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Ameliorating effect of quercetin against UV radiation-induced damage in *Drosophila melanogaster*

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ABSTRACT: Quercetin is a plant flavonoid found in various fruits, leaves such as tea, vegetables and has been extensively studied due to its antioxidative, anticancer, anti-inflammatory and anti-neurodegenerative effects. UV radiation is harmful for human being as it may cause several complications such as skin cancer. Fruit fly (*Drosophila* sp.) has long been used as an arthropod model for genetics related studies. In the present study, the protective effect of quercetin is evaluated against UV-C radiation induced damage using *Drosophila melanogaster*. Pre-treatment with quercetin (10 μ M) recovered the shortened lifespan caused by UV radiation and has also increased eclosion rate and the dose of quercetin is lower than the previously reported doses of other flavonoids. Flies subjected to moderate dose of UV radiation showed distinct abnormal characters such as incomplete abdominal pigmentation, curly wings or outstretched wings, whereas quercetin pretreatment showed no such abnormal characters or mutant phenotypes. There is a considerable amount of change in the enclosed adult fly size, pupal size and pupal migration distance as well. Gel electrophoresis study of salivary gland DNA of *D. melanogaster* demonstrates the efficacy of quercetin in conferring protection to DNA against UV radiation-induced damage. Therefore, it can be concluded that quercetin may act as an effective protective agent against UV radiation-induced damage.

Keywords: Quercetin; Flavonoid; UV radiation; Radioprotection; Fruit fly; Eclosion.

1. INTRODUCTION

Flavonoids are a class of plant secondary metabolites. A few of them are considered as dietary flavonoids. It is present in various plants such as onions, apples, tea leaves and many other fruits and vegetables. One of the most common and extensively studied plant flavonoids is quercetin (Figure 1). It has been found that the consumption of fruits and vegetables which are rich in quercetin is associated with positive health effects [1-3]. This chemical has been extensively studied due to its antioxidative [4], anti-cancer [5], anti-inflammatory [6], radioprotective [7-8] and anti-neurodegenerative effects [9].

Fruit fly (*Drosophila* sp.) has long been used as an arthropod model for genetics related studies. In last few decades, such model organism has made it possible to identify various pathways that regulate lifespan of

the flies [10]. Low dose irradiation has been found to change the life span of *Drosophila* depending on the genotype [11].

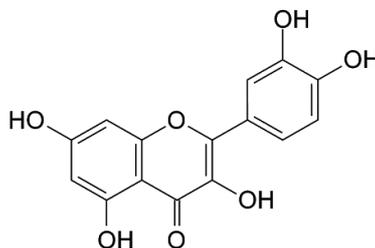


Figure 1. Chemical structure of quercetin.

Ultraviolet (UV) radiation is one of the most common types of non-ionizing radiation. It is a part of the electromagnetic spectrum of sunlight. Although the energy carrying capacity of UV radiation is very low and this radiation cannot remove electron or ionize molecules easily but they produce charged ions while passing through the matter. In general, there are three types of UV rays according to their wavelength viz. UV-A, UV-B and UV-C [12]. Among them UV-C is the most damaging and harmful one, having wavelengths of 100-280 nm. All of the UV-C rays and almost 90% of the UV-B (280-315 nm) are absorbed by the atmospheric ozone layer. Remaining 10% of the UV-B and most of the radiation in the UV-A (315-400 nm) reach the earth's surface. UV-B can only penetrate up to epidermis of human skin. Shorter exposure to UV-B helps in the synthesis of vitamin D in our skin; while longer exposure can cause skin burn, skin damage, skin cancer, eye damage [13-14]. UV-A can make its entry starting from epidermis making all the way to hypodermis layer easily. This radiation can cause skin ageing, wrinkling and in most cases, damage keratinocytes which are present in the basal layer of epidermis [12, 15]. Most of the skin cancers are caused by this UV-A radiation [13]. UV-C has germicidal activity [16]. However, unintentional overexposure or accidental exposure to UV-C causes skin redness and eye irritation [17-18]. Hence, indeed UV radiation is harmful for human being and can cause severe complications including skin cancer.

2. METHODOLOGY

2.1. Fly culture

All experiments are performed using wild type *D. melanogaster*. These flies are maintained and raised in the laboratory stock centre on cooked food composed of standard maize powder or cornmeal, brown sugar, Baker's yeast or CSY medium (cornmeal 31.25 g, brown sugar 31.25 g, Baker's yeast 18.75 g), agar agar (6.25 g), propionic acid, nipagin dissolved in ethanol (1 ml). The incubator is maintained at 24°C with 40% humidity [19].

2.2. Quercetin treatment

After moderate boiling and thorough mixing, the food is cooled, poured into glass vials of 3 cm diameter, and are allowed to cool and harden in room temperature. For quercetin treated culture sets i.e. Q, Pre-Q and Post-Q, 10 µM of quercetin (Sigma-Aldrich) is added prior to hardening of culture medium i.e. food. Each vial is filled with 5 ml of culture medium and is plugged with sterilized cotton. Five different set of cultures are made – control (C), quercetin treated (Q), UV treated (UV), quercetin treatment after UV exposure (Post-Q) and quercetin treatment prior to UV exposure (Pre-Q). All experiments are performed in triplicates. For each experimental set i.e. C, Q, UV, Post-Q and Pre-Q, a total of 2100 flies are used.

2.3. Exposure to UV radiation

For the UV and Post-Q culture sets, each of the samples is subjected to UV exposure for a period of 60 seconds. The source of UV used is UV-C (180 J/m^2). For the Pre-Q culture set, larvae are grown in culture medium containing quercetin. Freshly eclosed flies from that generation are allowed to lay eggs overnight. From those eggs when third instar larvae are obtained, those larvae are treated with UV rays for a period of 60 seconds.

2.4. Pupation rate, pupal size and pupal migration distance

The newly formed pupae are counted once a day from the onset of pupariation and also the larvae that pupariated on the medium are also counted. Pupae developed from all the five different culture sets are measured using slide calipers. Each pupa is marked on the vial wall and counted. The sizes of the pupae obtained from those larvae are measured using slide calipers. The three larval instars show two very important behaviours: they migrate towards the culture media which is referred to as feeding stage and the mature third instar larva usually have a propensity to migrate towards the cotton plug prior to pupal stage. This migration distance of the larvae from the culture media towards cotton plug is measured for all five culture sets.

2.5. Ecdysis rate, fly size and microscopic observation

The third instar larvae of fruit fly from the five different culture sets are subjected to irradiation at several doses in order to determine the optimal dose to analyze the effects of quercetin. From the total number of pupae obtained from the previous experiment are allowed to develop into adult fly. The total numbers of adult eclosed flies are counted. The size of each freshly eclosed flies are measured. Each fly is observed under binocular microscope (Magnus MS24, India) for any abnormal morphological characteristics.

2.6. DNA damage assay from salivary gland of *Drosophila*

DNA was isolated from the salivary gland of the third instar larvae of five different culture sets and was subjected to agarose gel electrophoresis (Genei mini sub system, India).

2.7. Statistical analysis

All obtained demographic data are presented as the mean \pm SEM and analyzed with Origin Pro 8 software.

3. RESULTS AND DISCUSSION

Quercetin, rutin and many other flavonoids have a high antioxidant activity and thus have been widely studied in the fields of food science and nutrition science [20-21]. Rutin is a natural flavonoid contained in fruits and vegetables and is one of the glycosylated derivatives of quercetin [22]. The radioprotective effects of quercetin and rutin against gamma radiation have been confirmed in Swiss albino mice previously [23-24]. Curcumin is an active component of turmeric just like quercetin of tea leaves, onion etc. This yellow coloured plant phenolic compound which possesses therapeutic properties has various health benefits [25-32]. In the present work, the probable radioprotective effects of bioflavonoid quercetin have been explored against UV radiation.

It is evident from the Figure 2 that the pupation rate of the Post-Q ($80.8 \pm 2.63\%$) and Pre-Q ($83.1 \pm 0.5\%$) flies are much higher than that of the UV treated larvae ($37.8 \pm 1.27\%$). The pupation rate of pre-quercetin treated flies is much higher than that of the only UV treated larvae.

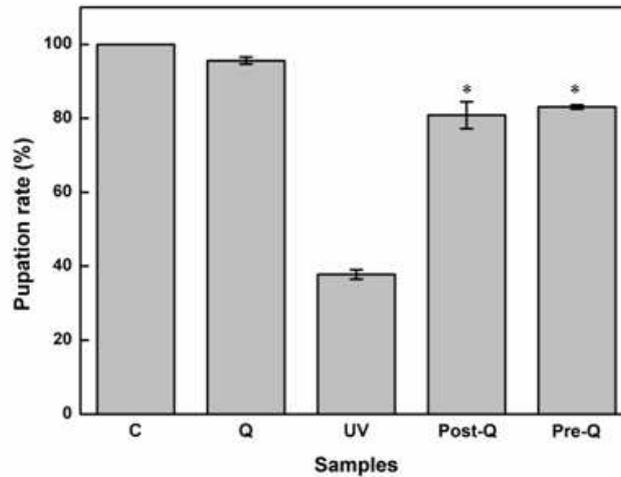


Figure 2. Effect of quercetin on pupation rate: Control (C), Quercetin (Q), UV treated, Post-Q and Pre-Q (* $p < 0.05$ as compared to radiation alone group).

It is also found that quercetin treated pupae showed a moderate increase in their size (Figure 3). Although the differences are minute but the pupae size of the Post-Q (2.3 ± 0.03 mm) and Pre-Q (2.46 ± 0.01 mm) treated pupae are comparatively higher than the pupae size of the UV treated (2.2 ± 0.03 mm) third instar larvae. The control pupa size in this case is 2.55 ± 0.04 mm. The pupae size experiment also reflects the same trend and supports the previous experiment, although the differences are not that much significant.

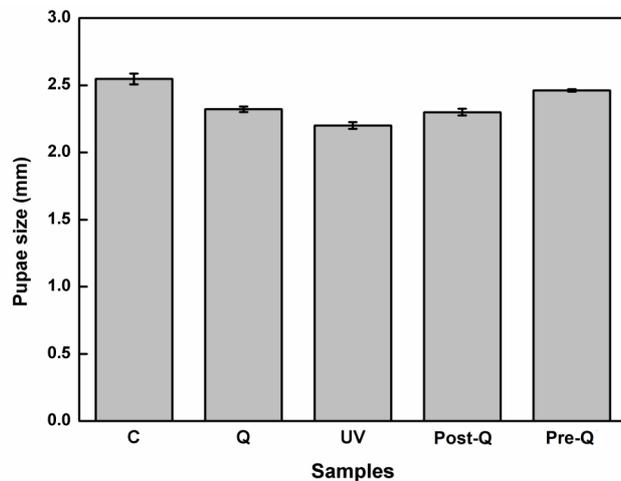


Figure 3. Effect of quercetin on the size of pupae: Control (C), Quercetin (Q), UV treated, Post-Q and Pre-Q.

Pupal migration distance from the culture medium is another parameter to assess the effect of low-dose radiation effects on fruit fly. The mean migration distance of control (C) and positive control (Q) are 7.0 ± 0.02 cm and 5.2 ± 0.11 cm respectively. There is a significant increase in the migration distance of the quercetin treated UV exposed larvae i.e. Post-Q (3.3 ± 0.09 cm) and Pre-Q (4.2 ± 0.16 cm) compared to the UV treated ones (1.2 ± 0.08 cm). The effect of UV radiation minimizes the migration rate of the pupae, which is successfully recovered by the pre-treatment of quercetin (Figure 4).

Larvae pre-treated with quercetin and developed into adult flies have showed significant increase in their mean eclosion rate. The mean eclosion rate of the quercetin control is $87.5 \pm 1.67\%$. There is a drastic decrease in the eclosion rate of UV treated larvae ($22.5 \pm 0.84\%$) whereas treatment with quercetin has recovered the eclosion rate to some extent. The eclosion rate of Post-Q larvae is $64.9 \pm 1.44\%$ and that of Pre-

Q larvae is $70.1 \pm 1.0\%$. The eclosion rate of fruit flies is recovered three times with the pre-treatment of quercetin than that of only UV treated flies (Figure 5). Quercetin reversed shortened lifespan of irradiated flies as well as increased the pupation and eclosion rate.

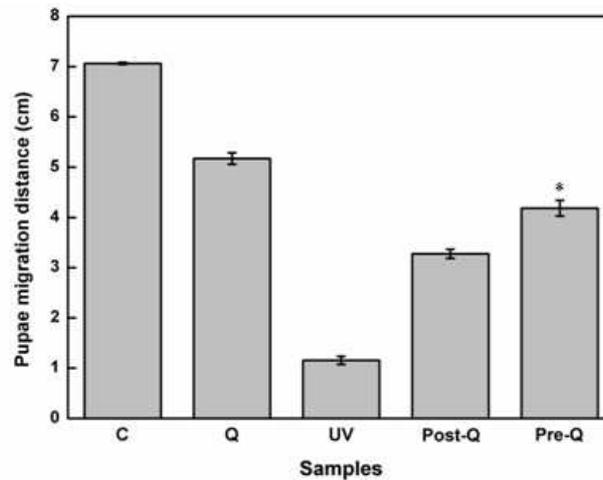


Figure 4. Effect of quercetin on the migration distance of *Drosophila* pupae: Control (C), Quercetin (Q), UV treated, Post-Q and Pre-Q (* $p < 0.05$ as compared to radiation alone group).

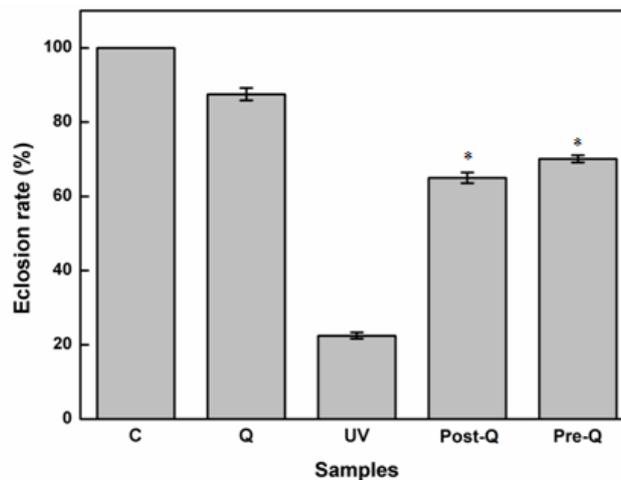


Figure 5. Effect of quercetin on eclosion rate: Control (C), Quercetin (Q), UV treated, Post-Q and Pre-Q (* $p < 0.05$ as compared to radiation alone group).

In enclosed fly size experiment (Figure 6), the size of control adult female flies are 2.5 ± 0.01 mm and of control male flies are 2.2 ± 0.01 mm. Size of the UV treated adult female flies are 2.3 ± 0.03 mm and of male flies are 1.7 ± 0.03 mm. Larvae fed with quercetin have showed a minute increase in fly size prior to UV exposure i.e. Pre-Q (2.4 ± 0.01 mm for female and 2.1 ± 0.03 mm for male). Fly size of the UV pre-exposed and quercetin post-treated larvae are 2.4 ± 0.01 mm for female flies and 1.9 ± 0.04 mm for male flies. In this study, the male flies show more severe effect of UV radiation than the female flies; however, in case of both the sexes, quercetin ameliorates the effects of radiation. This sex specific radiation resistance may be due to the presence of only one X chromosome in males, whereas two X are present in case of female flies.

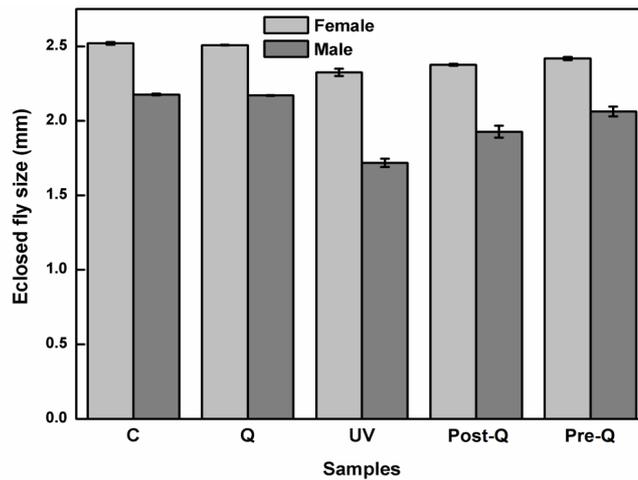


Figure 6. Effect of quercetin on the size of enclosed adult *Drosophila*: Control (C), Quercetin (Q), UV treated, Post-Q and Pre-Q.

When third instar larvae are exposed to UV radiation for a period of 60 seconds, and are allowed to develop into adult flies they showed some unusual characters such as incomplete abdominal pigmentation, curly wings, outstretched wings; whereas quercetin pre-treatment showed no such abnormal characters or mutant phenotypes. From the Figure 7 (a-d), it is evident that freshly eclosed female fly which is UV treated at its larval stage have showed incomplete abdominal pigmentation at the left lateral side of the body. Figures 7 (e, g, h) depicts UV treated male flies that have incomplete abdominal pigmentation. In the Figure 7 f, there is a male fly in which one wing has not emerged probably during its eclosion. Among two flies in the Figures 7 (i, j), Figure 7 (i) shows normal male fly which has its wing running parallel to its body axis; whereas in the Figure 7 (j), the male fly shows outstretched wings i.e. a mutant phenotype along with incomplete abdominal pigmentation. In quercetin treated sets, no such abnormal flies are detected. Figures (k, l) show normal female fly and normal male fly respectively.

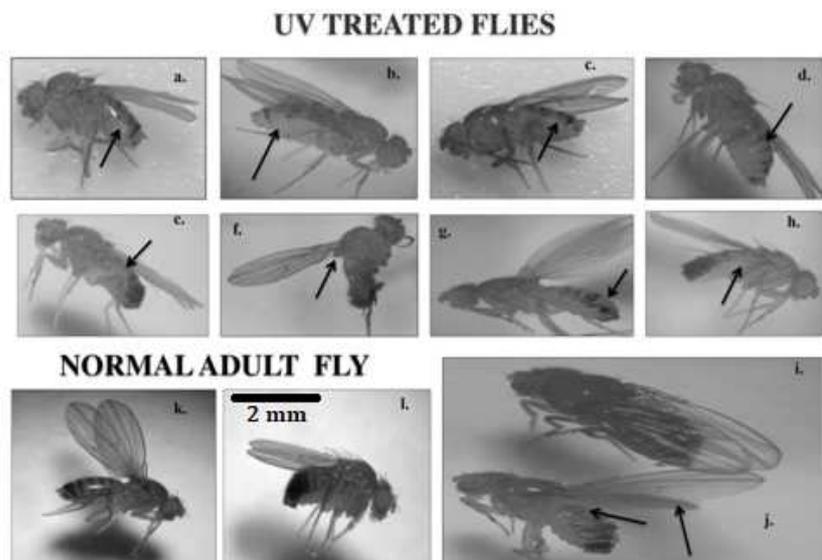


Figure 7. UV-induced deformities of *D. melanogaster*: (a-d) incomplete abnormal pigmentation in female flies; (e,g,h) incomplete abdominal pigmentation in male flies; (f) single winged male fly; (i) normal male fly; (j) deformed wing and incomplete abdominal pigmentation in male fly; (k) normal female fly; (l) normal male fly. All arrows indicate the abnormal characteristics.

Isolated DNA from the salivary gland of *Drosophila* is subjected to agarose gel electrophoresis (Figure 8). Lane 1 and 2 shows DNA isolated from control (C) and positive control larvae (Q). Lane 3 shows salivary gland DNA of UV treated larvae (UV). Lane 4 and 5 represents DNA from Post-Q and Pre-Q salivary glands of larvae, respectively. Salivary gland DNA isolated from the UV treated larvae when are subjected to agarose gel electrophoresis moves further than the DNA of Post-Q and Pre-Q larvae. The control DNA (lane 1) has similar position with respect to DNA of Pre-Q larvae (lane 5), which indicates the protective effect of quercetin against UV radiation induced DNA damage.

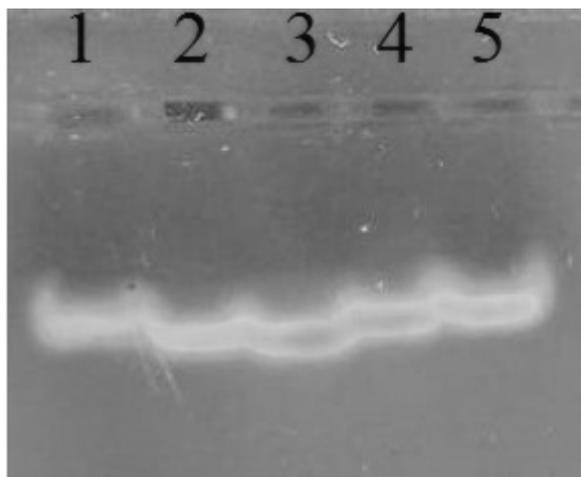


Figure 8. Effect of quercetin on salivary gland DNA of *Drosophila*. Lane 1 – control, Lane 2 – quercetin treated sample, Lane 3 – UV treated sample, Lane 4 – Post-Q sample, Lane 5 – Pre-Q sample.

An important risk factor in skin carcinogenesis is solar UV radiation. High energetic photons are capable of interacting with DNA and it also induces DNA damage. In order to determine the effect of UV radiation on SKH-1 mice in relation to DNA damage, a study was conducted, which revealed the increase in single strand breaks [33]. Bulky photodimers are generated at di-pyrimidine sites due to solar UV radiation. It also induces single strand breaks and various types of oxidative lesions [34]. UV radiation is capable of inducing single strand breaks in the nuclear DNA of humans as well [35]. A variety of mutagenic and cytotoxic DNA lesions such as cyclobutane pyrimidine dimers (CPD), 6-4 photoproducts (6-4PPs) and DNA strand breaks are generated due to one of the powerful mutagenic agent i.e. UV radiation [36]. Single strand breaks and oxidised bases are generated due to UV-A radiation [37]. Relatively low dose of UV ray induces the formation of DNA strand breaks [38]. CPDs and 6-4PPs are the two type of UV-B induced DNA damage [39]. Single strand breaks are generally predominant just after UV irradiation [40]. Such studies confirm that UV radiation can induce the formation of DNA strand breaks along with other molecular changes. Hence, the present study on salivary gland DNA of fruit fly successfully proves the protecting effect of quercetin against UV radiation induced DNA damage.

Moreover, it is already reported that curcumin pre-treatment of concentration 100 μM mitigates the effects of gamma irradiation on *Drosophila* [27]. But, quercetin pre-treatment has recovered the shortened eclosion rate caused by UV radiation at comparatively lower dose of quercetin (10 μM) than the dose of curcumin as reported by Seong et al. [27]. It confirms the efficacy of quercetin as a potent radioprotector. The present study unravelled the UV protective potential of quercetin on *D. melanogaster*. From the results, it is evident that quercetin has the efficacy to render UV protection on fruit flies. However, this effort to promote

quercetin as antioxidant supplement and probable radioprotector against UV radiation-induced damage warrants further *in vivo* studies with clinical trials for the benefit of human being.

Authors' Contributions: Conceived idea and designed the experiments: SP, DM. Performed the experiments: SM, DM, SP. Analyzed the data: SP, DM, MB. Contributed reagents/materials/analysis tools: DM, SP. Contributed to writing of manuscript: SP, DM, MB, SM. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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