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Article

The effect of ultraviolet radiation and the antioxidant curcumin on the longevity, fertility, and physical structure of *Drosophila melanogaster*: Can we defend our DNA?

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SUMMARY

Ultraviolet (UV) radiation is known to alter DNA structure and impair cellular function in all living organisms. One proposed mechanism of injury involves the production of harmful free radicals. The DNA damage caused by UV radiation can lead to a myriad of medical issues, but there is limited research on potential rescue interventions. However, studies have suggested that naturally occurring antioxidants may exert their positive influence on an organism by reducing oxidative injury, which leads to the purpose of this project: to study the effects of UV radiation and determine whether antioxidant-enriched nutrition can combat the potential deleterious effects of UV radiation on *Drosophila melanogaster*. We hypothesized that UV radiation would diminish the lifespan and fertility of *Drosophila*, as well as causing physical abnormalities. We also predicted that *Drosophila* cultured in the presence of media enriched with the antioxidant curcumin would have enhanced lifespans and fertility. Finally, we hypothesized that raising *Drosophila* with curcumin-enriched media, would diminish the negative impact of UV radiation on the organism's longevity and fertility. We found that UVB (320nm) radiation caused a 59% decrease in the *Drosophila* lifespan and mutagenic effects on flies' physical appearance, but did not significantly affect fertility. Curcumin significantly prolonged lifespan and enhanced fertility for both UV- and non-UV-exposed flies. Therefore, we conclude that curcumin can prolong lifespan, enhance fertility, and mitigate the deleterious effects of UV radiation on *Drosophila*. Our research demonstrates that we can harness the positive potential of natural antioxidants and use them as weapons in our war against radiation-induced diseases, including conditions like cancer.

INTRODUCTION

The genetic code, inscribed in the DNA of each species, is vital to the unique and efficient functioning of every living organism [1]. However, this genetic code is not securely protected. External forces may influence our genetic instructions and cause devastating diseases such as cancer. One such environmental force is ultraviolet (UV) radiation, which is on the rise due to depletion of atmospheric ozone [2]. Ultraviolet radiation can cause both direct and indirect damage to the DNA of living organisms [3]. Cells do have some repair mechanisms to fix the damage, but these pathways are not always completely successful [3]. For example, the form of UV radiation known as UVB (290-320 nm wavelength), can damage living cells and alter the molecular structure of DNA by causing physical breaks or mutations in DNA structure [4], a phenomenon that is implicated in carcinogenesis. UV radiation can also cause indirect DNA damage by creating free radicals. Free radicals are molecules that are highly reactive due to the presence of unpaired electrons. Such hydroxyl radicals can attack the DNA backbone and bases, potentially causing cells to die or develop mutations. [3]. By causing damage to cells and DNA, free radical build-up can lead to cancer and other diseases.

Just as the environment can negatively influence our genetic information, some naturally occurring protective factors have also been identified. One such tool is a set of compounds called antioxidants [5]. Endogenous antioxidants are those which living organisms may produce for the sole purpose of neutralizing free radicals by donating electrons while exogenous antioxidants are extracted from external sources such as food and supplements. Beta-carotene, lycopene, and vitamins A, C, and E are primary forms of exogenous antioxidants. Studies in the 1990s reported that individuals with a low intake of antioxidant-rich food were at a greater risk for developing chronic diseases, including cancer [5]. However, studies that looked at the relationship between antioxidants and conditions like cardiovascular disease and cancer did not find convincing evidence for antioxidants' protective effects. It is important to note, however, that those trials were of short duration, were conducted in people with existing diseases, and utilized artificially manufactured antioxidant supplements [5].

Prior research has looked at UV-radiation-induced DNA damage in animal models, including *Drosophila melanogaster*, or the simple fruit fly, and shown that such radiation can have harmful effects on flies' lifespan, fertility and physical structure [6, 7]. Research has also shown that antioxidants such as curcumin can extend the *Drosophila* lifespan [8]. *Drosophila* also has a short, simple reproductive cycle lasting about 8-14 days, so several generations can be observed in weeks [8]. Curcumin is a natural, organic antioxidant found in

turmeric, a spice which is consumed and used for medicinal purposes in Asia [9]. Curcumin has been shown to scavenge free radicals such as the superoxide anion and to exhibit anti-cancer properties [9].

Based on a thorough review of the scientific literature, we posed the following questions: If UV radiation damages DNA at least in part by creating free radicals, and antioxidants are effective neutralizers of those free radicals, can we use one agent to protect us from the harmful effects of the other? If an organism is provided with antioxidant-rich nutrition, will this diet make it less likely to succumb to the harmful effects of UV radiation? To our knowledge, these questions have never been answered by the scientific community. We chose to study these questions using *Drosophila melanogaster* for several reasons. They are small and inexpensive to maintain, and 75% of the genes that cause disease in humans are also found in the fruit fly [10].

We designed an experiment to study the effects of UV radiation and antioxidant-enriched nutrition on *Drosophila* and to study whether the potential beneficial effects of antioxidant treatment can mitigate the harmful effects of UV radiation. We hypothesized that: 1) flies exposed to UV radiation will show reduced lifespan and fertility, as well as physical abnormalities; 2) flies cultured with curcumin-enriched media will show increased lifespan and fertility; and 3) flies cultured with curcumin-enriched media will be protected against the negative impact of UV radiation on longevity and fertility. As a result of these experiments, we concluded that treatment with the antioxidant curcumin increases the fertility of fruit flies, both in UV- and non-UV-exposed flies. UV radiation caused devastating mutagenic effects to the physical structure in both parent and offspring generations, including tumors and crippling wing mutations. UV radiation also caused a significant decrease in lifespan, but this effect was counteracted by treatment with curcumin. Additionally, curcumin treatment increased *Drosophila* lifespan and even had the ability to mitigate the effect of UV radiation on lifespan. Therefore, curcumin has the ability to act as a preventative measure against ultraviolet radiation and resulting health risks in flies.

RESULTS

We placed *Drosophila* cultures into four experimental groups: control, UV-exposure only, curcumin-enriched media only, and UV exposure with curcumin-enriched media. The UV group was exposed to UVB (320 nm) radiation in a dark room for three minutes. We transferred flies into fresh vials every four days and recorded numbers of alive flies. To assess fertility, we anesthetized flies and separated them by sex. Five flies of each sex were placed into each vial. We used the same four experimental groups. We then counted the larvae and pupae on days 5 and 10 similar to previously published methods [6]. To study physical structure, 4-5 flies were anaesthetized and their physical characteristics were observed. After UV exposure, we examined flies for any mutagenic effects on both first and second generations.

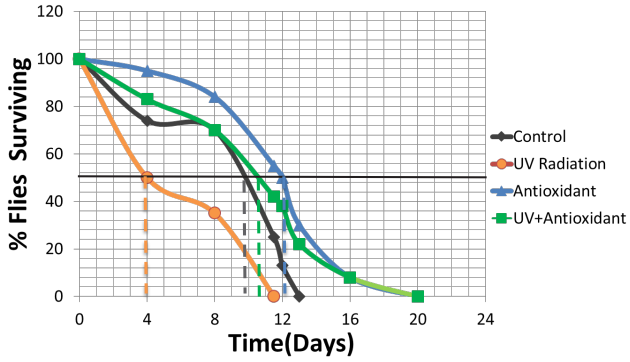

Figure 1. *Drosophila* longevity of all experimental groups. This graph displays the relative survival rates for all experimental groups as well as the 50% Cohort Survival (n = 20-25 flies per vial). UV radiation alone caused a significant decrease in lifespan, which was associated with an extremely steep slope. The antioxidant nutrition was somewhat able to mitigate this effect.

At each time point studied, the antioxidant group did the best in terms of survival and the UV-exposed group did the worst (**Figure 1**, **Table 1**). Importantly, flies that were given curcumin-enriched media but were also exposed to UV radiation lived longer than control flies, but not as long as those who were given the same media but not exposed to radiation. This demonstrates that the antioxidant was able to partially protect the *Drosophila* from radiation-induced injury.

We assessed fertility by counting larvae and pupae on days 5 and 10. By day 10, the antioxidant group had many more offspring (mean = 44.7, SEM = 21.1) than either the control (mean = 7.0, SEM = 3.2) or UV-exposed (mean = 8.7, SEM = 3.5) groups (**Table 2**, **Figures 2-3**). On day 10, we also observed that the UV + antioxidant group (mean = 40.0, SEM = 22.0) had greater survival than the control or UV-only groups (**Table 2**, **Figure 2-3**). We also found that the *Drosophila* that were raised on antioxidant-rich media had far more pupae than those without. In summary, UV radiation did not significantly decrease the reproductive potential of *Drosophila*. We also found that antioxidant treatment led to

Table 1. Time taken for 50% of flies to die.

Experimental Condition	Mean (Standard Error)	
	Day 5	Day 10
	Larvae and Pupae	Larvae and Pupae
No UV, no antiox.	0.7 (0.58)	7.0 (3.21)
+UV only	0.3 (0.33)	8.7 (3.48)
+Antioxidant	3.7 (1.40)	44.7 (21.07)
+Antioxidant, +UV	1.7 (1.67)	40.0 (22.01)

Table 2. Effect of UV radiation and curcumin of *Drosophila* fertility.

Experimental Condition	Days
No UV, no antiox.	9.7
+ UV only	4.0
+ Antioxidant only	10.5
+Antioxidant, +UV	12.0

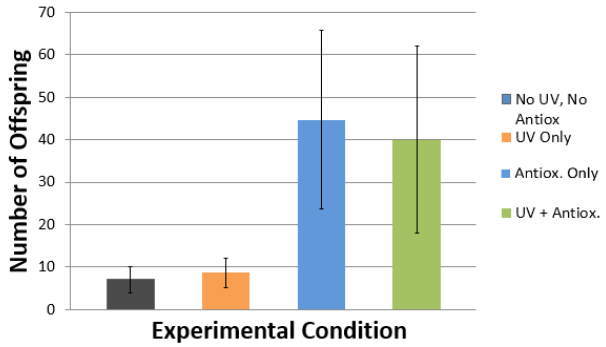

Figure 2. Fertility results on day 10 . Shown here is the average number of offspring (larvae/pupae) per experimental group on Day 10. UV radiation did not influence fertility. However, antioxidant nutrition significantly enhanced fertility in flies with and without exposure to UV radiation. (5 male and 5 female flies per vial)

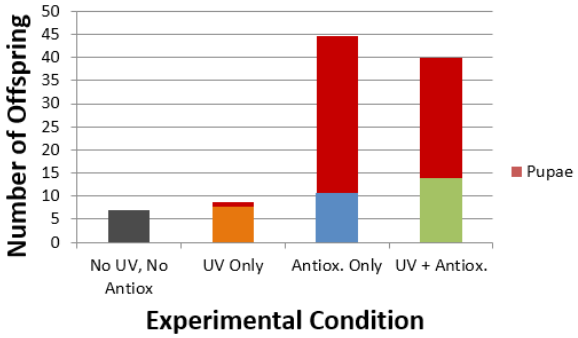

Figure 3. Fertility-larvae and pupae day 10. This figure displays the distribution of offspring among larvae and pupae for each experimental group. The antioxidant nutrition groups seemed to have an accelerated life cycle with more pupae present on day ten compared to larvae. (5 male and 5 female flies per vial)

an increased number of offspring, even in flies exposed to UV radiation, but this effect was not statistically significant (p = 0.2464, two-way ANOVA).

In terms of physical structure, *Drosophila* exposed to UV radiation showed a darkened exoskeleton. The offspring of UV-exposed flies showed many more mutagenic effects (**Figure 4**), including vestigial and curved wing structures, which are well-established mutations that map to chromosome 2 [11]. We also found abnormal head growths and tumors. In addition, we observed that second-generation larvae and pupae had very translucent body chambers compared to the more-opaque controls, perhaps making them more susceptible to environmental insults.

DISCUSSION

The purpose of this experiment was to determine the effects of UV radiation and antioxidant (curcumin)-rich nutrition on the phenotype of *Drosophila melanogaster*, as well as to assess whether antioxidant treatment could diminish the harmful effects of UV radiation on the organism. Our hypotheses were partially supported by our results. UV radiation appeared to decrease *Drosophila* lifespan based on the form of the longevity curves, however, this was not subjected to a test of statistical significance. UV radiation did cause mutagenic effects on their physical structure. However, UV radiation did not decrease the fertility of *Drosophila*. Antioxidant-rich nutrition, in the form of curcumin-enriched media, appeared to increase *Drosophila* lifespan and fertility but the latter effect was not statistically significant. Although not subject to statistical testing, our data suggested that curcumin treatment may enable UV-exposed flies to live much longer than both flies who only received UV radiation and control flies. Lastly, curcumin treatment increased the number of larvae and pupae produced, even after the flies been exposed to UV radiation. We recognize that our quantitative data is preliminary and not subject to rigorous statistical testing, however, it does suggest an interesting trend it terms of the harmful effects of UV radiation and the potentially beneficial effects of the antioxidant, curcumin. Due to the fact that *Drosophila* share 75% of the genes that cause diseases in humans, the data collected in this experiment would be clinically relevant to a degree, but with some discrepancies due to the natural differences between *Drosophila* and humans. A clinical trial or further research in mammalian systems would be useful in order to solidify the validity between our data and its effects on humans.

The decrease in *Drosophila* lifespan due to UV radiation may be caused by DNA damage and the induction of cancer, as has been previously described [10]. It could also reflect tissue injury and aging mechanisms caused by excessive free radical production [11]. Prolongation of lifespan by curcumin supports prior literature [9], and our data shows, for the first time, that this naturally-derived antioxidant can even mitigate the negative effects on lifespan induced by UV radiation. UV radiation did not affect *Drosophila* fertility, possibly indicating that the rays did not affect gonadal cells or that the DNA repair mechanisms within the reproductive system were more proficient. Antioxidant treatment may enhance

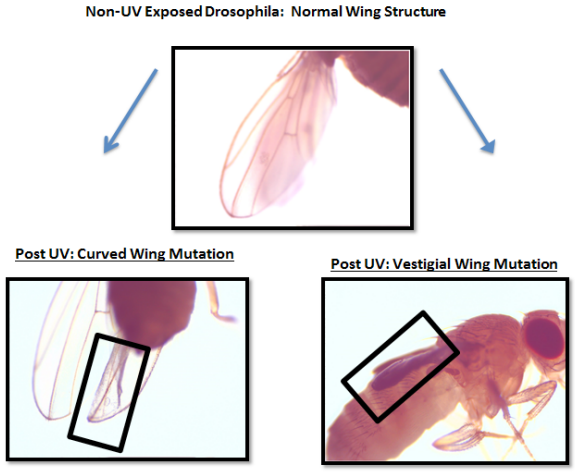

Figure 4. The effects of UV radiation on *Drosophila* physical structure. These figures display the phenotypic effects of UV radiation on *Drosophila*. Dichaite and vestigial wing mutations (in the F1 generation) were produced in response to exposure (n = 15-20 flies per group).

fertility, perhaps by increasing the overall health and vigor of the organism and also indirectly by prolonging lifespan and allowing a longer reproductive time frame. UV radiation produced mutagenic effects on the *Drosophila* physical structure as previously described, especially with regards to wing development [7].

Our findings are extremely exciting and promise a potential breakthrough in the prevention of radiation-induced diseases such as cancer. Our research demonstrates that we can utilize the positive potential of natural antioxidants in our war on cancer. It is possible that antioxidants exert their positive effects by protecting against injury within various organ systems and by scavenging mutation-causing free radicals, which can be formed naturally or under the influence of radiation. The findings of our study have broad implications for the design of preventive approaches against environmental hazards such as UV radiation. Clinical research and human studies are vital to furthering our understanding of this protective phenomenon and its applicability to public health. It would also be extremely informative to study the effect of antioxidant dosages and determine a minimum and maximum effective dosage, as well as toxicity at large doses if applicable.

Our study is limited by a small number of fruit flies, due to space and budget limitations. Future studies should analyze the corresponding DNA changes associated with the phenotypic effects so that we can better understand carcinogenesis. Research in this field is critically necessary to help scientists design novel prevention strategies for devastating diseases, including cancer. While we must continue pursuing research to find a cure for cancer, we should be equally committed to stopping cancer before it even gets started.

METHODS

Experiment 1: Dependent Variable = Longevity

Wild-type *Drosophila melanogaster* were acquired from Carolina Biological Company and maintained at room temperature under normal lighting conditions. Eight vials of culture media were prepared and labeled, two for each of the following experimental conditions: 1) No UV exposure or curcumin treatment; 2) UV exposure only; 3) curcumin treatment; and 4) UV exposure and curcumin treatment. Four vials contained curcumin-enriched culture medium (100 mg curcumin/1 g culture medium). USDA-certified organic curcumin was obtained from Micro Ingredients Company. 15-20 drosophila were placed in each vial.

A 4-watt (254/354 nm) UV lamp was used to irradiate two experimental groups (UV only and UV + antioxidant) in a dark room for three minutes. All vials were placed in a small incubator and maintained at a temperature of 25-28ºC. The adult flies were transferred to new vials every four days to avoid including their offspring in the longevity count. During each transfer, we recorded the dead flies in the old vial, living flies in new vial, and the percentage of flies remaining alive in each of the experimental groups. Data were tabulated and graphed on survival curves.

Experiment 2: Dependent Variable = Fertility

Twelve vials of culture media were prepared and labeled, three for each of the four experimental groups described above. Six vials contained curcumin-enriched culture medium (1 mg curcumin/1 g culture medium). A Drosophila Kit from Carolina Biological was obtained in which sex is matched to eye color: red-eyed flies were female and white-eyed flies were male. Flies were carefully anaesthetized using FlyNap (Carolina Biological) and separated into two groups by gender. Five male and five female flies were placed into each vial. Six vials were irradiated as described above. The larvae and pupae were counted in each vial five and 10 days after UV exposure. Mean offspring and standard error were calculated for each group, and developmental distribution of offspring was noted.

Experiment 3: Dependent Variable = Physical Structure

We examined 4-5 wild-type fruit flies under a microscope for characteristics such as body shape, eye color, and wing anatomy. Microscopic photographs were taken in order to document the standard physical structure. From the same wild-type culture, we collected flies to set up four vials, two each for no-UV and for UV-exposed *Drosophila*. Two vials were exposed to UV radiation as described above. We observed flies for the next three-to-four weeks. After one week, when larvae became visible, we transferred the parental generation to a new vial and studied these flies for physical abnormalities. After three weeks, we studied the second generation for mutagenic effects. Again, we used the FlyNap anesthetizer to immobilize the flies so that they could be observed and photographed under the microscope.

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