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Research Article

NEUROPROTECTIVE ROLE OF UMBELLIFERONE IN THE *DROSOPHILA* MODEL OF ROTENONE-INDUCED PARKINSON'S DISEASE

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ABSTRACT

Umbelliferone (UMB) is a coumarin derivative present ubiquitously in plants and demonstrated to have neuroprotective effects in rodent models of focal cerebral ischemia-reperfusion, MPTP-induced PD, sporadic AD and chronic unpredictable mild stress-induced depression. However, the neuroprotective role of UMB against an experimental model of PD has not yet been investigated. The aim of the present study is to examine the protective effect of UMB against rotenone-induced reduction in lifespan, cognitive impairment and oxidative stress in *Drosophila melanogaster*. Flies were divided into control, rotenone-treated, rotenone + UMB (25, 50 and 100µM) and UMB alone-treated. The duration of lifespan, cognitive (thermotaxis, smell and taste chemotaxis and phototaxis) and behaviour studies (open-field test and negative geotaxis) and biochemical (ROS, TBARS, SOD, catalase and GSH) assays were performed. UMB co-exposure attenuated the rotenone-induced reduction in lifespan, locomotion and cognitive impairment and oxidative stress in flies through its potent antioxidant properties. Our results indicated the protective role of UMB against rotenone-mediated neurotoxicity in flies could be partially due to its potent antioxidant function. Moreover, further *in vivo* and *in vitro* studies are needed to understand the precise neuroprotective mechanisms.

Keywords: *Drosophila melanogaster*, Rotenone, UMB, Oxidants, Antioxidants, Behaviour.

INTRODUCTION

Drosophila melanogaster (fruit fly) has developed as an *in vivo* model to study the mechanism of neurodegeneration. The fruit fly is used as a PD model due to the physiological, biological and neuronal features (presence of a small cluster of dopaminergic (DAgic) neurons (~130 DAgic neurons) similar to mammals and also homogenous to five out of six genes like DJ-1, UCH-L1, parkin, PINK1 and LRRK2 that mimic the sporadic form of PD (Whitworth *et al*., 2006). In addition, fruit flies also resemble human beings by its response to L-DOPA exposure (Cannon *et al*., 2009). The European Center for the Validation of Alternative Models approves the fruit fly as the model organism because it fulfils the 3Rs protocol (Hirth, 2010). Additionally, this organism is beneficial because it is commonly used to screen and evaluate numerous therapeutic compounds and phytoextracts in a fast and simple method (Chaudhuri *et al*. 2007; Sudati *et al*. 2013). Recently, *Drosophila* is used as a model for the successful discovery of three FDA-approved drugs (nadolol, fosfanol and levonordefrin) for therapy of Huntington's disease (Desai *et al*., 2006). For PD, more studies on the effect of paraquat and rotenone / genetic manipulations or their combinations on *Drosophila* models have been carried out (Feany and Bender, 2000). PD patients have 2.5 times or more chance of exposure in their life span with these two commonly used pesticides (Spivey, 2011). Nowadays, rotenone exposure can occur not only for occupational people but also for non-occupational individuals due to its presence in the environment (Tanner *et al*., 2011). It belongs to the rotenoid family, which contains a dihydro-γ-pyrone ring that is responsible for the toxicity (Lawana and Cannon, 2020). This neurotoxin can pass through the blood–brain barrier, inducing PD

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symptoms in flies (Johnson and Bobrovskaya, 2015) by obstructing the activity of mitochondrial complex-1electron transport chain (Wang *et al*., 2020) and enhancing the formation of reactive oxygen species. Reduced synthesis of ATP along with enhanced formation of the ROS (Siima *et al*., 2020) and destroys the dopaminergic neurons, resulting in cognitive dysfunctions and motor symptoms resembling clinical PD (Chia *et al*., 2020).

Currently available PD drugs act either by stimulating the dopaminergic system or inhibiting the cholinergic and glutamatergic system, thereby offering brief relief to the patients (Taddei *et al*., 2017). Most of them restore the dopaminergic function temporarily, without protecting against the neurodegeneration process. Moreover, the drug efficacy is varied for individuals (Akram *et al*., 2017) and provokes side effects (Oertel and Schulz, 2016; Olanow, 2008). As oxidant-antioxidant imbalance is a key component in PD pathogenesis, antioxidants with potent radical scavenging activity may be useful in the management of PD (Puspita *et al*., 2017). Numerous plant extracts or its based compounds with potent antioxidant properties are reported to attenuate the toxic effects of oxidative stress induced by ROS in PD. Umbelliferone (UMB) is a coumarin derivative found ubiquitously in some plants such as coriander, carrot, wild celery, etc. (Ali *et al*., 2020). Previous studies demonstrated the anticancer, antimicrobial, antioxidant, antidiabetic and anti-inflammatory properties of UMB (Karakaya *et al*., 2019; Mazimba *et al*., 2017). In addition, it has the ability to penetrate the BBB, making it a capable agent in the therapy of neurodegenerative diseases. The neuroprotective effects of UMB in experimental models of focal cerebral ischemia-reperfusion (Wang *et al*., 2015), MPTP-induced PD (Subramaniam and Ellis, 2013), sporadic AD (Hindam *et al*., 2020) and unpredictable chronic mild stress-mediated depression (Qin *et al*., 2017; Su *et al*., 2016) were reported. However, the effect of UMB on the rotenone-induced *Drosophila* model of PD has not yet been investigated.

METHODS AND MATERIALS

Drosophila melanogaster stock and culture

The Oregon R strain (wild-type) D. melanogaster was obtained from CCMB, Hyderabad, India. They were reared in *Drosophila* standard cornmeal-sugar medium under standard conditions (Arumugam *et al*., 2018).

Treatment schedule

Dose-dependent study - Phase I

The control and experimental flies were grouped into control, rotenone (500 µM) (Surya *et al*., 2025), UMB (25 µM) + rotenone, UMB (50 µM) + rotenone, UMB (100 µM) + rotenone and UMB (100 µM) administered via food medium for 7 days. The maximal protective dose of UMB was fixed after analysing the life span test on the 7th day.

Study with fixed dose - Phase II

The flies were grouped into control, rotenone (500 µM), UMB (50 µM) + rotenone and UMB (50 µM). On the 8th day, the control and experimental flies were analysed for several behavioural assays. Then, flies were sedated, homogenised using phosphate buffer, centrifuged, and supernatants were separated and utilised for oxidant and antioxidant assays (Surya *et al*., 2025).

Open field test

15 flies were kept in a petridish that was separated into sections (1 cm × 1 cm squares) using grid lines and were observed for 1 minute (Hirth, 2010). The movement was calculated by counting the no. of squares crossed in three trials.

Negative geotaxis

15 control and experimental flies were kept at the bottom of a glass vial (15 cm × 1.5 cm), which was plugged with a cotton. The vial was gently tapped to allow them to fall to the bottom. The time taken for each D. melanogaster to reach the top was noted. Three trials were done and data were calculated as mean ± SEM (Amulya and Subramanian., 2022).

Smell chemotaxis

15 control and experimental flies were kept in the bottom of a glass vial that was labelled into 3 (I, II & III) compartments. The vial was plugged (compartment I) with benzaldehyde (100 nM) soaked cotton. The flies found in compartments I, II & III were counted. Three trials were performed and the data was calculated as mean ± SEM (Amulya and Subramanian., 2022).

Taste chemotaxis

15 control and experimental flies were kept in the bottom of a glass vial that was labelled into 3 (I, II & III) compartments. The vial was plugged (compartment I) with sucrose (0.1%) soaked cotton. The flies found in compartments I, II & III were counted. Three trials were performed and the data was calculated as mean ± SEM (Amulya and Subramanian., 2022).

Phototaxis

15 control and experimental flies were kept in the bottom of a glass vial, which was plugged with a cotton. The flies were allowed to adapt darkness by keeping them in a dark condition for half an hour. Each vial was connected with another vial and divided and labelled into 3 (I, II & III) compartments. A light source was placed near the compartment I for a minute. The flies found in compartments I, II & III were counted. Three trials were performed and the data was calculated as mean ± SEM (Amulya and Subramanian., 2022).

Thermotaxis

A vial was taken and heated (45ºC) at one end. The other end was attached with another vial using adherent tape. Both vials were sectioned into 3 compartments (I, II& III), with compartment I representing the heated area. 15 control and experimental flies were kept in the bottom of a glass vial (III compartment). The flies found in compartments I, II & III were counted. Three trials were performed and the data was calculated as mean ± SEM (Amulya and Subramanian., 2022).

Estimation of biochemical variables

Thiobarbituric Acid Reactive Substances (TBARS) Assay

To the whole-body homogenate, acetic acid buffer, sodium dodecyl sulfate and thiobarbituric acid were added, heated in a water bath, cooled and OD was observed at 532 nm. The concentrations were quantified as µmol MDA per mg of protein (Ohkawa *et al*.,1979).

Protein carbonyl

To whole body homogenate, 20% trichloroacetic acid was added to remove proteins. Then allowed to react with 2,4-dinitrophenylhydrazine, leading to dinitrophenylhydrazone complex formation. This complex was subsequently dissolved in 6 M guanidine hydrochloride, and OD was measured at 375 nm (Dalle-Donne *et al*., 2003).

Superoxide Dismutase (SOD) Activity Assay

To 10 µL of tissue homogenate, quercetin in dimethyl formamide, N,N,N′,N′-tetramethyl ethylenediamine and sodium phosphate buffer containing EDTA were added. Then, the absorbance was measured at 406 nm and expressed as U^a/mg of protein (Franco *et al*., 2009).

Catalase Activity Assay

To the tissue homogenate, phosphate buffer containing EDTA, H_2O_2 and Triton X-100 were added. The OD was measured at 240 nm and expressed in U^b/mg of protein (Paula *et al*., 2014).

Reduced Glutathione (GSH) Content Assay

0.1 M formic acid was added to the tissue homogenate and centrifuged to precipitate the proteins. The supernatant was mixed with o- phthalaldehyde and kept in a room temperature for half an hour and OD was measured at 345 nm and 425 nm (Rao *et al*., 2016).

Determination of Drosophila Reactive Oxygen Species (ROS)

100 µL of protein isolated from tissue homogenate was treated with H2DCFDA in a 96 well plate and kept for 45 minutes in room temperature. The fluorescence was measured at 490 and 522 nm (Filaferro *et al*., 2022).

RESULTS AND DISCUSSION

Rotenone exposure (500 µM) induced ~ 50% death in flies as compared to control flies (Figure 1). The lifespan of the *Drosophila* co-exposed to rotenone and UMB was enhanced dose- dependently as compared to toxin alone-exposed flies. Our results indicated that the exposure to rotenone significantly reduced the lifespan of flies, whereas co-administration of various concentrations of UMB (25, 50 and 100 Mm in the diet) used in this study, prolonged the lifespan of flies in a dose-dependent manner as compared to control flies. This demonstrates that UMB could have anti-aging property through extending the survival of the flies. A review by Chen *et al*., (2024) suggested that the extracts of cocoa, broccoli, and blueberry and dietary phytochemicals such as curcumin, quercetin, fisetin, resveratrol, epicatechin, epigallocatechin-3-gallate, urolithin A, sesamin, sulforaphane and spermidine enhanced the lifespan of *Drosophila.* No changes in the survival rate were present in between UMB alone-exposed flies as compared to control flies.

The movements of control and experimental flies were measured by the open field test (Figure 2). Significant reduction in the number of crossings (decreased movement) was found in rotenone-exposed flies as compared to control flies. The open-field test is used to analyze the exploratory behavior of the flies in a novel environment, where a diminished number of peripheral and central crossings along with reduced rearing and grooming activities, were observed in the flies exposed to rotenone. Co- treatment of UMB significantly enhanced the movement of rotenone-treated flies as compared to rotenone-treated flies alone. The negative geotaxis assay is used to measure the climbing activity of control and experimental flies. The increased climbing time (seconds) i.e., diminished negative geotaxis behaviour, was found in rotenone-administered flies as compared to normal ones (Figure 3). The decreased climbing time (seconds) i.e., enhanced negative geotaxis, was found in UMB and rotenone co-exposed flies, indicating the neuroprotective role of UMB. Changes in the activity of AChE are responsible for reduced locomotion and activities in the open field test (Fernandes *et al*., 2021). Previous studies (Kumar *et al*., 2020 and Farombi *et al*., 2019) indicated that modified activities of AChE were present in the rotenone-exposed flies. Our results showed that UMB ameliorated the rotenone-mediated reduction in the locomotion and activities of the flies. Herbet *et al*. (2026) found that the administration of UMB reduced the AChE activity and the behavioral impairments induced by scopolamine. No significant changes in movement were observed between the UMB alone-exposed and control groups.

The fruit flies are behaviorally prone to temperature (thermotaxis) (Hamada *et al*., 2008), light (phototaxis) (Hardie, 2012), tastants (taste chemotaxis) (Weiss *et al*., 2011), and chemical odors (smell chemotaxis) (Touhara and Vosshall, 2009). The attractive and repulsive responses may be determined by observing towards or outwards movement of flies depending on the intensity and nature of the stimulus (Vang *et al*., 2012). These stimuli are sensed

by vision (phototaxis), odour (smell chemotaxis), taste (taste chemotaxis) and thermosensors (thermotaxis). In the phototaxis assay, normal flies are found mainly in compartment I, where the light source is kept (attraction movement) (Figure 4A). In the thermotaxis experiment, control flies move or tend to move away from the hot surface (compartment I) to other compartments (repulsion movement) (Figure 4B). Rotenone-exposed flies showed a significant reduction in both the attractant and repulsion movement (diminished phototaxis and thermotaxis), as compared to control flies. But there is a deterioration in these movements in rotenone+ UMB-treated flies as compared to rotenone-exposed flies. There are no significant alterations found in between the UMB alone treated flies as compared to control flies.

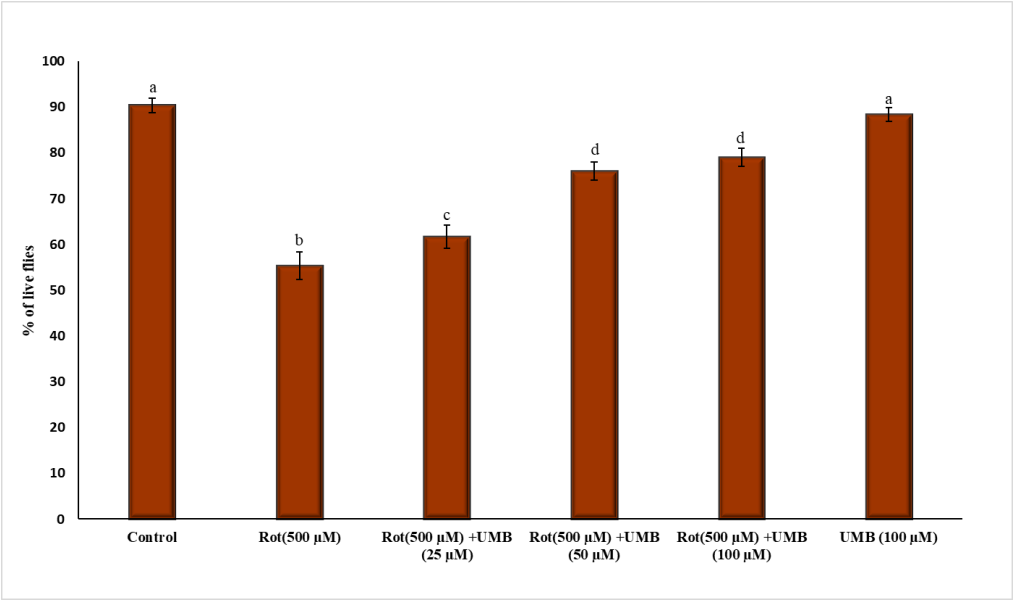


Figure 1. Effect of UMB (dose-dependent) on rotenone-induced viability in flies. Values are expressed as mean ± SEM; *n*=15, values not sharing the same alphabet differ significantly (p<0.05); ANOVA followed by DMRT.

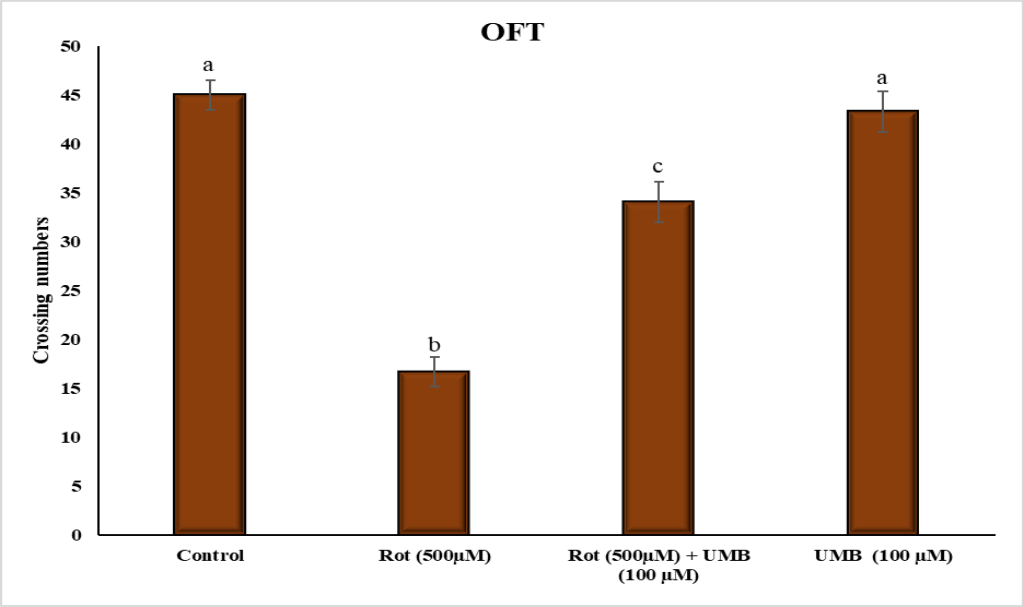


Figure 2. Effect of UMB on rotenone-induced locomotor deficit in flies. All the values are expressed as mean ± SEM; *n*=15, values not sharing the same alphabet differ significantly (p<0.05); ANOVA followed by DMRT.

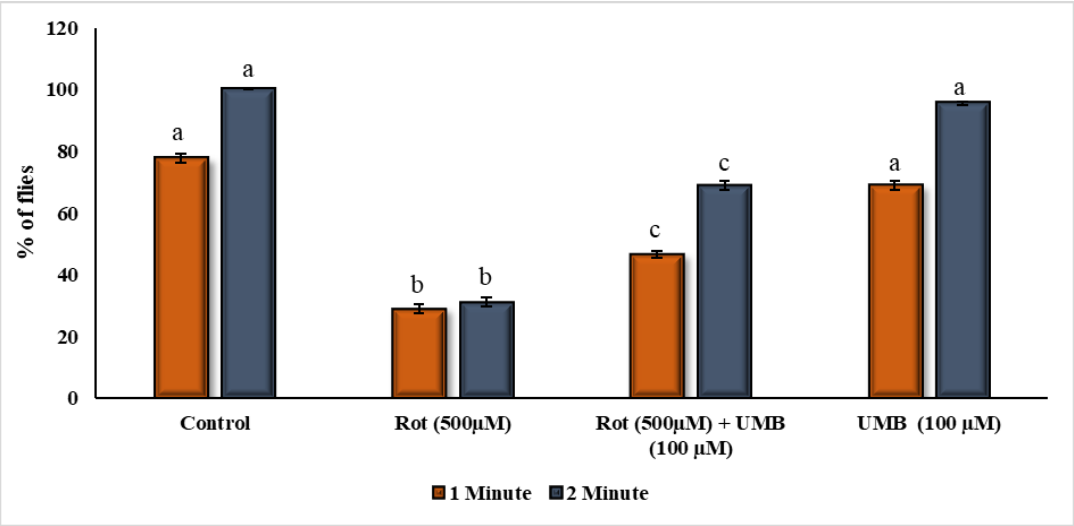
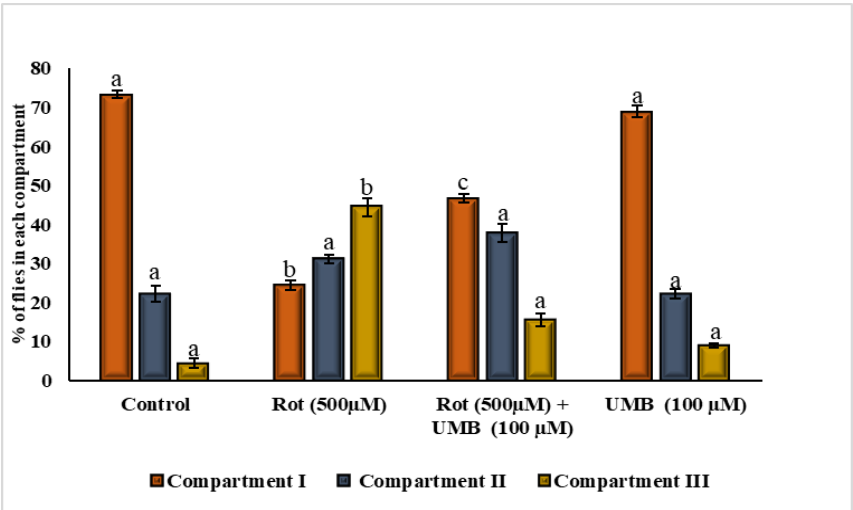


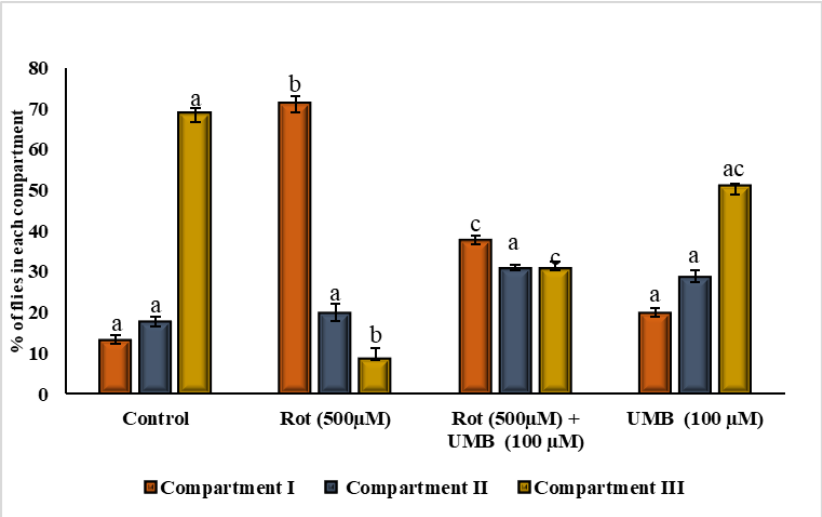
Figure 3. Effect of UMB on rotenone-induced movement deficit in flies. All the values are expressed as mean ± SEM; *n*=15, values not sharing the same alphabet differ significantly ($p<0.05$); ANOVA followed by DMRT.

The olfactory and gustatory functions of control and experimental flies were determined by smell and taste chemotaxis. Normal flies did not like the chemical smell of the benzaldehyde that is placed in compartment I and tended to move away from compartment I to compartments II or III (smell chemotaxis) as compared to rotenone-treated flies. Most of the rotenone-treated flies were found in compartment I ($p<0.05$). Significantly enhanced movements were found in UMB+ rotenone-treated flies to compartment III ($p<0.05$) as compared to rotenone-alone exposed flies (Figure 4C). Normal flies like the taste of the sucrose that is placed on compartment I and tend to move closer to the cotton plug dipped in sugar solution. Rotenone-alone treated flies showed the lesser mobility towards compartment I ($p<0.05$). The response of UMB cotreated rotenone flies was similar to the movement of control flies ($p>0.05$). There are no significant alterations in gustatory function found in between UMB alone and control flies (Figure 4D).

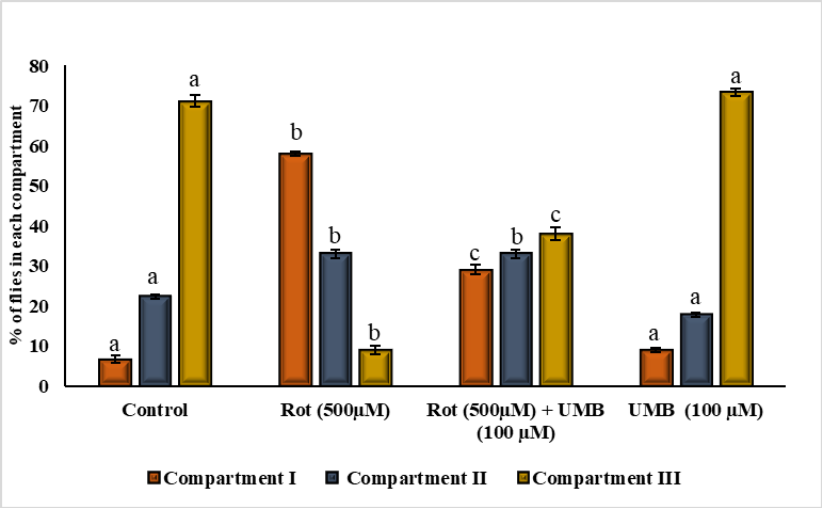
A. Phototaxis



B. Thermotaxis



C. Smell Chemotaxis



D. Taste chemotaxis

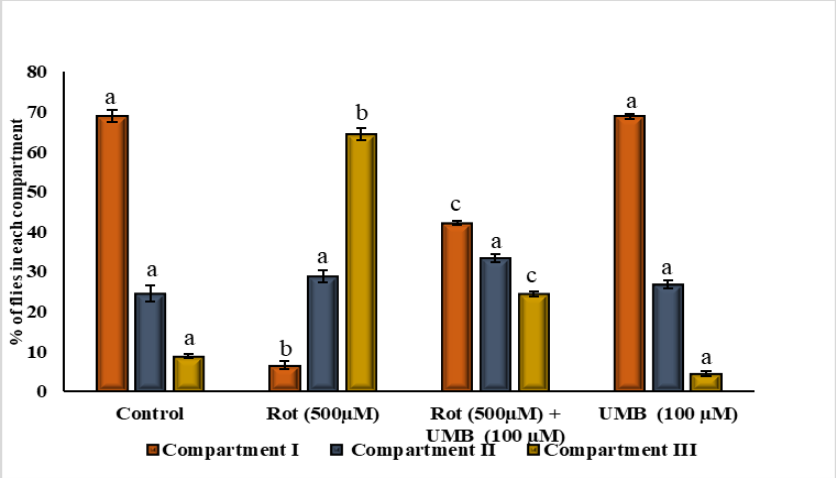
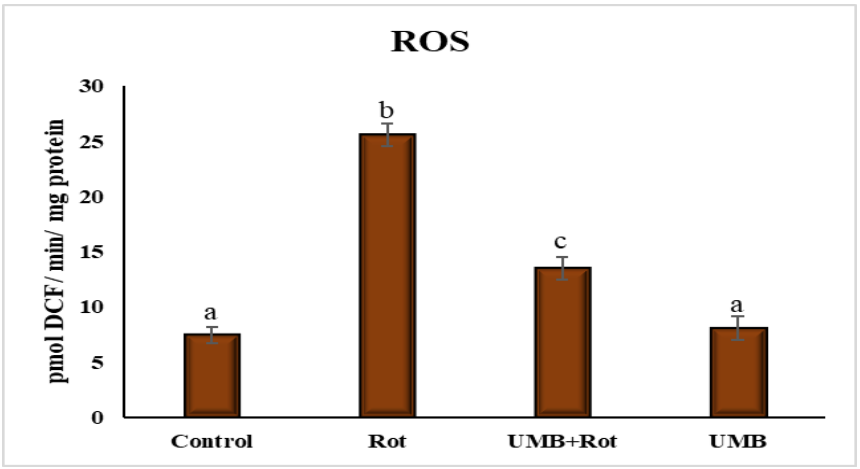
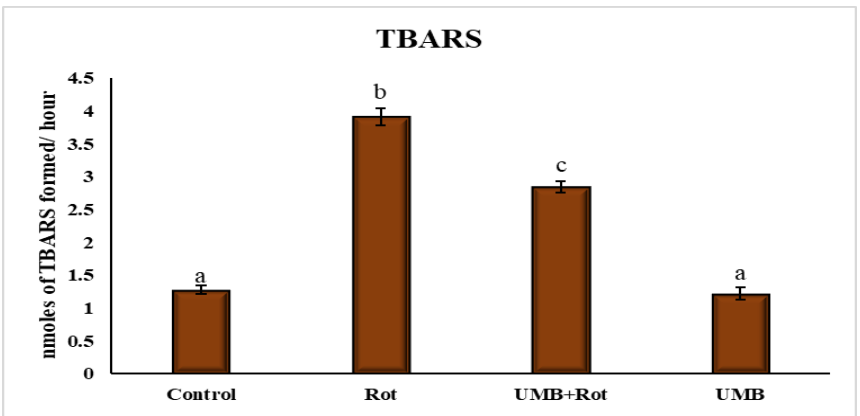


Figure 4 (A, B, C, D): Effect of UMB on rotenone-induced cognitive deficits in flies. All the values are expressed as mean ± SEM; n=15, values not sharing the same alphabet differ significantly (p<0.05); ANOVA followed by DMRT.

Similar to mammals, DA neurotransmission not only regulates the movement in flies but also controls other behaviors such as memory, sleep, olfaction and learning (Yamamoto and Seto 2014). The flies consist of an easily detectable set of dopaminergic (DA) neurons, that are present as symmetrically dispersed clusters in the brain (Whitworth *et al*., 2006, Mao and Davis 2009). The group II flies showed cognitive impairments, which is evidenced by loss of attractant behaviour towards taste chemotaxis and aversive behaviour towards smell chemotaxis, thermal and light stimuli as rotenone induces degeneration of dopaminergic neurons, which leads to the depletion of dopamine. The PD flies exposed to UMB may attenuate dopamine depletion, which is evidenced by the improvement in smell and taste chemotaxis, phototaxis and thermotaxis. Seong *et al*. (2019) demonstrated that the administration of UMB and its derivatives enhanced the levels of dopamine by inhibiting the activity of monoamine oxidase B, which is involved in the breakdown of dopamine. There are no significant alterations found in the UMB alone exposed and control groups.

Rotenone exposure in flies enhanced the levels of ROS, TBARS and protein carbonyls (Figures 5 A, B and C) compared with control flies. UMB administration significantly reduced the pesticide-induced oxidative stress-related indices as compared to rotenone-alone treated flies. There are no significant alterations in the levels of ROS, TBARS and protein carbonyls found in between UMB-alone exposed flies as compared to the control group. Rotenone enhances the oxidative imbalance, which results in the generation of Lewy bodies, causing injury to dopaminergic neurons (Singh *et al*., 2022). Therefore, in the study, the levels of thiobarbituric acid reactive species (TBARS), ROS and PCC were studied in RT-induced PD flies. Rotenone exposure inhibited the activity of mitochondrial complex I, which could enhance the generation of TBARS, ROS and PCC in Drosophila which is corroborated with results of our previous study (Surya *et al*., 2025). Here, it is found that the exposure of UMB significantly attenuated the levels of TBARS in rotenone-treated flies. A previous study by Cárdenas *et al*., (2025) showed that the UMB-rich *Parastrephia quadrangularis* extract significantly inhibited the TBARS levels in RT-induced fly models, which is consistent with our study. Normally, protein carbonylation occurs by the conjugation of the amine group of amino acids with an aldehyde (Blakeman *et al*., 1995) that enhances the half-life of proteins. Proteins transformed by ROS are accumulated during various neurodegenerative conditions, including PD mainly due to reduced protease activity (Dalle-Donne *et al*., 2005). Hence, the quantification of the levels of carbonyl group indicates the amount of oxidative stress that occurs in several diseased conditions (Dimock *et al*., 2001). UMB significantly inhibited the levels of PCC in rotenone exposed flies due to its antioxidant properties.





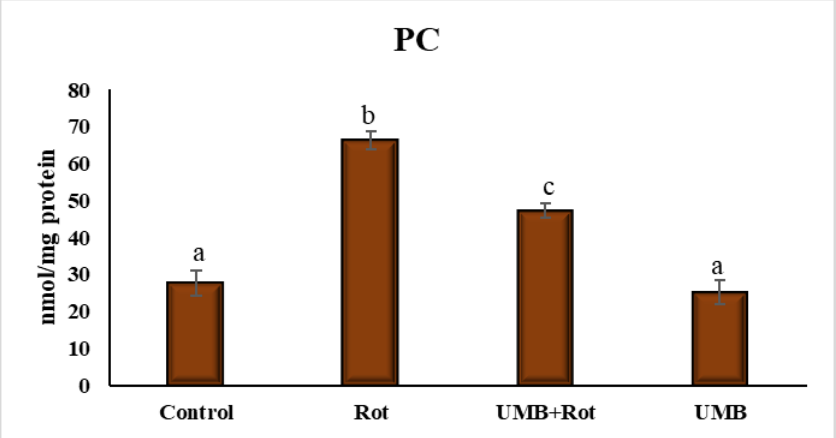
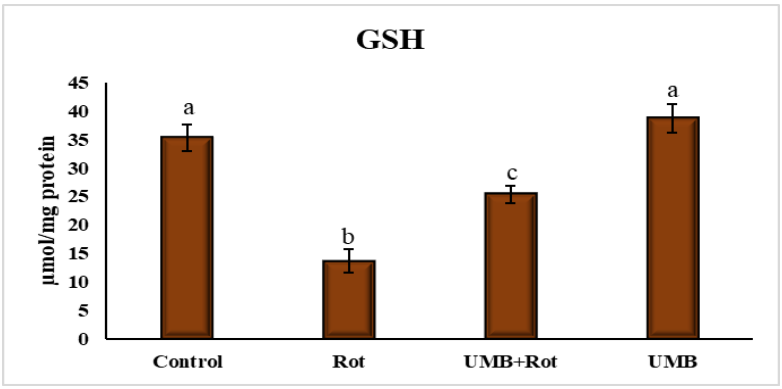
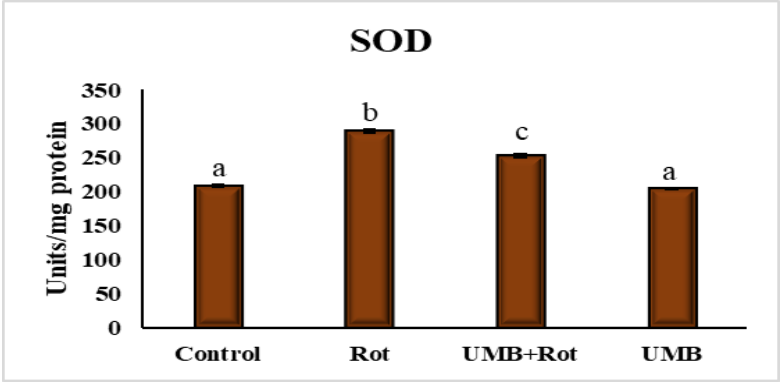


Figure 5 (A, B, C). Effect of UMB on rotenone-induced levels of oxidants in flies. All the values are expressed as mean ± SEM; n=30, values not sharing the same alphabet differs significantly (p<0.05); ANOVA followed by DMRT.

Rotenone exposure in flies diminished the levels of GSH (Figure 6A) and amplified the activities of SOD and catalase (Figure 6B and C) as compared to normal flies. Co-administration of UMB tends to bring the levels of GSH and activities of SOD and catalase to near normal owing to its potent antioxidant properties. In the present study, we observed a marked induction of oxidative imbalance in rotenone-exposed flies along with the significant enhancement in the activities of enzymatic antioxidants like SOD and catalase, which may be due to a protective or compensatory mechanism (Hosamani, 2009). These enzymatic antioxidants may offer protection against several toxic effects of reactive oxygen species induced by pesticides (El-Demerdash, 2012). SOD plays a key role in the removal of the more toxic superoxide anion into less toxic H_2O_2 by the dismutation reaction (Droge, 2002). Catalase is a primary antioxidant enzyme that decomposes H_2O_2 into H_2O in combination with GPx (Cheng *et al*., 1981). GSH is a ubiquitous, soluble, non-enzymatic antioxidant tripeptide that is present abundantly in the cellular system. It can detoxify ROS by reacting directly with other non-enzymatic antioxidants or by acting as a substrate for GPx and GST. Several studies have reported the potent antioxidative nature of UMB and its capability to modulate the activities of antioxidant enzymes and GSH levels, which may support the research findings (Özbağcı *et al*., 2025; Zhang *et al*., 2023; Yang *et al*., 2023).





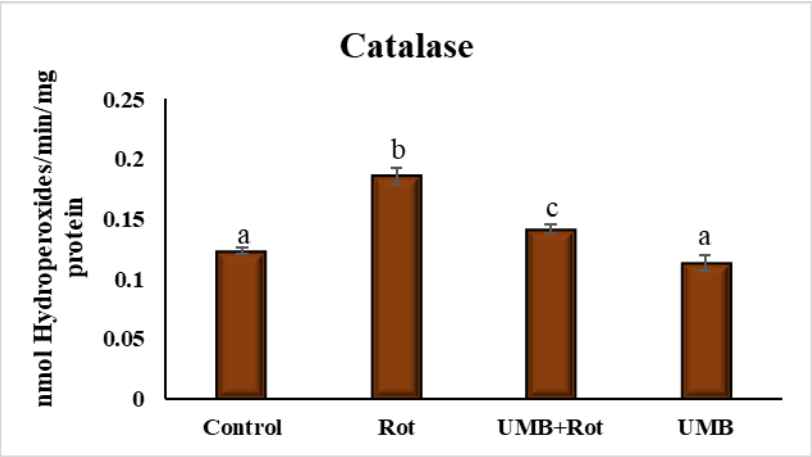


Figure 6 (A, B, C). Effect of UMB on rotenone-induced reduction in the levels and activities of antioxidants in flies. All the values are expressed as mean ± SEM; *n*=30, values not sharing the same alphabet differs significantly ($p<0.05$); ANOVA followed by DMRT.

CONCLUSION

Our study showed that the rotenone exposure diminished the longevity, locomotion and cognition and increased oxidative imbalance in flies. UMB co-administration ameliorated both locomotion and cognition, partly due to its potent antioxidant functions. However, further *in vitro* and *in vivo* studies are needed to know the molecular mechanism and prove the neuroprotective role of UMB.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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