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Regular Article

Protective effect of bischalcone derivative in *Drosophila melanogaster* against electron beam radiation

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In this paper the protective effect of *(2E, 5E)* – 2,5-bis (3-methoxy-4-hydroxy-benzylidene) cyclopentanone bischalcone derivative (Curcumin analogue, CA), on electron beam radiation induced oxidative stress in *Drosophila melanogaster* adult flies. Curcumin (CU) was taken as standard. The CA pre treated and irradiated flies were screened for wing shape abnormalities in F1 and F2 generations. There was considerable decrease in the wing shape abnormality frequency in the case of CA fed irradiated flies compared to control.

Key words: antioxidant, *bischalcone,* electron beam radiation, wing shape abnormalities,

The development of effective radioprotectors and radio recovery drugs is of great importance in view of their potential application during both planned radiation exposure (e.g. radiotherapy) and unplanned radiation exposure (e.g. in the nuclear industry, natural background radiation emanating from the earth or other sources. These drugs are also likely to be useful in nuclear warfare to provide protection to personnel. Over the past 50 years, research in the development of radioprotectors worldwide has focused on screening a plethora of chemical and biological compounds (Rajesh Arora *et al*, 2005)

Appropriate antioxidant intervention seems to inhibit or reduce free radical toxicity and thus offer protection against radiation. A number of dietary antioxidants have been reported to decrease free radical attack on biomolecules. Curcumin, commonly called diferuloyl methane, is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa.* Traditionally the rhizome is used for many ailments because of its wide spectrum of pharmacological activities. Curcumin chemically is a bis-α, β-unsaturated β-diketone that exhibits keto-enol tautomerism (Sugiyama *et al*, 1996). Curcumin is known for its antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities. It also has hepatoprotective and nephroprotective activities, suppresses thrombosis, protects against myocardial infarction and has hypoglycemic and antirheumatic properties

(Jovanovic *et al*, 1999). Moreover, various animal model and human studies have shown that curcumin is extremely safe even at very high doses. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent. Poor aqueous solubility, relatively low bioavailability and intense staining color of curcumin have been highlighted as major problems; and consequently search for a ''super curcumin'' without these problems and with efficacy equal to or better than that of curcumin is ongoing (Preetha *et al*, 2008). So a synthetic bischalcone (CA) [*(2E,5E)*-2,5-bis (3-methoxy-4-hydroxy-benzylidene) cyclopentanone has been synthesized. In this study, we have chosen to employ the fruit fly *D. melanogaster* as a model system to study the protective efficacy of bischalcone. We studied wing shape abnormalities in F1 and F2 generation in different treatment groups.

MATERIALS AND METHODS

Preparation of wheat cream agar medium:

D. *melanogaster* (Oregon K) adult males (8-10 days old) were obtained from Drosophila stock centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, Karnataka, India. The flies were maintained at 22 ± 1°C and 70-80% relative humidity, fed on a standard wheat cream agar medium seeded with yeast. The medium was prepared according to standard protocol. 100 ml of the media contains 10g wheat flour, 10g jaggery, 1g agar agar, 0.75ml propionic acid (antifungal agent) and few granules of yeast were added. After 24 hours flies were transferred to fresh media bottles to avoid sticking of flies to media. Whenever required, the flies were exposed to the fumes of diethyl ether in a small airtight glass container for 1 min (Darshan Raj *et al*, 2011) for observation under stereozoom and for other studies.

Preparation of compounds for feeding the flies:

The compounds CA and CU [1mg, 2mg, 4mg/ml] were dissolved in 0.5% dimethyl sulfoxide (DMSO) and used as stock solutions. 0.5% DMSO was used as control. The concentration of compounds used was 200 and 400µg/ml. Both standard and test compound were introduced into the medium at semisolid state and mixed well and was allowed to solidify. 30 adult males were introduced into the vials containing media (Ashadevi *et al*. 2001)

Safety evaluation of compounds:

Initially the toxicity of 0.5% DMSO was evaluated by rearing the flies in media with 0.5% DMSO (solvent control) and without solvent (Normal control). Further the flies were fed on a medium containing CA and CU at 200, 400 µg /mL. Lethality due to compounds and solvent DMSO (0.5%) was monitored by counting dead flies every 24h up to 7 days and data was expressed in terms of percentage mortality (Darshan Raj *et al*, 2011). There was no significant mortality observed in the flies reared on medium containing 0.5% DMSO, CA and CU. The control-I flies were fed with regular wheat cream agar medium for 7 days.

Study design

Control-I Wild type male flies (Wheat cream agar medium)

Control-II Wild type male flies (Compound unfed) + 1.5Gy

Group 1

1a) 200µg/ml of CU fed flies + 1.5Gy

1b) 400µg/ml of CU fed flies + 1.5Gy

Group 2

2c) 200µg/ml of Bischalcone CA fed flies + 1.5Gy

2d) 400µg/ml of Bischalcone CA fed flies + 1.5Gy

Irradiation:

The male flies of 8-10 days old were taken for studies. They were irradiated using electron beam with 1.5 Gy dose at Microtron Accelerator centre at Mangalore University, Mangalore, Karnataka, India. Polypropylene tubes of 65x25mm size and width 2 mm thick were used to expose flies to electron beam radiation. After irradiation

flies were introduced into fresh vials containing standard wheat cream agar medium (Darshan Raj *et al*, 2011)

Identification and isolation of mutants

The flies were irradiated at 1.5Gy using electron beam on seventh day as shown in the study design. The male flies from each group were mated with non irradiated virgins. F_1, F_2 generations were examined to identify and isolate phenotypic abnormalities with respect to body size, eyes, head, thorax, wing shape and wing venation. In each culture vial, 3 pairs of flies were kept for 3-4 days and were then discarded. The number of flies comprising the F_1 generation were recorded to check for any dominant lethals. The F_1 flies were inbred to obtain F_2 generation to score for sex-linked recessive lethal. In the same way wing abnormalities were also screened in flies. The Parallel control populations were also maintained for making comparisons (Muhammad, 2001).

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA). The values are Mean ± S.D computed from all the three replicates. Prism software (Ver. 3.0) was used for statistical analysis. p-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The flies fed with CA and CU for 7 days as mentioned in study plan were screened for wing abnormalities. Perusal of the results (Fig.1) revealed that CU and CA were not showed any wig abnormalities on *D. melanogaster* at worked concentrations of compounds.

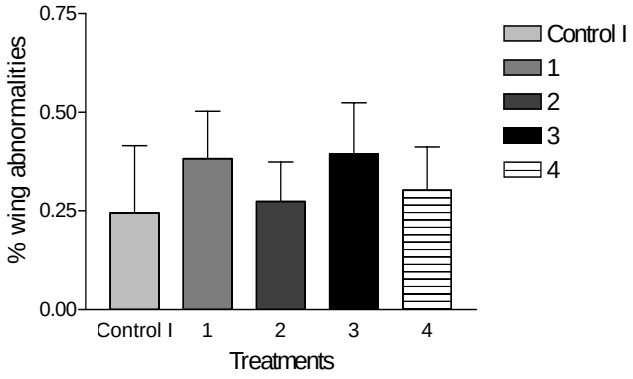
The flies fed with CU and CA for 7 days were irradiated using electron beam (as per study design). These flies were screened for wing abnormalities and their F_2 progenies were screened for phenotypic recessive mutations (wing abnormalities). The CU and CA fed, and unfed irradiated flies showed cut, wrinkled and notch like wing abnormalities (Fig. 2). The F_1 progeny of control-II showed 17.89 ± 0.45% cut, wrinkled and notch like wing abnormalities, whereas in case of CU and CA fed irradiated flies (group 1 and 2), the frequency of wing abnormalities were found to be significantly reduced (Fig.3). To confirm whether the wing abnormalities observed were a consequence of a mutation or developmental disorder, the F_1 flies were allowed to inbreed. In F_2 generation the abnormalities continued both in controls and compounds fed irradiated flies group. In case of control flies 7.485±1.763% were found to possess wing abnormalities, whereas 400 µg/ml CU and CA fed flies produced 1.783±0.05 and 2.147±0.742 % wing abnormalities respectively. The proportions of flies with wing abnormalities were comparatively higher in case of compound unfed irradiated flies than that of compound fed flies (Fig. 3).

Mutations in *colt* gene cause semi-lethality and death of a high proportion of first-instar larvae of *Drosophila*. The other process affected by *colt* is the one which takes place at the time of eclosion and leads to the unfolding and expansion of the wings. This process is found to be severely impaired in *colt* adult escapers which are characterized by partially unfolded wings with the loss of venation and reduced size, as well as poor viability and sterility. Genes involved in wing morphogenesis are known to encode diverse products such as mitochondrial metabolic enzymes, cell surface proteins and proteins involved in discrete signalling pathways. *Colt* encodes a putative mitochondrial carrier protein of unknown solute specificity. The description of *colt* has thus indicated how a gene presumably involved in a mitochondrial metabolic process can specifically affect wing morphogenesis, by altering the differentiation of the wing epithelial cells and by blocking the unfolding and expansion of the wing after eclosion (Hartenstein *et al.* 1997). The CA and CU

screened for toxicity studies revealed that they are non toxic at the concentrations used. Screening for wing abnormalities was carried out for all treatments of flies. Such screening result showed that wing abnormalities in F_1 progeny of control I was found to be 0.36±0.10%. The 400µg/ml CU and CA fed flies group showed 0.25±0.02%, 0.34±0.05% respectively. In F_2 generation also no such significant abnormalities found. This study supports that compound treated were non mutagenic at worked concentrations.

In case F_1 generation of control II flies showed 17.89±4.50% abnormalities of wing shape. The wing shape abnormality continued in F_2 generation also. Reduction in wing shape abnormalities in compound fed flies group in both the groups' shows that compounds may provide protection to radiation induced damage.

(A)



(B)

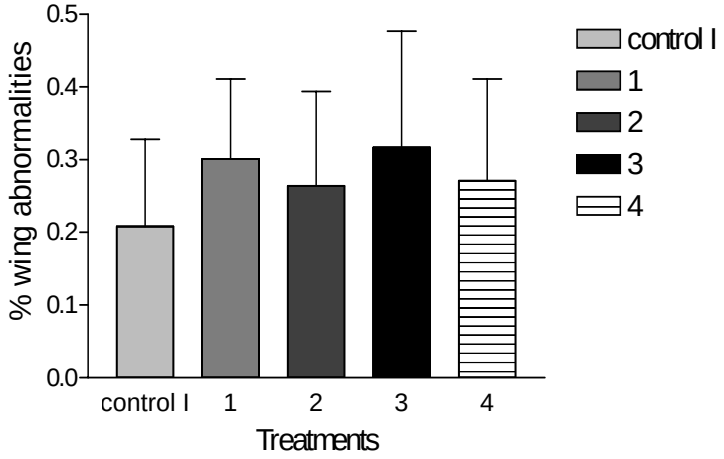


Figure 1. Wing abnormalities of Batch I flies. (A) F_1 generation (B) F_2 generation. In Fig (A) and (B) control represents compound unfed flies. 1 and 2, represents CU treated flies at 200,400 µg/ml respectively; 3 and 4 represents CA treated flies at 200,400 µg/ml concentrations respectively. Result expressed in % of wing abnormalities.

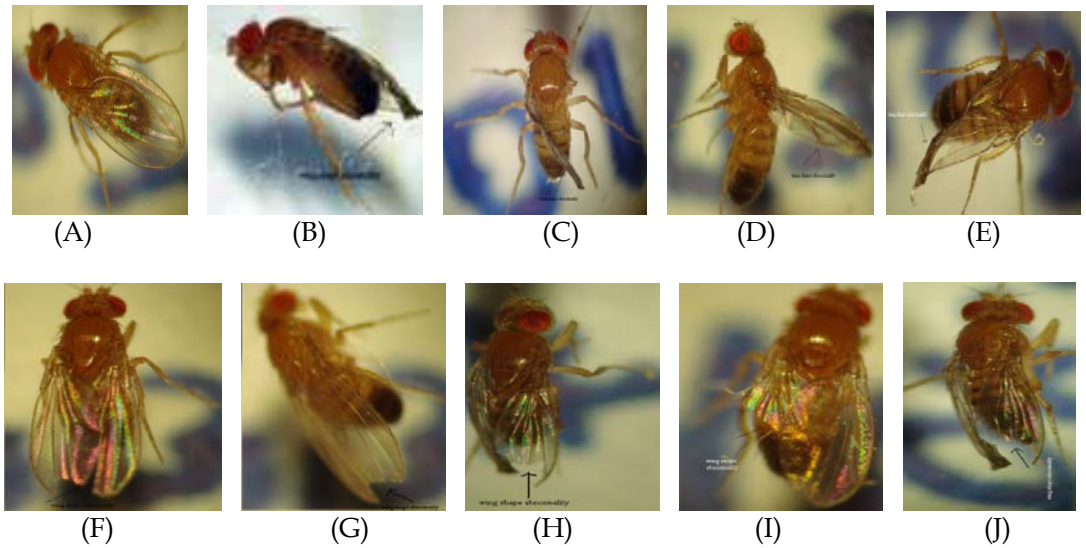
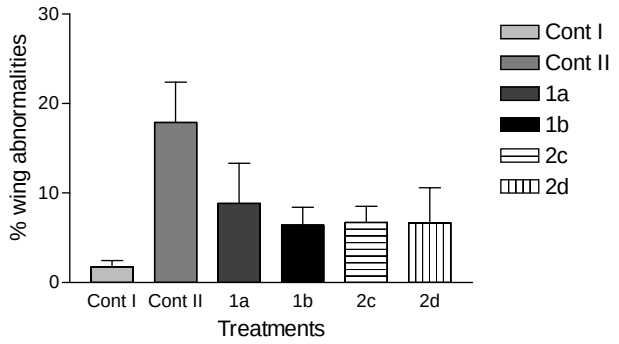


Figure 2. Different types of wing abnormalities. (A) Normal, (B) to (E) Wrinkled wing, (F) to (J) Cut wing.

(A)



(B)

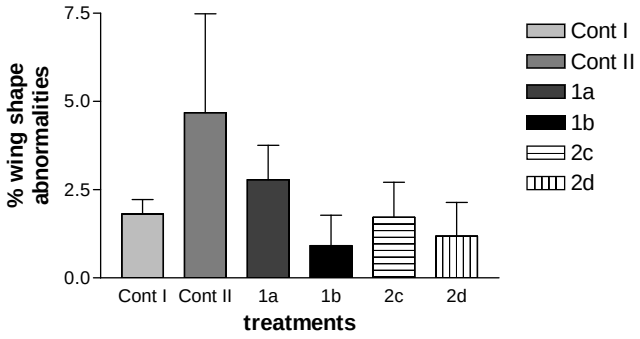


Figure3. Percentage of flies with wing abnormalities in F_1 (A) and F_2 (B) generations. The numbers on X axis represent treatments as mentioned in study design.

These fly stocks with wing abnormalities are being maintained in the lab and at present, even after five generations, flies from irradiated unfed batch are found to possess more wing abnormalities. Whereas in the compound fed flies. However, pair mating between flies of similar phenotype have to be conducted to elucidate the pattern of inheritance. Further, standard procedures will be followed for chromosomal localization of mutation.

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