Protective effects of tea polyphenols and β-carotene against γ-radiation induced mutation and oxidative stress in *Drosophila melanogaster*

Isha Nagpal and Suresh K. Abraham*

**Abstract**

**Background:** The commonly consumed antioxidants β-carotene and tea polyphenols were used to assess their protective effects against γ-radiation induced sex-linked recessive lethal (SLRL) mutation and oxidative stress in *Drosophila melanogaster*. Third instar larvae and adult males of wild-type Oregon-K (ORK) were fed on test agents for 24 and 72 h respectively before exposure to 10Gy γ-irradiation. The treated/control flies were used to assess the induction of SLRLs. We also evaluated antioxidant properties of these phytochemicals in the third instar larve.

**Results:** Different stages of spermatogenesis in adult males showed a decrease in γ-radiation induced SLRL frequencies upon co-treatment with test agents. A similar trend was observed in larvae. Furthermore, a significant increase in antioxidant enzymatic activities with a decrease in malondialdehyde content was observed.

**Conclusion:** β-carotene and tea polyphenols have exerted antigenotoxic and antioxidant effects in *Drosophila*. This study demonstrated the suitability of *Drosophila* as an alternative to mammalian testing for evaluating the antigenotoxic and antioxidant activity of natural products.

**Keywords:** *Drosophila melanogaster*, γ-radiation, Tea polyphenols, β-carotene, Sex-linked recessive lethal mutations, Antioxidant enzymes, Alternative to mammalian testing

**Background**

Radiations and certain chemical agents induce DNA lesions, which may cause genomic instability and activate cancer generation. Exposure to such mutagens disturbs ROS homeostasis leading to overproduction of ROS which enhances DNA damage. In the modern world scenario, exposure to such harmful radiation has become more frequent. Several studies showed that ionizing radiation (such as X-rays, γ-rays) cause lethal mutation in the germ cells of *D. melanogaster* [1–3].

Chemopreventive phytochemicals from commonly consumed fruits, vegetables and beverages are well documented for inducing antimutagenic and/or antioxidant activity by different mechanisms [4]. The most common radioprotective mode of action of phytochemicals implicate chelation and scavenging of free radicals generated during exposure to mutagens [5] and upregulation of mRNAs of antioxidant enzymes like catalase, glutathione S-transferase [6, 7] leading to overexpression of such antioxidant enzymes in vivo, and rescuing cells from oxidative stress and DNA damage [8].

β-carotene (BC) belongs to the carotenoid family with potential to scavenge singlet oxygen species/free radicals [9]. It is a natural precursor of vitamin A, which is most efficiently converted into vitamin A as compared to other provitamins [10]. BC is a potent antioxidant, and studies demonstrate that BC significantly reduces radiation-induced DNA damage including γ-radiation [11] and UV radiations [12]. BC is reported to protect against chromosomal damage, induction of micronuclei [13] and lipid peroxidation [14, 15].

Tea is the most common beverage consumed as green tea or black tea. Both forms of tea contain active ingredients commonly called as tea polyphenols which are
strong antioxidants and exert antimutagenic potentials [16–19]. Radioprotective effects of tea polyphenols against harmful effects of radiations are widely reported in various in vitro and in vivo studies [20–24]. Studies have demonstrated that epigallocatechin gallate (EGCG), a bioactive phytochemical present in green tea can reduce UV radiation induced DNA damage in cultured human cells and peripheral leucocytes [25].

In toxicological research, animal models are widely used. However, its cost and licensing law issues limit its use in large-scale drug screening process [26]. To minimize the use of higher animals, use of alternative models have gained much importance. *Drosophila*, an invertebrate model with its extensively studied genome, shows more than 70% of gene homology with humans [27, 28]. The similarity of metabolic pathways between *Drosophila* and mammals has encouraged the use of *Drosophila* in the context of screening and evaluating the antimutagenicity especially for detecting somatic and germ cell mutations of pure and crude mixtures of various compounds [29–35]. This model has fewer ethical concerns due to which it is included in the recommendations of European Center for Validation of Alternative Methods (ECVAM) and Organization for Economic Cooperation and Development (OECD) for genotoxicity testing that promotes to reduce, refine or replace (3Rs) the use of vertebrate models for laboratory experiments [36, 37].

In *Drosophila*, genotoxic damage can be detected either by performing the somatic mutation and recombination test (SMART) or the sex-linked recessive lethal test (SLRL). The sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster* is widely used for detection of genetic lesions in germ cells [38]. It can be used to study the genotoxic effects of various environmental toxicants [39–41]. This test can be used to screen recessive lethal mutations at almost 800 different loci on the X chromosome, which represents 80% of the X chromosome and one-fifth of the entire *Drosophila melanogaster* genome [42]. Since *Drosophila* model exhibits the significant activity of xenobiotic drug metabolizing enzymes like cytochrome P-450 and aryl hydrocarbon hydroxylase [43, 44], the SLRL test can be used to detect mutagens and promutagens with very short half-lives [45–47]. Promutagens are mostly activated in spermatids [48], and SLRL can be used to analyze induced mutations in specific stages of germ cells [49].

Considering the well-established antioxidant properties of BC and TP in other in vivo models, the present investigation was initiated with the main objective of assessing whether or not these test agents show a similar level of antimutagenic and antioxidant potential in the *Drosophila* model. In accordance with these objectives, the experiments were carried out to study: (a) antigenotoxic potentials of BC and TP against γ-radiation-induced SLRL mutation in the germ cells of both larvae and adult flies of *D. melanogaster*; (b) analysis of antioxidant capacity (in terms of antioxidant level/activity) following induction of oxidative stress and its modulation with phytochemicals.

**Methods**

**Drosophila stocks**

The experiments were carried out using Muller-5 or Basc stock In(1)Sc8 ScRR + S, ScScw/B) and Oregon-K (wild type). These stocks were obtained from *Drosophila* stock center, Department of Zoology, Mysore University (India). Flies were cultured on the standard *Drosophila* culture medium [2].

**Chemicals**

The chemicals used in the present studies were polyphenol 60 from green tea (CAS No.138988–88-2), and β-carotene (CAS No.7235–40-7) obtained from Sigma-Aldrich Company, India. All the other used reagents and chemicals were of analytical grade, procured from local sources.

**Irradiation**

Culture vials containing adult flies and larvae of *Drosophila* were exposed to 10Gy γ-radiations at a dose rate of 1.8Gy/min in a gamma chamber (source 60Co, 204 TBq, 5500 Ci) obtained from Bhaba Atomic Research Centre (BARC), Mumbai, India. The dose rate was determined using Fe2+/Fe3+ dosimetry.

**Test concentrations and treatment**

*Drosophila* larvae and adult flies were fed on different concentrations of test agents. The concentrations which did not show lethality or delay in development of larvae were selected for the experiments.

**Test for detecting sex-linked recessive lethal mutation**

**Adult feeding experiments**

Four days old adult male flies (ORK) were starved for 6-8 h and transferred into a glass vial containing filter paper soaked in the test agents mixed with 10% sucrose solution which was also used as a negative control. The filter paper was renewed once every 12 h. After feeding on test solutions for 72 h, the control/ treated flies were irradiated, and each irradiated male was allowed to mate with 5 virgins *Basc* females for three days resulting in brood I. After three days, the same male fly was allowed to mate with another set of 5 virgin females (Muller-5) to produce brood II. The same pattern was repeated until brood IV to check the antimutagenic effect of test agents in postmeiotic (spermatozoa and spermatids), meiotic (spermatocytes) and premeiotic (spermatogonia) cells against the group treated with γ-irradiation alone [50].
**Larval feeding experiments**

Inseminated females (ORK) were allowed to lay eggs on the culture medium for 8-10 h. After this, the females were discarded, and 3 days later, the larvae were transferred to glass vials containing test solution mixed with instant *Drosophila* culture medium. After 24 h, one batch of third instar larvae was harvested and irradiated to check the enzymatic activity. The emerging one-day old adult males were mated individually with a set of 3 to 5 Basc females in order to conduct SLRL test [47].

For both the adult and larval feeding experiments, the inseminated females (one female/vial) from the F₁ generation were used to raise the F₂ generation which was scored for the absence of wild-type males indicating the occurrence of SLRL mutation [51].

**Assays for oxidative stress markers**

Biochemical assays were carried out for evaluating oxidative stress in control and treated third instar larva. For this purpose, larval tissue homogenates were prepared following the method by Singh et al. [52]. Protein content in cytosolic and microsomal samples was estimated by the method of Lowry et al. [53] using Folin’s reagent and BSA as the external standard. Protein concentration was expressed as mg/ml homogenate. GSH content was measured by the method of Ellman et al. [54] with some minor modifications using 5 mM CDNB as substrate. The formation of CDNB-GSH conjugate was measured by monitoring the absorbance for 3 min at 340 nm in a time interval of 3 s. GST activity was expressed as nmol CDNB reduced/min/mg larval protein. Glutathione S-transferase (GST) activity was measured by the method of Habig et al. [56] with some minor modifications using 5 mM CDNB as substrate. The activity of SOD was determined as described by Nishikimi et al. [57] with minor modifications as described by Singh et al. [52]. One unit of enzymatic activity is defined as the protein concentration required for inhibiting the chromogen production by half in a time interval of 1 min, and the SOD activity is expressed as U/min/mg protein. Malondialdehyde (MDA) content was measured as a marker of lipid per-oxidation (LPO) by the method of Okhawa et al. [58]. MDA was assayed using tetra ethoxy propane as an external standard. It was expressed in nmol MDA formed/h/mg protein.

The data is presented as percentage change by using the formula:

Mean (sample) − Mean (Control)/Mean (Control) x 100

**Statistical analysis**

The experimental values are represented as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by post hoc tests for multiple comparisons among the treated groups and control using Newman’s Keuls test. Pearson’s correlations were also calculated and analyzed using Prism computer program (GraphPad version 5.0, San Diego, CA, USA). Kastenbaum and Bowman tables were used for validation of results obtained from SLRL test [59, 60], which are further analyzed by the Z Test for difference in proportions.

**Results**

Induction of SLRLs by 10Gy γ-radiation in *Drosophila* larvae and adult flies and its modulation by BC and TP

In our study, we examined the antimutagenic potential of BC and TP against γ-radiation-induced SLRL mutation. As compared to negative control group, no significant difference was observed in the mutation frequencies of individual treatment of TP and BC group. However, the mutation frequencies significantly increased with the positive control group (10Gy γ-radiation).

We observed a significant reduction in the incidence of mutation frequencies with phytochemical pre-treatment against γ-radiation induced mutation in the germ cells of larvae as well as adult flies of *D. melanogaster*. The results obtained in terms of % lethals from the adult and larval SLRL experiments with the two test compounds are summarized in Tables 1 and 2 respectively.

Data from adult feeding experiments (Table 1) demonstrates that BC (0.5%) + γ-radiation has significantly reduced the mutation frequency induced by 10 Gy γ-irradiation in all the germ cell stages (Brood I-IV) from 2.966 to 0.775% (73.85% reduction, p ≤ 0.01) followed by γ-radiation + TP (1%) which is 2.966 to 0.782% (73.64% reduction, p ≤ 0.01). Results obtained from larval feeding experiments (Table 2) showed γ-radiation + TP (1%) has significantly reduced the mutation from 2.053 to 1.160% (43.51% reduction, p ≤ 0.01) followed by γ-radiation + BC (0.25%), in which the reduction was from 2.053 to 1.217% (40.72% reduction, p ≤ 0.01).

**Modulation of oxidative stress levels in third instar larvae of *D. melanogaster***

As compared to negative control group, the amounts of anti-oxidant enzymes (GSH, GST, CAT) slightly but significantly increased and the LPO level decreased when treated with TP 1%, and GSH increased with BC 0.25%. However, the amounts of anti-oxidant enzymes significantly decreased and the LPO level increased when treated with γ-radiation (10Gy).
| Treatment                              | Brood | No. of X-Chromosomes scored | Lethals (n) | Lethals (%) |
|----------------------------------------|-------|----------------------------|-------------|-------------|
| 10% Sucrose (Negative Control)         | I     | 913                        | 1           | 0.110       |
|                                        | II    | 837                        | 2           | 0.239       |
|                                        | III   | 682                        | 1           | 0.147       |
|                                        | IV    | 554                        | 1           | 0.181       |
|                                        | I-IV  | 2986                       | 5           | 0.167       |
| TP 1%                                  | I     | 781                        | 2           | 0.256       |
|                                        | II    | 746                        | 1           | 0.134       |
|                                        | III   | 614                        | 1           | 0.163       |
|                                        | IV    | 517                        | 0           | 0.000       |
|                                        | I-IV  | 2658                       | 4           | 0.150       |
| BC 0.25%                               | I     | 869                        | 1           | 0.115       |
|                                        | II    | 684                        | 2           | 0.292       |
|                                        | III   | 577                        | 0           | 0.000       |
|                                        | IV    | 461                        | 1           | 0.217       |
|                                        | I-IV  | 2591                       | 4           | 0.154       |
| BC 0.5%                                | I     | 889                        | 2           | 0.225       |
|                                        | II    | 702                        | 1           | 0.142       |
|                                        | III   | 693                        | 0           | 0.000       |
|                                        | IV    | 415                        | 1           | 0.241       |
|                                        | I-IV  | 2699                       | 4           | 0.148       |
| BC 1%                                  | I     | 902                        | 2           | 0.222       |
|                                        | II    | 819                        | 1           | 0.122       |
|                                        | III   | 674                        | 1           | 0.148       |
|                                        | IV    | 529                        | 1           | 0.189       |
|                                        | I-IV  | 2924                       | 5           | 0.171       |
| γ-radiation                            | I     | 752                        | 31          | 4.122       |
|                                        | II    | 732                        | 27          | 3.689       |
|                                        | III   | 717                        | 15          | 2.092       |
|                                        | IV    | 665                        | 12          | 1.805       |
|                                        | I-IV  | 2866                       | 85          | 2.966       |
| γ-radiation + TP 1%                    | I     | 825                        | 8           | 0.970       |
|                                        | II    | 782                        | 5           | 0.639       |
|                                        | III   | 645                        | 5           | 0.775       |
|                                        | IV    | 562                        | 4           | 0.712       |
|                                        | I-IV  | 2814                       | 22          | 0.782       |
| γ-radiation + BC 0.25%                 | I     | 810                        | 7           | 0.864       |
|                                        | II    | 711                        | 6           | 0.844       |
|                                        | III   | 512                        | 4           | 0.781       |
|                                        | IV    | 499                        | 3           | 0.601       |
|                                        | I-IV  | 2532                       | 20          | 0.790       |
| γ-radiation + BC 0.5%                  | I     | 788                        | 7           | 0.888       |
|                                        | II    | 651                        | 5           | 0.768       |
|                                        | III   | 582                        | 4           | 0.687       |
were treated with γ-radiation + BC (0.25%) (17.52% increase) followed by γ-radiation + BC (0.5%) (17.52% increase). γ-radiation + BC (0.25%) has reduced the MDA content by 56.5% followed by 54.4% reduction in γ-radiation + BC (0.5%) (Table 3).

Correlation between different stress parameters
A correlation was drawn between the different sets of oxidative stress markers (GSH, GST, CAT, SOD, and LPO) when the larvae were exposed to test agents (TP and BC) individually as well as in combination with γ-radiation. A significant negative correlation of MDA content (lipid peroxidation) and positive correlation of GSH content was observed with different sets of antioxidant enzymes (GST, CAT, and SOD) (Tables 4 and 5).

Discussion
Ionizing radiations are deleterious in nature and cause direct and indirect DNA damage. In recent years, human exposure to harmful levels of radiations has increased due to radiation based clinical diagnosis, and radiotherapy for cancer treatment [61]. Also, studies have shown that radiotherapy is responsible for “bystander effect” [62]. Ionizing radiation starts a cascade of events which leads to free radical generation [63]. DNA damage can occur in many different ways, amongst which adduction of free radicals with DNA is most common under stressful conditions [64]. Hence, to prevent free radical associated DNA damage, the most common strategy is to quench the generated free radicals. In the present study, we observed the antimutagenic and antioxidant activity of TP and BC against γ-radiation induced mutation and oxidative stress in the larvae and adult flies of D.melanogaster.

For testing γ-radiation induced mutation in the germ cells, SLRL test was performed to assess radioprotective effects of TP and BC in both larvae as well as adult flies.
of *D. melanogaster*. The maximum reduction of mutation frequencies in larvae was observed with γ-radiation + TP (1%), followed by γ-radiation + BC (0.25%) when compared to 10Gy γ-radiation. In the case of adult germ cells, a significant reduction was observed in all the successive germ cell stages (i.e. Brood I to IV) when flies were treated with γ-radiation + BC (0.5%) followed by γ-radiation + TP (1%). TP and BC have shown antigenotoxic activity against various chemical carcinogens in *Drosophila* [65, 66]. Significant protective effects of TP and its active constituents have been demonstrated against γ-radiation induced DNA damage in human lymphocytes [67] and also in splenocytes [68] and blood leucocytes [68] of mice. Similarly, BC showed protective effects against damage induced by X-rays and γ-rays in germ cells and somatic cells of rats and mice [11, 13, 69].

Radiation induced reactive oxygen species (ROS) is a major cause of oxidative stress to cells. Oxidative stress arises due to the imbalance of free radical generation and the antioxidant defense mechanisms. TP and BC are potent scavengers of free radicals. Our result show that pre-treatment with TP and BC has inhibited the reduction in antioxidant enzyme levels (GSH, GST, SOD, and CAT) caused by exposure to γ-radiation. Furthermore, this pre-treatment has rescued the cells from damage caused by lipid peroxidation. These findings are in agreement with in vivo and in vitro studies in which pre-treatment with TP and its active constituents led to a reduction in LPO levels [70] with a concomitant increase in intracellular levels of GSH, GST, CAT and SOD [68, 71–75]. A similar trend was observed for BC in humans [76, 77] and experimental animals [11].

Administration of these two phytochemicals (TP and BC) has reduced γ-radiation induced oxidative stress up to >50%. Furthermore, the antioxidant activity results are correlating well with the SLRL data we obtained. Since TP and BC are strong antioxidants, the most

### Table 3: Modulatory effects of TP and BC against γ-radiation induced oxidative stress on third instar larvae of *D. melanogaster* (ORK)

| Parameters | γ-GSH | γ-GST | γ-CAT | γ-SOD | γ-LPO |
|------------|-------|-------|-------|-------|-------|
| Control    | 62.69 ± 3.66 | 37.39 ± 4.75 | 54.56 ± 4.30 | 3.56 ± 0.17 | 2.48 ± 0.30 |
| TP 1%      | 76.56 ± 2.73 | 46.28 ± 2.29 | 64.68 ± 2.89 | 3.95 ± 0.25 | 1.74 ± 0.21 |
| BC 0.25%   | 71.87 ± 1.58 | 43.67 ± 3.72 | 53.01 ± 2.65 | 4.07 ± 0.10 | 2.07 ± 0.20 |
| BC 0.5%    | 64.32 ± 3.24 | 41.12 ± 2.08 | 57.53 ± 4.50 | 3.74 ± 0.11 | 2.24 ± 0.22 |
| BC 1%      | 64.26 ± 1.27 | 35.99 ± 4.79 | 51.91 ± 3.14 | 3.59 ± 0.21 | 2.08 ± 0.23 |
| γ-radiation| 49.41 ± 1.89 | 23.63 ± 2.94 | 31.08 ± 5.90 | 1.45 ± 0.34 | 5.70 ± 0.30 |
| γ-radiation + TP (1%) | 61.97 ± 2.31 | 36.12 ± 2.55 | 53.44 ± 4.08 | 2.89 ± 0.22 | 3.94 ± 0.17 |
| γ-radiation + BC (0.25%) | 58.07 ± 1.80 | 34.28 ± 1.63 | 51.34 ± 2.56 | 2.93 ± 0.26 | 2.48 ± 0.29 |
| γ-radiation + BC (0.5%) | 57.51 ± 2.18 | 36.28 ± 4.03 | 50.71 ± 4.28 | 2.51 ± 0.27 | 2.60 ± 0.23 |
| γ-radiation + BC (1%) | 53.27 ± 2.67 | 29.67 ± 3.08 | 38.52 ± 4.47 | 2.20 ± 0.24 | 2.84 ± 0.21 |

Effect of TP and BC on γ-radiation induced oxidative stress parameters.

| Parameters | γ-GSH | γ-GST | γ-CAT | γ-SOD | γ-LPO |
|------------|-------|-------|-------|-------|-------|
| Control    | 62.69 ± 3.66 | 37.39 ± 4.75 | 54.56 ± 4.30 | 3.56 ± 0.17 | 2.48 ± 0.30 |
| TP 1%      | 76.56 ± 2.73 | 46.28 ± 2.29 | 64.68 ± 2.89 | 3.95 ± 0.25 | 1.74 ± 0.21 |
| BC 0.25%   | 71.87 ± 1.58 | 43.67 ± 3.72 | 53.01 ± 2.65 | 4.07 ± 0.10 | 2.07 ± 0.20 |
| BC 0.5%    | 64.32 ± 3.24 | 41.12 ± 2.08 | 57.53 ± 4.50 | 3.74 ± 0.11 | 2.24 ± 0.22 |
| BC 1%      | 64.26 ± 1.27 | 35.99 ± 4.79 | 51.91 ± 3.14 | 3.59 ± 0.21 | 2.08 ± 0.23 |
| γ-radiation| 49.41 ± 1.89 | 23.63 ± 2.94 | 31.08 ± 5.90 | 1.45 ± 0.34 | 5.70 ± 0.30 |
| γ-radiation + TP (1%) | 61.97 ± 2.31 | 36.12 ± 2.55 | 53.44 ± 4.08 | 2.89 ± 0.22 | 3.94 ± 0.17 |
| γ-radiation + BC (0.25%) | 58.07 ± 1.80 | 34.28 ± 1.63 | 51.34 ± 2.56 | 2.93 ± 0.26 | 2.48 ± 0.29 |
| γ-radiation + BC (0.5%) | 57.51 ± 2.18 | 36.28 ± 4.03 | 50.71 ± 4.28 | 2.51 ± 0.27 | 2.60 ± 0.23 |
| γ-radiation + BC (1%) | 53.27 ± 2.67 | 29.67 ± 3.08 | 38.52 ± 4.47 | 2.20 ± 0.24 | 2.84 ± 0.21 |

### Table 4: Correlation between oxidative stress parameters.

Correlation between different sets of oxidative stress markers (GSH, GST, SOD, CAT and LPO) when the larvae were exposed to TP and combination of TP (1%) + γ-radiation (10Gy)

| GSH | GST | CAT | SOD | LPO |
|-----|-----|-----|-----|-----|
| GSH | 1   |     |     |     |
| GST | 0.989 | 1 |     |     |
| CAT | 0.959 | 0.990 | 1 |     |
| SOD | 0.925 | 0.966 | 0.975 | 1 |
| LPO | -0.927 | -0.955 | -0.948 | -0.990 | 1 |

### Table 5: Correlation between different sets of oxidative stress markers (GSH, GST, SOD, CAT and LPO) when the larvae were exposed to varying doses of BC and combination of BC (0.25%, 0.5%, 1%) + γ-radiation (10Gy)

| GSH | GST | CAT | SOD | LPO |
|-----|-----|-----|-----|-----|
| GSH | 1   |     |     |     |
| GST | 0.938 | 1 |     |     |
| CAT | 0.820 | 0.917 | 1 |     |
| SOD | 0.965 | 0.934 | 0.896 | 1 |
| LPO | -0.743 | -0.842 | -0.863 | -0.826 | 1 |
plausible explanation for the observed antimitogenic and antioxidant activity against γ-radiation could be the free radical scavenging property of these dietary phytochemicals. The antioxidant activities of TP and BC are attributed to their chemical nature. Chemically, TP contains phenolic structure that promotes electron sharing with free radical and exhibits the process of electron resonance dissociation [78], while BC contains conjugated alkyl structure enabling it to trap and stabilize peroxy free radicals and thereby reduce damage to the cell and cell membrane [79].

An important observation from the present study is the absence of a dose response for the antigenotoxic and antioxidant activity of BC. The highest dose of BC (1%) showed a lower level of antimitogenicity and antioxidant activity as compared to the other two doses (BC 0.25%) and (BC 0.5%). These observations suggest the possibility of a biphasic effect of BC that showed protection when taken at dietary levels but may have adverse effects when consumed in higher doses [80–82].

The present work further highlights the utility of Drosophila for evaluating the genotoxicity and antigenotoxicity of single as well as crude mixtures of dietary phytochemicals. The suitability of Drosophila for detecting antigenotoxic effects of coffee, a complex mixture of bioactive compounds, was first demonstrated in our laboratory [83, 84] using the somatic mutation and recombination test (SMART). Subsequently, many publications have shown the suitability of Drosophila for detecting antigenotoxic effects of crude mixtures of natural compounds [85–87]. Our studies were further strengthened by the similarity between the results of Drosophila and other in vivo and in vitro systems on antimitogenic effect and antioxidant activity of TP and BC. To the best of our knowledge, so far no study has been performed using SLRL test to assess the protective efficacy of BC and TP against γ-radiation-induced mutation and oxidative stress in the germ cells of larvae and adult flies of D. melanogaster.

Conclusion
In conclusion, our present work has demonstrated the important role Drosophila assays can play in assessing the antimitogenic and antioxidant activity of pure compounds and crude mixtures of dietary phytochemicals in different germ cell stages. The similarity and correlation of experimental data between Drosophila and in vivo mammalian assays further reveal the usefulness of this test system for extrapolation to mammals indicating that the Drosophila test system is a favourable candidate to be considered as an alternative to mammalian testing.

Acknowledgements
Authors would like to thank University Grants Commission-RESOURCE NETWORKING and Department of Science and Technology- PURSE for the funding.

Funding
This work was funded by University Grants Commission-RESOURCE NETWORKING and Department of Science and Technology- PURSE (New Delhi).

Availability of data and materials
Not applicable.

Authors’ contributions
SKA designed the experiments. IN performed all the experiments. Authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References
1. Beckman C, Roy RM, Sproule A. Modification of radiation-induced sex-linked recessive lethal mutation frequency by tocopherol. Mutat Res. 1982;105:73–7.
2. Abraham SK, Singh SP, Kesavan PC. Buthionine sulfoximine mediated enhancement of γ-radiation induced mutation frequency in Drosophila melanogaster. Mutat Res. 1993;301:255–9.
3. Mazur Barnett S, Mróz ER. Effect of γ-irradiation pretreatment on radiation-induced genetic damage in Drosophila melanogaster. Mutat Res. 1989;212:173–9.
4. Suth YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003;3:768–80.
5. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4:118–26.
6. Paul P, Unnikrishnan MK, Nagappa AN. Phytochemicals as radioprotective agents - A review. Indian J Nat Prod Resour. 20112:137–50.
7. Jagetia GC. Radioprotective potential of plants and herbs against the effects of ionizing radiation. J Clin Biochem Nutr. 2007;40:74–81.
8. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology. 2003;189:1–20.
9. Hayatsu H, Arimoto S, Negishi T. Dietary inhibitors of mutagenesis and carcinogenesis. Mutat Res. 1993;280:42–9.
10. Gul K, Kak A, Singh AK, Singh P, Youssuf B, Wani AA, et al. Chemistry, encapsulation, and health benefits of β-carotene - A review. Cogent Food Agric. 2015;1:1018696.
11. El-Habit OH, Saada H, Azab KS, Abdel-Rahman M, El-Malah D. The modifying effect of β-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. Mutat Res. 2000;466:179–86.
12. Sinha RP, Hader DP. UV-induced DNA damage and repair: a review. Photochem Photobiol Sci. 2002;1:225–36.
13. Abraham SK, Sarma L, Kesavan PC. Protective effects of chlorogenic acid, curcumin and β-carotene against γ-radiation-induced in vivo chromosomal damage. Mutat Res. 1993;303:109–12.
14. Allard JP, Royall D, Kurian R, Muggli R, Jeejeebhoy KN. Effects of beta-carotene supplementation on lipid peroxidation in humans. Am J Clin Nutr. 1994;59:884–90.
15. Hosseini F, Naseri MKG, Badavi M, Ghaifani MA, Shahbazian H, Rashidi I. Effect of beta carotene on lipid peroxidation and antioxidant status following renal ischemia/reperfusion injury in rat. Scand J Clin Lab Invest. 2010;70:259–63.

Abbreviations
BC: β-carotene; CAT: Catalase; GSH: Glutathione content; GST: Glutathione S-transferase; LPO: Lipid peroxidation; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TP: Tea Polyphenon-60
16. Rice-Evans C. Plant polyphenols: free radical scavengers or chain-breaking antioxidants? Biochem Soc Symp. 1995;61:103–16.

17. Kuroda Y, Hara Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res. 1995;343:69–97.

18. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenic activity. J Agric Food Chem. 1995;43:27–32.

19. Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol. 2011;82:1807–21.

20. Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, Lebovohl M. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radic Biol Med. 1999;26:1427–35.

21. Tobi SE, Gilbert M, Paul N, McMillian TJ. The green tea polyphenol, epigallocatechin-3-gallate, protects against the oxidative cellular and genotoxic damage of UV-A radiation. Int J Cancer. 2002;102:439–44.

22. Kim J, Hwang JS, Cho YK, Han Y, Jeon YJ, Yang KH. Protective effects of (−)-epigallocatechin-3-gallate on UV-A- and UVB-induced skin damage. Skin Pharmacol Appl Skl Physiol. 2001;14:79–93.

23. Uchida S, Ozaki M, Suzuki K, Shikita M. Radioprotective effects of (−)-epigallocatechin 3-o-gallate (green-tea tannin) in mice. Life Sci. 1995;50:1417–52.

24. Feng Q, Tonii Y, Uchida K, Nakamura Y, Hara Y, Osawa T. Black tea polyphenols, theaflavins, prevent cellular DNA damage by inhibiting oxidative stress and suppressing cytokine PS100 1A1 in cell cultures. J Agric Food Chem. 2002;50:213–20.

25. Morley N, Clifford T, Salter L, Campbell S, Gould D, Curnow A. The green tea polyphenol epigallocatechin gallate and green tea can protect human cellular DNA from ultraviolet and visible radiation-induced damage. Photodermatol Photoimmun Photomed. 2005;21:15–22.

26. Bhat J. Drosophila researchers focus on human disease. Nat Genet. 2007;39:589.

27. Lindsley DL, Zimm GM. The genome of Drosophila melanogaster. San Diego: Academic Press; 1992.

28. Oliveira O, Zimmering S, Arceo C, Guzman J, De La Rosa ME. Evidence for the protective effect of ascorbic acid (vitamin C) in treatment with y-rays and chromium (VI) oxide (CrO3) in somatic cells of Drosophila. Mutat Res. 1995;346:19–21.

29. Stanimirović-radak M, Andjelković M. Studying genotoxic and antimutagenic effects of plant extracts in Drosophila test systems. Bot Serbica. 2016;40:21–8.

30. Mladenović M, Matić S, Stanić S, Soljić J, Mihailović V, Stanković N, et al. Combining molecular docking and 3-D pharmacophore generation to enclose the in vivo genotoxicity of naturally occurring aromatic compounds: myricetin, quercetin, rutin, and rosmarinic acid. Biochem Pharmacol. 2013;86:37–50.

31. Sortibrán ANC, Téllez MGO, Arnaiz RR. Assessment of the genotoxic and antigenotoxic activity of some essential oils evaluated by wing mosaic and the sex-linked recessive lethal test. Adv Tech Biol Med. 2015;S1:2379–86.

32. Botas J. Drosophila researchers focus on human disease. Nat Genet. 2007;39:589.

33. Stanić V, Delić G, Mihailović M, Bogojević D, Soljić J. Study of genotoxicity and antigenotoxicity of Cotinus coggyria (S) extract by Kastenbaum-Bowman test. Arch. für Genet. 1975;48:158–21.

34. Lee WR, Abrahamsson S, Valencia R, von Halle ES, Würgler FE, Zimmemg S. The sex-linked recessive lethal test for mutagenesis in Drosophila melanogaster. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res. 1985;123:183–79.

35. Abrahamsson S, Würgler FE, De Jongh C, Meyer HU. How many loci on the X-chromosome of Drosophila melanogaster can mutate to recessive lethals? Environ Mutagen. 1980;44:57–53.

36. Baars AJ. Biotransformation of xenobiotics in Drosophila melanogaster and its relevance for mutagenicity testing. Drug Metab Rev. 1980;11:191–221.

37. Gladstone M, Tin T. Chemical genetics and drug screening in Drosophila cancer models. J Genet Genomics. 2011;38:497–504.

38. Yang J, McCann C, Woods DJ, Telfozz S, Greenwood KG, ffrench-Constant RH, et al. A Drosophila systems approach to xenobiotic metabolism. Physiol. Genomics. 2007;30:223–31.

39. Würgler FE. Mutagenicity testing with Drosophila. Arch Toxicol. 1980;67:87–87.

40. Würgler FE, Graf U. Mutagenicity testing with Drosophila melanogaster. Basic Appl. Mutagen. Boston: Springer US; 1985. p. 343–72.

41. Kirkland DJ, editor. Statistical evaluation of mutagenicity test data. Cambridge: Cambridge University Press; 1989.

42. Vogel EW. A Comparison of genotoxic activity in somatic tissue and in germ cells of Drosophila melanogaster. In: Chu EHY and Generoso WM, editors. In: Mutation, Cancer, and M Cafemation. New York: Plenum Press; 1984. p. 233–55.

43. Würgler FE, Sobels FH, Vogel EW. Drosophila as an assay system for detecting genetic changes. In: Kilby EBL, Legator M, Nichols W, Ramel C, editor. Handbook of mutagenicity test procedures. Amsterdam: Elsevier; 1984. p. 335–73.

44. Laü S. Chromosome exposure. Nat Struct Mol Biol. 2007;14:794.

45. Singh MP, Reddy MM, Mathur N, Saxena DK, Chowdhuri DK. Induction of hsp70, hsp60, hsp80 and oxidative stress markers in benzene, toluene and xylene exposed Drosophila melanogaster: role of ROS generation. Toxicol Appl Pharmacol. 2009;235:226–43.

46. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–75.

47. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–7.

48. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47:389–94.

49. Habig WH, Pabst MJ, Jakoby WB. Glutathione S transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249:730–9.

50. Nishikimi M, Appaji Rao N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972;46:849–94.

51. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analy Biochem. 1979;95:351–8.

52. Kastenbaum MA, Bowman KO. Tables for determining the statistical significance of mutation frequencies. Mutat Res. 1979;90:527–49.

53. Würgler FE, Graf U, Bechtold W. Statistical problems connected with the sex-linked recessive lethal test in Drosophila melanogaster. I. The use of the Kastenbaum-Bowman test. Arch. für Genet. 1975;45:158–78.

54. Goldstein M, Kastan MB. The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annu Rev Med. 2015;66:129–43.

55. Haraki S, Kotsinas A, Chronopoulous E, Kletas D, Georgakilas A, Gorgoulis VG. The role of oxidative DNA damage in radiation induced bystander effect. Cancer Lett. 2013;356:43–51.

56. Iyer R, Lehert BE. Effects of ionizing radiation in targeted and nontargeted cells. Arch Biochem Biophys. 2000;376:14–25.

57. Dzdaroglu M, Jaruga P. Mechanisms of free radical-induced damage to DNA. Free Radic Res. 2012;46:382–419.

58. Hayatsu H, Inada N, Kukatani T, Arimoto S, Negishi T, Mori K, et al. Suppression of genotoxicity of carcinogens by (−)-epigallocatechin gallate. Prev Med. 1992;21:370–6.

59. Das CD, Araujo BC, Dutra ES, Nepomuceno JC. Protective effects of β-carotene against the genotoxicity of doxorubicin in somatic cells of Drosophila melanogaster. Genet Mol Res. 2009;8:1367–75.

60. Davari H, Haddad F, Mohammari A, Farhad Rahimi M, Ghavamnasiri MR. Study of radioprotective effect of green tea against gamma irradiation using micronucleus assay on binucleated human lymphocytes. Iran J Basic Med. 2012;15:1026–31.

61. Richi B, Kale RK, Tiku AB. Radio-modulatory effects of Green Tea Catechin EGCG on pBR322 plasmid DNA and murine splenocytes against gamma-radiation induced damage. Mutat Res. 2012;747:62–70.
69. Salvadori DMF, Ribeiro LR, Xiao Y, Boei JJ, Natarajan AT. Radioprotection of β-carotene evaluated on mouse somatic and germ cells. Mutat Res. 1996;356:163–70.

70. Nanjo F, Honda M, Okushio K, Matsumoto N, Ishigaki F, Ishigami T, et al. Effects of dietary tea catechins on alpha-tocopherol levels, lipid peroxidation, and erythrocyte deformability in rats fed on high palm oil and perilla oil diets. Biol Pharm Bull. 1993;16:1156–9.

71. Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: a literature review. Chin Med. 2010;5:13.

72. Xinlin WEI, Ying LIU, Xiao J, Wang Y. Protective effects of tea polysaccharides and polyphenols on skin. J Agric Food Chem. 2009;57:757–62.

73. Frei B, Higdon J. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr. 2003;133:3275S–84S.

74. Lin Y, Cheng C, Lin Y, Lau Y, Juan I, Lin J. Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione s-transferase in rats. Animals. 1998;8561:1893–9.

75. Das DK, Sinha M, Khan A, Das K, Manna K, Dey S. Radiation protection by major tea polyphenol, epicatechin. Int J Hum Genet. 2013;1:3:59–64.

76. Kasperczyk S, Dobrakowski M, Kasperczyk J, Ostaslawska A, Zalejska-Fiolk J, Birink E. Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers. Toxicol Appl Pharmacol. 2014;282:6:36–41.

77. Ben-Amotz A, Yatziv S, Sela M, Greenberg S, Rachmilevich B, Shwarzman M, et al. Effect of natural beta-carotene supplementation in children exposed to radiation from the Chernobyl accident. Radiat Environ Biophys. 1998;37:187–93.

78. Chen ZY, Chan PT. Antioxidative activity of green tea catechins in canola oil. Chem Phys Lipids. 1996;82:163–72.

79. Konopacka M, Widel M, Rzeszowska-Wolny J. Modifying effect of vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in mouse cells. Mutat Res. 1998;417:85–94.

80. Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res. 2007;55:207–16.

81. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med. 1996;334:150–5.

82. The Alpha-Tocopherol Beta Carotene Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 1994;330:1029–35.

83. Abraham SK. Antigenotoxicity of coffee in the Drosophila assay for somatic mutation and recombination. Mutagenesis. 1994;9:383–6.

84. Abraham SK, Graf U. Protection by coffee against somatic genotoxicity in Drosophila role of bioactivation capacity. Food Chem Toxicol. 1996;34:1:1–14.

85. Graf U, Abraham SK, Guzmán-Rincón J, Würgler FE. Antigenotoxicity studies in Drosophila melanogaster. Mutat Res. 1998;402:203–9.

86. Patenkovic A, Stamenkovic-Radak M, Banjanac T, Andjelkovic M. Antimutagenic effect of sage tea in the wing spot test of Drosophila melanogaster. Food Chem. 2009;47:180–3.

87. Costa WF, Nepomuceno JC. Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of Drosophila melanogaster. Environ Mol Mutagen. 2006;47:18–24.