintained using the CAFE method, where they were fed with liquid food. In this method, the viability of the flies was recorded daily to monitor their longevity. The data obtained from the three groups, including the positive control, the treated group, and the negative control, were plotted and compared in a graph (Figure 8). This graph allowed for a visual comparison of the longevity of flies from the different groups over time.

Based on the graph, the positive control group (Actin5C-GAL4 × Oregon R) exhibited the longest lifespan, reaching 30 days. In contrast, the flies treated with S3-18 (Actin5C-GAL4 × UAS-Aβ42) had a lifespan of 24 days, and the negative control group (Actin5C-GAL4 × UAS-Aβ42) lived for 18 days.

Throughout the 30-day period, the survivability of the positive control group gradually declined. Similarly, the survivability of the flies treated with S3-18 also decreased slowly, reaching a significant drop on the 24th day. In contrast, the negative control group maintained its survivability at 100% until the 13th day, after which it experienced a sharp decrease in survival within five days. Comparing the treated group (S3-18) with the negative control, it becomes evident that the treated group exhibited a longer lifespan. This indicates that S3-18 had a positive effect on improving the longevity of flies expressing Aβ42.

DISCUSSION

Rough Eye Phenotype (REP) assay

Drosophila treated with S3-18, S5-52, S3-30, and S5-44 displayed improved eye morphology compared to the negative control group. These findings suggested that these compounds had neuroprotective effects on the flies expressing Aβ42. On the other hand, the eye morphology of flies treated with S4-34 and S2-12, ranked 5th and 6th respectively, closely resembled that of the negative control. This indicated that these two coumarin derivatives exhibited either weak or no neuroprotective effects against Aβ42 expression.

The severity of the eye phenotype correlated with the extent of neuronal cell loss (Lenz et al., 2013). Coumarin derivatives that showed stronger neuroprotective effects were able to rescue more neuronal cells from death, resulting in a lower severity of the observed eye phenotype (REP) in the flies. This was particularly relevant in this case, as neuronal death was triggered by the accumulation of Aβ42 plaques, a significant hallmark of Alzheimer's disease (AD). The accumulation of Aβ42 led to neurodegeneration, causing stress in the neurons and ultimately resulting in their death (Crews & Masliah, 2010; Hardy, 2009; Pandey & Nichols, 2011; Sarkar et al., 2016; Tare et al., 2011).

As a result, S5-44 was identified as having the most potent inhibition of Aβ42 aggregation, as flies treated with this derivative displayed the best eye morphology among all the tested compounds. Considering the critical role of Aβ42 peptide accumulation in AD pathology, inhibiting the assembly of Aβ monomers into aggregate structures has emerged as a promising strategy for AD treatment.

In recent years, coumarins, which are found in numerous plant species, have garnered significant attention due to their diverse biological properties associated with neurological diseases, particularly Alzheimer's disease (AD) (Hamulakova et al., 2017). Their potential has led to the consideration of coumarin as a valuable scaffold in designing drugs for AD treatment. Notably, coumarin derivatives have shown promise in preventing misfolded Aβ aggregation. Specific studies have highlighted the potency of certain coumarin derivatives, such as compound 13 and 15, in inhibiting Aβ aggregation (Huang et al., 2015). Interestingly, it was suggested that incorporating a hydroxyl group into the para position of the side chain-phenyl ring may enhance inhibitory activity against Aβ42 self-induced aggregation. Conversely, a reduction in the carbon nitrogen double bond appeared to diminish the inhibition of Aβ42 self-aggregation (Huang et al., 2015). This variation in structural modifications could account for the wide range of neuroprotective effects demonstrated by the coumarin derivatives investigated in this study.

In addition to these findings, other studies have also revealed the active inhibition of Aβ self-aggregation by certain coumarin derivatives. For example, compounds 4y and 4w exhibited the ability to actively inhibit Aβ self-aggregation. Furthermore, the conjugates of 7-hydroxycoumarin linked in position 4 to tacrine demonstrated Aβ aggregation inhibition as well (Hamulakova et al., 2017).

Another study investigated coumarin-pargyline hybrids, particularly compound 4x, and found that they exhibited remarkable inhibitory activities against Aβ42 aggregation (Yang et al., 2009). The research suggested that the stability of the 4x-Aβ complex resulted from π-π stacking interaction and hydrogen bond interactions (Yang et al., 2009). The coumarin derivatives used in this study may undergo similar interactions with Aβ, thereby contributing to their inhibition of Aβ aggregation.

In this particular study, the assessment of the reduced eye phenotype (REP) in flies was conducted qualitatively, relying on visual inspection of eye phenotypes and manual scoring. However, this method was limited by its lack of high sensitivity and accuracy. To address this limitation, a more robust approach could involve the use of a quantitative assessment of eye morphology in flies, such as the Flynotyper software. This software offers a quantitative measure that can effectively detect and differentiate between various levels of defects in the fly eye. By employing this more advanced quantitative method, researchers can obtain more precise and reliable data, enhancing the accuracy of their findings and enabling a more comprehensive analysis of the effects of coumarin derivatives on the flies' eye morphology.

To further investigate the ability of coumarin derivatives to inhibit Aβ aggregation, various methods can be employed, such as the Thioflavin T (ThT) fluorescence assay, 1-anilinonaphthalene-8-sulfonic acid (ANS) fluorescence assay, turbidity assay, and native gel electrophoresis. These techniques provide valuable insights into the interactions between coumarin derivatives and Aβ peptides, helping to elucidate the mechanisms of inhibition.

Additionally, molecular docking studies can be conducted to gain a deeper understanding of how coumarin derivatives interact with Aβ peptides at the molecular level. These computational studies can provide valuable information about the binding modes and affinity of the coumarin derivatives to Aβ, complementing the experimental findings.

Furthermore, to validate the neuroprotective effects of the coumarin derivatives used in this study, it would be beneficial to apply these derivatives in treatment on mammalian models of Alzheimer's disease. By using mammalian AD models, researchers can observe the effects of the coumarin derivatives in a more clinically relevant context and assess their potential as therapeutic agents for neurodegenerative diseases.

Overall, employing these various methods and approaches would offer a comprehensive and multi-faceted investigation into the neuroprotective properties of coumarin derivatives and their potential as a treatment strategy for Alzheimer's disease.

Lifespan analysis

Among the various coumarin derivatives, S3-18 was selected to assess its effectiveness in improving the longevity of *Drosophila*. The positive control group displayed the longest lifespan, which was 30 days. In contrast, the lifespan of flies fed with S3-18 and the negative control was 24 days and 18 days, respectively. Previous studies had shown that the lifespan of Aβ42 flies was significantly reduced (Finelli et al., 2004; Iijima et al., 2004). However, flies treated with S3-18 demonstrated a longer lifespan compared to the negative control group. These findings strongly suggested that S3-18 had a positive impact on the longevity of flies expressing Aβ42, potentially exerting a beneficial effect on the health and lifespan of these Drosophila models.

D. melanogaster serves as a model organism extensively used in the study of age-related diseases, and its typical mean lifespan ranges from 2 to 3 months (Helfand & Rogina, 2003). In this specific study, the positive control group exhibited the longest lifespan, which was recorded as 30 days. This reduced lifespan in the positive control group was consistent with a previous study that indicated flies fed using the CAFE (Capillary Feeder) method had a shorter lifespan (Lee et al., 2008). The shorter lifespan observed in the CAFE method could be attributed to environmental stress and the nature of the diet provided.

In the CAFE assay, evaporation of liquid food might occur, leading to condensed liquid food forming at the tips of the pipette tips. This could result in a lower accessibility of food by the flies when compared to solid food. The specific type of food used can also have a substantial impact on the flies' lifespan. For instance, some commonly used fly food, such as yeast extract instead of lyophilized whole brewer's yeast, can significantly shorten the flies' lifespan (Bass et al., 2007).

In this study, S3-18 demonstrated the ability to extend the lifespan of *Drosophila*. However, it's worth noting that another study reported that coumarin, the parent compound of S3-18, was unable to extend the lifespan of *Drosophila*. The difference in outcomes between these studies could potentially be attributed to variations in

the chemical structures of coumarin and coumarin derivatives like S3-18. The study suggesting that coumarin was not able to extend lifespan also noted that coumarin might have potential toxicity (Abraham et al., 2010; Cohen, 1979).

Additionally, it's essential to consider that the *Drosophila* strains used in the two studies differed. The previous study utilized w 1118 and JIV strains, which are distinct from the UAS-Aβ42 line used in this current study. Genetic variations and specific characteristics of each *Drosophila* strain could contribute to differing responses to coumarin derivatives, potentially explaining the contrasting results.

Indeed, the reliability of the results obtained from the longevity assay with S3-18 may have been compromised due to the insufficient number of flies used at the beginning of the experiment. To ensure more robust and dependable data, it is recommended to use a larger sample size. Specifically, employing at least 100 flies in each group would enhance the statistical power and accuracy of the study. Additionally, to account for potential variations and increase confidence in the findings, conducting three replicates of the experiment would be beneficial.

CONCLUSION

The use of natural products holds promise as a potential alternative for treating neurodegenerative diseases like Alzheimer's disease (AD). These products offer the advantage of alleviating AD symptoms with potentially fewer or no side effects compared to synthetic drugs. Coumarin, being a versatile compound, allows for various chemical substitutions at different sites in its structure, making it a valuable tool for designing different derivatives, which could have implications for drug discovery.

However, further studies are necessary to assess the effectiveness of coumarin derivatives in other vertebrate model organisms to better understand their neuroprotective functions. Exploring their effects in more complex biological systems will provide crucial insights for potential translational applications.

One of the intriguing possibilities is the development of coumarin derivatives as potential drugs for targeting Aβ42-mediated neurodegeneration observed in AD. If successful, these derivatives could hold significant therapeutic potential, offering hope for the treatment of neurodegenerative diseases and improving the lives of those affected by these conditions. Continued research in this area could bring us closer to discovering novel and effective treatments for AD and other related disorders.

REFERENCES

Bass, T. M., Grandison, R. C., Wong, R., Martinez, P., Partridge, L., & Piper, M. D. (2007). Optimization of dietary restriction protocols in Drosophila. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *62*(10), 1071 – 1081.

Botas, J. (2007). Drosophila researchers focus on human disease. Nat Genet, *39*(5), 589-591.

Blennow, K., de Leon, M. J., & Zetterberg, H. (2006). Alzheimer's disease. Lancet (London, England), *368*(9533), 387–403.

Fernandez-Funez, P., Sanchez-Garcia, J. & Rincon-Limas, D. (2013) Unraveling the basis of neurodegeneration using the Drosophila eye. In: Molecular genetics of axial patterning, growth and disease in the Drosophila eye (A. Singh & M. Kango-Singh, eds.), pp 271 – 293. New York: Springer.

Hamulakova, S., Janovec, L., Soukup, O., Jun, D., & Kuca, K. (2017). Synthesis, in vitro acetylcholinesterase inhibitory activity and molecular docking of new acridine-coumarin hybrids. Int J Biol Macromol, *104*(Pt A), 333-338.

Hardy, J. (2009). The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *Journal of Neurochemistry*, *110*(4), 1129 – 1134.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, *297*(5580), 353 – 356.

Huang, M., Xie, S. S., Jiang, N., Lan, J. S., Kong, L. Y., & Wang, X. B. (2015). Multifunctional coumarin derivatives: monoamine oxidase B (MAO-B) inhibition, anti-beta-amyloid (Abeta) aggregation and metal chelation properties against Alzheimer's disease. Bioorg Med Chem Lett, *25*(3), 508 – 513.

Iijima, K., Liu, H. P., Chiang, A. S., Hearn, S. A., Konsolaki, M., & Zhong, Y. (2004). Dissecting the pathological effects of human Abeta40 and Abeta42 in Drosophila: a potential model for Alzheimer's disease. Proc Natl Acad Sci U S A, *101*(17), 6623 – 6628.

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Raubenheimer, D. (2008). Lifespan and reproduction in Drosophila: new insights from nutritional geometry. Proc Natl Acad Sci USA, *105*(7), 2498 – 2503.

Lenz, S., Karsten, P., Schulz, J. B., & Voigt, A. (2013). Drosophila as a screening tool to study human neurodegenerative diseases. J Neurochem, *127*(4), 453 – 460.

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, *443*(7113), 787 – 795.

Pandey, U. B., & Nichols, C. D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacol Rev, *63*(2), 411 – 436.

Rubin, G. M. (2000). Biological annotation of the Drosophila genome sequence. Paper presented at the Novartis Foundation symposium.

Sarkar, A., Irwin, M., Singh, A., Riccetti, M., & Singh, A. (2016). Alzheimer's disease: the silver tsunami of the 21(st) century. Neural Regeneration Research, *11*(5), 693 – 697.

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med, *8*(6), 595 – 608.

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med, *1*(1), a006189.

Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Selkoe, D. J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med, *14*(8), 837 – 842.

Stocker, H., & Gallant, P. (2008). Getting started: an overview on raising and handling Drosophila. Methods Mol Biol, *420*, 27 – 44.

Tanzi, R. E., & Bertram, L. (2005). Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell, *120*(4), 545 – 555.

Tare, M., Modi, R. M., Nainaparampil, J. J., Puli, O. R., Bedi, S., Fernandez-Funez, P., Singh, A. (2011). Activation of JNK signaling mediates amyloid-ss-dependent cell death. PLoS One, *6*(9), e24361.

Wang, J., Gu, B. J., Masters, C. L., & Wang, Y. J. (2017). A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. Nat Rev Neurol, *13*(10), 612 – 623.

William, W. J., Carvalho, G. B., Mak, E. M., Noelle, N., Fang, A. Y., Liong, J. C., Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. Proceedings of the National Academy of Sciences, *104*(20), 8253-8256.

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., Zetterberg, H. (2016). Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol, *15*(5), 455 – 532.

Wittmann, C. W., Wszolek, M. F., Shulman, J. M., Salvaterra, P. M., Lewis, J., Hutton, M., & Feany, M. B. (2001). Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles. Science, *293*(5530), 711 – 714.