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Neuroprotective activity of Curcumin against paraquat induced oxidative stress markers in *Drosophila melanogaster*.

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Introduction

Neurodegenerative disorders are an important source of morbidity and suffering for mankind. The roles of free-radical-mediated oxidative injury in acute insults to the nervous system including stroke or trauma, as well as in chronic neurodegenerative disorders, are being increasingly recognized. It is known that oxygen is an essential molecule for survival of the majority of living

organisms. Oxidative stress is the harmful condition that occurs when there is an excess of free radicals and/or a decrease in antioxidant levels. The evidence to date for oxidative stress in Parkinson's disease, Schizophrenia, Alzheimer's disease, and other neurodegenerative diseases is strongly persuasive. Clinical studies have shown that a number of events associated with Alzheimer's are capable of stimulating production of free radicals and depletion of antioxidant levels. Tackling of the free radical involvement offers a novel therapeutic target in the study of neurodegenerative disorders. Strategies aimed at limiting free radical production oxidative stress and damage may slow the progression of neurodegenerative diseases (Singh *et al.,* 2004).

Curcumin, commonly called diferuloyl methane, is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. It exhibits antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (Anand *et al.,* 2008). We have tested the antioxidant property of Curcumin in *Drosophila melanogaster.*

Materials and Methods

Culturing of flies

D. melanogaster (Oregon K) adults (8-10 days old) were obtained from *Drosophila* stock centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, Karnataka, India. Fly populations were built up and maintained at 22 ± 1°C and 70-80% relative humidity, fed on a standard wheat cream agar medium seeded with yeast.

Safety evaluation of compound

In the present set of experiments, the test flies were fed on a medium containing Curcumin at 100, 200, and 300 µg/ml concentrations of Curcumin dissolved in 0.5% DMSO.

The toxicity of 0.5% DMSO was checked in the medium by culturing the flies in media with and without 0.5% DMSO. Since there was no mortality in the flies reared on medium containing 0.5% DMSO, further studies were carried out to find out whether Curcumin is causing mortality in the experimental batches.

In each culture vial, 4 ml of food with or without Curcumin was added. Lethality due to compounds was monitored by counting dead flies every 24 h up to 7 days in the vials containing Curcumin, and data were expressed in terms of percentage mortality.

Preparation of compound for feeding the flies

Curcumin was dissolved in 0.5% dimethyl sulfoxide (DMSO) and was used as control. The compounds were introduced into the medium in a semisolid state, mixed well, and allowed to solidify. 50 adult flies were introduced into the vials containing media.

Whole body homogenate preparation

0.1M Sodium-phosphate buffer (pH 7.4) was used for preparing whole body homogenates. The flies (30 nos.) from control and tested groups were used for this purpose. After homogenizing, the samples were centrifuged at 2500 ×g for 12 min at 4°C. The supernatant was filtered through nylon mesh (pore size, 10 µm) and used for biochemical assays (Smith *et al.,* 1978).

Paraquat exposure and concentrations

In a preliminary study, flies were exposed to paraquat at concentrations of 20, 25, 30, and 35 mM for 96 h to determine lethality. However, to assess the neuroprotective effects of Curcumin, only

one concentration of Paraquat (25 mM) was employed. For these studies, paraquat exposed flies were supplemented with Curcumin (300 µg/mL) in the diet and were tested for the modulatory effect of Curcumin on paraquat induced lethality, locomotor dysfunctions, and oxidative impairments.

Paraquat resistance test

Three to five days old adult flies were fed with control food or food containing the compounds for a period of 7 days. Then 50 flies were fed with the test compound Curcumin and the control were starved for 6 h to make sure that no food remained in the digestive tract so that none of the compounds would alter the uptake of paraquat. Afterwards, the flies were transferred to vials containing only filter paper soaked with 25 mM paraquat in 5% sucrose solution (Hosamani *et al.*, 2010). This concentration of paraquat was selected, because it enables us to study the flies for a period of 24 h or more after treatment. Survival was determined at 24 h and at 48 h later. Surviving flies were used for preparing samples for biochemical assays. Each assay was repeated thrice (Anand *et al.*, 2008).

Biochemical investigations

Following the exposure to various treatments, the flies were mildly anesthetized using diethyl ether in a small airtight glass container for 1 min. Quantification of oxidative markers *viz*., MDA (Ravikumar and Muralidhara, 2009), GSH (Ernesto *et al.*, 2006) and activities of a few of the antioxidant enzymes were made using the whole body homogenates. Protein estimation was done using samples obtained after homogenization (Wolf, 1994).

Results and Discussion

Our aim was to elucidate antioxidant and neuroprotective efficacy of Curcumin *in vivo*. *Drosophila* is an excellent *in vivo* system for testing the therapeutic compounds due to its relatively short life span wherein the adult flies appear to show many of the manifestations of cellular senescence observed in mammals. Oxidative stress plays an important role in governing the life span of the fly. The results revealed that Curcumin has protective action against paraquat induced oxidative stress in *Drosophila*. There was a significant induction of oxidative stress among flies exposed to paraquat (25 mM), which was evidenced by the marked elevation in MDA and further a significant change in the activities of antioxidant enzymes such as CAT, SOD suggested an increased generation of ROS (Feany *et al.*, 2000).

Ameliorative effects of Curcumin on paraquat induced oxidative stress markers in whole body homogenates of adult flies fed with Curcumin supplemented diet for 7 days showed significant diminution in MDA levels. In Curcumin treated flies homogenate, the level of MDA was found to be 3.47 ± 0.25 nmol malondialdehyde/mg protein (Figure 1) as against the paraquat treated flies that showed 12.34 ± 0.51 nmol malondialdehyde/mg protein. Severe depletion in cellular GSH levels upon paraquat exposure in *Drosophila* adds further evidence that a state of oxidative stress exists *in vivo*, which may lead to mitochondrial damage, increase in free radical generation, and peroxidation of membrane lipids (Barclay *et al.*, 2000). A moderate increase in GSH level was evident among flies treated with Curcumin (40.325 ± 1.78 µg GSH/mg protein), although paraquat exposure caused a significant decrease in GSH level, on co-exposure with Curcumin; flies were able to restore the depleted GSH levels (Figure 2), thus clearly suggesting the ability of Curcumin to up-regulate levels of GSH.

Conclusion

Based on biochemical investigations, we have found that dietary feeding of Curcumin to *Drosophila* for a short duration has the propensity to attenuate paraquat induced oxidative stress owing to its antioxidative nature and its ability to modulate the activities of antioxidant defenses, such as reduced GSH and antioxidant defenses. Additional evidence, *viz*., lower incidence of paraquat induced mortality and higher resistance to paraquat among flies pretreated with Curcumin, clearly support such a mechanism. Further, its antioxidant property was clearly evident by its ability to significantly abrogate paraquat induced oxidative stress, by depleting the lipidperoxidation product malanoldialdehyde.

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***Drosophila polymorpha* life cycle.**

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Introduction

Many studies on the subject of the *Drosophila* life history have revealed that much of the observed interspecific variability can be explained by genetic interaction and ecological traits (Markow and O'Grady, 2006; Prasad and Joshi, 2003). The life cycle is one of the most important factors that determine the *Drosophila* life history.

There is considerable interspecific variation in each of the *Drosophila*'s life cycle stages, making this type of fly a quite versatile model for life history studies (Jennings, 2011). Thorough knowledge of each developmental stage of *Drosophila* could clarify some evolutionary questions, such as the mechanisms underlying morphological differentiation, and also the ecological results during the speciation process. Our study expands upon the *Drosophila polymorpha* life cycle, from egg to adult. Furthermore, a comparison is made with other species of *Drosophila*, an important factor that leads to a better understanding of evolution within the genus.

Increasing numbers of studies on sexual isolation of *Drosophila* have ensured that there are many inter-specific differences in the reproductive biology for this group that contribute to the speciation process (Coyne *et al*., 1994).