Original Research Article

Neuroprotective Potential of Eugenol in Polyglutamine-Mediated Neurodegenerative Disease Using Transgenic *Drosophila* Model

Dose-Response:
An International Journal
October-December 2024:1–10

DOI: 10.1177/15593258241291652
journals.sagepub.com/home/dos
Sage

Anjalika Chongtham[1,2] and Namita Agrawal[1]

Abstract

Polyglutamine (PolyQ) diseases including Huntington's disease are devastating neurodegenerative disorders characterized by progressive neuronal loss and motor dysfunction. PolyQ pathology involves multiple cellular events and phytochemicals with multi-target mechanisms hold promise to treat these diseases with least side effects. One such promising phytochemical is Eugenol, which possesses antioxidant and anti-inflammatory properties, potentially targeting disrupted cellular pathways in PolyQ diseases. The present study investigated the effects of Eugenol on neurodegeneration and motor dysfunction in transgenic *Drosophila* models of PolyQ diseases. In this study, the robust pseudopupil assay was performed to analyze adult photoreceptor neuron degeneration, a marker of widespread degenerative events. Furthermore, the well-established crawling and climbing assays were conducted to evaluate progressive motor dysfunction in the PolyQ larvae and flies. This study found that Eugenol administration at disease onset or after progression reduced PolyQ disease phenotypes, particularly, neurodegeneration and motor dysfunction in a dose-dependent manner and with no side effects. Thus, this study suggests that Eugenol could be a viable candidate for developing treatments for PolyQ diseases, offering a multi-target approach with the potential for minimal or no side effects compared to conventional therapies.

Keywords

Eugenol, neurodegenerative disease, polyglutamine (PolyQ) disease, Huntington's disease, *Drosophila*

Introduction

Neurodegenerative diseases are a group of disorders characterized by the progressive degeneration of the structure and function of the nervous system, particularly the neurons. These diseases often result in the gradual decline of cognitive function, movement, and other neurological functions. Most prevalent neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), fronto-temporal dementia (FTD), Huntington's disease (HD) and spinocerebellar ataxias (SCAs).[1] Although age has been the foremost contributing factor for the onset and progression of neurodegenerative disorders affecting millions globally, recent advancements in the field have revealed genetic influence as another factor.[2] Due to the involvement of multiple molecular pathways in these diseases, discovering safe and effective drug for their treatment has proven difficult. Therefore, despite significant efforts made in this field, there is no cure for neurodegenerative diseases. Hence, ongoing research aims to understand the etiology and pathophysiology of this distressing disease so that a potential treatment strategy can be developed in order to manage or relieve the unpleasant symptoms and disease progression.

Polyglutamine (PolyQ) related disease family encompasses at least nine heritable disorders, including HD, SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17.[3] Each of these disorders

[1] Department of Zoology, University of Delhi, Delhi, India
[2] Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Received 19 February 2024; accepted 11 September 2024

Corresponding Author:
Namita Agrawal, Department of Zoology, University of Delhi, Room-306, Delhi 110007, India.
Email: nagrawaluci@gmail.com

results from abnormal expansion of a cytosine-adenine-guanine trinucleotide (CAG) repeat, coding for a glutamine tract (PolyQ) present in the wild-type protein. In healthy individuals, PolyQ tract is between 30 and 35, however, in patients or carriers, it is above 40 and can reach to any number. Although PolyQ protein is ubiquitously expressed, its abnormal expansion leads to several malfunctions such as proteolytic cleavage, conformational change in the pathogenic proteins, protein aggregation, cell death, transcriptional and metabolic disturbances, that eventually lead to neuronal death. PolyQ disorders share several overlapping pathogenic features, such as late onset; progressive degeneration of vulnerable subsets of neurons; inverse correlation between CAG repeat length and disease onset; and the presence of mutant protein aggregates in selective degenerative neurons.[4,5] The PolyQ diseases manifest as movement disorders, cognitive abnormalities and neuropsychiatric disturbances. Among the PolyQ diseases, HD is the most common inherited neurodegenerative disorder. HD is an autosomal dominant disorder caused by an increased number of CAG repeats (>35) located in the first exon of the gene coding for Huntingtin (Htt) protein.[6] Anomalous expansion of the CAG repeat affects multiple cellular processes and gradually damages brain cells through several mechanisms. Htt is a ~350 kDa protein expressed ubiquitously but the highest level of Htt protein is found in the brain, due to the which normal functions such as anti-apoptotic activity, vesicle trafficking and neuronal pathways are altered in disease condition.[7]

PolyQ pathogenesis is complex, involving disruption of multiple cellular functions and processes, for example, transcription, post-translational modifications, oxidative stress and mitochondrial function.[8,9] With the advancement in research, there have been numerous additions to the raveled story of PolyQ diseases and one such noteworthy mention is the systemic effects of the PolyQ diseases especially in HD. Several reports in HD state that metabolic dysregulation and inflammation lead to a compromised systemic immune system, and all of these indicate the peripheral comorbidities of the neuronal disorder.[10,11] Given the myriads of cellular insults triggered by expanded PolyQ domain, developing effective therapeutic strategies appear a daunting prospect. To date, no cure or disease-modifying treatment exists for PolyQ diseases and most of the currently available treatments such as precise target-based drug designing is known to have undesirable complications or side effects thereby degrading the quality of life further. Therefore, developing novel and effective therapeutics targeting multiple cellular events intended to suppress or prevent disease progression without side effects is warranted. Administrations of phytochemicals (plant-derived bioactive compounds) that target multiple cellular processes are assumed to attain greater and more promising therapeutic efficacy as compared to mono-targeted agents or synthetic drugs. With the increased awareness and development of new techniques, extraction and purification procedures have become easy leading to a growing sector of phytochemicals. Scientific studies have proven a direct relationship with human health and consumption of phytochemicals such as isoprenoids, carotenoids, saponins and dietary fibers produced by plants in the crude form. In plants, phytochemicals act as a natural defense system against pathogen attacks and help in coping up with the stress induced by physical or chemical damage.[12] These phytochemicals possess multiple health benefits, as they may serve as antioxidants and enhance immune response and cell-to-cell communication, allowing the body's built-in defenses to work more efficiently. One such bioactive compound is Eugenol (4-allyl-2-methoxyphenol), a naturally occurring volatile phenolic molecule found majorly in aromatic oil extracted from cinnamon, cloves, basil, bay leaves, oregano and thyme.[13] It is considered as a non-mutagenic, non-carcinogenic and recognized safe by the Food and Drug Administration (FDA). Eugenol has been very well investigated for its pharmacological activities and has been reported to show potent antioxidant, anti-inflammatory, anti-depressant, anti-analgesic, DNA-protective and neuroprotective action.[13-16] Eugenol can easily bypass the blood brain barrier and penetrate the brain when consumed orally due to its hydrophobicity. Eugenol protected mice from 6-hydroxydopamine (6-OHDA)-induced PD. It prevented 6-OHDA-mediated reduction in dopamine level in the mouse striatum by inhibiting lipid peroxidation and stimulating glutathione (GSH) and L-ascorbate (Asc) generating systems.[17] It also inhibited Ca^{2+} and protected PC-12 cells from amyloid-β-induced cytotoxic effect.[18] In 5× familiar AD (5×FAD) mouse model, Eugenol administration mitigated cognitive dysfunction, reduced neuronal loss and amyloid-β deposition.[19] Despite its demonstrated benefits for other neurological disorders, its potential to treat PolyQ diseases such as HD remains largely unexplored. *Drosophila* models of PolyQ diseases including HD mimic several pathological features such as late onset, progressive accumulation of abnormal protein aggregates, motor neuron dysfunction followed by degeneration and finally decreased lifespan.[20-22] The present study aimed to investigate the therapeutic effects of Eugenol on PolyQ disease symptoms using transgenic *Drosophila* models, which express expanded PolyQ or mHtt peptide in neurons. This study specifically investigated whether administering Eugenol at the onset of the disease or after its progression could attenuate key hallmark features of PolyQ diseases, such as neurodegeneration and motor dysfunction, in a dose-dependent manner.

Materials and Methods

Reagents

Eugenol was obtained from Sigma Aldrich, St. Louis, MO, USA, Product No. W246719. FD & C Blue Dye No. 1 was obtained from Sigma Aldrich, St. Louis, MO, USA, Product No. 861146.

Drosophila *Stocks and Rearing Condition*

Expression of transgene containing polyglutamine repeats was carried out by using the GAL4/upstream activating sequence (UAS) system.[23] The polyglutamine stocks used in the present study were w; P{UAS-Httex1p Q20}, w; P(w^{+mC} = UAS-Q48+myc/flag) and w; P{UAS-Httex1p Q93}4F1.[24,25] These flies were crossed to the pan-neuronal *elav* driver w; P{$w^{+mW.hs}$ = GawB}elavC155. Different concentrations of Eugenol (10 μM, 20 μM, 30 μM, and 40 μM) were mixed in standard cornmeal/sugar/agar fly medium for rearing larvae or flies. Controls were grown in food without Eugenol at 25°C. All cultures were kept on a 12 h:12 h light:dark cycle.

Feeding Assay

Taste and smell are vital sensory modalities which enable animals to evaluate and distinguish nutritious and valuable food from toxic substances. To understand if dosage effect observed by feeding different concentrations of Eugenol is indeed due to different dosage with the same amount of food intake, we monitored feeding behavior of larvae. A colorimetric estimation of food intake was performed as previously described[26,27] to monitor feeding behavior in non-pathogenic Httex1p Q20 and diseased Httex1p Q93 larvae. Blue dye (FD & C Blue Dye No. 1) along with Eugenol of various concentrations (10 μM, 20 μM, 30 μM and 40 μM) were added to yeast paste that was placed at the center of a petri dish (100 mm diameter × 10 mm height) containing 3.3 % (weight/volume) agar. Two groups of 10 wandering third instar larvae were collected, gently washed with distilled water, and then fed blue yeast paste either without or with varying doses of Eugenol. After 2 h of feeding, each group of larvae were washed thoroughly in distilled water, dried and then homogenized. After homogenization of larvae in PBS and centrifugation at 17,115×g for 10 min, absorbance was measured at 625 nm using a spectrophotometer.

Crawling Assay

To evaluate motor function in diseased condition, Httex1p Q93 and Q48 larvae were fed with food supplemented with different concentrations of Eugenol and a measure of motor function called the 'crawling assay' was performed as previously described.[26] A wandering third instar larva was placed into a track (2 mm width × 30 mm length × 5 mm depth) created in a petri dish (100 mm diameter × 10 mm height) containing 3.3% agar, and the distance travelled by individual larva in 30 s was recorded. The experiment was repeated two times for a total of 10 larvae (n = 10) per condition.

Climbing Assay

To monitor locomotor activity of adult Httex1p Q20, Httex1p Q93 and Q48 flies fed with Eugenol a well-established climbing assay was performed as previously decribed.[25,26] Briefly, a group of 10 adults were tapped gently to the bottom of a vertical glass tube (2.2 cm diameter) and number of flies that were able to climb up a height of 10 cm within 15 s was recorded. For each condition, the experiment was repeated 6 times for two groups of 10 flies (n = 20).

Pseudopupil Analysis

Pseudopupil analysis is a measure of neuronal loss that allows characterization of the photoreceptor neurons by visualizing rhabdomeres in the ommatidia of the fly compound eye. HD neurotoxicity can be monitored by measuring the loss of visible photoreceptor neurons in the eye[24] and photoreceptor loss can be used as a quantitative marker for the *in vivo* assessment of neurodegeneration.[25] Pseudopupil analysis was performed as described before.[25] The decapitated head of anesthetized adult fly was mounted in a drop of nail polish on a microscope slide. The eyes were then examined using Nikon Eclispse (Ni-E) microscope with 50X oil objective. At least 300 ommatidia in 6 flies were scored per condition and the number of visible rhabdomeres per ommatidium was counted for each condition.

Statistics

Statistical analysis was done through SPSS software (IBM SPSS Statistics 22). Normality of data was assessed using Shapiro-Wilk tests and equal variance by Levene's test. For normally distributed data, statistics were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. If not normally distributed, data were analyzed by Kruskal-Wallis test followed by Mann-Whitney U test using Bonferroni correction for adjusting probability.

Results

Evaluation of Feeding Behavior of Larvae

When orally administered, all doses of Eugenol, including the higher ones, did not alter the food intake of larvae expressing either non-pathogenic Httex1p Q20 (Figure 1A) or pathogenic Httex1p Q93 (Figure 1B) in neuronal populations under the control of the pan-neuronal elav-Gal4 driver. This indicates that the observed responses to different concentrations of Eugenol are not due to variations in feeding behavior but are indeed attributable to the different drug concentrations.

Eugenol Effectively Muffles Photoreceptor Neurodegeneration

The pseudopupil assay was conducted on day 7 post-eclosion to assess the impact of Eugenol on the integrity of ommatidia in flies expressing non-pathogenic Httex1p Q20, as well as pathogenic Httex1p Q93 and Q48 peptides

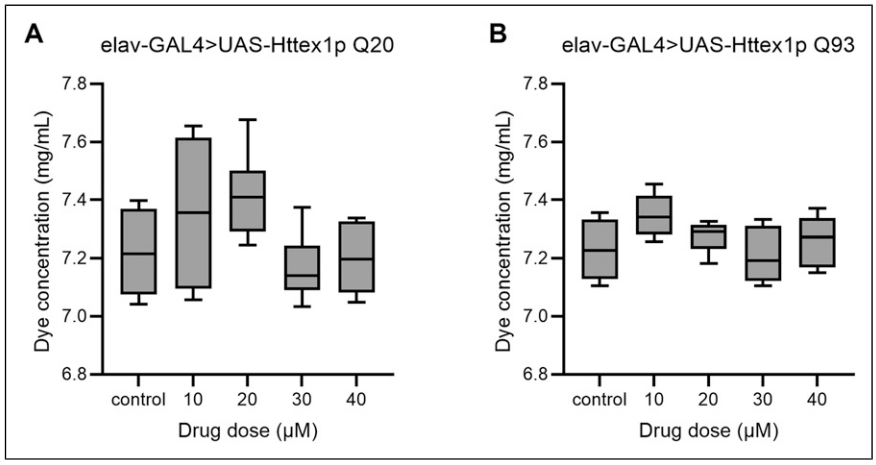

Figure 1. Eugenol doses have no effect on food intake. Various doses of Eugenol were fed to third instar larvae. No differential feeding behavior was observed in non-pathogenic (A) Httex1p Q20 as well as pathogenic (B) Httex1p Q93 larvae. Two groups of 10 larvae were assayed (n = 20) for 6 spectrophotometer readings. The box plots denote the median, minimum and maximum values. Data was analyzed using an analysis of variance (ANOVA).

driven by elav-Gal4. Httex1p Q20 flies fed with Eugenol-supplemented food (at concentrations of 10, 20, 30, or 40 μM) showed normal, seven well-organized rhabdomeres in each ommatidium (Figure 2A). In contrast, under pathogenic conditions with Httex1p Q93 and Q48, feeding flies 20 μM Eugenol either from the larval stage (Figure 2B–G) or after eclosion (Figure 3A–F) significantly mitigated photoreceptor neurodegeneration.

Eugenol Improves Impaired Locomotor Function in Drosophila *PolyQ Model*

Dietary Eugenol reduced motor dysfunction of Httex1p Q93 and Q48 larvae in a dose-dependent manner, with a notable improvement in larval crawling ability at a 20 μM dose (Figure 4A and B). To evaluate motor function in adults, climbing ability was assessed at days 1, 3, and 7 post-eclosion. Eugenol had no effect on control Httex1p Q20 flies (Figure 5A). However, 20 μM Eugenol administered to Httex1p Q93 or Q48 flies either from the start of larval life (Figure 5B and C) or later at the adult stage (Figure 5D and E) effectively mitigated progressive motor dysfunction by day 7 post-eclosion.

Discussion

PolyQ disorders including HD belong to a larger family of protein conformation diseases caused by mutations or cellular events which lead to protein misfolding and accumulation of abnormal structural forms of disease-specific protein. Although the clinical features and pattern of neurodegeneration differ among PolyQ diseases, growing evidence suggests that these diseases share common pathological features and toxic cellular processes.[28,29] Abnormal structural forms of disease-specific proteins promoted by PolyQ expansion are postulated to be central to pathogenesis. PolyQ diseases are associated with abnormal protein aggregates which reflect the intracellular accumulation of mutant PolyQ proteins. Progression of disease leads to characteristic symptoms which generally appear at midlife and progressively worsen until death. The main physiological and pathogenic features include elevated oxidative stress, mitochondrial dysfunction, neuroinflammation, dysfunction and loss of neurons and synapses.[28-32] Mutant PolyQ proteins are ubiquitously expressed but selective degeneration of neurons occurs in specific brain regions. From a therapeutic perspective, it is encouraging that many of the protein conformation diseases share important pathogenic features as therapeutic strategy for one disease may be effective in other diseases. Unfortunately, there are currently no treatments which can cure, prevent disease onset or slow down the course of disease. Despite significant advances in basic and clinical research, etiology and pathophysiology of most of these conditions remain largely obscure. Therefore, neurodegenerative diseases represent a scientific challenge and a growing public health issue with no disease-modifying therapies. To date, only a few symptomatic treatments are available, and these conventional therapies are often accompanied by debilitating side effects. Given the current lack of effective treatments, developing therapeutic strategies using natural products known for their pharmacological safety and minimal or no side effects is of paramount importance. Scientific innovations and extensive research have shown that the diverse array of bioactive compounds found in natural products represents a valuable resource for drug development.

Research over the last decade has demonstrated that bioactive components or phytochemicals present in spices can modulate inflammatory pathways and may exert

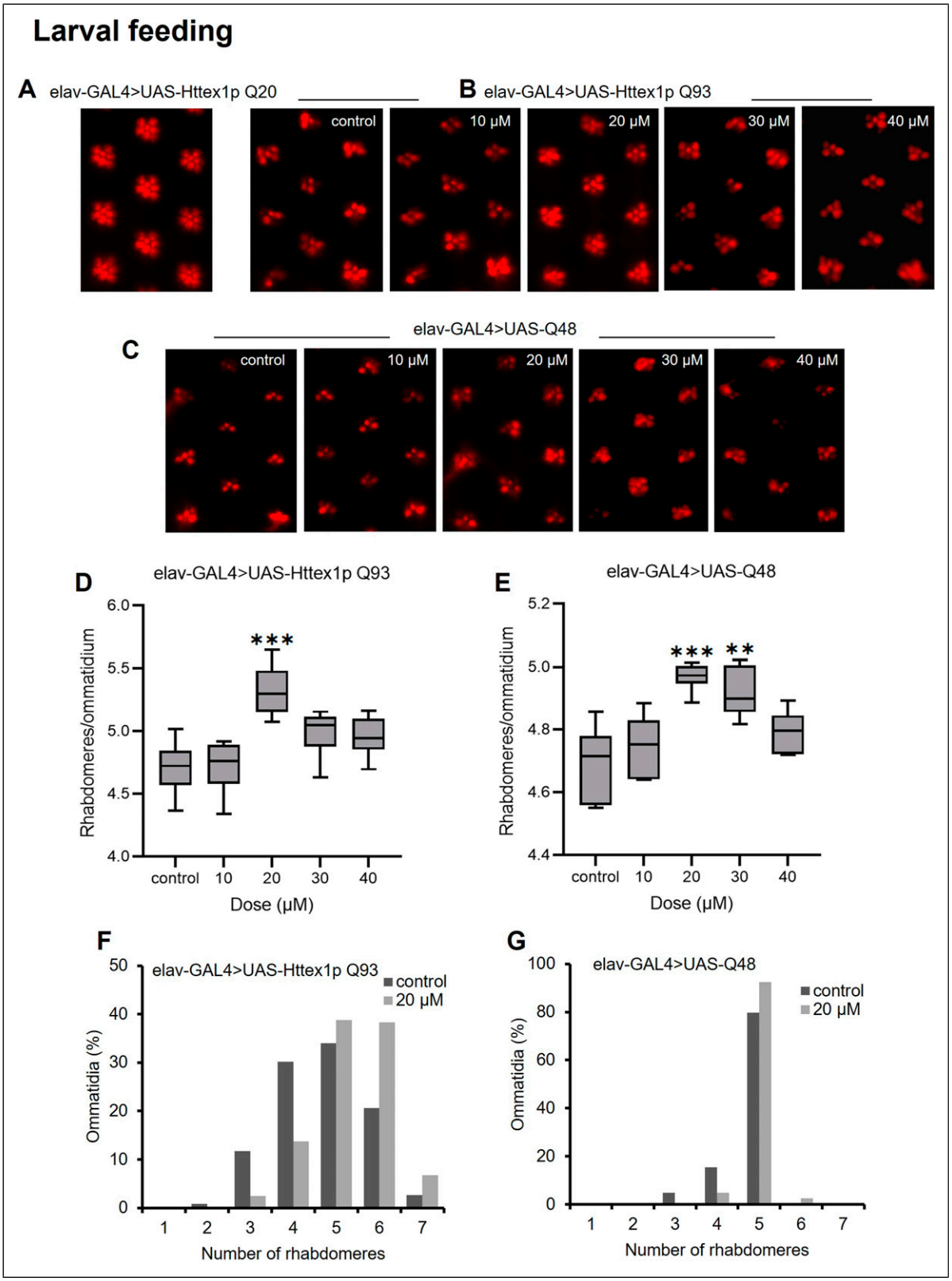

Figure 2. Administration of Eugenol at the disease onset suppresses photoreceptor degeneration in PolyQ flies. No neurotoxicity was observed in 7-day-old-non-pathogenic Httex1p Q20 flies (A). An effective dose of 20 μM Eugenol significantly suppressed neurodegeneration, when fed since early larval stage in pathogenic Httex1p Q93 (B, D, F) and Q48 flies (C, E, G) at 7-day post eclosion. We quantified these data as the number of rhabdomeres per ommatidium (D, E) and the distribution of the percent of ommatidia containing the specified number of photoreceptors (F, G). For each condition, 6 flies were assayed and at least 300 ommatidia were scored. The box plots show the median, minimum and maximum values. Data analysis was performed using an analysis of variance (ANOVA) followed by Tukey's post hoc test, ***, $P < 0.001$; **, $P < 0.01$, compared with control.

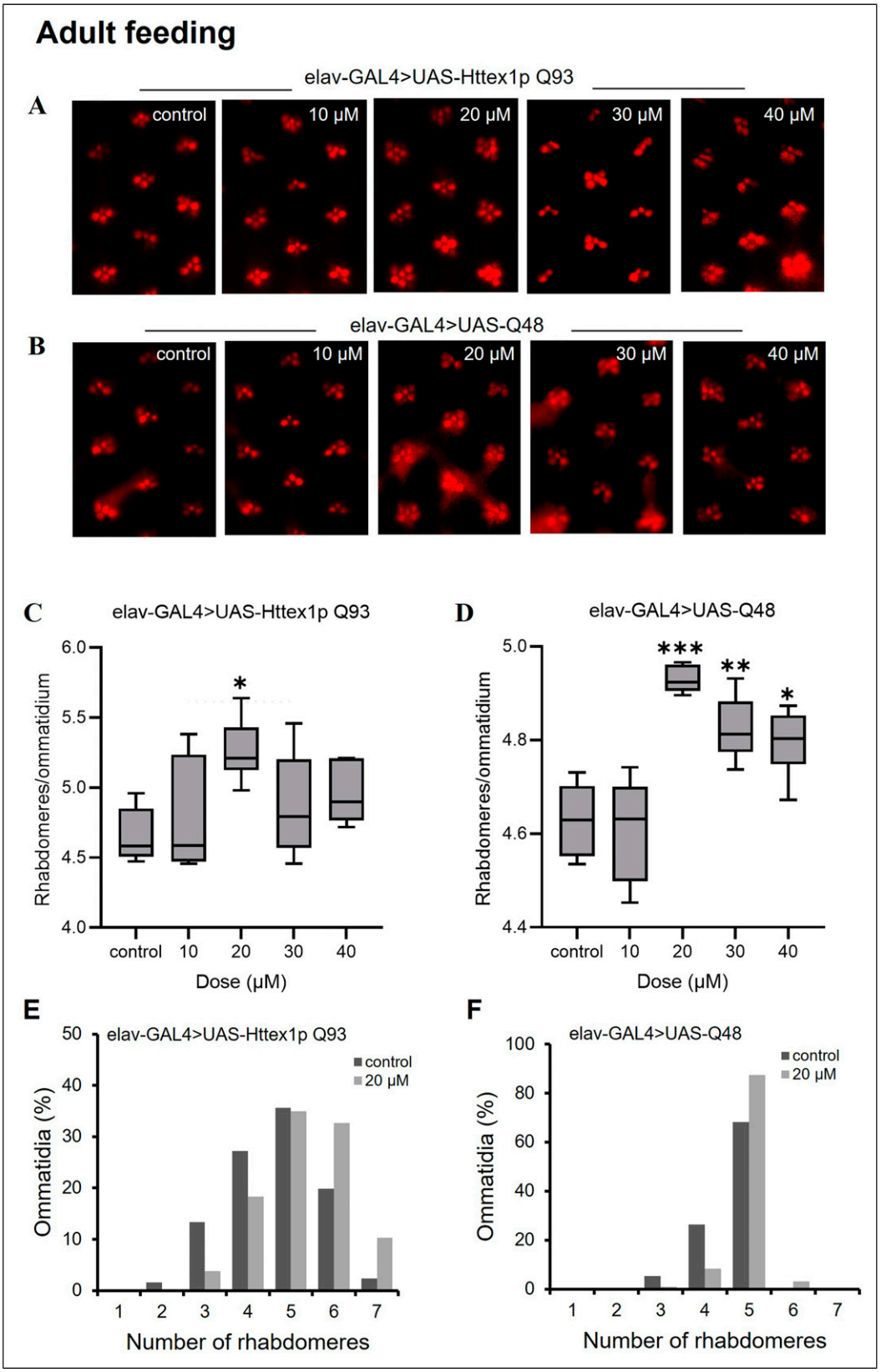

Figure 3. Suppression of photoreceptor neurodegeneration in PolyQ flies by Eugenol after disease progression. Feeding Eugenol at an effective dose of 20 μM only during adult stage suppressed photoreceptor neurodegeneration in Httex1p Q93 (A, C, E) and Q48 (B, D, F) flies. Data was analyzed by calculating the number of rhabdomeres per ommatidium (C, D) and the distribution of the percent of ommatidia containing the specified number of photoreceptors (E, F). A total of 6 flies were assayed per condition and at least 300 ommatidia were scored. The box plots show the median, minimum and maximum values. Data was analyzed by an analysis of variance (ANOVA) followed by Tukey's post hoc test, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$, compared with control.

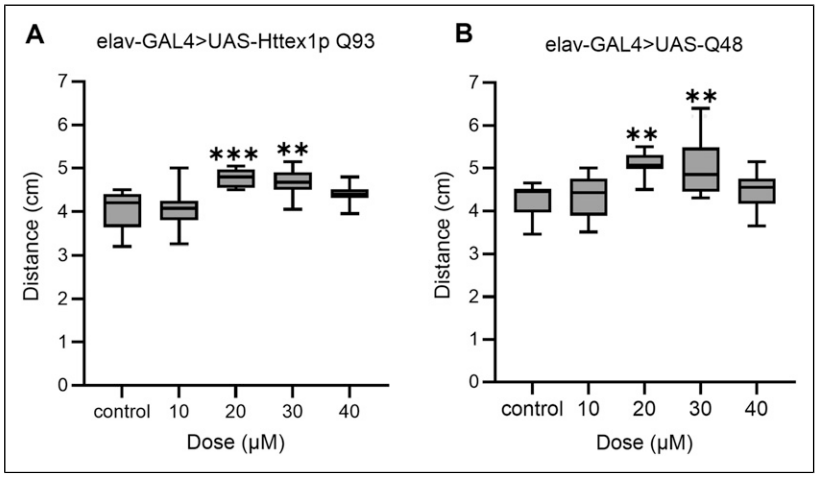

Figure 4. Administration of Eugenol improves locomotor dysfunction of PolyQ larvae. Feeding 20 μM Eugenol significantly suppressed impaired motor function of Httex1p Q93 (A) and Q48 (B) larvae. For each condition, the crawling ability of 10 larvae (n = 10) was monitored and the experiment was repeated 2 times for each larva. The box plots show the median, minimum and maximum values. Data analysis was done using an analysis of variance (ANOVA) followed by Tukey's post hoc test, ***, $P < 0.001$; **, $P < 0.01$, as compared to control.

neuroprotection. Notably, the incidence of neurodegenerative diseases is lower among Asian populations who regularly consume spices compared to Western countries.[33] Accumulating evidence suggests that regular intake of phytochemicals can lead to long-term health benefits, including improved mental and physical efficiency, enhanced neuronal survival, reduced inflammation, and a strengthened antioxidant system.[34,35]

The natural phenolic compound 'Eugenol' possesses potent antioxidant, anti-inflammatory, cardiovascular, analgesic and local anesthetic properties. Its antioxidant effects have been demonstrated in various conditions, including neuropathy, nephrotoxicity, neurotoxicity, chronic inflammation, and cancer.[15,36,37] Eugenol has been shown to enhance glutathione-related enzyme activity, inhibit 5-lipoxygenase activity and protect neurons against excitotoxic and oxidative damage.[38-40] Previous studies strongly support Eugenol's therapeutic potential in treating AD and PD.[16-18] Given its wide range of therapeutic properties and outstanding safety profile, 'Eugenol' holds significant promise for treating multiple neurodegenerative diseases without causing undesirable side effects.

Some of the most common measures of neurodegeneration and neuronal dysfunction include assessing the integrity of photoreceptor cells in the fly compound eye, as well as monitoring larval crawling and adult climbing ability. The adult compound eye consists of a hexagonal array of approximately 750 facets, the lenses of the unit eyes, or ommatidia. Each ommatidium is composed of 9 neuronal cells (8 photoreceptors and 1 mechanosensory) and 11 support cells including the pigment and cone cells. The photoreceptor cells produce highly reticulated membranes called rhabdomeres which carry light-gathering rhodopsins. In the fly eye, each ommatidium typically contains a trapezoidal arrangement of eight rhabdomeres, with seven of them being visible.[20] Overt degeneration of photoreceptor neurons is a well-accepted measure of PolyQ mediated neuronal degeneration. The progressive loss of photoreceptor neurons in flies expressing Httex1p Q93 or Q48 peptide can be observed using the pseudopupil assay which involves scoring of the visible rhabdomeres by shining light through the back of head.[24] Although progressive neuronal loss is undoubtedly a major hallmark of HD, impaired motor neuron function also occurs in disease pathogenesis. Expression of normal repeat Httex1p Q20 peptide has no cytotoxic effect on its own while flies which express expanded Httex1p Q93 or polyQ peptide show progressive motor inability with age due to impaired functioning of motor neurons.[20,22] This study investigated the effects of Eugenol on photoreceptor degeneration and motor dysfunction of transgenic *Drosophila* expressing expanded Httex1p Q93 or Q48 peptide under the control of *elav*-Gal4 driver. As common measures of compromised motor neuron function at different developmental stages, we monitored larval crawling and adult climbing ability in pathogenic condition. The current study shows that administration of Eugenol does not result in adverse effects in non-pathogenic flies expressing Httex1p Q20 peptide; however, feeding Eugenol since larval stage or only during adult period after disease progression significantly subdues photoreceptor degeneration and impaired motor function of diseased Httex1p Q93 and Q48 flies. This study results clearly demonstrate Eugenol as a safe, potent suppressor of toxic PolyQ protein-induced neurodegeneration and motor dysfunction. While this study reveals the potential benefits of Eugenol in mitigating PolyQ disease symptoms, a limitation is the lack of investigation into the underlying molecular mechanisms, such as its effects on protein aggregation, interactions with quality control pathways, and its antioxidant and anti-inflammatory

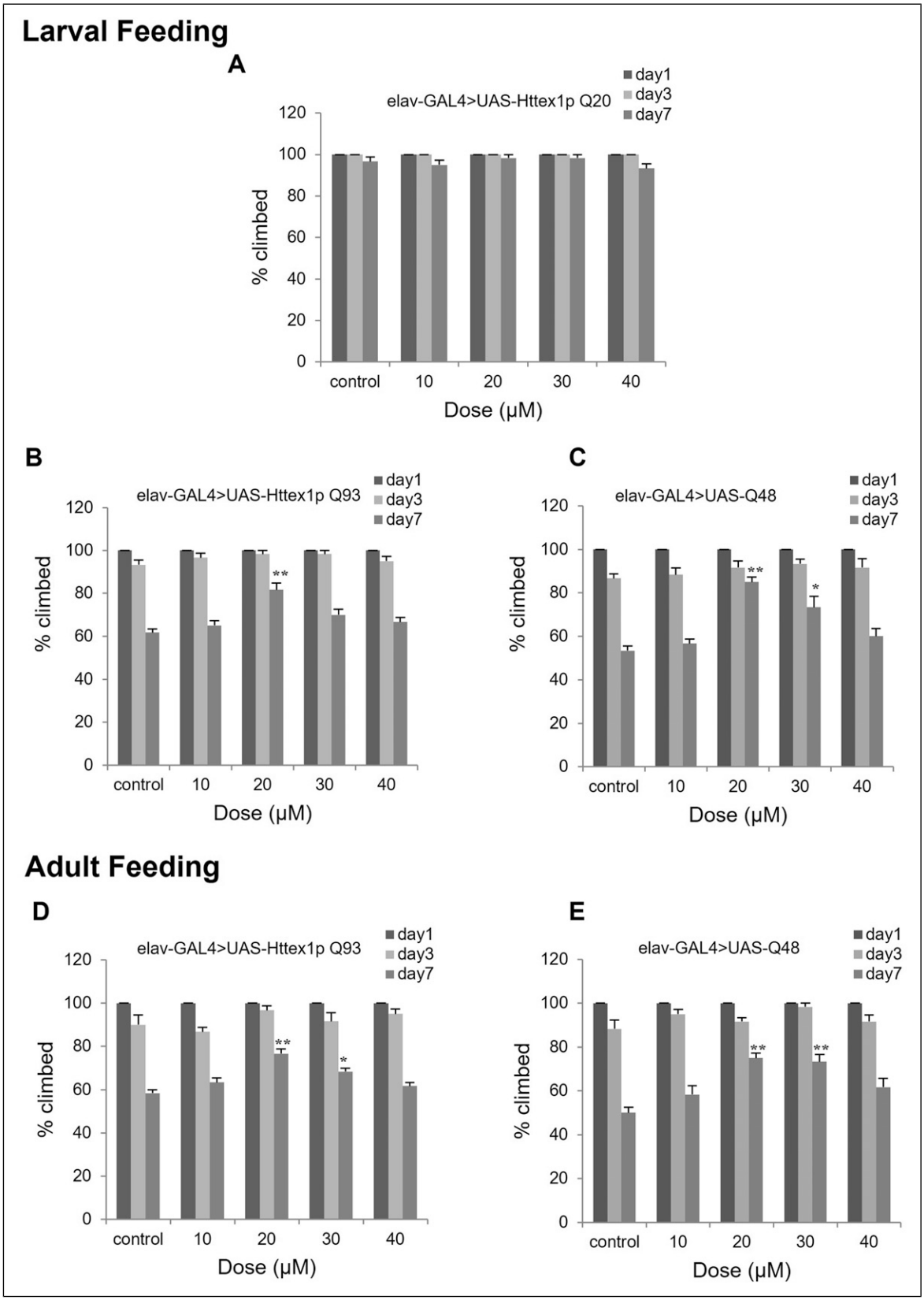

Figure 5. Eugenol ameliorates locomotor dysfunction of PolyQ flies. (A) Eugenol had no effect in non-pathogenic Httex1p Q20 condition. Feeding Eugenol since larval period (B) and (C), or only during adult life (D) and (E), markedly suppressed locomotor dysfunction of diseased Httex1p Q93 and Q48 flies at 7-day post eclosion. The climbing ability of 2 groups of 10 flies (n = 20) were assayed per condition for a total of 6 trials. Data was analyzed using Kruskal-Wallis test followed by Mann-Whitney test with Bonferroni correction to adjust *P*-values; values represent mean ± SEM (**, $P < 0.01$; *, $P < 0.05$, compared with control).

properties. Further, to gain deeper mechanistic insights into the observed reduction in neurodegeneration and motor dysfunction, future studies should incorporate molecular analyses such as gene expression analysis, protein interaction studies, and analysis of specific signaling pathways. However, it is essential to note that in HD, symptomatic issues such as cognitive and motor deficits are the most problematic and of major concern. In this study, we are strongly suggesting a systematic dosage study and suppression of behavioral problems in HD, focusing on addressing these critical symptomatic issues.

Conclusion

In summary, the current study demonstrates that 'Eugenol administration' at disease onset or even after progression significantly ameliorated PolyQ induced neurodegeneration, cytotoxicity and compromised neuronal function. Thus, Eugenol can be an effective and promising agent for the treatment of multiple neurodegenerative diseases including HD.

Acknowledgments

N.A. and A.C. acknowledge University Grants Commission (UGC) for the financial assistance provided in the form of research fellowship (Ref. No. SMS/02/139/ZOO/2012-13/22211). N.A. and A.C. thank Prof. J. Lawrence Marsh, University of California, Irvine, CA, USA for the generous gifts of PolyQ and GAL4 driver lines. N.A. and A.C. thank Jassika Gupta and Mansi Yadav, Department of Zoology, University of Delhi for reviewing the manuscript draft.

Author Contributions

N.A. and A.C. designed the experiments and analyzed the data. A.C. performed the experiments. Manuscript writing and reviewing was done by both the authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the DU/DST-PURSE GRANT, R & D GRANT from the University of Delhi, and University Grants Commission (UGC).

Ethical statement

Ethical approval

This study used only *Drosophila* models which did not require ethical approval.

ORCID iD

Anjalika Chongtham https://orcid.org/0000-0001-8437-3899

References

1. Lamptey RNL, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A review of the common neurodegenerative disorders: current therapeutic approaches and the potential role of nanotherapeutics. *Int J Mol Sci*. 2022;23(3):1851.
2. Perrone F, Cacace R, van der Zee J, Van Broeckhoven C. Emerging genetic complexity and rare genetic variants in neurodegenerative brain diseases. *Genome Med*. 2021;13(1):59.
3. Costa MD, Maciel P. Modifier pathways in polyglutamine (PolyQ) diseases: from genetic screens to drug targets. *Cell Mol Life Sci*. 2022;79(5):274.
4. Lieberman AP, Shakkottai VG, Albin RL. Polyglutamine repeats in neurodegenerative diseases. *Annu Rev Pathol*. 2019;14:1-27.
5. McLoughlin HS, Moore LR, Paulson HL. Pathogenesis of SCA3 and implications for other polyglutamine diseases. *Neurobiol Dis*. 2020;134:104635.
6. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993; 72(6):971-983.
7. Cattaneo E, Zuccato C, Tartari M. Normal huntingtin function: an alternative approach to Huntington's disease. *Nat Rev Neurosci*. 2005;6(12):919-930.
8. Xiang C, Zhang S, Dong X, Ma S, Cong S. Transcriptional dysregulation and post-translational modifications in polyglutamine diseases: from pathogenesis to potential therapeutic strategies. *Front Mol Neurosci*. 2018;11:153.
9. Gkekas I, Gioran A, Boziki MK, Grigoriadis N, Chondrogianni N, Petrakis S. Oxidative stress and neurodegeneration: interconnected processes in PolyQ diseases. *Antioxidants*. 2021; 10(9):1450.
10. Nambron R, Silajdžić E, Kalliolia E, et al. A metabolic study of Huntington's disease. *PLoS One*. 2016;11(1):e0146480.
11. Jia Q, Li S, Li XJ, Yin P. Neuroinflammation in Huntington's disease: from animal models to clinical therapeutics. *Front Immunol*. 2022;13:1088124.
12. Kaur S, Samota MK, Choudhary M, et al. How do plants defend themselves against pathogens-Biochemical mechanisms and genetic interventions. *Physiol Mol Biol Plants*. 2022;28(2): 485–504.
13. Taleuzzaman M, Jain P, Verma R, Iqbal Z, Mirza MA. Eugenol as a potential drug candidate: a review. *Curr Top Med*. 2021;21: 1804–1815.
14. Barboza JN, da Silva Maia Bezerra Filho C, Silva RO, Medeiros JVR, de Sousa DP. An overview on the anti-inflammatory potential and antioxidant profile of Eugenol. *Oxid Med Cell Longev*. 2018;2018:3957262.
15. Nisar MF, Khadim M, Rafiq M, Chen J, Yang Y, Wan CC. Pharmacological properties and health benefits of Eugenol: a comprehensive review. *Oxid Med Cell Longev*. 2021;2021: 2497354.
16. Ulanowska M, Olas B. Biological properties and prospects for the application of Eugenol-A review. *Int J Mol Sci*. 2021;22(7): 3671.

17. Kabuto H, Tada M, Kohno M. Eugenol [2-methoxy-4-(2-propenyl)phenol] prevents 6-hydroxydopamine-induced dopamine depression and lipid peroxidation inductivity in mouse striatum. *Biol Pharm Bull*. 2007;30(3):423–427.
18. Irie Y, Keung WM. Rhizoma acori graminei and its active principles protect PC-12 cells from the toxic effect of amyloid-beta peptide. *Brain Res*. 2003;963(1–2):282–289.
19. Jung MJ, Kim N, Jeon SH, Gee MS, Kim JW, Lee JK. Eugenol relieves the pathological manifestations of Alzheimer's disease in 5×FAD mice. *Phytomedicine*. 2023;118:154930.
20. Marsh JL, Pallos J, Thompson LM. Fly models of Huntington's disease. *Hum Mol Genet*. 2003;12(Spec No 2):R187–R193.
21. Marsh JL, Thompson LM. Can flies help humans treat neurodegenerative diseases? *Bioessays*. 2004;26(5):485–496.
22. Bolus H, Crocker K, Boekhoff-Falk G, Chtarbanova S. Modeling neurodegenerative disorders in *Drosophila melanogaster*. *Int J Mol Sci*. 2020;21(9):3055.
23. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. 1993;118(2):401–415.
24. Steffan JS, Bodai L, Pallos J, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. *Nature* 2001;413(6857):739–743.
25. Agrawal N, Pallos J, Slepko N, et al. Identification of combinatorial drug regimens for treatment of Huntington's disease using *Drosophila*. *Proc Natl Acad Sci U S A*. 2005;102(10):3777–3781.
26. Chongtham A, Agrawal N. Curcumin modulates cell death and is protective in Huntington's disease model. *Sci Rep*. 2016;6:18736.
27. Aditi K, Shakarad M, Agrawal N. Altered lipid metabolism in *Drosophila* model of Huntington's disease. *Sci Rep*. 2016;6:31411.
28. Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a *Drosophila* model of Huntington's disease. *Proc Natl Acad Sci U S A*. 2004;101(9):3224–3229.
29. Slepko N, Bhattacharyya AM, Jackson GR, et al. Normal-repeat-length polyglutamine peptides accelerate aggregation nucleation and cytotoxicity of expanded polyglutamine proteins. *Proc Natl Acad Sci U S A*. 2006;103(39):14367–14372.
30. Paulson HL, Bonini NM, Roth KA. Polyglutamine disease and neuronal cell death. *Proc Natl Acad Sci U S A*. 2000;97(24):12957–12958.
31. Blum ES, Schwendeman AR, Shaham S. PolyQ disease: misfiring of a developmental cell death program? *Trends Cell Biol*. 2013;23(4):168–174.
32. Amor S, Peferoen LA, Vogel DY, et al. Inflammation in neurodegenerative diseases–an update. *Immunology*. 2014;142(2):151–166.
33. Kannappan R, Gupta SC, Kim JH, Reuter S, Aggarwal BB. Neuroprotection by spice-derived nutraceuticals: you are what you eat. *Mol Neurobiol*. 2011;44(2):142–159.
34. Nisar A, Jagtap S, Vyavahare S, et al. Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. *Front Pharmacol*. 2023;14:1177050.
35. Velmurugan BK, Rathinasamy B, Lohanathan BP, Thiyagarajan V, Weng CF. Neuroprotective role of phytochemicals. *Molecules*. 2018;23(10):2485.
36. Zari AT, Zari TA, Hakeem KR. Anticancer properties of Eugenol: a review. *Molecules*. 2021;26(23):7407.
37. Padhy I, Paul P, Sharma T, Banerjee S, Mondal A. Molecular mechanisms of action of Eugenol in Cancer: recent trends and advancement. *Life*. 2022;12(11):1795.
38. Yoshimura M, Amakura Y, Yoshida T. Polyphenolic compounds in clove and pimento and their antioxidative activities. *Biosci Biotechnol Biochem*. 2011;75(11):2207–2212.
39. Kar Mahapatra S, Chakraborty SP, Majumdar S, Bag BG, Roy S. Eugenol protects nicotine-induced superoxide mediated oxidative damage in murine peritoneal macrophages in vitro. *Eur J Pharmacol*. 2009;623(1–3):132–140.
40. Prasad NS, Raghavendra R, Lokesh BR, Naidu KA. Spice phenolics inhibit human PMNL 5-lipoxygenase. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70(6):521–528.